

The Netherlands 9-13 June



**EGF**  
**2024**

# Why grasslands?

*Edited by*

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W. Voskamp-Harkema  
A. van den Pol-van Dasselaar



Volume 29  
Grassland Science in Europe

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Proceedings of the 30<sup>th</sup> General Meeting  
of the European Grassland Federation  
Leeuwarden, the Netherlands  
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*Published by*

The Organising Committee of the 30<sup>th</sup> General Meeting of the European Grassland Federation, on behalf of the Dutch-Flemish Society for Grassland and Fodder Crops (NVWV); Steve Bikostraat 300, 3573 BH Utrecht.

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eISBN: 978-90-903-8494-8 / eEAN: 9789090384948

*Abstract submission and evaluation by*



*Editing and production by*

Koninklijke Brill BV  
Plantijnstraat 2  
2321 JC Leiden  
The Netherlands  
[www.brill.com](http://www.brill.com)



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*Distributed by*

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# Foreword

We would like to welcome all delegates of the 30<sup>th</sup> General Meeting of the European Grassland Federation to Leeuwarden, the Netherlands. The Dutch-Flemish Society for Grassland and Fodder Crops (NVWV) is very pleased to organise this meeting for you. Together with partners and many volunteers from the Netherlands and Flanders, we have been preparing for your arrival in Leeuwarden.

Why grasslands? This question lies at the heart of our meeting here in Leeuwarden, June 2024. The role of animals in net food security is a topic of ongoing debate. Europe's vast grassland areas are facing unprecedented threats, with urbanization, conversion to other crops, and other factors leading to their gradual disappearance. The loss of these grasslands may also result in loss of the benefits these grasslands provide. The General Meeting of the European Grassland Federation in 2024 will address this crucial issue, exploring why grasslands are important.

There will be five subthemes:

- Grasslands: What? What is the role of grasslands in net food security?
- Grasslands: How? How do we balance ecosystem services?
- Grasslands: Which? Which methods can be used to monitor, evaluate and steer grassland management?
- Grasslands: Where? Where should we focus on which ecosystem services?
- Grasslands: Whom? For whom are grasslands important?

In the concluding session at the end of the meeting, the question “Why grasslands?” will be answered based on the contributions of the participants.

At EGF2024, we anticipate lively debates, insightful presentations, interesting mid-conference tours and social activities, and above all fruitful exchanges of ideas among researchers, practitioners, and stakeholders from across Europe and beyond. Together, we will explore the multifaceted roles of grasslands and seek innovative solutions to the challenges they face.

We would like to thank all authors for their contributions and the large group of reviewers for their essential support. We extend our gratitude to the Scientific Committee, the Organising Committee, all our sponsors, partners, and volunteers who have generously contributed to the organization of this event. Their support is invaluable and greatly appreciated.

We encourage you to actively contribute to the meeting and we wish that the 30<sup>th</sup> General Meeting of the European Grassland Federation will lead to many new insights and connections!

Dr. Agnes van den Pol-  
van Dasselaar

*President, European  
Grassland Federation*

Dr. Cindy Klootwijk

*Chair Scientific  
Committee*

Dr. Wiepk Voskamp-  
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# “Why grasslands?”: Insights from Farm Case Studies in the Netherlands and Flanders

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## Abstract

The specific use of grasslands depends on factors such as location, land management practices and local policies. In the Netherlands and Flanders, grasslands are mainly used for livestock farming, especially dairy farming. The population density in these regions has led to increased competition for land. In both the Netherlands and Flanders there has been a trend of scaling up and intensification in dairy farming, to meet the growing and changing demand for food and animal protein production. This trend has been driven by high costs for land and labour and low costs for fertilizer and concentrates. Due to increasing competition for land and intensification, grasslands and grazing practices are under pressure in the Netherlands and Flanders. This may lead to additional losses, as grasslands not only provide high quality roughage, but also a variety of ecosystem services. The positive impact of grasslands through its services are gaining attention among Dutch and Flemish dairy farmers. Here we present the results of a survey conducted on ten commercial dairy farms in the Netherlands and Flanders that use grassland and perform grazing. The farms were not selected to be representative, but they do provide a portrait of the variable landscape in the region. Based on the multifaceted benefits of grasslands that resulted from the survey we emphasize the importance of preserving grasslands and grazing in the Netherlands and Flanders, fitting in with the theme of the conference “Why grasslands?”

**Keywords:** ecosystem services, Farm Case Studies, Flanders, grassland use, the Netherlands

## Introduction

The aim of this paper is to provide insight into key developments around grasslands in the Netherlands and Flanders, i.e. the Dutch-speaking part of Belgium. The Netherlands and Flanders are the scope of the Dutch-Flemish Society for Grassland and Fodder Crops (NVWV, [www.nvww.nl/en](http://www.nvww.nl/en)) that organised the 30<sup>th</sup> General Meeting of the European Grassland Federation in 2024. Flanders and the Netherlands have much in common, not only from a linguistic point of view, but also from a historical, geographical/geological/ pedological, demographic and economic point of view.

This paper consists of four parts. First, the literature is consulted to provide an overview of the developments in the past decades. Van den Pol-van Dasselaar *et al.* (2015) and Reheul *et al.* (2017) provided an overview of developments in the Dutch and Flemish grass-based sectors up to the respective years of their publications. Policy changes in the dairy sector, such as the abolition of milk quotas, the introduction of phosphate quotas and the phasing-out of derogation, have further impacted the industry. The derogation allowed dairy farms in the Netherlands under certain circumstances to exceed the standard EU application limits of nitrogen (N) from organic manure. Klootwijk *et al.* (2016) and Alderkamp *et al.* (2024) have analysed the impact of these changes on the dairy sector. Second, the current status of grassland-based farms in the Netherlands and Flanders is outlined. Third, farm profiles of ten commercial dairy farms in the Netherlands and Flanders are used to illustrate grassland use in the local context. Finally, the overview of developments in the sector and the examples of dairy farms are used

to demonstrate the ecosystem services that grasslands provide in the Netherlands and Flanders, which fits well with the conference theme “*Why grasslands?*”

## Recent developments in grassland use in the Netherlands and Flanders

Van den Pol-van Dasselaar *et al.* (2015) showed a trend towards scaling up and intensification on Dutch and Flemish dairy farms to meet the growing and changing demand for food and animal protein production. This trend was driven by high costs for land and labour and low costs for fertilizer and concentrates and led to huge changes in the dairy sector. In the period 1965–2015, the average number of dairy cows per farm in the Netherlands increased tenfold to around 85, the average milk production per cow doubled to just over 8000 kg milk cow<sup>-1</sup>, and the average milk production per ha of total farmland tripled to around 15 000 kg ha<sup>-1</sup>. At the same time, the number of dairy farms declined tenfold to around 18 000 (Van Dijk *et al.*, 2015). The increase in dairy cows per farm, milk production per cow, and milk production per hectare highlights the intensification in dairy farming. High-output dairy farms in the Netherlands and Flanders are characterized by high nitrogen (N) and phosphorus (P) flows, which require efficient nutrient management. Oenema and Oenema (2022) showed the nitrogen and phosphorus use efficiencies (NUE and PUE) in Dutch grassland-based dairy farms after correction for externalization of purchased animal feed and exported animal manure. The corrected NUE varied from 31–33% and the PUE from 44–78% when related to feed efficiency. Van den Pol-van Dasselaar *et al.* (2015) emphasized that challenges and constraints related to nutrient management, grazing, and societal demands need to be addressed for sustainable and profitable farming. They stressed the need for sustainable intensification of dairy farming, taking into account environmental, economic and societal aspects, and highlighted the importance of grassland in addressing these challenges.

The abolition of the European Union’s milk quota system in 2015 has provided a further impetus for the expansion and intensification of the dairy sector. Milk quotas were introduced in 1984 to reduce the oversupply of European milk that affected milk prices at global level. In 2015 they were abolished due to substantial growth in global demand for milk. The effects of the abolition of the European milk quota system in 2015 were examined by Klootwijk *et al.* (2016). The abolition allowed farmers to increase milk production, which also raised concerns about environmental impacts, such as nitrogen and phosphate pollution, and greenhouse gas emissions. The Netherlands, with its high livestock density, faced the challenge of complying with the European Nitrates Directive, which led to the introduction of the so-called “Dairy Act” in 2015 (Klootwijk *et al.*, 2016). This act aimed to support the growth of the dairy sector while limiting phosphate production. Due to the introduction of this Act, further significant growth in farm intensity was deemed to be unlikely (Klootwijk *et al.*, 2016). The study of Klootwijk *et al.* (2016) further showed that several factors, including manure policy, costs, and phosphate quotas, were expected to limit the growth of Dutch dairy farms after quota abolition to a potential increase in farm intensity (in litres milk ha<sup>-1</sup>) of 4–20%.

Currently (2024), stricter nitrogen (N) policies are implemented including the phasing out of the derogation. The derogation allowed dairy farms in The Netherlands to exceed the application standard of 170 kg N ha<sup>-1</sup> from organic manure. Alderkamp *et al.* (2024) investigated the potential impact of these stricter N policies on a typical Dutch dairy farm using a linear programming model with economic optimization. They considered the abolition of the derogation, which is part of the 7<sup>th</sup> Nitrates Action programme, in their model. The results indicated changes in farm dynamics, such as an increase in the share of maize land and a decrease in the number of dairy cows per farm. The results of Alderkamp *et al.* (2024) show that stricter N policies lead to a lower farm income, mainly due to lower revenues from milk yield and higher costs for manure disposal. These economic factors, alongside environmental and legal considerations, play a crucial role in decision-making on the farm.

The study by Alderkamp *et al.* (2024) also evaluated the effects of using grass-clover swards on dairy farms as a strategy to mitigate negative economic consequences of stricter N policies. The use of grass-clover swards showed the potential to partly compensate for the negative economic impacts (50–78%) of derogation phasing out, while at the same time reduce greenhouse gas emissions (2–6%). However, there were trade-offs between economic and environmental objectives, underlining the complexity of decision-making for dairy farmers. The study acknowledged uncertainties and constraints, including the influence of variations in feed availability and quality, market price fluctuations, and challenges associated with implementing grass-clover swards, such as maintaining the desired legume share in the sward.

Prospects for a sustainable intensification of grass and forage crops are given by Reheul *et al.* (2017). Given the scarcity and elevated price of land and labour in the region of Flanders and the Netherlands the concept “more knowledge per ha” offers the best opportunities to improve the eco-efficiency of grass and forage crops. Levers proposed for sustainable intensification are, firstly, cropping system based on ley arable rotations. Especially in regions where the share of forage maize in the arable land is important, ley-arable rotations offer opportunities to reduce the use of mineral N and herbicides (Van Eekeren *et al.*, 2023). Secondly, taking profit of genetic progress in maize and grass and clover varieties can improve feed autonomy and reduce environmental impact. In forage maize breeding, starch content and cell-wall digestibility steadily increased over the last decades. Moreover, very early maturing varieties were bred, which allowed catch crops (Italian ryegrass or rye) to be established after harvesting maize, thus reducing N leaching after maize harvest. In grass breeding, varieties are bred with an improved ability for growing with clover, which could result in more productive and more stable grass-clover leys (Cougnon *et al.*, 2024). In drought resistant species like tall fescue (*Festuca arundinacea*) and cocksfoot (*Dactylis glomerata*), breeding resulted in varieties with an improved feeding value. Thirdly, an efficient and modern mechanisation allowing a timely organisation of forage harvest safeguards the quality of the harvested forage. It can also limit the environmental impact of activities like, e.g., slurry application.

## Current status of grass-based farms in the Netherlands and Flanders

### *Grass-based farms in the Netherlands*

In 2023, there were 3.8 million cattle in the Netherlands (CBS, 2024). The main categories of cattle were dairy cows (1.6 million), youngstock on dairy farms (1.0 million) and veal calves (1.0 million). The number of dairy cows peaked shortly after the abolition of the milk quota in 2015, after which the number of dairy cows decreased to its current level of 1.6 million. In addition to cattle, there are 0.8 million sheep and 0.6 million goats and 0.1 million horses and ponies in the Netherlands. In 2023, only 14 000 of the original 18 000 dairy farms in 2015 were left, i.e. a decrease of 20% in less than 10 years. The average number of dairy cows per farm increased to 110 cows plus accompanying youngstock and the average milk yield to more than 9000 kg cow<sup>-1</sup>. Currently, 34% of all dairy farms milk with a robotic milking system (KOM, 2024).

Around 55% of the Utilised Agricultural Area (UAA) in the Netherlands is devoted to grasslands and forage crops. This area is slowly decreasing due, e.g., to urbanisation. In 2023, the area of permanent grasslands was approximately 670 000 ha, the area of temporary grasslands was 200 000 ha and the area of natural grasslands was 90 000 ha. The area of forage crops amounted to 195 000 ha. The majority of this forage crop area, i.e., 180 000 ha, was used for silage maize (CBS, 2024). The total area of grasslands has decreased in the last decade by around 5% and the total area of forage crops by 15%. The average area of a Dutch dairy farm is around 64 hectares of land for grassland and fodder crops (Agrimatie, 2024).

The temperate maritime climatic conditions favour abundant grass growth. The annual average DM yield of perennial ryegrass in Value for Cultivation and Use (VCU) trials in the Netherlands was 12.5 t ha<sup>-1</sup>



in the period 1975–2015. Between 1990 and 2016, the average annual grass DM yield on dairy farms was 11.1 t ha<sup>-1</sup> (Schils *et al.*, 2020). After 2016 the average grass production on dairy farms decreased towards 10 tonnes DM ha<sup>-1</sup>, mainly due to variation in weather conditions. In the dry year 2018, for example, production was just over 8 tonnes, while in the good grass year 2021, production was again 11 tonnes DM ha<sup>-1</sup> (Agrimatie, 2024).

The majority of dairy farms (83%) practise some form of grazing of dairy cows during the season (ZuivelNL, 2023). The grazing season usually lasts from April to October, and the average number of grazing days per year is 160 days for dairy cows that graze. It is common practice to provide supplemental feeding to the animals in the barn during the grazing season. The figures on grazing for young stock are somewhat lower: 39% of dairy farms graze young stock of less than one year old, and 62% graze young stock older than one year. When focusing on the animals that graze, there are also large differences between dairy cows and young stock. The annual number of grazing hours is 1300–1350 hours per season for dairy cows, 2300–2400 hours per season for young stock younger than one year, and 3200–3400 hours per season for young stock older than one year (CBS, 2024). As the increase in cow numbers per farm during the last decades was not followed by an equal increase in the amount of grassland per farm that is accessible for grazing, the amount of grazing is negatively correlated with the size of the farm. The percentage of grazing dairy farms was at its lowest in 2014 with 78%. Thereafter it increased again till 2020 and has been rather stable since then. The increase since 2014 was mainly due to larger farms (>100 dairy cows) that started grazing again. The increase in grazing was supported by grazing premiums, development of new simple grazing systems (like the New Dutch Grazing), and support from research, education, advice and society.

#### *Grass-based farms in Flanders*

In 2023, there were 1.25 million cattle in Flanders, including 350 000 dairy cows and 147 000 beef cows, mainly from the Belgian blue breed (Statbel, 2024). The total cattle population decreased by around 70,000 animals during the past decade, as the proportion of young stock also decreased significantly. The number of cattle farms is steadily decreasing, while the average farm size is increasing proportionally. On average, farms that specialised in cattle had 157 cattle-units on their farm in 2022 (Statbel, 2024). Despite the strong decrease in the number of beef cows (–30 % in the last 10 years), beef cows remain relatively important in Flanders compared to the Netherlands: 5831 farms or 25% of all active farms in Flanders had beef cattle in 2022. As a result of the abolition of the milk quota in 2015, the dairy sector became specialised. The number of dairy cows increased by around 60 000, whereas the number of farms with dairy cattle decreased with 18% (since 2012). There were 4593 farms with an average of 77 dairy cows in Flanders in 2022; 2609 of these were specialised dairy farms having on average 106 dairy cows.

To supply these cattle with feed, around 350 000 ha or over 50% of the available agricultural land is used to grow fodder crops. In 2023, this included over 160 000 ha of permanent grasslands, 52 000 ha of temporary grasslands and 117 000 ha of maize for silage. This distribution has remained relatively stable for several years. The average size of Flemish farms was 28 ha, whereas farms specialised in cattle have an average size of 58 ha.

#### *Grasslands under pressure in the Netherlands and Flanders*

The area of grasslands and arable land is decreasing year by year as more and more agricultural land disappears from the sector (Verhoeve, 2015). In Flanders, 180 000 ha of the 780 000 ha designated as agricultural land are currently used for other purposes such as pastures for hobby animals, gardens, recreational areas, housing, gardens, and nature. Horses in particular occupy an increasing share of the grasslands. The number of horses in Flanders is approaching 200 000 animals, requiring approximately 150 000 hectares of grassland (De Morgen, 2024). The shrinking agricultural area and the competition

between sectors is driving up the price of agricultural land, as there are many interested buyers. As a result, Flanders ranks third in the European ranking of prices per hectare of agricultural land with an average price of approximately €65 000 ha<sup>-1</sup> (after Malta and the Netherlands). In the Netherlands, the average price in 2022 was approximately € 85 000 ha<sup>-1</sup>, with very large differences between regions, ranging from €65 000 to €150 000 (Eurostat, 2024).

Increasing management intensity makes grazing a greater challenge on many farms. In addition, more extreme weather conditions in the context of global climate change pose an additional challenge for the production of grass of sufficient quality. Nevertheless, there are several trends that encourage dairy farmers to engage in grazing. Several dairies pay a premium of about 1–2 euro per 100 kg milk to dairy farms who graze their cows. This has led many dairy farmers to rediscover grazing and there is a great demand for knowledge about grazing on a modern dairy farm. Next to a grazing premium there are also dairies who pay for a plus on animal welfare, the reduction of GHG emission per kg of milk and biodiversity measures. For the latter a Biodiversity Monitor with Key Performance Indicators (KPIs) has been developed for dairy farming which is implemented by the dairy industry in an independent standard (Erisman *et al.*, 2016; Van Ekeren *et al.*, 2015; Van Laarhoven *et al.*, 2018).

## Grassland use on ten commercial grass-based dairy farms in the Netherlands and Flanders, a case study

### *The ten dairy farms*

To give a better insight into the grassland use on commercial grass-based dairy farms, we interviewed ten dairy farmers in the Netherlands and Flanders which have a special relationship with grass. We asked several questions like “How is the grassland used?” and “Why grasslands?” Figure 1 shows the location of the selected farms in the Netherlands and Flanders.



Figure 1. Locations of the selected farms in the Netherlands and Flanders.

The farms were not selected to be representative of dairy farms in the Netherlands and Flanders, but they do provide a portrait of this variable landscape in the region:

- F1 is a farm in the north of the Netherlands on a clay soil. The farm has implemented special grassland management to support meadow birds.
- F2 is also located in the north of the Netherlands on a clay soil. This farm only feeds grass as roughage in the ration.
- F3 is located in the west of the Netherlands on a peat soil near Amsterdam. The farm has implemented special grassland management to support meadow birds. In addition, the farm has many visits of guests from the region who are educated on the farm.
- F4 is located in the south of the Netherlands on a sandy soil. This farm is part of the so-called ‘Cows and Opportunities’ network - a group of farmers who are very committed to reducing emissions to the environment.
- F5 is a farm in the east of the Netherlands on sandy soil that conducts practical research into clover, species-rich grasslands and water management. The animals on this farm graze but are also fed fresh grass in the barn.
- F6 is an organic farm in the west of the Netherlands on peat soils which, like F2, only includes grass as roughage in the ration.
- F7 is an organic farm located in the south of the Netherlands on a sandy soil. It uses the “short grass (i.e. kurzrasen)” grazing system to ensure high-quality grass intake and to maintain white clover in the grass-clover sward. Kurzrasen is a continuous grazing system, in which the sward height is always kept between 3 and 5 cm.
- F8 is a dairy farm in Flanders with good arable land and permanent grasslands which are frequently flooded in winter, making their management very challenging.
- F9 is an organic dairy farm in Flanders on a sand/clay soil. It uses a dual-purpose breed and was nominated for the most beautiful pasture in Flanders in 2023.
- F10 is located in the centre of Flanders on a sandy soil. This farm, just like F1, F3 and F6, has no arable land.

#### *HOW are the grasslands used?*

The ten farms dairy farms are characterised in Table 1. The available area of the farms is between 35 and 100 ha and the number of dairy cows per farm varies accordingly. Most of the farms also have an area available for the cultivation of arable crops. The arable crops are mostly fodder crops e.g. silage maize, other cereals for whole crop silage and/or fodder beets (farms F4, F5, F7, F8 and F9; not shown), but sometimes the crops are grown for external use or for sale (e.g. grains on farms F2 and F8, sugar beet on F9; not shown). Grassland management varies from farm to farm. Table 1 shows the share of permanent grasslands, extensively managed grasslands and the use of temporary grasslands with grass-clover mixtures, multi-species and Italian ryegrass (*Lolium multiflorum*).

#### *WHAT do the animals eat – is fresh grass included in the ration?*

Table 2 shows the ration, the average crude protein content of the ration, and details on the grazing system used. All farms practise grazing. F3, F6 and F9 apply continuous grazing. F8 practises siesta grazing, but the grazed grass is only a limited is not a considerable part of the ration. F8 changed grazing management when they started to use a commercially available 3-NOP (3-nitrooxypropanol) product to reduce enteric methane emission by the cows. Also, F4 mentioned only 5 hours of grazing time per day. Although the cows have constant access to pasture in summer, this is the expected time the cows actually spend on pasture due to the milking system (automatic milking system; AMS). Farm F6 also uses an AMS, but its estimate of actual grazing time is higher.

Table 1. Characteristics of the ten selected farms: number of dairy cows, milk production per cow per year, farm intensity in L per hectare, and land use in hectares: total, arable, grassland, permanent, temporary grass-clover or species-rich, *Lolium multiflorum* as catch crop and extensively managed.

	No. dairy cows	Milk (cow <sup>-1</sup> year <sup>-1</sup> )	Intensity (l ha <sup>-1</sup> )	Total (ha)	Arable (ha)	Grassland (ha)	Permanent grassland (ha)	Temporary: Grass-clover or species-rich grassland (ha)	<i>Lolium multiflorum</i> as catch crop (ha)	Extensively managed grassland (ha)
F1	199	8 000	15.8	101		101	101			Much, but not officially registered
F2	125	8 000	14.1	71	9	62	51	11.5		
F3	134	9 340	14.5	81		81	70			13
F4	130	11 000	26.0	55	10	45	18	25		
F5	188	9 580	21.7	83	18	65	44	21		1.5
F6	52	8 360	10.4	42		42	42			
F7	93	6 450	6.0	100	16	84	56	26.5		27
F8	120	12 070	24.5	59	31	28	24		10	
F9	75	4 000	3.0	100	27	73	60	15	-	35–40
F10	90	9 800	25.2	35		35	11	21.5		2.5

Table 2. Ration and grazing characteristics: % fresh grass, % grass silage, % maize silage, % concentrates+by-products, protein in the ration (all expressed in DM and as year-round average), grazing system used, grazing hours per day and grazing days per year.

	Fresh grass (%)	Grass silage (%)	Maize (%)	Concentrates/by-products (%)	Protein in ration (g (kg DM) <sup>-1</sup> )	Grazing system	h day <sup>-1</sup>	days year <sup>-1</sup>
F1	20	30		50	131	Rotational grazing, "mosaic management"	9	190
F2	25	50		25	158	Strip grazing	12	245
F3	20	37	9	34	158	Rotational grazing	15 <sup>1</sup>	210
F4	25	20	35	20	154	Compartmented continuous grazing	5	180
F5	16	26	18	40	157	Strip grazing and feeding fresh grass in the barn	7	224
F6	40	40		20	241 <sup>2</sup>	Rotational grazing	18	210
F7	35	40	10	15	145	Short grazing i.e. "Kurzasen"	14	241
F8	3	23	34	40	164	Siesta grazing	3	180
F9	50	50		n.a.	165	Rotational grazing (twice per day)	20	220
F10	15	35		50	187	Rotational grazing (daily)	8	150

<sup>1</sup>May-Sept: 20 h<sup>-1</sup> day<sup>-1</sup>; until May and from Sept–Nov: appr 10 h day<sup>-1</sup>.

<sup>2</sup>Recent silage analyses indicated that protein in ration was strongly reduced (100-point reduction).

### *WHAT are the trade-offs to the environment?*

To estimate trade-offs to the environment, N-surplus per ha, and CO<sub>2</sub> emissions per kg fat and protein-corrected milk were used. In the Netherlands, these characteristics are calculated annually as part of the 'annual nutrient cycle assessment' (ANCA) (Table 3). The losses to the environment vary greatly and depend on the type of farm (organic versus conventional), intensity of the farm, but also on the type of soil. Especially the high N-mineralisation from the peat soil (F3) influences the N-surplus ha<sup>-1</sup>. Most farms apply some practices to reduce emissions to the environment. The addition of water during slurry application is very common, which leads to a reduction in ammonia emissions depending on the application method.

Furthermore, most farms reduce the protein content of the ration to reduce nitrogen excretion, try to clean the barn floor regularly and use grazing as a method to reduce NH<sub>3</sub> emissions. A commercially available 3-NOP product is used on F8 and F10 to reduce CH<sub>4</sub> emissions.

### *WHICH ecosystems services are provided? - biodiversity characteristics and other services*

The interviewed farmers declared in the interviews that they do not only produce milk but deliver also other (ecosystem) services (Table 4). Grass-clover and species-rich grasslands are often mentioned and used for several reasons. The first reason is the nitrogen fixation that leads to increased protein content of the forage and decreased use of artificial fertilizers (F2, F4, F5, F6, F9, F10). Other reasons are drought tolerance of the species (F1, F5, F9, F10), biodiversity (F5, F9) and cow health (F5, F9).

### *WHICH advantages and limitations of grasslands do the farmers in our survey experience?*

The farmers in our survey see a number of advantages of grassland. The main advantage is that grassland provides a complete forage that is well suited to cows (F1, F9) and can be grown on soils where other crops are (barely) possible (F1, F3, F8). Grassland also ensures fodder production on dry sandy soils under the current climatic challenges (F10). In addition, fodder and protein production is sufficient for the cows – grazing provides direct utilization of energy and protein - and it makes it possible to harvest protein for the cows on their own farm (F2, F3, F5, F6, F7, F10). One farmer also stated that the N mineralization after ploughing a grassland is a great advantage in the crop rotation because it reduces the nitrogen requirement for the following maize crop (F7). Grasslands also ensure a healthy soil life and high biodiversity in the agricultural landscape and blend well into the landscape. They can help to sequester carbon and reduce emissions (F5, F7, F9, F10). Farmers also like to see cows grazing (F4, F5). Finally, the ability to choose between grazing and mowing is appreciated because it offers ease, safety and freedom (F9).

Table 3. Sustainability characteristics and emissions.

	N-surplus (kg ha <sup>-1</sup> )	CO <sub>2</sub> (kg FPCM) <sup>-1</sup>	Practices to reduce emissions (NH <sub>3</sub> /CH <sub>4</sub> )
F1	114	1054	Low fertilizations; water to in slurry, low CP in ration, grazing, regular floor shovelling
F2	136	1158	Lower slurry gifts, water to in slurry, low CP in ration, regular floor shovelling
F3	282 <sup>1</sup>	1379 <sup>2</sup>	Reduced emission stable; low CP in ration, water to in slurry
F4	110	867	Reduced emission floor, water to in slurry
F5	75	981	Low mineral fertilization, longer grazing period, increasing fresh grass, low CP in ration
F6	27	1115	Low CP in ration, grazing, regular floor shovelling
F7	34	1210	Grazing, low stocking rate
F8	n.a.	n.a.	Use of commercially available 3NOP product to reduce CH <sub>4</sub> emissions
F9	n.a.	n.a.	Continuous grazing (to the max)
F10	n.a.	n.a.	Calculation of rations, reduced emission stable, low emission technique (slurry application), use of commercially available 3NOP product to reduce CH <sub>4</sub> emissions

<sup>1</sup> Including mineralization of peat soil: corrected N-surplus: 83. <sup>2</sup> Corrected CO<sub>2</sub> emission: 1028.

Table 4. Ecosystem services and sustainability characteristics on the different farms, as and only if specifically mentioned by the farmers.

		Farms
Biodiversity	Field borders	F4
	Botanical management around ditches	F1, F3, F6
	Bird management and/or extended mowing	F1, F3, F6, F10
	Endangered species conservation program	F8, F9
	Species-rich grasslands	F1, F4, F5, F6, F9
	Management / use grassland on nature areas	F3, F4, F7, F10
	Hedges	F2, F5, F8
Mineral efficiency	Trees	F8
	Legumes	F1, F2, F4, F5, F9, F10
	Adapted fertilization	F2, F4, F5, F6, F9, F10
	Extended grazing	F1, F5
C sequestration	Reduced imports	F7, F9
	Permanent grassland (>10 years)	F1, F2, F5
Pesticide reduction		F5, F6, F7, F9
Energy transition	Solar panels	F1, F2, F3, F5, F6
Cooperation with regional cooperatives		F1, F4, F5, F7, F8, F9, F10
Farmer – citizen – initiatives	Local sales such as milk, ice cream, meat	F1, F3, F9
	Tourism / catering / event location	F3, F8
	Education	F1, F3, F5, F6, F7, F8
	Research	F5

Farmers also pointed out disadvantages, e.g. the feed value is not always suitable for highly productive dairy cows (F1) and the productivity of grasslands is often more variable than that of maize (F4). It is not always possible to graze during longer periods of rain (F2). Drought can also be a problem (evaporation, high water consumption (F4, F5, F7)). In some situations, mineralization is too high, resulting in a high protein content in the grass and making it difficult to offer a balanced ration – this could become even more difficult in the future as inputs (also due to the loss of the derogation) decrease and extensive management increases (F3, F4). The quality of the grass can be unpredictable (also due to weather conditions) and is therefore not always easy to manage (F3, F8, F10). Fertilization and harvesting are time-consuming (~money) (F5, F9) and the renewal of permanent grasslands with poor botanical composition is complicated (F8).

#### *Recommendations from farmer interviews: insights for further research*

Farmers were asked to indicate which topics were important to them and should be addressed in future research. A very important aspect was how to keep (permanent) grassland productive and palatable with less fertilization (F2, F3, F4, F6, F10). Nitrogen regulations are becoming stricter, leading to a reduction in the amount of animal manure to be applied. This will also lead to a reduction of minerals other than N: e.g. K, P and S. Further wishes for research into the use of animal manure (F3) and the comparison of nitrogen leaching between animal manure and artificial fertilizers (F5) is linked to this topic.

It was also pointed out that water storage and water management are important (F4, F5) and that grass mixtures are needed that are more persistent and resistant to drought (F10). Furthermore, the rehabilitation of very wet and frequently flooded soils is a problem (F10) and there should be grass species available for less optimal growing conditions (F8). Finally, farmers would like to have more information

on tools for optimal grazing (F10), on 100% grass-fed milk production (F9) and on the added value of grassland ecosystem services, especially in terms of the economic value (F1).

## **WHY grasslands?**

In the interviews we asked the opinion of the farmers on the role of grasslands on their farm. Some quotes are given below:

Farmer F1: *“Grassland is important in our management, for the cows, but also for the whole area and for the meadow birds living on the grasslands.”*

Farmer F2: *“Grassland is the base of our management and delivers energy and protein for our livestock. Furthermore, it is important for soil quality and health. Variation in grass growth, grass quality and grazing is not a problem, but has to be handled as a part of the system.”*

Farmer F3: *“Grassland is an inseparable part of the peat grassland landscape. Furthermore, the management of birds on grassland and biodiversity near ditches are also important on the landscape. The close proximity of the city gives extra potential for tourism.”*

Farmer F7: *“Grassland is the license to produce for dairy farming, because of positive impacts on soil quality and (soil) biodiversity, and the possibility to reduce emissions by grazing (NH<sub>3</sub>). Grass is an important protein source and is important for the landscape.”*

These quotes underscore the farmers' recognition of the ecosystem services provided by grasslands. The significance of grasslands lies in their ability to offer various ecosystem services, as depicted in Figure 2 (Schils *et al.*, 2022). Figure 2 shows that permanent grasslands are more beneficial for most ecosystem services than croplands and temporary grasslands (indicated by the bullet points in the outer circle). For example, permanent grasslands provide high soil quality (bullet points run-off, soil loss, bulk density), water regulation (hydraulic activity), carbon sequestration and biodiversity (threatened species). Semi-natural grasslands are not included in the figure, but are among others beneficial for water use, N<sub>2</sub>O and NH<sub>3</sub> emissions, biodiversity and landscape enhancement. Temporary grasslands perform worse than permanent grasslands for most ecosystem services. Croplands are generally less valued for ecosystem services, except for yield and energy.

## **Conclusions**

The case study was meant to showcase examples of different grassland management use and as a portrait of grass-based dairying in the Netherlands and Flanders. Since the farms have not been selected to be representative of the sector, conclusions should be read having the goal of the case study in mind. The diversity among grassland farms in the Netherlands and Flanders is notable, as illustrated through interviews with ten grass-based dairy farmers. Many of these farmers have adopted various strategies to enhance ecosystem services, for example, incorporating grass-clover mixtures and species-rich grasslands to mitigate nitrogen input and bolster drought resistance. Several farmers have highlighted the growing challenges in sustaining dry matter yield and forage quality in their grasslands, attributed to stricter nitrogen regulations also impacting other vital nutrients like potassium and phosphorus. Moreover, given the prevailing climatic challenges, there is a need to reassess the cultivation and management practices of grasslands and forage crops. Additionally, there is an urgent demand from society for ecosystem services delivery, such as biodiversity conservation, landscape preservation, and water retention. However, their economic valuation remains a subject of exploration.

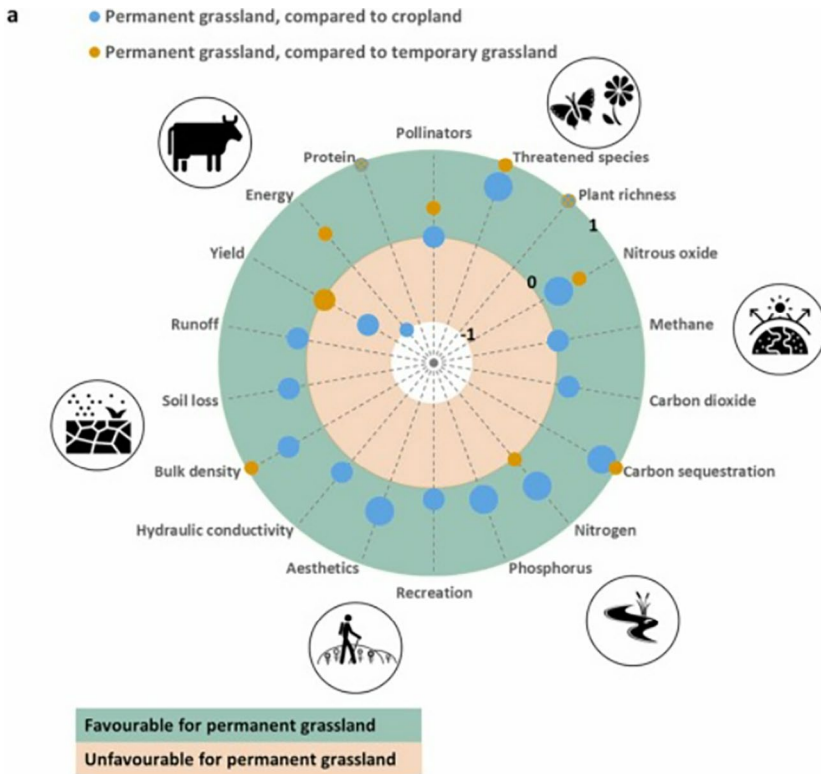


Figure 2. Comparison between land use types for indicators of ecosystem services, permanent grassland compared to cropland and temporary grassland. The boundary between the outer and inner shaded zones represents a mean score of 0. The shaded outer zone represents a favourable score for permanent grassland (moving outwards, the mean score increases from 0 to 1), the shaded inner zone represents an unfavourable score (moving inwards, the mean score decreases from 0 to -1). Dot size indicates number of underlying cases (small: <5 cases, medium: 5–9 cases, large: >9 cases). Source: Schils *et al.* (2022).

In the future, grasslands in the Netherlands and Flanders are expected to face pressure due to increasing competition for land within the agricultural sector (e.g. arable land) and outside the agricultural sector (e.g. urbanisation). Regarding the question of WHY grasslands, farmers cite, next to a high quality roughage for their cows, numerous reasons, including the promotion of biodiversity (birds, insects, soil organisms, plant species), enhancement of soil quality, water retention capabilities, landscape aesthetics (which contribute to tourism), protein yield, feed value, and the overall well-being of cows and farmers. These multifaceted benefits underscore the importance of preserving grasslands in both the Netherlands and Flanders. Considering the increasing competition for land, a payment scheme to value these ecosystem services might support preserving these grasslands and their ecosystem services.

## Acknowledgement

We would like to acknowledge the ten dairy farmers for their cooperation.

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**Theme 1.**

**WHAT?**

**What is the role of grasslands  
in net food security?**



# What is the role of grasslands under a feed-no-food scenario?

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## Abstract

The current food system is transgressing planetary boundaries, which define a safe operating space for humanity on Earth. An emerging solution is adopting a circular food system, where waste is minimized and competition between food and feed resources is mitigated. Central to this concept is the ‘feed-no-food’ principle, which highlights the increased importance of grasslands as the primary feed source and, consequently, the basis for most animal-sourced products. This paper investigates the potential of grasslands to maximize protein production under a ‘feed-no-food’ scenario, focusing on increasing milk production from grass, given its higher protein conversion ratio compared to meat. Our findings suggest that in some cases, it is feasible to reduce livestock numbers to the carrying capacity of the grasslands while still producing more bovine protein than currently achieved. This can be accomplished through the strategic use of dual-purpose cattle, which offer a higher milk/meat ratio. Additionally, the study examines the transfer of nutrients from grasslands to croplands. The results indicate that while this nutrient transfer is beneficial, it cannot support a fully organic food system.

**Keywords:** circular food system, nitrogen cycle, dietary changes, grass-based protein production

## Introduction

Our food system is encountering a systemic problem: an increasing part of the population adopts an unhealthy diet produced in a food system that trespasses on the safe operating space for humanity, known as the planetary boundary (Campbell *et al.*, 2017; Gerten *et al.*, 2020), including biosphere integrity, land-system change, biochemical flows and climate change. While the environmental impact of livestock is highly heterogeneous (Poore and Nemecek, 2018), globally, part of this transgression can be related to livestock: livestock relies on 26% of earth’s land area, which represents 77% of the agricultural land (FAOstat), emits 60% of food system emission (Costa *et al.*, 2022) and monogastrics consumes 76 % of the human edible feed globally (Mottet *et al.*, 2017).

There is no silver bullet to keep the food system within planetary boundaries, and several options, including dietary changes towards healthier, more plant-based diets, improvements in technologies and management, and reductions in food loss and waste, must be combined (Springmann *et al.*, 2018).

For Europe, it was shown that a circular food system, a food system in which waste is minimized and recycled, can contribute to making the food system more sustainable (Van Zanten *et al.*, 2023) while producing enough healthy food within a self-sufficient European food system. Under global food shortages, savings in agricultural land could be used to feed an additional 767 million people outside the EU (+149%. In such a system, livestock’s role is to transform non-edible production, such as grass, or waste products of the food system, into valuable protein for human consumption (Van Zanten *et al.*, 2019). Termed the “feed no food” principle, this approach is anticipated to result in a substantial reduction in monogastric animals being fed primarily on the small amounts of available agricultural waste, while ruminants are expected to rely on grass, which is not edible to humans. The change of diet is particularly significant in this scenario, as current EU diets contain about 30% of meat, milk and eggs, which corresponds to 58% of the protein supply (based on FAO stat - Food and Diet / Apparent intake in 2020). About 20% of total meat consumed in Europe is beef (Hocquette *et al.*, 2018). Note that ‘Other’ encompasses other animal sourced food, such as animal fat or meat in processed food.

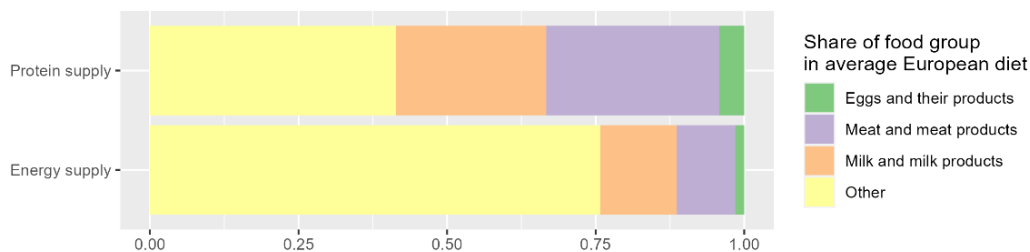


Figure 1. Share of meat, milk and egg in European diets in terms of energy and protein supply (Source FAOstat Food and Diet/Apparent intake in 2020).

This paradigm shift towards a circular food system enhances the significance of grasslands, both permanent and temporary, as source of proteins. Permanent grasslands cover 34% of the European Union's agricultural area and can almost only be used exclusively for food production through ruminant conversion of grass biomass to milk and meat. In addition, they provide other ecosystem services; e.g., they regulate water flow, prevent soil erosion, sequester carbon, and provide habitats for non-plant organisms such as insects needed for crop pollination (Richter *et al.*, 2021) which are then comparable across different ES and easily understandable to decision makers. However, a comprehensive synthesis of methods to measure ES indicators in grasslands, a central element of many landscapes around the globe, is still lacking, hampering the implementation of grassland ES-multifunctionality surveys. To identify suitable and recommendable methods, we reviewed the literature and evaluated labor intensiveness, equipment costs and predictive power of all methods. To facilitate the translation of biophysical ES into monetary terms, we further provide an overview of available methods for the economic valuation of ES. This review resulted in a toolbox comprising 85 plot-scale methods for assessing 29 different ES indicators for 21 provisioning, regulating, supporting or cultural ES. The available methods to measure ES indicators vary widely in labor intensiveness, costs, and predictive power. Based on this synthesis, we recommend 1. Temporary grasslands, typically composed of grass-legume leys, are widespread across all agro-ecologies of Europe (Ballot *et al.*, 2022) whereas current dominant cropping systems are known to rely only on a few crops species – like cereals in the European Union (EU). While they appear to compete with food production, they actually are playing crucial function in crop rotations particularly organic ones, by fixing nitrogen thereby reducing the need for N-fertilizers while contributing to soil health and suppressing weeds thus allowing farmers to forgo pesticides and herbicides. Temporary grasslands, therefore, should be seen as an integral and essential practice linked to food production rather than competing with food.

The primary objective of this paper is to explore how grassland can contribute to a healthy food system within planetary boundaries under a 'feed-no-food' scenario, by maximizing protein production while providing nitrogen that can be applied to crops.

### Maximizing returns from grasslands in a 'feed-no-food' scenario at a food system level

Under the 'feed-no-food' scenario, grasslands become the main source of feed and, therefore, the main source of animal-based products, including animal-sourced food and manure in the food system.

An efficient food system needs to maximize the amount of animal production from grassland, and particularly, proteins. Alexander (2016) shows that milk exhibits significantly higher protein conversion efficiency than meat, and cattle overall have a better protein conversion efficiency than other ruminants (Rouillé *et al.*, 2023). This suggests that using grassland to produce milk rather than meat will allow the production of more protein from the same land. However, not all grasslands achieve the necessary feeding

value for dairy cows, i.e. with a high enough concentration of metabolizable or net energy and crude protein or low fibre concentration. Schils *et al.* (2022) show that higher nitrogen input, be it through fertilizer or legumes, tends to increase both yields and crude protein content, suggesting that only grassland that is used more intensively achieves yields and qualities to support dairy cows. Less intensive, and therefore lower quality, grass cannot be used for dairy but can be used for fattening animals or for small ruminants. An efficient use of grassland will, therefore, support both milk and meat production depending on the quality of the grass.

Optimizing between dairy and meat production can be achieved with a coupled dairy-meat production system, also known as dual-use cattle or dairy-based beef (de Vries *et al.*, 2015; Mosnier *et al.*, 2021). This can be achieved with dual-purpose cattle (Brito *et al.*, 2021), as well as with specialized milk breeds in combination with sexed semen for the replacement unit and the use of beef-oriented semen for the additional calves (Butler and Holden, 2018).

In addition to providing animal protein, livestock also play an essential role in the food system by contributing nitrogen, supporting both crop growth and soil health. Livestock do not inherently create nitrogen: animal feed contains nitrogen that is absorbed to produce milk and meat or excreted as manure. Consequently, livestock provide an efficient means to transfer the nitrogen contained in the grassland to the cropland when the manure is stored and then applied to crops.

### **Calculating the protein and manure production capacity of European grasslands**

Calculating the available grass for Europe involved a comprehensive integration of various datasets. The CORINE land cover map (CLC2018) was employed to determine the extent of cropland and permanent grassland. The proportion of temporary grassland in crop rotations was computed based on Ballot (2022) whereas current dominant cropping systems are known to rely only on a few crops species &ndash; like cereals in the European Union (EU, reporting the proportion of temporary grassland over a crop rotation across Europe based on satellite images. While grassland yield data from Smit *et al.* (2008) which is not available. This paper presents and analyses spatially explicit data of grassland productivity and land use across regions in Europe. Data are extracted from various regional, national and international census statistics for Europe, extending eastwards to the Ural Mountains. Regional differences in grassland productivity are analysed considering selected climatic and agronomic parameters and are compared with the remotely sensed normalised difference vegetation index (NDVI) were assumed to be the same across all the grassland type, the percentage of the grass harvested was assumed to be different for different grassland qualities to account the difference in yield per type of grassland. Temporary grassland as well as permanent grassland identified as pasture in CORINE was assumed to be intensively used and 80% of the grass was harvested, while natural grassland, defined in CORINE as not getting fertilized other than from grazing animals, was assumed to have a 40% harvest rate, implying that natural grassland produces about half that of intensively managed grassland. This is in line with Swiss standard gross margin data (Agridea, 2023). This allows the calculation of the total supply of grass available in Europe.

To compute the grass demand, a representative dual-purpose cow unit was modelled, including the dairy cow, its replacement unit, as well as the beef fattening, assuming that calves not needed for the replacement will be fattened for 10 months. Milk productivity and replacement rate were based on Swiss herd-book data of the last 10 years, using a Simmental breed with an annual replacement rate of 28%, corresponding to an average productive life of 3.6 years (Bieber *et al.*, data not shown).

The amount of grass required to feed the dairy cow, replacement heifer, and the fattening, were based on nutritional requirements for these animals to achieve the milk production found in the herd-book data, as well as the weight gain from standard grass-fed fattening animal (Gruber *et*

*al.*, 2006). Nutritional values of grassland were taken from feedbase (Boltshauser *et al.*, 2012). The productivity parameter computed based on this production unit are found in Theme 1. WHAT? What is the role of grasslands in net food security?.

Nitrogen excreted by the dual purpose dairy cow production unit was computed following IPCC guidelines chapter 10 (IPCC, 2019). We also calculate whether the resulting N in manure would allow sufficient nitrogen from grassland to be transferred to cropland by computing a soil nitrogen balance on cropland and grassland. To do so, N demand from crop was based on N content of the crops and its residue. Crop yields were taken from Eurostat for the baseline. The N applied in the baseline, included the application of manure, fertilizer and nitrogen from legumes. N from manure was calculated by using Eurostat livestock statistics multiplied by excretion rates provided by de Vries (2021) partly driven by increased nitrogen (N and the share applied to cropland (after losses and allocation to grassland) based on Einarsson (2020), also fertilizer amount available in Eurostat were applied.

In addition, an organic ‘feed-no-food’ scenario was calculated. In this scenario, the total amount of available grass was divided by the yearly intake of grass to get the number of dual-purpose dairy cow production unit. The amount of manure available for allocating to cropland was computed based on the N excretion rate shown in Table 1. No artificial fertilizers were applied and legume fixation remained unchanged. On the nitrogen demand side, crop yield would be reduced by 15% based on Knapp and van der Heijden (2018) to reflect optimal organic yields.

## Results

The analysis first quantified the production of animal source protein from dual-purpose cows in European grasslands under a ‘feed-no-food’ scenario, which included pasture, natural grasslands and temporary grasslands. The findings in Figure 1a show a reduction of bovine animals in most European countries. Exceptions are Latvia and Ireland, showing an increase in bovine population. The increase in Ireland’s bovine population under the ‘feed-no-food’ scenario is attributed to the fact that today the grassland use is dominated by a sheep population, offering potential for a shift towards more bovine on grasslands. Shifting to dual-purpose dairy systems depends on the suitability of grasslands for dairy. If unsuitable, there may not be enough dairy-origin animals for fattening. In such cases, sheep, which tend to have lower environmental impact compared to beef (Poore and Nemecek, 2018), offer a more viable solution. In contrast, most other countries are projected to have a reduction in bovine numbers, with the Mediterranean region and Denmark facing the most significant declines. The significant decrease in the Mediterranean’s bovine population in a ‘feed-no-food’ scenario is due to the prevalent practice of indoor

Table 1. Parametrization of the representative dual-purpose cow production unit.

Parameter per dairy production unit (1 dairy cow+share of replacement+share of fattening on a yearly base)	
Replacement rate	28.6%
Dairy cow body weight	632 kg
Yearly intake of grass in DM	7406 kg DM year <sup>-1</sup>
Yearly milk production	4936 litres
Yearly share of meat production from calf fattening	220 kg
Yearly share of meat slaughter of dairy cow	84 kg
Yearly excretion rate	124 kg N

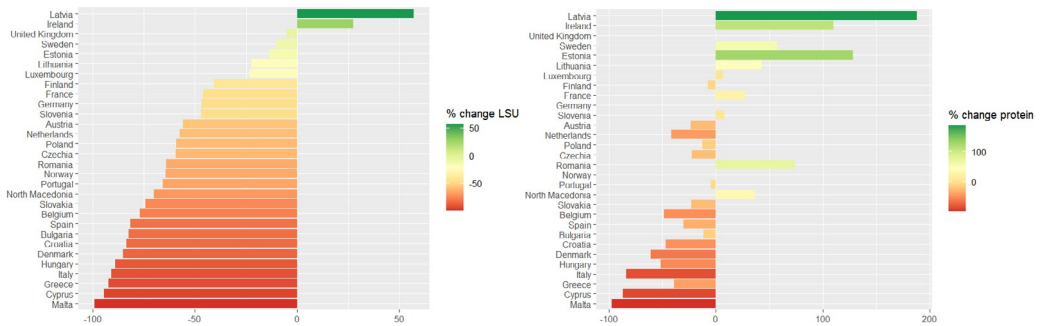


Figure 2. Change in percent of LSU in a 'feed-no-food' scenario in percent compared to the current situation (left) and the change in percent of bovine protein produced compared to the current situation (right).

keeping and concentrate feeding of dairy animals in this region, which would not be feasible under the scenario's constraints.

In the analysis of protein production from grasslands under a 'feed-no-food' scenario, we observed a notable increase in countries such as Ireland and Latvia, which aligns with their increased bovine livestock units (LSU) (Figure 2, right). Conversely, regions like the Mediterranean, Denmark and the Benelux countries exhibited a decrease, correlating with their reduced animal numbers. However, interesting phenomena were observed in France, Germany and Romania. Despite a reduction in bovine LSU, these countries showed an increase in protein production from grasslands compared to the current situation. This counter intuitive outcome can be attributed to the existing milk/meat production ratio in these countries as shown in Figure 2. In the current situation, these countries have a lower milk-to-meat ratio than in the 'feed-no-food' scenario, with dual-purpose breeds that prioritizes milk over beef production. Milk yields more protein per hectare of grassland, which effectively compensates for the productivity loss associated with dual-purpose cattle, compared to separate beef and dairy systems that rely partially on concentrates and maize silage, which compete with food production.

The second part of the analysis examined the implications of nitrogen transfer from grasslands to croplands assuming organic farming scenario in the 'feed-no-food' scenario. The soil nitrogen balance under current conditions (with applied fertilizers and assumed current yields) to a 'feed-no-food' scenario

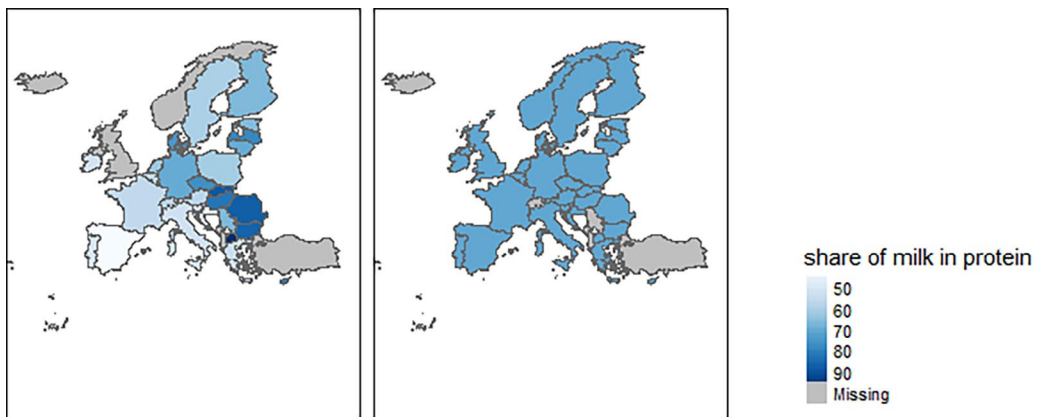


Figure 3. Share of milk protein in the total bovine protein production in the current situation (left) and in the 'feed-no-food' scenario with dual-purpose cattle (right).



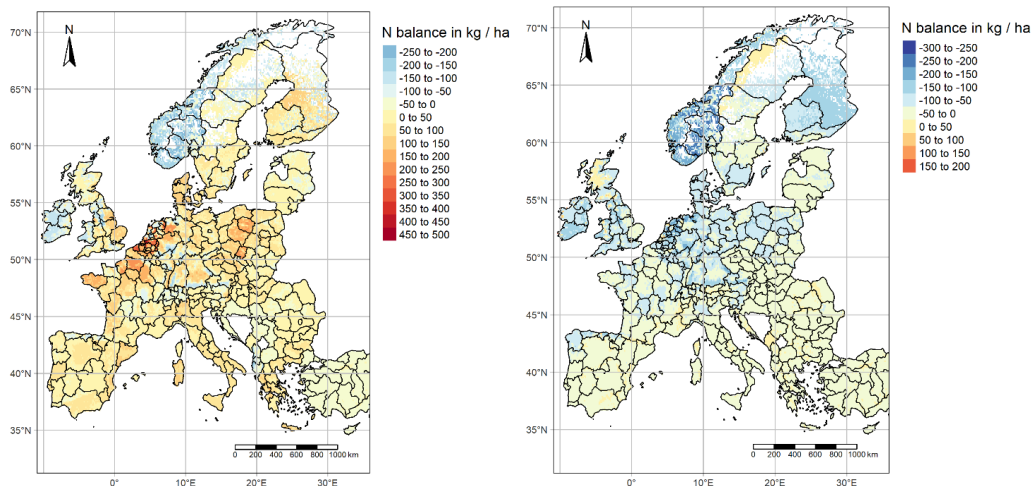


Figure 4. Soil nitrogen balance on crop and grassland, in the current situation (left) and in an organic ‘feed-no-food’ scenario (right).

that assumes organic crop yields (a 15% reduction from current yields) without the use of synthetic fertilizers were computed and shown in Figure 4.

Our analysis of the current nitrogen soil balance in Europe, considering existing livestock numbers, fertilizer use, and crop yields, reveals a widespread nitrogen surplus across most regions. Notable exceptions to this trend include Ireland and the Scandinavian countries, which do not exhibit a similar surplus.

Transitioning to a ‘feed-no-food’ scenario, where dual-purpose cows are the sole livestock fed from grasslands, and have similar grazing times to that currently observed (based on Einarsson 2020), and no artificial fertilizers are applied, results in a paradigm shift to a Europe-wide nitrogen deficit. This deficit might be slightly overestimated, as our model did not account for monogastric animals fed on food system waste; nonetheless, this distortion is minimal since a ‘feed-no-food’ scenario aiming to stay within planetary boundaries would inherently reduce waste to minimal levels, leaving limited scope for monogastric feeding. The nitrogen deficit is particularly marked in regions such as the Benelux countries, which currently boast the highest crop yields. Conversely, the deficit is less severe in the Mediterranean region, where crop yields are typically lower. This shows that under a feed-no-food scenario, nitrogen transfer from grassland to cropland will not be sufficient to produce current organic yield, necessitating innovative approaches to nutrient management and agricultural practices to mitigate the potential decline in food security and maintain ecosystem health.

## Discussion

While our approach offers an overview of a ‘feed-no-food’ scenario in Europe, it is important to acknowledge its limitations. Firstly, the study assumes a Swiss dual-purpose cow as a representative breed for the entirety of Europe. This generalization overlooks regional variations in cattle breeds and their respective productivity characteristics. Future research should aim to refine this aspect by tailoring the production parameters to the specific breeds present in each region, thereby enhancing the accuracy of our projections. Secondly, our analysis relies on grassland yield data from Smit *et al.* (2008), which, being somewhat dated and at a national level, may not accurately reflect current grassland productivity across diverse European landscapes. Obtaining more recent and detailed data on grassland productivity

is crucial for a more precise estimation of the sustainable bovine population in Europe under the ‘feed-no-food’ scenario.

Despite these methodological limitations, the study provides several important insights into the potential dynamics of a ‘feed-no-food’ scenario in European agriculture. Firstly, this study shows that the number of bovine animals that can be sustainably supported on grasslands is generally lower than the current bovine population; up to reduction of almost 100% in Malta and Cyprus, and with few exceptions, namely Latvia and Ireland. This trend is not surprising, considering that most current bovine systems, whether dairy or beef, rely at least partially on concentrates and/or maize silage, which are in direct competition with human food resources. However, it is important to note that a reduction in animal numbers, and the lower productivity of grass-fed dual-purpose cattle compared to more intensive systems, does not necessarily equate to a decrease in protein production from grasslands. Intriguingly, this study suggests the potential for increased protein production, particularly evident in countries where the milk/meat production ratio is traditionally lower. Systems focusing on dual-purpose dairy, with an emphasis on a high milk/meat ratio, capitalize on the more efficient protein conversion rate of milk from grass as compared to meat. This aspect introduces a promising perspective on how strategic adjustments in livestock management could enhance the overall protein yield from grasslands, thereby contributing to a more sustainable and efficient use of agricultural resources.

Secondly, this study brings into focus the indispensable role of grasslands as a source of nitrogen, particularly through nitrogen fixation from legumes in grassland as well as the application of manure in crop systems. This aspect is important for a more sustainable agriculture, where the dependency on synthetic fertilizers is increasingly being questioned and efforts are made to minimize or eliminate their use. Grasslands, when managed effectively, can become a key player in the natural cycle of nutrients and providing essential nitrogen to crops, which is critical for their growth and productivity by supporting soil health. Yet in the feed-no-food scenario, the amount of nitrogen transferred from grassland to cropland through manure will not cover the N required by crops in a fully organic system. This shortfall highlights the necessity of incorporating complementary strategies that can augment the nitrogen supply in a more holistic manner, such as increasing the use of nitrogen-fixing legumes in crop rotations or the recycling of nutrients from human excreta.

## **Conclusion**

In the context of a circular food system embracing the ‘feed-no-food’ principle, the significance of grasslands is set to rise markedly. Grasslands will become crucial in supplying a range of animal-sourced products, from protein-rich foods which, in small quantities, are integral to healthy diets, to organic manure that enhances soil health. To maximize the potential of grasslands, a strategic shift is needed towards prioritizing milk production over meat production. This study illustrated this by modelling a grass-fed dual-purpose cow, relying solely on the locally available grasslands.

However, it is important to recognize the limitations of the feed-no-food strategy, particularly in relation to organic farming. While the manure derived from these grasslands, through the grazing of livestock, is a valuable resource, this study suggests that it alone may not suffice to meet the nutrient demands of a fully organic food system. This gap highlights the necessity for additional sustainable practices and innovations in organic farming. Developing a deeper understanding the interplay of grasslands with other agricultural practices is essential to understand how to move to a more sustainable food system.

## Acknowledgements

This research has been part of the PATHWAYS project, funded by the European Union's Horizon 2020 Research and Innovation Programme under grant agreement No 101000395.

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# What can we do to improve the contribution of grassland to net food security?

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## Abstract

Intensification of animal production systems over the last 20+ years has increased the consumption of human edible protein by livestock, leading to increased competition for and cost of human edible protein. Grassland based agriculture, in a large part, supports ruminant production systems, which convert human inedible proteins (grassland) into human edible meat and dairy products of high protein and other nutrient value. Grassland-based systems have conversion efficiencies of 2.5 to almost 4 in terms of kg human edible protein produced for each 1 kg human edible protein consumed by livestock. Grassland offers a range of other ecosystems services including supporting plant and animal biodiversity, water resource management (e.g. water retention, provision of flood plains, filtering), carbon storage and sequestration, promoting soil health, sustaining rural communities, and contributing to rural culture and landscape management. Sustainable grassland management addresses the requirements of grassland to contribute to global net food security, particularly through ruminant production, as well as environmental conservation and management.

**Keywords:** grasslands, ruminant production, food-feed competition, food security

## Introduction

Grassland covers a large proportion of the land area globally, much of which is unsuitable for crop production. Provision of food from grassland-based production systems plays an important role in global food security, through conversion of a low-cost human inedible food source (grassland) by ruminants into high quality food for human consumption, and in many cases without competing for land for crop production (Van Kernebeek *et al.*, 2016). In marginal areas, grassland yields are generally higher and crop failures less severe than those of directly human edible crops. In these regions grassland is a significant contributor to food production through ruminants, and the milk and meat produced by them. As the global population continues to grow, increased food production is required from the same land area and, in some regions such as Europe, declining land area as a result of competition with other land uses such as bioenergy production coupled with reduced inputs. The consequences will be increasing food costs, reduced food supply in some regions, increased carbon footprint of animal feed and food as more is imported, intensification of livestock production systems and loss of grassland for food production which will ultimately have negative impacts on various environmental parameters and ecosystem services.

Demand for protein foods continues to grow globally. On a per capita basis, global meat consumption is expected to grow by 2% by 2032 compared to the base period of 2020–2022, driven by income and population growth (OECD-FAO, 2023). Milk and meat from ruminants are important sources of amino acids, fatty acids and other nutrients of key importance for human health (e.g. Leroy *et al.*, 2022; O'Callaghan *et al.*, 2016; Walther *et al.*, 2022). Improving the contribution of grasslands to net food security is challenging, requiring a combination of sustainable management practices which allow the production of grassland for feed production while minimising the impact on the ecosystem and the environment.

Research has identified many factors that can improve grassland production and utilisation including incorporating legumes (e.g. Clavin *et al.*, 2017; Egan *et al.*, 2018; McClearn *et al.*, 2019), improving grazing management (O'Donovan and Delaby, 2015), ensuring that the ruminants grazing the grassland are well adapted (Delaby *et al.*, 2021) and development of tools to help anticipate and contribute to decision making (e.g. PastureBase Ireland; Hanrahan *et al.*, 2017). Improved grassland use must be implemented without damaging and, where possible, having positive effects on the ecosystem in which they exit. This type of improved grassland use/management, which combines grassland with ruminants, is sometimes referred to as agro-ecology or sustainable intensification. Many policy drivers are influencing the sustainability of grassland-based systems. These are sometimes complimentary and sometimes antagonistic. European agricultural policy has a large focus on robust and resilient food systems while reducing the impact of agriculture on climate change and environmental degradation (The EU Green Deal Farm to Fork Strategy) and addressing biodiversity decline. Although food production is now largely at a global scale, food security is an important consideration in many regions. The potential of grassland to improve its contribution to net food security is variable across Europe, depending on soil type, soil fertility, land prices, landscape and topography, meteorological conditions (especially temperature and rainfall), tradition and skill of local farmers, production chain and food processors.

## **Role of animal-based protein for human nutrition**

### *Inedible to edible protein*

The sources of nutrition from which animal-based proteins are produced (e.g. home grown, imported, using human food for animal feed) are important and contribute to the sustainability of grassland-based production systems. Ruminants (e.g. cattle, sheep, goats), due to their complex digestive system, can efficiently convert low-quality forages to protein rich products for human consumption (Clauss *et al.*, 2010). More recently, in many regions globally, ruminant production systems are becoming increasingly reliant on feed crops produced on arable land. This is resulting in food-feed competition as arable land increasingly produces feed for livestock rather than food for humans. The level of food-feed competition varies between and within regions (Leroy *et al.*, 2022), influenced by many factors including production costs, product price, scale of operations, logistics of feed supply (e.g. access to harbour for importation) and availability of resources. In many cases, ruminant production systems have moved to non-traditional regions/areas where forage production is low for specific local reasons including, but not limited to, excess or not enough rainfall, extreme temperatures (high or low), soil type, topography and costs. Lack of grassland (forage) requires that these livestock are fed crops (e.g. silage maize and soya) meaning livestock are directly competing with humans for cereals and other human edible crops including grains, vegetables and potatoes.

Worldwide, livestock are net contributors to the production of human edible protein (e.g. Leroy *et al.*, 2022; Mottet *et al.*, 2018). The net supply of edible protein by livestock can be considered using a number of metrics including feed conversion ratio (FCR; Wilkinson, 2011), total protein efficiency (Laisse *et al.*, 2018), net protein efficiency (Laisse *et al.*, 2018), edible protein conversion ratio (EPCR; Laisse *et al.*, 2019; Mosnier *et al.*, 2021; Wilkinson, 2011) and land-use ratio (LUR; van Zanten *et al.*, 2016). Globally, all ruminant production systems, including feedlot systems, require about 0.6 kg human-edible protein per kg of human edible protein produced (Mottet *et al.*, 2017). Grassland based ruminant production systems perform well in terms of converting feed protein to food protein. Laisse *et al.* (2018) showed that the net protein efficiency (conversion of human edible protein in the feed to human edible protein products) of grassland based dairy cattle production receiving low concentrate input is up to 2.57 in France, while Hennessy *et al.* (2021) reported a net protein efficiency of 4 for Irish grassland based dairy cattle production systems (Figure 1).

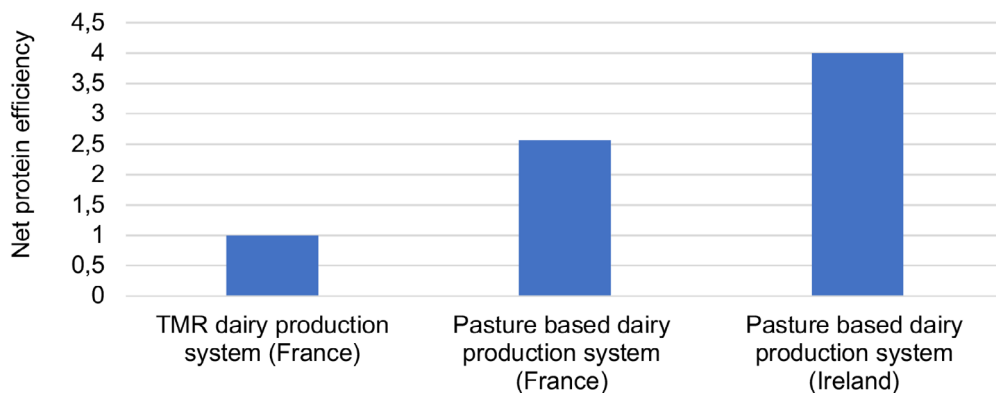


Figure 1. Net protein efficiency of dairy production systems (kg human edible protein produced (kg human edible protein consumed by livestock)<sup>-1</sup>) of dairy production systems in France (indoor feeding TMR and grassland-based; Laisse *et al.*, 2018) and Ireland (grassland based; Hennessy *et al.*, 2021).

### *Animal products: high quality protein source*

A recent report by the FAO identifies the important contribution of terrestrial animal-sourced food (milk, meat, eggs) to healthy diets for improved nutrition and health outcomes (FAO, 2023). Terrestrial animal-source foods provide energy and many essential nutrients, such as protein, fatty acids and several vitamins and minerals that are less common in other food types (FAO, 2023). An adequate supply of protein in the diet is defined as the ability of the diet to supply proteins to meet the nutritional demands of the body and depends not only the quantity of protein provided by the diet but also the quality of the proteins in terms of essential amino acids and their digestibility (Ertl *et al.*, 2016; Neumann *et al.*, 2002; WHO *et al.*, 2007). It is generally recognised that animal proteins are of higher nutritional quality for humans than plant proteins, due largely to their digestibility and amino acid composition, particularly the essential amino acids (Day *et al.*, 2022) that must be supplied by food as they cannot be synthesised in adequate quantities in the body. The digestible indispensable amino acid score (DIAAS) is a measure of protein quality. Ertl *et al.* (2016) found that the DIAAS of food from Austrian dairy systems is up to 1.87 times greater than that of the potentially human-edible protein content of the feed input to the dairy systems. Hennessy *et al.* (2021) reported that the DIAAS of dairy systems in Ireland was up to 4.55 and suckler beef systems was up to 3.45 times greater than that of the potentially human-edible protein input fed to cattle. The high DIAAS in Ireland is largely driven by the high quantity of grass in the diet of livestock in dairy (O'Brien *et al.*, 2018) and beef systems. Walther *et al.* (2022) compared the composition of full fat milk and plant-based beverages (marketed as milk substitutes) and found that the protein quality of milk was outstanding compared with all plant-based drinks and exhibited higher calculated DIAASs. The biologically higher value of milk compared to plant-based beverages was demonstrated by a study with over 5000 Canadian toddlers showing that children consuming milk were significantly taller than those using plant-based alternatives (Morency *et al.*, 2017).

Milk and meat are rich sources of many other essential nutrients (e.g. Leroy *et al.*, 2023; Walther *et al.*, 2022) of key importance for human health. Many are considered crucial for the human brain, including iron, zinc, and vitamin B12 and if not supplemented, these nutrients are either obtained exclusively from animal-sourced foods or are more bioavailable in those foods (Leroy *et al.*, 2023). Beal and Ortenzi (2022) estimated that the bio availability of iron and zinc in ruminant meat is 2 and 1.7 times, respectively, greater than that of pulses (e.g. beans, peas, lentils). Meat also supplies many of the other B vitamins, including niacin and thiamine, that can be limited in micronutrient-poor diets based on non-fortified cereal staples (Leroy *et al.*, 2023).

The longer chain forms of omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are only found in marine organisms and land herbivores, are critical components of human nutrition in terms of cell membrane structure and tissue health (especially for the brain, heart, and retina). They also play a role in cardiovascular health and mitigation of chronic inflammation (Leroy *et al.*, 2023). The fatty acid profile of grassland fed milk and meat is generally more desirable than that of produce from livestock fed high concentrate diets. Milks from grass-based diets have higher proportions of unsaturated fats and higher unsaturation, health-promoting, and desaturase indices than livestock fed medium or low levels of grass (Timlin *et al.*, 2023). Grass-fed milks have high levels of omega-3 and conjugated linoleic acids compared to indoor total mixed ration feeding systems (O'Callaghan *et al.*, 2016; Timlin *et al.*, 2023).

#### *Risk of reduced grassland for food production*

More recently, bioenergy production is now competing for biomass usually used to provide feed and food as countries transition from fossil fuels to greener, renewable energy sources (Muscat *et al.*, 2020). The EU currently has a binding target of 42.5% renewable energy by 2030 (EU Renewable Energy Directive EU/2018/2001 amended in 2023 EU/2023/2413). Bioenergy produced from agricultural, forestry and organic waste feedstock continues to be the main source of renewable energy in the EU, accounting for about 59% of renewable energy consumption in 2021 and agricultural biomass made up 8% of that bioenergy (EC, 2021). In parts of Europe, e.g. France and Germany, bioenergy is in direct competition with animal feed for maize silage (Delaby, pers. commun.). In Denmark, pulp from biorefinery, which includes grass, suitable for animal feed is being diverted to anaerobic digestion (Eriksen, pers. commun.). In other regions, energy companies are paying high levels rent for land on which to place solar panels (solar farms) making land prices beyond the reach of livestock farmers. Utilising grassland area, and indeed arable area, for energy and fuel production ultimately reduces the quantity of food produced by agriculture.

#### *Can Green Biorefineries benefit net food security?*

Recently, there is increased interest in the use of green biomass from grasslands not just as ruminant feed but in novel more versatile ways (Gaffey *et al.*, 2023). Given the high yield potential of grasslands, this may result in a situation where grasslands and the ecosystem services they provide can be utilised more widely (Jørgensen *et al.*, 2022). The development of grass-based food products is, at least within Europe, limited due to the Novel Foods Act that requires authorization of novel food materials prior to the release to markets (EFSA, 2024). However, experimentation to use grass protein (Møller *et al.*, 2021) or even the fibre fractions (Csatári *et al.*, 2022) of the green biomass as human food components has been conducted and may become reality in the near future. While waiting for direct human consumption of grass protein and other nutrients, green biorefineries can promote net food security from grassland by providing novel feed ingredients that replace human edible components of livestock diets. Extracted grass protein is currently commercially produced in Denmark based on positive results obtained in feeding trails for pigs and poultry (Stødkilde *et al.*, 2020, 2023). In Finland, a low-cost approach of feeding mechanically separated juice from ensiled grass to growing pigs as part of their liquid feed resulted in similar pig performance and meat quality compared to feeding conventional feed components (Keto *et al.*, 2021). However, the extraction of protein requires high investment and operation costs, and yields of protein have remained low. This can partly be compensated by the fact that simultaneously with feed production, the green biorefineries produce several other commodities such as pulp for animal feed, fibres, animal bedding, biochemicals, nutraceuticals, bioactive compounds, biogas and biochar (Gaffey *et al.*, 2023).

### **Improving net food security from grassland**

Sustainable grassland-based ruminant production systems must take cognisance of the impacts of grazing and grassland management on the wider ecosystem, and while contributing to net food security, can

be used to address many of the sustainability challenges relating to agriculture. Sustainable grassland production systems mean different things in different regions. In temperate climates, where a long grass growing season occurs, including Ireland, parts of the UK, North-West Europe, New Zealand, parts of Australia and parts of South America, grazed grass contributes significantly to milk and meat production, while in other regions, grassland provides the main source of forage for indoor feeding systems or for long winter periods (e.g. Dillon *et al.*, 2005; O'Brien *et al.*, 2018; Virkajärvi *et al.*, 2015). However, ultimately sustainable grassland systems provide a low-cost forage for ruminant production systems whether fed *in-situ* via grazing or conserved as hay, haylage or silage. Grassland-based milk and meat production systems make use of human inedible forages and marginal land not suitable for growing food crops to produce human edible protein to sustain a growing global population. While there can be negative impacts of some ruminant production systems, well managed grassland systems can contribute positively to ecosystem management and address sustainability challenges for agriculture, while simultaneously making use of inedible feed and marginal land for food production (Leroy *et al.*, 2022; Petermann and Buzhdygan, 2021; Thompson *et al.*, 2023).

### *Grass leys*

Sustainable grassland management maintains healthy grassland ecosystems, supports livestock production, and promotes environmental sustainability. Even within cropping areas, short term grassland leys play an important role in the rotation. There is a renewed interest in mixed livestock and cropping farming (e.g. De Wit *et al.*, 2006; Sekaran *et al.*, 2021). In many regions, grass and livestock are being introduced or reintroduced to tillage and cropping systems as part of the rotation (Van Eekeren *et al.*, 2023). Grassland plays an important role in maintaining crop production by contributing to soil organic matter (OM) content and providing a better balance for crop rotations, contributing to reduced reliance on fertiliser and pesticide use. This is (only) possible with grassland and the ruminants who feed on grassland and recycle nutrients back into the soil through urination and defecation in grazing systems and slurry and farmyard manure in other ruminant production systems, while producing food from that grassland.

### *Grazing management*

Achieving high levels of production and optimum utilisation from grazed grassland requires the implementation of good grazing practices. Developing a grazing plan that considers factors such as stocking rate, feed demand, forage growth rates, annual grassland production, and seasonal variations in grassland production delivers sustainable systems. Adjusting grazing intensity based on these factors prevents overgrazing and overuse of the pasture resources. Implementing appropriate grazing management systems to optimise the utilization of high-quality feed will increase animal production through grazing. There are several types of grazing systems, each tailored to suit the specific environmental, soil and herd characteristics of the farm/region, as well as the management objectives. Some of the common grazing systems include continuous grazing systems, mob grazing, rotational grazing, strip grazing and cell grazing (Allen *et al.*, 2011). Well managed grazing systems promote grass quality and allow for effective utilisation of grassland, usually permitting adequate regrowth periods while minimising tissue turnover, e.g. 21-day grazing rotation in mid-season in perennial ryegrass swards in temperate regions ensures plants reach the three-leaf stage and are grazed before herbage senescence commences (e.g. Turner *et al.*, 2006). Grazing livestock recycle nutrients through dung and urine deposition and thereby promote soil fertility and plant growth.

Quantifying the capacity of farmland to grow grass is an important consideration in promoting sustainable grazing systems. Measuring weekly, seasonal, and annual grass production allows farmers to determine appropriate stocking rates and grassland management strategies (O'Donovan *et al.*, 2020). Decision tools/systems are available in many countries to support grazing management, these include PastureBase Ireland ([www.pbi.ie](http://www.pbi.ie); Ireland; Hanrahan *et al.*, 2017), the MoSt Grass Growth model



(Ireland; Ruelle *et al.*, 2018), Pâture Plan (France), and Beweidingswijzer (<https://webapplicaties.wur.nl/software/beweidingswijzer/>; The Netherlands).

### *Sward species*

Selecting the best plant species for grazed grassland contributes to yield, quality and nutrient use efficiency. Optimising the yield of grassland from a given land area provides more feed for livestock. Species evaluation (and breeding programmes) provide important information for farmers who are sowing leys, reseeding grassland and enhancing grassland. Tools like the Pasture Profit Index (McEvoy *et al.*, 2011; Tubritt *et al.*, 2021) and the Forage Value Index (Chapman *et al.*, 2017) provide a ranking of perennial ryegrass cultivars in terms of production, quality, and persistence. Similar indexes/tools are not currently available in all countries/regions or for other grassland species, but national recommended lists are valuable resources providing data on species performance at region/country level.

Incorporating legumes into grassland systems has positive impacts on herbage production and quality. Red and white clovers are of particular importance in European grassland systems. The biological N fixation capacity of legumes reduces the requirements for chemical nitrogen (N) fertiliser input. Andrews *et al.* (2007), Enriquez-Hidalgo *et al.* (2018) and Dineen *et al.* (2020) are amongst numerous authors who have shown that herbage production can be maintained or increased when there is white clover in the sward, even with reduced chemical N fertiliser input. Red clover has a significant capacity to produce large quantities of high-quality silage with little to no chemical N fertiliser input (e.g. Clavin *et al.*, 2017; Holohan *et al.*, 2022; Marshall *et al.*, 2017) making it an important contributor to grassland-based production systems. Incorporating legumes in grassland have been shown to maintain or increase herbage quality at lower N fertiliser application rates compared to grass swards and subsequently increase animal production. For example, Thomson *et al.* (1985) and Egan *et al.* (2018) reported similar herbage crude protein content on grass-white clover swards receiving lower chemical N fertiliser input compared to grass-only swards, and Leach *et al.* (2000) and Riberio Filho *et al.* (2003) found that digestibility of grass-clover swards was greater than that of grass-only swards at low chemical N fertiliser input.

Sown multispecies swards are important in cutting and grazing systems across Europe. In terms of herbage production, there are conflicting data regarding the benefits of multispecies swards relative to grass-only swards, with many attributing the herbage production benefits to the legume component of the sward (Jaramillo *et al.*, 2021; Moloney *et al.*, 2021) and others finding benefits only under cutting (e.g. Jing *et al.*, 2017). McCarthy *et al.* (2020), in a meta-analysis, reported milk production benefits of multispecies swards compared to grass-only swards, while Bryant *et al.* (2017) found no benefits of multispecies swards on milk production. The benefits of components of multispecies swards, however, are limited by the persistence of species, particularly herbs and some legumes in grazed grassland (Gilliland, 2022).

### *Manipulation of grass to concentrate ratio in dairy cow diets*

Manipulating the grass silage to concentrate ratio in dairy cow diets (e.g. Ferris *et al.*, 2001 (concentrate proportion 0.10–0.80 on a dry matter (DM) basis); Sairanen *et al.*, 2022 (concentrate proportion 0.34–0.54 on a DM basis); Steinshamn and Thuen, 2008; (concentrate proportion 0–0.39 on a DM basis)) influences both milk yield and the quantity of that milk produced from grass. These examples show that there is great flexibility in the proportion of grass-based feeds in dairy cow diets. The large variation observed in different European production systems is thus defined based on other factors such as biophysical conditions affecting the relative competitiveness of grass *vs.* other feed production possibilities, regulations (e.g. organic production), traditions, and importantly, the price of different feeds relative to milk price. From a net food production perspective, there is much scope to reduce the human edible proportion in dairy cow diets, if sufficient incentives are obvious for the farmers. To make informed decisions in ration formulation, knowledge of the feeding value and potential DM intake of different

feeds, and the subsequent milk output is required. An important concept is the substitution rate, which describes the reduction in intake of grass silage offered *ad libitum* when concentrate input is increased. Huhtanen *et al.* (2008), in a meta-analysis, found that the substitution rate was, on average  $-0.47$ , but varied depending on the feeding scenario (Figure 2). The authors concluded that the substitution rate is quadratic, i.e. the reduction of silage intake is greater at higher concentrate inclusion levels (Figure 2a). In addition, when silage intake potential is high (both nutritional and preservation quality), the substitution rate is greater than with lower quality grass silage (Figure 2b).

## Challenges for grassland-based agriculture

Agriculture, including grassland-based systems, faces many sustainability challenges including economic, social and environmental aspects. The key economic sustainability challenge is around the provision of a living income for farmers/farming families. Social sustainability challenges include the wider society benefits including sustaining rural communities, landscape management, employment/availability of labour, health and wellbeing of the farmer and their family. Environmental sustainability challenges include water quality, greenhouse gas emissions, ammonia emissions, biodiversity, carbon sequestration/loss/emissions. The Nitrates Directive (91/676/EEC) and Water Framework Directive are targeting improved water quality. The EU Biodiversity Strategy addresses biodiversity decline and will be supported by the Nature Restoration Law. The European Climate Law (Regulation 2021/1119) is focused on reducing net greenhouse gas emissions and achieving climate neutrality. In contributing to global food security, agriculture must strive to minimise environmental impact. In addition to herbage production and provision of feed for livestock, grasslands offer a diverse array of ecosystems services that are not provided or are limited in arable and other crop systems.

### Reducing inputs and losses agriculture

Recycling organic manures produced on farm or importing manures reduces the requirement for chemical fertilisers (N, phosphorus and potassium) for grassland production. Applying slurry and manure using methods and at times of the year that minimise loss of N through volatilisation ensures that N is retained for plant growth (e.g. Lalor and Schulte, 2008). Covering slurry storage tanks, reducing slurry pH via acidification and shortening manure storage time are some of the means of reducing greenhouse gas (GHG) emissions from slurry. Adapting the type of chemical N fertiliser used to low emissions fertiliser, e.g. replacing calcium ammonium nitrate fertiliser with urea or urea combined with a urease inhibitor such as NBPT, 2-NPT or NBPT+NPPT reduces ammonia emissions (e.g. Forrester *et al.*, 2017).

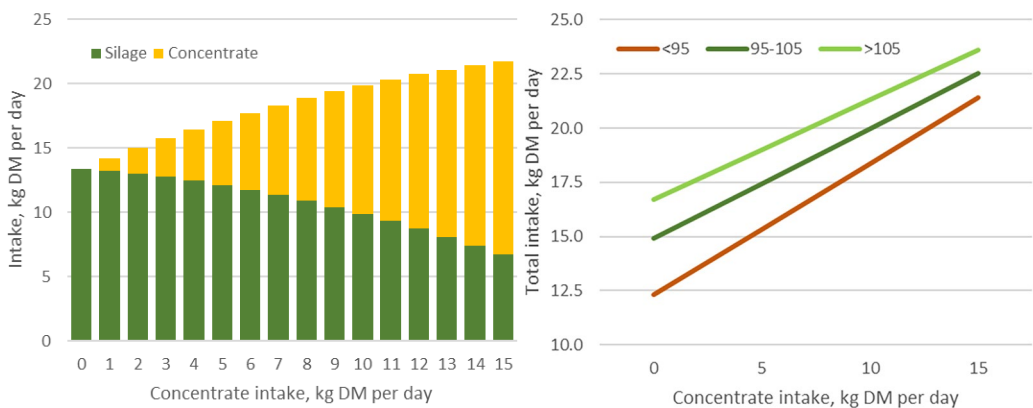


Figure 2. Substitution rate is higher at (a) high concentrate inclusion levels and (b) when grass silage has a high DM intake index (Huhtanen *et al.*, 2007). The relationships are based on Huhtanen *et al.* (2008).

Incorporating legumes reduces the requirement for chemical N fertiliser application in intensive grazing systems from 250+ kg N ha<sup>-1</sup> to less than 150 kg N ha<sup>-1</sup> (e.g. Egan *et al.*, 2018; Murray *et al.*, 2024; Andrews *et al.*, 2007). Plantain in multispecies swards can reduce urinary N content (Bryant *et al.*, 2017; Marshall *et al.*, 2021) which may result in reduced nitrate leaching. In New Zealand, a number of large research projects are exploring the benefits of incorporating plantain in grassland swards to reduce nitrate loss (e.g. Navarrete *et al.*, 2022; Rodriguez *et al.*, 2020).

The use of technology to increase the precision of chemical N fertiliser application (e.g. GPS, identification of N hotspots), decisions support tools to estimate herbage availability and growth rates, remote sensing of herbage mass and rapid detection of soil nutrient status are likely to provide greater opportunities to reduce chemical N fertiliser use in the future.

### *Mitigating greenhouse gas emissions*

Grassland based milk production systems can have lower methane emissions per unit of livestock compared to indoor total mixed ration (TMR) systems (Robertson and Waghorn, 2002; O'Neill *et al.*, 2011). This can be due to lower milk production in grazed systems (O'Neill *et al.*, 2011), and the effect may be seasonal, with lower emissions when grass quality/digestibility is high, e.g. in spring (Lahart *et al.*, 2024). The forage to concentrate ratio in ruminant diets is highly variable, as discussed previously. Although diets with higher concentrate proportion produce less methane per day or per kg energy corrected milk yield (Jiao *et al.*, 2014; Lovett *et al.*, 2005), the human edible feed ratio is greater, and the source of the components of the ration can increase the carbon footprint of the product.

Improving the quality of the grassland consumed by grazing livestock has positive impacts in terms of methane emissions. Grassland forage quality can be increased through improved grassland management resulting in lower pre-grazing herbage mass (Wims *et al.*, 2010), seasonal effects (Lahart *et al.*, 2024; O'Neill *et al.*, 2011) or sward composition, namely inclusion of white clover in the grassland sward (Enriquez-Hidalgo *et al.*, 2014; Lee *et al.*, 2004). Selecting animals suited to grassland and forage-based systems will ensure good feed conversion efficiency, reducing methane emissions per kg food product. Lahart *et al.* (2024) found that higher genetic merit dairy cows (based on the Irish Economic Breeding Index), bred for pasture-based systems in Ireland, had lower methane emissions than the national average dairy cow. There is conflicting evidence in the literature in terms of the benefits, if any, of multispecies swards in reducing methane emissions (e.g., Loza *et al.*, 2021; Ramirez-Restrepo and Barry, 2005), and the impact appears to be influenced in particular by the presence/absence of herbs and the species of herbs, as well as maturity and grazing intensity. Herron *et al.* (2021) found that grass-clover systems with reduced chemical N fertiliser input reduced the global warming potential of pasture-based milk production systems by 5.4%, driven by reduced chemical N fertiliser inputs and increased milk production from the same land area compared to a grass-only high chemical N fertiliser input system. Multispecies swards containing legumes reduce nitrous oxide emissions (Cummins *et al.*, 2021; Luo *et al.*, 2018) through reduced chemical N fertiliser input by up to 41%.

### *Carbon sequestration*

Grasslands play an important role in increasing carbon (C) sequestration (or maintaining it in regions where there is already high soil organic carbon stocks (SOC)). Quantifying soil C sequestration and changes in SOC stocks is challenging as data is required over long periods. Up to 11% of the carbon applied in slurry to grassland is retained in the soil, building SOC (Jensen *et al.*, 2022). Introducing grass-clover leys to the rotation in long term arable ground for two years in a six-year rotation increased SOC, more rapidly in the initial years of a long-term study by Jensen *et al.* (2022). Deeper rooting herbs associated with sown multispecies swards may result in increased carbon sequestration (e.g., Fornara and Tillman, 2008). In a large review, Conant *et al.* (2017) found that, improving grassland management

(grazing management practices, fertilisation, incorporating legumes and improving grassland species, as well as irrigation where used) and converting land use from crops to grassland increased soil C stocks at rates of 0.105 to more than 1 Mg C ha<sup>-1</sup> year<sup>-1</sup>. Other authors, including Soussana *et al.* (2004), conclude that long term grassland is important for increasing C sequestration, and for conserving C stocks, and soil disturbance in grassland swards, such as required when converting to arable/crops should be minimised to avoid loss of C stocks.

#### *Water quality and management*

Acknowledging that agriculture has, in the recent past, had a negative impact on water quality, grassland is an important land use in terms of mitigating nutrient loss to water. Nitrogen loss is a big risk to water quality. Within grassland systems there are numerous sources of N including fertiliser, organic manures, soil N, biological N fixation, and dung and urine deposition, all providing N to satisfy the demands of productive grassland. However, N supply can be surplus to sward requirements for a variety of reasons and can potentially be leached. Strategic fertiliser application, taking consideration of sward demands, weather conditions, presence/absence of legumes, can reduce the risk of surplus N. Poor soil fertility limits the capacity of grassland to utilise N, contributing to the potential for loss (e.g. Lawniczak *et al.*, 2016). Intensively, well managed grassland can effectively use fertiliser N, even at relatively high application rates with limited additional leaching. Fontaine *et al.* (2023) reported no additional leaching from grass-clover leys receiving up to 150 kg fertiliser N ha<sup>-1</sup>, and at 200 kg N ha<sup>-1</sup> around 5% of additional fertiliser-N was leached. Increasing N use efficiency, precision grassland management, reducing N surplus by reducing N inputs (purchased feed and fertiliser), and increasing the N products (milk and meat) sold from the farm are important farm level actions to reduce nitrate leaching (Murphy *et al.*, 2024). Riparian margins and buffer zones offer mitigation, especially for N and phosphorus likely to be lost in runoff and overland flow. Grasslands mitigate against flooding and soil erosion (Milazzo *et al.*, 2023). They act as natural sponges, absorbing water during heavy rainfall events, reducing and slowing overland flow and subsequently flooding downstream. The extensive root systems in grassland help with water infiltration and grassland cover reduces opportunities for soil erosion during flooding events. Although pesticides are used on grassland, they are generally used at the time of sward renewal/reseeding or when significant weed encroachment occurs, so that the ecotoxicity pressure is smaller than from most other crops. Leys within crop rotations provide break crops, reducing the risk of pests and diseases in cereal and other crops reducing pesticide use and therefore the risk of runoff to water ways.

#### *Biodiversity and landscape management*

Milk and meat production often conjures images of livestock grazing in green landscapes. Grasslands are an integral part of our landscapes and grazing livestock deliver significant management of those grasslands. Managed grasslands provide scenic landscapes and offer recreational opportunities (e.g. walking, hiking) not available from other agricultural systems. They contribute to species richness within landscapes and play an important role in helping address the biodiversity crisis. Managed grasslands avoid scrub or forest encroachment which can result in plant species loss (e.g. Pornaro *et al.*, 2013). A range of habitats within swards and on the margins occur in grassland farms. Short and tall grass areas in grazed grassland, extensively managed grassland, old permanent pasture, multispecies swards, meadows, and legume-rich swards are amongst the habitat types provided by grasslands. In addition, grassland farms often have a range of other habitats including hedgerows, trees, small woodlands, ponds, wetland/peat areas, old farmyard buildings and structures, historical ruins, extensively managed areas, all providing habitats for a range of flora and fauna. Riparian margins, often established to reduce nutrient and pesticide losses to waterways, can be rich biodiversity habitats supporting flora and fauna. Increasing the species incorporated in sown grasslands has a role in reversing biodiversity decline. Incorporating a range of grass, herb and legume species increase the plant species richness, enhancing biodiversity both above and below ground (e.g. Grange *et al.*, 2021).

## Conclusion

As the global population continues to grow, and the demand for human edible protein increases, grasslands play a key role in global net food security through provision of a low cost, human inedible feed source for milk and meat production. Grassland ruminant production systems have a high net protein efficiency compared to other feeding systems that have more grains and other crops and provide food (milk and meat) with a high digestible indispensable amino acid score for human nutrition. The land area under grassland is generally marginal and not suitable for crops for food production for local/site specific reasons. Managing grasslands to optimise the production and quality of feed for ruminant production systems contributes to net food security will also providing a wide range of ecosystem services and addressing and providing solutions to the many sustainability challenges faced by agriculture.

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# Intake and growth of steers offered perennial ryegrass and perennial ryegrass-red clover silage

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## Abstract

A moderate average daily gain (ADG) of 0.6 kg day<sup>-1</sup> is targeted for dairy-beef weanlings during their first winter indoors. Deviations from this target can influence animal growth when returned to pasture. The objective of this study was to determine the intake, growth and subsequent compensatory growth at pasture of dairy-beef steers offered either perennial ryegrass (PRG) or perennial ryegrass-red clover (RCGS) silage during their first winter indoors. Seventy-two steers were assigned to PRG ( $n=36$ ) or RCGS ( $n=36$ ) silage treatments. Perennial ryegrass-red clover silage had a clover proportion of 0.87. Silage was offered *ad libitum*, plus 1.3 kg DM hd<sup>-1</sup> d<sup>-1</sup> of concentrate. Steers fed RCGS silage showed higher DMI and ADG by 1.4 kg and 0.12 kg, respectively, compared to PRG ( $P<0.05$ ). However, elevated intake of RCGS led to increased residual feed intake, reducing feed conversion efficiency ( $P<0.001$ ). Although non-significant ( $P>0.05$ ), RCGS-fed steers were 9 kg heavier after the winter indoors but showed no difference by the end of the second grazing season. The PRG steers achieved a compensatory growth index of 1.0, suggesting RCGS's higher animal growth over-winter growth restricts subsequent gains the following grazing season.

**Keywords:** red clover, silage, dry matter intake, average daily gain, compensatory growth

## Introduction

Forage legumes, such as red clover, can contribute substantially to pasture-based ruminant production systems due to their ability to fix atmospheric nitrogen (N), thus reducing the reliance on chemical N fertiliser. Given the increasing need to improve farm gate N balance because of rising fertiliser and feed costs and environmental constraints, red clover can offer significant benefits to beef production systems. Swards with a high content of red clover (75% on a dry matter (DM) basis) are capable of fixing 24–36 kg N (Mg DM produced)<sup>-1</sup> (Peoples and Baldock, 2001, Peeters *et al.*, 2006), meaning swards of high red clover proportion and DM production are potentially fixing in excess of 200 kg N ha<sup>-1</sup> annually. Animal performance is generally increased when animals consume red clover-grass silage compared with grass silage, primarily due to increased dry matter intake (DMI) potential. Beef cattle consuming red clover-grass silage are known to have an increased DMI when compared with animals consuming grass silage (Steen and McIlmoyle, 1982). Red clover generally contains a greater ratio of indigestible fibre: digestible fibre (0.27 vs. 0.19, respectively) than grass silage (Halmenmies-Beauchet-Filleau *et al.*, 2014). Although the extent of digestion is reduced for red clover-grass silage when compared with grass silage, the rate of digestion of the digestible fibre is faster (Kuoppala *et al.*, 2009). This facilitates a faster rate of passage, lower rumen fill and thus increased DMI. Red clover has a higher concentration of crude protein (CP) compared to grass. Consequently, as the proportion of red clover reduces relative to grass in silage swards, there is a corresponding reduction in silage CP concentration (Clavin *et al.*, 2017). However, despite higher DMI and expected animal performance from silages containing red clover, little is known about its impact on feed efficiency and compensatory growth potential of dairy-beef cattle. The objective of this study was to determine the intake, growth and compensatory growth of dairy-beef steers offered either PRG or RCGS silage during their first winter indoors.

## Materials and methods

Experimental silages were harvested during summer 2022 from secondary regrowth on 20 July, allowing 58 days of regrowth, using a mower conditioner and allowed to wilt for 36 h, precision chopped and ensiled in a walled silo for PRG and RCGS treatments. Both sward types contained tetraploid perennial ryegrass varieties of similar heading date and sward age (27 May to 5 June heading dates), with RCGS swards having the addition of a late heading diploid RC variety, across five individual paddocks (approx. 1 ha each) per treatment. Immediately after the primary harvest, 57 kg and 0 kg inorganic N ha<sup>-1</sup> were applied to PRG and RCGS swards, with each sward type receiving 26 kg and 111 kg inorganic P and K ha<sup>-1</sup>, respectively. Annually, PRG and RCGS swards received a total of 208 kg and 0 kg inorganic N ha<sup>-1</sup> and produced 15.6 and 19.3 Mg DM ha<sup>-1</sup>, respectively. Seventy-two spring 2022 born dairy-beef steers (Angus×Holstein Friesian or Aubrac×Holstein Friesian) were randomly assigned to one of two silage treatments: (1) perennial ryegrass (PRG) ( $n=36$ ), (2) perennial ryegrass-red clover (RCGS) ( $n=36$ ). Animals assigned to treatments were balanced by genotype, sire, liveweight and age. All animals were group penned on a slatted floor surface, offered *ad libitum* access to silage and supplemented with 1.3 kg DM hd<sup>-1</sup> day<sup>-1</sup> of barley-based concentrate, comprised of 862 g kg<sup>-1</sup> rolled barley, 60 g kg<sup>-1</sup> soyabean meal, 50 g kg<sup>-1</sup> cane molasses and 28 g kg<sup>-1</sup> vitamins and minerals. Individual feed intakes were recorded daily using an electronically controlled Calan-gate feeding system for 60 days after undergoing an acclimatisation period of 35 days. Animal liveweight was measured fortnightly and muscle and fat depth measured ultrasonically at the beginning and end of the housing period. Silage and concentrate was sampled twice weekly over the winter period to determine DM content and chemical composition. The data from this study were analysed using a mixed model with the fixed effects considered being animal genotype and age, and silage treatment. To determine RFI mid-test metabolic liveweight, ADG and fat depth were included as additional fixed effects. Random effects considered were pen and animal sire.

## Results and discussion

Despite the same harvest date and management, PRG silage exhibited superior *in vitro* digestibility compared to RCGS, while having lower CP content ( $P<0.001$ ) (Table 1). Despite the lower digestibility of RCGS, it contributed to higher DMI and ADG ( $P<0.001$ ). The lower digestibility of RCGS suggests the potential for a faster passage rate through the digestive system, leading to reduced rumen fill. This, in conjunction with higher CP intake, likely contributes to the improved animal intake and growth characteristics observed.

Although RCGS was grown with fewer inputs and resulted in higher animal performance over the winter, its inclusion resulted in lower feed conversion efficiency as it increased RFI by 0.29 kg DM day<sup>-1</sup>, thus the economic efficiency of this additional gain needs to be examined further. The high DMI characteristic, CP and ADG of RCGS suggests that concentrate could be eliminated or reduced for dairy-beef cattle over this first winter indoors while maintaining a moderate growth of 0.6 kg day<sup>-1</sup>.

As a result of the higher ADG, RCGS steers were 9 kg heavier at the end of the winter period. However, this difference was found to be non-significant ( $P>0.079$ ), likely attributable to a type II error stemming from increased variance in animal liveweight and the limited sample size per treatment. Despite the higher initial weight of RCGS steers at the onset of the second grazing season, both treatment groups reached similar liveweights by season end. Notably, PRG steers demonstrated a compensatory growth index of 1.0, signifying their superior recovery in growth compared to RCGS steers when provided with higher nutritive value pasture during the grazing season, a direct consequence of achieving a moderate ADG during the winter period indoors.

Table 1. Red clover proportion and chemical composition of experimental silages.

Variable	PRG	RCGS	Concentrate
Red clover proportion	0.00	0.87	–
pH	3.6±0.09	4.1±0.09	–
Dry matter digestibility (g (kg DM) <sup>-1</sup> )	719±9.5	629±9.5	–
Digestible organic matter (g (kg DM) <sup>-1</sup> )	642±10.9	544±11.5	–
Organic matter digestibility (g (kg DM) <sup>-1</sup> )	704±10.7	602±10.7	–
Water soluble carbohydrates (g (kg DM) <sup>-1</sup> )	15.4±3.12	8.8±3.27	–
Ash (g (kg DM) <sup>-1</sup> )	91±1.9	93±1.9	58±1.2
Crude protein (g (kg DM) <sup>-1</sup> )	133±3.4	153±3.4	140±4.7
Neutral detergent fibre (g (kg DM) <sup>-1</sup> )	412±32.4	458±32.4	152±6.6
Acid detergent fibre (g (kg DM) <sup>-1</sup> )	319±7.2	335±7.2	48±1.5

Table 2. Feed intake, feed efficiency and growth characteristics per silage treatment.

	PRG	RCGS	SE	P-value
No. of animals	36	36	–	–
1 <sup>st</sup> winter indoors				
Dry matter intake (kg DM day <sup>-1</sup> )	6.2	7.6	0.126	0.001
Residual feed intake (kg DM day <sup>-1</sup> )	-0.14	0.15	0.073	0.001
Initial liveweight (kg)	286	286	3.7	0.104
Final liveweight (kg)	337	346	3.8	0.079
Average daily gain (kg day <sup>-1</sup> )	0.56	0.68	0.035	0.021
2 <sup>nd</sup> grazing season				
Initial liveweight (kg)	339	346	3.5	0.114
Final liveweight (kg)	494	493	5.0	0.929
Average daily gain (kg day <sup>-1</sup> )	0.95	0.91	0.02	0.135

## Conclusion

Despite lower digestibility of RCGS, it maintains higher animal performance than PRG, although reducing feed efficiency. This demonstrates the opportunity for RCGS in beef systems to reduce reliance on inorganic N and concentrate, creating economic and environmental benefits. However, RCGS silages should be targeted towards priority animal groups, with limited compensatory growth opportunity and who can fully avail of increased intake and growth potential.

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# Grasslands' yield gap and its impact on the contribution to food security of dairy farms

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## Abstract

Grassland yield is highly correlated with dairy systems' net productivity, i.e. its contribution to food security. To estimate optimal net productivity per farm, we developed different scenarios of grass growth using LINGRA-N-plus model with three mowing regimes (4 cuts, 5 cuts and grazing) and three levels of fertilisation (90 kg N ha<sup>-1</sup>, 225 kg N ha<sup>-1</sup> and 360 kg N ha<sup>-1</sup>). For nine dairy farms located in the Liege grassland region (Belgium), we calculated the optimal net productivity reached with these grass productions and an eventual energy supplement. We compared it with their current net productivity for the years 2016 to 2020. Farms showed a high variability in current net productivity but had very similar optimal net productivities, indicating a margin of improvement linked to farm management. At 1 livestock unit (LU), performances of net productivity were similar to current performances at an average of 1.7 LU+70 kg mineral N ha<sup>-1</sup>. The use of 360 kg N ha<sup>-1</sup> induced up to 204% of net productivity but it reduced N fixation, N efficiency and N self-sufficiency. An energetic supplementation induced, in this case, a maximum of +7% of net productivity. This work can help farmers and advisers reflect on current farm management to improve contribution to food security.

**Keywords:** livestock systems, grass-based, feed/food competition, grass growth simulation

## Introduction

The competition of livestock for human-edible feedstuffs and agricultural land availability is seen as a hindrance to future food security (Mottet *et al.*, 2017) providing manure and draught power, and generating income. But they also consume food edible by humans and graze on pastures that could be used for crop production. Livestock, especially ruminants, are often seen as poor converters of feed into food products. This paper analyses global livestock feed rations and feed conversion ratios, with specific insight on the diversity in production systems and feed materials. Results estimate that livestock consume 6 billion tonnes of feed (dry matter. Net productivity ((produced human-edible food–consumed human-edible feedstuffs)/non-human-edible area) is a metric representing the contribution to food security of animal systems that has been developed to integrate those two aspects simultaneously (Battheu-Noirfalise *et al.*, 2023). The maximum value of net productivity that dairy systems can reach is constrained by local pedo-climatic conditions, impacting grassland yields, and sustainable management such as fertilisation. The aim of this study was to test contrasted fertilisation and mowing regime to estimate grassland yield and its impact on potential net productivity on some commercial farms.

## Materials and methods

We calculated the current net productivity of nine specialised dairy farms (Liege grassland region, Belgium) on the basis of farm accounting data with the methodology proposed in Battheu-Noirfalise *et al.* (2023). Potential grass yields ( $\tau$  DM ha<sup>-1</sup>) and its CP-content (g kg DM<sup>-1</sup>) were determined using the LINGRA-N-plus model (Giannitsopoulos *et al.*, 2021) with water and N constraints for each farm-specific pedoclimatic conditions. The comparison was done for the years 2016 to 2020. We tested three contrasted mowing (4 cuts (4C), 5 cuts (5C), and grazing (G)) and three fertilisation regimes representative of contrasted stocking rates (90 kg N ha<sup>-1</sup> approx. 1 livestock unit (LU) ha<sup>-1</sup>, 225 kg N ha<sup>-1</sup> approx. 2.5 LU ha<sup>-1</sup> and 360 kg N ha<sup>-1</sup> approx. 4 LU ha<sup>-1</sup>). One LU is defined as one dairy cow

of 5790 l cow<sup>-1</sup> year<sup>-1</sup>. The amount of symbiotic-N fixation was estimated as a negative correlation of the N fertilisation (Limbourg, 1998) and resulted in an additive fertilisation of 46 kg N ha<sup>-1</sup> for 1 LU ha<sup>-1</sup>. The crude protein (CP) content of grass equalled the N content in harvested grass multiplied by 6.25, while the energy (VEM) content of grass was estimated as a function of the CP-content based on a regression of Walloon grass samples from 2010 to 2020 (REQUASUD licence A07/2023). The milk production per cow permitted by the CP-content and by the VEM-content of grass were both estimated using the formula of Cuvelier *et al.* (2021). If there was a VEM-deficit in the grass, we tested two energetic supplementations based on feed with contrasted human-edible protein fraction (hePF): wheat (1258 VEM and 110 g CP kg DM<sup>-1</sup>, hePF=66%) and sugar beet pulp (1025 VEM and 95 g CP kg DM<sup>-1</sup>, hePF=0%). The number of cows was determined as the grass DM yield divided by the ingestion capacity of cows, which was calculated as a function of the VEM or CP-limited milk production (Cuvelier *et al.*, 2021). Heifers were supposed to be calving at 24 months with a herd renewal of 25% and to eat on average 7.5 kg DM of grass per day. Silage losses and grazing losses were of 20% and 15% of the produced grass DM, respectively. Net productivity was calculated as the sum of the protein of the produced milk (number of cows \* milk production) and meat from the herd renewal minus the amount of human-edible protein of wheat if applicable. Optimal net productivity was calculated as the mean between the net productivity resulting from 4C, 5C or G, representing summer feeding, and from 4C or 5C representing winter feeding as grazing was considered impracticable in the winter.

## Results and discussion

Mean grass yields per treatment ranged from 7.2 (4C:1LU) to 13.6 (5C:4LU) t DM ha<sup>-1</sup> while mean CP-content ranged from 125 (5C:2.5LU and G:1LU) to 152 (G:4LU) g kg DM<sup>-1</sup> (Table 1). The N efficiency was the lowest for the highest fertilisation and lowest mowing frequency treatments, specifically 4C:4LU (65%) and 5C:4LU (69%). The N self-sufficiency was the highest for G:1LU (121%) and still higher than 100% for the two other 1LU treatments.

On average, the farms had grassland yields of 8.4±1.9 t DM ha<sup>-1</sup>, current net productivity of 212±65 kg CP for a stocking rate of 1.7±0.3 LU ha<sup>-1</sup> (equivalent to 153±27 kg N of manure) and an additional dose of 70±69 kg N ha<sup>-1</sup> of mineral N was provided. Using a linear model, the decomposition of variance showed that the individual farm was responsible for 30% of the variability of current net productivity. However, potential net productivity was not influenced by the farm, due to very similar pedo-climatic conditions. Therefore, Figure 1 shows the potential net productivity averaged between farms. Based solely on grass, the 1 LU-fertilisation shows performances of potential net productivity similar to the farm sample, ranging from 95 to 103%. Grossly, performances for 2.5 LU show an increase of 140–150%

Table 1. Mean (SD) of grass yield, CP-content, N efficiency and supported LU for the different treatments defined by 4 cuts (4C), 5 cuts (5C) and grazing (G), and 1, 2.5 and 4 LU ha<sup>-1</sup>.

Treatment	4C:1LU	4C:2.5LU	4C:4LU	5C:1LU	5C:2.5LU	5C:4LU	G:1LU	G:2.5LU	G:4LU
Yield	7.2	10.3	13.3	7.4	11.2	13.6	8.2	10.1	12.5
(t DM ha <sup>-1</sup> )	(1.3) <sup>f</sup>	(0.9) <sup>d</sup>	(1.6) <sup>ab</sup>	(0.4) <sup>ef</sup>	(0.8) <sup>c</sup>	(2.0) <sup>a</sup>	(0.5) <sup>e</sup>	(0.6) <sup>d</sup>	(1.8) <sup>b</sup>
CP-content	129	130	136	128	125	142	125	142	152
(g kg DM <sup>-1</sup> )	(8) <sup>d</sup>	(6) <sup>d</sup>	(2) <sup>c</sup>	(13) <sup>d</sup>	(11) <sup>d</sup>	(3) <sup>b</sup>	(6) <sup>d</sup>	(9) <sup>b</sup>	(11) <sup>a</sup>
N efficiency (%)	87	76	65	89	79	69	102	86	72
	(11) <sup>b</sup>	(4) <sup>cd</sup>	(7) <sup>f</sup>	(7) <sup>b</sup>	(3) <sup>c</sup>	(9) <sup>ef</sup>	(4) <sup>a</sup>	(3) <sup>b</sup>	(7) <sup>de</sup>
N self-sufficiency (%)	102	56	43	106	62	44	121	56	42
	(19) <sup>b</sup>	(6) <sup>d</sup>	(5) <sup>e</sup>	(9) <sup>b</sup>	(6) <sup>c</sup>	(6) <sup>e</sup>	(10) <sup>a</sup>	(4) <sup>d</sup>	(6) <sup>e</sup>

Different letters show significant differences between treatments.



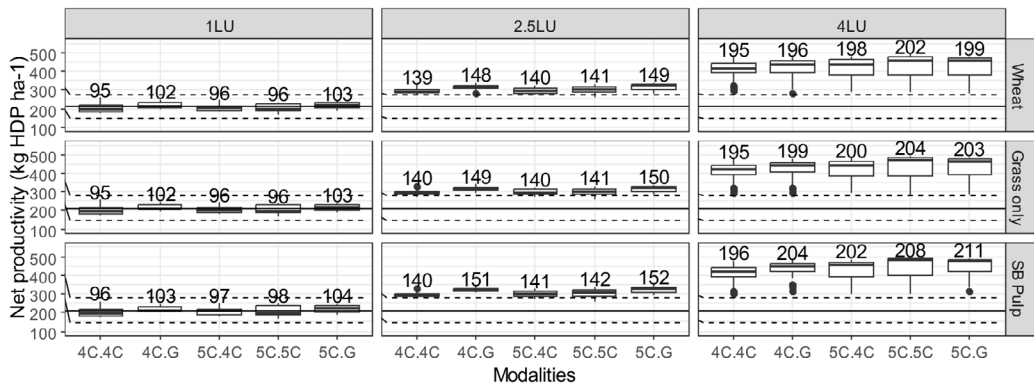


Figure 1. Optimal net productivity with modalities being the mean of a winter and summer treatment. The plain and dashed line represent the mean  $\pm$  SD of the current net productivity of the farm sample. Percentages above modalities compare optimal to current net productivity. HDP, Human Digestible Protein.

of the farm sample and the 4 LU show an increase of 195–204% of the net productivity. The differences between grass only and the use of an energy supplement are the highest for 5C.G:4LU with an increase of 7% with the use of sugar beet pulp and a reduction of 5% when considering the use of wheat.

## Discussion and conclusions

Results of the grass growth model appear consistent with Walloon permanent grassland yield data but the model has to be calibrated further. As farms showed high variability in current net productivity but very similar optimal net productivities, it offers perspectives in terms of improvement linked to farmer's management. Moreover, the model shows that with a lower stocking rate (1 LU), farms could potentially reach the same net productivity as they do currently, based solely on grass and on well managed heifers, frequent mowing for silage making and efficient grazing. However, this gap is also probably linked to N losses in farm manure management. Higher fertilisation induced higher net productivities (up to 204% of current performances for 360 kg N ha<sup>-1</sup>) but impacted N fixation, N efficiency and N self-sufficiency. Full grass diets are hard to achieve due to the limited ingestion ability of cows, and the use of an energetic supplementation induced, in this case, a maximum of +7% of net productivity with the use of non-human-edible feedstuffs. This work presents a locally adapted assessment that can help farmers and advisers reflect on current farm management to improve contribution to food security. This work does not consider further economic or environmental aspects.

## Acknowledgements

The first author is a recipient of a Ph.D. grant of the Fund for Scientific Research (FNRS). The authors thank D. Knoden (Fourrages Mieux) and B. Wyzen (Élévéo) for their inputs.

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# Land-use efficiency of grass-based versus maize-based dairy cattle to protein production in France

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## Abstract

Livestock use 56% of agricultural land in France. With the aim of preserving resources for food production, the question of the efficient use of land by dairy cattle arises. The Land Use Ratio (LUR) has been proposed for measuring land-use efficiency. It compares the potential for plant protein production on land used for livestock production with the animal protein produced on the same land. A LUR value lower than 1 indicates that animal production is more efficient to produce protein than plant production on a given land area. The LUR was applied to 12 case studies of French dairy cattle farms contrasted in their feeding system: grass-based, mixed and maize-based. Grass-based systems use more land per kg protein produced ( $78.6 \pm 13.6 \text{ m}^2 \text{ kg}^{-1}$ ) than maize-based systems ( $33.6 \pm 5.4 \text{ m}^2 \text{ kg}^{-1}$ ). But, compared to plant production, as grass-based dairy systems use significant proportion of non-arable grassland, they are more efficient in terms of land use for protein production than maize-based systems (LUR  $0.91 \pm 0.62$  vs  $2.29 \pm 0.63$ , respectively). Taking into account the edible fraction of plant and animal proteins and their respective digestible indispensable amino-acid score increases the land used efficiency of dairy systems whatever the system.

**Keywords:** feed–food competition, land use, efficiency, protein, dairy cattle, grass

## Introduction

In a context of limited resources, ruminant production is criticized for its low feed conversion efficiency and high use of land. Indeed, livestock uses 56% of the agricultural area in France, but 55% of this area are permanent grasslands, that are for a large part non-arable and contribute to C storage and biodiversity. Moreover, more than 75% of the diet of dairy cattle in France is not edible for humans (grass, forages, by-products) (Laisse *et al.*, 2018). To assess the land use efficiency, a common metric used is the area needed to produce one kg of plant or animal product (Nijdam *et al.*, 2012). The Land Use Ratio (LUR) that compares the potential for plant protein production on land used for livestock production with the animal protein produced on the same land was proposed (van Zanten *et al.*, 2016), and refined to take into account the quality of protein for human nutrition in plant and animal source foods (Hennessy *et al.*, 2021). Using the LUR at the farm scale, the aim of this study was to evaluate the land use efficiency for protein production of French dairy cattle systems differing in the proportion and type of grasslands.

## Materials and methods

Twelve case-studies of dairy farms distributed in 4 French regions, Auvergne-Rhone-Alpes ( $n=3$ ), Pays de la Loire ( $n=3$ ), Bretagne ( $n=2$ ) and Hauts de France ( $n=4$ ) were used. They were classified into 3 groups according to the respective proportions of grass and maize forage in the diet (Table 1). Proteins produced by the farming systems in milk and meat were considered, as well as all land utilised for animal production within the farm and outside corresponding to the feeds purchased. The calculation of LUR proposed by van Zanten *et al.* (2016) and Hennessy *et al.* (2021) were applied at the farm scale, using the following equation:

$$\text{LUR} = \frac{\sum_{i=1}^n \sum_{j=1}^m (\text{LO}_{ij} \times \text{HDP}_j)}{\text{HDP of 1 kg of ASF}}$$

where  $LO_{ij}$  is the land area occupied to cultivate the amount of feed ingredient  $i$  ( $i=1, n$ ) on the type of land used  $j$  ( $j=1, m$ ) that are needed to produce 1 kg of human digestible protein (HDP) of animal source food (ASF).  $HDP_j$  is the amount of plant digestible protein that could be produced per year on land type  $j$ . The land used in dairy cattle systems were distributed in 3 types  $j$ : (1) permanent grasslands that were considered as non-arable ( $HDP_j=0$ ); (2) arable land within the farm area (temporary grasslands and crops for concentrate feeds produced on the farm) and (3) arable land outside the farm for purchased concentrate feeds. We estimate  $HDP_j$  on arable land within and outside the farm from the mean production of a 6-year crop rotation combining wheat, maize, faba bean, rapeseed, wheat and lupin.  $HDP_j$  values were estimated to 278 and 806  $\text{kg ha}^{-1} \text{ year}^{-1}$  for organic and conventional systems respectively. LUR calculations were conducted according the original method in digestible protein (van Zanten *et al.*, 2016) and the adjusted method (Hennessy *et al.*, 2021) correcting the plant and animal protein production by the human-edible fraction and by the protein quality using the digestible indispensable amino-acid score (DIAAS) (FAO, 2013).

## Results and discussion

The two-thirds of land used for animal production in grass-based systems are permanent grasslands that we considered as non-arable, whereas they represent only around 20% of land used in mixed and maize-based systems (Table 1). As the milk production level of the cows in grass-based systems is lower than in mixed and maize-based systems, they require more land per kg protein produced. Consequently, they produce less digestible protein or “edible animal protein  $\times$  DIAAS” per hectare. But, the potential of plant protein production on the land used in grass-based systems is lower than the animal protein produced (108.1 vs 126.6  $\text{kg ha}^{-1}$ ). In contrast, digestible animal protein produced in mixed and maize-based systems is lower than the potential of digestible plant protein production (Table 1).

LUR values calculated according van Zanten *et al.* (2016) show that only two grass-based systems are more efficient than plant production ( $LUR < 1$ ) for protein production (Figure 1). When LUR calculation integrates the edible fraction of protein and their quality through the DIAAS (Hennessy *et al.*, 2021), LUR values are lower than 1 for all grass-based systems, 2 mixed and 2 maize-based systems, indicating these systems as more efficient than plant production. This study highlights that, although the LUR calculations rank the systems in the same order, the results highly depend on assumptions made on (1) the non-arable feature of permanent grasslands, (2) the potential level of plant production on the land used for protein or other nutrients, and (3) the relative nutritional quality between animal and plant products.

Table 1. Characteristics of the 12 dairy farms case-studies and their animal protein production land-use efficiency compared to the potential plant protein production one.

	Grass-based (n=4)	Mixed (n=4)	Maize-based (n=4)-
Feeding system	>80% grass 0% maize	<80% grass 10–20% maize	<60% grass >20% maize
Total land used for animal production (ha)	75.6 $\pm$ 13.6	75.2 $\pm$ 25.9	94.2 $\pm$ 28.4
Milk production (kg cow <sup>-1</sup> year <sup>-1</sup> )	5618 $\pm$ 674	6412 $\pm$ 731	8086 $\pm$ 1313
Non-arable land used (%)	66.3 $\pm$ 27.6	21.8 $\pm$ 32.9	20.1 $\pm$ 14.5
Land used to produce animal protein ( $\text{m}^2 \text{ kg}^{-1}$ )	78.6 $\pm$ 13.6	53.0 $\pm$ 11.4	33.6 $\pm$ 5.4
Digestible animal protein produced ( $\text{kg ha}^{-1}$ )	126.6 $\pm$ 28.5	184.4 $\pm$ 33.5	287.9 $\pm$ 42.5
Potential digestible plant protein produced ( $\text{kg ha}^{-1}$ )	108.1 $\pm$ 65.6	509.0 $\pm$ 306.3	643.9 $\pm$ 116.6
Edible animal protein produced $\times$ DIAAS ( $\text{kg ha}^{-1}$ )	153.8 $\pm$ 34.7	224.2 $\pm$ 40.5	350.1 $\pm$ 51.2
Potential edible plant protein produced $\times$ DIAAS ( $\text{kg ha}^{-1}$ )	51.1 $\pm$ 32.9	223.5 $\pm$ 126.5	276.6 $\pm$ 50.1

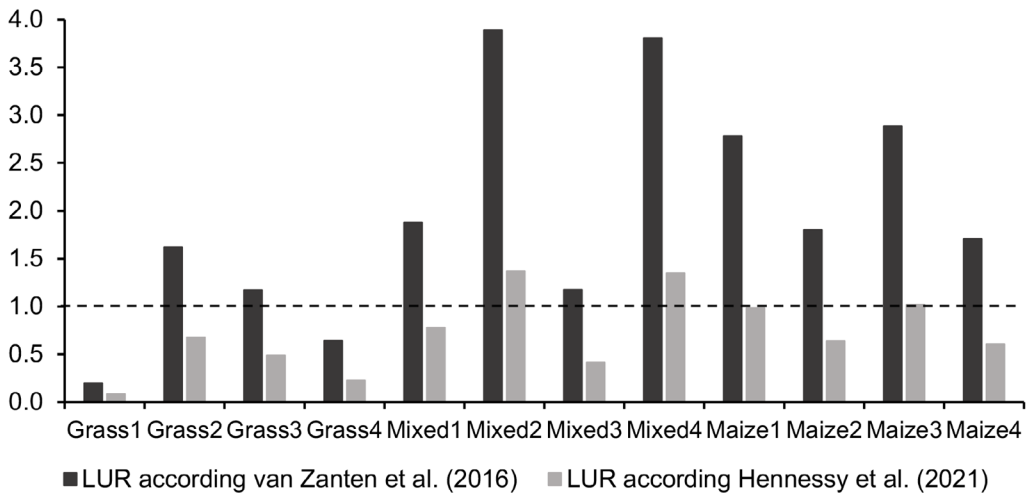


Figure 1. Land use ratio (LUR) for grass-based, mixed and maize-based dairy systems calculated according van Zanten *et al.* (2016) or Hennessy *et al.* (2021).

## Conclusion

Grass-based systems can be an efficient utilization of land for protein production for humans, in particular when they use non-arable grasslands. Metrics to assess land-use efficiency need to be further investigated to be generalized to the diversity of animal production systems.

## Acknowledgement

We thank the GIS Avenir Elevages (<https://www.gis-avenir-elevages.org/>) for the financial support of Margot Allix's engineering internship.

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# Microclimate, grass growth and herbage quality of peat grassland under free field photovoltaic modules

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## Abstract

Exploring the compatibility of solar energy and grass production on peat soil grasslands is needed to identify suitable solutions for energy provision and agricultural land use. In this context, a field experiment was established with permanent grassland on peat soil in North Germany. A free field photovoltaic system had been installed two years before. Results of the first full growing season are shown. To analyse the impact of the photovoltaic modules, measurements were done in three different sections: (i) an adjacent free grassland not affected by modules, (ii) under the solar modules (SM), (iii) in between SM. Eight sensors in each section collected data on soil moisture and surface temperature. Grass samples in a three-cut system were taken to measure herbage mass and quality. Results indicate changes in grassland microclimate due to SM and showed slightly lower herbage production underneath the modules and altered forage nutrition, with higher protein and lower sugar concentration. The ongoing research will assess herbage production variability with climate and solar module impacts on peat soil water retention and grass-water use. The obtained results are promising to combine energy production and livestock nutrition in the future.

**Keywords:** photovoltaic, herbage, sensors, peat, microclimate

## Introduction

The use of photovoltaic systems on agricultural lands is of growing interest and could lead to a beneficial combination of sustainable energy and agricultural production (Weselek *et al.*, 2019). This raises questions regarding the impact of solar modules (SM) on microclimatic conditions and the quantity and quality of forage production. This study aimed to analyse surface temperature, soil moisture, biomass, crude protein and crude sugar on a free-field photovoltaic system on peatland using transects in a free area, under SM, and in the aisle between SM as treatments.

## Materials and methods

The study was conducted at the Solarpark Lottorf (54°44'55.5" N, 9°56'78.1" E), Schleswig-Holstein, Germany. SM rotate on a single-axis and are 2000 mm long, with a maximum edge height of 640 mm, respectively, 2170 mm above the surface and a distance of 4000 mm to the next row of SM. From April 2023 on, 48 TMS-4 sensors (TOMST, Prague, Czech Republic) were put into the ground to measure soil moisture and temperature at -6cm, +2cm and +15cm. For the experimental design, three different treatments were selected: (i) an adjacent free area without SM, (ii) beneath the SM, and (iii) the aisle between a group of SM (Figure 1). A transect of 4 m × 10 m was established for each treatment.

Within these transects, sensors were inserted into the ground at the midpoint, with approximately one-metre spacing. Beneath SM (ii), a transect extended precisely under two rows of SM, with two sensors each placed between the solar cells, directly beneath the solar cells, and at the left and right edges of the solar cells. This design was replicated at a second location within the solar park. Two of the 48 sensors did not record data correctly. Sensors operated with a 15-minute measurement interval. Concurrently with the sensor measurements, to simulate a three-cut system, grass samples were cut at ca. 7 cm above

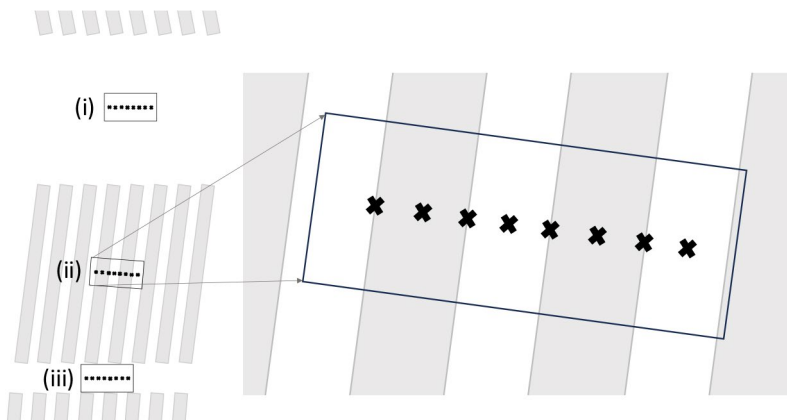


Figure 1. Schematic view of one experimental block with (i) free, (ii) under SM, (iii) aisle; expanded picture of sensor placement under SM.

ground on three different dates (24 May, 26 July and 28 September) within a 30 cm×60 cm frame east of each sensor in each transect (48 grass samples each day). The sensor data were aggregated and averaged for each cutting date. After drying, the grass samples were subjected to Near-Infrared Spectroscopy (NIRS) for forage analysis. To compare the median and variability among treatments we applied boxplots and calculated the coefficient of variation (CV).

## Results and discussion

The surface temperature (surface T) was visibly lower at the first cutting date (Figure 2). Fluctuations within a treatment, especially towards the third cutting date, were minimal. Direct exposure to sunlight in the free area could contribute to a higher temperature range (Vervloesem *et al.* 2022). Accordingly, the surface temperature was lower under SM at all three cutting dates. The coefficient of variation (CV) is consistently lower or equal to 0.05 (Table 1). An accompanying study by Hamidi *et al.* (these proceedings) explores how this influence of covered or uncovered grassland affects sheep behaviour in lying and active time. In June and July (second cut), soil moisture (vol. moisture) was lower across all three treatments. Although the measured range shows a wide span, moisture values are slightly higher in the free area on all three dates. The change in soil moisture across cutting dates is similar; however, the second experimental block was noticeably wetter in comparison to the first, which could account for the wide range of soil moisture. Within the SM treatment, soil moisture directly under, at the edges and in the gap between SM had quite different values (data not shown). CV of the free area is particularly smaller on the second date (0.332) compared to the other treatments (aisle 0.549, modules 0.488). Biomass production (dry matter, DM) was slightly reduced under SM. Related CV in the free area is higher at all three cutting dates (1: 0.625, 2: 0.519, 3: 0.441). Armstrong *et al.* (2016) showed that plant biomass is up to four times higher in uncovered areas. Under SM crude protein (XP) shows higher values and reaches a median of 20% on the third cutting date. CV for XP in the free area is approximately 0.05 for the first two dates, with all other measurements below 0.15. Crude sugar (XZ) content is lower under SM with medians at 5-6% on all three cutting dates. The highest CV is observed for the first cut in the aisle (0.308) and, the lowest CV for the second cut in the free area (0.144). Further research should consider the differences within each treatment and the possible change in biodiversity, especially grass species.

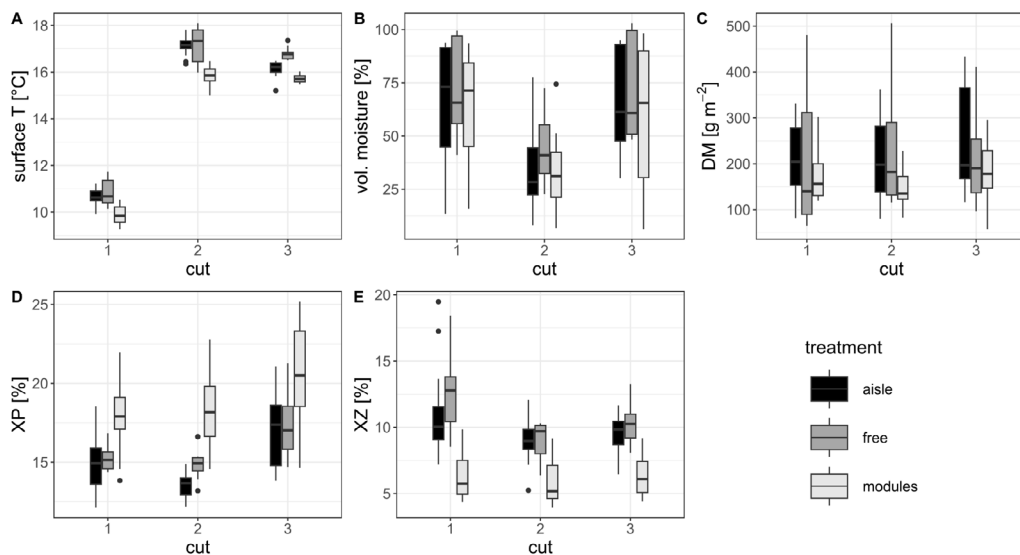


Figure 2. Boxplots of treatments (aisle, free, modules) for A: average surface temperature (surface T), B: average volumetric moisture (vol. moisture), C: dry matter (DM), D: crude protein (XP), E: crude sugar (XZ) on each cutting day (1: 24 May, 2: 26 July, 3: 28 September).

Table 1. Coefficient of variation for surface temperature (T), vol. moisture (vM), dry matter (DM), crude protein (XP) and crude sugar (XZ) for each treatment on each cutting day (1: 24 May, 2: 26 July, 3: 28 September).

	aisle 1	free 1	modules 1	aisle 2	free 2	modules 2	aisle 3	free 3	modules 3
T	0.031	0.051	0.041	0.022	0.043	0.025	0.021	0.014	0.011
vM	0.411	0.309	0.407	0.549	0.332	0.488	0.373	0.328	0.526
DM	0.373	0.625	0.328	0.485	0.519	0.299	0.425	0.441	0.359
XP	0.121	0.046	0.122	0.055	0.058	0.138	0.143	0.112	0.154
XZ	0.308	0.210	0.271	0.178	0.144	0.275	0.157	0.150	0.236

## Conclusion

We found influences on surface temperature, soil moisture, biomass production, crude protein and crude sugar levels by solar modules (SM), calling for further research to understand the underlying mechanisms for the impact of SM on grassland microclimate and vegetation.

## Acknowledgement

Thanks to the supporters of the experiment, especially to Dag Frerichs and Holger Reimer.

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# Greenhouse gas emissions and feed-food competition on Swiss dairy farms

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## Abstract

Negative environmental impacts from livestock need to be reduced. However, animal-sourced proteins will remain important for global food supply, as animals are able to convert biomass not suitable for human consumption to high value proteins. Animals compete directly with human food supply when fed human-edible feedstuffs. Feeding cows with concentrated feeds may decrease GHG-intensity; however, it likely increases feed-food competition. To investigate these conflicts of interest, we assessed GHG emissions and the human edible protein conversion ratio (edible protein in feeds/edible protein in animal products, ePCR) of 87 Swiss dairy cow farms. The GHG emission intensity was 0.70–1.21 kg CO<sub>2</sub>eq (kg energy corrected milk)<sup>-1</sup>. The ePCR ranged from 0.04 to 1.14. Correlation between GHG-intensity and ePCR was low, implying that low GHG-intensity does not contradict low feed-food competition. Human-edible protein production per kg of CO<sub>2</sub>eq ranged from –4.6 to 32.5 g CP. As a novel approach, we propose to calculate environmental footprints based on net human edible protein supply.

**Keywords:** net human food supply, environmental footprint, human edible feed conversion ratio

## Introduction

Ruminant production systems are associated with important negative environmental impacts, such as emissions of greenhouse gases (GHG) and eutrophication (Beal *et al.*, 2023). Whereas environmental impacts need to be significantly reduced, milk and meat are expected to remain important for global food supply (FAO and GDP, 2019). Ruminants will play a central role in the future food supply, as they are able to convert biomass not suitable for human consumption (such as roughage or by-products from food-processing) to high value proteins. However, net contribution of animal sourced proteins to human food supply should be critically assessed, as animals may compete directly with human food supply when fed with human-edible feedstuffs and indirectly when arable land is used for feed production instead of producing food crops (van Zanten *et al.*, 2022). Trade-offs between reducing product-related environmental footprints and the ability to upcycle non-human edible feeds manifest with respect to concentrate feeding. Concentrated feedstuffs such may offer a strategy to decrease GHG-intensity. Feeding cereals and maize to cows, however, is likely to increase feed-food competition (Ineichen *et al.*, 2023). Hence, we investigated potential conflicts of interest between GHG-intensity and feed-food competition on Swiss dairy cow farms.

## Materials and methods

Eighty-seven Swiss dairy farms located in different production zones across Switzerland were assessed in 2021. Farm managers supplied farm data such as live weight (LW) of the cows, feed rations and manure management practices. Milk production and milk contents were supplied by the Swiss milk trade database. Dry matter (DM) intake was computed according to Swiss feeding recommendations that rely mainly on milk performance level and LW of the cows (Jans *et al.*, 2015). Farm-specific reproduction figures were taken from the Swiss stock movement database. The number of culled cows and the number of replacement stock were determined for each farm based on the three-year average replacement rate of dairy cows. Meat outputs consisted of culled cows and surplus calves before fattening. All computations followed a lifecycle assessment (LCA) approach where the system boundary was set to cradle-to-farm-

gate. The GHG emissions were computed applying the KLIR model (Köke *et al.*, 2021) that primarily follows IPCC-guidelines. Total GHG emissions were expressed by applying the GWP-100 metric (IPCC, 2021) and were allocated to milk and meat according to an updated biophysical allocation approach (Ineichen *et al.*, 2022).

Direct feed-food competition was assessed by computing the human-edible protein conversion ratio (ePCR) as a ratio of human edible crude protein (CP) in feeds divided by the human edible CP in milk and meat, as described in Ineichen *et al.* (2023). However, slight modifications were implemented. All feeds not considered as roughage according to the Swiss regulation on direct payments (Federal council, 2013) were considered as concentrate feeds. Human-edible proportions of rapeseed and sunflower cakes were set to 0, as protein extraction from these products are currently not realized in relevant amounts. To comply with the mass balance after dehulling and husking, edible proportions of non-hulled and non-husked spelt, barley and oat were slightly decreased to 0.48, 0.33 and 0.42, respectively. The edibility of corn gluten meal was aligned with the edible proportion of protein in the maize kernel (0.70). Potential differences in the amino acid composition of plant and animal sourced proteins and hence the value for human nutrition was not considered. Linear correlation was tested using the 'Hmisc' (Harell, 2023) package in R studio version 4.2.1 (R Core Team, 2022).

## Results and discussion

Average annual milk performance per cow was 7820( $\pm 1566.4$ ) kg energy corrected milk (ECM) with a concentrate feed input of 109( $\pm 55.2$ ) g (kg ECM)<sup>-1</sup>. Human-edible protein feed intake was 114( $\pm 69.1$ ) kg CP cow<sup>-1</sup>, whereas human-edible protein output (milk and meat) was 237( $\pm 48.1$ ) kg CP cow<sup>-1</sup>, resulting in a net human-edible CP supply (output-input) of 122 ( $\pm 42.5$ ) kg CP cow<sup>-1</sup>. Remarkably, net CP supply of two of the 87 farms was negative (-16 and -39 kg CP cow<sup>-1</sup>), meaning that these farms did not contribute to net human food supply. Thus, ePCR ranged from 0.04 up to 1.14, with an average of 0.45 (Figure 1).

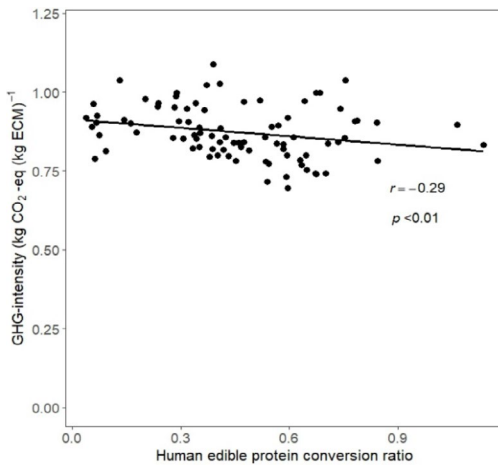


Figure 1. Relationship between human-edible protein conversion ratio (ePCR) and GHG-intensity on 87 Swiss dairy farms.

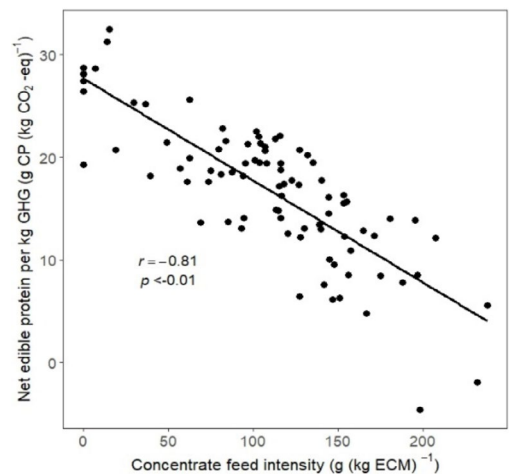


Figure 2. Net human edible protein production per kg of CO<sub>2</sub>eq of 87 Swiss dairy farms in relation to concentrate feed intensity.

The correlation between ePCR and GHG was negative but weak (Figure 1). To our knowledge, the relationship between GHG emissions and feed-food competition has not yet been discussed in the literature.

As two farms failed to contribute to net human food supply, GHG emissions per kg of net edible protein ranged from  $-520$  up to  $207$ , with an average of  $58 \text{ kg CO}_2\text{eq (kg net edible CP)}^{-1}$ . However, reporting negative emissions is not very meaningful as in this context it does not refer to negative emissions but rather to negative net edible protein production. Thus, we propose to report the reciprocal value, which ranged from  $-4.6$  to  $32.5$  and  $16.8 \text{ g CP (kg CO}_2\text{eq)}^{-1}$  on average. This significantly correlates with the concentrate intensity ( $\text{g concentrate (kg ECM)}^{-1}$ ) (Figure 2), indicating that dairy farms relying on roughage produced more net human-edible protein per kg of GHG emitted.

## Conclusion

Low product-related GHG emissions from Swiss dairy farms do not necessarily imply high feed-food competition. We propose combining the concepts of net contribution to human food supply with environmental impact assessment. However, as in some animal production systems the production of human edible proteins may be lower than the amount that was used for feeding the animals, the contribution to net human food supply can be negative. We thus suggest expressing emissions from net edible protein production as g net human edible CP per kg of  $\text{kg CO}_2\text{-eq}$  emitted rather than vice versa.

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# Drought impact on dynamics of red clover and birds-foot trefoil ratio in mixtures

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## Abstract

Changes in agricultural policy and climate put a strong pressure to (re)add alternative species to leys contributing to biological diversity. We aimed at characterizing the potential and compatibility of birds-foot trefoil as an alternative legume species and comparing it with red clover. The experiment included grazing, forage and universal type of multispecies mixtures (3 or 4 species), containing main (red clover and white clover) and minor (birds-foot trefoil) legume species. The germination percentage of legumes was lower than expected in all mixture types, ranging from 44% to 22.8%, with the highest percentage of legumes observed in the mixture consisting of red clover, perennial ryegrass and meadow fescue. Mixtures with birds-foot trefoil were the most affected by spring and summer drought, resulting in survival of only 8.5–14.20% of legume plants. Moist early autumn weather was favourable for mixtures with red clover, with legumes increasing up to 41.50%. The botanical composition change was affected by summer drought as well as competitive companion grass species, which should be carefully considered when composing the mixtures.

**Keywords:** legumes, grasses, minor species, *Lotus corniculatus*, botanical composition

## Introduction

For a long time, productivity has been the main indicator for assessing grasslands, finally resulting in the use of a limited number of species in sown grasslands. One way to diversify industrialized farming is by introducing minor species, especially legumes, which are considered to offer many social and ecological benefits (Voisin *et al.*, 2014). In addition, legumes can help to increase herbage quality by supplementing it with proteins. Moreover, legume-rich leys could be one of the most attractive ways to reduce reliance on agrochemical inputs and increase agrobiodiversity and thus help implementing the principles of EU Green Deal's "Biodiversity" and "Farm to Fork" ([https://commission.europa.eu/strategy-and-policy/priorities-2019-2024/european-green-deal\\_en](https://commission.europa.eu/strategy-and-policy/priorities-2019-2024/european-green-deal_en)). Changes in agricultural policy along with the changing climate makes (re)considering the addition of alternative species, such as tall fescue (*Festuca arundinacea* Schreb.) and birds-foot trefoil (*Lotus corniculatus* L.) that are able to withstand harsh weather conditions and still produce herbage. Though such mixtures will not deliver the highest amount of energy, they recover more rapidly after stress and provide at least a certain amount of forage (Kanapeckas *et al.*, 2011). In addition, birds-foot trefoil is known for its ability to shift nitrogen excretion pathways from the volatile urine to the more stable form in dung, and to increase animal productivity, and also reduce methane emissions, not forgetting its tolerance to traffic (Casler & Undersander, 2018). This study aimed at characterizing the potential of birds-foot trefoil as an alternative legume species for the mixtures as well as its compatibility, comparing it with red clover.

## Materials and methods

The study was performed in Dotnuva, Lithuania (55°23' N, 23°57' E) during 2022–2023. The trial was set up in 8.25 m<sup>2</sup> plots using a randomized complete block design in 3 reps. The experiment included the following factors: (1) grazing, hay/silage and universal type of grassland, (2) two legume species, (3) five partner grass species, (4) trinary (two grass + one legume species) and quaternary (three grass + 1 legume or 2 grass + 2 legume species) mixtures and (5) main (*Trifolium* spp) and minor (*Lotus corniculatus*)

species (Table 1). The seed viability was tested on Petri dishes and seed mixtures were composed based on the germination rate where legume proportion was 50%. The cultivars and breeding lines were selected based on their superior productivity and Lithuanian origin: *Trifolium pratense* cv. Arimaičiai, *Lotus corniculatus* breeding line, *Trifolium repens* cv. Nemuniai, *Lolium perenne* cv. Elena DS, *Festuca pratensis* cv. Alanta, *Festuca arundinacea* cv. Monas, *Festulolium* cv. Punia DS, *Phleum pratense* cv. Žolis.

Plots were harvested at emergence of legume first-flowers, and subsequent harvests - at 40–50-day intervals. The number of germinated plants was calculated 30 days after sowing as the number of plants/m<sup>2</sup>. The botanical composition of mixtures was determined by weighing herbage at the first and last cut of the season, by estimating comparative weight of functional groups. The summer of 2022 was hot and moist, while winter of 2022–2023 was changeable. There was snow and freezing temperatures in December, but from January average temperatures were around zero with no snow cover. Spring was dry and rainfall scarcity continued throughout summer, but the last 10 days of August and the rest of the autumn were moist. The statistical analysis was implemented in the open-source R statistical environment (version 4.3.1; R Core Team, 2023). Analysis of variance and post-hoc tests were conducted using R package 'agricolae' (de Mendiburu and Yaseen, 2020). To estimate environment effect on DMY, ANOVA and subsequently post hoc Tukey HSD test was applied.

## Results and discussion

The percentage of germinated plants was evaluated in July 2022, considering seed viability as the only factor influencing germination success. Although the weather conditions were hostile for germination, the evaluated germination percentage of legumes was lower than expected in all types of mixtures, ranging from 44% to 22.8%. The highest percentage of legumes was calculated in the mixture F2 consisting of red clover, perennial ryegrass and meadow fescue. Universal (U1) and grazing type (G1) mixtures containing birds-foot trefoil were also characterized by high proportion of legume, respectively 42% and 41%. The lowest legume germination (22.8%) was observed in the G2 mixture containing red and white clovers, tall fescue and *Festulolium*. There were no significant differences between the two legume species used and type of mixture. The differences in expected and observed proportion of germinated legumes could be due to factors we did not consider, for instance soil physical and chemical properties. Seed size could also be an important factor, because the mixture seeds are sown at the same depth despite their size. A hostile winter of 2022–2023 was mild allowing the mixtures to overwinter well but subsequent thriving of the mixtures was inhibited by spring drought which continued throughout the summer. Drought effect on the first cut yield and legume/grass ratio in the mixtures was significant, resulting in twice or even three-fold reduction of the legume percentage (Figure 1).

The mixtures that suffered most were G1, U1 and F1, containing birds-foot trefoil, where legume percentage was 8.5–14.20% and only single plants were observed. In late summer, a high amount of precipitation boosted the emergence of legumes enabling them to compete with the grasses. Therefore,

Table 1. Species composition of grass-legume mixtures

Legume	Partner grass species		
	Forage	Universal	Grazing
	<i>L.perenne</i> + <i>F.pratensis</i>	<i>F.arundinacea</i> + <i>Festulolium</i> + <i>Ph.pratensis</i>	<i>F.arundinacea</i> + <i>Festulolium</i> + <i>T.repens</i>
<i>L. corniculatus</i>	F1	U1	G1 (without <i>T.repens</i> )
<i>T. pratense</i>	F2	U2	G2

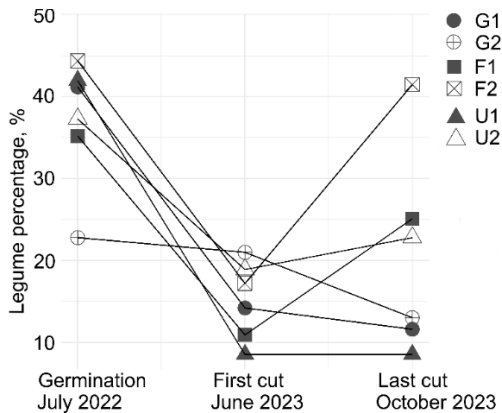


Figure 1. The change of botanical composition of mixtures, where G indicates grazing type mixture. U, universal; F, forage. The number represents legume species used in the mixture: 1, birds-foot trefoil; 2, red clover.

mixtures F2, F1 and U2 recovered after drought significantly ( $p > 0.05$ ) better than the remaining ones, and the highest percentage of legumes was observed in the mixture with red clover. Whereas the proportion of trefoil in U1 and G1 mixtures remained the same, it even decreased in the mixture with red and white clover G2, revealing highly competitive features of *Festulolium* and suggesting a careful consideration of companion species when composing mixtures.

## Conclusion

Red clover outperformed birds-foot-trefoil in trinary and quaternary mixtures with grasses. The proportion of trefoil in the mixtures was affected not only by drought conditions but also by high competitiveness of *Festulolium*. To receive the added nutritive value that birds-foot-trefoil can offer, the mixture composition should be modified by including less-aggressive companion grass species and/or by adding a higher proportion of birds-foot-trefoil seeds.

## Acknowledgement

The work was funded by the long-term research programme ‘Genetics, biotechnology and breeding for plant biodiversity and innovative technologies’ implemented by LAMMC.

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# Sustainability assessment of three highly contrasting farming systems using the IDEA4 method

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## Abstract

Sustainable agriculture must meet the objectives of agroecological, social, and economic transition to embed the challenges of sustainable development. Recent research addresses a lack of studies on sustainability based on dimensions and attributes assessment at the farm level. This paper proposes a three-dimensional (agroecological, socio-territorial and economic) and five-attribute (territorial embeddedness, self-sufficiency, productive and reproductive abilities, overall responsibilities, and robustness) assessment at the farm level using the IDEA4 methodological framework. The study surveyed 47 farms in the Centre-Val de Loire region, France. A hierarchical clustering based on agricultural unit and productive features was applied. Three farming systems were identified: pasture-based farms, mixed crop-livestock farms, and cereal farms. Sustainability results indicated that pasture-based farms are 1.4-fold more sustainable in their agroecological practices and 1.2-fold more in their social activities and territorial embeddedness than cereal farms. Mixed crop-livestock farms and pasture-based farms show a higher self-sufficiency and overall responsibility than cereal farms, but they appear to be more resilient in their scores for the three dimensions and attributes of sustainability. This study advocates for the major place of livestock and pasture-based systems in fostering sustainable agriculture. However, these results are closely linked to the study area and the multi-criteria method employed.

**Keywords:** sustainable farming systems, farm level assessment, multidimensional analysis, sustainability attributes

## Introduction

Modern agricultural systems lead to a decline in ecosystem diversity and integrity, as well as direct harm to human health (Pretty, 2018). One key to limiting the negative externalities is to assess agricultural performances at the farm level. Recently, Chopin *et al.* (2021) various management changes have been proposed. Different tools, with varying characteristics, sustainability framing and indicators, have been used to evaluate the impact of these changes on sustainability. Here, we review 119 tools for farm sustainability assessment and compare their use, sustainability dimensions, themes and types of indicators used for biodiversity conservation, farm viability and gender equity. Our main findings are that (1) pointed out that the number of peer-reviewed publications assessing farming systems sustainability has increased exponentially in recent years. However, they revealed that only 2% of all peer-reviewed papers address sustainability based on dimensions and attributes of sustainable development. A major contribution to improving agricultural sustainability relies on considering the objectives of sustainable agriculture based on the three dimensions and the five attributes of sustainable development (FAO, 2019). Here, the author proposes a combined three-dimensional and five-attribute assessment of sustainability at the farm level in the French region of Centre-Val de Loire. The released Farm Sustainability Indicator version 4 (IDEA4) methodological framework was used (Zahm *et al.*, 2023). A total of 47 farms were surveyed with the IDEA4 grid and grouped into three farming systems according to a clustering process based on farm biotechnical features.

## Materials and methods

This study was conducted in the Centre-Val de Loire region, France. On-farm data were collected through four-hour semi-guided interviews using the IDEA4 grid. A total of 47 farms were surveyed. The farming

systems were constructed using a typology approach based on continuous variables (Nowak *et al.*, 2013; Pépin *et al.*, 2021). Relevant variables such as utilised agricultural area (ha), both cropland and grassland area (ha), livestock unit (LU), livestock density (LU·ha<sup>-1</sup>), working unit, and the treatment frequency index were selected. A literature review was conducted to identify farming systems. To investigate farming systems sustainability, the released IDEA4 method was used. It consists of a multi-criteria assessment of farm sustainability using a dual evaluation approach based on the three dimensions of sustainable development (agroecological, socio-territorial and economic) and the five attributes (territorial embeddedness, self-sufficiency, productive and reproductive abilities, overall responsibility, and robustness) of sustainable farming systems. The first assessment approach is based on a grid of 53 indicators aggregated in an ascending classification structured according to the three dimensions of sustainable development. The assessment is quantitative and based on sustainability units aggregated by addition and thresholding rules (Zahm *et al.*, 2023). The second approach characterises the level of sustainability of the five attributes using the same 53 indicators. These indicators are organised based on a hierarchical tree specific to each attribute. The aggregation of indicators is ascending, qualitative and hierarchical within each attribute (Zahm *et al.*, 2023). Clustering was built on a principal component analysis, which expresses most of the inertia within the first and the second dimension. A two-way ANOVA test with a Bonferroni correction was performed for the three-dimensional data. Attribute scores were aggregated and divided by the maximal scores of each indicator that structures each attribute. A Chi-square test of independence was applied to the attributes.

## Results and discussion

The clustering output highlights three different farming systems: cereal farms without livestock (CFs;  $n=9$ ), mixed crop-livestock farms (MCLFs;  $n=22$ ) and pasture-based farms (PBFs;  $n=16$ ). Among them, PBFs and MCLFs have agroecology scores 1.4-fold and 1.3-fold higher than CFs, respectively. Scores for the socio-territorial dimension show that PBFs have the best scores over MCLFs and CFs, with no significant differences (Fig. 1a). The economic dimension scores highest for CFs, although the scores for the three farming systems are more nuanced. Attribute results (Fig. 1b) indicate that MCLFs and PBFs are 1.4-fold and 1.3-fold more self-sufficient and 1.6-fold and 1.7-fold more embedded in their territories than CFs. However, both CFs and MCLFs have equal abilities on producing and reproducing goods and services. Overall responsibility is higher for PBFs and MCLFs. All three systems exhibit similar robustness scores.

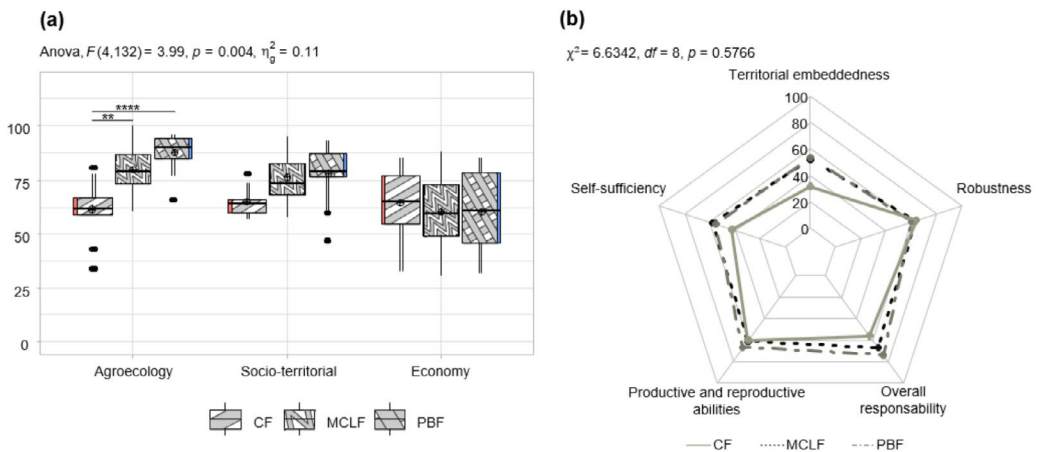


Figure 1. Sustainability score of (a) three-dimensions and (b) five-attributes (%). CFs cereal farms; MCLFs mixed crop-livestock farms; PBFs pasture-based farms.  $P < 0.00001$ \*\*\*\*;  $P < 0.001$ \*\*.



Compared to other studies, these results are aligned with those reported in the literature. Kremen & Merenlender (2018) pointed out that CFs have low developed biodiversity due to open landscapes and monoculture at the field level. They store low carbon and degrade water quality and ecosystems. In contrast, livestock farms have sustainability attribute scores two to 3-fold higher, particularly for pollinator preservation and soil fertility. Both studies emphasise the significance of diversified agricultural farming and livestock systems as a lever for sustainability performance. Ripoll-Bosch *et al.* (2012) pasture-based sheep farming systems are mostly located in marginal/High Nature Value areas. These production systems are multifunctional, and their economic, environmental and social roles are equally important and recognised by policy makers and by society. However, the number of animals and holdings is decreasing, and there is great uncertainty regarding the reproducibility of these farming systems, which depends on many internal and external farm factors and their interactions. The aim of this paper was to perform a comprehensive assessment of sustainability in different sheep farming systems in north eastern Spain using the MESMIS framework. We followed a case-study approach to perform an in-depth investigation of 4 sheep meat and dairy farms with different intensities of reproduction management. Critical points of sustainability, including weaknesses and opportunities, were obtained using a participatory process with stakeholders (farmers and technical advisers highlight that meat production features high multidimensional score in environmental and social dimensions. Sustainability attributes suggest a great robustness and resilience for meat production systems with scores ranging around 50%. This paper suggests a similar trend for the environmental and social pillars as well as self-sufficiency attribute, but this study's result for the robustness attribute is 1.3-fold lower for analogous farming systems.

## Conclusion

This paper highlights that livestock and pasture-based farming systems are the most effective in terms of agroecology and social dimensions. Although cereal systems without livestock are economically viable, the lack of diversification remains a challenge for their sustainability. Assessing attributes allows for a better understanding of the drivers in such systems, and livestock and pasture-based systems are among the keys to achieving self-sufficiency and overall responsibility. This is an initial step towards assessing the sustainability of livestock and cereal farming. It is advisable to remain objective regarding results, as they may vary with different multi-criteria evaluation methods and study areas.

## Acknowledgements

The author warmly thanks the 47 farmers surveyed for their confidence in sharing their practices. Many thanks to the French Centre-Val de Loire Rural Development Program and the European Agricultural Fund for Rural Development for supporting this work.

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# Italian ryegrass or silage rye as precrop for silage maize in Flanders

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## Abstract

Italian ryegrass (*Lolium multiflorum*) is often used as a catch crop in Flanders and can be grown for a 1-cut harvest before installing silage maize as the successive crop. Silage rye (*Secale cereale*) can occupy the same place in a crop rotation, but can be harvested earlier in the spring. This allows for earlier installation of silage maize, which reduces its vulnerability to drought stress. A field trial, an experiment at farmers' field scale, and one animal feed trial were conducted to test silage rye as a possible alternative precrop in maize cultivation. The harvest date was chosen as the optimum for rye and about two weeks earlier than the harvest date that most farmers chose for Italian ryegrass in the region of the field trials. The results of the field trial suggest that, in comparison to Italian ryegrass, silage rye harvested at the optimal time results in higher dry matter yields (DMY); however, this was not confirmed in the experiment at field scale. Plant analysis and the animal feed trial revealed that silage rye is richer in fibre, poorer in energy, and tends to lead to a non-significant reduction in milk production.

**Keywords:** silage rye, Italian ryegrass, dry matter yield, fodder quality, milk yield

## Introduction

Italian ryegrass is a common catch crop in Flanders (Belgium), because it can be sown until late November and generates an additional cut in the spring prior to installation of silage maize. An early spring N fertilization combined with a cut in early May results in a highly productive sward with excellent fodder quality. However, extreme weather conditions in the last decade have resulted in either (i) postponing the ryegrass cut due to excessively wet conditions, or (ii) drought conditions that lead to excessively dry soils and the exhaustion of soil moisture by the ryegrass. Both result in disastrous DMY of the succeeding maize crop. An increasing number of farmers are therefore considering installation of silage rye instead of Italian ryegrass, as silage rye can be harvested one or two weeks earlier. Fodder quality and feed intake of silage rye were investigated in two experiments. The objectives of this study were to compare (i) DMY of four rye and two Italian ryegrass varieties at three N fertilization levels (exp. 1) and (ii) the fodder quality of rye vs. Italian ryegrass based on a chemical determination of fodder quality (exp. 2a) and (iii) milk production when feeding silage rye vs. Italian ryegrass based on an in vivo feed experiment (exp. 2b).

## Materials and methods

*Exp. 1:* A field-scale experiment with a randomized complete block design with two factors (variety and N fertilization) and three blocks was installed on October 19, 2019 on a sandy soil (SOC 1.4% and  $\text{pH}_{\text{KCl}}$  5.4) after the harvest of silage maize. The varieties were sown at the density advised by the seed company. Rye varieties: R1 (silage type, 78 kg ha<sup>-1</sup>), R2 (catch crop type, 80 kg ha<sup>-1</sup>), R3 (silage type, 90 kg ha<sup>-1</sup>), R3 (grain type, 75 kg ha<sup>-1</sup>); Italian ryegrass varieties: I1 (982 germinating seeds m<sup>-2</sup> or 40 kg ha<sup>-1</sup>) and I2 (a very early variety, 1400 germinating seeds m<sup>-2</sup> or 57 kg ha<sup>-1</sup>). The fertilization levels were 0–50–100 kg N ha<sup>-1</sup>, applied on March 4, 2020. The trial was harvested with a Haldrup plot harvester on April 27, 2020, at the optimal time of silage rye harvest (flag leaf growth stage). DMY of each replicate were calculated after drying a subsample in a forced-draft oven at 70°C for 72 hours.

*Exp. 2a:* A farmer's field (5.8 ha) on a sandy loam soil (SOC 1.8% and  $\text{pH}_{\text{KCl}}$  6.2) was split in two partitions after the harvest of silage maize. One half was sown with silage rye (80 kg ha<sup>-1</sup>) and one half with Italian ryegrass (40 kg ha<sup>-1</sup>), both on September 20, 2020. Both partitions were fertilized with 70

kg N ha<sup>-1</sup> CAN (calcium ammonium nitrate) in the first week of March. Both partitions were cut on April 25, 2021, at the optimal moment for silage rye harvest (flag leaf growth stage) and when the Italian ryegrass was in the 2<sup>nd</sup> node stage. After prewilting, the material was ensiled in plastic covered silage bales on April 28. The Italian ryegrass was tedded once; the rye was not. Fodder quality was determined on a chemical basis on samples taken from the bales prior to starting Exp. 2b.

*Exp. 2b:* In 2022 a trial was set up with 24 high producing dairy cows. Maize silage, Italian ryegrass or silage rye and pressed beet pulp (50/43/7 on DM) mixed with soybean meal (10.5% for Italian ryegrass, 9.2% for silage rye), corn meal (2% for silage rye) and feed urea (0.5% for Italian ryegrass and 0.3% for silage rye) were fed ad libitum. Both diets were supplemented with (rumen protected) soybean meal and balanced concentrates to meet 105% of the energy and protein requirements of each individual cow and to attain a rumen degraded protein balance of 180 g day<sup>-1</sup>. Dry matter intake (DMI) and milk production were registered daily, whereas milk composition was determined in the last week of each period.

## Results and discussion

In Exp. 1 the DMY of rye increased more with increased N fertilization than Italian ryegrass (Figure 1). At the fertilization levels of 50 and 100 kg N ha<sup>-1</sup> the DMY of all rye varieties was significantly higher (ANOVA,  $p < 0.05$ ) compared to both Italian ryegrass varieties. At harvest, several plots with rye in the field trial showed some plants with appearance of the first spikes, while the Italian ryegrass was still at an earlier stage. This indicates that rye should be harvested (2–3 weeks) earlier than Italian ryegrass, thus enabling earlier installation of the maize crop. Caution is needed when postponing the harvest of rye, however. Observations of the field trial indicated that after appearance of the flag leaf in rye, the growth stages can follow very quickly. The harvest window for Italian ryegrass therefore appears to be larger.

In Exp 2a Italian ryegrass achieved a higher DMY than silage rye, in comparison to Exp. 1 (Table 1). Because the silage rye showed a quite thin crop at the beginning of the spring, it was presumed that the tillering of the silage rye was suboptimal and this caused some loss in yield potential. The DM% of silage rye was 36.8%, which is >10% below that of Italian ryegrass, but it was sufficient to make a good ensiled product. The difference in DM% is undoubtedly connected to the tedding of the Italian ryegrass. There

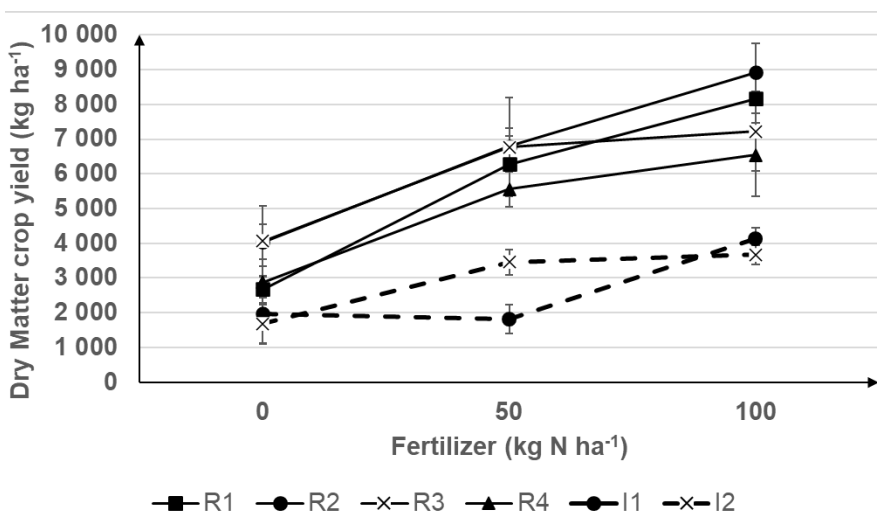


Figure 1. The mean DMY of the rye and Italian ryegrass varieties at different N fertilization levels. The error bars indicate the standard error.

was only a small difference between the ash content of the two ensiled products. While silage rye has a less closed sward and is therefore more susceptible to incorporation of soil mineral material, the absence of a tedding step in silage rye prevented an increase in ash content. Both silage rye and Italian ryegrass showed far lower than average crude protein content for practical conditions. The silage rye product has a higher stem/leaf ratio than Italian ryegrass, which resulted in less sugar and VEM and more crude fibre than Italian ryegrass. Although the silage rye contained more crude protein, the protein/energy balance pushed the DVE content to a higher level for Italian ryegrass. Despite the DMY and low crude protein content, the feed value measurements (VEM and DVE) were close to those recorded on samples from farmers. However, we have no further database with which to compare fodder quality of silage rye.

Table 1. Fodder quality parameters of the silage fodder from Exp. 2a.

	DM (%)	DMY (kg ha <sup>-1</sup> )	Crude protein	Ash	Crude fibre	Sugar	VEM	DVE
Silage rye	36.8	2 165	138	93	257	149	974	74
Italian ryegrass	51.3	3 924	125	104	263	263	1 025	84

VEM, feed units milk; DVE, protein digestible in the intestines. Crude protein, ash, crude fibre, sugar VEM and DVE are all in g (kg DM)<sup>-1</sup>.

Despite the higher DMI in Exp. 2b, with an Italian ryegrass based ration and a trend towards higher milk production, the differences in milk production are not significant (Table 2). Both nitrogen and feed efficiency are significantly higher for a silage rye based ration, however.

Table 2. LS means of production parameters ( $\pm$ SEM) in Exp. 2b. Significant differences between ration are indicated with a different letter ( $p < 0.05$ ).

	Total DMI	Roughage DMI	Milk production	Nitrogen efficiency (%)	Feed efficiency (%)
Silage rye	23.1 $\pm$ 0.4 <sup>a</sup>	16.9 $\pm$ 0.5 <sup>a</sup>	35.1 $\pm$ 1.2 <sup>a</sup>	32.3 $\pm$ 0.7 <sup>b</sup>	1.55 $\pm$ 0.04 <sup>b</sup>
Italian ryegrass	24.4 $\pm$ 0.4 <sup>b</sup>	18.3 $\pm$ 0.5 <sup>b</sup>	36.2 $\pm$ 1.2 <sup>a</sup>	30.4 $\pm$ 0.7 <sup>a</sup>	1.48 $\pm$ 0.04 <sup>a</sup>

Milk production comprises fat and protein corrected milk, feed efficiency is kg milk production per kg DM fed. Total DMI, roughage DMI and milk production are in kg day<sup>-1</sup>.

## Conclusion

Based on the field trial, silage rye can reach higher DMY earlier in spring than Italian ryegrass. However, this was not confirmed in an experiment at farmers' field scale. Earlier harvest of the precrop is equal to an earlier installation of the succeeding silage maize crop. Silage rye tends to be richer in fibre and poorer in energy than Italian ryegrass when harvested at the end of April when silage rye achieves the flag leaf stage and Italian ryegrass is in 2<sup>nd</sup> node stage. Although the DMI is lower for a silage rye based ration, and milk production tends to be higher for an Italian ryegrass based ration, the differences in milk production are not significant.

# Yield and shoot traits of five tropical grasses in response to N and distance to trees

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## Abstract

Trade-offs may occur in the adaptive responses of species to shading and nitrogen (N), and functional traits can help to explain the consequences of these responses for species performance. Our objective was to gain understanding of the mechanisms between traits of five C<sub>4</sub> perennial grasses determining above-ground dry matter yield (DMY) when both resources, light and N, vary. Forage grasses were grown in six shading conditions (full sunlight vs. five positions between *Eucalyptus dunnii* rows) with two N levels (0 vs. 300 kg N ha<sup>-1</sup> year<sup>-1</sup>). Path analysis was used to explore the relationship between DMY, shading levels, N nutrition index and shoot traits. DMY increased between 126 to 569 g DM m<sup>-2</sup> with N fertilization. Increased shading reduced DMY by 6.9 to 12.5 g DM m<sup>-2</sup> for each 1% of increase in shading. DMY was modulated by shoot traits, but with different responses according to species, highlighting different strategies to cope with changes in light and N availability.

**Keywords:** functional traits, perennial grasses, silvopastoral, species strategies

## Introduction

Functional shoot traits can explain how different species respond to resource variation and the consequences for species performance. Light intensity and N availability seem to be the most important factors influencing growth of C<sub>4</sub> forage species, particularly in silvopastoral systems (Paciullo *et al.* 2016). Few studies have combined these two factors to explore the role of intraspecific trait variability in productivity. Quantifying the link between the environment and plant traits is important to understanding the consequences of changes in resource availability, but such studies rarely extend into the cultivated C<sub>4</sub> species. Our objective was to understand the mechanisms relating shoot traits to above-ground DMY production when both light and N vary.

## Methods

The experiment was located at the IDR-Paraná, Ponta Grossa-PR, Brazil. The local climate is subtropical humid. The study included five grasses widely used for Brazilian livestock: *Axonopus catharinensis*, *Cynodon* spp. hybrid Tifton 85, *Hemarthria altissima* cv. Flórida, *Megathyrsus maximus* cv. Aruana and *Urochloa brizantha* cv. Marandu. These species were planted in pure stands in two side-by-side experiments, i.e., in the full sun (4.5 m<sup>2</sup> plots) and under *E. dunnii* trees (100 m<sup>2</sup> plots). Liming, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O were applied according to soil analyses. *Eucalyptus* trees were planted in 2007 (267 trees ha<sup>-1</sup>) in a double-row arrangement (4×3×21 m). The impact of two N levels (0 and 300 kg N ha<sup>-1</sup> year<sup>-1</sup>), five positions in relation to the trees vs. full sun system were evaluated, with three replicates. The plots were mechanically cut when light interception (LI) by the swards reached 95%. Canopy height (H) was determined at harvest, and 50% of this height was harvested. DMY above cutting height was determined by clipping herbage in 0.25 m<sup>2</sup> per plot and position. Annual DMY was the sum of mass from all cuts between 18 October 2011 and 28 May 2012. Shoot traits were assessed in 10 tillers collected at random per plot, when LI reached 95%. Sheath length and number of leaves (NL) were measured first. Leaf length, fresh mass and leaf area (LA) of the youngest fully expanded leaf lamina of each tiller were measured after tiller rehydration. Laminas were dried at 60 °C, weighed and leaf dry matter content (LDMC) and specific leaf area (SLA) were calculated. Tiller density (TD) was the number of tillers in 0.0625 m<sup>2</sup>. Leaf area

index (LAI) was calculated as LA×NL×TD. Samples of laminas and herbage harvested for DMY were analysed via near-infrared reflectance spectroscopy for crude protein (CP). Leaf N content (LNC) was calculated as CP/6.25 and LNC on fresh mass basis (LNC<sub>F</sub>) as LNC×LDMC. Nitrogen nutrition status was estimated using the NNI method (Lemaire and Gastal 1997). DMY was analysed with a mixed model including species, N fertilization, position, and their interactions as fixed effects. Block, whole plot and strips were included as a random effect. Model analysis with DMY as a dependent variable was performed using the percentage of shading at each position, LAI, H, leaf weight ratio (LWR), LNC<sub>F</sub>, NNI and SLA as independent variable, as well as the factor position and interactions of these variables with species factors. The best model was defined using stepwise model selection with the “stepAIC” function of the “MASS” package for R (R Core Team 2022). All statistical analyses were performed with R within the RStudio IDE.

## Results and discussion

The species × N levels × position interaction was significant ( $P < 0.05$ ) for DMY (Table 1). All species showed an increase in DMY with N, except *H. altissima*. No significant differences were observed on DMY between full sun and moderate shading (i.e., P3, where tree root competition is minimized) for any species and N levels.

Path analysis highlighted a positive and significant effect of N, mediated by NNI, and a negative effect of shading on DMY (Figure 1). The fact that the final model included the factor position relative to the trees indicate that other direct mechanisms, unrelated to shading (e.g., competition for water or nutrients between trees and understorey  $C_4$  plants), were important in determining production. SLA was the third most important variable explaining DMY, because by increasing the area of a given unit of leaf biomass, the interception of light is increased, especially under low-light conditions. Some traits had different impact on DMY depending on species, as indicated by significant interactions (e.g., LAI × species and shading × species, Figure 1). An increase in LAI was associated with an increase in DMY for *M. maximus* (+8.6 g DM m<sup>-2</sup> for each unit of LAI) and *U. brizantha* (+3.4 g DM m<sup>-2</sup> for each unit of LAI). LAI is a vital growth parameter for yield because it determines light capture by the crop. However, for the other

Table 1. Shading level (%) and annual dry matter yield (g m<sup>-2</sup>) for each species, nitrogen level and position between tree rows and at full sun.

	P1	P2	P3	P4	P5	Full sun
Shading	50±4.0	39±0.79	21±2.1	28±2.5	31±2.7	0
0 kg N ha <sup>-1</sup> year <sup>-1</sup>						
Ac	302±151.1a	449±151.1a	660±151.1a	618±151.1a	358±151.1a	582±123.4a
Cs	318±123.4b	605±123.4ab	549±123.4ab	338±123.4b	253±123.4b	821±123.4a
Ha	500±123.4b	953±123.4b	1381±123.4a	690±123.4b	525±123.4b	962±123.4ab
Mm	320±151.1a	358±151.1a	565±151.1a	601±151.1a	425±151.1a	756±123.4a
Ub	444±123.4bc	530±123.4bc	672±123.4ab	440±123.4bc	298±123.4c	1043±123.4a
300 kg N ha <sup>-1</sup> year <sup>-1</sup>						
Ac	533±151.1b	824±151.1ab	1052±151.1a	924±151.1ab	583±151.1b	1271±123.4a
Cs	736±123.4d	1044±123.4bc	1281±123.4ab	927±123.4bcd	641±123.4d	1667±123.4a
Ha	639±123.4b	925±123.4ab	1250±123.4a	1051±123.4a	991±144.9ab	911±123.4ab
Mm	573±123.4b	1001±123.4a	1193±123.4a	1167±123.4a	875±123.4b	1371±123.4a
Ub	1115±123.4ab	1207±123.4ab	1348±123.4a	1363±123.4a	923±144.9b	1119±123.4ab

Positions 1 and 5, closest to the tree's rows; P3, the central position between trees rows; P2 and P4, the intermediate positions. *Axonopus catharinensis* (Ac), Tifton 85 (Cs), *Hemarthra altissima* (Ha), *Megathyrus maximus* (Mm) and *Urochloa brizantha* (Ub). Within rows, means followed by the same letter are not significantly different according to Tukey test ( $P < 0.10$ ).

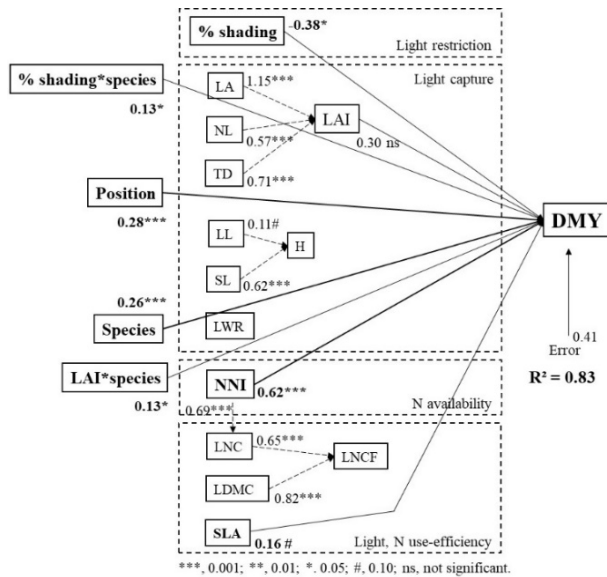


Figure 1. Path analysis model illustrating relations between traits, species, position between tree rows, nitrogen nutrition index (NNI) and shading with DM yield (DMY). Path coefficients higher than 0.10 are in bold. Arrows for DMY were included for variables selected in stepAIC procedure. LA, leaf area; NL, number of leaves; TD, tiller density; LL, leaf length; SL, sheath length; LWR, leaf weight ratio; LNC, leaf N content; LDMC, leaf dry matter content; SLA, specific leaf area; LAI, leaf area index; H, canopy height; LNCF, LNC per unit of fresh matter.

species, the relationship between LAI with DMY response was negative, notably for *H. altissima* (-11.3 g DM m<sup>-2</sup> for each unit of increase in LAI). DMY decreased with increasing shading for all species, ranging from -10.1 (Tifton 85) to -12.5 g DM m<sup>-2</sup> (*A. catharinensis*) per 1% of shading, except *U. brizantha*, whose slope (-6.9 g DM m<sup>-2</sup> per 1% increase in shading) was not significant different from zero ( $P=0.16$ ).

## Conclusions

Pathways related to light restriction and capture, N availability and light and N -use efficiency were associated with DMY response to the factors studied, explaining more than 80% of the variability in DMY. The tolerance to shading in decreasing order was *U. brizantha*, greater than *H. altissima*, *A. catharinensis*, *M. maximus* and Tifton 85.

## Acknowledgement

The first author is grateful to CNPq for the fellowship (202925/2019-6).

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# Mineral concentrations in grasses and legumes change during a 2-week growth period in summer

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## Abstract

In ruminant diets, forage is an important natural source of minerals. Data on P, Ca, Mg and K concentrations in 4 grasses and 4 legumes were collected weekly, during 2-week periods in August in 2007 and 2008 (following cuts taken 36 days before (2007) and 41 days before (2008), in an experiment where a silage cutting regime was practised). In August, concentrations of Ca and Mg were higher in legumes than in grasses. The rates of change in P, Ca and Mg concentrations during a 2-week growth period differed among species. Outcomes are discussed in relation to cattle nutrition requirements, risk for declined concentrations of minerals at delayed harvests, and options for designing grassland mixtures for sustainable agricultural systems.

**Keywords:** mineral, grass, legume, season, animal requirement

## Introduction

In ruminant diets, forage is an important natural source of minerals. Knowledge about the mineral concentration of grassland species at silage cuts during the season is desired to balance supplements in indoor feeding rations in order to meet the requirements of the planned livestock production. Insight in changes in concentrations of P, Ca, Mg and K during prolonged growth is relevant as, in practice, harvests can be delayed. Therefore, a field experiment was conducted on a sandy soil with 7 two-species forage mixtures that were harvested five times in 2007 (14 May, 14 June, 16 July, 21 August and 9 October) and four times in 2008 (21 May, 2 July, 12 August and 9 October) (Elgersma and Sørensen, 2016). In May and August, the dynamic development of components of feed value were investigated by sampling at the optimum harvest date  $\pm$  one week (Elgersma and Sørensen, 2018). The aim of this experiment was to study macromineral concentrations of grasses and legumes, grown in mixtures, during a 2-week growth interval in summer (August). We expected that concentrations of minerals would be higher in the legume species than in the grasses. We hypothesized that decline rates of minerals during this 2-week period would be similar among species.

## Materials and methods

Perennial ryegrass (*Lolium perenne*) was sown with each of four forage legumes: red clover (*Trifolium pratense*), lucerne (*Medicago sativa*), birdsfoot trefoil (*Lotus corniculatus*) and white clover (*Trifolium repens*); white clover was also sown with hybrid ryegrass (*Lolium boucheanum*), meadow fescue (*Festuca pratensis*) and timothy (*Phleum pratense*), respectively. The 7 two-species grass-legume mixtures were sown in 2006 in a cutting trial with 4 replications in Foulum, central Jutland, Denmark. Hand-separated samples of 4 grasses and 4 legumes were collected weekly, during a 2-week period in August in 2007 and 2008, following preceding cuts taken 36 and 41 days before, respectively, in an experiment where a silage cutting regime was practised and harvests took place on 14 May, 14 June, 16 July, 21 August and 9 October in 2007 and 1 May, 2 July, 12 August and 9 October 2008. In August, weekly changes in components of feed value were investigated by sampling at the optimum harvest date  $\pm$  one week (for details see Elgersma and Sørensen, 2018). All legumes were grown with perennial ryegrass as companion species. All grasses were grown with white clover a companion species. These samples were also used in this study. Samples

were digested with a mixture of nitric acid and perchloric acid according to the AOAC procedure no. 996.16. Elements (phosphorus (P), calcium (Ca), magnesium (Mg) and potassium (K)) were determined using ICP-MS on an X-Series II instrument from Thermo Fischer (Bremen, Germany). Due to technical problems, K concentrations were not measured in 2007. Effects of species, year and species  $\times$  year as fixed factors ( $n=2$ ) were analysed using the GLM procedure of the SAS statistical package. Values of the slopes of linear regression lines were calculated for each parameter for each 2-week harvest interval. Duncans Multiple Range test was used for multiple comparison of means at  $P=0.05$ .

## Results and discussion

Differences occurred among the 8 species in concentrations of Mg and Ca in August (Table 1). As expected, legume species had higher Mg and Ca concentrations than grass species ( $P<0.0001$ ). Among legumes, red clover had the highest Mg concentration ( $P<0.0001$ ) and lucerne had the lowest Ca concentration ( $P<0.0001$ ). Among grasses, timothy had the lowest Mg concentration ( $P<0.0001$ ) and had a lower Ca concentration than perennial ryegrass. Red clover was the only species that met the cow Mg requirement. The Ca requirement for cows was met in all legumes, but in none of the grasses. Rates of change in concentrations of Mg and Ca during the 2-week growth period differed among the 8 species (Table 1). The Mg concentration did not change in white clover, the ryegrasses and meadow fescue; its decline rate was highest ( $P<0.001$ ) in red clover and it was higher ( $P<0.001$ ) in timothy than in the other grasses and white clover. The Ca concentration did not change in white clover ( $P<0.0001$ ) whereas it declined in all other species, at a similar rate of decline.

For most grass-legume mixtures, delayed harvest may thus pose a risk for animal nutrition if no mineral supplementation is provided to the silage produced from these mixtures.

Table 1. Concentrations (g (kg DM)<sup>-1</sup>) of magnesium (Mg), phosphorus (P), calcium (Ca) and potassium (K) and their rate of change (Rate) (g (kg DM)<sup>-1</sup> week<sup>-1</sup>) during 2-week growth intervals in eight species (S) that were grown in two-species grass-legume mixtures.

Species	Concentration				Rate			
	Mg	P	Ca	K <sup>1</sup>	Mg	P	Ca	K <sup>1</sup>
WC	2.35 b <sup>2</sup>	3.31 d	14.86 a	20.8 cd	0.01 a	0.06 ab	0.48 a	0.32 a
RC	3.26 a	3.00 e	13.97 a	19.5 d	-0.31 d	-0.19 abc	-1.09 c	-0.85 ab
LU	2.24 b	2.60 f	10.26 c	20.5 cd	-0.10 abc	-0.21 bcd	-0.40 b	-0.72 ab
BT	2.36 b	2.55 f	13.33 b	14.4 e	-0.15 bc	-0.26 cd	-0.28 b	-1.60 ab
PR	1.98 c	5.83 a	6.03 d	32.5 a	-0.00 a	0.09 a	-0.28 b	-0.03 ab
HR	1.88 c	5.14 b	4.91 de	30.9 ab	0.01 ab	-0.00 abc	-0.34 b	-1.51 ab
MF	1.79 c	4.28 c	5.33 de	29.4 b	-0.02 a	-0.03 abc	-0.10 b	-2.76 b
TI	1.44 d	3.23 de	4.11 e	23.4 c	-0.19 c	-0.39 d	-0.32 b	-2.72 b
Sign. <sup>2</sup>	S	<0.0001	<0.0001	<0.0001	<0.001	<0.05	0.0001	NS
	Y	NS	<0.0001	<0.0001	n.a.	<0.005	NS	n.a.
	S $\times$ Y	NS	<0.01	NS	n.a.	NS	NS	n.a.
SE	0.06	0.08	0.51	0.90	0.04	0.09	0.14	0.83
NRC <sup>3</sup>	2.2	3.5	6.2	6.0				

Data are derived from weekly cuts in August in each of two harvest years<sup>1,2</sup>. Species abbreviations: Legumes: WC, white clover; RC, red clover; LU, lucerne; BT, birdsfoot trefoil (BT). Grasses: PR, perennial ryegrass; HR, hybrid ryegrass; MF, meadow fescue, TI, timothy. Effects of year (Y) on concentrations and rates are also shown. SE, standard error; n.a., not applicable.

<sup>1</sup> K concentrations were only determined in year 2.

<sup>2</sup> Within a column, values without a common superscript are significantly different; P values are shown; NS, not significant.

<sup>3</sup> Dairy cow requirement (g (kg DM)<sup>-1</sup>) in rations (NRC, 2001).

Differences occurred among the 8 species in concentrations of P and K (Table 1). Legume species had lower ( $P<0.0001$ ) concentrations of P than most grass species and lower ( $P<0.0001$ ) concentrations of K than all species. Among legumes, white clover had the highest P concentration ( $P<0.0001$ ) and that of red clover was higher ( $P<0.0001$ ) than of lucerne and birdsfoot trefoil. Among grasses, perennial ryegrass had the highest P concentration ( $P<0.0001$ ) and that of both ryegrasses was higher ( $P<0.0001$ ) than of other grasses, while timothy had the lowest ( $P<0.0001$ ) P concentration. The P requirements for cows were only met in the ryegrasses and meadow fescue. Rates of change in concentrations of P during the 2-week growth period differed ( $P<0.05$ ) among the 8 species (Table 1). Timothy had the fastest ( $P<0.05$ ) decline in P concentration and the decline rates in P concentration in lucerne and birdsfoot trefoil were higher ( $P<0.05$ ) than in other species. In white clover and the ryegrasses, the P concentration did not decline during the 2-week growth period. The rate of decline in K concentration tended to higher in meadow fescue and timothy than in white clover. The K requirements for cows were exceeded in all species, in particular in the grasses.

## Conclusion

Concentrations of Ca and Mg were higher, and P and K concentrations were lower in legume species than in grass species. Decline rates of mineral concentrations during 2-week growth periods in August differed among species. Red clover showed the fastest decline in Mg and Ca concentrations while timothy showed the fastest decline in P concentration. Delayed harvest in August of grass mixtures with red clover, lucerne or birdsfoot trefoil would have led to lower mineral concentrations in the resulting silages.

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# Restoration of relict farmland to improve the environment and food security in Ukraine

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## Abstract

The non-systematic and gradual transformation of up to 80% of grasslands into intensive arable crop production in Ukraine has resulted in a severe decline in the ecological state of the areas involved. Soil erosion is particularly problematic, affecting some 30% of arable land. To counter these adverse effects, studies were conducted in the Forest Steppe area of Ukraine, focusing on the (re-) introduction of mono- and mixed cultures of grass and leguminous plants on relict farmland. The impact on productivity, feed quality and plant biodiversity during the regeneration was studied. To this goal, accumulation of and root dry biomass, anti-erosion effect, nitrogen fixation, cellulolytic activity of soil microorganisms under different fertilization strategies, and mowing frequency were determined. The (re-) establishment of alfalfa (*Medicago sativa*) and mixtures of it with grass (*Bromopsis inermis* and *Festuca orientalis*) over 2019–2022 on 23–34-year-old relict farmland, lead to an increase in dry matter yield (for fodder or green manure) by 2.5–3.0 times and dry root biomass by 1.6–1.7 times. Cellulolytic activity in the soils increased by 3–4%. These effects are mainly due to nitrogen fixation by alfalfa and application of 90 kg N ha<sup>-1</sup>. The conclusion is that (re-)introduction of these crops has a clear anti-erosion effect and may improve the environment and enhance safeguarding of food security in our country.

**Keywords:** biodiversity, aboveground biomass, fallow, productivity, restoration methods

## Introduction

In Ukraine the increased, unsystematic ploughing of grasslands for intensive cultivation of arable crops (in places up to 80% or more) has led to a deterioration in the stable feed base of livestock and the development of soil erosion on up to 30% of arable land and even 60–70% in some small river basins. It has also lead to an increase in relict farmland (Kurhak, 2010). All this, combined with the constant excessive use of pesticides, fertilisers and other chemicals, leads not only to the pollution of water sources, including drinking water, but also to the deterioration of species biodiversity, in particular the disappearance of valuable plant species. Thus a lot of good pasture land was lost. It is known that perennial grasslands, as conservation objects, even on steep slopes, reliably protect soils from erosion and protect rivers and other reservoirs from siltation and pollution (Goreth *et al.*, 2021; Kiss *et al.*, 2022). But in Ukraine their share is only 13% of the total agricultural land use, while the standard for stable land use is 30% (Kurhak, 2010). International research into the restoration of relict farmlands has been conducted for some time (Goreth *et al.*, 2021; Kiss *et al.*, 2022), but scarcely in Ukraine (Kurhak *et al.*, 2023). Therefore, a study was performed to find ways of restoring relict farmlands and measures necessary for their effective use on grey forest and dark grey soils of the Forest-Steppe of Ukraine.

## Materials and methods

Field experiments were performed in 2019–2022 to obtain grassland restoration on relict grassland, situated in Chabany, Kyiv region (Forest-Steppe of Ukraine), that was left without cultivation for 23–34 years before, in three different ways: A) spontaneous self-seeding (since 1997) as control; B) spontaneous

self-seeding with seeding of a wild grass-legume mixture (15 species) collected from a virgin plot, and C) reseeded with alfalfa (*Medicago sativa*) and mixtures of it with the grasses *Bromopsis inermis* and *Festuca orientalis*). Experiments were replicated four times, size of the plots was 25 m<sup>2</sup> (for plots under 1 and 2) and 30 m<sup>2</sup> (plots under 3). The following factors were determined (Bogovin *et al.*, 2017): (a) productivity of mono- and mixed cultures of grass and leguminous plants, measured by weighing forage mass and calculating dry matter yield (DMY) and energy content per hectare; (b) species composition of cultures mentioned under (a), according to the geobotanical description of herbivores; (c) relative erosion resistance of cultures mentioned under (a) by measuring erosion of a representative monolith of turf (soil, size of 20×20×20 cm<sup>3</sup>), determining root dry matter (RDM) - by the calculated method and the ratio of DMY/RDM; (d) cellulolytic activity of the soil (decomposition of linen) in a 0–20 cm soil layer; (e) the amount of N<sub>2</sub> fixed (kg N ha<sup>-1</sup>) in each crop was calculated by multiplying the proportion of e total N uptake originating from N<sub>2</sub> fixation (%Ndfa) with total crop N content (N concentration × crop biomass) accumulation. Fodder nutritional value and (number of) species involved and the erosion resistance were determined according to Bogovin *et al.* (2017).

## Results and discussion

Results of restoration long-relict farmland under spontaneous regeneration and with or without sowing mixtures of wild grasses and legumes and defined alfalfa-grass seed mixtures and with or without mowing of the vegetation are summarized in Table 1.

Productivity between the different treatments ranged from 2.63–6.18 t ha<sup>-1</sup> of forage dry matter and 24.5–60.6 GJ ha<sup>-1</sup> of energy content. Highest productivity was in variant B5 (Table 1, relict soil resown with wild grass-legume species) and annual application of N<sub>90</sub>P<sub>40</sub>K<sub>70</sub>). The productivity was significantly higher than treatment A5, where no extra sowing was applied. Species richness varied from 40–70 species between treatments. The highest number of species was found in treatments without fertilizer and 2× mowing in experiment A and B. In experiment A, *Calamagrostis epigejos* dominated, and in experiment B *Festuca valesiaca* dominated. Mineral fertilization had a negative effect on species richness in all cases.

Table 1. Development of forage productivity, quality and plant diversity on 23–34-year-old relict farmland, with spontaneous regeneration (A, control) and spontaneous regeneration with seeding of a wild grasses-legume seed mixture (B) (experiments from 2020–2022, average).

Treatment	DMY (t ha <sup>-1</sup> )	Energy (GJ ha <sup>-1</sup> )	Feed value, point <sup>1</sup>	No. of plant species
A. Relict farmland with spontaneous regeneration (plots established in 1997)				
A1. Without mowing	–	–	3.4	45
A2. 1× mowing without fertilizers	2.63	24.5	3.6	44
A3. 1× mowing + N <sub>90</sub> P <sub>40</sub> K <sub>70</sub>	4.26	39.6	4.4	40
A4. 2× mowing without fertilizers	3.00	29.4	4.2	54
A5. 2× mowing + N <sub>90</sub> P <sub>40</sub> K <sub>70</sub>	5.46	53.5	4.9	38
B. Relict farmland with spontaneous regeneration and sowing of a wild grass/legume mixture (seeds obtained from plots established in 1985)				
B1. Without mowing	–	–	–	70
B2. 1× mowing without fertilizers	3.31	30.8	3.4	62
B3. 1× mowing + N <sub>90</sub> P <sub>40</sub> K <sub>70</sub>	4.45	41.4	4.5	52
B4. 2× mowing without fertilizers	3.46	33.9	4.0	70
B5. 2× mowing + N <sub>90</sub> P <sub>40</sub> K <sub>70</sub>	6.18	60.6	4.9	55
Least significant difference (LSD <sub>05</sub> )	0.24	–	0.2	3

<sup>1</sup>Index of fodder value in points: 8, highest; 7, high; 6, sufficient; 5, good; 4, medium; 3, rather low; 2, low; 1, very low; 0, no feed value, harmful; –1, poisonous.

Restoration of relict farmlands, both by sowing of alfalfa alone and by sowing it together with grass species (experiment C), lead to an increase in DMY (for fodder or green manure by 2.5–3.0 times) and RDM (by 1.6–1.7 times), in cellulolytic activity (3–4%), and in the erosion resistance (1:20 min). Symbiotic nitrogen fixation contributed 184–203 kg N ha<sup>-1</sup> when alfalfa was introduced (Table 2). N<sub>90</sub>P<sub>40</sub>K<sub>70</sub> fertilisation increased the proportion of the grasses *Arrhenatherum elatius*, *Elytrigia repens*, and also (experiment A only), *Poa angustifolia*.

Table 2. Influence of re-seeding of *Medicago sativa* alone or in combination with *Bromopsis inermis* and *Festuca orientalis* on the productivity, soil cellulolytic activity and erosion resistance of relict farmlands (average for 2019–2022; variant 3 served as internal control)

Crop species sown and seed application (kg ha <sup>-1</sup> )	DMY (t ha <sup>-1</sup> )	RDM (t ha <sup>-1</sup> )	Cellulolytic activity of the soil (%)	Erosion resistance (min) <sup>1</sup>	N fixation (kg ha <sup>-1</sup> )
<i>Medicago sativa</i> , 18	9.08	8.53	15	8:32	203
<i>Medicago sativa</i> , 10 + <i>Bromopsis inermis</i> , 8 + <i>Festuca orientalis</i> , 6	9.00	9.17	15	9:53	184
<i>Bromopsis inermis</i> , 15 + <i>Festuca orientalis</i> , 14	3.24	7.29	11	8:33	–
<i>Bromopsis inermis</i> , 15 + <i>Festuca orientalis</i> , 14 + N <sub>90</sub>	5.50	8.58	15	10:05	–
LSD <sub>05</sub>	0.40	0.47	2	0.25	–

<sup>1</sup>Time of erosion of a soil monolith (turf) by a uniform stream of water.

## Conclusion

Relict farmlands of Ukraine should be restored to grassland as a source of grass fodder, environmental protection, particular soil (erosion resistance) and plant biodiversity (species richness). Restoration experiments using spontaneous regeneration as well as additional sowing a mixture of wild grass and legume seeds or a defined alfalfa and grass mixture showed that especially in the latter case the (re)-introduction of these crops had a clear anti-erosion effect and may improve the environment and enhance food security in our country.

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# Grasslands: an asset to secure livestock feed in the face of heatwaves? A literature search

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## Abstract

Research in forage production is crucial in the face of the challenges posed by global change in securing livestock feed. Two important but understudied questions are the impact of repeated heatwaves on forage production and how plant diversity can mitigate this threat. Heatwaves are expected to become more frequent, longer and more intense. A literature search was conducted to gather knowledge and detect information gaps. We found that heatwaves can cause an impairment in growth activities. However, the extent of mass reduction in temperate herbs is controversial. Resistance to heatwaves has been widely demonstrated, but is species dependent. Some species have the ability to recover, but this is overlooked. Some species are capable of storing and retrieving information from previous exposures to improve future responses, a mechanism that should be considered in forage management. Plant diversity therefore may offer an alternative to the search for the best resistance and/or resilience in forage production, although this remains unproven. A multi-species grassland would have a greater potential to ensure production than monospecific crop because diversity ensures at least some successful responses. Further research should focus on the diversity of responses, and to predict which plant composition can ensure livestock feed during repeated heatwaves.

**Keywords:** grassland, heat-wave, disturbance, extreme event, plant ecology, climate change

## Introduction

Grasslands in temperate climates are maintained through grazing and mowing practices to provide sufficient and high-quality fodder for livestock. These grasslands provide a multitude of environmental benefits, such as promoting biodiversity or sequestering carbon in soil (Schils *et al.*, 2022). Global change poses several threats to the functioning of grasslands, making it essential to investigate forage production to secure livestock feed. This communication discusses the impact of recurring heatwaves on temperate grasslands, a relatively unexplored aspect of climatic change (Breshears *et al.*, 2021), despite its relevance based on the IPCC reports. Additionally, we explore the potential for plant diversity to alleviate this climatic threat, especially considering the decline of diversity in European grasslands due to intensified management practices (Schils *et al.*, 2022).

A heatwave occurs when the maximum temperature reaches above the 90<sup>th</sup> percentile for three or more consecutive days (Perkins-Kirkpatrick and Gibson, 2017). Heatwave events influence grasslands in the short-term (days), but may influence mid-term dynamics (years). Heatwave events are unpredictable and differ from warm seasons, which are regular climatic features of ecosystems. Hence, they should be viewed through the lens of disturbance ecology as extreme climatic events, and not as sustained stressful periods for which the involved adaptations differ completely (Breshears *et al.*, 2021). Their impact may be determined by the sensitivity of plant communities (White *et al.*, 2000), but the timing, intensity and duration of the heatwave (Qu *et al.*, 2020), along with the coupling of other events such as droughts (De Boeck *et al.*, 2016; Hoover *et al.*, 2014) and the nutrient availability in soils (Wang *et al.*, 2008) may condition the response of grasslands to heatwaves.

## Methodology

A literature search of articles in Web of Science was conducted to answer whether temperate grasslands are an asset to secure livestock feed during heatwaves. Our search string was AB=((heatwave\* OR heat-wave\*) AND (herb\* OR grass\* OR plants)).

## Results and discussion

We found 850 original research and review papers dealing with heatwaves and herbaceous species. Among them, only 14 studied the direct impact on grasslands. The communication focused on reported impacts, herbaceous species responses, and the roles of plant diversity.

Heatwaves have a variety of impacts on grassland plants, ranging from cellular to community levels. When temperature rises above plant thermotolerance threshold, there is an increase of leaf conductance and transpiration to lower temperature but leading to water loss (Breshears *et al.*, 2021). It results in the cessation or reduction of growth activity, and may even be lethal (Breshears *et al.*, 2021). Some studies on temperate grassland species found a decrease in aboveground biomass (Wang *et al.*, 2008; White *et al.*, 2000), while others did not report any significant reduction after heatwaves (Dreesen *et al.*, 2012; Hoover *et al.*, 2014; Mainali *et al.*, 2014). Root growth appears to be stimulated in grassland species during heatwaves (Dreesen *et al.*, 2012; Mainali *et al.*, 2014). Certain species exhibit an increase in leaf abscission during or following heatwaves (Dreesen *et al.*, 2012; Qu *et al.*, 2020).

Grassland species exhibit different responses to heatwaves, either resistance during the extreme event or the ability to recover afterwards. Most of the reported mechanisms involve resistance during heatwaves (i.e. thermotolerance). A certain robustness in morphology and anatomy is suitable for resisting heatwaves, such as prostrate forms or leaves with thick cuticles (Qu *et al.*, 2020). At the cellular level, heat shock proteins (HSPs) play a crucial role in reducing or repairing damage, particularly in the photosynthetic pathway (Davies *et al.*, 2018). Plants with improved water use efficiency are better in resisting heatwaves as they are likely to compensate for water loss resulting from increased heat stress-induced transpiration (French *et al.*, 2017). Leaf shedding is considered as a mechanism to improve resistance by reducing heat load and water loss (Dreesen *et al.*, 2012; Qu *et al.*, 2020). Leaf abscission can contribute in the reduction or cessation of growth during heatwaves, but it could be a mechanism to replace less resistant leaves with more resistant ones (French *et al.*, 2017). Additionally, strategies of fast-growing and strategies of rapid reproductive phenology are reported (Qu *et al.*, 2020), as strategies for a quickly recovery in disturbed environments. Plant resilience, which is currently less explored than resistance, appears nevertheless particularly relevant for grassland communities dedicated to producing forage over time.

Heatwaves may precede other extreme events, rather than being an isolated event. Therefore, the frequency of these events should be considered. Plants possess the ability to acclimate to these recurring events (Breshears *et al.*, 2021). This ability is based on stress memory (Bruce *et al.*, 2007). Grassland species can store and retrieve information obtained from previous exposures. This enables them to respond to future heatwaves (White *et al.* 2000), by developing new thermotolerant leaves or producing more HSPs, among other memory acclimation-related processes (Davies *et al.*, 2018).

The literature highlights the diversity of responses to heatwaves among temperate grassland species. This diversity of responses leads to shifts in plant composition in the short and medium terms (Wang *et al.*, 2008; White *et al.*, 2000), depending on the impacts and factors described above. The presence of species with different responses to heatwaves in communities may increase the resilience of grasslands at the community level and then of productivity under different scenarios of heatwaves. But many questions remain unanswered. What is the extent of the impact of successive summer heatwaves on grassland



species? Which species are resistant, sensitive or resilient? Are different responses related to resource gradient theory or functional groups? Which species can acclimate to be more resistant or resilient to heatwaves? Can species interactions within community affect plant responses? Can we predict which functional assemblage will ensure forage production during heatwaves?

## Conclusion

Even if grasslands represent an asset for feeding livestock in the context of global change, heatwaves jeopardize forage production. The main mechanisms by which herbaceous species cope with heatwaves are poorly understood. Further research is needed to guide towards the best grassland composition that will ensure livestock feed during heatwaves.

## Acknowledgements

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 101034329. Recipient of the WINNING Normandy Program supported by the Normandy Region.

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# Impact of clover inclusion on feed intake and milk production in a tall fescue-dominated ration

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## Abstract

Red clover is known to increase dry matter intake (DMI) and might counteract lower DMI in tall fescue-rich rations. Therefore, we evaluated DMI and milk yield (MY) when red clover was incorporated into an ensiled tall fescue- (*Festuca arundinacea* Schreb) rich ration or in a diploid perennial ryegrass- (*Lolium perenne* L.) rich ration. The trial involved 30 dairy cows, divided in 6 groups. Each group received subsequently one of three treatments in a balanced Latin-square design (1; diploid perennial ryegrass (Lp2), 2; diploid perennial ryegrass plus clover (Lp2+Tp), 3; tall fescue plus clover (Fa+Tp)). In the clover groups, 40% of the grass silage was substituted with red clover (Tp). We hypothesised that substituting part of the grass by red clover results in greater DMI, while MY remains comparable. Statistically significant difference in daily DMI between the clover rations (23.4 kg Lp2+Tp and 23.9 kg Fa+Tp) and the ration without clover (22.7 kg Lp2) ( $P < 0.05$ ) was shown, due to higher roughage DMI although the daily fat and protein corrected milk yield (FPCMY) (34.6 kg Lp2, 34.9 kg Lp2+Tp, 34.6 kg Fa+Tp) and milk composition (except for urea) were not affected. Thus, Fa+Tp resulted in similar DMI as Lp2+Tp, while maintaining overall dairy cow productivity.

**Keywords:** dairy cow, dry matter intake, milk yield, red clover, tall fescue, perennial ryegrass

## Introduction

Extreme climatic events, such as prolonged summer droughts and intense winter rainfall, are projected to undergo a notable rise in frequency and severity across North-West Europe. The escalating occurrence of such periods also puts at risk the production of high-quality forage grass, a crucial component of dairy cow rations. To safeguard the functionality of grasslands and ensure sustainable forage production, it becomes imperative to diversify European grassland ecosystems with species that are adapted to the new, challenging growth conditions posed by extreme heat, drought, and flooding events (Voltaire *et al.*, 2014). One such species with promising attributes is tall fescue (*Festuca arundinacea* Schreb.). Research conducted by Cougnon *et al.* (2014) revealed that tall fescue yields are 20-30% higher than ryegrass, with a comparable crude protein content with equivalent fertilizer application. This is attributed to tall fescue's deep and extensive root system, which grants it robust drought resistance (Cougnon *et al.*, 2017) and substantial yield potential. However, it is crucial to acknowledge certain limitations of tall fescue, such as its relatively low digestibility and limited voluntary intake by grazing animals (Cougnon *et al.*, 2014). When heifers were fed sole grass silages of different grass species, the lowest dry matter intake (DMI) was attributed to heifers fed tall fescue and late-cut ryegrass. This can be attributed to its high NDF concentration which promotes a high rumen physical fill (Parninan-Khajehdizaj *et al.*, 2023). Johansen *et al.* (2017) conducted a meta-analysis comparing feed intake, milk production, milk composition and organic matter digestibility (OMD) in dairy cows fed different grass and legume species. Although this study found no significant differences among the included grass species in terms of DMI, milk production, milk composition or OMD, it also showed that only few observations were available and acknowledged that literature is scarce on this subject. Interesting multiple studies have shown that adding clover to a ration can affect the roughage intake by 10% (Dewhurst, 2013). The objective of this study was to investigate if DMI and milk yield (MY) are affected by grass species and clover inclusion in the ration.

We hypothesized that substituting part of the tall fescue by red clover results in greater DMI, while MY remains comparable to Lp2 diets.

## Material and methods

This trial was conducted in 2022 at the cattle farm of ILVO (Melle, Belgium). Tall fescue (cv. Ticho), diploid perennial ryegrass (cv. Melspring) and red clover (cv. Global) were grown in pure stands in Melle (50°58'N, 3°46'E). The field was established in April 2019. In 2021 five cuts were harvested. The first two cuts were ensiled one above the other in a pit for this experiment. In the grass-clover silages, approximately 40%, on dry matter (DM) basis, of red clover was included on top of every cut. After pre-wilting on the field for 24-36h, the DM content of the harvested grass varied from 27% to 51% and from 25% to 30% for the red clover depending on factors such as yield, grass species and weather conditions. Thirty high-producing Holstein cows were included in a balanced 3x3 Latin-square design with 3 dietary treatments and 3 periods of 4 weeks. The 5 primiparous and 25 multiparous were  $134.7 \pm 51$  days in milk (DIM),  $691 \pm 75.2$  kg body weight (BW),  $2.8 \pm 1.26$  lactations and had a milk yield (MY) of  $36.6 \pm 4.39$  kg day<sup>-1</sup> at the start of the experiment (average  $\pm$  standard deviation (SD)). The roughage diet contained 31% corn silage, 60% pre-wilted grass(-clover) silage and 9% of pressed beet pulp, on DM basis, and was mixed with soybean meal, rumen protected soybean meal (covasoy), corn meal and rolled barley. This was fed as a total mixed ration through roughage intake control bins. All diets were completed with a balanced concentrate feed in the concentrate boxes to meet the cows' requirements. Three experimental diets were compared: diploid perennial ryegrass (Lp2), diploid perennial ryegrass plus clover (Lp2+Tp), and tall fescue plus clover (Fa+Tp) (Table 1). The cows were milked twice a day in a herringbone milking parlour. BW of the cows was recorded twice a day after milking. Milk samples were collected at 4 consecutive milkings in the last week of every experimental period. MY was corrected for fat and protein content (FPCMY) according Subnel *et al.* (1994). All statistical analyses were performed using the statistical software program R (version 4.0.4, [www.r-project.org](http://www.r-project.org)). Differences were considered significant at  $P \leq 0.05$ .

## Results and discussion

The total DMI was found to be significantly different between the Lp2 diet (22.7 kg day<sup>-1</sup>) and the two diets with clover (23.4 kg day<sup>-1</sup> Lp2+Tp and 23.9 kg day<sup>-1</sup> Fa+Tp) (Table 2). The difference can be attributed to a lower roughage intake, i.e. 13.6 kg day<sup>-1</sup> for Lp2 versus 14.6 kg day<sup>-1</sup> for Lp2+Tp and 14.8 kg day<sup>-1</sup> for Fa+Tp. Furthermore MY, milk composition and total milk solids were not different between treatments, although milk urea significantly varied between treatments. Methane emissions weren't different between the three treatments. The higher DMI in this study is in line with the study of Dewhurst *et al.* (2013). The fact that production figures were not different confirmed our hypothesis that the lower DMI and MY when using tall fescue might be overcome by adding clover to the roughage ration.

## Conclusion

The inclusion of clover provides an effective strategy to counteract the lower intake that is described when using ensiled tall fescue in diets for dairy cows, while maintaining overall dairy cow productivity. This study shows that including red clover is an opportunity for farmers to use tall fescue, which is a more drought tolerant grass species, in their cultivation plan to maintain the productivity and quality of the roughage. Also nutritionists will benefit from these results which will provide them extra knowledge on how to maintain and improve feed intake in rations with tall fescue.

## Acknowledgements

We thank Flanders Innovation and Entrepreneurship (VLAIO, Belgium) for funding the KLIMGRAS project and especially the staff of the ILVO dairy farm for their appreciated help with this experiment.

Table 1. Composition of rations (% on dry matter base) for the three different treatments.

	Treatment		
	Lp2	Lp2+Tp	Fa+Tp
Silage	35.3	37	32.8
Maize silage	18.7	19.5	22.2
Pressed beet pulp	5.9	6	6.9
ILVO concentrate	26.5	29.6	29.3
Maize meal	3.6	2.4	2.3
Rolled barley	1.8	1.5	1.7
Soybean	7.7	0.04	0.11
Covasoy	0.2	3.3	3.7

Table 2. Dry matter intake (DMI), milk production (FPCMY), milk solids and CH<sub>4</sub> emissions of 30 dairy cows.

	Treatment			SEM
	Lp2	Lp2+Tp	Fa+Tp	
Total DMI (kg)	22.7 <sup>b</sup>	23.4 <sup>a</sup>	23.9 <sup>a</sup>	0.332
Roughage DMI (kg)	13.6 <sup>b</sup>	14.6 <sup>a</sup>	14.8 <sup>a</sup>	0.275
FPCMY (kg cow <sup>-1</sup> )	34.6 <sup>a</sup>	34.9 <sup>a</sup>	34.6 <sup>a</sup>	0.715
Fat (%)	4.3 <sup>a</sup>	4.3 <sup>a</sup>	4.3 <sup>a</sup>	0.084
Protein (%)	3.6 <sup>a</sup>	3.6 <sup>a</sup>	3.6 <sup>a</sup>	0.044
Urea (mg l <sup>-1</sup> )	203 <sup>a</sup>	165 <sup>c</sup>	180 <sup>b</sup>	4.77
CH <sub>4</sub> (g day <sup>-1</sup> )	527 <sup>a</sup>	522 <sup>a</sup>	538 <sup>a</sup>	12.9

Values with a different superscript are significantly different ( $P < 0.05$ ).

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# Is milk $\delta^{13}\text{C}$ a suitable sustainability indicator for grassland-based feeds in dairy herd diets?

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## Abstract

As the global population expands rapidly, sustainably produced foods are absolute necessities to ensure net food security. Milk protein, as a complete protein, is a major contributor towards human nutrition and one of the highest ranked foods in terms of nutrient-to-calorie ratio for numerous amino acids, calcium, phosphorus and riboflavin. Grassland-based feeding (GBF) is a sustainable feeding strategy in countries with abundant grasslands, as dairy cows have the unique ability to transform human inedible fibre fractions into high quality milk proteins. We aimed to evaluate the efficacy of  $\delta^{13}\text{C}$  as an indicator for the proportion of GBF (GBF%) in a dairy herd's diet. We collected 217 milk samples, over one calendar year, from 21 dairy farms selected to represent a wide range of feeding strategies based on contrasting proportions of GBF (28 – 99 %). Based on mixed-model multiple regression, we observed a statistically significant relationship between GBF% and  $\delta^{13}\text{C}$ . As GBF% increased, milk  $\delta^{13}\text{C}$  values decreased. These findings may be of value as part of a future 'sustainability-index' tool for the direct measurement of GBF dairy production.

**Keywords:** sustainable, grass-fed, bovine, SIRA (stable isotope ratio analysis)

## Introduction

To feed a population of 10 billion in 2050 it is essential to master the trade-offs between sustainability and food security (Vågsholm *et al.*, 2020). Milk protein, as a complete protein containing all 9 essential amino acids, is a major contributor towards human nutrition (White and Gleason, 2023).

Dairy production from grassland-based systems is a sustainable feeding strategy in temperate countries with abundant natural and semi-natural grasslands, as dairy cows have the unique ability to transform human-inedible grassland fibre fractions into high quality milk proteins without competing with human-edible crops such as maize. Camin *et al.* (2008) used stable isotope ratio analysis to demonstrate that for each 10% increase of maize in the diet, the  $\delta^{13}\text{C}$  milk casein values increased by 0.7–1.0‰. With a probable future 'sustainability-index' in mind, we aimed to test the efficacy of  $\delta^{13}\text{C}$  values of whole milk as an indicator for the proportion of GBF (GBF%) in dairy herd diets. This may well aid researchers and policy makers in measuring the sustainability of future food systems.

## Materials and methods

This study was conducted in Switzerland over one year (January to December 2020) to reflect and incorporate seasonal variations in dairy herd feeding strategies. Twenty-one dairy farms were selected to represent a wide range of feeding strategies based on contrasting proportions of GBF% (28–99%; Figure 1). Total GBF intake was obtained and verified using a series of cross-checks. In the first step, average dry matter (DM) intake was calculated on herd level according to energy corrected milk production, live-weight, days in milk, and parity (Jans *et al.*, 2017). Average DM requirements were compared to farmer-reported rations surveyed during a telephone interview and an on-farm visit of each farm. Farmers reported monthly intakes of grass silage, hay, whole-plant green maize, concentrates and others feeds as percentages of the total ration. The remainder was attributed to grazed herbage intake. The reported

quantities fed were then compared to the reported quantities supplied by means of a balance sheet. The Swiss Feed Database (Agroscope, 2016) was employed to calculate energy and protein ( $NE_L$ ,  $APD_E$  and  $APD_N$ ) contents of the feed components produced and fed on farm. Lastly, the total amounts of  $NE_L$ ,  $APD_E$  and  $APD_N$  were calculated for the reported ration and compared with the average requirements for the lactating herd according to Jans *et al.* (2017); a discrepancy of no more than 10% was considered plausible for inclusion in the study.

Chilled (4°C) tank milk samples (50 ml) were collected from each farm, twice per month, and frozen within 24 hours at -20°C until analysis. For  $^{13}C/^{12}C$  ratio analysis, monthly samples were pooled in equal proportions and vortexed for one minute (Vortex Genie 2, Scientific Industries, Bohemia, NY, USA) before pipetting a 5 µl aliquot into ultra-clean tin cups (4.6 mm). Samples were dried at 60°C for 24 hours before combustion on an elemental analyzer (EA1100, Carlo Erba Instrumentation, Milan, Italy) interfaced (Conflo III, Finnigan MAT, Bremen, Germany) to an isotope ratio mass spectrometer (Delta Plus, Finnigan MAT) operating in continuous flow mode. Samples were measured against a working standard  $CO_2$  reference gas (purity=4.5; Westfalen, Münster, Germany) calibrated against the secondary isotope standard, IAEA-CH6 (sucrose; International Atomic Energy Agency (IAEA), Vienna, Austria.). A solid internal laboratory standard (SILS, a finely ground protein powder) was run as a control after every 10<sup>th</sup> sample (precision of the SILS for the measurement period=0.11‰). The carbon isotope ratio is expressed in per mil (‰) against the international standard, Vienna Pee Dee Belemnite, as:

$$\delta = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000$$

where  $R$ =ratio of  $^{13}C$  to  $^{12}C$ .

Statistical analyses were performed with R version 4.3.0 (R Core Team, 2023). To evaluate the suitability of  $\delta^{13}C$  as a possible indicator for GBF%, a linear mixed effects model was applied (lmerTest; Kuznetsova *et al.*, 2017). The best model was selected using the lowest Akaike Information Criterion from a subset of 6 models based on the initial model, depicted as:

$$Y = b_0 + b_1 \times \text{Diet}_{\text{GBF}\%} + b_2 \times \text{Latitude} + b_3 \times \text{Longitude} + b_4 \times \text{Month} + b_5 \times \text{Month} \times \text{Diet}_{\text{GBF}\%} + b_6 \times F + e$$

where  $Y = \delta^{13}C$ ;  $b_0$ =overall mean;  $b_1, 2, \dots, 6$ =regression coefficients of the observed effects of GBF ( $\text{Diet}_{\text{GBF}\%}$ ), farm location (Latitude and Longitude), month (as a categorical variable) and the interaction between the month and GBF ( $\text{Month} \times \text{Diet}_{\text{GBF}\%}$ ). Farm ( $F$ ) was included as a random effect, and  $e$  as the random error of the model. This allowed for farm effects to be modelled by a random intercept. To correct GBF effects for farm location, the latitude and longitude of each farm were included in the initial model as continuous explanatory variables.

## Results and discussion

Whole milk  $\delta^{13}C$  values ranged from -30.9 to -20.7 ( $\pm 2.4$ ) ‰ across all farms, months and feeding strategies. Regression analysis revealed a statistically significant effect of GBF% on milk  $\delta^{13}C$  values ( $P < 0.001$ ). The higher the GBF% in the herd's diet, the more negative the  $\delta^{13}C$  value was of the milk (Figure 2). This is because C3 plants (temperate grasses and herbage) that fix C using the Calvin cycle are characterised by lower  $\delta^{13}C$  values (ca. -26‰) than C4 plants (maize and tropical grasses) (ca. -12‰) that use the Hatch-Slack pathway (Szapak *et al.*, 2013) which is then reflected in the milk. Our results suggest that the  $\delta^{13}C$  value of whole tank milk may be a suitable starting point for the determination of the GBF% in the diets of dairy herds in temperate climates.

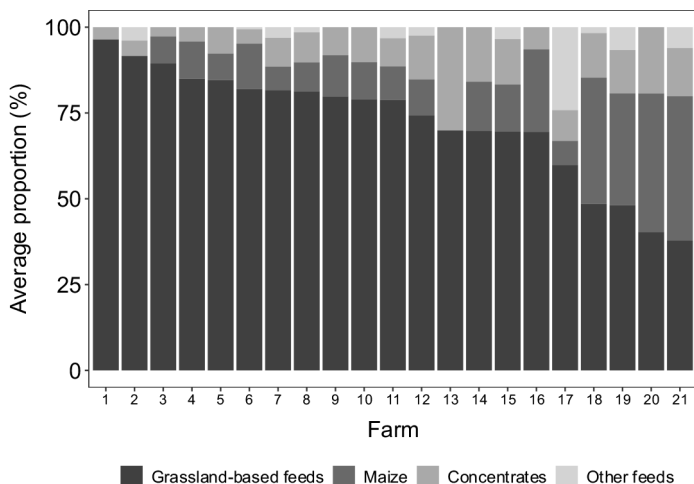


Figure 1. Average annual ration composition per farm, arranged in descending order of GBF%.

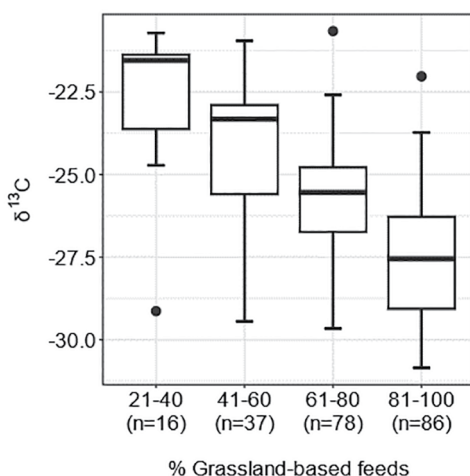


Figure 2. Effect of GBF%, in 20% increments, on the  $\delta^{13}\text{C}$  of tank milk (n=total number of observations).

## Conclusions

Although  $\delta^{13}\text{C}$  is currently not routinely used as a monitoring and evaluation tool for milk, it may be of value as part of a future 'sustainability-index' tool for the direct measurement of grassland-based dairy production in temperate climates despite the cost. Future research should concentrate on larger, international datasets to validate and progress this notion.

## Acknowledgements

The authors would like to thank J. Braun (BFH, HAFL), J. Mäder and N. Matt for the collection of the on-farm data, Dr. S. Ineichen (BFH, HAFL) for sample preparation and Dr. R. Schäufele (TUM) for the milk isotope analysis. This project was funded by Innosuisse, Berne, Switzerland (32078.1 IP-LS).

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# Beef production systems with dairy×beef heifers based on forage and semi-natural grasslands

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## Abstract

Beef production on semi-natural grasslands provides human food and nature conservation of biodiverse habitats. This study examined animal performance and carcass characteristics in four different breed combinations of dairy (Swedish Red (SR) and Swedish Holstein (SH))×beef heifers after Angus (Ang) or Charolais (Ch) sires allocated to a High or Low production system using semi-natural grasslands. The High system involved a moderately high indoor feed intensity, only one summer on pasture, and slaughter at 20 months of age, whereas Low system involved low indoor feed intensity, two summers on pasture and slaughter at 27 months. Liveweight gain was higher for Ch than Ang and higher for High than Low. Ch carcasses had a higher weight and conformation score than Ang, whereas Ang had higher scores of fatness and marbling. Low heifers had lower fatness score than High heifers. ChLow heifers were leaner than the three other groups. The AngHigh, ChHigh, AngLow and ChLow heifers grazed 0.36, 0.33, 1.32 and 1.14 ha, respectively. The results indicate that dairy × beef heifers can be used for nature conservation grazing of semi-natural grasslands and at the same time reach market-oriented carcasses.

**Keywords:** feed intensity, grazing, semi-natural pasture, crossbreeding, carcass

## Introduction

Biodiverse natural grasslands show a strong deteriorating trend in the EU, including in Sweden (European Environment Agency, 2020). The greatest threat to the biodiversity of grasslands is cessation of agricultural management and subsequent overgrowth (European Environment Agency, 2020). Business opportunities for beef production on semi-natural grasslands have increased in Sweden in recent years, as there is now a certification system and a steadily increasing demand. In addition farmers can receive economic support to keep grazing livestock on semi-natural grasslands. Dairy×beef heifers are suitable for beef production on semi-natural grasslands and have increased in number in Sweden in recent years. The aim of this study was to investigate the effects of dam breed, sire breed, and intensity of production system on animal performance and carcass characteristics in dairy × beef heifer production based on forage and semi-natural grasslands.

## Materials and methods

The experiment was conducted at the SLU Götala Beef and Lamb Research Centre, Skara, in south-western Sweden during the years 2019–2022. In total, 72 dairy×beef heifers were followed from weaning to slaughter in an experiment with a 2×2×2 factorial design, comparing two sire breeds (Angus (Ang) and Charolais (Ch)), two dam breeds (Swedish Red (SR) and Swedish Holstein (SH)) and two production systems (High and Low), both including grazing of semi-natural grasslands. System High involved a moderately high feed intensity during two indoor periods with an intermediate grazing period (108 days, 740 kg liveweight ha<sup>-1</sup>) and slaughter at 20 months of age. System Low involved a low feed intensity during three indoor periods with two intermediate grazing periods (286 days, 715 kg liveweight ha<sup>-1</sup>) and slaughter at 27 months. At the start of the study, all animals were fed *ad libitum* of total mixed rations consisting of grass-clover silage, rolled barley, rolled peas, and rapeseed meal. Heifers in the Low system were fed forage *ad libitum* as the sole feed from 225 kg until slaughter. The forage composed of grass-clover silage during the remaining part of indoor period 1 and the fully indoor periods 2 and 3, with pasture herbage during the grazing periods in between. Heifers in the High system were fed 20% rolled

barley and 80% grass-clover silage during their indoor period 2 until slaughter. Heifers in the Low system grazed during summers 2020 and 2021, whereas the only grazing period for the heifers in the High system occurred in 2021. The pasture consisted of permanent, unfertilized semi-natural grassland, dominated by *Deschampsia cespitosa* (tufted hairgrass), with *Festuca rubra* (red fescue) also prominently present. Sward height at the end of the grazing periods was visually determined to be short enough not to accumulate litter and hence qualified for agri-environmental payments for preserving grasslands biodiversity.

The data were analyzed with the Mixed procedure in SAS (2018), with production system, sire breed, and dam breed as fixed factors, their interactions, and the individual animal nested within pen. Means were compared pairwise using LSD<sub>0.05</sub>-tests and denoted as significant at  $p < 0.05$ , and as a tendency for significance at  $0.05 < p < 0.10$ .

## Results and discussion

Sire breed and production system influenced performance and carcass characteristics significantly more than dam breed did. Liveweight gain from weaning to slaughter was higher for Ch than Ang and higher for High than Low (Table 1). Ch carcasses had a higher weight, conformation score, and proportion of high-valued retail cuts than Ang, whereas Ang had higher scores of fatness and marbling (Table 1), which is in accordance with previous studies (e.g. Huuskonen *et al.*, 2013; Eriksson *et al.*, 2020). The Low system gave carcasses with a lower fatness score than the High system (Table 1), which also has been found previously (Hessle *et al.*, 2007). There were interactions indicating ChLow being leaner than the other groups, having lower fatness score than AngLow, whereas no breed effect was found in the High system. ChLow also comprised a higher proportion of retail cuts than ChHigh, whereas no effect of system was found in the Ang heifers.

SH heifers had higher overall daily liveweight gain from weaning until slaughter (0.93 *vs.* 0.90 g;  $p=0.0156$ ) and a lower proportion of bone in the hindquarter (19.0 *vs.* 19.6 %,  $p=0.0202$ ) than SR heifers.

Although the heifer groups did not all gain weight on pasture, all contributed to the management of semi-natural grasslands. Managed grassland area per heifer was 0.36, 0.33, 1.32 and 1.14 ha for AngHigh, ChHigh, AngLow and ChLow heifers, respectively. These figures correspond to every kg of meat produced resulted in 18, 15, 58 and 46 m<sup>2</sup> grazed semi-natural grassland for AngHigh, ChHigh, AngLow and ChLow heifers.

## Conclusion

The results indicate that dairy×beef heifers can be used for nature conservation grazing of semi-natural grasslands and reach acceptable performance and carcass characteristics. In such production, lower indoor feed intensity combined with higher slaughter age result in a larger grazed area and leaner carcasses than higher indoor feed intensity combined with lower slaughter age, where the carcasses might be too fat. However, at low feed intensity crossbred heifers of late-maturing beef breeds, such as Charolais, may need to be kept to higher slaughter age than in this study in order to fully utilize their growing potential and deposit fat.

Table 1. Daily feed intake, liveweight gain, feed efficiency (in metabolizable energy) and carcass characteristics of dairy×beef heifers ( $n=18$ ) with two sire breeds (Sire, Ang is Angus, Ch is Charolais) in two production systems with a semi-high feed intensity and 20 months of slaughter age (High) or a low feed intensity and 27 months of slaughter age (Low).

Item	High		Low		SE	Level of significance		
	Ang	Ch	Ang	Ch		System	Sire	Sys × Sire
Indoor period 1								
Dietary intake (kg dry matter)	7.19 <sup>a</sup>	7.29 <sup>a</sup>	5.97 <sup>b</sup>	6.28 <sup>b</sup>	0.14	<0.0001	ns	ns
Dietary intake (% of liveweight)	3.01 <sup>a</sup>	2.73 <sup>b</sup>	2.90 <sup>ab</sup>	2.86 <sup>ab</sup>	0.04	ns	0.0114	0.0585
Liveweight gain (kg day <sup>-1</sup> )	1.20	1.21	1.15	1.17	0.02	0.0772	ns	ns
Feed efficiency <sup>a</sup> (MJ kg gain <sup>-1</sup> )	77.2 <sup>a</sup>	74.5 <sup>a</sup>	62.8 <sup>b</sup>	64.3 <sup>b</sup>	1.0	<0.0001	ns	ns
Grazing period 1								
Liveweight gain (kg day <sup>-1</sup> )	-0.16 <sup>a</sup>	-0.09 <sup>a</sup>	0.58 <sup>b</sup>	0.65 <sup>b</sup>	0.03	<0.0001	0.0773	ns
Indoor period 2								
Dietary intake (kg dry matter)	12.63 <sup>a</sup>	13.37 <sup>a</sup>	10.27 <sup>b</sup>	10.06 <sup>b</sup>	0.24	<0.0001	ns	ns
Dietary intake (% of liveweight)	2.53 <sup>a</sup>	2.42 <sup>a</sup>	2.12 <sup>b</sup>	1.97 <sup>b</sup>	0.04	<0.0001	0.0359	ns
Liveweight gain (kg day <sup>-1</sup> )	1.50 <sup>a</sup>	1.56 <sup>a</sup>	0.89 <sup>b</sup>	0.82 <sup>b</sup>	0.03	<0.0001	ns	ns
Feed efficiency <sup>a</sup> (MJ kg gain <sup>-1</sup> )	101.8 <sup>a</sup>	101.7 <sup>a</sup>	124.8 <sup>b</sup>	130.5 <sup>b</sup>	2.9	<0.0001	ns	ns
Grazing period 2								
Liveweight gain (kg day <sup>-1</sup> )	–	–	0.31	0.37	0.27	–	ns	–
Indoor period 3								
Dietary intake (kg dry matter)	–	–	12.2 <sup>a</sup>	13.2 <sup>b</sup>	0.27	–	0.0239	–
Dietary intake (% of liveweight)	–	–	1.81	1.88	0.03	–	0.0910	–
Liveweight gain (kg day <sup>-1</sup> )	–	–	1.25	1.35	0.08	–	ns	–
Feed efficiency <sup>a</sup> (MJ kg gain <sup>-1</sup> )	–	–	116.1	117.7	5.1	–	ns	–
From weaning to slaughter								
Liveweight gain (kg day <sup>-1</sup> )	0.99 <sup>a</sup>	1.04 <sup>b</sup>	0.80 <sup>c</sup>	0.83 <sup>c</sup>	0.01	<0.0001	0.0153	ns
Slaughter								
Carcass weight (kg)	322 <sup>c</sup>	348 <sup>b</sup>	352 <sup>ab</sup>	372 <sup>a</sup>	4.6	0.0011	0.0084	ns
Conformation <sup>a</sup>	6.7 <sup>a</sup>	7.3 <sup>b</sup>	6.6 <sup>a</sup>	7.1 <sup>ab</sup>	0.12	ns	0.0055	ns
Fatness <sup>b</sup>	10.4 <sup>a</sup>	10.1 <sup>a</sup>	9.8 <sup>a</sup>	8.6 <sup>b</sup>	0.15	0.0005	0.0020	0.0401
Marbling <sup>c</sup>	3.3 <sup>a</sup>	2.0 <sup>bc</sup>	2.7 <sup>ab</sup>	1.5 <sup>c</sup>	0.18	0.0561	0.0001	ns
Cutting up								
Retail cuts <sup>d</sup> (% of hindquarter)	35.3 <sup>c</sup>	37.5 <sup>b</sup>	36.3 <sup>bc</sup>	40.3 <sup>a</sup>	0.29	0.0006	<0.0001	0.0458
Trim fat (% of hindquarter)	17.0 <sup>a</sup>	13.7 <sup>b</sup>	15.2 <sup>ab</sup>	9.6 <sup>c</sup>	0.50	0.0013	<0.0001	ns
Bone (% of hindquarter)	18.6 <sup>c</sup>	19.2 <sup>bc</sup>	19.3 <sup>b</sup>	20.2 <sup>a</sup>	0.16	0.0033	0.0076	ns

Least square mean, pooled standard error of the mean (SE), and significance of the main effects of sire breed, production system and their interaction. Effects of dam breed not reported in the table.

<sup>a</sup> EUROP system: 6=0+, 7=R-, 8=R.

<sup>b</sup> EUROP system: 8=3, 9=3+, 10=4-.

<sup>c</sup> Visually determined in *Musculus longissimus dorsi* between the 10<sup>th</sup> and 11<sup>th</sup> ribs on a scale 1=lean and 5=well-marbled.

<sup>d</sup> High-value retail cuts; strip loin, fillet, topside, outside round, eye of round, top rump and rump steak.

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# Potential of grass silage as a source of nutrients in poultry production

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## Abstract

Feed costs constitute over 60% of total expenses in organic layer poultry production, with feed protein supply being a significant concern. Alfalfa-based dehydrated silage pellets are mainly diets composed of leaves (ABSP). This is a non-conventional protein source that could enhance profits by reducing feed costs and ensuring consistent availability. This experiment studied the effects on the performances of Novogen Brown light layers of a commercial control diet replaced with 10% ABSP. After a 21-day trial, the new diet (ABSP) had improved the laying rate, yolk colour of eggs, feed conversion rate,  $\omega-3$  (PUFAs) and  $\omega-6/\omega-3$  ratio ( $P<0.05$ ) while the body weight and egg weight were degraded with the substitution of the ABSP in the diet ( $P>0.05$ ). Laying rate showed a tendency to increase ( $P=0.06$ ). These findings suggest that ABSP can replace at least 10% of the feed in organic layer diets without compromising production parameters negatively.

**Keywords:** alfalfa, silage, pellet, soybean, organic layer

## Introduction

The growing demand for animal protein-based products by the human population is increasing the competition between humans and livestock for food resources, and it is necessary to find other feed resources for animals. The use of good-quality forages for organic farming could be a partial and sustainable source of nutrients in organic layer hen farming and could represent an option to be used as poultry feeds to overcome the problem of high cost of soybean meal and therefore contributing to reducing the costs of production (Laudadio *et al.*, 2014; Tufarelli *et al.*, 2018). Alfalfa-based dehydrated silage pellets (ABSP) are a non-conventional protein resource consistently available and that could address this challenge. The objective of the present study was to replace part of a commercial diet with ABSP as an alternative ingredient and source nutrients in the feed of organic laying hens. We hypothesized that a moderate substitution of a commercial organic diet of layers with ABSP will not negatively affect the performance and egg parameters of laying hens. Although grasses and legumes already have been used as feed in organic farming, there is limited information on the effects of silage forage on the performance of laying hens raised in organic layer system.

## Materials and methods

The trial was conducted with forty organic Novogen Brown Light at the Center for Agronomic Technologies (CTA) of Strée-Modave (Belgium). Layer hens were divided into two groups, and 4 sub-groups each, according to a fully randomized block design. The treatment diet consisted of a 10% substitution of commercial organic feed with alfalfa-based dehydrated material and was given to the Experimental group. The control diet consisted of the same organic commercial feed and was distributed to the Control group. Dehydrated silage pellet was produced from unwrapped bale forage which was manually sorted in such a way to remove the fibrous fractions and the alfalfa-based dehydrated silage fraction was mainly made of leaves which were then ventilated at 50°C in an oven for one day.

The experimental house consisted of eight boxes (4 m long, 1.25 wide and 2.8 high), arranged by 4 and separated by a corridor measuring 4 m by 1.90 m. The air inside the building was common to all the batches, and the lighting system provided 5 to 10 lumen at the hen level with a photoperiod of 16 h light for the duration of the trial. On day 0 of the experiment, over a period of 4 weeks, egg weight was measured daily with an electronic scale (precision 0.01 g), and laying rate was measured as the ratio of daily laying rate percentage. Feed intake was measured in accounting the differences between the distributed and refusals feeds in each week on each sub-group of 5 hens. Each week, the weight gain of each hen was measured to calculate the average weekly weight gain of each batch. On the days 7 and 21, egg physico-chemical parameters were measured according to the protocol described by Moula *et al.* (2010), after random selection of 3 eggs per box. The statistical analysis was carried out using a general linear model (Myers *et al.*, 2012)  $Y_{ij} = \mu + \alpha_i + \beta_j + \epsilon_{ij}$  where  $Y_{ij}$ =studied variable,  $\mu$ =overall mean  $\alpha_i=i^{\text{th}}$  treatment effect ( $i=1$  to 2),  $\beta_j=j^{\text{th}}$  replication effect ( $j=1$  to 4),  $\epsilon_{ij}$ = residual error.

## Results and discussion

The alfalfa-based silage pellet composition was 87% dry matter, 24.2% crude protein, 4.9% fat, 14% ash, 20% crude fibre; and, 0.80%, 0.49%, 0.06%, 36.9% and 2872 kcal kg<sup>-1</sup> of calcium, phosphorous, nitrogen-free extract and metabolizable energy, respectively. Both groups experienced weight gain during the experimental period, but the control group exhibited significantly higher weight and average daily gain (ADG) values than the treatment group ( $P<0.001$ ). The ABSP degraded the egg weight and ADG ( $P<0.001$ ). There were notable distinctions between the two groups in terms of feed intake, and feed conversion ratio: feed intake was lower and feed conversion ratio higher in Experimental group ( $P<0.05$ ; Table 1).

The incorporation of ABSP into the laying hens' diet tended to increase the laying rate, a reduction in feed intake and average daily gain as well as a slight reduction in egg weight. The fermented-state of the incorporated forage may have contributed to the animals' ability to tolerate dietary fibres, or the presence of bacteria (e.g., *Lactobacillus* spp.) following forage fermentation may have benefitted the animals and could have helped to redirect nutrients towards egg production. The laying rate is higher than that reported by Englmaierová *et al.*, (2019), where they found a drop in laying rate using a 4% alfalfa rate in laying hen feed. A higher laying rate could be attributed to phytoestrogenic ingredients present in

Table 1. Performances of production and physico-chemical parameters on eggs of laying hens receiving a diet with a substitution of 10% on ABSP

Parameter	Control	Experimental	SEM	P value
Initial weight (g)	1823	1749	26.6	0.05
Final weight (g)	1922	1776	30.7	0.002
Weight gain (g)	99	27	12.7	0.001
ADG (g day <sup>-1</sup> )	4.8	1.3	0.6	0.001
Egg weight (g)	57.9	55.8	0.37	0.001
Feed Intake (g)	147.3	129.6	46.8	0.001
FCR	2.89	2.51	0.044	0.001
Laying rate (%)	90.3	93.3	1.05	0.06
Yolk colour	7.7	10.4	0.17	0.001
$\omega$ -3 (PUFA)	3.3	4.6	0.25	0.001
$\omega$ -6/( $\omega$ -3) ratio	6.95	5.34	0.4	0.001

ADG=Average Daily Gain; FCR=Feed Conversion Ratio;  $\omega$ -3=Omega 3; PUFA=Polyunsaturated fatty acid;  $\omega$ -6=Omega 6

forage, such as apigenins, luteolins, and coumestrol (Seguin *et al.*, 2004; Tucak *et al.*, 2018), which possess a chemical structure similar to phytoestrogens. These compounds exhibit oestrogenic and antioestrogenic effects, potentially increasing the laying rate in hens. A highly significant difference in yolk colour was observed between the two groups. The results suggest that ABSP enhanced egg quality through increased PUFA  $\omega-3$  and  $\omega-6/\omega-3$  ratio in eggs from the treatment group ( $P < 0.01$ ).

## Conclusion

The results demonstrate that ABSP in the diet can positively influence the production performance of organic hens, such as laying rate, feed conversion ratio, yolk colour and  $\omega-3$  and  $\omega-6/\omega-3$  ratio (PUFA). However, the incorporation of ABSP in the diet showed negative effects on egg weight and average daily gain of the hens. Alfalfa-based silage pellets could have the potential to enhance income for organic poultry farms in the future. Further research is essential to assess the optimal utilization of ABSP in formulating layer feed.

## Acknowledgements

We thank the CTA, the University of Liège and the Government of Rwanda through the World Bank's Priority Skills for Growth Program (PSG) and Ministry of Education for providing the financial support for the realization of this research.

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# Including plantain in grazing mixtures supports milk production of dairy cows in early lactation

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## Abstract

Ribwort plantain (*Plantago lanceolata* (PL)) can reduce nitrogen (N) loss from dairy cows; however, few studies have examined its effect on milk performance over an extended period. The objective was to investigate the effect of including PL in a spring pasture sward of perennial ryegrass and white clover for early lactation grazing dairy cows on milk production and composition, urine N concentration, and rumen pH. Twenty-four dairy cows were allocated to one of two pasture treatments in a randomised complete block design: perennial ryegrass-white clover (RC) and a perennial ryegrass-white clover-PL (RCP) sward containing circa 52% PL on a DM basis. The average intake of concentrate was 5.64 kg of DM cow<sup>-1</sup> day<sup>-1</sup>. The study lasted 14 weeks. Cows grazing RCP produced milk with significantly lower milk fat (3.97 vs. 4.28 g kg<sup>-1</sup>,  $P < 0.05$ ) and protein concentrations compared to RC (3.33 vs. 3.48 g kg<sup>-1</sup>,  $P < 0.05$ ). However, no difference was observed in milk yield or milk solids (fat+protein) yield between RC and RCP (29.21 vs. 30.26 kg cow<sup>-1</sup> day<sup>-1</sup>; 2.25 vs. 2.22 kg cow<sup>-1</sup> day<sup>-1</sup>,  $P > 0.05$ , respectively) or urine N concentration (0.48 vs. 0.49 g (kg N)<sup>-1</sup>,  $P = 0.81$ , respectively). Rumen fluid pH was significantly lower for RCP compared to RC (6.45 vs. 6.68;  $P < 0.05$ ). Plantain inclusion maintained milk yield; however, it did not reduce urinary N concentration of dairy cows in early lactation.

**Keywords:** plantain, nitrogen, dairy cow, pasture, early lactation

## Introduction

Pasture-based milk production systems in Ireland comprise a diet of grazed perennial ryegrass (*Lolium perenne* (PRG)) with concentrate supplementation. A low level of nitrogen (N) recovery in milk occurs when grass accounts for over 70% of the cows' dry matter intake (Whelan *et al.*, 2012). Increasing concentrate supplementation levels with grazed PRG pasture reduces N excretion in lactating dairy cows (Reid *et al.*, 2015) but incurs added costs (Ramsbottom *et al.*, 2015). Therefore, identifying grazing mixtures that simultaneously support milk production and reduce N loss by the dairy cow is important. Ribwort plantain (*Plantago lanceolata* (PL)) grazed as a monoculture or with PRG and white clover (*Trifolium repens* (WC)) has reduced urinary N excretion, without impacting milk production in early lactation (EL) over 10 days (Box *et al.*, 2017). Further investigation into the effect on milk production throughout EL when the dairy cows' energy requirements is at their peak are required. Thus, the objective of this study was to investigate the effect of including PL in a PRG and WC grazing sward on milk production and composition, urine N concentration, and rumen fluid pH of dairy cows over 14 weeks in EL.

## Materials and methods

A grazing study was conducted at University College Dublin, Lyons Farm, Co. Kildare, Ireland during Spring 2023. Eighteen multiparous and six primiparous cows were blocked based on parity and assigned to one of two pasture treatments in a randomized complete block design ( $n = 12$ ). The treatments were a PRG-WC (RC) and PRG-WC-PL (RCP) pasture sward. Both pasture swards were proportionally sown in April and August 2022 with the varieties AberGain and AberChoice selected for PRG; Buddy and Iona for WC; and Tonic for PL. Both treatment groups were balanced for previous 305-day milk production and composition data, calving date, and body weight. Cows were milked twice daily at



07:00 and 16:00 hours with their concentrate allocation split evenly between milkings, averaging 5.64 kg of DM cow<sup>-1</sup> day<sup>-1</sup>. Milk yield measurements and composite milk samples were collected from one successive evening and morning milking each week. Rumen fluid samples for pH analysis were obtained by oesophageal sampling on the evenings of day 3 in weeks 4, 7, 10 and 13 of the study. Urine samples were collected at each a.m. and p.m. milking of days 1, 3 and 5 in week 10.

Cows grazed in separate paddocks and were offered a fresh pasture allocation (15 kg of DM cow<sup>-1</sup> above 4 cm) every 24 hours. To determine pre-grazing herbage mass in the paddock, three 0.25 m<sup>2</sup> quadrats were cut to 4 cm and weighed. Each cut was pooled together, and duplicate sub-samples were taken for dry matter (DM) determination, chemical analysis, and botanical composition. Effects of treatment, week, parity, days in milk, treatment × week as fixed effects, and cow as random effect were included in the model using the MIXED procedure of SAS® Studio (edition 3.81).

## Results

The mean pre-grazing herbage mass was 1965 ± 700 kg DM ha<sup>-1</sup> for RC and 1921±544 kg DM ha<sup>-1</sup> for RCP. The mean post-grazing herbage mass (cut above 4 cm) for RC and RCP was 265±139 and 264±125 kg DM<sup>-1</sup> ha, respectively. The RCP sward was predominantly PL (52%) and PRG (28%), whilst the RC sward was dominated by PRG (87%; Table 1).

Milk yield and milk solids (fat+protein) yield (29.21 vs. 30.26 kg cow<sup>-1</sup> day<sup>-1</sup>; 2.25 vs. 2.22 kg cow<sup>-1</sup> day<sup>-1</sup>, *P* > 0.05, RC and RCP, respectively) did not differ in response to dietary treatment (Table 2). Cows grazing RCP produced significantly lower milk fat and protein (3.97 and 3.33 g kg<sup>-1</sup>) concentrations compared to RC (4.28 and 3.48 g kg<sup>-1</sup>, *P*<0.05); however, no difference was observed in milk lactose concentration (*P*=0.39). There was no difference in urine N concentration between RC and RCP (0.48 vs. 0.49 g kg<sup>-1</sup>, *P*=0.81). Cows grazing RCP had significantly lower rumen pH compared to RC (6.45 vs. 6.68; *P*<0.05, respectively). Significantly lower DM content and higher ash content were measured in RCP compared to RC pasture (149 vs. 186 g kg<sup>-1</sup>; 107 vs. 84 (kg DM)<sup>-1</sup>; *P*<0.001, respectively).

Table 1. Botanical composition (as %) and chemical composition (g kg<sup>-1</sup> DM) of feeds offered.

Item	Concentrate	Pasture treatment		SEM	<i>P</i> -value
		RC <sup>1</sup>	RCP <sup>2</sup>		
Proportion on a DM basis (%)					
Perennial ryegrass		87	38	3.752	<0.0001
White clover		3	2	0.746	0.52
Plantain stem		–	6	1.738	–
Plantain leaf		–	46	5.113	–
Unsown		7	4	1.274	0.13
Dead		3	4	1.739	0.32
Chemical composition					
Dry matter (g kg <sup>-1</sup> )	889	187	149	4.447	<0.001
Composition DM (g (kg DM <sup>-1</sup> ))					
Ash	75	84	108	3.123	<0.001
Crude protein	152	178	190	7.788	0.28
Neutral detergent fibre	244	439	400	8.279	0.003
Acid detergent fibre	104	211	19	5.237	0.09

<sup>1</sup>Perennial ryegrass and white clover.

<sup>2</sup>Perennial ryegrass, white clover and plantain.

The RCP pasture had lower neutral detergent fibre (NDF) content compared to RC (400 vs. 439 g kg<sup>-1</sup> DM,  $P=0.003$ , respectively). No differences were observed in crude protein or acid detergent fibre concentrations between pasture treatments ( $P > 0.05$ ).

Table 2. The effect of treatment on milk production and composition, urine N concentration and rumen pH.

Item	Pasture treatment		SEM	P-value
	RC <sup>1</sup>	RCP <sup>2</sup>		
Milk yield (kg cow <sup>-1</sup> d <sup>-1</sup> )	29.21	30.26	1.141	0.42
Milk solids (kg cow <sup>-1</sup> d <sup>-1</sup> )	2.25	2.22	0.082	0.71
Fat (g (kg milk <sup>-1</sup> ))	4.28	3.97	0.108	0.0499
Protein, (g (kg milk <sup>-1</sup> ))	3.48	3.33	0.042	0.02
Lactose, (g (kg milk <sup>-1</sup> ))	4.70	4.67	0.027	0.39
Urine N, g kg <sup>-1</sup>	0.48	0.49	0.033	0.81
Rumen pH	6.68	6.45	0.056	<0.01

<sup>1</sup>Perennial ryegrass and white clover.

<sup>2</sup>Perennial ryegrass, white clover and plantain.

## Discussion

This study found no difference in milk yield or milk solids yield, consistent with shorter-term studies in EL with dairy cows grazing PL with PRG and WC (Box *et al.*, 2017) or as a monoculture (Navarrete *et al.*, 2022) compared to a PRG and WC mixture. The lower NDF content in RCP pasture may explain the lower rumen pH and subsequent lower milk fat concentration of the RCP dietary treatment (Kharitonov, 2022). Similar to Navarrete *et al.* (2022), no difference in urinary N concentration was observed. The high amount of concentrate in the cows' diet in this study may be an explanation for the lack of effect.

## Conclusion

Including ribwort PL (53% on a DM basis) in a PRG and WC pasture sward did not show any difference in milk yield or urine N concentration in EL. Although rumen fluid pH was within an optimal range, it was reduced with PL inclusion. Therefore care is needed to prevent any further decrease in rumen pH, which may reduce milk fat concentration and rumen health.

## Acknowledgements

This project was funded by the Department of Agriculture Food and the Marine's Competitive Research Funding Programme (2021R482).

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# Grass-based feeding strategies for organic growing-finishing pigs

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## Abstract

Freshly cut grass-clover (GC) as roughage and biorefined GC protein has been proposed to mitigate the negative climate impact of European pig production. This study aimed to document the effect on productivity of GC-based feeding strategies for organic growing-finishing pigs from 30-110 kg during temperate summer and winter conditions. 135 piglets were randomly assigned to diets with combinations of concentrate (soy press cake or biorefined GC protein) and roughage (freshly cut GC, GC silage or GC pulp silage). Substituting soy with biorefined GC did not impact feed intake, daily gain, or feed conversion ratio (FCR). A higher meat percentage was obtained during the summer trial compared to winter (62.8% vs 61.9%,  $P=0.02$ ). Lysine content in the GC concentrate could be reduced by 10% without negative effects on animal performance. The FCR was similar across all diets, but higher during the winter trial (2.87 vs 2.48 kg feed (kg gain)<sup>-1</sup>,  $P=0.01$ ). Freshly cut GC provided more crude protein (CP) to the pigs than GC silage and pulp (2.45, 1.88, 1.23 g day<sup>-1</sup>, respectively  $P<0.01$ ). The GC-based concentrate can maintain the productivity of organic growing-finishing pigs during summer and winter conditions and may thus be a sustainable alternative to soy.

**Keywords:** biorefining, grass-clover, organic pigs, alternative feeding, feed utilisation

## Introduction

Reports indicate that organic pork production is associated with a 7–22% higher GHG emission than conventional production. The development of alternative feeding strategies is of utmost importance to make organic pork production more environmentally competitive. One potential solution is to increase the proportion of grass-clover (GC) in the feed uptake (Halberg *et al.*, 2010). Under temperate conditions, GC is typically used as roughage in the form of silage and can contribute significantly to the amino acid (AA) supply of organic sows (Eskildsen *et al.*, 2020a). Biorefined GC protein has been suggested as a high-quality alternative to imported feed protein (van der Heide *et al.*, 2021). Exploring feeding strategies based on GC-based concentrate and roughage could therefore be a potential mitigation strategy for pork production; however, it is essential to ensure high feed utilization. This study aimed to investigate the effect of GC-based feeding strategies on the productivity of organic growing-finishing pigs during summer and winter conditions and to calculate the protein contribution from GC-based roughages. Moreover, as the sustainability of organic pig production is challenged by excess protein feeding to secure AA supply, the aim was to evaluate the effect of reducing the concentrate protein level.

## Materials and methods

The forage used for GC roughages and biorefined concentrate protein was composed of perennial ryegrass (*Lolium perenne* L., 50% variety ‘Saqui’, 15% ‘Thegn’), 25% red fescue (*Festuca rubra rubra* ‘Gondolin’), and 10% white clover (*Trifolium repens* L., ‘Rivendel’) (DLF, Denmark). In autumn 2021, GC was cut, wilted for two days, chopped, and ensiled. During May-June 2022 and August-October 2022, GC was harvested at 7 cm stubble height (Maksigrasser, GT 140, Future Grass Technolog) after 5-6 weeks of regrowth and pressed into a pulp, which was subsequently ensiled, and a green juice. Soluble protein was extracted from the green juice using heat precipitation and dried into GC protein batches (Stødkilde *et al.*, 2021). During July-October 2022 freshly cut GC was brought directly from the field

to the pig barn. Seventy-five and 60 weaned piglets (Duroc×(Landrace×Yorkshire); 50% gilts and 50% barrows, ten weeks of age) were randomly assigned to combinations of concentrates and roughages in a summer (July-October 2022) and a winter (January-April 2023) trial, respectively (Table 1). For both summer and winter trials, three pens with five pigs each were allocated to a feed combination.

The pigs were housed in indoor pens with an outdoor run, mimicking organic production conditions, and were fed *ad libitum* with concentrate until reaching a maximum of 3 kg pig<sup>-1</sup> day<sup>-1</sup>. Any feed residues were registered at pen level. Roughage was provided *ad libitum*. The pigs were weighed individually at the initiation of the trials and before slaughter, when blood samples were also taken from the jugular vein of representative pigs (3–7 pigs per diet) and analysed for creatinine and urea (Eskildsen *et al.*, 2020b). Meat percentage was determined at slaughter. Data were analysed using a one-way linear model with the Proc Glimmix procedure (SAS version 9.4). For data related to feed intake, the pen was the experimental unit; for weight, the individual pig was the experimental unit.

## Results and Discussion

Substituting soy with biorefined GC protein did not affect pig performance (Table 2) thus confirming previous studies (Stødkilde *et al.*, 2021, 2023). A higher concentrate intake was observed during the winter trial resulting in a higher FCR ( $P=0.01$ ) compared to the summer trial ( $P=0.01$ ) which may be attributed to a higher energy need for thermoregulation. The meat percentage was higher during the summer trial ( $P=0.02$ ) confirming the additional energy need during winter. No differences in plasma urea/creatinine ratio were observed (data not shown), indicating similar utilization of the feed CP. Importantly, it was found that lysine levels could be reduced by 10% when feeding biorefined GC protein in combination with freshly cut GC without compromising animal performance or meat percentage. As feed production accounts for most of the negative impact of pork production (Stødkilde *et al.*, 2023) and feed prices increase, the results will have environmental, economic and welfare benefits for the production. Roughage is expected to provide nutrients to the pigs which are currently not accounted for during feed formulations (Eskildsen *et al.*, 2020a). In the present study, the average daily roughage intake was 252–470 g pig<sup>-1</sup> with the intake of freshly cut GC being higher than GC silage and pulp ( $P<0.01$ ). A tendency of higher intake during winter ( $P=0.06$ ) was seen. Roughage provided 7.69–15.3 g CP pig<sup>-1</sup> day<sup>-1</sup> with fresh GC generally contributing the most CP ( $P>0.01$ ). Freshly cut GC supplied the animals with additional CP enabling a reduction of at least 10% CP in concentrate during summer. However, as GC composition and quality fluctuate during the growth season, the CP contribution from freshly cut GC will also vary and may necessitate a corresponding variation in concentrate CP. The roughages provided more CP in the winter trial compared to the summer trial ( $P<0.01$ ) due to a higher CP content in the winter GC silage. During winter, the allocation of GC pulp enables efficient utilization of the residual fraction from the production of biorefined GC protein. During the growth season, the focus can thus be on protein production without compromising the winter supply of the required roughage, hereby enhancing the circularity of GC-based feeding strategies.

Table 1. Grass-clover (GC)-based feeding strategies.

Concentrate main protein source (total lysine content)	Roughage		
	Freshly cut GC	GC silage	GC pulp
Soy press cake (70 g (kg Lys) <sup>-1</sup> )	Summer	Summer+Winter	Winter
Biorefined GC protein (70 g (kg Lys) <sup>-1</sup> )	Summer	Summer+Winter	Winter
Biorefined GC protein (63 g (kg Lys) <sup>-1</sup> )	Summer	–	–

Table 2. Performance of pigs on grass-clover (GC)-based feeding strategies.

	Concentrate			Roughage			Season	
	GC (70 g (kg Lys) <sup>-1</sup> )	GC (63 g (kg Lys) <sup>-1</sup> )	Soy (70 g (kg Lys) <sup>-1</sup> )	Freshly cut GC	Silage	Pulp	Summer	Winter
Daily concentrate intake (kg pig <sup>-1</sup> )	2.40	2.31	2.35	2.27	2.38	2.50	2.29 <sup>b</sup>	2.47 <sup>a</sup>
Daily gain (g pig <sup>-1</sup> )	932	937	944	932	934	952	930	948
FCR (kg feed (kg gain) <sup>-1</sup> )	2.81	2.39	2.56	2.48	2.66	2.89	2.48 <sup>b</sup>	2.87 <sup>a</sup>
Meat (%)	62.4	63.2	62.1	63.1	62.0	62.0	62.8 <sup>a</sup>	61.9 <sup>b</sup>
Daily roughage intake (g pig <sup>-1</sup> )	318	428	317	470 <sup>a</sup>	252 <sup>b</sup>	276 <sup>b</sup>	365	287
Daily roughage CP intake (g)	11.9	13.9	11.7	15.3 <sup>a</sup>	11.8 <sup>b</sup>	7.69 <sup>c</sup>	11.3 <sup>b</sup>	12.6 <sup>a</sup>

Values are averages and should be compared only within 'concentrate', 'roughage' or 'season'. Any statistically significant differences (effect of concentrate, roughage, or season) are indicated with superscripts and *p*-values presented in the main text. FCR, feed conversion ratio; CP, crude protein.

## Conclusions

The study presents evidence that GC can be successfully included in the diets for organic growing-finishing pigs, during summer and winter, in the form of biorefined protein in the concentrate and as different types of roughage, thereby enabling alternatives to the current feeding strategy. At the same time, lysine content can be reduced when fed in combination with fresh GC. Biorefined protein from GC may thus be sustainable alternative to soy.

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# Effect of daily grazing time of dairy cows. I: Milk yield and composition

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## Abstract

A feeding trial was conducted in Galicia (NW Spain) to evaluate the effect of variable spring-grazing time on dairy cow's productivity and milk composition. Five groups of six cows each were randomly assigned to five treatments (daily hours of access to pasture) of 0, 4, 9, 15 and 22 h. While in the barn, the cows had free-access to a total mixed ration (TMR) composed of grass silage, maize silage and concentrate. Average daily dry matter intake (DMI), milk yield, production efficiency and body weight (BW) recovery of cows decreased significantly with increasing grazing time. The highest values of milk fat, protein and lactose were observed in the confined, TMR-fed group without access to pasture, and these values decreased significantly as grazing time increased. The results obtained corroborate the bibliography that points out the limitations of grass as the only food to achieve high milk production, placing the production ceiling between 25 to 30 kg of milk day<sup>-1</sup>, which is related to the deficit in the energy contribution in systems based on the consumption of fresh grass.

**Keywords:** grazing time, milk yield, milk composition

## Introduction

In the last decades, milk production in developed countries has evolved towards more intensive feeding models, with larger farms and more dependence on purchased external inputs. As the production system intensifies, the cows have reduced or no access to pasture, being permanently stabled and fed with complete rations based on silage and concentrates. Under these TMR feeding conditions, milk production per cow clearly exceeds what is possible to obtain in grazing systems (Gulati *et al.*, 2018). However, grazed pasture is the cheapest source of nutrients for dairy cows and should form the basis of profitable low inputs animal production systems in Europe (Peyraud and Delagarde, 2013), produces high quality milk (O'Callaghan *et al.*, 2016) and grasslands with their multifunctional roles provides a good basis for developing more sustainable production systems in the long term (Peyraud *et al.*, 2010). The objective of this study was to evaluate the effect of variable spring-grazing time on dairy cow's productivity and milk composition.

## Materials and methods

The experiment was carried out at the Centro de Investigaciones Agrarias de Mabegondo (CIAM) research station farm (Galicia, NW Spain, 43°15' N, 8°18' W, 100 m above sea level) from 15 March 2021 to 20 July 2021. Five treatments, consisting of different daily time of access to pasture: 0 (T0), 4 (T4), 9 (T9), 15 (T15) and 22 h (T22). Grazing took place on a temporary perennial ryegrass-white clover sward and the trial had a duration of 16 weeks, with a preliminary adaptation period of 3 weeks. Cows were managed with electric fencing to enable strip grazing of the paddocks with fresh grass after each milking, with an *ad libitum* forage allocation (an average of 20 kg cow<sup>-1</sup> day<sup>-1</sup>). Following a completely randomized design with six replicates (cows) per each treatment, a total of 30 lactating Holstein Friesian dairy cows were used in the experiment and grouped by calving date (27.3±7.3 days), parity (2 primiparous group<sup>-1</sup>), milk production (35.4±7.4 kg), BW (610.9±72.2 kg) and body condition score

(BCS) ( $2.6 \pm 0.27$ ). While in the barn, the cows had free access to a TMR composed of maize silage (39.6%), grass silage (22.9%) and a commercial concentrate (37.5%), unique for all treatments except for T22, which had pasture as a sole feed. Cows' milk yield was recorded daily and individual milk samples were taken every two weeks corresponding to a consecutive morning and evening milking. Daily TMR intake per cow was recorded automatically using electronic feeders and cows were weighed every three weeks. Pasture intake of cows was estimated by difference between total intake calculated by an equation of NRC (2001) for lactating Holstein cows based on milk yield, milk fat, body weight and week of lactation and the recorded TMR intake. Milk samples were immediately stored at 4°C and transported to the official regional interprofessional milk laboratory (Laboratório Interprofesional Galego de Análise do Leite, LIGAL) where they were subjected to routine FT MIR analysis (milk composition) using a MilkoScan™ FT6000 (Foss Electric A/S, Hillerød, Denmark). Data were analysed using PROC GLM from SAS package (SAS Institute, 2009).

## Results and discussion

The average efficiency values (Table 1), expressed as milk yield  $\text{DMI}^{-1}$ , were positively related to the consumption of a TMR in the barn, with values of 1.44, 1.40, 1.36, 1.30 and 1.23  $\text{kg milk kg DMI}^{-1}$  for T0, T4, T9, T15 and T22, respectively, all values being significantly different from each other. Similarly, corrected milk yield varied between 41.0  $\text{kg cow}^{-1} \text{day}^{-1}$  for T0 and 29.8  $\text{kg cow}^{-1} \text{day}^{-1}$  for T22, showing the limitations of grass as the only food to achieve high milk production, which is related to the limitation of energy input in grazing-based systems.

Table 1. Dry matter intake, efficiency, milk yield, milk composition and body weight.

	Treatments					P
	T0	T4	T9	T15	T22	
Dry matter intake and efficiency						
DMI ( $\text{kg cow}^{-1}$ )	26.1 <sup>a</sup>	25.3 <sup>b</sup>	24.6 <sup>b</sup>	23.4 <sup>c</sup>	22.4 <sup>d</sup>	***
Efficiency ( $\text{milk yield DMI}^{-1}$ )	1.44 <sup>a</sup>	1.40 <sup>b</sup>	1.36 <sup>c</sup>	1.30 <sup>d</sup>	1.23 <sup>e</sup>	***
Milk yield ( $\text{kg cow}^{-1} \text{day}^{-1}$ )						
Milk yield	36.5 <sup>a</sup>	35.1 <sup>ab</sup>	33.4 <sup>b</sup>	31.1 <sup>c</sup>	28.4 <sup>d</sup>	***
Corrected milk yield	41.0 <sup>a</sup>	38.4 <sup>b</sup>	36.0 <sup>c</sup>	32.6 <sup>d</sup>	29.8 <sup>e</sup>	***
Milk composition ( $\text{g kg}^{-1}$ )						
Fat	42.7 <sup>a</sup>	42.2 <sup>a</sup>	41.3 <sup>a</sup>	39.3 <sup>b</sup>	38.7 <sup>b</sup>	***
Protein	34.0 <sup>a</sup>	32.7 <sup>b</sup>	30.5 <sup>c</sup>	30.2 <sup>c</sup>	30.4 <sup>c</sup>	***
Lactose	48.7 <sup>a</sup>	48.2 <sup>b</sup>	47.8 <sup>c</sup>	47.1 <sup>c</sup>	47.8 <sup>c</sup>	***
Urea ( $\text{mg l}^{-1}$ )	318 <sup>a</sup>	295 <sup>b</sup>	256 <sup>c</sup>	244 <sup>cd</sup>	226 <sup>d</sup>	***
Body weight (kg)						
BW week 1	602	620	596	638	637	
BW week 16	663	666	636	647	647	
Body condition score						
BCS week 1	2.63	2.63	2.71	2.79	2.50	
BCS week 16	2.90	2.83	2.79	2.71	2.50	

Corrected milk yield corrected: 3.5% fat and 3.2% protein; T0: 0 hours of access to pasture; T4: 4 hours of access to pasture; T9: 9 hours of access to pasture; T15: 15 hours of access to pasture; T22: 22 hours of access to pasture; Figures affected by different letters in the same row are significantly different.



When cows are fed only grass, production per cow is in the range of 20–27 kg day<sup>-1</sup>, usually being limited by energy availability (Fulkerson and Trevaskis, 1997). The values recorded in the experiment fit with bibliographic observations, since the animals were in the first half of lactation. On the other hand, the ratio of protein to that of non-structural carbohydrates is normally too high in grasses, so an energy supplement allows optimizing the use of N in the pasture (Bargo *et al.*, 2003).

The hours of access to pasture (or, alternatively, to the TMR ration offered in the barn) significantly modified the milk composition, with the highest fat, protein and lactose values observed in the treatment fed exclusively in the barn without access to pasture, with decreasing values as the grazing time increased. The values for the extreme groups (T0 vs. T22) were 42.7 vs 38.7 g kg<sup>-1</sup> fat, 34.0 vs 30.4 g kg<sup>-1</sup> protein and 48.7 vs 47.8 g kg<sup>-1</sup> lactose. Urea content remained within normal limits, although a downward trend was observed in this value as the time of access to pasture increased, from 318 mg l<sup>-1</sup> for T0 to 226 mg l<sup>-1</sup> for T22. The values for T0 are quite high, likely because the TMR was too high in degradable protein and the value for T22 can be considered as normal without excess in degradable nitrogen. BW recovery was +60.3 kg, +45.5 kg, +39.6 kg, +9.1 kg and +10.6 kg for T0, T4, T9, T15 and T22, respectively, and the change in BCS was +0.27, +0.20, +0.08, -0.08 and no change for T0, T4, T9, T15 and T22 respectively. The results reflect the greater energy intake derived from the consumption of TMR and the higher level of activity of the animals during grazing.

## Conclusion

The results obtained corroborate the bibliography that points out the limitations of grass as the only food to achieve high milk production, placing the production ceiling between 25 to 30 kg milk day<sup>-1</sup>, which is related to the deficit in the energy contribution in systems based on the consumption of fresh grass.

## Acknowledgement

The research was funded by Axencia Galega da Calidade Alimentaria (FEADER 2020-066A project)

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# Effect of daily grazing time of dairy cows. II: Milk fatty acid profile

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## Abstract

Although it is well known that feeding cows with fresh grass increases the content of beneficial fatty acids (FA) in milk, it was considered of interest to analyse the effect of increasing grass consumption on the FA profile in cow's milk. Five groups of six cows each were randomly assigned to five treatments, consisting of a different number of daily hours of access to pasture (0, 4, 9, 15 and 22 h) during the spring. During the time that cows stayed in the barn, free-access to a total mixed ration (TMR) composed of grass silage, maize silage and concentrate was allowed. Biweekly milk samples from each cow were analysed by gas chromatography to identify and quantify the FA profile. The proportion of saturated FA (g (kg total FA)<sup>-1</sup>) decreased linearly from 734 for the 0 h treatment to 637 for the 22 h treatment, where the cows did not consume the TMR mixture. The contents of the milk samples in certain unsaturated and polyunsaturated FAs considered bioactive increased significantly with grazing time. The results show how the increase in fresh grass intake is related to a FA profile considered more favourable from the point of view of cow's milk consumption in the human diet.

**Keywords:** grazing time, fatty acids

## Introduction

Dairy products are an important source for many vital nutrients including high quality protein, energy, and many essential minerals and vitamins, being included in recommendations for a healthy, well-balanced diet by public health organisations around the world (Rice *et al.*, 2013). Additionally, new indicators related to ethical aspects such as animal welfare and environmental impact (Luykx and van Ruth, 2008) are gaining public concern. In general, it is considered that outdoor management systems with grazing animals are perceived by consumers as more respectful of animals and the environment (Weinrich *et al.*, 2014). For example, Burow *et al.* (2013) mention better animal welfare of animals that spend a greater number of hours on pasture, while Conant *et al.* (2013) point out the role of grasslands in carbon sequestration. In addition, it is well known that higher proportions of fresh grass or grass silage in diet improved the milk FA profile by increasing the levels of polyunsaturated FA (PUFA) regarded as having a positive effect on human health, making this product more attractive as a part of so-called healthy diets (e.g., Dewhurst *et al.*, 2006). The objective of this experiment was to analyse the effect of increasing fresh grass consumption on the FA profile in cow's milk.

## Materials and methods

The experiment was carried out at the Centro de Investigaciones Agrarias de Mabegondo (CIAM) research station farm (Galicia, NW Spain, 43°15'N, 8°18'W, 100 m above sea level) during spring, from mid-March 2021 to mid-July 2021. Following a completely randomized design, 30 Holstein Friesian lactating cows were distributed in five homogeneous groups regarding calving date (27.3±7.3 days), parity (2 per primiparous group), milk production (35.4±7.4 kg), body weight (610.9±72.2 kg) and body condition score (2.6±0.27). Groups were randomly allocated to one of five treatments (daily time of access to a ryegrass-white clover pasture): 0 (T0), 4 (T4), 9 (T9), 15 (T15) and 22 h (T22). The trial duration was of 16 weeks, with a preliminary adaptation period of 3 weeks. Cows were managed

with electric fencing to enable strip grazing of the paddocks with fresh grass after each milking with an *ad libitum* forage allocation (an average of 20 kg cow<sup>-1</sup> day<sup>-1</sup>). While in the barn, the cows had free access to a TMR composed of maize silage (39.6%), grass silage (22.9%) and a commercial concentrate (37.5%), unique for all treatments except T22, without access to the barn. Cows' milk was sampled every two weeks by taking individual milk samples from a consecutive morning and evening milking. Samples were immediately stored at 4°C and transported to the official regional interprofessional milk laboratory (LIGAL) where they were immediately frozen (-20°C) until posterior FA analysis. A composite sample of the two milkings per cow of every sampling day was analysed by gas chromatography (GC-FID) following the LIGAL standard procedures ISO 14156/IDF 172 and ISO 15885/IDF 184. Data were analysed using the PROC GLM SAS package (SAS Institute, 2009).

## Results and discussion

The diet consumed by cows markedly affects the FA profile of milk, corroborating the results commonly found in other studies. The results of the experiment (Table 1) clearly show that a longer grazing daily time is related to a more favourable milk FA profile, with the differences between treatments being statistically significant for all the variables considered.

The quantity of saturated FA (g (kg total FA)<sup>-1</sup>) decreased by almost 100 g kg<sup>-1</sup>, from 734 g kg<sup>-1</sup> for T0, where the animals did not graze, to 637 g kg<sup>-1</sup> for T20, where the animals had continued access to pasture, reducing almost linearly for successive increases in grazing time. Instead, the proportion of unsaturated FA clearly increased with grazing time, with values of 302 g kg<sup>-1</sup> and 43.9 g kg<sup>-1</sup> for the monounsaturated FA (MUFA) and PUFA, respectively, in the milk of the T22 (continuous access to pasture) which were reduced to values of 224 g kg<sup>-1</sup> and 28.2 g kg<sup>-1</sup> for the MUFA and PUFA of the T0 (permanently stabled).

Table 1. Milk fatty acid profile ( $n=48$  observations per treatment).

	Treatments					P
	T0	T4	T9	T15	T22	
Main FA groups (g kg <sup>-1</sup> total FA)						
Saturated	734 <sup>a</sup>	716 <sup>b</sup>	689 <sup>c</sup>	653 <sup>d</sup>	637 <sup>e</sup>	***
Monounsaturated	224 <sup>d</sup>	238 <sup>c</sup>	262 <sup>b</sup>	294 <sup>a</sup>	302 <sup>a</sup>	***
Polyunsaturated	28.2 <sup>d</sup>	32.0 <sup>c</sup>	34.6 <sup>c</sup>	37.5 <sup>b</sup>	43.9 <sup>a</sup>	***
Omega-6 total FA	16.7 <sup>a</sup>	15.6 <sup>a</sup>	15.5 <sup>a</sup>	12.3 <sup>b</sup>	10.7 <sup>c</sup>	***
Omega-3 total FA	5.0 <sup>e</sup>	6.7 <sup>d</sup>	7.6 <sup>c</sup>	8.5 <sup>b</sup>	10.9 <sup>a</sup>	***
Conjugated Linoleic Acid (CLA) total	6.6 <sup>e</sup>	9.8 <sup>d</sup>	11.5 <sup>c</sup>	16.8 <sup>b</sup>	22.3 <sup>a</sup>	***
Individual FA (g kg <sup>-1</sup> total FA)						
Trans-vaccenic (C18:1t11)	9.2 <sup>e</sup>	15.5 <sup>d</sup>	20.3 <sup>c</sup>	26.9 <sup>b</sup>	38.3 <sup>a</sup>	***
Linoleic (C18:2c9c12 n6)	13.9 <sup>a</sup>	12.7 <sup>ab</sup>	12.4 <sup>b</sup>	9.4 <sup>c</sup>	7.3 <sup>d</sup>	***
Alpha-linolenic (C18:3c9c12c15 n3)	2.9 <sup>e</sup>	4.6 <sup>d</sup>	5.5 <sup>c</sup>	6.3 <sup>b</sup>	8.9 <sup>a</sup>	***
Conjugated Linoleic Acid (CLA) c9t11	4.8 <sup>e</sup>	7.8 <sup>d</sup>	9.3 <sup>c</sup>	13.9 <sup>b</sup>	18.0 <sup>a</sup>	***
FA ratios						
Omega6:Omega3	3.4 <sup>a</sup>	2.3 <sup>b</sup>	2.1 <sup>c</sup>	1.5 <sup>d</sup>	1.1 <sup>e</sup>	***
(t11:t10) C18:1	3.3 <sup>d</sup>	5.2 <sup>c</sup>	6.1 <sup>c</sup>	10.5 <sup>b</sup>	15.1 <sup>a</sup>	***

T0, 0 hours of access to pasture; T4, 4 hours of access to pasture; T9, 9 hours of access to pasture; T15, 15 hours of access to pasture; T22, 22 hours of access to pasture. Values with different letters in the same row are significantly different.

When considering the profile of individual milk FA, the beneficial effect of increasing grazing time on the milk FA composition was clearly demonstrated. Milk from cows that grazed for a longer period of the day showed higher values of trans-vaccenic (TVA), alpha-linolenic (ALA) and CLA compared to the groups of animals not fed with fresh grass, while, conversely, the linoleic (LA) value was lower in cows that grazed longer. The ratio Omega6:Omega3 decreased as the number of hours on the pasture increased, which is related to a positive effect on human health, as well as a higher (*t11:t10*) C18:1 ratio, which ranges in this experiment between 3.3 for T0 and 15.1 for T22, increasing as the hours of grazing increase. These effects showed a linear trend and, on average, for each hour of access to pasture milk concentration of TVA, CLA and ALA increased by 1.27, 0.59 and 0.25 g (100 g total FA)<sup>-1</sup> (TFA) whilst LA diminished by 0.30 g (100 g TFA)<sup>-1</sup>. In a similar fashion, the ratio *t11:t10* C18:1 was also reduced by 0.53 per additional hour of access to pasture and the Omega6:Omega3 ratio was reduced by 0.09 g (100 g TFA)<sup>-1</sup>.

## Conclusion

The results show that increasing the proportion of fresh grass in the diet of dairy cows causes the increase of the concentration in milk of bioactive FA and that pasture feeding is related to a FA profile considered more favourable from the point of view of cow's milk consumption in the human diet.

## Acknowledgement

The research was funded by Axencia Galega da Calidade Alimentaria (FEADER 2020-066A project)

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# Grasslands for a greater protein autonomy: lessons from Cap Protéines programme

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## Abstract

French ruminant farms have a high level of global protein autonomy (approximately 75%), mainly because of a significant contribution of fodder and grass. Protein autonomy increases with the level of grass consumption. Dairy cattle, ewes and goats are the least autonomous systems because of their high dependence on imported concentrates and especially soya. The use of soya is currently a controversial topic because of the ways it is produced (GMO, use of pesticides forbidden in the EU, deforestation, carbon footprint). In France, the consumption of soya is estimated to reach 3.8 Mt (44% of which are consumed by ruminants, with 93% of imported volumes). To decrease these imports, the Plan for Protein Sovereignty launched by the French Government in 2020 aims at doubling areas with oilseeds and fodder legumes to feed ruminants by 2030. As part of this plan, Cap Protéines is the R&D programme looking for protein autonomy. In two years (2021–2022), Cap Protéines identified and promoted 330 French farms with a high score of self-sufficiency in protein and carried out a wide set of experiments on fodder production and on animal feeding and produced many references, tools and communication supports to help self-sufficiency.

**Keywords:** grasslands, protein, autonomy, France, soya

## Introduction

While French ruminant farms rely heavily on fodder and grass for a significant proportion of their protein needs, a substantial dependence on imported protein-rich materials persists. France annually imports 3.5 million tonnes of soybean meal, with 1.5 million tonnes earmarked for ruminants, especially dairy cattle, ewes and goats with elevated nutritional demands (Pavie *et al.*, 2022). However, the origin of this imported soy, mainly from South America, presents environmental and ethical challenges, including deforestation in the Amazon rainforest, GMO utilization, and the application of EU-prohibited pesticides. To address these concerns, the Cap Protéines programme advocates for enhancing protein autonomy by reducing reliance on imported soy and intensifying the utilization of grass (Hardy *et al.*, 2023).

In December 2020 the French government initiated the Plan for Protein Sovereignty, incorporating Cap Protéines as a research and development initiative. Supported by Terres Inovia (oilseeds research institute) and Institut de l'élevage (livestock research institute), the programme comprises over 80 agronomic and zootechnical experiments, monitoring 330 pilot farms, and creating 20 demonstration platforms in agricultural high schools to boost protein autonomy in ruminant farms and territories.

## Materials and methods

Cap Protéines focused on various experiments to enhance protein production and utilization in livestock farming. Agronomic levers were explored through demonstration and trial platforms, emphasizing crop forage, protein crops, and cereal mixtures for self-consumption. Notably, experiments underscored the profitability of raising ruminants on grass, with practices such as winter grazing shown to improve food and protein autonomy. Some of the experiments especially concern grassland (Table 1).

Table 1. Some of Cap Protéines experiments on grassland.

Experimental farm (region)	Experimental modality	Years
Trévarez (Bretagne)	Grazing of dairy cows in summer on grassland rich in legumes (80% clover) vs fodder corn and rapeseed meal	2021 and 2022
La Blanche Maison (Normandie)	Grazing of dairy cows with rapid rotation in autumn and winter vs free access	2022
Poisy (Auvergne Rhône-Alpes)	Grazing of pure legume by dairy cows	2021
Thorigné d'Anjou (Pays de la Loire)	Summer grazing of suckler cows on drought-resistant legume-based grassland	2022
Les Bordes (Centre-Val de Loire)	Spring pasture for suckler cows finishing on multi-species grassland	2021 and 2022
Les Etablières (Pays de la Loire)	Less rapeseed meal and more grass for young beef	2021–2022
Le Mourier (Nouvelle-Aquitaine)	Cellular grazing in spring for sheep	2022
Jalogny (Bourgogne-Franche-Comté)	Use rotational grazing to supplement male calves born in autumn	2021

## Results and discussion

In many cases, experiments have shown that raising ruminants with grass is economically as profitable, or even more profitable for farmers, than other feeding systems. For example, experimental farms in the western part of France showed that winter grazing practices improved food and protein autonomy. It is also a way to save litter, fodder, concentrates as well as working time (Brocard *et al.*, 2024). The experimental farm of Poisy showed that grassland with legumes makes it possible to extend the grazing period of dairy cows in summer when the drying conditions limit the growth of grasses (Berchoux *et al.*, 2022). For suckler cattle, grazed grass is the first lever of protein autonomy. Experiments confirm that it is possible to save concentrates by favouring the grazing of cull cows or grazers (Buteau *et al.*, 2022). An experimental cattle farm in Jalogny conducted a comparative study on two grazing practices for male Charolais calves born in the autumn. The implementation of optimized rotational grazing during the spring season resulted in a reduction of one-third in the concentrates provided to the calves, all while maintaining the desired commercial outcomes. Furthermore, refraining from supplementing pasture led to substantial savings of 100 kg of concentrates per calf by strategically delaying the sale and weaning process for an additional 15 days (Douhay *et al.*, 2022). Improved grassland management plays a pivotal role in enhancing the protein self-sufficiency of livestock systems. The optimal utilization of this protein reservoir, readily accessible to animals, hinges on ensuring the availability of leafy grass with a high content of total crude protein throughout the grazing season. The potential for protein production and valorization ranges between 1 to 2 tons of crude protein per hectare per year, depending on pedoclimatic conditions and the types of ground cover (Pierre *et al.*, 2023).

The network of 330 French farms with high protein autonomy serves as a practical model, emphasizing the feasibility of raising ruminants while minimizing protein material imports. For each of these farms, a technical, economic, environmental and autonomy analysis was carried out and a 4-page document was written. Those 330 protein-independent breeders have used a wide range of levers but are focusing on the production of quality grass and legumes (Miquel *et al.*, 2022).

Cap Protéines also developed or adapted tools for breeders and advisers. The software, digital platforms or smartphone applications are the following: Devautop (a protein autonomy diagnostic tool - [devautop.fr](https://devautop.fr)), HappyGrass (grassland management smartphone application — [happygrass.fr](https://happygrass.fr)), Optim'AL (economic optimization software for dairy cow's feeding — [optimal-vl.fr](https://optimal-vl.fr)), Perpet (grassland evaluation serious game) and My Luzerne (alfalfa cultivation smartphone application).

To transfer knowledge to breeders and future breeders in order to raise their awareness of protein autonomy, Cap Protéines organized demonstration days and shared technical references in different media ([cap-proteines-elevage.fr](http://cap-proteines-elevage.fr) website, books, press articles, social networks, video bank, etc.).

## Conclusion

Cap Protéines has played a pivotal role in accumulating experiences, digital tools, and testimonials on enhancing protein autonomy in French ruminant farms. Cap Protéines starts to communicate and raise awareness among farmers and advisers about protein autonomy. But the barriers to change are real: habits, more complex strategies, limited economic interests... We need to keep a strong mobilization on the theme (experiments / demonstrations) and maintain massive communication. Cap Protéines has underscored that grass is a primary lever for improving the protein autonomy of ruminant farms.

## Acknowledgements

The research received financial support from the France Relance Programme. The authors express gratitude to the 120 technical partners and development organizations, including Inosys-Réseaux d'Élevage, F@rmXP Network, Arvalis, Itab, Armefflor, Chambre d'agriculture France, Eliance, Civam, BTPL, AFPF, AFZ, Semaé and Cerience.

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# Winter grazing for dairy and beef animals: an opportunity to grasp?

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## Abstract

With the influence of climate change, grass growth is changing: less grass in summer, more in autumn and winter, an earlier start in spring. At the same time, bovine farms must increase their feed autonomy to face the challenge of fluctuations of input costs and prices. One way to improve protein self-sufficiency may be winter grazing. Four experimental farms in western France implemented a 2-year experiment with various categories of animals including growing animals and lactating cows. These experiments showed the high quality of the winter grass (crude protein of 18-21%). The grass growth ranged between 5 and 30 kg DM day<sup>-1</sup>ha<sup>-1</sup> between November and March. On average 0.5 to 1 t DM ha<sup>-1</sup> of grazed grass was valorized by the animals during the winter. The growth reached by all categories of animals that remained outside with little or no buffer feed are consistent with the expectations for such heifers or bulls. The group of dairy cows that had access to grazing for 3 h per day significantly produced +2 kg of milk cow<sup>-1</sup> day<sup>-1</sup> compared to the control group that remained in the barn with the same diet at trough. More experiments must be implemented to investigate all potential management options.

**Keywords:** climate change, winter grazing, dairy cows, heifers, young bulls

## Introduction

Because of climate change, grass growth is changing: less grass in summer, more in autumn and winter, an earlier start in spring. At the same time, bovine farms must increase their feed autonomy to face the challenge of fluctuations of input costs and prices (Hardy *et al.*, 2024). French dairy farms usually produce cereal crops and maize silage to provide energy for dairy diets but lack sources of proteins (protein concentrate being the most expensive ingredient of the diets). One way to improve protein self-sufficiency may be winter grazing. It is particularly the case for organic dairy farms producing milk in winter when local protein resources are scarce (Madeline *et al.*, 2016). However, there are few references available. Hence, an experimental programme was set up in western France during the winters 2021–2022 and 2022–2023 to assess the potential impacts on grass and animals of winter grazing.

## Materials and methods

Three experimental farms in western France implemented repeated tests with various categories of animals (dairy or beef heifers, young bulls, lactating cows) to test the feasibility of winter grazing and assess the quality of winter grass. The weather conditions of the two winters were very different: a very dry and mild winter in 2021–2022 followed by a very wet one in 2022–2023.

### *Winter grazing with Normande young bulls*

Two batches of Normande breed cattle were compared: one batch in rotational grazing (small parcels) and one batch in continuous or “free” grazing (large parcel). Rotational grazing involves the implementation of a cellular grazing system allowing rotation during the winter period. For free grazing, the animals had access to a large parcel throughout the winter period: from late November to mid-February. The growth facilitated by winter grazing was quantified for both batches through weigh-ins at the beginning and end of the winter grazing period. Regarding the vegetation aspect, a monitoring of the vegetation was established to record densities, growth, and nutritional value of the grass during the winter period. The



stocking rates were 1 Livestock Units (LU) ha<sup>-1</sup> in 2021 and 1.3 LU ha<sup>-1</sup> in 2022 for free grazing, and 1–8 LU ha<sup>-1</sup> instantaneous in 2021 and 1.3–10 LU ha<sup>-1</sup> instantaneous in 2022.

#### *Winter grazing with gestating dairy heifers*

Twelve gestating Holstein heifers, four months into gestation at the beginning of the trial and expected to calve in the spring, were divided into two homogeneous groups: one group managed through rotational grazing on ryegrass-white clover parcels, typically grazed by dairy cows during the peak season, and another group housed in a barn, fed with grass silage. The heifers were managed in small groups of 6 or 7, with a low instantaneous stocking rate of approximately 3 LU ha<sup>-1</sup> (0.3 LU ha<sup>-1</sup> grazed in total over the winter). The trial took place over two consecutive winters: from November 4, 2021 to February 9, 2022, and from November 14, 2022 to February 8, 2023; totalling 97 and 85 trial days. The heifers were weighed at the beginning, middle, and end of the trial. Rotational grazing was conducted on 9 to 11 paddocks averaging 1.2 hectares, with an average grass height at entry of 8.5 to 9 cm and an objective of exiting at 4 cm (measured with a plate-meter). For the grazing group, a grass sample was collected for nutritional analysis before each paddock entry. Each silage bale distributed to the housed group was also analysed. Herbage intake was estimated based on the growth achieved (INRA 2018 equations), and silage intake was determined from dry matter and the weight of the distributed bales.

#### *Winter grazing of dairy cows*

The trial involved 50 crossbred cows (Holstein×Jersey×Normande) with 40% primiparous cows, in a robotic milking system. The cows were divided into two groups: a control group without grazing, 100% housed, and an experimental group grazing for 3 hours per day, receiving the same ration at the feed bunk as the control group. The trial lasted for 8 weeks from December 7, 2022 to February 1, 2023, with two pre- and post-experimentation periods of 3 weeks each. The winter ration for both groups consisted of 5 kg Dry Matter (DM) of corn silage, unlimited grass silage (and therefore in variable quantities), 1.5 kg DM of alfalfa silage, and 2.5 kg DM of grain cereal mixture fed at the milking robot. Rotational grazing was conducted on 12 hectares, with 1 to 2 days per paddock, adjusting the electric fence based on the available grass quantity, totalling two grazing cycles during the trial. The instantaneous stocking rate was 50 LU ha<sup>-1</sup> as during the rest of the year.

## **Results and discussion**

#### *Winter grazing with Normande young bulls*

During the first winter, the winter grazing period extended over 78 days, allowing the cattle to utilize 4.8 tonnes of DM (1.1 tonnes DM ha<sup>-1</sup>) of grass in continuous grazing, equivalent to 8.8 kg DM per animal per day. In rotational grazing, 5.4 tonnes DM were utilized (1.25 tonnes DM ha<sup>-1</sup>), with 8.6 kg DM per animal per day. In the second winter, the results were similar for the quantity of grass utilized in free grazing, with 4.8 tonnes DM (1.13 tonnes DM ha<sup>-1</sup>; 9.5 kg DM animal<sup>-1</sup>day<sup>-1</sup>), compared to rotational grazing, where 4.7 tonnes DM were utilized (1.09 tonnes DM ha<sup>-1</sup>; 9.2 kg DM animal<sup>-1</sup>day<sup>-1</sup>). The calculation of Average Daily Gains (ADG) during the trial periods showed good growth performance for the cattle, averaging 660 g day<sup>-1</sup> for 24-month-old cattle in the first winter and nearly 980 g day<sup>-1</sup> in the second year. The grass crude protein concentration averaged 16% per kg DM in the continuous group, versus 18.2% for the rotational group.

#### *Winter grazing with gestating dairy heifers*

The growth of gestating heifers at pasture averaged 900 g day<sup>-1</sup> over the two winters and thus met the objectives. This is not statistically different from that of barn-housed heifers consuming silage (table 1). Based on the growth achieved and the INRA 2018 equations, heifers at pasture consumed 9.1 to 9.9 kg of grass DM per heifer per day, compared to 9.2 to 11.1 kg DM of silage for the housed group. Hence, 5 to

6 tonnes of silage and 3.4 tonnes of straw were saved thanks to winter grazing. The grazed grass averaged 21.2% of crude protein per kg of dry matter during the trial.

### Winter grazing of dairy cows

With a grazing duration of 3 hours per day over 8 weeks, the grazing group reduced its consumption of grass silage at the feed bunk by 0.9 kg DM cow<sup>-1</sup>day<sup>-1</sup> compared to the control group. The intake of grazed grass, estimated based on the UF (Feed Unit) and PDI (Digestible Protein) requirements for milk production and maintenance needs (INRA 2007 equation), is estimated to be around 2 kg DM cow<sup>-1</sup>day<sup>-1</sup>. The winter grazing group achieved better milk performance (+2 kg cow<sup>-1</sup>day<sup>-1</sup>) than the 100% housed group (Figure 1). No clear trends appear in terms of fat content, protein content, robot milking frequency, or animal health. Moreover, the grazing group seems to have lost less weight at the end of winter than the experimental one. The grazed grass averaged 20.1% of crude protein per kg DM during the trial. This trial will be repeated over two more winters.

## Conclusion

Winter grazing can be a lever in the face of climate change and an opportunity to bring balance to protein content in a winter diet for growing animals or lactating cows, especially in agrobiology, although the absence of impact on soil trampling and vegetation recovery in spring must be confirmed. Moreover, whatever the type of animal, the impact on working time and conditions must be assessed with more accuracy.

## Acknowledgement

These experiments were part of Cap Protein project funded by France Relance Program.

Table 1. Experiment 2. Growth of heifer and grass use per year and batch (medians).

	Winter 2021–2022		Winter 2022–2023	
	Grazing	Haylage	Grazing	Haylage
No. of heifers	6	6	6	6
Start weight (kg)	483	512	462	475
End weight (kg)	578	610	539	541
ADG (g day <sup>-1</sup> )	881	990	903	942
DMI (kg day <sup>-1</sup> )	9.1	11.1	9.9	9.2
Total intake (kg DM day <sup>-1</sup> )	5.3	6.0	5.0	4.7

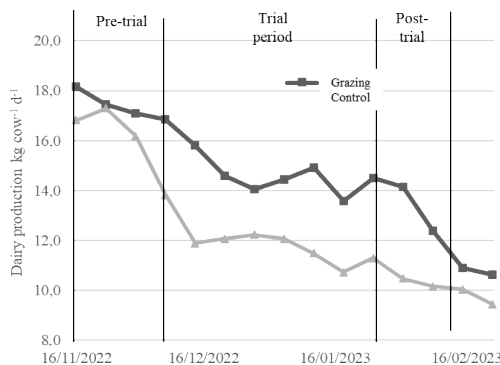


Figure 1. Experiment 3. Dairy production of control and experimental group, winter 2022–2023 (2×16 cows).

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# Herbage nitrogen yield in grass and grass-white clover swards receiving zero nitrogen

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## Abstract

Nitrogen fertilisers are a major contributor to ammonia and greenhouse gas emissions through their inputs in grazed herbage systems. Nitrogen use efficiency can be increased through reduced nitrogen inputs to grazing systems but herbage and animal production needs to be maintained. The objective of this study was to evaluate the herbage nitrogen yield from grass-only and grass-white clover herbage receiving zero nitrogen inputs. The study consisted of zero nitrogen plots established in 2020 and 2021 within grazed paddocks of either grass or grass-white clover swards at Teagasc, Clonakilty Agricultural College, Cork, and Teagasc, Moorepark, Fermoy, Cork, Ireland in 2021. The plots received no chemical or organic N and were not grazed for the duration of the grazing season. Plots were relocated within paddock between grazing seasons in Clonakilty. The grass-only sward plots yielded 7404 kg DM ha<sup>-1</sup> per year and 193 kg N ha<sup>-1</sup>. The grass-white clover swards yielded significantly higher herbage (9837 kg DM ha<sup>-1</sup> per year) and N (291 kg N ha<sup>-1</sup>) than the grass-only swards ( $P < 0.001$ ). The results provide an indication of background N mineralization of 193 kg N ha<sup>-1</sup> and biological N fixation of 98 kg N ha<sup>-1</sup> within grass-only and grass-clover swards receiving zero nitrogen.

**Keywords:** nitrogen fertiliser, environment, mineralization, biological nitrogen fixation

## Introduction

Inorganic nitrogen (N) fertiliser is a cornerstone input of intensive farming systems globally, including those dominant in temperate grasslands. Nitrogen fertiliser is one of the most efficient ways to increase production during periods of the year when N from N<sub>2</sub>-fixation or mineralization is inadequate to meet plant demand (Whitehead, 1995). However, the Haber-Bosch process of manufacturing inorganic N consumes roughly 58 MJ of energy and emits large amounts of greenhouse gas (GHG) at the same time contributing to approximately 1% of the total global GHG emissions. Industrial production of 1 kg of inorganic N, equates to 2.25 kg of CO<sub>2</sub> being emitted (Lüscher *et al.*, 2014). It is also estimated that for every 100 kg of N applied to the soil approximately 1 kg of N<sub>2</sub>O is emitted to the atmosphere but can be as low as 0.5 kg N<sub>2</sub>O-N with reduced N application rates (Kanter *et al.*, 2020). The level of N<sub>2</sub>O emitted is critical as N<sub>2</sub>O is 298 times more potent than CO<sub>2</sub> and persists in the atmosphere for a lengthy time (>100 years; Kingston-Smith *et al.*, 2010). Therefore, with increasing environmental targets to be met there is a requirement to maximise naturally occurring N resources from mineralization and fixation. The objective of this study was to evaluate the herbage nitrogen yield from grass-only and grass-white clover herbage receiving zero nitrogen inputs.

## Materials and methods

The experiment was carried out at two sites in the south of Ireland, Clonakilty Agricultural College, Cork (51°63' N, 08°85' E) in 2020 and 2021 and Teagasc Moorepark, Cork (52.16° N, 8.24° W) in 2021. The soil type was sandy loam at Clonakilty and loam at Moorepark. The study consisted of zero nitrogen plots (5×5 m) established within 2 representative grazed paddock blocks per site of either grass

(perennial-ryegrass) or grass-white clover swards (perennial-ryegrass, medium leaf white clover variety) with two replicates per paddock and all paddocks sown in 2012. There was a total of four paddocks at each site. The plots received no chemical or organic N and were not grazed for the duration of the grazing season. Plots were relocated within paddock between grazing seasons in Clonakilty. Grass-only paddocks received 230 kg N ha<sup>-1</sup> per year and grass-clover paddocks received 150 kg N ha<sup>-1</sup> year<sup>-1</sup> in the form of urea + N-(n-Butyl)thiophosphoric triamide (NBPT). Fertiliser was spread early February and post each grazing event until the chemical N deadline in September each year. Herbage yield was measured prior to grazing both within the paddock and the plot. Pre-grazing yield in the paddock was measured by harvesting two strips (5 m×1.2 m) within the paddock to a height of 4 cm using an Etesia mower (Etesia UK, Warwick, UK). Herbage yield was measured within the plot using a quadrat (0.5 x 0.5 m) and hand shears at the same time as the paddock was yielded prior to grazing. A 100 g subsample was collected and dried at 60°C for 48 hours to determine dry matter from both paddocks and plots. These samples were milled through a 1 mm screen using a Cyclotech 1093 Sample Mill (Foss, Hillerød, Denmark) on removal from the oven and scanned under near infrared reflectance spectrometry (NIRS) for estimation of the crude protein (CP) content. The CP content was expressed as N content by dividing by 6.25. Statistical analysis was undertaken using PROC MIXED liner mixed model in SAS (version 9.4). Terms included in the model were site-year, sward type, rotation (repeated), plot ID (subject) and their subsequent interactions. Individual plot was the experimental unit. Site-year was included in the model as all sites were not included every year within the dataset. Tukey's test was used to determine differences between treatment means.

## Results and discussion

The grass-only sward plots yielded a mean 7404 kg DM ha<sup>-1</sup> year<sup>-1</sup> and 193 kg N ha<sup>-1</sup> across site-years. The grass-white clover swards yielded significantly more herbage (9,837 kg DM ha<sup>-1</sup> per year) and N (291 kg N ha<sup>-1</sup>) than the grass-only swards ( $P<0.001$ ; Table 1). The grass-only paddocks yielded 13,386 kg DM ha<sup>-1</sup> resulting in a yield difference 5982 kg DM ha<sup>-1</sup> compared to the grass-only plot receiving zero N. This indicates a response to fertiliser of 26 kg DM per kg N applied (230 kg N applied). The grass-clover paddocks yielded 12 979 kg DM ha<sup>-1</sup>, resulting in a yield difference of 3142 kg DM ha<sup>-1</sup> compared to the grass-clover plot receiving zero N. The grass clover plots also had higher N content (+0.5%;  $P<0.001$ ) and total herbage N yield (+98 kg N;  $P<0.001$ ) than the grass-only plots. The 193 kg N ha<sup>-1</sup> provides an estimation of N mineralization from the organic matter in the soil at the sites. The additional 98 kg N ha<sup>-1</sup> year<sup>-1</sup> present in the grass-clover swards could be estimated as biological nitrogen fixation. Figure 1 illustrates the herbage yield by rotation with an increase for grass-clover swards beginning in rotation 2 and remaining higher for the rest of the grazing season as clover content increases in the sward as typical for grass-clover swards.

Table 1. Effect of sward type (ST) and rotation (R) on nitrogen (N) yield of herbage across site-years in grass-only and grass-clover sward plots receiving zero N.

	ON (Grass only)	ON (Grass clover)	SE	ST	R	ST×R
Herbage yield (kg DM ha <sup>-1</sup> )	978	1280	39.3	<0.001	<0.001	0.022
Daily N yield (kg N)	0.73	1.17	0.057	<0.001	<0.001	0.035
N content (%)	2.50	3.01	0.070	<0.001	<0.001	0.028
Total herbage yield (kg DM ha <sup>-1</sup> )	7404	9837	336.5	<0.001	–	–
Total herbage N yield (kg N ha <sup>-1</sup> )	193	291	12.6	<0.001	–	–

ON, receiving zero nitrogen.

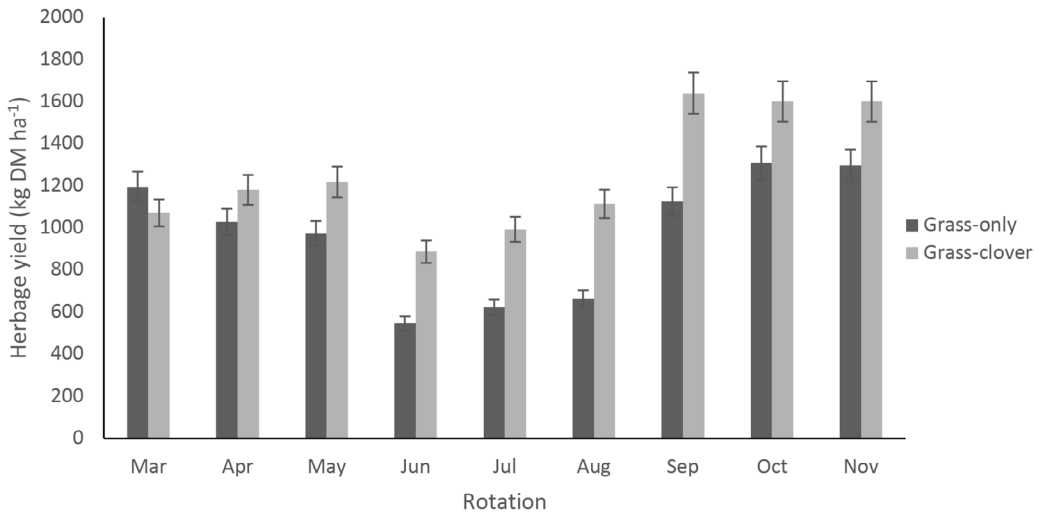


Figure 1. Mean herbage yield by rotation for grass-only and grass-clover cutting swards receiving zero nitrogen over three site-years (2020–2021).

The results provide an indication of the levels background N mineralization and biological N fixation within grass-only and grass-clover swards receiving zero nitrogen.

## Conclusion

This study provides an indication of background N mineralization and biological N fixation within PRG-only and PRG-WC swards are available to the plant. This information requires further measurements and investigation to give a more comprehensive insight into precision N applications that could reduce applications of chemical N fertiliser in grass-only and especially grass-clover grazing systems.

## Acknowledgement

This research was funded by VistaMilk SFI Research Centre.

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# Effect of white clover in perennial ryegrass swards with reduced nitrogen inputs on milk production

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## Abstract

Grazed grass is the cheapest feed available for dairy cows in temperate regions; thus to maximise profits dairy farmers must utilise this high quality feed where possible. To reduce cost inputs and environmental impacts of inorganic nitrogen (N) use, there is renewed interest in the incorporation of legumes, white clover in particular, in grazing systems. The objective of this study was to reduce chemical N fertiliser input to grass-based milk production systems in Ireland by incorporating white clover into grassland swards. Three grazing treatments were used for this study: grass-only swards receiving 200 kg N ha<sup>-1</sup> and grass-white clover swards receiving 100 or 150 kg N ha<sup>-1</sup> annually. Twenty cows were assigned to each treatment and swards were rotationally grazed at a stocking rate of 2.5 cows ha<sup>-1</sup>. In the first year of the study, cows grazing the grass-white clover treatments had greater production in terms of milk yield (+257 kg) and milk solids yield (+35 kg) compared with cows grazing the grass-only treatments. There was no treatment effect on herbage production. This significant increase in milk production suggests the inclusion of white clover in grazing systems can be effectively used to increase milk production and reduce N fertiliser inputs and their environmental impacts.

**Keywords:** grazing, nitrogen fertiliser, environment, milk solids

## Introduction

In grass-based systems, to reduce input costs and environmental impacts of inorganic nitrogen (N) use and to increase farm gate N use efficiency (NUE), there is renewed interest in the incorporation of legumes, and white clover (*Trifolium repens* L. (WC)) in particular, in perennial ryegrass (*Lolium perenne* L. (PRG))-based production systems (Lüscher *et al.*, 2014). Perennial ryegrass-only based grazing systems are highly efficient and low-cost but are ultimately dependent on high N fertilizer levels (>200 kg N ha<sup>-1</sup>) to achieve high levels of herbage production (Enriquez-Hidalgo *et al.*, 2016). White clover can offset some of these N requirements through its ability to biologically fix atmospheric N to facilitate grass production (Crush, 1987). An increase in milk production from cows grazing PRG-WC swards has also been observed and can be attributed to an overall increase in herbage dry matter (DM) intake from PRG-WC swards and to the high nutritional value of WC (Murray *et al.*, 2023). The objective of this study was to determine the effect of WC inclusion in PRG swards and N fertiliser rate on milk production of spring-calving grazing dairy cows.

## Materials and methods

The experiment was carried out at Moorepark Teagasc, Fermoy, Co. Cork, Ireland from February 2023 to November 2023. The experiment contrasted two sward types (PRG-only and PRG-white clover) receiving varying levels of fertiliser. This resulted in three separate grazing treatments; a PRG-only sward receiving 200 kg N ha<sup>-1</sup> (Gr200), a PRG-white clover sward receiving 150 kg N ha<sup>-1</sup> (Cl150) and PRG-white clover sward receiving 100 kg N ha<sup>-1</sup> (Cl100). There were 20 cows per treatment and each treatment was stocked at 2.5 cows ha<sup>-1</sup>. Each farmlet was 6.8 ha consisting of 12 paddocks each. Cows were assigned to treatment based on calving date, parity, pre-experimental milk yield and economic breeding index.

Cows remained in their treatments for the entire grazing season. Treatments were rotationally grazed from early-February to mid-November for 8 rotations and the target post-grazing sward height was 4 cm. Nitrogen fertiliser applications were applied according to Table 1, using urea +NBPT. Each farmlet was walked weekly to monitor average farm cover and when surpluses arose they were removed in the form of baled silage. If a feed deficit occurred across all treatments then all treatments were supplemented with concentrate, on average, 576 kg (2 kg per day on average) fresh weight of concentrate was fed across all treatments. If a feed deficit occurred in an individual treatment then cows were supplemented with conserved forage produced from within that treatment (416 kg DM cow<sup>-1</sup> for all treatment groups). Pre-grazing herbage mass in each paddock was determined twice weekly by harvesting two strips using an Etesia mower (Etesia UK Ltd., Warwick, UK) in the area to be grazed next. Pre- and post-grazing heights were measured daily using a rising plate meter (Jenquip, Fielding, New Zealand). Sward WC content was measured before grazing in each paddock in each rotation by cutting 15 random grab samples to 4 cm with a Gardena hand shears, separating the sample into PRG and WC fractions and drying at 90°C for 16 hours. Milk yield was recorded daily and milk composition weekly by taking milk samples from a consecutive evening and morning milking. Data from 60 cows over one year (60 variables) were available for analysis. Grazing characteristics (Table 2) were analysed using Proc MIXED linear model in SAS (version 9.4). Terms included in the model were paddock (subject), rotation (repeated), WC treatment and fertiliser rate treatment. Milk data were also analysed using Proc MIXED in SAS (version 9.4). Terms included in the model were sward type treatment, fertiliser rate treatment and parity.

## Results and discussion

There was no effect of sward type or N fertiliser application rate on any grazing characteristics (Table 2). There was also no reduction in production for the two WC treatments receiving less chemical N compared to the Gr200 treatment, similar to Egan *et al.* (2018).

White clover inclusion had a significant ( $P < 0.001$ ) positive effect (Table 3) on milk solids production, whereas N fertiliser rate had no effect. The Gr200 treatment produced 5,601 kg milk and 480 kg milk solids (kg fat+protein) cow<sup>-1</sup>, in comparison with the PRG-WC swards that produced on average 5858 kg milk and 515 kg milk solids cow<sup>-1</sup>. The difference in milk solids production between the PRG-only and PRG-WC treatments occurred due to the higher milk yield produced rather than a difference in fat or protein content, which is similar to previous results reported (Egan *et al.*, 2018). Nitrogen fertiliser rate had no significant effect on milk production or composition over the total lactation.

Table 1. Nitrogen fertiliser application strategy (kg N ha<sup>-1</sup>).

Rotation/Date	Gr200	C150	C100
February	28	28	28
Mid-March	28	28	19
15 <sup>th</sup> April (2 <sup>nd</sup> rotation)	20	20	19
6 <sup>th</sup> May (3 <sup>rd</sup> rotation)	20	13	9
27 <sup>th</sup> May (4 <sup>th</sup> rotation)	17	9	0
17 <sup>th</sup> June (5 <sup>th</sup> rotation)	17	9	0
8 <sup>th</sup> July (6 <sup>th</sup> rotation)	17	9	0
29 <sup>th</sup> July (7 <sup>th</sup> rotation)	17	9	0
19 <sup>th</sup> August (8 <sup>th</sup> rotation)	17	9	9
Mid-September	19	16	16
Total	200 kg	150 kg	100 kg



Table 2. Effect of sward type (ST) and nitrogen fertiliser rate (FR) on grazing characteristics.

	Gr200	Cl150	Cl100	SE	ST	FR
Pre-grazing herbage mass (kg DM ha <sup>-1</sup> )	1599	1422	1498	76.1	0.077	0.217
Pre-grazing height (cm)	11.78	11.31	11.36	0.325	0.266	0.546
Post-grazing height (cm)	4.42	4.39	4.43	0.040	0.782	0.786
Clover content (%)	-	19.0	19.1	1.03	-	0.886

Gr200, PRG-only sward receiving 200 kg N ha<sup>-1</sup>; Cl150, PRG-white clover sward receiving 150 kg N ha<sup>-1</sup>; Cl100, PRG-white clover sward receiving 100 kg N ha<sup>-1</sup>; SE, standard error.

Table 3. Effect of sward type (ST) and fertiliser rate (FR) inclusion on full lactation milk production.

	Gr200	Cl150	Cl100	SE	ST	FR
Days in milk	287	290	288	3.5	0.652	0.855
Milk yield (kg cow <sup>-1</sup> )	5601	5824	5892	138.3	0.129	0.301
Milk solids yield (kg cow <sup>-1</sup> )	480 <sup>a</sup>	511 <sup>b</sup>	518 <sup>b</sup>	11.9	0.023	0.072
Fat content (g kg <sup>-1</sup> )	50.4	51.6	52.1	0.12	0.577	0.320
Protein content (g kg <sup>-1</sup> )	36.3	36.9	36.5	0.05	0.713	0.540
Lactose content (g kg <sup>-1</sup> )	47.1	47.1	46.8	0.02	0.501	0.751

See footnote to Table 2 for abbreviations.

## Conclusion

The inclusion of WC in PRG swards had a significant positive affect on milk solids production. Reducing N fertiliser rate from 200 to 150 or 100 kg N ha<sup>-1</sup> with white clover in the sward did not affect herbage or milk production. The inclusion of white clover will reduce N surpluses on farms while increasing milk solids production and, in turn, increasing farm profits per ha.

## Acknowledgement

This research was funded by the Irish Dairy Levy administered by Dairy Research Ireland.

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# Yield potential of tall fescue compared to perennial ryegrass in Belgium

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## Abstract

Compared to perennial ryegrass (Lp), tall fescue (Fa) is praised for its great yield potential, both in dry and wet years. However, data quantifying the yield difference between these two species are scarce. Based on data from the Belgian value for cultivation and use trials in the period 2015–2022 we assessed yield potential of both species in years with contrasting climatic conditions and on four different locations with contrasting soil types. The 20% greater yield potential of Fa compared to Lp was confirmed. This difference increased to 27% in dry years on deep, loamy soils. These results strengthen the idea that in both normal and drier years, tall fescue has a great potential to make forage production more resilient in a changing climate.

**Keywords:** drought, dry matter yield, rooting depth

## Introduction

Compared to perennial ryegrass (*Lolium perenne* (Lp)), tall fescue (*Festuca arundinacea* (Fa)) is praised for its good drought tolerance and its great yield potential both in dry and wet years. In a trial performed in the period 2010–2012 in Melle, Belgium, yield potential of Fa (15 610 kg dry matter ha<sup>-1</sup> year<sup>-1</sup>) was 20% greater than perennial ryegrass (12 698 kg dry matter ha<sup>-1</sup> yr<sup>-1</sup>) (Cougnon *et al.*, 2014). Similar yield differences were found in Germany (Becker *et al.*, 2020). The greater root biomass in deeper soil layers of Fa compared to Lp (Cougnon *et al.*, 2017) allows tall fescue to maintain its growth for longer during dry periods. As intra-annual precipitation variation increases as a result of climate change, the yield difference between Fa and Lp is expected to increase. Hence, it is not clear whether Fa maintains its better drought tolerance on shallow, sandy soils. The aim of the research reported in the present paper is to answer the following questions: (i) Does the yield difference between Lp and Fa increase in dry years? (ii) Is the yield difference between Lp and Fa influenced by trial location? (iii) Does the difference between Lp and Fa increase with the sward age? To answer these questions, we analysed a dataset of the Belgian ‘value for cultivation and use’ (VCU) trials for both species, in the period 2014–2022, including dry and wet years and contrasting soil textures (deep, loamy *versus* shallow, sandy).

## Materials and methods

The Belgian VCU trials for forage grasses were performed on four locations in Belgium (Table 1). For Lp and Fa, separate trials were sown per species, in the spring (of y0) and yield was measured in the three years following the establishment year (y1, y2 and y3). In the spring of the following year (y1) the trial was duplicated, and yield is measured in y2 and y3. All these trials were randomised complete block designs with four replicates. Sowing density was 1400 germinative seeds m<sup>-2</sup> for both species. Annual fertilisation was around 300 kg N ha<sup>-1</sup> and 350 kg K<sub>2</sub>O ha<sup>-1</sup>. The aim was to harvest five cuts per year. Harvesting was done with a Haldrup forage harvester. Although Fa and Lp were tested in different trials, the management (cutting frequency, fertilisation) was the same and trials sown in the same year were sown on the same field.

Precipitation and potential evapotranspiration from crop canopy over a 30-year period (from 1992 to 2022) were obtained from the Agri4Cast resources portal of the European Commission Joint Research Centre ([agri4cast.jrc.ec.europa.eu](http://agri4cast.jrc.ec.europa.eu)). For each location, the grid point closest to the trial field was used.

Additionally, the standardised precipitation-evapotranspiration index (SPEI-3) was calculated by first taking the rolling average of the water balance - the difference between the precipitation and the potential evapotranspiration - with a moving window of 3 months. Second, the water balance was standardised by subtracting the 30-year mean for each day of year, and dividing by the standard deviation. A trial year was classified as 'dry' when the average SPEI-3 drought index during the growing season — from the beginning of March to the end of September — was lower than  $-0.5$ . This was the case for the years 2017, 2018, 2019, 2020 and 2022. Normal or wet years with an average SPEI-3 higher than  $-0.5$  were 2014, 2015, 2016 and 2021. Soil textures and annual precipitation characteristics from 2015 to 2022 on the four trial locations are summarised in Table 1.

For Lp, we included both diploid and tetraploids from the late and intermediate group. We only considered the data from the reference varieties which were harvested for at least 8 out of 9 trial years.

Analysis of variance (ANOVA) was calculated using a linear mixed effects model. The model used for statistical analysis included total annual dry matter yield (Y), grass species (S), a categorical variable for drought (D), growth location (L), sward age (A) as fixed factor and harvest year (H) and grass species variety (S:V) as random factors.

$$\log(Y) \sim S \times D + S \times L + S \times A + (I | H) + (I | S:V)$$

The response variable yield was log-transformed so that the model residuals met normality, which was assessed using a qqplot and the Shapiro-Wilk test ( $P=0.71$ ). All statistical analyses were performed using R (R Core Team, 2016). The R package lmerTest was used for calculation of the model. The estimated marginal means on the linear mixed effects model, conditioned on species was calculated using the emmeans function from the emmeans package.

## Results and discussion

The estimated marginal means of dry matter yield, conditioned on species for the three fixed factors drought (D), age (A) and location (L), is shown in Table 2. In dry years and in wet years, Fa was more productive than Lp. The main effect of D was not significant ( $P=0.11$ ), but the  $S \times D$  interaction was significant ( $P < 0.001$ ) indicating that Lp was significantly more affected by drought than Fa, reflecting the superior drought tolerance of Fa compared to Lp. The absence of significantly lower yields in "dry" growing seasons, based on the precipitation over the whole growing season, indicates that grass, regardless of the species, is a very resilient crop. Additionally, more than half of the potential grass production is produced in the first two cuts, before drought occurs. Furthermore, post-drought compensatory growth after drought periods, mainly explained by an improved N nutrition through increased mineralisation (Schärer *et al.*, 2023), can also partly compensate for the lower yield in dry periods.

Table 1. Soil type and precipitation during the study period in the trial locations of the Belgian VCU trials.

Location	Soil texture	Precipitation (mm)		
		Min	Max	Mean
Poperinge	Sandy loam	565 (2022)	759 (2014)	656
Merelbeke	Sandy loam	523 (2022)	834 (2016)	648
Bassevelde	Sandy	561 (2018)	762 (2017)	676
Ravels	Sandy	516 (2018)	784 (2016)	663

Table 2. The estimated marginal means of dry matter yield conditioned on species for the three fixed factors drought (D), age (A) and location (L).

		Fa (kg ha <sup>-1</sup> )	Lp (%)	Fa/Lp
Drought (D)	Not dry	16 647	14 328	116
	Dry	17 501	14 186	123
Sward age (A)	1	18 958	16 647	114
	2	16 984	13 494	126
	3	15 522	13 095	119
Location (L)	Bassevelde (sand)	16 984	14 328	119
	Merelbeke (sandy loam)	17 327	14 045	123
	Poperinge (sandy loam)	18 398	14 472	127
	Ravels (sand)	15 678	14 328	109

The main effect of sward age was a strong negative effect on both Lp and Fa ( $P < 0.001$ ), but the age effect was greater for Lp than for Fa ( $P < 0.001$ ). The estimated marginal means in Table 2 indicate that the decline is strongest after the first production year, especially for Lp, which is in accordance with the greater persistence of Fa compared to Lp.

Both the main effects of location and the interaction effect between location and species were significant ( $P < 0.001$ ), indicating that the location affected both species, but they were not affected in the same way. The yield difference between Fa and Lp was greater in locations with a deep, loamy soil compared to locations with a shallow, sandy soil. We hypothesise that Fa cannot take advantage of its deeper roots on shallow sandy soils, losing its competitive advantage compared to Lp on these soils. This corresponds with the observation that wild populations of tall fescue are less frequent in the area of Flanders with sandy soils compared to regions with sandy loam or loamy soils (own observations).

## Conclusion

The higher yield potential of Fa compared to Lp was confirmed. In drier years and after the first production year, and also on the locations characterised by a loamy, deeper soil, the difference between both species was exacerbated. This study confirms the interest of tall fescue in the face of a changing climate.

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# Changes in herbage productivity of winter fodder in the first cut over four years

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## Abstract

Grasslands are a vital component of the food supply chain, providing feed for livestock, contributing to soil health, promoting biodiversity, and offering climate-related benefits. Maintaining the health and productivity of grasslands is essential for global food security. Harvested swards are an important winter fodder for livestock. To study the effect of N fertilization and different plant species composition on expected feedstock for winter forage, grass-legume swards with four perennial grasses and four legumes at loamy soil at the Lithuanian Research Centre for Agriculture and Forestry were investigated, with ten different grass-legume mixtures ( $n=4$ ) over 2018-2022. Both fertilization ( $N_{60}$ ) and the different species composition ( $N_0$ ) of grasses had a significant effect on the dry matter yield of the first cut of swards ( $p<0.05$ ). The four-year average data revealed that nitrogen fertilizer significantly increased yields in the first cut, resulting in an average biomass gain increase of 25.5%. Additionally, there is potential for a 48% higher yield in monocultures and a 21% increase in grass-legume swards.

**Keywords:** biodiversity, environment, herbage yield,  $N_2$  fixation

## Introduction

The consistent distribution of fodder throughout the season ensures that dairy cows have continuous access to nutritious substances. This is particularly crucial in animal husbandry, where animals often depend on grasses and fodder to maintain good health and productivity. It can also influence the quality of feed, as new and fresh grasses tend to be more nutritious. Therefore, uniform distribution ensures that animals receive a diverse and high-quality diet throughout the entire season (Ponnampalam and Holman, 2022). Consequently, in the preparation of silage, the first cut of grass allows for the collection of young, fresh grass parts, which are often highly nutritious, thus improving the quality of feed and contributing to enhanced animal nutrition and productivity (Weiby *et al.*, 2022). The aim of this study was to evaluate the effect of fertilizer application ( $N_{60}$  and  $N_0$ ) on the yield of the first cut of different grass-legume communities across multiple years.

## Materials and methods

A study was carried out during 2019–2022 at the Lithuanian Research Centre for Agriculture and Forestry. The experiment was established in early spring 2018 on loamy soil type: Cambisols ( $pH_{KCl}$  6.9, total N of 0.25%,  $P_2O_5$  of 22.0 mg (100 g)<sup>-1</sup>,  $K_2O$  17.3 mg (100 g)<sup>-1</sup>,  $C_{org}$  1.76%). Each plot was 15 m<sup>2</sup> (1.5×10 m) to define two monocultures and ten mixtures with different species composition (G1=perennial ryegrass, G2=× *Festulolium*, G3=meadow fescue, G4=timothy, L1=white clover, L2=red clover, L3=lucerne, L4=sainfoin); legume contained 40% and grasses 60% in the mixtures. The sward was managed by cutting from three to five times each year. Nitrogen fertilizer was applied each year at the rate of 150 kg N ha<sup>-1</sup> (60 kg ha<sup>-1</sup> in spring and 45 after 1 and 2 cut), except for the sowing year 2018. During each harvest, the biomass of aboveground vegetation in each plot was evaluated. This process involved cutting the entire plot to a height of 5 cm and recording the fresh weight. A subsample of this material was then selected, and its fresh weight was measured. Subsequently, the material underwent drying at 105°C for 24 hours until a constant weight was reached to determine the dry matter (DM). To analyse

the effects of the treatment, an analysis of variance two-way factorial ANOVA was conducted at the 5% probability level ( $p < 0.05$ ).

## Results and discussion

A two-factor analysis of variance (ANOVA) showed that different sward composition (grass-legume mixtures), fertilization and factors interaction had a significant effect on changes in herbage yield in 2020 and 2021; except 2019 and 2022 (Table 1).

Analysing the yield of the first cut, it was determined that throughout the experiment, the most productive sward consisted of white clover, lucerne, perennial ryegrass, *×Festulolium*, meadow fescue and timothy (Figure 1), except for the year 2020, which was characterized by being very wet and warm, without extreme weather conditions. The average first cut yield of this sward over a four-year period was 3990 kg ha<sup>-1</sup>, which was 9.5% lower than the yield of the most productive fertilized monoculture sward of *×Festulolium*. The productivity of individual swards varied each year when using mineral nitrogen fertilizers, indicating that biomass increment is more dependent on different climatic conditions, like drought or heat stress. In contrast, when not using mineral nitrogen fertilizers, the productivity trend of individual swards was maintained.

Mixtures containing lucerne (N<sub>0</sub>) exhibited one of the highest productivities, with an average four-year first cut yield reaching 3574 kg ha<sup>-1</sup>. However, it was observed that species diversity in the sward already showed yield differences in the first cut; however, one additional species of leguminous grass in the mixture could increase yields by up to 490 kg ha<sup>-1</sup> more than the additional three leguminous grass species. It was also observed that the number of grasses species played an important but not significant role in the yield productivity when comparing two and four species in mixtures. It was determined that a potential average increase of 353 kg ha<sup>-1</sup> in yield is achievable using four grasses species in mixtures with additional leguminous grasses: lucerne, sainfoin, or together with lucerne, sainfoin, and with red clover. However, in mixtures where only red clover was additionally used, this trend was not observed.

Using N<sub>150</sub>, the most productive sward in the first two years of use was the monoculture sward, with an average yield of 6177 kg ha<sup>-1</sup>, but in the last two years of use, the productivity of this sward decreased significantly, with an average yield of 2648 kg ha<sup>-1</sup>, compared to the most productive sward of different species compositions with eight grass-legume species, with an average yield of 3588 kg ha<sup>-1</sup>. This shows that monoculture sward may have a limited persistence and a very unstable yield over the years.

Table 1. Results of two-factor analysis of variance of the aboveground biomass of multi-species swards in 2019–2022.

Year	2019	2020	2021	2022
Factor A (grass–legume mixtures)	ns	*	*	*
Factor B (fertilization)	*	*	*	ns
Interaction A × B	ns	*	*	*

Values with asterisks indicate significant differences ( $p < 0.05$ ), ns indicates non-significant difference; the data from 1 cut is provided.

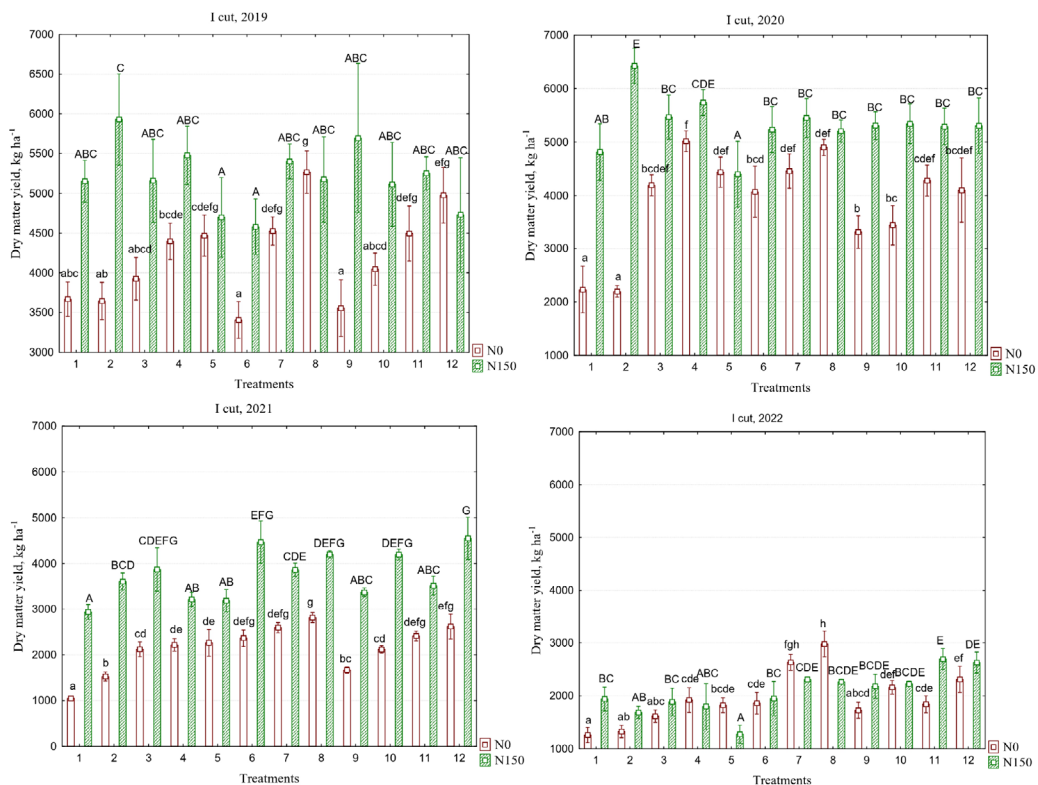


Figure 1. Herbage yield in 2019–2022 years in the first cuts. Treatments: 1—G1, 2—G2, 3—L1+L2/G1, 4—L1+L2/G2, 5—L1+L2/G1+G2, 6—L1+L2/G1+G2+G3+G4, 7—L3+L1/G1+G2, 8—L3+L1/G1+G2+G3+G4, 9—L4+L1/G1+G2, 10—L4+L1/G1+G2+G3+G4, 11—L1+L2+L3+L4/G1+G2, 12—L1+L2+L3+L4/G1+G2+G3+G4. Different lowercase letters indicate significant differences between the treatments (N<sub>0</sub>) and uppercase letters (N<sub>150</sub>); p < 0.05.

## Conclusion

The comparison of mixtures of different species compositions, showed a potential difference in yield of 1426 kg ha<sup>-1</sup> (N<sub>0</sub>) and 913 kg ha<sup>-1</sup> (N<sub>150</sub>) is possible. Therefore, when formulating sward mixtures, it is crucial to optimize the species composition because data indicates that it is not the number of species that drives the increase in sward productivity, but rather the compatible coexistence of different species of grasses and legumes in the mixture.

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# Increasing soil phosphorus content increased spring barley yield while it had no effect on grass yield

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## Abstract

Spring barley in Finland benefits from P fertilisers more than grasses. However, the interaction between crop, P fertilisation and soil P status on the yield response remains unclear. Our aim was to investigate the effect of soil P status and its interaction with P fertiliser application on the yields of both annual cereal and perennial grasses. The experiment was conducted in Central Finland in 2020–2023 in a research field with low P status ( $P_{AAC}$ , measured by acid ammonium acetate, pH 4.65). Medium and high soil P statuses were created by superphosphate fertilisation and ploughing in 2017–2018, using a Latin square (3\*3) design. The main plot was the soil P status, the sub-plot was the plant (timothy ley and spring barley), and the sub-sub-plot was the mineral P fertiliser application (0, 10, 20 and 40 kg P ha<sup>-1</sup> year<sup>-1</sup>). Yield, P content of soil, and P balance were measured. In barley, a high rather than low soil P status resulted in a higher yield, and P fertiliser application increased yield at a low P status, while no effect on the grass yield was observed. New methods are required for barley cultivation in grass rotation.

**Keywords:** barley, grass, phosphorus, soil, yield

## Introduction

In Finland, arable land is dominated by grass (40%) and spring barley (20%), crucial for livestock feed. Reduced phosphorus (P) fertilisation since the mid-1990s has reduced the soil P status, raising concerns about productivity. The 150-year experiments at Rothamsted, UK show that even with abundant P fertilisation, a low P status yields less than a high P status, with less fertilisation. Spring barley benefits more from P fertilisers than grasses, but the interaction between P fertilisation and soil P status on yield response is unclear. Our research aims to investigate these effects on annual cereal and perennial grass yields.

## Materials and methods

The experiment was conducted in Central Finland in 2020–2023 on a clay field with a low soil P status measured by the acid ammonium acetate ( $P_{AAC}$ , pH 4.65). Medium and high P statuses were created by P fertilisation and ploughing in 2017–2018, using a Latin square (3 P status\*3 replicates) design. The main plot was the soil P status, the sub-plot was the plant (timothy ley and spring barley), and the sub-sub-plot was the mineral P fertiliser application (0, 10, 20 and 40 kg P ha<sup>-1</sup> year<sup>-1</sup> in Yara Superphosphate P20). In 2020, timothy (*Phleum pratense*, cv. Nuutti) plots were established using barley as a cover crop. Timothy had three cuts per season with P fertilisation in the spring on the soil surface. Spring barley (*Hordeum vulgare*, cv. Kaarle) was harvested as grain. Grass received 240 kg N ha<sup>-1</sup> and 67 kg K ha<sup>-1</sup>, while barley received 100 kg N ha<sup>-1</sup> and 53 kg K ha<sup>-1</sup>. Grass dry matter yield (DMY) and barley yield, along with P concentration, were measured at each harvest. Soil  $P_{AAC}$  were analysed in the spring of 2020 and autumn of 2023. P balance was determined by the difference between P fertilisation and P uptake. Statistical analyses were calculated using the MIXED procedure of SAS 9.4. In the case of yields, barley and grass were calculated separately.



## Results and discussion

In 2020, cool conditions until mid-June enhanced barley growth, despite uneven rainfall. The 2021 growing season was consistently warmer, but long dry periods seriously weakened the growth of barley. In 2022 and 2023, temperature degree sums from sowing to late June were close to average but ended up higher, while precipitation remained near average. Grass cumulative DM yield from 2021 to 2023 was 27 970 kg ha<sup>-1</sup> (7890+10 570+9510 kg DM ha<sup>-1</sup> respectively), and it remained unaffected by P fertilisation or soil P status. Barley yields reacted significantly to the soil P status. A high soil P status yielded 27% higher cumulative grain yield than a low soil P status, regardless of the amount of P fertilisation (Figure 1). In the low soil P status, 20P and 40P produced 19% and 24% higher cumulative yields than 0P fertilisation, respectively. However, no P fertilisation effect on yield was observed in medium and high P statuses.

A P deficit in cumulative P balance from two to four times greater was formed in medium and high soil P statuses than in low soil P status (Table 1). The P balance of grass was highly negative, whereas it was positive in barley. P fertilisation significantly mitigated the negative P balance. The decrease in soil P concentration indicated by the “difference 2023–2020  $P_{AAC}$ ” was logically aligned with the cumulative P balance. Surprisingly, despite a positive P balance in barley and in fertilisation treatment 40P, the difference in  $P_{AAC}$  between 2023 and 2020 remained negative. There was also an interaction between soil P status and fertilisation in the  $P_{AAC}$  2023: in low P status of soil, fertilisation had no impact on  $P_{AAC}$ . However, in medium P status of soil, 0P and 10P fertilisation resulted in  $P_{AAC}$  levels of 4.6 and 4.3 mg l<sup>-1</sup> lower than P40 respectively. In high P status of soil, 0P and 10P fertilisation led to  $P_{AAC}$  levels 6.0 and 4.8 mg l<sup>-1</sup> lower than P40 respectively, with 0P also being 3.7 mg l<sup>-1</sup> lower than P20 (SEM 0.77).

The decline in  $P_{AAC}$  is aligned with farm-level trends. Based on meta-analyses, low soil P status is expected to decrease barley and grass yields (Valkama *et al.* 2009). However, this experiment and a recent fertilisation experiment have shown no grass yield response to P (Kykkänen *et al.*, 2018). The yield

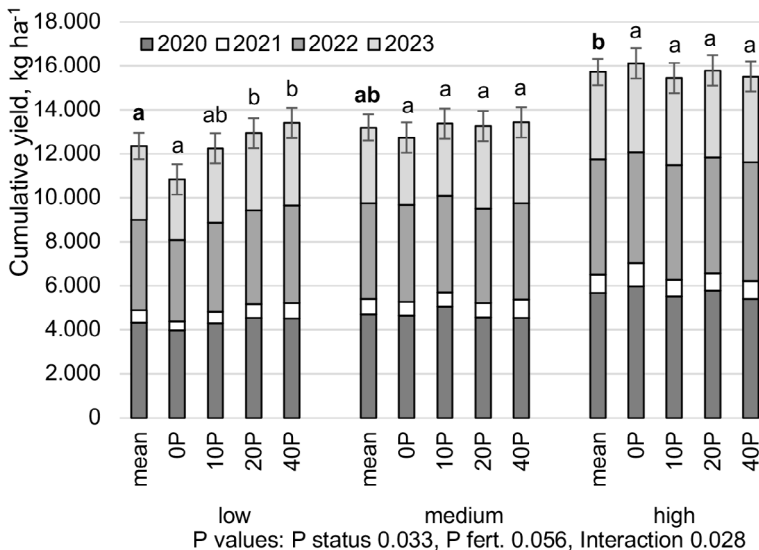


Figure 1. Cumulative barley yields (15% moisture, kg ha<sup>-1</sup>) in low, medium, and high soil P statuses on average (mean,  $n=12$ ) and using P fertiliser levels 0, 10, 20, and 40 kg P ha<sup>-1</sup> year<sup>-1</sup> (0P, 10P, 20P and 40P;  $n=3$ ). Means of each P status (bold letters) and means of each P fertiliser level on each P status separately marked with a different letter differ significantly at  $p \leq 0.05$  (Tukey's test). Error bars are standard error of means (SEM).

Table 1. Soil  $P_{AAC}$  ( $\text{mg l}^{-1}$ ) at the end of the experiment (autumn 2023), the change of soil  $P_{AAC}$  (autumn 2023–spring 2020), and cumulative P balance ( $\text{kg ha}^{-1}$ ) 2020–2023.

Plant	P status	P fert.	Difference		Cumulative 2020–2023
			2023	2023–2020	P balance ( $\text{kg ha}^{-1}$ )
			$P_{AAC}$ ( $\text{mg l}^{-1}$ )	$P_{AAC}$ ( $\text{mg l}^{-1}$ )	
Grass			11.0 <sup>a</sup>	-4.8 <sup>a</sup>	-39 <sup>a</sup>
Barley			11.1 <sup>a</sup>	-2.8 <sup>b</sup>	15 <sup>b</sup>
SEM			1.51	0.81	1.2
	Low		5.0 <sup>a</sup>	-1.2 <sup>c</sup>	-5 <sup>b</sup>
	Medium		11.1 <sup>ab</sup>	-4.2 <sup>b</sup>	-11 <sup>b</sup>
	High		17.1 <sup>b</sup>	-6.0 <sup>a</sup>	-19 <sup>a</sup>
SEM			1.64	0.82	1.5
		0	9.2 <sup>a</sup>	-5.0 <sup>a</sup>	-79 <sup>a</sup>
		10	9.9 <sup>a</sup>	-4.7 <sup>ab</sup>	-41 <sup>b</sup>
		20	11.7 <sup>b</sup>	-3.7 <sup>b</sup>	-2.7 <sup>c</sup>
		40	13.5 <sup>c</sup>	-1.9 <sup>c</sup>	75 <sup>d</sup>
SEM			1.52	0.83	1.1
P values	P status		0.018	<0.001	0.002
	Plant		0.85	<0.001	<0.001
	P fert.		<0.001	<0.001	<0.001
		P status×plant	0.40	0.086	0.22
		P status×P fert.	0.018	0.054	0.067
		Plant×P fert.	0.50	0.021	0.35
		P status×plant×P fert.	0.96	0.30	0.25

SEM=standard error of the mean. Means marked with a different letter differ significantly at  $p \leq 0.05$  (Tukey's test).

response of spring barley to P fertilisation suggests that P requirement of barley temporarily exceeded the soil's capacity to release P from reserves.

## Conclusion

A higher soil P status increased spring barley yield whereas it did not affect grass ley yield. Grass can therefore also take P from soil efficiently at low soil  $P_{AAC}$  levels. In grass rotations including barley, the specific P requirements of barley should be considered. New management methods, e.g. precision farming, intercropping, nutrient management, and plant breeding are required to meet the P needs of barley.

## Acknowledgements

The study was financially supported by Natural Resources Institute Finland (Luke; 2020), the Ministry of Agriculture and Forestry of Finland (Makera), and Yara Suomi Ltd (FOMARE project 2021–2024).

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# Potential health value of alternative plant resources explored as feed for ruminants

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## Abstract

To face current challenges, ruminant feeding systems have to adapt the use of common resources or develop the use of alternative ones. This study explored the potential of alternative plant resources that could be used on farms to provide nutrients with health-promoting abilities for ruminants, i.e. tree leaves (Lutèce elm, common ash, goat willow, white mulberry, Italian alder, black locust), duckweeds, reeds and grass from orchards. Samples were collected in summer 2022 and assayed for condensed tannin, tocopherol, carotenoid, total polyphenol contents and antioxidant activity (DPPH assay). Tree leaves except white mulberry had the highest total polyphenols (67.1 vs 10.9 mg eq gallic acid (g DM)<sup>-1</sup> for other resources) and DPPH values (118 vs 25 mg eq trolox (g DM)<sup>-1</sup> for others). Black locust was the richest in tannins and carotenoids whereas goat willow was the richest in tocopherols. DPPH values were positively correlated with total polyphenols ( $r=0.93$ ) and tocopherols ( $r=0.66$ ), in agreement with their significant antioxidant activity. To conclude, several tree leaves seem to be good sources of metabolites with health-promoting abilities for ruminants.

**Keywords:** tree leaves, reeds, duckweed, polyphenols, antioxidant, carotenoids, tocopherols

## Introduction

Ruminant feeding is facing new and strong challenges due to climate change, environmental footprint of ruminant production and feed-food competition. To face these challenges, ruminant feeding systems have to adapt the use of common resources or develop the use of new resources. Increasing the proportion of pasture in the diet is limited by grassland availability. The use of alternative forages to replace or combine with main forages could fill this grass shortage. Unusual resources available on farms, such as tree leaves, grass from orchards or marsh could constitute good alternative resources for ruminants, having interesting nutritive value (Mahieu *et al.*, 2021), and may also present other benefits as they contain secondary metabolites that are likely to improve animal health and animal product quality (Poutaraud *et al.*, 2017). The aim of this study was to explore the potential of 10 unusual plant resources to be used to provide nutrients with health-promoting abilities for ruminants.

## Materials and methods

Three representative samples of each resource were collected and analyzed. Tree leaves (Lutèce elm, *Ulmus* cv. Nanguen; common ash, *Farinas excelsior* L.; white mulberry, *Morus alba*; Italian alder, *Alnus cordata* (Loisel); black locust, *Robinia pseudoacacia* L.; and goat willow, *Salix caprea* L.) were collected at Lusignan; grass from orchards at Saint-Marcel-Les-Valence; reeds (*Phragmites australis* (Cav.) Trin. ex Steud.) and duckweeds (*Lemna minuta* Kunth and *Lemna gibba* L.) at Saint Laurent de la Prée. All samples were collected in July 2022 except for black locust and goat willow which were collected in August 2022. Plant samples were quickly stored at -20°C, freeze-dried and ground before analysis. Different indicators (Maxin *et al.*, 2018) of animal health potential were assayed in the samples: carotenoids,

tocopherols, total polyphenols (TP) and condensed tannins (CT). Carotenoids and tocopherols were analysed as described in Maxin *et al.* (2020) and CT were determined by the colorimetric HCl–butanol method. A purified CT extract of sainfoin was used as a standard. TP content was determined by the Folin–Ciocalteu method. Results were expressed as mg of gallic acid equivalent per g dry matter (mg eq GA (g DM)<sup>-1</sup>). The antioxidant activity of samples was estimated via the measurement of the free-radical scavenging activity of DPPH\* (2,2-diphenyl-1-picrylhydrazyl). The results were expressed as mg trolox (Tx) equivalent per g DM. Anova was performed to test differences between resources on the indicators assayed (resource as effect, Minitab® version 21). Pearson’s correlation tests were also performed to assess the relationships between TP, CT, carotenoids and tocopherol contents and antioxidant activity.

## Results and discussion

The TP content varied highly with resources and was significantly higher for the tree leaves than for other resources (Table 1). These values obtained for tree leaves were higher than values previously observed for highly diversified natural grassland (Graulet *et al.*, 2012). In agreement with Mahieu *et al.* (2021), black locust had considerably higher CT content (166 mg (g DM)<sup>-1</sup>) than other resources (<49.1 mg (g DM)<sup>-1</sup>), these values being in the range of values previously observed for legume species (Maxin *et al.*, 2020). High dietary CT content (>50 mg g<sup>-1</sup>) is known to have detrimental effects on animal intake (Min *et al.*, 2003). Therefore, its inclusion in the diet should be limited to avoid adverse effects. Significant differences in total tocopherols among the 10 resources were observed. Goat willow and Italian alder had the greatest contents whereas duckweed and grass from orchards had the lowest contents. All resources were richer in  $\alpha$ -tocopherol (>86% of total tocopherols, data not shown) than  $\gamma$ -tocopherol.

A total of 9 carotenoid compounds were identified and quantified in all resources (Table 2). Lutein, violaxanthin and all-E- $\beta$ -carotene were quantitatively the major carotenoids in all resources. These carotenoid profiles are consistent with those observed for natural pastures and legume species (Graulet *et al.*, 2012; Maxin *et al.*, 2020). Black locust was the richest whereas Lutèce elm was the poorest in carotenoids. Antioxidant activity assayed with DPPH method varied from 15.2 to 203.3 mg Tx eq g<sup>-1</sup> DM, underlining important difference in antioxidant supply between resources. DPPH values were positively correlated to TP ( $r=0.93$ ,  $P<0.001$ ), total tocopherols ( $r=0.66$ ,  $P<0.001$ ) and CT ( $r=0.48$ ,  $P=0.008$ ). This suggests that polyphenols and tocopherols present in these resources have a significant contribution in the whole antioxidant activity whereas CT would contribute to a lesser extent.

Table 1. Total polyphenol, condensed tannins and total tocopherol contents, and antioxidant activity of the 10 alternative resources explored.

	TP (mg GA eq (g DM) <sup>-1</sup> )	CT (mg sainfoin CT eq (g DM) <sup>-1</sup> )	DPPH (mg Tx eq (g DM) <sup>-1</sup> )	Total tocopherols (mg (g DM) <sup>-1</sup> )
Black locust	61.2 <sup>b</sup>	166.0 <sup>a</sup>	116.6 <sup>b</sup>	0.28 <sup>d</sup>
Common ash	52.8 <sup>b</sup>	2.0 <sup>d</sup>	77.8 <sup>c</sup>	0.60 <sup>bc</sup>
Duckweed <i>L. gibba</i>	10.4 <sup>d</sup>	1.4 <sup>d</sup>	26.4 <sup>d</sup>	0.10 <sup>d</sup>
Duckweed <i>L. minuta</i>	9.7 <sup>d</sup>	2.3 <sup>d</sup>	30.2 <sup>d</sup>	0.20 <sup>d</sup>
Goat willow	84.2 <sup>a</sup>	32.6 <sup>bc</sup>	134.5 <sup>b</sup>	1.16 <sup>a</sup>
Grass Orchards	14.0 <sup>cd</sup>	2.6 <sup>d</sup>	28.7 <sup>d</sup>	0.13 <sup>d</sup>
Italian alder	90.9 <sup>a</sup>	11.3 <sup>cd</sup>	122.6 <sup>b</sup>	1.01 <sup>a</sup>
Lutèce elm	89.8 <sup>a</sup>	49.1 <sup>b</sup>	203.3 <sup>a</sup>	0.64 <sup>b</sup>
Reeds	9.9 <sup>d</sup>	1.7 <sup>d</sup>	15.2 <sup>d</sup>	0.32 <sup>cd</sup>
White mulberry	23.6 <sup>c</sup>	1.5 <sup>d</sup>	50.2 <sup>cd</sup>	0.31 <sup>cd</sup>
SEM	4.20	8.5	12.6	0.11
<i>P</i> value (species)	<0.001	<0.001	<0.001	<0.001

TP, total polyphenols; CT, condensed tannins. Values in the same column with different superscript are different ( $P<0.05$ )

Table 2. Content in the 5 main carotenoids<sup>1</sup> of the 10 resources explored

$\mu\text{g g}^{-1}\text{ DM}$	Antheraxanthin	Lutein	All E- $\beta$ -carotene	Neoxanthin	Violaxanthin
Black locust	86.7 <sup>ab</sup>	544.7 <sup>a</sup>	163.9 <sup>a</sup>	112.0 <sup>a</sup>	228.5 <sup>a</sup>
Common ash	35.2 <sup>cde</sup>	213.3 <sup>cd</sup>	72.0 <sup>cd</sup>	38.6 <sup>bc</sup>	68.1 <sup>d</sup>
Duckweed <i>L. gibba</i>	47.6 <sup>bcdde</sup>	225.7 <sup>cd</sup>	47.2 <sup>d</sup>	44.7 <sup>bc</sup>	167.1 <sup>abc</sup>
Duckweed <i>L. minuta</i>	39.0 <sup>cde</sup>	498.4 <sup>ab</sup>	81.1 <sup>bcd</sup>	86.6 <sup>ab</sup>	222.4 <sup>a</sup>
Goat willow	68.1 <sup>abc</sup>	392.5 <sup>abc</sup>	129.7 <sup>ab</sup>	76.7 <sup>abc</sup>	112.1 <sup>bcd</sup>
Grass Orchards	35.3 <sup>cde</sup>	262.3 <sup>cd</sup>	69.3 <sup>cd</sup>	50.0 <sup>bc</sup>	113.5 <sup>bcd</sup>
Italian alder	14.9 <sup>e</sup>	392.1 <sup>abc</sup>	119.8 <sup>abc</sup>	62.0 <sup>acd</sup>	63.5 <sup>d</sup>
Lutèce elm	26.2 <sup>de</sup>	170.3 <sup>d</sup>	66.3 <sup>cd</sup>	32.3 <sup>c</sup>	76.8 <sup>cd</sup>
Reeds	64.8 <sup>abcd</sup>	283.2 <sup>bcd</sup>	97.3 <sup>bcd</sup>	54.6 <sup>bc</sup>	164.7 <sup>abc</sup>
White mulberry	95.8 <sup>a</sup>	334.1 <sup>abcd</sup>	95.3 <sup>bcd</sup>	62.6 <sup>abc</sup>	194.7 <sup>ab</sup>
SEM	14.1	76.0	18.5	17.4	32.2
P-value (specie)	<0.001	<0.001	<0.001	<0.001	<0.001

<sup>1</sup>The 4 other minor carotenoids quantified were zeaxanthin, lutein epoxide, 13cis $\beta$ -carotene and 9cis $\beta$ -carotene. Values in the same column with different superscript are different ( $P < 0.05$ ).

## Conclusion

The contents in secondary metabolites and antioxidant activity differed greatly between the resources explored. Several tree leaves would be good sources of secondary metabolites and antioxidants for ruminants. However, *in vivo* trials are required to assess the quantities animals can ingest of these resources and confirm their positive interest for animal health.

## Acknowledgements

This study is part of the ‘SourceN’ project funded by the INRAE cross-disciplinary research program METABIO. The authors acknowledge Noémie Porte and Josiane Portelli for their skills in performing laboratory analysis.

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# Effect of formic acid treatment of grass liquid fraction on protein separation efficiency

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## Abstract

Increased self-sufficiency in feed protein production is needed in European countries for economic, environmental and political reasons. Grass can produce high protein yields per hectare. In a green biorefinery, the protein rich liquid can be separated from the grass biomass. A low-cost method to separate the protein from the grass liquid would be useful. This study investigated the effect of formic acid addition (0, 4, 6, 8 and 10 l Mg<sup>-1</sup>) to grass liquid followed by natural sedimentation on protein separation efficiency. The experiment was conducted in 500 ml bottles for up to 21 days. In the control treatment, natural fermentation occurred, and organic acids (lactic acid, acetic acid) were produced that lowered the pH. Results showed that the sediment accounted for, on average, 228 g kg<sup>-1</sup> (SD 2.7) of the original fresh weight across formic acid treatments and 196 g kg<sup>-1</sup> for the control. The formic acid addition increased the recovery of true protein from 800 to 946 g kg<sup>-1</sup> in the sediment, but N recovery decreased with increased level of formic acid. This low-cost method could be used in concentrating the fresh grass liquid from biorefinery for further processing or for direct use as a semi-liquid feed.

**Keywords:** biorefinery, grass protein, liquid feed, sedimentation, sustainability

## Introduction

There is a growing necessity for self-sufficiency in feed protein production in Europe driven by environmental, economic and political reasons. In forages, the soluble nutrients are bound within the fibre matrix of plant cell walls and are mostly unavailable for monogastric animals. Through biorefinery, the green biomass can be separated into a protein rich liquid fraction and a fibrous solid fraction. The protein-rich liquid can be fed to animals directly, but its high water content limits its inclusion rate in diets and causes high transportation costs, restricting its use to liquid feeding systems. To increase protein density, the liquid fraction can be heated to coagulate the protein, mechanically processed to separate the coagulated protein, and then dried. The dry concentrate is easy to transport and can be incorporated to diets of all animals (pigs, poultry and ruminants), but the separation process is expensive (the heat treatment, mechanical separation and final drying require specialised equipment and have high energy consumption), and losses occur at all steps of processing. The aim of this preliminary study was to evaluate a low-cost low-input method to concentrate the protein in the liquid fraction of fresh grass biomass by coagulating the soluble protein with addition of formic acid and to use natural sedimentation process to concentrate the protein content in a small volume of liquid.

## Materials and methods

This study was performed using liquid fraction from pure timothy grass (*Phleum pratense*) harvested in second cut (31 July 2023) in Jokioinen, Finland (60°48' N 023°29' E). The green biomass was separated into liquid and solid fractions using a pilot scale single screw press (MAS SP300 filter press, Smicon, Haderslev, Denmark), and the liquid yield was 27.5% on fresh matter basis. Five levels (0, 4, 6, 8, 10 l Mg<sup>-1</sup>) of formic acid addition were tested with three replicates for each level of addition. The experiment was carried out in 0.5-l glass bottles. Each bottle was filled with 500 g of grass juice and corresponding addition of formic acid (0, 0.2, 0.3, 0.4 and 0.5 ml of pure formic acid). The bottles were closed with rubber plugs and metal nuts. After closing, the bottles were shaken to mix the additive, and the shaking

was repeated after 6 and 24 h. The bottles with formic acid treatment were allowed to stand in dark place with room temperature for 21 days and the bottles without formic acid (control) were opened after 6 days to reduce the effect of natural fermentation on the pH of the liquid fraction.

After sedimentation, two distinct layers could be observed: a transparent brown liquid layer on the top and a green non-transparent layer on the bottom of the bottles. When bottles were opened, the clear brown liquid at the top was removed using vacuum to avoid mixing with the sediment. The brown liquid was analysed for pH, total N and true protein. Then the sediment was weighed, mixed, and analysed for dry matter and ash content. The data were analysed by ANOVA using the mixed procedure of SAS (version 9.4) with a model including the main effect of formic acid addition. The linear and quadratic trends of formic acid addition levels were evaluated using orthogonal polynomial contrasts.

## Results and discussion

The sedimentation could be observed already 2 h after the addition of formic acid. After four days, no more progress in the sedimentation process was visually observed in the formic acid treated vessels. In control vessels, the sedimentation could be observed after 12 h and no progress in sedimentation was observed after 5 days. On average 228 (SD 2.7) and 196 g kg<sup>-1</sup> (SD 34.6) of starting weight of the liquid was recovered as protein rich sediment for the formic acid and control treatments, respectively. The extraction rate of crude protein was the highest (Table 1) for control (630 g kg<sup>-1</sup>) and it decreased linearly with increasing level of formic acid addition. It has previously been shown that soluble protein is coagulated by acid treatment (Näsi, 1983), but also that strong acid will hydrolyse protein (Tsugita *et al.*, 1982).

The decreased recovery of N with increased level of formic acid addition may indicate that part of the soluble protein was hydrolysed. This statement is supported by the pH data (linear and quadratic effect) showing that the pH dropped from on average 4.4 in control vessels to 3.2 with the highest level of formic acid addition (Table 1). The pH of the control vessels indicated that substantial natural fermentation occurred, and organic acids (lactic acid, acetic acid) were produced that lowered the pH. The recovery of true protein in the sediment (linear and quadratic effect) increased from 800 g kg<sup>-1</sup> in control vessels to on average 946 g kg<sup>-1</sup> (SD 5.0) in formic acid treatments. The dry matter recovery in sediment (Table 1) increased in linear and quadratic manner from 309 in control to on average 437 g kg<sup>-1</sup> (SD 10.7) in formic acid treated samples, indicating that a relatively low level of formic acid addition could already be sufficient. The ash recovery in sediment (Table 1) was on average 285 g kg<sup>-1</sup> (SD 19.3) across all treatments, which was lower than that of protein. This would reduce the ash load of the feed thus allowing for higher inclusion rates in pig diets, where high potassium concentrations can be problematic (Keto *et al.*, 2021; Rinne *et al.*, 2018)).

Table 1. Effect of formic acid addition of grass liquid fraction on extraction rates of crude protein, dry matter and ash into the sediment.

	Formic acid (l Mg <sup>-1</sup> )					SEM	P-value	
	0	4	6	8	10		Linear	Quadratic
Brown liquid pH	4.41	3.70	3.49	3.33	3.21	0.01	<0.001	<0.001
Extraction rates (g kg fresh liquid) <sup>-1</sup>								
Dry matter	309	442	435	437	435	6.9	<0.001	<0.001
Ash	301	283	280	299	261	6.8	0.017	0.600
Crude protein	630	580	540	510	500	10.8	<0.001	0.187
True protein	800	942	953	947	942	18.5	<0.001	0.004

SEM, standard error of the mean.

The formic acid treatment also offers other benefits in addition to protein sequestration. Many commercial silage additives are based on formic acid (Muck *et al.*, 2018), so it will contribute to both the sediment and the brown juice hygienic quality and stability. Additionally, formic acid is commonly used in pig liquid feeding systems to preserve the feed and to improve the gut health of the animals (Nguyen *et al.*, 2020). If formic acid-containing protein feed is used, it will reduce the need for separate formic acid addition, which would be an additional benefit and cost saving.

## Conclusion

Formic acid-assisted natural sedimentation method could be very useful in concentrating the fresh liquid from biorefinery for further treatment or for direct use as a semi-liquid feed. More research is needed to optimize the dose of formic acid for protein sedimentation in the liquid fraction from a green biorefinery.

## Acknowledgement

This work was conducted as part of GrassProtein project funded by the Finnish Ministry of Agriculture and Forestry, MAKERA, (VN/7679/2021).

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# Grass for biorefinery: Effects of N fertilization and harvest time on liquid yield and composition

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## Abstract

Grasses contain a considerable amount of protein. Using the green biorefinery concept, protein can be mechanically separated from the biomass to the liquid fraction. This approach would increase self-sufficiency in feed protein production and contribute to the environmental and economic sustainability of animal production. The main aim of this experiment was to study the effect of the N-fertilizing level and harvest time of grass biomass on yield and composition of the liquid fraction. An experiment was set up to collect grass biomass from first and second cuts. Both cuts were harvested three times at one-week intervals. The first cut was fertilized with three levels of N (100, 130 and 160 kg ha<sup>-1</sup>) in spring, and the second cut with two levels of N (110 and 140 kg ha<sup>-1</sup>). The biomass was separated into solid and liquid fractions using a laboratory-scale twin-screw press. With increasing level of N fertilizer, the dry matter concentration of grass decreased, and the liquid and N yields increased. The liquid yields varied from 3085 to 7764 and from 7365 to 13 983 kg ha<sup>-1</sup> and the liquid N yields from 8.3 to 19.1 and from 17.0 to 29.1 kg ha<sup>-1</sup> in the first and second cut, respectively.

**Keywords:** green biorefinery, liquid-solid separation, regrowth, primary growth, N recovery

## Introduction

Grasses contain a considerable amount of protein. Furthermore, grassland provides many positive ecosystem services. Using the concept of green biorefinery, the protein can be mechanically separated from the biomass to the liquid fraction. Protein in the liquid fraction can be used to feed pigs and poultry, but also after further processing it could even be used in novel human foods. Green biorefineries would increase self-sufficiency in feed protein production but also the environmental and economical sustainability of animal production. The yield and quality of protein in liquid and fibre fraction from biorefinery may vary depending on weather conditions, N fertilizing level and time of harvesting of the green biomass. The aim of the current experiment was to study the effect of N fertilizing level and harvest time of pure timothy grass (*Phleum pratense*) on yield and composition of the liquid fraction from the primary growth and first regrowth.

## Materials and methods

The green biomass of pure timothy grass grown in Jokioinen, Finland (60°48' N 023°29' E) was used from first and second cut in the year 2023. Both cuts had three harvest times at one-week intervals (at 5, 12 and 19 June in the first cut, and 25 and 31 July and 7 August in the second cut). Additionally, the first cut was fertilized in spring with 3 levels of N (100, 130 and 160 kg ha<sup>-1</sup>). The area for the second cut experiment was harvested on 15 June and then fertilized with 2 levels of N (110 and 140 kg ha<sup>-1</sup>). There were 3 replications for each treatment in each harvest time. The growing conditions were cool and dry for the first cut but humid and warm for the second cut. From each experimental plot, 15 m<sup>2</sup> was harvested using a forage plot harvester (Haldrup, Ilshofen, Germany). The harvested biomass was weighed and analysed for dry matter (DM) and feed quality. The samples for biorefinery were frozen within 2 h after harvest. Prior to the processing, the samples were allowed to thaw for 48 h at +4°C. Biomass was separated into solid and liquid fractions using a laboratory-scale twin-screw press (Angel Juicer, Busan, South Korea) by processing 500 g of each sample. The liquid yield was recorded, and samples of the liquid

fraction were analysed for DM, ash, total N and true protein contents. Experimental data were analysed by ANOVA using the mixed procedure of SAS (version 9.4) with a model that contained fixed effects of harvest time, N fertilizer level and their interaction separately for both cuts. Effects were considered significant at  $P < 0.05$ .

## Results and discussion

The cool and dry growing conditions for the first cut resulted in low fresh yield (6000–14 000 kg ha<sup>-1</sup>) and dry matter yield (1800–3200 kg DM ha<sup>-1</sup>) compared to typical yields of around 5000 kg DM ha<sup>-1</sup> in experiments for the first cut (Luke, 2024; Termonen *et al.*, 2020). The DM concentration in the first harvest ranged from 250 to 300 g kg<sup>-1</sup>. Humid and warm condition during the growth of the second cut resulted in higher and more typical yields (fresh yield range of 13 000 to 23 000 kg ha<sup>-1</sup> and DM yield 3100–5000 kg ha<sup>-1</sup>) and lower DM concentration in the yield (200–240 g kg<sup>-1</sup>). With increasing level of N fertilizer, the DM of fresh grass decreased and the liquid yield per kg biomass and per hectare increased (Table 1).

Table 1. Effect of N fertilizer level and harvest time on grass composition and liquid yield.

Harvest time	N fertilizer (kg ha <sup>-1</sup> )	Grass DM (g kg <sup>-1</sup> )	Liquid yield (g kg <sup>-1</sup> )	Liquid DM (g kg <sup>-1</sup> )	Liquid ash (g (kg DM) <sup>-1</sup> )	Liquid yield (kg ha <sup>-1</sup> )	Liquid N yield (kg ha <sup>-1</sup> )
First cut							
June 5	100	299 <sup>ab</sup>	528 <sup>abcd</sup>	184 <sup>a</sup>	88 <sup>d</sup>	3085 <sup>e</sup>	8.3 <sup>e</sup>
	130	290 <sup>b</sup>	539 <sup>abc</sup>	178 <sup>a</sup>	86 <sup>d</sup>	3295 <sup>e</sup>	10.3 <sup>cde</sup>
	160	289 <sup>b</sup>	546 <sup>ab</sup>	171 <sup>a</sup>	98 <sup>cd</sup>	3439 <sup>de</sup>	11.9 <sup>cd</sup>
June 12	100	314 <sup>a</sup>	491 <sup>d</sup>	189 <sup>a</sup>	97 <sup>cd</sup>	3739 <sup>de</sup>	8.4 <sup>ce</sup>
	130	297 <sup>ab</sup>	503 <sup>cd</sup>	175 <sup>a</sup>	101 <sup>cd</sup>	4306 <sup>cd</sup>	10.8 <sup>cde</sup>
	160	294 <sup>ab</sup>	516 <sup>bcd</sup>	176 <sup>a</sup>	99 <sup>cd</sup>	4714 <sup>c</sup>	12.9 <sup>bc</sup>
June 19	100	265 <sup>c</sup>	553 <sup>ab</sup>	135 <sup>b</sup>	116 <sup>bc</sup>	6502 <sup>b</sup>	13.4 <sup>bc</sup>
	130	262 <sup>c</sup>	561 <sup>a</sup>	128 <sup>b</sup>	124 <sup>ab</sup>	6908 <sup>ab</sup>	16.3 <sup>ab</sup>
	160	250 <sup>c</sup>	567 <sup>a</sup>	118 <sup>b</sup>	143 <sup>a</sup>	7764 <sup>a</sup>	19.1 <sup>a</sup>
SEM		6.4	9.2	7.5	4.1	315.7	1.18
Second cut							
July 25	110	244 <sup>a</sup>	555 <sup>b</sup>	96 <sup>a</sup>	201	7365 <sup>b</sup>	17.0 <sup>b</sup>
	140	224 <sup>ab</sup>	576 <sup>ab</sup>	91 <sup>a</sup>	185	8693 <sup>b</sup>	24.1 <sup>ab</sup>
July 31	110	206 <sup>b</sup>	631 <sup>a</sup>	82 <sup>b</sup>	171	12100 <sup>a</sup>	23.1 <sup>ab</sup>
	140	198 <sup>b</sup>	635 <sup>a</sup>	73 <sup>cd</sup>	190	13983 <sup>a</sup>	29.1 <sup>a</sup>
August 7	110	225 <sup>ab</sup>	578 <sup>ab</sup>	78 <sup>bc</sup>	165	12816 <sup>a</sup>	22.4 <sup>ab</sup>
	140	218 <sup>ab</sup>	603 <sup>ab</sup>	70 <sup>d</sup>	190	13939 <sup>a</sup>	26.9 <sup>a</sup>
SEM		6.9	12.1	1.4	13.5	504.7	1.46
<i>P</i> -value							
First cut vs second cut		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
First cut	Harvest time	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	N fertilizer	0.002	0.036	0.006	0.003	<0.001	<0.001
Second cut	Harvest time	0.002	<0.001	<0.001	0.485	<0.001	0.007
	N fertilizer	0.060	0.115	<0.001	0.405	0.004	<0.001

Values with the same letter in a column are not significantly different ( $P < 0.05$ ) based on Tukey's test. If there are no differences in Tukey test, letters are removed. SEM, standard error of mean.

The liquid yield varied between 491 and 567, and from 555 to 635 g kg<sup>-1</sup> original fresh biomass in first and second cut, respectively. The total liquid yield varied from 3085 to 7764 kg ha<sup>-1</sup> and from 7365 to 13 983 kg ha<sup>-1</sup> in first and second cut, respectively. The ash content of liquid ranged from 86 to 143 and from 165 to 201 g kg<sup>-1</sup> DM in first and second cut, respectively (Table 1). Marked effect of N fertilizer level and harvest time was observed for almost all measured parameters in first cut (Table 1). The highest N yields (19.1 and 16.3 kg ha<sup>-1</sup>) were observed in last harvest for the two highest N levels (160 and 130 kg ha<sup>-1</sup>, respectively). In the second cut, the highest N yield was observed at second harvest with the highest N fertilizer level (29.1 kg ha<sup>-1</sup>). Due to dry growing conditions in the early summer, the impacts of increased N fertilizer rates on fresh matter, DM and N yields were much smaller than anticipated. The N yield balance (difference between N applied in fertilizer and harvested) was strongly positive which indicates that N fertilizer was only partially utilised. Usually, the balance is close to zero. Positive N yield balance will reduce the N yield in biomass and the N yield in liquid fraction. The true protein yield (Figure 1A) from first cut increased with increasing N fertilizer level and with harvest time and reached 12.7 kg ha<sup>-1</sup> at the last sampling time (19 June) with N fertilizer of 160 kg ha<sup>-1</sup>. In the second cut, the true protein yield (Figure 1B) followed the same pattern as N yield by increasing with N fertilizer level, reaching the maximum of 16.3 kg ha<sup>-1</sup> at the second harvest time.

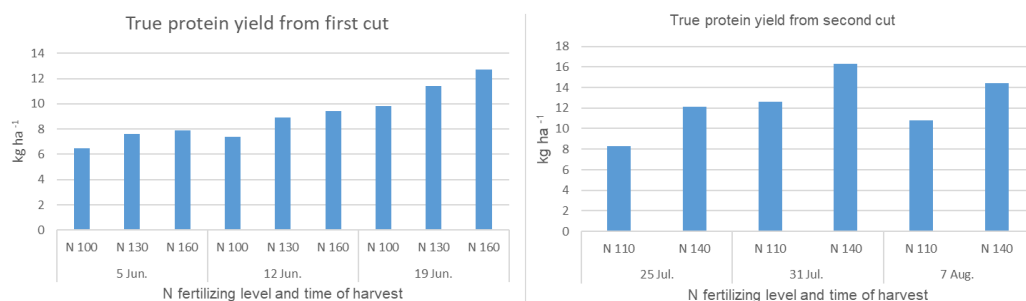


Figure 1. Effect of N fertilizer level and harvest time of timothy grass N yield as true protein in first (A) and second (B) cut.

## Conclusion

The harvest time and N fertilizing level strongly affected the N yield in liquid fraction and the true protein yield from liquid fraction per hectare. The observed yields were low, but by optimizing the harvest time and N fertilizing level, could be manipulated. In a biorefinery set-up, the other fractions of the grass biomass will also have added-value use so that the competitiveness of the process does not solely depend on the liquid protein extraction.

## Acknowledgement

This work was conducted as part of GrassProtein project funded by the Finnish Ministry of Agriculture and Forestry / Makera (VN/7679/2021).

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# Energy requirements and energy supply of dairy cows during early lactation in pasture-based systems

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## Abstract

The objective of the current study was to measure energy requirements and intake profiles for grazing dairy cows in early lactation. Cows were offered a daily herbage allowance to achieve a post-grazing sward height of 4 cm with 3 kg concentrate/cow/day. They were offered silage supplementation when necessary during periods of high rainfall which reduced intake due to difficult grazing conditions. Individual energy (UFL; unité fourragère lait) requirements were calculated based on energy required for milk production, maintenance and growth (< 40 months); UFL supply was calculated based on measured dry matter intake and the UFL content of the feeds. Week of lactation and parity had a significant effect on UFL requirement, primiparous (parity 1) animals had the lowest UFL requirement (15.9 UFL cow<sup>-1</sup> day<sup>-1</sup>), followed by second (parity 2) and third parity (parity 3) animals (17.8 and 19.0 UFL cow<sup>-1</sup> day<sup>-1</sup>, respectively). The UFL supply followed the same trend with parity 1, 2 and 3 animals consuming 12.0, 15.4 and 16.4 UFL cow<sup>-1</sup> day<sup>-1</sup>, respectively. This difference in energy intake and demand resulted in cows being in a state of negative energy balance and led to bodyweight loss of 1.05 kg/cow/day over the first 12 weeks of lactation.

**Keywords:** spring grassland management, energy balance, dry matter intake

## Introduction

In early lactation, it is essential to meet the nutritional requirements of dairy cows in order to achieve production potential and good health and fertility (Jørgensen *et al.*, 2016; Mekuriaw, 2023). Energy requirements increase rapidly during early lactation as milk production increases (Ingvartsen and Anderson, 2000). Intake capacity is also lower as dry matter intake (DMI) is reduced during late pregnancy (Mekuriaw, 2023) and changes in metabolic status to support the onset of lactation (Ingvartsen and Anderson, 2000). This difference in actual energy supply and energy requirement to support milk production and maintenance can lead to cows entering a state of negative energy balance (NEB), which can lead to bodyweight (BW) loss (Ingvartsen and Anderson, 2000).

## Materials and methods

The experiment was carried out at the Teagasc Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Ireland over a 12-week period across two years (2021 and 2022). A total of 80 spring-calving dairy cows (20 primiparous and 60 multiparous) were randomised and placed on trial as they calved based on breed (Holstein Friesian (HF) and Jersey×Holstein Friesian (JeX)), parity (2.3±0.86, means±SD), calving date (15 February±19 days SD), pre-experimental milk production (18.4±3.75 kg cow<sup>-1</sup>) and BW (547±69.9 kg). Animals were also balanced within individual parity groups. Grazing began on 1 February in both years and the stocking rate was 2.6 livestock units (LU) ha<sup>-1</sup>. Animals were offered an average daily herbage allowance to achieve a post grazing sward height of 4 cm (12 kg DM cow<sup>-1</sup> day<sup>-1</sup>) plus 3 kg concentrate cow<sup>-1</sup> day<sup>-1</sup>. Daily herbage allowance was calculated each day using measured pre-grazing herbage mass (measured using an Etesia mower to cut 2 strips per paddock) to a target of 4 cm and was adjusted daily depending on the previous day's post-grazing sward height which was measured using a rising plate meter (Jenquip, Feilding, New Zealand). Silage supplementation (of

known energy concentration) was offered when necessary due to inclement weather with an average of 3 kg DM silage cow<sup>-1</sup> day<sup>-1</sup> offered throughout the experiment. Milk yields were measured daily and milk composition and BW were measured weekly. Individual DMI was measured on week 2, 4, 6, 8, 10 and 12 of the experiment using the N-alkane technique as described by Mayes *et al.* (1986) which was used to calculate energy intake using the net energy system (Jarrige, 1989) with the UFL intake of grass, silage and concentrates. Energy requirement was based on UFL required for milk production, maintenance (which accounted for energy required for grazing and walking), growth (<40 months) and BW change (Faverdin *et al.*, 2007). Energy balance for individual animals was calculated as the difference between estimated energy requirements and estimated energy intake. Data were analysed using PROC MIXED in SAS 9.4 (SAS Institute, Cary, NC, USA). Week of lactation (WOL), breed, parity, year and associated interactions were included as fixed effects, animal was included as a random effect.

## Results and discussion

Similar to Ingvartsen and Anderson (2000), there was a rapid increase in energy requirement from week 1 to 3 of lactation for parity 1, 2 and 3 animals (+0.8, +0.9 and +1.3 UFL cow<sup>-1</sup>, respectively), followed by a decrease of 0.33 UFL/cow/week until week 12. There was an interaction between WOL and parity (Figure 1): UFL requirement was the same for parity 2 and 3 animals on week 1 and 2; however, parity 3 animals had significantly greater UFL requirement from week 3 onwards due to greater UFL requirements for milk production and maintenance. There was also an interaction between WOL and breed as UFL requirements for HF animals were greater for week 1 and 2 of lactation, while JeX animals had a greater UFL requirement thereafter (+0.5 and +0.4 UFL cow<sup>-1</sup> day<sup>-1</sup>). This may be due to HF cows mobilising more energy during early lactation compared to Jerseys (Friggens *et al.*, 2007).

Parity 3 animals had the greatest UFL intake, followed by parity 2, and parity 1 animals had the lowest UFL intake (16.4, 15.4, and 12.0 UFL cow<sup>-1</sup> day<sup>-1</sup>, respectively) (Figure 2), which was caused by greater TDMI for parity 2 and 3 animals (16.8 and 17.7 kg DM cow<sup>-1</sup> day<sup>-1</sup>, respectively) compared to parity 1 animals (13.2 kg DM/cow/day). The UFL intake increased by 0.65 UFL cow<sup>-1</sup> week<sup>-1</sup> from week 2 to 6 and reduced to +0.29 UFL cow<sup>-1</sup> from week 7 to 12 of lactation. In the current study, cows lost an average of 88 kg between week 1 and 12. The difference in UFL requirement and intake lead to parity 1 animals being in a state of NEB until week 12 (-3.2 UFL cow<sup>-1</sup> day<sup>-1</sup>), while parity 2 and 3 animals were in NEB until week 10 (-3.0 UFL cow<sup>-1</sup> day<sup>-1</sup>). The negative effects of NEB and the resulting BW loss can be more pronounced in pasture-based systems (Vance *et al.*, 2012).

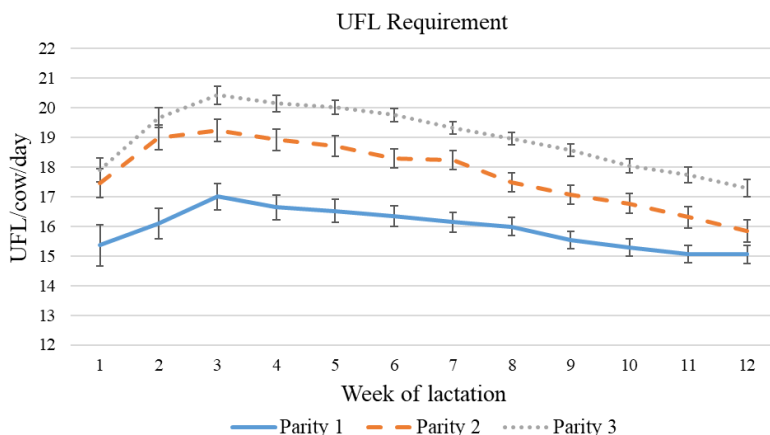


Figure 1. The interaction between parity and week of lactation on the UFL requirement of dairy cows from week 1 to week 12 of lactation. Data represent mean values, whiskers standard error.

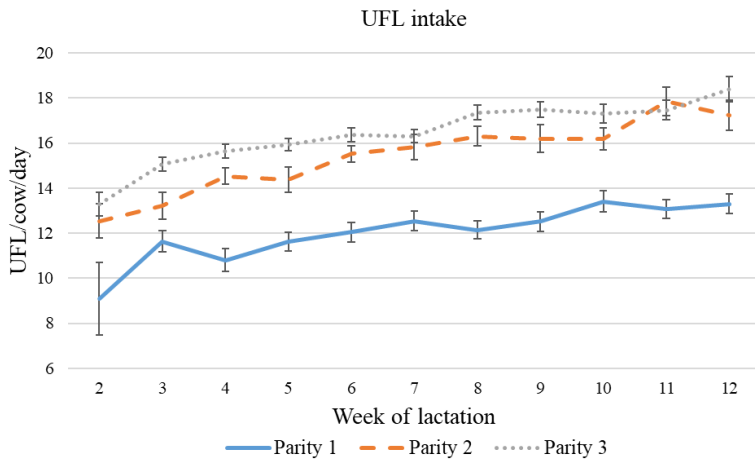


Figure 2. The interaction between parity and week of lactation in the UFL intake per cow and day. The data represent mean values, whiskers standard error.

## Conclusions

In a pasture-based production system, dairy cows were in a state of NEB from week 1 to week 11 of lactation, which led to a reduction in BW during the first 12 weeks of lactation. Parity 1 animals remained in a state of NEB longer than parity 2 and 3 animals due to the partitioning of energy towards growth. The UFL intake and requirements vary significantly between parities as UFL requirements for milk production and maintenance increased with parity; however, breeds did not differ in UFL intake or UFL requirement.

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# Effects of foliar fertilization on sward yield and quality of grass-legume mixtures

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## Abstract

Foliar fertilizers applied onto grass-legume mixtures can play an important role in grassland-based production systems. There are many products on the market that can be used as foliar fertilizers and biostimulants. However, there is a need to evaluate their beneficial effects in different forage crops and sites. The experiment was established in 2019 in Brody Experimental Station of PULS in a two-factor field experiment with two mixtures (grass-clover and grass-lucerne) and five foliar fertilization treatments (composed by Plonvit Z fertilizer and Tytanit biostimulant). During the experimental years (2020–2021) the herbage biomass in the whole plot was harvested three times per year to determine annual dry matter yield. The botanical composition of the first regrowth was determined by manual separation of sampled biomass. Concentrations of protein, sugar, crude ash, phosphorus, calcium and magnesium in herbage were also determined. It was found that the foliar application of Plonvit Z fertilizer and Tytanit biostimulant resulted in an increase in annual dry matter yield, regardless of the mixtures, by on average of 14–18%; however, the increase was only statistically significant in the first cut of 2020. Their application only had a small effect on the sward botanical composition but had no significant effect on herbage quality.

**Keywords:** grass-legume mixture, foliar fertilizers, dry matter yield, herbage quality

## Introduction

The need to optimise and improve the use of fertilisers is a part of sustainable forage production on grasslands (Huyghe *et al.*, 2014). One form of fertilization that has recently gained importance is foliar application of fertilizers, mainly consisting of microelements and biostimulants (Li *et al.*, 2022) representing numerous products on the market. While biostimulators and microelements are widely investigated in arable crops, research on the topic in grasslands is limited and inconsistent. The aim of this study was to determine the effects of Plonvit Z fertilizer and the Tytanit plant growth biostimulant on the sward botanical composition, yield and herbage quality of two selected grass-legume mixtures.

## Materials and methods

A study was carried out during 2020–2021 at Brody Experimental Station (52°43'24" N, 16°30'31" E) of PULS. The experiment was established in early spring 2019 on Luvisols soil (pH<sub>KCl</sub> 5.9, total N 0.13%, P<sub>2</sub>O<sub>5</sub> 29.4 mg (100 g)<sup>-1</sup>, K<sub>2</sub>O 17.9 mg (100 g)<sup>-1</sup>, Mg 5.8 mg (100 g)<sup>-1</sup>) on 10 m<sup>2</sup> (1 × 10 m) plots in a two-factor field experiment in which two mixtures (grass-clover and grass-lucerne) and five foliar fertilization (FF) treatments for each regrowth in BBCH 22–29 were investigated (Table 1). Chemical composition of the foliar fertilizer Plonvit Z included (in g dm<sup>-3</sup>): 195 N, 26 MgO, 59 SO<sub>3</sub>, 0.18 B, 11.7 Cu, 10.4 Fe, 14.3 Mn, 0.065 Mo, 13 Zn and 0.26 Ti. Tytanit® is a patented yield biostimulant from Intermag, containing 8.5 g dm<sup>-3</sup> Ti in the form of a unique molecule aTIUM®. Foliar fertilization was performed with a manual sprayer and the amount of working liquid was 300 l ha<sup>-1</sup>.

Table 1. Experimental factors and their levels used in the study.

Experimental factor	Factor level	Description
1. Grass-legume mixture	Grass-clover	<i>Lolium perenne</i> (Lp) 2n late 15%, <i>Festulolium braunii</i> (Fb) 15%, Lp 4n early 10%, Lp 4n medium 10%, <i>Lolium hybridum</i> (Lh) 10%, <i>Festuca pratensis</i> (Fp) 10%, <i>Phleum pratense</i> (Php) 10%, <i>Festuca arundinacea</i> (Fa) 5%, <i>Trifolium pratense</i> (Tp) 10%, <i>Trifolium repens</i> (Tr) 5%
	Grass-lucerne (more resistant to drought)	<i>Lolium perenne</i> Lp 28%, <i>Lolium multiflorum</i> (Lm) 10%, <i>Phleum pratense</i> Php 15%, <i>Dactylis glomerata</i> (Dg) 10%, <i>Festuca arundinacea</i> Fa 10%, <i>Festuca rubra</i> (Fr) 10%, <i>Festuca trachyphylla</i> (Ft) 5%, <i>Agrostis gigantea</i> (Ag) 2%, <i>Trifolium pratense</i> Tp 5%, <i>Medicago sativa</i> (Ms) 5%
2. Foliar fertilization	1	Without foliar fertilization
	2	Plonvit Z 2.0 l ha <sup>-1</sup>
	3	Tytanit 0.4 l ha <sup>-1</sup>
	4	Plonvit Z 2.0 l ha <sup>-1</sup> +Tytanit 0.4 l ha <sup>-1</sup>
	5	Plonvit Z 2.0 l ha <sup>-1</sup> +Tytanit 0.4 l ha <sup>-1</sup> +additives (solution of urea 5.0 kg ha <sup>-1</sup> and magnesium sulphate 5.0 kg ha <sup>-1</sup> )

The sward was managed by cutting three times each year. Basic fertiliser was applied each year: N – 90 kg ha<sup>-1</sup> (30 kg ha<sup>-1</sup> in spring and after 1<sup>st</sup> and 2<sup>nd</sup> cut), P – 60 kg ha<sup>-1</sup>, K – 90 kg ha<sup>-1</sup>. The yearly mean temperature and total precipitation for 2020 and 2021 in Brody were 8.5°C, 599 mm and 10.4°C, 396 mm, respectively. The botanical composition of the first regrowth was determined by manual separation of harvested biomass. For each plot, biomass of aboveground vegetation was measured at each harvest. This was done by cutting the whole plot to a height of 5 cm and determining the fresh weight of the sward. A subsample of this material was taken, its fresh weight was determined and the material was dried at 65°C to constant weight to measure dry matter (DM). The samples collected for DM were ground to pass through a sieve of 1 mm of mesh size and used for forage quality analysis. Concentration of crude protein (CP) based on (total N content by Kjeldahl)×6.25 (only in 1st cut), sugars (WSC) using colorimetric method by Dubois, crude ash (Ash), calcium (Ca), magnesium (Mg) and phosphorus (P) using standard analytical methods in dry matter of herbage were analysed. Statistical analyses of the data were done by a two-way ANOVA considering grass-legume mixture and foliar fertilization as factors for each cut and year separately. The differences between means were evaluated using the Tukey’s HSD test ( $P < 0.05$ ).

## Results and discussion

During the two-year study, the sward botanical composition (Figure 1) of the mixtures interacted in accordance with the expected species biology.

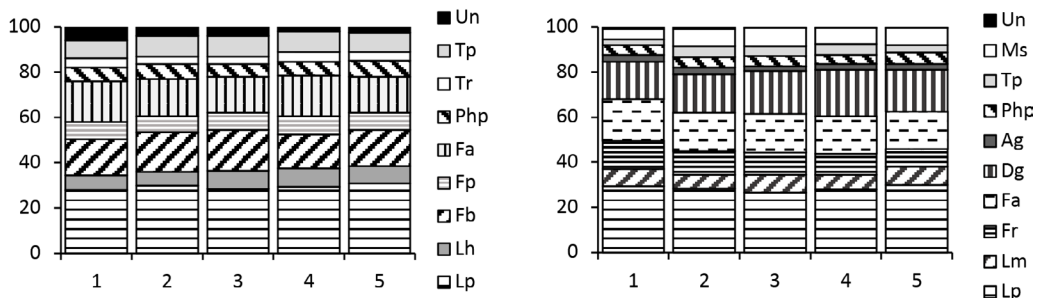


Figure 1. Effect of foliar fertilization treatments (x-axis) on sward botanical composition (y-axis; in %) of grass-clover (left) and grass-lucerne (right) mixture averaged for first regrowth over years 2020-2021. (1, without FF; 2, Plonvit Z; 3, Tytanit; 4, Plonvit Z+Tytanit; 5, Plonvit Z+Tytanit+additives).



In both mixtures, unsown species/weeds (Un) invasion was higher in the second than in the first year. However, we observed considerably lower invasion of weeds in the grass-clover mixture in the treatments Plonvit Z+Tytanit and Plonvit Z+Tytanit+additives compared to other FF treatments. In the grass-lucerne mixture the proportion of Ms was higher by 2-6% in FF treatments compared to the treatment without FF.

In the first cut of 2020, we found a significant difference ( $P<0.01$ ) in sward DM yield between FF treatments in both mixtures (Table 2). In treatments without FF and Plonvit Z, a significantly lower DM yield was obtained compared to the Tytanit, Plonvit Z+Tytanit and Plonvit Z+Tytanit+additives treatments in grass-clover and -lucerne by 23% and 30%, respectively. The positive effect of Tytanit with combination with mineral nitrogen fertilizer on DM yield of Fb was also reported by Malinowska *et al.* (2020). In the remaining cuts in the study years, we observed a tendency to increase the DM yield as a result of the use of FF compared to the control treatment by 18.2% (grass-clover) and 13.8% (grass-lucerne), however, this was not statistically confirmed.

Analysing the effects of FF on herbage quality, we observed only some trends in the concentration of the selected elements of chemical composition of grass-legume mixtures. The herbage of grass-legume mixtures treated with FF had higher CP, ash and P (grass-lucerne) contents and lower Ca and WSC (grass-lucerne) contents. The differences between no FF vs. FF treatments were not statistically significant.

Table 2. Sward DM yield ( $\text{t ha}^{-1}$ ) of grass-legume mixtures in three cuts of 2020 and 2021.

FF	Grass-clover						Grass-lucerne					
	2020			2021			2020			2021		
	1	2	3	1	2	3	1	2	3	1	2	3
1	3.800 a	2.930	2.350	4.150	2.427	1.815	3.360 a	1.810	1.310	4.797	2.528	1.605
2	3.880 ab	3.530	2.640	5.589	2.869	2.426	3.710 ab	1.560	1.280	4.254	2.488	1.583
3	4.540 c	3.370	2.440	5.841	2.710	2.217	4.350 c	1.680	1.640	6.153	2.913	2.029
4	4.940 c	3.500	2.230	5.313	2.469	2.234	4.770 c	1.880	1.460	5.363	2.733	2.313
5	4.640 c	3.720	2.070	4.864	2.670	1.912	4.670 c	2.070	1.320	5.633	2.466	1.815
P	<0.01	ns	ns	ns	ns	ns	<0.01	ns	ns	ns	ns	ns

ns, non significant; FF, foliar fertilization: 1, without FF; 2, Plonvit Z; 3, Tytanit; 4, Plonvit Z+Tytanit; 5, Plonvit Z+Tytanit+additives. Different letters indicate significantly different means ( $P<0.05$ ).

## Conclusions

The foliar application of Tytanit biostimulant and Plonvit Z fertilizer in combination with Tytanit as well as this treatment with additives in grass-legume mixtures resulted in an increase of dry matter yields in the first cut but not in the following regrowths of both years. As a result of the use of Plonvit Z and Tytanit, favourable changes were observed in the botanical composition of the sward, consisting in lower invasion of weeds in the grass-clover mixture and a higher proportion of lucerne in the grass-lucerne mixture, which did not have a significant impact on the herbage quality. Study on the assessment of the effects of using foliar fertilization on grassland should be continued.

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# Sustainability of rose veal in organic beef production

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## Abstract

While organic beef production has been growing in Belgium, most of the organically produced weaned calves are sold in the conventional sector, and methane emissions related to meat production are discussed critically as contributing to climate change. In addition, the contribution of meat production to food security is regularly questioned. In this context, we evaluated the sustainability of grassland-based rose veal production as an alternative to bull fattening. A herd of 11 dual-purpose Belgian Blue suckler cows and their calves was monitored in 2018, 2019 and 2020 in Libramont, Belgium. Calving took place between February and May. A rotational grazing system with eight paddocks of 0.6 ha was implemented from May to mid-November. Calves were weighed monthly and the supplement feed intake (spelt and organic concentrates) weighed daily. Grass intake and quality were quantified through sward height and growth measurements and using NIRS on quadrat samples. Methane emissions of cows and calves were measured using automated head chambers. The tested system was net efficient with a production of human edible protein that is four times higher than the amount of human edible protein consumed by the animals.

**Keywords:** cow, calf, methane, food security, grazing

## Introduction

In 2022, 12.7% of Wallonia's agricultural land was under organic certification, with grasslands covering 73% of the organic agricultural land. EU targets are to reach 25% of agricultural land under organic certification by 2030, but the quantity of suckler cows has been stable since 2017 (Beaudelot *et al.*, 2023). One major break is the low share of organic young cattle valorisation under organic certification, with low and variable bovine production sold to the organic market (Mailleux and Engel, 2020).

Generally, beef production is criticised for its contribution to global warming, mainly through enteric methane emissions, while methane emissions in EU are expected to be reduced by 30% by 2030. The role of beef in food security is also questioned. While ruminants utilise forages and by-products that are not edible for humans, studies have also pointed to the use in beef production of cereals and other feeds that are in direct competition with human food (Mosnier *et al.*, 2021).

In this context, organic rose veal production appears as a potential alternative that could be more sustainable. Rose veal is a young bovine, male or female, fed with their mothers' milk and progressively supplemented with forages and concentrate feeds. Slaughtered before eight months, they produce a light-pink meat, different from beef products found on current local market.

The trial presented here aims at providing some references for the sustainability evaluation of grass-based rose veal production, with a focus on the animal performances, the contribution to food security and their contribution to climate warming through methane emissions, which are, to our knowledge, not present in the literature for grazing cow-calf pairs.

## Materials and methods

The trial was performed during three years with a herd of 12, 11 and 11 dual-purpose Belgian Blue suckler cows in 2018, 2019 and 2020, respectively. Calving took place between February and May. The grazing

season lasted from early May to mid-November. A rotational grazing system was implemented for the suckling cow-calf pairs, using eight paddocks of 0.6 ha. Calves were supplemented with 1.3 kg DM day<sup>-1</sup> of a mix of spelt and organic concentrates during 132 days on average. Calves were weighed and the grass intake was quantified through measurement performed every Thursday and when changing of the paddock. Those measurements consisted in at least 50 sward surface height and three quadrat (40cm x 40cm) samples. Grass quality was determined by infrared analyses (NIRS). Methane emissions were monitored using two automated head chamber systems (AHCS), also known as Greenfeeds (C-Lock, Rapid City, SD, USA), in 2019 and 2020. Access to AHCS and supplementary feed was separated for calves and cows. For each animal, methane emissions recorded during AHCS visits were averaged monthly.

The studied system included 391 days of cow life (calving interval) and 7.5 months of calf life. To estimate the contribution of this system to food security, we have evaluated the net protein efficiency by dividing the human edible protein production of the cow-calf system by the human edible protein consumption of the system (Laisse *et al.*, 2019), considering that the protein content of the rose veal produced is 190 g (kg LW)<sup>-1</sup>. The land used by the system to produce one kg of carcass has also been computed with a distinction between tillable and non-tillable land.

## Results and discussion

Across the grazing season, average grass intake was estimated as 10.5±0.85 (n=3 years) kg DM per day per cow-calf pair. The observed daily weight gain of the calf was 1.1±0.09 and 1.2±0.15 kg LW day<sup>-1</sup> for the females and males, respectively. Among the male calves, 16 out of 20 were slaughtered and sold as rose veal at 229 days of age with a live weight of 330 kg and a carcass weight of 203 kg (dressing percentage of 61.4%).

Measuring methane emission of animals on pasture is challenging. Over a one-month period, only 50% of the animal-month combination visited the AHCS more than 20 times (Figure 1, left). Methane emissions, with values above 50 g CH<sub>4</sub>/day, for calves older than 3 months, are correlated with the age of the calves ( $R^2=0.60$ ,  $p<0.01$ , Figure 1, right). For cows there were 39 month-animals with more than 20 visits. A mean methane emission of 297±34 g CH<sub>4</sub>/day was recorded. This is compatible with the observed methane emission by grazing lactating beef cows (McCaughey, 1999) and by grazing pregnant heifers with high herbage allowance (Orcasberro *et al.*, 2021). These values are also compatible with emissions of early pregnant Charolaise cows grazing heading timothy herbage (273.3±28.7 g CH<sub>4</sub> day<sup>-1</sup>) but higher than values observed for these animals grazing timothy at other stages of development (Pinares-Patiño, 2003). The enteric methane emissions were summed up to approximately 13 kg CH<sub>4</sub> and 116 kg CH<sub>4</sub> for the calf and the cow, respectively, or 17.8 kg eq CO<sub>2</sub> per kg carcass of rose veal, while considering only enteric methane emissions of the cow-calf system.

This system is a net protein producer thanks to its low feed concentrate use. Cows are fed from grass (except during AHCS visits where they received about 17 kg of organic concentrates per cow per year). Indeed, the system produces more than four times the amount of human edible protein feed. In terms of land use, the system uses 1.7 m<sup>2</sup> of tillable land and 36 m<sup>2</sup> of permanent grassland per kg of carcass produced. In terms of land use, these performances are similar to the Irish grass-based systems, and have higher net protein efficiency than the other systems observed by Mosnier *et al.* (2021).

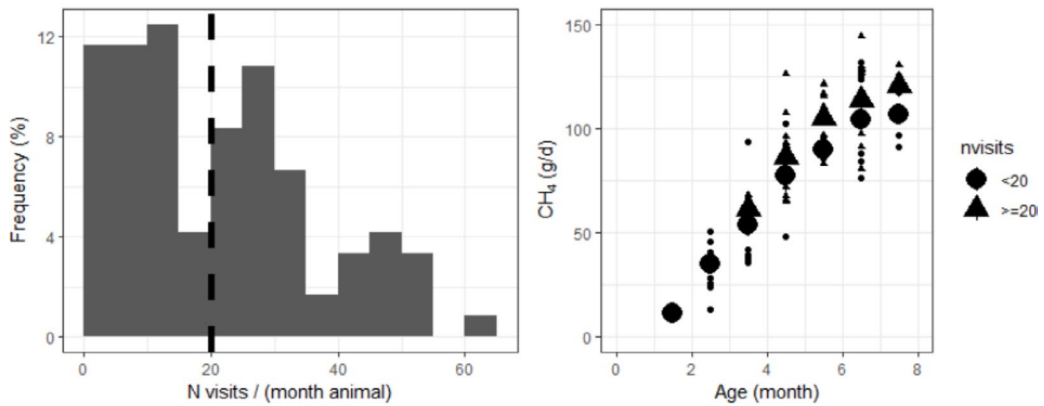


Figure 1. Left: Histogram of the number of visits of the calves to the AHCS per animal-month. Right: Mean emission in  $\text{g day}^{-1}$  measured for animals visiting more (triangles) or less (circles) than 20 times over a month. The big triangles and circles are the mean over the different calves of these two groups.

## Conclusion

The study provides references of intake, methane emissions and contribution to food security of grass-based organic rose veal production, including methane measurement on young calves and mother cows. The rose veal production system benefits from being a net protein producer thanks to the utilisation of permanent grasslands and the low use of concentrates. Further analysis based on the LCA methodology should be applied to evaluate the contribution of the complete system to climate change.

## Acknowledgement

The present research was funded by the Walloon research programme in Organic agriculture (BIO2020).

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# Effect of silage inclusion and silage species on milk production of late lactation, grazing dairy cows

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## Abstract

The objective of this study was to investigate the effect of silage inclusion and silage species on the milk production of late lactation, spring-calving, grazing dairy cows. Forty-five dairy cows were blocked based on pre-experimental milk production, parity and breed and randomly assigned to one of three dietary treatments. The three dietary treatments were: grazed pasture during the day and during the night (GP); pasture during the day and 8 kg of dry matter (DM)  $\text{cow}^{-1} \text{day}^{-1}$  of perennial ryegrass (PRG)-silage fed during the night (GS); and pasture during the day and 8 kg DM  $\text{cow}^{-1} \text{day}^{-1}$  of PRG-red clover-silage fed during the night (GRCS). All groups were supplemented with 2.69 kg DM  $\text{cow}^{-1} \text{day}^{-1}$  of concentrate. The study consisted of a two-week covariate period, one week for diet transition and seven weeks of data collection. Cows fed GP had higher milk yield, milk protein concentration and milk solids yield when compared with cows fed GS and GRCS ( $P < 0.001$ ). There was no effect of silage species (i.e. GS vs. GRCS) on milk production. The inclusion of silage into the diet of late lactation, grazing dairy cows reduced milk and milk solids production, regardless of the silage species.

**Keywords:** red clover, silage, pasture, late lactation, dairy cow

## Introduction

The European Green Deal has set a goal to reduce nutrient losses to the environment by 50% and to reach a 20% reduction in inorganic nitrogen (N) fertiliser use by 2030. Perennial ryegrass (*Lolium perenne* L.; PRG) monocultures are highly dependent on inorganic fertiliser to maintain high levels of herbage production. When red clover (*Trifolium pratense* L.) was included into PRG silage swards and grown without inorganic N application, the same herbage biomass was produced as PRG swards receiving a 412 kg N  $\text{ha}^{-1} \text{year}^{-1}$  application (Clavin *et al.*, 2017). In Ireland, maximising the proportion of pasture in the lactating dairy cow's diet has been shown to increase profitability and sustainability. However, at times, pasture supply and inclement weather can lead to the requirement to supplement the animal's diet with silage. During these situations, in order to maintain pasture in the diet, cows typically graze during the day and are offered silage at night. In indoor feeding systems, the effects of feeding red clover-silage to dairy cows have been widely investigated. Results suggest that red clover-silage has a faster rate of fermentation, a faster particle breakdown and transits more rapidly through the rumen, resulting in increased dry matter intake (DMI) and improved milk production when compared to PRG-silage (Dewhurst *et al.*, 2009; Steinshamn, 2010). However, there are only a limited number of studies investigating the effect of feeding red clover-silage when pasture comprises a substantial part of the diet. Therefore, the objectives of this study were to investigate the effect of: (1) silage inclusion in the diet of grazing dairy cows; and (2) the silage species (PRG-silage vs. PRG-red clover-silage) on spring-calving dairy cow production in late lactation.

## Materials and methods

This study was conducted at the Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland, from mid-September to the end of November 2022. Forty-five Holstein Friesian and Holstein Friesian×Jersey dairy cows, averaging (mean±SD) 215±25 days in milk and 495±48 kg of body weight, were blocked based on pre-experimental milk production, parity and breed. The cows were then randomly assigned to one of three dietary treatments ( $n=15$ ). The three dietary treatments were: (1) grazed pasture during day and night (GP); (2) grazed pasture during the day and 8 kg of dry matter (DM)  $\text{cow}^{-1} \text{day}^{-1}$  of PRG-silage fed at night (GS); and (3) grazed pasture during the day and 8 kg DM  $\text{cow}^{-1} \text{day}^{-1}$  of PRG-red clover-silage fed during the night (GRCS). The experiment consisted of a two-week covariate period, one week of diet acclimation and seven weeks of data collection. Silage swards were cut on the 18<sup>th</sup> of July, 2022 (2<sup>nd</sup> cut), at a pre-cutting herbage mass of 3,985 and 4,325 kg DM  $\text{ha}^{-1}$  for the PRG and the PRG-red clover swards, respectively. The PRG-red clover-silage comprised 28% red clover. During the experiment, the quantity of silage offered was adjusted daily for DM concentration, based on silage samples taken four times a week. Weekly silage samples were also taken, dried and milled for subsequent chemical analyses. All cows were supplemented with 2.69 kg DM  $\text{cow}^{-1} \text{day}^{-1}$  of a 16% crude protein concentrate (on a DM basis). When grazing, the cows were allocated a 12-h residence time, targeting a compressed residual sward height of 4 cm. Individual milk yields were recorded daily, and milk composition weekly. Weekly milk solids yields (fat yield+protein yield) were then calculated. Body weight (BW) was recorded weekly and body condition score (BCS) was assessed at the same occasion, using a 1 to 5 scale with 0.25 increments. The data were analysed using a mixed model in R, version 4.2.3 (R Core Team, 2023). Treatment, week, parity, breed and the interaction between treatment and week were included in the model as fixed effects. Cow was included as a random effect with a covariate adjustment applied for each cow. Treatment effect was considered as significant at  $P \leq 0.05$ .

## Results and discussion

The inclusion of silage in the diet reduced milk yield, milk protein concentration and milk solids yield (GS and GRCS vs. GP;  $P < 0.001$ ; Table 1). However, silage inclusion did not have an effect on milk fat concentration ( $P = 0.618$ ). Cows fed GRCS produced a similar fat yield as the cows fed GS and GP, whereas the cows fed the GS diet produced a lower fat yield than the cows fed GP ( $P < 0.05$ ). Similarly, O'Brien *et al.* (1996) observed reduced milk solids yield from silage supplementation, while maintaining daily milk yield. These results are in contradiction with Reid *et al.* (2015) who reported no difference in milk yield and milk solids production when silage was incorporated into the diet of late lactation grazing dairy cows (up to 6 kg DM  $\text{cow}^{-1} \text{day}^{-1}$ ), despite a reduction in DMI.

In the present study, silage species (i.e. GS vs. GRCS) did not affect milk production (Table 1), which is in agreement with Irawan *et al.* (2024). However, the outcomes of this study are in contradiction with Steinshamn (2010), who observed increased milk production from cows fed red clover-silage when compared with cows fed grass-silage. A potential explanation could be related to the fact that the cows within the current study were fed a restricted 8 kg DM of silage at night when compared to the indoor fed, *ad libitum* conditions investigated in Steinshamn (2010). The feed restrictions were applied in order to achieve high pasture utilization, a key target within a pasture-based system. A further explanation could be the low clover content of the PRG-red clover-silage in the current experiment (28%) compared with the RC silages investigated in Steinshamn (2010) (>90%). There was no effect of treatment on BW; however, cows fed GP had a lower BCS than cows fed the GRCS diet ( $P < 0.05$ ).

Table 1. Effect of silage supplementation and silage species on milk production, milk composition, BW and BCS of grazing dairy cows in late lactation

Item	GP	GS	GRCS	SEM	P-value
Milk yield (kg day <sup>-1</sup> )	14.5 <sup>a</sup>	13.0 <sup>b</sup>	13.7 <sup>b</sup>	0.31	<0.001
Protein (g kg <sup>-1</sup> )	44.4 <sup>a</sup>	41.8 <sup>b</sup>	42.0 <sup>b</sup>	0.53	<0.001
Fat (g kg <sup>-1</sup> )	58.5	60.0	59.2	0.87	0.618
Protein yield (kg day <sup>-1</sup> )	0.63 <sup>a</sup>	0.54 <sup>b</sup>	0.57 <sup>b</sup>	0.01	<0.001
Fat yield (kg day <sup>-1</sup> )	0.83 <sup>a</sup>	0.78 <sup>b</sup>	0.81 <sup>ab</sup>	0.02	<0.05
Milk solids yield (kg day <sup>-1</sup> )	1.45 <sup>a</sup>	1.32 <sup>b</sup>	1.37 <sup>b</sup>	0.03	<0.001
BW (kg)	521	527	520	4.98	0.192
BCS	2.94 <sup>a</sup>	3.05 <sup>ab</sup>	2.99 <sup>b</sup>	0.02	<0.05

BW, body weight; BCS, body condition score; GP, grazed pasture; GS, pasture during the day and PRG-silage at night; GRCS, pasture during the day and PRG-red clover-silage during the night; SEM, standard error of the mean. Within-row means with different superscripts are significantly different among treatments ( $P \leq 0.05$ ).

## Conclusion

Silage inclusion in the diet of late lactation grazing dairy cows reduced milk production performance. When pasture supply and grazing conditions allow it, silage supplementation in late lactation should be limited. Silage species did not have an effect on milk production in this experiment. Future work should investigate the response of lactating dairy cows fed *ad libitum* silage with a higher red clover content within the context of a pasture-based system.

## Acknowledgement

This experiment was funded by Teagasc Core Funding and Dairy Research Ireland.

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# Effect of pasture type on dairy-beef heifer production efficiency

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## Abstract

Increasing sward diversity by incorporating legumes or herbs into swards of perennial ryegrass (*Lolium perenne* L. (PRG)) has many desirable benefits such as increasing herbage nutritive value and reducing reliance on inorganic nitrogen (N) fertiliser. The aim of this experiment was to determine the effect of pasture type on slaughter performance of early maturing dairy-beef heifers. The three pasture treatments were: PRG, receiving 150 kg N ha<sup>-1</sup>, CLOVER (*Trifolium repens* and *Trifolium pratense*), receiving 75 kg N ha<sup>-1</sup>, and multispecies swards (MSS) consisting of PRG, red clover, white clover, plantain (*Plantago lanceolata*), and chicory (*Cichorium intybus*) receiving 75 kg N ha<sup>-1</sup>. No differences were observed for total herbage dry matter (DM) production between treatments. A greater proportion of grass-clover (0.86) or MSS (0.75) heifers were drafted from pasture only, compared to 0.68 for PRG heifers, which required higher concentrate input and housing. Animals offered CLOVER and MSS produced a significantly heavier carcass (250 vs. 243 kg) than PRG ( $P < 0.05$ ), due to improved lifetime performance, which allowed these animals to be drafted for slaughter at a targeted fatness (8 to 9 fat score) at a younger age ( $P < 0.05$ ). Carcass conformation was unaffected by pasture-type ( $P > 0.05$ ; 5.1 on average across treatments on a 15-point scale).

**Keywords:** dairy-beef, heifers, clover, multi-species swards, animal performance

## Introduction

In Ireland, dairy-beef heifers have the highest probability of failing to meet 'overall' carcass specifications compared to dairy-beef steers or suckler bred steers and heifers (Kenny *et al.*, 2020). This reduced ability to meet carcass specification has likely contributed to the relatively high slaughter ages of these heifers. Research has shown that younger slaughter ages are possible during the 'second' grazing season or during a shorter indoor feeding period, although at a lower carcass weight compared to steers or suckler bred beef cattle. Carcass output and the level of inputs needed can be optimised by grazing highly productive and high nutritive value pastures. Perennial ryegrass (*Lolium perenne* L. (PRG)) is the most commonly sown grass species in Ireland. However, PRG pasture is highly dependent on the application of chemical nitrogen (N) for growth, which can have a negative impact on ground water quality and gaseous emissions. One of the key factors in addressing the sustainability challenges associated with ruminant livestock production is reducing reliance on inputs of chemical fertilisers. Clover-based swards have shown many benefits in terms of sward nutritive value, animal intake and performance, and increased biological fixation of N (Enriquez-Hidalgo *et al.*, 2018). Similarly, multi-species swards (MSS) containing clover have shown potential to increase sward DM production under reduced chemical N application rates (Grace *et al.*, 2018). However, the herbage composition of more diverse swards changes within and across years, nonetheless, there is limited data available on the persistency of mixed swards in livestock production systems. Thus, the performance of dairy-beef cattle consuming contrasting pasture types requires further investigation.

## Materials and methods

In 2021 and 2022, 105 and 108 beef × dairy heifer calves, respectively, were purchased at approximately 20 weeks of age and were assigned to one of three pasture treatments: (1) PRG-only, receiving 150 kg total N ha<sup>-1</sup>, (2) CLOVER (red and white; *Trifolium repens* and *Trifolium pratense*), receiving 75 kg total N

ha<sup>-1</sup>, and (3) MSS (PRG, red and white clover, plantain (*Plantago lanceolata*) and chicory (*Cichorium intybus*)) swards receiving 75 kg total N ha<sup>-1</sup>. The sire breeds were Hereford and Angus and all progeny were from Holstein-Friesian dams. The calves were balanced across treatments based on breed, date of birth (mean 16 February), and live weight (mean 159±6.18 kg at arrival on farm). All treatments were stocked at 2.5 LU ha<sup>-1</sup> and produced 182 kg organic N/ha. The online tool “PastureBase Ireland” was used as an aid for grazing management for each pasture treatment. During the first grazing season, calves were supplemented with 1 kg of concentrate (fresh weight basis) daily and fresh herbage was offered every 48 hours. Swards were rotationally grazed, targeting a pre-grazing herbage mass of 1300 to 1600 kg DM ha<sup>-1</sup> for calf and yearling heifers, respectively. Pre-grazing herbage mass was measured in each paddock prior to grazing by harvesting two strips using an Etesia mower (Etesia UK, Warwick, UK). The target post-grazing sward height was 5 cm for all pasture treatments and this was measured using a rising platometer (Jenquip, Feilding, New Zealand). Botanical composition was measured prior to each grazing for the CLOVER and MSS swards, by cutting and separating samples into grass, legume and herb fractions, followed by drying to determine the DM proportions. Calves were housed indoors in November, when grazing conditions deteriorated or when target closing farm cover (450 kg DM ha<sup>-1</sup>) was achieved. During the first winter period, weanlings were offered silages *ad libitum*, from their respective pasture treatment, in addition to 1.25 kg concentrate hd<sup>-1</sup> day<sup>-1</sup>. Yearlings were turned out to pasture in early March, and were weighed fortnightly over the grazing season and drafted for slaughter when they reached a target fat score of between 8 and 9, determined by body condition scoring. Carcass conformation and fat scores were determined using the EUROP grid classification system. Any heifers not slaughtered off grass were housed in October, and commenced their ‘finishing’ diet of *ad libitum* silage and 4.0 kg concentrate hd<sup>-1</sup> day<sup>-1</sup> until slaughter. Data were analysed using SAS 9.4 using Proc Mixed with fixed effects considered being treatment, time (year or rotation), sire, genotype, paddock and associated interactions. Random effects included in the model were paddock and sire.

## Results and discussion

There were no significant differences observed for pre-grazing herbage mass, pre-grazing height or post-grazing height ( $P>0.05$ ). The PRG, CLOVER and MSS pastures produced similar DM yields of 11 900, 11 500 and 11 400 kg of DM ha<sup>-1</sup>, respectively. Over the entire grazing season, the average clover content (red and white clover) was 22% and 21% for the CLOVER and MSS pastures, respectively. Despite an additional application of 75 kg N ha<sup>-1</sup> to the PRG treatment compared to the CLOVER and MSS treatments (i.e. 150 vs. 75 kg N ha<sup>-1</sup>), the similar annual DM yields for the three pasture types implies that the inclusion of legumes and improved species diversity can reduce the need for chemical N application.

The effect of pasture type on animal liveweight gain and slaughter performance are presented in Tables 1 and 2. The CLOVER and MSS treatments lifetime average daily liveweight gain (ADG) advantage over that of the PRG treatment is in line with other studies (Boland *et al.*, 2022). Although the PRG treatment performed similarly to the other two pasture treatments during the first winter period, performance at pasture was lower than that achieved by the MSS group during the first and second grazing season and lower than the CLOVER during the second grazing season (Table 1). This resulted in overall lower lifetime performance and a greater ( $P<0.05$ ) age at slaughter for the heifers consuming the PRG herbage (Table 2). Overall, a greater number of heifers were slaughtered off pasture for the CLOVER and MSS treatments, compared to the PRG treatment (0.86 vs. 0.75 vs. 0.68). This was likely due to superior sward nutritive value from the incorporation of clover and herbs. Thus, the indoor finishing concentrate requirement was lower for the CLOVER (25 kg) and MSS (34 kg) treatments compared to PRG (62 kg), which represents a significant saving in costs associated with feed and housing. Despite more PRG heifers requiring housing and higher concentrate inputs to get to a fat score of between 8 and 9, they were still significantly leaner than CLOVER and PRG heifers ( $P<0.05$ ), being half a fat grade lower. The

CLOVER and MSS heifers were heavier at slaughter ( $P<0.05$ ) resulting in a heavier carcass at a reduced slaughter age to the PRG heifers.

Table 1. Effect of pasture treatment on live weight gain of dairy-beef heifers slaughtered from pasture.

	PRG	CLOVER	MSS	SEM	Significance
ADG (kg day <sup>-1</sup> )					
1 <sup>st</sup> grazing season	0.61 <sup>a</sup>	0.62 <sup>a</sup>	0.79 <sup>b</sup>	0.052	***
1 <sup>st</sup> winter	0.65	0.65	0.68	0.031	NS
2 <sup>nd</sup> grazing season	0.81 <sup>a</sup>	0.92 <sup>b</sup>	0.87 <sup>b</sup>	0.019	***
Lifetime	0.74 <sup>a</sup>	0.78 <sup>b</sup>	0.79 <sup>b</sup>	0.010	**

SEM, standard error of the mean; NS, not significant ( $P>0.05$ ); \*\* $P<0.01$ ; \*\*\* $P<0.001$ .

Table 2. Slaughter performance of dairy-beef heifers slaughtered from pasture, managed on perennial ryegrass (PRG) plus red and white clover swards (CLOVER), multispecies swards (MSS) and PRG-only swards (PRG).

	PRG	CLOVER	MSS	SEM	Significance
Age (months)	19.6	19.2	19.2	6.5	NS
Slaughter weight (kg)	482 <sup>a</sup>	492 <sup>b</sup>	490 <sup>b</sup>	5.4	*
Kill-out (%)	50	51	51	0.1	NS
Carcass weight (kg)	243 <sup>a</sup>	250 <sup>b</sup>	249 <sup>b</sup>	2.7	*
Conformation score (1–15)	5.0	5.2	5.2	0.11	NS
Fat score (1–15)	8.0 <sup>a</sup>	8.5 <sup>b</sup>	8.6 <sup>b</sup>	0.19	**

SEM, standard error of the mean; NS, not significant ( $P>0.05$ ); \*  $P<0.05$ ; \*\*  $P<0.01$ .

## Conclusion

Reduced chemical N fertiliser use, improved lifetime ADG and carcass weight of cattle are key mechanisms for improving both profitability and environmental footprint for pasture-based dairy-beef production. The incorporation of clover into swards of PRG offers farmers an opportunity to improve efficiency, while also striving to meet sectoral climate targets.

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# Effect of pasture species and inorganic nitrogen fertilisation on intake, digestibility and milk production of cows

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## Abstract

The objective of this experiment was to investigate the effect of pasture species and inorganic nitrogen application rate on the milk production, dry matter intake (DMI) and organic matter digestibility (OMD) of spring-calving dairy cows. In spring and autumn, 12 rumen cannulated Holstein Friesian cows were blocked based on pre-experimental milk production and randomly assigned to one of four dietary treatments. The four dietary treatments were: (1) perennial ryegrass (PRG) receiving 25 kg of inorganic N ha<sup>-1</sup> cut<sup>-1</sup> (PRG-L); (2) PRG receiving 50 kg of inorganic N ha<sup>-1</sup> cut<sup>-1</sup> (PRG-H); (3) PRG-white clover receiving 0 kg of inorganic N ha<sup>-1</sup> cut<sup>-1</sup> (WC-L); and (4) PRG-white clover receiving 25 kg of inorganic N ha<sup>-1</sup> cut<sup>-1</sup> (WC-H). During the spring experiment, cows fed WC-L produced lower milk yield, milk protein concentration and milk solids yield when compared to cows fed PRG-L and PRG-H. Cows fed WC-L had the lowest OMD and tended to have the lowest DMI. No effect of treatment on milk outcomes was observed during the autumn experiment. During the autumn, cows fed PRG-H and WC-H had higher OMD when compared with cows fed PRG-L and WC-L. These results highlight important findings for best practices of management in future low inorganic N grazing systems.

**Keywords:** dairy cow, digestibility, white clover, chemical nitrogen, perennial ryegrass

## Introduction

In Ireland and other regions of North Western Europe, grazed pasture is the lowest-cost feedstuff available for ruminant production systems. Such pastures predominately consist of perennial ryegrass (*Lolium perenne* L. (PRG)); however, there has been a renewed interest in the inclusion of forage legumes such as white clover (*Trifolium repens* L. (WC)) into PRG swards as a means to improve the nutritive value of the pasture and to provide low-cost nitrogen (N) through biological N fixation. Furthermore, the European Green Deal has set a target for a 20% reduction in agriculture inorganic N use by 2030. The capability of legumes such as WC to fix N will be essential to maintain productive pastures while implementing this policy. The inclusion of WC has been demonstrated to support higher dry matter intake (DMI) in grazing dairy cows when measured using the n-alkane technique, translating into improved milk production when compared with PRG swards (Egan *et al.*, 2018; McClearn *et al.*, 2019). Organic matter digestibility (OMD) is a key variable in estimating the energy content and overall nutritive value of pasture herbage. Organic matter digestibility can be influenced by a number of factors, such as sward composition; for example, Hurley *et al.* (2021) reported increased *in vivo* OMD in stall-fed sheep when fed WC. Grassland management factors, such as inorganic N application rate, can also influence sward OMD and milk production (Delegarde *et al.*, 1997). As a consequence of the renewed interest in WC, the pressure to reduce inorganic N application rates and the limited availability of lactating dairy cow studies on this topic in the literature, it is important to evaluate the differing combinations of these variables on animal outcomes. Therefore, the objective of this experiment was to investigate the effect of pasture species and inorganic N application rate on the DMI, OMD and milk production of lactating dairy cows during spring and autumn.

## Materials and methods

The experiment was conducted at the Teagasc Animal and Grassland Research and Innovation centre, Moorepark, Fermoy, Co. Cork, Ireland (52°16' N, 8°25' W). Twelve rumen cannulated multiparous Holstein Friesian dairy cows were blocked based on pre-experimental milk production and body weight and then randomly assigned to one of four dietary treatments. The dietary treatments were: (1) PRG-L; (2) PRG-H; (3) WC-L; and (4) WC-H. The experimental design was an incomplete crossover and the experiment was performed on two occasions, during the spring (April to May) and autumn (September to early November) of 2022. Each experiment consisted of a 14-day dietary adaptation period, prior to an 8-day sample collection period. Cows were then reallocated to another dietary treatment for a second 14-day dietary adaptation period, prior to the final 8-day sample collection period. Inorganic N, if applicable, was applied approximately 21 days prior to harvesting of the respective swards. The cows were housed in individual stalls for the 8-day sample collection periods to assess individual DMI and facilitate the total collection of urine and faeces. During these periods, herbage was harvested twice daily at 0800 and 1500 h and was offered 8 times a day with the herbage refrigerated at 4°C between feedings to minimise respiration. The quantity of herbage offered to each cow was recorded and a subsample was taken twice daily for dry matter (DM) and chemical composition analysis. All cows also received 0.88 kg of DM day<sup>-1</sup> of a standard concentrate. White clover content on each subplot was determined as described by Egan *et al.* (2018) prior to harvesting. On each day of collection, total faecal excretions were weighed, mixed and duplicate subsamples were taken for DM and chemical composition analysis. Procedures described by Hurley *et al.* (2021) were used to measure OMD. Cows were milked twice daily with milk yields recorded and milk samples collected at each milking. Milk samples were subsequently analysed for fat and protein. Data were analysed in SAS using a mixed model procedure. Treatment and period were included as fixed effects and cow was included as a random effect. Significance was considered at  $P \leq 0.05$  and trends were considered at  $0.05 < P \leq 0.10$ .

## Results and discussion

During the spring, cows fed WC-L had lower milk yield, milk protein concentration and milk solids yield when compared to cows fed PRG-L and PRG-H (Table 1). Cows fed WC-L also had the lowest OMD and tended to have the lowest DMI. These outcomes are in agreement with Delagarde *et al.* (1997) who demonstrated that when lower inorganic N rates were applied to swards, cows consuming such swards reduced their DMI, OMD and milk production. Although WC can fix large quantities of atmospheric N, the lower clover content (approx. 18%) at this time of the year may not have been able to compensate for the absence of inorganic N application. During the autumn, there was no effect of treatment on any milk production outcome indicating that at higher WC contents (approx. 53%) milk production performance can be maintained with lower inorganic N applications rates. Both Egan *et al.* (2018) and McClearn *et al.* (2019) observed higher milk production performance when cows consumed white clover; however, Fitzpatrick *et al.* (2022) observed no difference. In the current experiment, although not significantly different, cows fed WC-H produced the greatest milk yield and milk solids yield. Cows fed WC-H also tended to have the greatest DMI. Interestingly, cows fed PRG-H and WC-H had higher OMD when compared with cows fed PRG-L and WC-L.

Table 1. Effect of pasture species and inorganic nitrogen on DMI, OMD and milk production of lactating dairy cows during the spring and autumn<sup>1</sup>

Item	PRG-L	PRG-H	WC-L	WC-H	SEM	P-value
<i>Spring</i>						
DMI, kg d <sup>-1</sup>	17.8 <sup>y</sup>	17.8 <sup>y</sup>	16.4 <sup>z</sup>	17.6 <sup>yz</sup>	0.55	0.09
OMD, g kg <sup>-1</sup> DM	813 <sup>a</sup>	816 <sup>a</sup>	799 <sup>b</sup>	814 <sup>a</sup>	3.93	<0.05
Milk yield, kg d <sup>-1</sup>	25.1 <sup>a</sup>	26.8 <sup>b</sup>	23.3 <sup>c</sup>	24.2 <sup>ac</sup>	1.00	<0.01
Milk protein, %	3.56 <sup>a</sup>	3.51 <sup>b</sup>	3.46 <sup>c</sup>	3.52 <sup>ab</sup>	0.06	<0.01
Milk fat, %	4.10	3.93	3.92	3.85	0.13	0.42
Milk solids, kg d <sup>-1</sup>	1.92 <sup>a</sup>	1.99 <sup>a</sup>	1.72 <sup>b</sup>	1.78 <sup>b</sup>	0.07	<0.01
<i>Autumn</i>						
DMI, kg d <sup>-1</sup>	19.8 <sup>yz</sup>	19.1 <sup>y</sup>	19.6 <sup>y</sup>	21.7 <sup>z</sup>	0.75	0.09
OMD, g kg <sup>-1</sup> DM	803 <sup>a</sup>	810 <sup>b</sup>	798 <sup>a</sup>	811 <sup>b</sup>	3.33	<0.01
Milk yield, kg d <sup>-1</sup>	17.5	17.4	18.5	19.5	1.16	0.32
Milk protein, %	4.15	4.14	4.15	4.20	0.11	0.91
Milk fat, %	4.64	4.96	4.37	4.60	0.21	0.16
Milk solids, kg d <sup>-1</sup>	1.53	1.57	1.55	1.70	0.09	0.15

DMI, dry matter intake; OMD, organic matter digestibility; PRG-L, perennial ryegrass (PRG) receiving 25 kg of inorganic N ha<sup>-1</sup> cut<sup>-1</sup>; PRG-H, PRG receiving 50 kg of inorganic N ha<sup>-1</sup> cut<sup>-1</sup>; WC-L, PRG-white clover (PRG-WC) receiving 0 kg of inorganic N ha<sup>-1</sup> cut<sup>-1</sup>; WC-H, PRG-WC receiving 25 kg of inorganic N ha<sup>-1</sup> cut<sup>-1</sup>; SEM, standard error of the mean. Within row means with different superscripts are significantly different, <sup>a-c</sup>( $P \leq 0.05$ ) and tends to differ at <sup>yz</sup>( $0.05 < P \leq 0.10$ ).

## Conclusions

Given the environmental and policy pressure to reduce inorganic N application rates, the incorporation of forage legumes into grazing systems is recommended. However, low or zero inorganic N application rates can result in reduced animal performance, DMI and OMD when sward white clover contents are low, particularly in spring. These findings highlight important considerations for the development of low inorganic N grazing systems.

## Acknowledgement

This project was funded by the Department of Agriculture, Food and the Marine's Competitive Research Funding Programme (2021R482).

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# Long-term analysis of climate and management effects on grassland yield

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## Abstract

This study investigates the impact of climate and management intensity on grassland yield in Austria, where grasslands are a crucial agricultural resource. The analysis examines the effects of temperature, precipitation, and various cutting regimes (two-, three-, and four-cut systems) on permanent grassland sites based on data from 20 locations over 20 years. Advanced linear mixed models analyse the interaction of these factors across different sites and years. The models show that higher temperatures generally decrease yield, whereas increased precipitation enhances them. The effect of the cutting regime on dry matter yield was also significant, indicating higher yields with greater utilisation intensity. The results further show a complex interaction between temperature, precipitation, management, site and yield. Considering the varying climate influences, the findings highlight the necessity of adapting grassland management practices to local conditions. This approach is vital in climate change, where flexible and site-adapted management strategies are essential for maintaining grassland productivity. The research underscores the need for advanced statistical methods to understand these complex interactions and develop effective management strategies.

**Keywords:** grassland management, yield, linear mixed model, temperature, precipitation

## Introduction

In Austria, grasslands are the most important agricultural land use type in terms of area, particularly in disadvantaged regions. They serve as a primary feed base for over 53 000 grassland farms and fulfil numerous ecological and socioeconomic functions (BML, 2022). Understanding the complex factors influencing grassland dry matter yield (DMY), especially climatic impacts, is crucial for sustainable and resilient land management (Pötsch, 2009). This study focuses on the impacts of temperature, precipitation and management intensity on DMY. Advanced statistical methods analyse the effects of location, year and their interaction to quantify the site- and management-specific influence of temperature and precipitation on DMY.

## Materials and methods

After the drought year 2001, the survey aimed to quantify the influence of temperature and precipitation on several permanent grassland sites in Austria (Figure 1). Data from 20 locations collected on a long-term experiment (2002 to 2021) are the basis for the analysis. Individual field experiments used a Latin square design with three replicates each. The study explored various management intensities, including two-, three-, and four-cut systems, and adjusted organic fertilisers accordingly. Linear mixed models specifically designed for evaluating agricultural experimental data collected across multiple locations and years were used for the analysis. These models account for the fixed effects of environmental and management factors and random variability due to specific site-year combinations. The first model accounts for the fixed effects of temperature, precipitation, management, and random variability at site levels and years nested within sites, making them particularly suited for multi-site, multi-year agricultural experiments. The second model includes random intercepts and slopes for cumulative temperature and precipitation within each cutting system \* site interaction level. This advanced mixed-effects model improves upon the basic model. It allows cumulative temperature and precipitation effects on DMY to

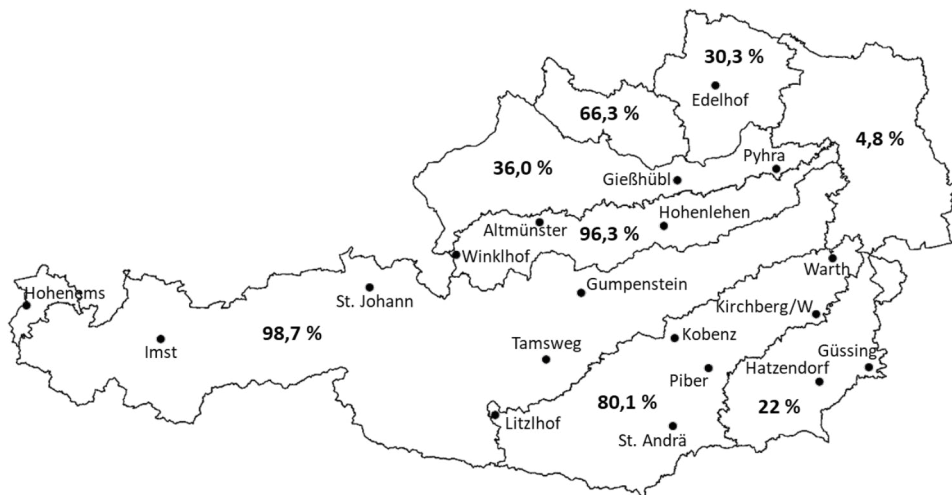


Figure 1. Grassland regions in Austria, according to Schaumberger (2024), with the percentage of grassland in the total agricultural area and the experimental sites.

vary depending on the specific cutting systems and the experimental sites. The specific intercepts and slopes enable the analysis of how climatic factors and management practices interact across different sites and management intensities. All statistical analyses and visualisations were performed using R version 4.3.1 (R Core Team, 2021). The mixed models were created using the ‘lme4’ package with the ‘lmer’ function (Bates *et al.*, 2015).

## Results and discussion

The analysis of experimental data shows a site-specific interaction between temperature, precipitation, management and DMY. Overall, the fixed effects poorly explained the variance of DMY ( $R^2=0.17$ ). However, incorporating random effects for individual sites and years nested within those sites significantly improved the model’s accuracy ( $R^2=0.75$ ). This improvement in model accuracy highlights the importance of considering site-specific and annual variations in such studies to understand growth dynamics comprehensively. After standardising both, cumulative temperature and precipitation to a mean of 0 and a standard deviation of 1, the observation showed that increased temperatures negatively affect yield ( $P<0.01$ ), while precipitation positively influences it ( $P<0.001$ ). Specifically, each standard deviation increase in cumulative temperature reduces yield by  $429 \text{ kg DM ha}^{-1}$ , suggesting potential stress effects due to temperature or associated water scarcity. In contrast, each standard deviation increase in precipitation raises yield by  $925 \text{ kg DM ha}^{-1}$ , underscoring the crucial role of plant-available water during the growing season for water-demanding permanent grassland. Besides climatic factors, the analysis revealed a significant effect of the cutting regime ( $P<0.001$ ) on DMY, showing higher yields at higher utilisation intensity ( $4 > 3 > 2$ ). Adjusted fertiliser applications according to the cutting frequency also contribute to this. Evaluating the mixed model with random slopes provides comprehensive insights into the diverse effects of climatic parameters across different sites (Figure 2). The interaction of the climate parameter with management and site characteristics demonstrates heterogeneous impacts on DMY. This underscores the importance and necessity of site-adapted grassland management. Such an approach highlights the need to tailor grassland management practices to the unique conditions of each location, considering the varying influences of climate on different sites.



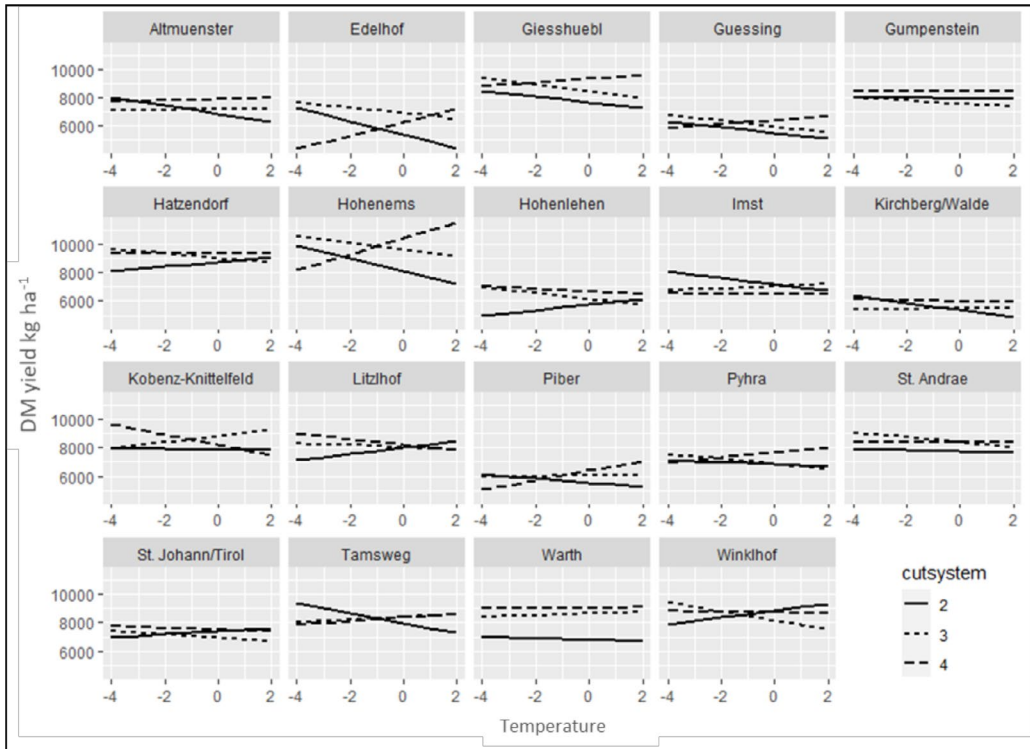


Figure 2. Influence of normalised cumulative temperature on dry matter yield (DMY) depending on the site and management intensity.

## Conclusion

The experiments' findings emphasise the importance of considering climatic and site-specific factors in grassland management. While climate factors like temperature and precipitation fundamentally influence yield, local conditions significantly modify these effects. Advanced statistical methods are necessary to analyse these complex interactions, considering the varying influences of climate and site interactions. Understanding these interactions in the context of climate change is crucial for developing adaptive management strategies to maintain grassland productivity. The results highlight the need for flexible grassland management, with timely adjustments to adapt to specific conditions, emphasising the importance of site-adapted management to maintain grassland productivity.

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# Effect of concentrate crude protein on milk production of early lactation, grazing dairy cows

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## Abstract

The objective of this experiment was to investigate the effect of concentrate supplement crude protein (CP) concentration on milk production in early lactation, grazing dairy cows. Ninety-two lactating Holstein Friesian dairy cows were blocked based on pre-experimental milk production and parity, and randomly assigned to 1 of 4 dietary treatments. Dietary treatments were pasture supplemented with: (1) a 17% CP concentrate (H); (2) a 13% CP concentrate (M); (3) a 9.5% CP concentrate (L), and (4) a 9.5% CP concentrate containing rumen-protected methionine and lysine (L-AA). All concentrate CP concentrations are stated on a dry matter basis. Cows were offered 2.67 kg of concentrate DM day<sup>-1</sup>. The experiment consisted of an initial 2-week covariate period, an 8-week experimental period, and a 4-week carry-over period. Cows fed the H diet produced more protein yield, fat yield and milk solids yield when compared to all other treatments. Milk solids yield was similar between cows fed M, L and L-AA. There was no effect of diet on milk fat and milk protein concentrations.

**Keywords:** dairy cow, milk production, concentrate supplement, grazing

## Introduction

Following the European Green Deal target of reducing emissions by 55% by 2030, the Irish Climate Action Plan (DAFM, 2020) encourages livestock farmers to reduce the level of crude protein (CP) in concentrates offered to grazing ruminants to a maximum of 15%. Irish spring pasture CP concentration is typically higher than the grazing dairy cows' requirement (ca. 18% vs. 15-17%, respectively); therefore, it is hypothesised that no additional feed CP is required. However, when dietary CP concentrations are reduced, metabolisable protein or specific amino acid (AA) have been demonstrated to limit animal performance (Zhang *et al.*, 2023). Several studies have investigated low concentrate CP supplementation to grazing dairy cows in the spring; however, results were equivocal and few studies investigated CP concentrations less than 15% CP (kg DM)<sup>-1</sup>. Therefore, the objective of this experiment was to investigate the effect of concentrate supplement CP concentration on milk production in early lactation, grazing dairy cows. The secondary objective was to investigate the effect of including rumen protected AA in low CP concentrates.

## Materials and methods

The experiment was conducted from March to June, 2023 at the Dairygold Research Farm (Teagasc Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland; 52°09' N; 8°16' W). The experiment consisted of an initial 2-week covariate period, an 8-week experimental period, and a 4-week carry-over period. Ninety-two lactating Holstein Friesian dairy cows were blocked based on pre-experimental milk production and parity and then randomly assigned to 1 of 4 dietary treatments ( $n=23$ ). The dietary treatments were pasture supplemented with: (1) a 17% CP concentrate (H); (2) a 13% CP concentrate (M); (3) a 9.5% CP concentrate (L); and (4) a 9.5% CP concentrate containing rumen-protected methionine (8.0 g day<sup>-1</sup> absorbable met) and lysine (7.2 g day<sup>-1</sup> absorbable lys; L-AA). Cows were offered 2.67 kg of concentrate DM day<sup>-1</sup> in the milking parlour. The main ingredient composition of the experimental concentrate supplements are in Table 1.

Table 1. Main ingredient composition of the experimental concentrate supplements

Ingredient (g/kg)	Experimental supplement			
	H	M	L	L-AA
Maize meal	270	270	270	270
Barley	100	100	100	100
Soybean meal	176	83	-	-
Beet pulp	163	207	246	246
Soya hulls	163	207	246	221
Molasses	80	80	80	80
Calcined magnesite	19	19	19	19
Rumen-protected amino acids	-	-	-	25

Dietary treatments were pasture supplemented with; a 17% CP concentrate (H); a 13% CP concentrate (M); a 9.5% CP concentrate (L); and a 9.5% CP concentrate containing rumen-protected amino acids (L-AA).

During the experimental period, cows were grazed separately on swards consisting predominantly of perennial ryegrass (*Lolium perenne* L.), with *ad libitum* access to fresh water. Cows were allocated a 12-hr residence time within each paddock or until a targeted post-grazing residual compressed sward height of 4 to 4.5 cm was achieved. Individual daily milk yields (kg) were recorded using electronic milk meters (Dairymaster, Causeway, Co. Kerry, Ireland) and later used to determine weekly milk production. Successive weekly p.m. and a.m. milk samples were analysed using a MilkoScan 7 (Foss Electric) to determine milk fat and protein, which were then used to calculate weekly milk solids yield (kg fat + kg protein). All data were analysed in a repeated measures model using the MIXED procedure of SAS version 9.4 (SAS Institute, Cary, NC, USA). The model included the fixed effects of treatment, week, their interaction and parity. An appropriate covariate adjustment was applied for each cow. The repeated measures analysis was based on week. Significance was considered at  $P \leq 0.05$ .

## Results and discussion

Cows fed H produced more milk yield when compared with cows fed L and L-AA (Table 2). Furthermore, cows fed H produced more protein yield, fat yield and milk solids yield when compared to all other treatments. There was no effect of treatment on milk fat and milk protein concentrations. These outcomes are in agreement with some studies (Doran *et al.*, 2023; Whelan *et al.*, 2012) but in contradiction with others (Burke *et al.*, 2008; Reid *et al.*, 2015). A challenge when comparing across studies is that there are a number of dynamic factors. Experiments differ in the investigated concentrate CP concentrations, the pasture CP concentrations, the level of concentrate supplementation and the season/stage of lactation examined. In the current experiment, further examination of the pasture nutritive value, dry matter intake and metabolic indicators of N status (i.e. plasma urea N and milk urea N) is required to understand the mechanisms leading to the reduced animal performance when low CP concentrates are fed.

Cows fed L-AA were similar to cows fed L except for protein yield (0.86 vs. 0.90 kg, respectively). This may indicate that early lactation grazing dairy cows are not limited by methionine or lysine supply. However, Whelan *et al.* (2012) demonstrated that the addition of rumen-protected methionine to a pasture-based diet negated the decrease in animal performance when cows were offered a low CP concentrate. In the current experiment, retrospective amino acid recovery and protection analysis of the L-AA concentrate demonstrated low recovery rates of the rumen-protected methionine source, indicating that the concentrate pelleting process may have disrupted the rumen-protection mechanism.

Table 2. Effect of concentrate crude protein concentration on milk production and milk composition of early lactation, grazing dairy cows

Item	Diet				SEM	P-value
	H	M	L	L-AA		
Milk yield (kg day) <sup>-1</sup>	26.7 <sup>a</sup>	26.2 <sup>ab</sup>	25.5 <sup>bc</sup>	25.2 <sup>c</sup>	0.30	< 0.05
Fat (%)	4.38	4.30	4.29	4.44	0.07	0.35
Protein (%)	3.50	3.46	3.53	3.43	0.04	0.16
Fat (kg day) <sup>-1</sup>	1.17 <sup>a</sup>	1.12 <sup>b</sup>	1.09 <sup>b</sup>	1.12 <sup>b</sup>	0.02	<0.01
Protein (kg day) <sup>-1</sup>	0.93 <sup>a</sup>	0.90 <sup>b</sup>	0.90 <sup>b</sup>	0.86 <sup>c</sup>	0.01	<0.001
Milk solids (kg day) <sup>-1</sup>	2.10 <sup>a</sup>	2.02 <sup>b</sup>	1.99 <sup>b</sup>	1.98 <sup>b</sup>	0.02	<0.001

Diet was pasture supplemented with 2.67 kg DM day<sup>-1</sup> of a: (1) 17% CP concentrate (H); (2) 13% CP concentrate (M); (3) 9.5% CP concentrate (L); and (4) 9.5% CP concentrate containing rumen-protected amino acids (L-AA).

<sup>abc</sup> P ≤ 0.05 for differences between means with different superscripts.

## Conclusion

Decreasing the concentrate CP concentration of early-lactation grazing dairy cows decreased milk production; however, there was no effect on milk fat and milk protein concentrations. These results suggest that producers should maintain concentrate CP concentrations of 15% kg<sup>-1</sup> of fresh weight during spring grazing. Further analysis is required to determine the nutritive value of the pasture fed in the current experiment. More studies are needed, as few pasture-based experiments have investigated concentrate CP concentrations below 15%. Finally, future experiments should investigate the optimum level of rumen-protected amino acid inclusion in concentrate feeds while being mindful of pelleting manufacturing processes.

## Acknowledgement

This project was funded by the Department of Agriculture, Food and the Marine's Competitive Research Funding Programme (2021R482).

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# Can a ley grazing dairy system compete with high yielding dairy farms in northern Germany in terms of environmental impact and eco-efficiency?

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## Abstract

High greenhouse gas emissions and nutrient surpluses from intensive dairy farming require new strategies to produce dairy more sustainably. Utilizing ley grasslands from organic crop rotations with ruminants can close nutrient cycles and provide high quality feed with minimal feed food competition. We assessed the environmental impact of two different dairy systems from a survey dataset of typical predominantly intensive indoor dairy farms in Schleswig Holstein, northern Germany (average SH) and an organic dairy system grazing leys integrated into a cash crop rotation (pasture ICLS). A cradle-to-farmgate life cycle analysis was conducted. Compared to the average SH, the pasture ICLS operated with substantially lower GHG emissions; 71% lower per ha and 51% lower per kg ECM; at a comparable level of land use efficiency and at 42% lower nitrogen surpluses per kg ECM. Although intensification and increasing milk yield per cow lowered product-based GHG emissions within the survey dataset, this did not reach the low level of the pasture ICLS, which was able to supply high quality products at a much higher level of eco-efficiency compared to high yielding indoor systems.

**Keywords:** dairy, pasture based, eco-efficiency, carbon footprint, system comparison

## Introduction

A future sustainable food system is required to feed the world while staying within the global system boundaries. Lowering the environmental impact of livestock production plays a key role within that transformational process. Studies argue that the role of livestock and ruminants in particular should be limited to convert non-human-edible biomass into human food, i.e. minimise feed food competition (Van Zanten *et al.*, 2018). This highlights the role of grasslands in future food security, which can, aside from providing biomass for ruminant feed, also provide multiple ecosystem services (Bengtsson *et al.*, 2019). Currently many efforts for lowering environmental impact aim at identifying greenhouse gas (GHG) mitigation measures in existing, intensive dairy systems where grass is often not the main feed. Such measures have limited potential (Arndt *et al.*, 2022) and may cause conflicts with other environmental indicators such as nutrient surpluses, feed food competition, land use efficiency or biodiversity and can therefore not entirely fulfil the above mentioned requirements. Hence, developing innovative dairy systems that combine low environmental impacts per unit product, i.e. eco-efficiency of production with minimal feed food competition is vital.

Ley pastures have been discussed as a way to reconnect livestock and arable farming in order to provide ecosystem services for crop production and society (Carvalho *et al.*, 2021; Sekaran *et al.*, 2021). Such innovative systems are, however, very rarely tested under environmental conditions where intensive dairying is predominant. The objective of this study was to benchmark and compare one example of an innovative ley grazing dairy system with typical intensive, specialised producers in northern Germany in terms of their eco-efficiency for GHG emissions, nutrient surpluses and land use.

## Materials and methods

One part of the dataset for the present study originated from a state-wide farm survey of dairy producers in the state Schleswig-Holstein, northern Germany (LKSH, 2021). The survey is conducted annually and covers a representative sample of economically best performing dairy farms in the state. Farms are predominantly intensive indoor systems (average of 9696 kg energy corrected milk (ECM) cow<sup>-1</sup>) with diets roughly containing one third of each of grass silage, maize silage and concentrates. The farm survey data was analysed based on the overall mean of the 209 farms (Average SH) and sub grouped by milk yield per cow (Producers SH; levels of <8500, 8500–9500, 9500–10 500 and >10 500 kg ECM cow<sup>-1</sup> year<sup>-1</sup>).

The other part of the dataset originated from an organic pasture-based dairy production as part of an integrated crop livestock system (Pasture ICLS) at Lindhof research farm, located in the east of Schleswig-Holstein (mean air temperature 8.7°C; mean annual precipitation 785 mm). A spring-calving (mid-January to March) Jersey herd with approx. 100 milking cows and young stock is managed in a 'low-input system' (6936 kg ECM cow<sup>-1</sup>), grazing on grass-clover leys as part of the organic cash crop rotation (2 to 3 years ley phase). From March onwards cows receive >90% of their feed budget from pastures. Swards are mainly established as undersowings into winter cereals and contain grass-clover-herb mixtures with high nutritive value. The data for both systems were taken from the accounting year 2020-21 (1 July 2020 to 30 June 2021).

Key indicators used for the environmental impact assessment were farm nitrogen (N) balances, land use efficiency (LUE) and GHG emissions, expressed in terms of global warming potential GWP100. GWP was calculated in an attributional life cycle assessment approach based on a spreadsheet model. The system boundary was the dairy farm for both systems including emissions from imported materials up until the farm gate (cradle to farmgate). For the pasture ICLS this meant that the cash crop production within the organic crop rotation was not part of the analysis. The functional unit was kg of ECM. LUE was calculated from land requirements for feed production on and off farm. Product-based emissions (product carbon footprint (kg CO<sub>2</sub>eq (kg ECM)<sup>-1</sup>; PCF), product N footprint (g surplus N (kg ECM)<sup>-1</sup>; PNF) and LUE (m<sup>2</sup> (kg ECM<sup>-1</sup>)) were calculated using an economic allocation (meat/milk) based on income data.

## Results and discussion

The seasonality of the pasture ICLS and cow genetics that are suitable for low input grazing allowed a maximised utilisation of high quality and low emission feed from ley pastures during lactation. Hence, emissions from manure management, feed imports and nitrous oxide emissions from soils were lower compared to the average SH. Despite the substantial difference in milk yield per cow, this resulted in a decrease in PCF of 51%, in PNF of 42% and in LUE of 4% compared to the average SH (Figure 1). Higher milk yield per cow in the producers SH resulted in lower product-based emissions and land use but higher emissions per ha (increase from 16.6 to 24.3 t CO<sub>2</sub>eq ha<sup>-1</sup> from lowest to highest producers SH group). Compared to average SH, the pasture ICLS was able to decrease both PCF and PNF to a lower level than the producers SH group with the highest milk yield per cow (Figure 1). Similar results were shown by Reinsch *et al.* (2021). Additionally, in the pasture ICLS trade-offs with other ecosystem services (e.g. clean water, clean air, biodiversity) are minimized while cash crop production substantially benefits from carry over effects of C and N (Taube *et al.*, 2023). Interpretation of results from a comparison with average farm survey data are not able to fully recognise potential mitigations in high yielding dairy systems, e.g. improved manure management. Nonetheless, the pasture ICLS demonstrates one example of highly eco-efficient dairying with little feed food competition. A complete assessment of the ICLS including the cash crop part of the rotation would highlight the overall benefits of this system and its contribution to building a future sustainable food system.

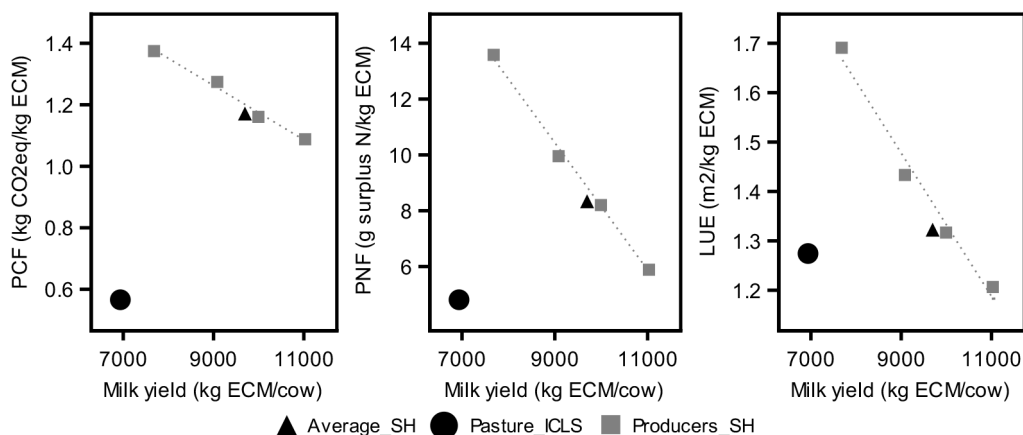


Figure 1. Effect of milk yield (kg ECM per cow) on the environmental indicators product carbon footprint (PCF; kg CO<sub>2</sub>eq (kg ECM)<sup>-1</sup>), product nitrogen footprint (PNF; g surplus N/kg ECM) and land use efficiency (LUE; m<sup>2</sup> (kg ECM)<sup>-1</sup>) in the pasture ICLS, the average SH and the survey dataset grouped by milk yield (Producers SH).

## Conclusion

Innovative dairy systems, as demonstrated here by the pasture ICLS, can be competitive in terms of eco-efficiency and milk output per unit land in regions where intensive dairying is typically predominant and soils are suitable for ley pastures. This was achieved by a system change towards seasonal pasture based production as part of an integrated crop livestock system. This provides more ecosystem services than just food production while using a lot less resources.

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# Long-term study on the effect of nitrogen fertilization on the growth of perennial ryegrass

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## Abstract

In Ireland, grazing systems provide the basis of sustainable livestock production in temperate regions, as grazed grass is the cheapest feed source for ruminant animals. Perennial ryegrass (*Lolium perenne* L.) underpins grassland production in Ireland and is the most important forage. The growth of perennial ryegrass occurs between February and November where grass growth is influenced by meteorological conditions varying from week-to-week and year-to-year. A long-term study was conducted at Teagasc, Moorepark, Ireland. A database was compiled from 2003–2023 illustrating the seasonal distribution of herbage production over 21 years. Nitrogen was applied weekly at three rates: 0, 150 and 300 kg N ha<sup>-1</sup>. Four series of plots for each treatment were harvested in rotation, spaced a week apart over the course of a growing season (February–November). Dry matter (DM) production averaged 5.25 t DM ha<sup>-1</sup> (0N), 8.95 t DM ha<sup>-1</sup> (150N) and 11.25 t DM ha<sup>-1</sup> (300N) over the periods examined.

**Keywords:** nitrogen, N response, seasonality

## Introduction

In Ireland, approximately 80% of a dairy cow's diet is made up of grazed or conserved forage (O'Donovan *et al.*, 2011). Grazed grass is the cheapest feed source available to livestock farmers and plays a vital role in the financial viability of family farms (Dillon *et al.*, 2005; Hanrahan *et al.*, 2018). Nitrogen (N) fertilization of perennial ryegrass plays an important role in providing sufficient herbage to meet feed requirements on farm. Nitrogen fertilizer is generally the most effective management input for manipulating herbage production within the limitations imposed by the environmental factors like soil type, temperature and moisture (Morrison *et al.*, 1974). In addition to these environmental factors, variations in yield and response to N fertiliser can be related to factors such as grass species and varieties, presence of a legume, frequency of defoliation, age of sward, season, and supply of other nutrients (Reid, 1970; Reid, 1978). Amongst these factors knowledge of the seasonal response is important as a tool for farm planning operations (Binnie *et al.*, 2000). This study examines the effects of chemical N fertilizer on herbage production at one site over a 21-year period.

## Material and methods

A cut-plot study was established in 2003 at Teagasc, Moorepark, Fermoy, Co. Cork, Ireland. Soil type is a free draining acid brown earth of sandy loam texture. Sixteen cut plots (1.2 m × 5 m) were rotationally cut once every 4 weeks (28 days). There were three nitrogen (N) treatments: 0 (0N), 150 (150N) and 300 kg N ha<sup>-1</sup> (300N) replicated 4 times. Fertilizer was applied by hand weekly to each plot in the form of calcium ammonium nitrate (CAN) at a rate of 5.6 kg N ha<sup>-1</sup> to the 150N treatment, 11.5 kg N ha<sup>-1</sup> for the 300N treatment and no N for the 0N treatment. Lime and fertilizer P, K and S were applied as required based on soil testing. One of each of the three treatment plots was cut every week, meaning that every 4 weeks each of the plots was cut, method of Corrall and Fenlon (1978). Plots were cut using an Etesia mower (Etesia Hydro 124D; Etesia) to a height of 4 cm above ground level. Mown herbage was



weighed and then a sample of herbage was taken from which 0.1 kg was weighed out and dried at 60°C for 72 h to determine dry matter (DM) content.

## Results and discussion

Total annual DM yield was 5.25 t DM ha<sup>-1</sup> for 0N, 8.95 t DM ha<sup>-1</sup> for 150N and 11.25 t DM ha<sup>-1</sup> for 300N. The seasonal distribution of DM yield may be as important in practice as the total yield obtained over the growing season. Seasonal production is shown in Figure 1. In this study the response to N fertiliser application was greatest in the May and June period.

There is a significant increase in yield across all seasons when nitrogen application is increased. This is in accordance with Whitehead (1995) who stated that the addition of increased levels of N has a direct impact on increased herbage production. Response to chemical N fertiliser application was 24.6 kg DM ha<sup>-1</sup> for treatments receiving 150N per year, while there was a response of 20 kg DM ha<sup>-1</sup> for swards receiving 300N (Figure 2).

## Conclusion

The application of chemical N fertiliser to grassland swards has a direct effect on the accumulation of more herbage within seasons and annually. Nitrogen fertilizer can be effective at manipulating the amount of herbage grown on farms.

## Acknowledgements

The financial support provided by the Teagasc Walsh Scholarship Programme, the SFIDAFM VistaMilk Research Centre (Grant no. 16/RC/3835) and the U-Protein Project funded by the Irish Department of Agriculture, Food and the Marine under the Food Institutional Research Measure (Grant no. 2019PROG702).

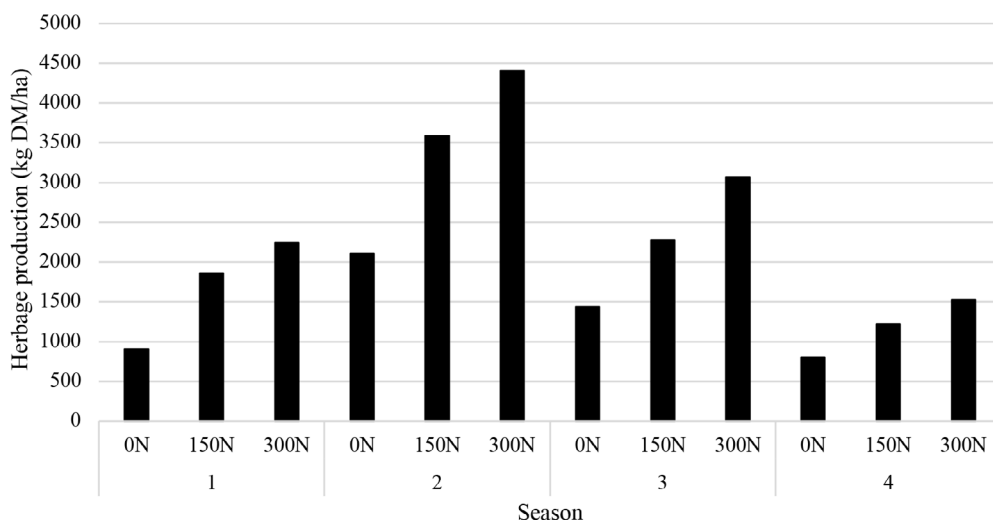


Figure 1. Average DM production of each N treatment (0, 150 or 300 kg N ha<sup>-1</sup>) over four seasons. Season (1) Spring (February–April). (2) Early Summer (May–June). (3) Late Summer (July–August). (4) Autumn (September–November).

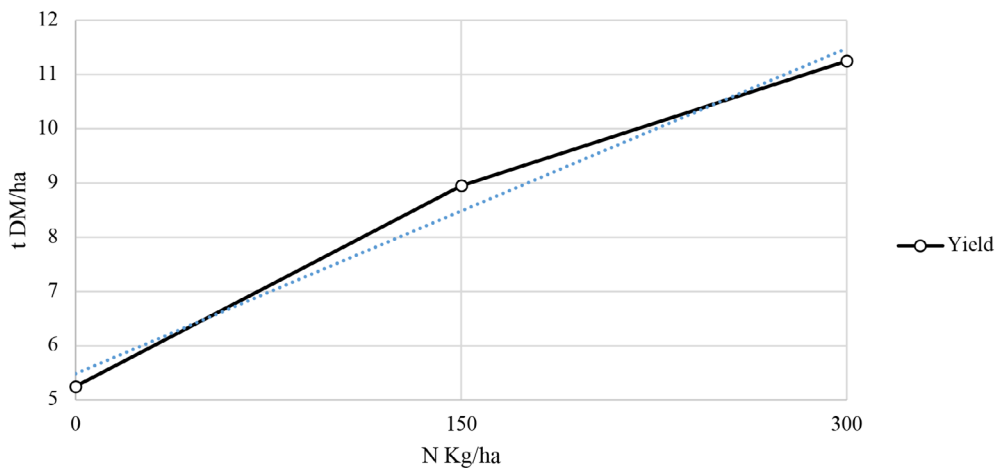


Figure 2. Total annual yield of N treatments.

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# Defining Grasslands' Role in Net Food Security: Policy ≠ Science

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## Abstract

The subject of grasslands' role in net food security encompasses several disciplines, but human nutrition must be pre-eminent. Estimations of dietary protein quality, bioavailability of nutrients from differing foodstuffs, and metabolic effects of energy sources should be the foundation of any consideration of food security and sustainability. Yet critical assumptions made when modelling food systems are frequently not based upon the highest quality scientific evidence. Dietary guidance and public health messaging are largely based upon the weakest-quality scientific evidence available while proposals for planetary diets ignore or misstate the available scientific evidence regarding the nutritive requirements for optimal human development and health. Metrics such as emissions per kilocalorie or yield of crude protein per hectare are misleading, minimizing the superior nature animal source foods (ASF) as sources of nutrition for humans. Production of high value foodstuffs from grasslands and ruminants offers unique ecological benefits compared to monogastric systems and the production of human-edible crops from cultivated grasslands. Prof. Friedrich Falke stated at the inaugural meeting of the International Grassland Congress in 1927 that '...feeding a population (of) a country by itself is the basis of public wealth, productivity and general well-being.' Any consideration of grasslands' role in the essential topics of food security sustainable food systems must be based upon the highest quality evidence available.

**Keywords:** nutrition, protein, carbohydrate, fat, meat, dairy

## Introduction

Estimates of sustainability must be multi-faceted: numerous societal, economic and environmental issues must be considered. In many policy discussions and most popular conversations, it is over-simplified, only focusing on a small number of environmental issues. A critical aspect of any consideration of food systems must be what constitutes a healthy diet and the nutritive values of the foodstuffs produced. Unrecognized by many outside the arenas, an active scientific debate has been underway within the human nutrition and medical health communities for almost a century. The uniquely fattening effects of carbohydrates was supplanted by the questionable belief that dietary fat led to heart disease (Taubes, 2007). The notion that naturally occurring saturated fats, primarily from animal source foods (ASF), caused heart disease led to dietary policies increasing plant lipid consumption (Teicholz, 2014). Faith-based notions of humanity's proper diet, combined with various social movements, lead to promotion of mostly- and entirely plant-only diets (Banta *et al.*, 2018; Lappé, 1965; Leroy and Hite, 2020). The premature adoption of one side in these debates has had impacts upon our grassland disciplines and communities. There is a significant divergence between high-quality scientific evidence and official dietary policies, to say nothing of marketing messages (Harcombe *et al.*, 2016; Ioannidis, 2018; Teicholz, 2015; Volek *et al.*, 2021). A lack of awareness of this diversity of scientific positions within the human nutrition and medical realm will impede efforts to improve food security, and impede grassland scientists' efforts to improve management and practice.

## Materials and methods

This work is intended as an introduction to this vital subject. Its aims are to present information that will be helpful to colleagues personally, to contribute to more robust conversations regarding food systems, and increase appreciation of grasslands in food systems.

## Results and discussion

More than 42% of humanity is malnourished (828 million calorically undernourished, 2.6 billion overweight or obese) (WHO, 2000). More than a fifth of all children under the five years of age are stunted due primarily to a lack of the nutrients best or solely provided by animal source foods (ASF) (Adesogan *et al.*, 2020). Stunting includes inhibited brain development as well as height. These children will not be able to achieve their potential. Animal source foods are essential for proper human development and function. Malnutrition, a major global public health problem in children and adults (Dukhi, 2020), is a pressing issue across all income-level countries, prevents sustainable development, and poses an environmental burden typically unrecognized. It is more than caloric insufficiency (WHO, 2000). One can be overfed and undernourished. 'Protein-Energy Malnutrition is by far the most lethal form of malnutrition' (WHO, 2000). Despite messages promoting the adoption of a 'plant-based' diet, humanity's diet is already plant-based. Plant source foods (PSF) provide the majority of calories and crude protein in humanity's food supply with wheat and rice (and products made from them) the two largest sources of energy and crude protein (FAO, 2020). Unsurprisingly, the essential amino acid lysine is globally limiting in humanity's food supply (Moughan, 2021). While childhood protein-energy undernutrition (PEU) or protein-energy malnutrition (PEM) is a widely discussed and accepted global problem, PEU in adults remains an under-recognized entity (Kapoor *et al.*, 2022). The discussion of crude protein (typically expressed simply as 'protein') is an inappropriate oversimplification (FAO, 2011; Moughan, 2021). The Digestible Indispensable Amino Acid Score (DIAAS) should be used to evaluate the human-utilizable essential amino acid yield between systems and environmental impacts (Tessari *et al.*, 2016; Moughan, 2021). Basing models and guidelines upon the Recommended Daily Allowance (RDA) of protein leads to flawed conclusions and poor health outcomes (Layman, 2004; Phillips *et al.*, 2016). Public messaging suggesting a need to reduce dietary protein intake is particularly concerning, given the preceding and evidence of increasing micronutrient deficiencies when calories from ASF fall below 30% (Nordhagen *et al.*, 2020) and less than 50% of protein is from ASF (Vieux *et al.*, 2022).

Diets with higher-than-recommended levels of ASF can correct the symptoms of metabolic illnesses (a form of malnutrition) (Ludwig *et al.* 2021), offering hope in arresting the current worldwide epidemic of diabetes and other metabolic diseases (Crofts, 2015).

The lack of ASF is due to many factors, including affordability and access. Ensuring that the essential ASF are available and affordable while producers realize a sustaining level of profit is another vital component of food security. Ruminant animals' function in circular grasslands systems is an essential component.

## Acknowledgements

The author wishes to express his deep appreciation for the opportunity to participate in the 30<sup>th</sup> European Grassland Federation General Meeting, the support of his family and colleagues, and for the wealth of information shared by colleagues across many disciplines. This work is entirely self-funded.

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# Effect of forage-to-concentrate ratio in dairy cow diets on estimated milk carbon footprint

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## Abstract

Three different scenarios were compared to assess the effects of concentrate proportion and the natural oil content of the dairy cow diet on the milk carbon footprint, including the feed production. The first two scenarios included grass silage and two levels of concentrate: 450 g (kg dry matter (DM))<sup>-1</sup> (Con45) or 350 g (kg DM)<sup>-1</sup> (Con35). Concentrates consisted of barley and rapeseed meal. The third scenario was a diet with concentrate proportion of 350 g (kg DM)<sup>-1</sup> composed of oats and rapeseed cake (Con35+) in order to test for the effect of oil inclusion from rapeseed cake. The concentrate proportion had no effect on modelled rumen methane production. The N<sub>2</sub>O emission was lower with Con35 than with Con45 due to smaller land use. The use of oil rich concentrate (Con35 vs Con35+) decreased methane production by 6% (kg energy corrected milk (ECM))<sup>-1</sup>. The differences in the milk carbon footprint between scenarios were relatively small, where the differences were 1.12, 1.03 and 1.01 kg CO<sub>2</sub>eq (kg ECM)<sup>-1</sup> for Con45, Con35, and Con35+. Respectively, the carbon footprint was 5.26, 4.66 and 4.60 CO<sub>2</sub>eq (kg ECM)<sup>-1</sup> when the feed was produced on pure peat soil.

**Keywords:** carbon footprint; land use; oil supplementation; methane; peatlands

## Introduction

Agricultural fields are a source of greenhouse gas (GHG) emissions. Cultivated land releases more GHG than grasslands. Grassland can also be a carbon sink (Saarinen *et al.*, 2019). GHG emissions consist mainly of carbon dioxide and nitrous oxide (CO<sub>2</sub> and N<sub>2</sub>O), depending on soil type and the amount of nitrogen fertilisation. Peatlands release CO<sub>2</sub> more than fifty to hundred times more than that released from mineral soils (IPCC, 2013). The decrease of field area per kg milk therefore also decreases the milk carbon footprint, especially if the reduction focuses on cultivated land and peatlands.

Vegetable oil in dairy cow feeding has been proved to mitigate rumen methane production (Martin *et al.*, 2010). However, a high amount of oil supplementation decreases fibre digestion, and the market price of oil is too high to be used cost-effectively in rumen methane production mitigation. According to Martin *et al.* (2010), the mitigating effect of oil has been 3.8% per 10 g added oil (kg diet DM)<sup>-1</sup>. If the net cost of 1 kg day<sup>-1</sup> oil supplementation were €1.5 day<sup>-1</sup>, the cost of a CO<sub>2</sub>eq tonne would be €640. Another way to add oil to the diet is to use rapeseed cake and oats in feeding. The daily oil supplementation level with these concentrates is small (diet fat content < 50 g (kg DM)<sup>-1</sup>), but it offers a cost-effective alternative to methane mitigation. The objective of this study was to quantify the effect of concentrate proportion and mild oil supplementation on the milk carbon footprint, taking LULUCF (Land Use, Land-use Change and Forestry) into account.

## Materials and methods

Three comparable scenarios including grass silage were modelled: a concentrate proportion of 45% with barley and rapeseed meal (Con45); a concentrate proportion of 35% (Con35); and a concentrate proportion of 35% with rapeseed cake and oats (Con35+). The grass yield was 6300 kg DM ha<sup>-1</sup>, and the grain yield was 2800 kg DM ha<sup>-1</sup>. The crop rotation included three years grass and one year grain. Rapeseed meal is a by-product of oil production and did not need cultivated area.

The production responses to concentrate supplementation were evaluated using the Lypsikki model (Huhtanen and Nousiainen, 2012), and rumen methane production according to Ramin and Huhtanen (2013). Emissions from peat soils and N<sub>2</sub>O emissions from nitrogen fertilisation were calculated according to IPCC (2013), and emissions from mineral soils according to Saarinen *et al.* (2019). The CFP of purchased feeds was fixed at 0.57 kg CO<sub>2</sub>eq (kg DM)<sup>-1</sup>. Small fractions of emission sources (fuel, electricity, outside transports, bedding material, lime, seeds) were common between scenarios and were assessed as 0.09 kg CO<sub>2</sub>eq (kg energy corrected milk (ECM))<sup>-1</sup>. The effect of land use change on the soil carbon content has been calculated with the Yasso model (Tuomi *et al.*, 2011). The allocation of the total carbon footprint was 85% for milk and 15% for meat.

## Results and discussion

The main result of the study was decreased land use with 1.2 kg lost in ECM yield when Con45 was replaced by Con35. The Con35 scenario compared to Con45 increased the milk production cost by 1.2 cent (kg ECM)<sup>-1</sup> taking the subsidies into account, thus making Con35 a less economic alternative. Subsidies included both hectare-based and milk production-based incomes. From an ethical and environmental perspective, grains and other human-edible feeds should be administered directly to human foods, and low concentrate feeding strategy needs to receive economic compensation from society. Currently, the subsidies are based on field area, resulting in a higher subsidy for grain than with grass production. This, with a relatively high milk production response to supplementation compared to the ratio of concentrate costs and milk price, encourages the use of grain in dairy cow feeding.

Emissions from mineral soil are small compared with the yield of milk per ha. The improvement in milk CFP with decreased concentrate proportion was 0.09 kg CO<sub>2</sub>eq (kg ECM)<sup>-1</sup>. The advance of a low grain diet increased to 0.70 kg CO<sub>2</sub>eq (kg ECM)<sup>-1</sup> when all crops were produced in peatlands. The emission factor for peatland is so high that it overcomes all other effects. Peatlands represent 10% of Finnish agricultural land, yet this estimate is more or less hypothetical. For example, the emission factor from peatland depends on the thickness of the turf, and one constant value does not describe all emissions well.

The concentrate proportion did not have marked effects on the modelled rumen methane intensity. Concentrate supplementation has decreased methane intensity (Bayat *et al.*, 2018) if the proportion of propionic acid in rumen fluid increases. Typically, the proportions of volatile fatty acids do not differ markedly when the change of concentrate proportion is moderate in a grass silage diet. In this study, the modelled result reflects this situation.

Oil supplementation decreased methane intensity by 4%, which is a little less than the expected 6% reduction according to Martin *et al.* (2010). Recent studies have reported an approximately 10% reduction in methane intensity when rapeseed meal and barley (oil content 30 g/kg DM) are replaced by rapeseed cake and oats (oil content 73 g (kg DM)<sup>-1</sup> Räsänen *et al.*, 2023).

The N<sub>2</sub>O emissions decreased with Con35 due to increased N use efficiency in feed production. Emissions per ha increase with Con35, but a decreased total field area overcompensates this effect. The amount of slurry nutrients was constant per cow, but the total amount of mineral fertilisation per kg milk was decreased. The mitigation of N<sub>2</sub>O was moderate, but the change was in the right direction.

Table 1. Effect of concentrate proportion of dairy cow diets on milk carbon footprint when feeds are produced on mineral or peat soil.

	Mineral soil			Peat soil	
	Con45	Con35	Con35+	Con45	Con35
Land use					
Grain (ha cow <sup>-1</sup> )	0.98	0.64	0.63	0.98	0.64
Grass (ha cow <sup>-1</sup> )	0.97	1.06	1.05	0.97	1.06
Milk yield (ECM day <sup>-1</sup> )	31.2	30.0	29.9	31.2	30.0
kg CO <sub>2</sub> eq (kg ECM) <sup>-1</sup>					
Methane	0.48	0.47	0.45	0.48	0.47
Fertilisation	0.37	0.35	0.33	0.37	0.35
Slurry storage	0.06	0.06	0.06	0.06	0.06
Plant residues	0.04	0.04	0.04	0.04	0.04
Purchased feeds	0.06	0.07	0.07	0.06	0.07
Other sources	0.08	0.08	0.08	0.08	0.08
LULUCF	0.06	-0.03	-0.03	4.22	3.65
Total sum	1.12	1.03	1.01	5.26	4.66

Con45, concentrate proportion 45% with barley and rapeseed meal; Con35, concentrate proportion 35% with barley and rapeseed meal; <sup>3</sup>Con35+, concentrate proportion of 35% with rapeseed cake and oats; ECM, energy corrected milk; LULUCF, land use and land use change, and forestry.

## Conclusions

The effect of concentrate proportion on the total milk carbon footprint was small when the feeds were produced on mineral soils. However, the combination of reduced grain use with oats and rapeseed cake saw a change in the right direction, with a 0.05 kg CO<sub>2</sub>e kg<sup>-1</sup> ECM decrease in the ECM carbon footprint. In practice, the most considerable factor in the milk carbon footprint remains the proportion of peat soils used in feed production.

## Acknowledgement

The authors acknowledge funding from the European Agricultural Fund for Rural Development.

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**Theme 2.**

**HOW?**

**How do we balance ecosystem  
services?**



# Balancing competing ecosystem services requires stakeholder involvement and actions on different spatial scales

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## Abstract

Ecosystem services (ES) are highly important for human wellbeing, but many grassland ES show trade-offs that are strengthened by management intensification. For example, high forage production conflicts with many cultural ES as well as the conservation of grassland biodiversity. Balancing these competing services is thus required to ensure that ES supply meets societal demand. This poses the question of how to achieve such a balance in the future. We discuss how involving stakeholders and implementing ES-enhancing actions at landscape, farm, and field scales can contribute to tackling this urgent question. First, multi-stakeholder approaches are required to assess prioritisation of ES to understand societal ES demand, to design multi-functional landscapes, and to motivate farmers to increase insufficiently supplied ES. Second, different actions need to be implemented across spatial scales, with the landscape being crucial to balance ES by spatial targeting of different grassland types. In addition, actions to enhance ES that are in short supply can and must be taken at farm and field scale. Therefore, all three spatial scales should be considered to balance competing grassland ES. Our synthesis provides not only a framework for improved balancing of ES, but also gives applied examples how this can be achieved.

**Keywords:** agri-environmental policies, ecosystem service trade-offs, land-use intensity, landscape management, multi-stakeholder surveys, synergies

## Introduction

Ecosystem services (ES) are defined as the goods and benefits humans derive from all sorts of ecosystems. They are crucially important for human wellbeing, and grasslands have been shown to be critical for ES supply in many regions of the world (Bardgett *et al.*, 2021; Bengtsson *et al.*, 2019; Power, 2010). Generally, ES can be categorised into *provisioning* (e.g., food, forage, fibre), *regulating* (e.g., erosion and stormwater control, carbon storage, habitat, biodiversity) and *cultural services* (e.g., aesthetics, recreation, heritage; Richter *et al.*, 2021). Ecosystem service multi-functionality describes the simultaneous production of many such ES (Allan *et al.*, 2015; Manning *et al.*, 2018). In recent times, the multi-functional role of agriculture in general, and of grasslands in particular, has repeatedly been emphasised by scientific, societal and political initiatives (Hart *et al.*, 2016; Nowack *et al.*, 2022). Nevertheless, many ES of permanent grassland are threatened and decreased by pressures such as land-use change and biodiversity loss (Allan *et al.*, 2015; IPBES, 2019). As a consequence, in Europe, ES supply does currently not match societal ES demand (Bengtsson *et al.*, 2019). One reason for this is different grassland ES competing with each other due to trade-offs, i.e., antagonistic relationships between two or more ES (Franzluubbers and Martin, 2022; Power, 2010). For example, high forage production conflicts with high cultural ES and biodiversity conservation (Figure 1). To match ES demand and supply in the future, such competing services need to be more effectively balanced.

Balancing competing ES, notably provisioning ES versus non-provisioning ES, is complicated. First, many non-provisioning services do not have a market value and are not directly addressed by agricultural policies. Second, different groups of stakeholders hold contrasting demands on ES supply. Indeed, agricultural and nature-conservation stakeholder groups may have different perceptions of ‘healthy’ versus ‘degraded’ grasslands in terms of the set of ES that should be delivered (Bardgett *et al.*, 2021; Klaus, 2023). Therefore, attempts to balance competing ES have to be based on a broad societal basis, which can only be achieved by involving all relevant stakeholders. These comprise all people or groups affecting or being affected by a change in ES supply (Peter *et al.*, 2021).

In this paper, we discuss options to balance competing ES and design multi-functional landscapes, requiring improved understanding of ES trade-offs and societal ES demand. We suggest that closing the gap between ES supply and demand requires targeted management actions at different spatial scales, i.e., landscape, farm and field. These three scales are all important for balancing competing ES due to scale-dependent opportunities and shortcomings. Finally, to enhance ES in short supply and to promote the uptake of these management actions, we argue for both improved collaboration between all involved stakeholders and also for policies that support farmers in producing ES that are in short supply because they do not have a market value.

## Ecosystem service trade-offs and bundles

Farming for grassland ES is faced with considerable field-scale trade-offs among ES, which need to be considered to deliver the whole set of societally-demanded services (Figure 1). Management intensity is known to play a major role in shaping these trade-offs (Lindborg *et al.*, 2022). For example, a key trade-off occurs from fertiliser inputs, which affect biotic and abiotic processes: While high fertilisation intensity promotes plant growth and thus forage production, this at the same time decreases the aesthetic quality and biodiversity of a grassland (Bengtsson *et al.*, 2019). In response to the fertilisation-driven differences in resources, communities of plant, animal and microbial taxa change their functional traits related to growth and nutrient capture (i.e., resource economics; Grigulis *et al.*, 2013; Neyret *et al.*, 2024). While slow-growing species with resource conservative traits dominate in nutrient-poor grasslands, fast growing and competitive species with exploitative traits, such as rapid nutrient uptake, dominate in nutrient-

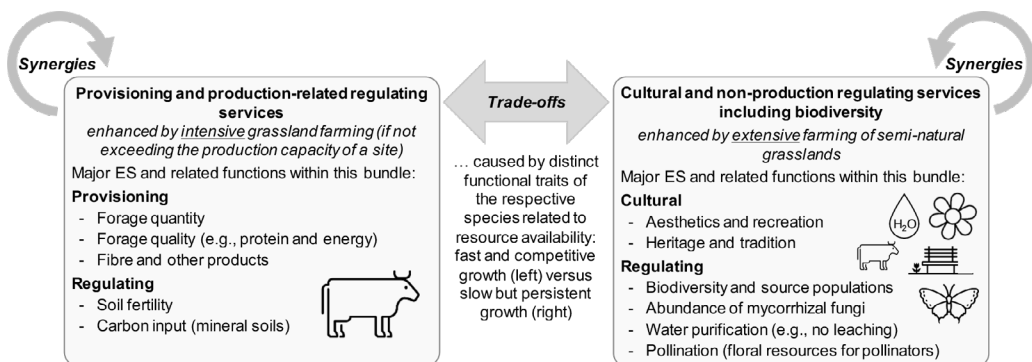


Figure 1. Trade-offs and synergies among ecosystem services (ES) related to intensive, productive versus extensive, semi-natural grasslands, which provide distinct bundles of ES (Lindborg *et al.*, 2022). Note that in intensive grasslands (left), many ES depend heavily on anthropogenic inputs (fertiliser, fuel, etc.), which are not considered part of the ES framework (Bethwell *et al.*, 2021). Therefore, ES from intensive grasslands need to be considered in contrast to the inputs required. This issue is much less relevant in less intensive and extensive grasslands (right). It is important to note that besides trade-offs between provisioning (intensive) and non-provisioning (extensive) grassland ES, many services primarily supported by extensive semi-natural grasslands are important for sustaining productivity on the landscape scale, e.g., pollination of crops.

rich conditions (Lavorel *et al.*, 2011). This management-induced functional distinction of grassland ecosystems results in distinct ES bundles, i.e., ES that occur together in space and time (Saidi and Spray, 2018). For the temperate zone, strong trade-offs between biodiversity and many cultural and regulating ES (first bundle) versus intensive food and forage production (second bundle) have been found (Figure 1). For instance, dry matter production is highest in sown temporary grasslands (leys), but somewhat lower in most intensive permanent grasslands, and lowest in extensive semi-natural grasslands, which constitute the backbone of traditional cultural landscapes and nature conservation (Lindborg *et al.*, 2022; Schils *et al.*, 2022). This clustering of many ES into a reduced number of ES bundles facilitates land-use decisions by reducing the complexity inherent to the multiple ES provided by grasslands, and it depicts an important tool for communicating ES supply and demand to stakeholders (Saidi and Spray, 2018).

## **Understanding ecosystem service demand to design multi-functional landscapes**

Improved understanding and joint consideration of ES demand, production (supply), and flow to society is needed to adequately balance ES (Neyret *et al.*, 2023). However, societal ES demand is difficult to assess and studies on the related socio-cultural dynamics are scarce (Peter, 2020). Currently, ES demand is best approximated via the prioritisation of ES by stakeholders, putting quantitative weightings to each ES. This requires comprehensive surveys of relevant stakeholder groups and their perceptions and values (Horcea-Miclu *et al.*, 2016). To move towards standardised analyses of inherently different ES, multi-criteria evaluation approaches of the benefits delivered by ES can be adopted (Manning *et al.*, 2018). Such interdisciplinary and participatory approaches also help understanding the gap between perceptions of ES across stakeholder groups, including the scientific community and the general public. Based on such surveys, the socio-cultural factors and worldviews shaping ES demand and supply can be understood (Peter *et al.*, 2021).

Although many grassland ES might not be sufficiently recognized by society, studies on the prioritisation of ES by stakeholders and the perception of citizens found almost all ES to be relevant when people were directly asked about them. Yet, significant differences were found between individuals depending on factors such as profession, education, socio-cultural context, age, and geographic location (e.g., Klaus *et al.*, 2022; Lamarque *et al.*, 2011; Peter *et al.*, 2021; van den Pol-van Dasselaar *et al.*, 2014). This highlights the complexity of interactions between culturally-defined worldviews and ES priorities of different groups. Contrasting stakeholder views also relate to short versus long-term gains and local versus global considerations, such as local disadvantage versus global benefit linked to a management decision. Previous studies found agriculture to mainly prioritise provisioning ES, while tourism tends to focus on cultural ES such as leisure activities and biodiversity (Peter *et al.*, 2021). In addition, Peter *et al.* (2021) identified so-called 'worldview types', which describe the link between prioritising certain ES and a specific socio-cultural worldview. Stakeholder groups, in which an individualistic and rather conservative worldview dominates, put greater value on provisioning ES and perceive nature as constant but unpredictable. In contrast, stakeholder groups that are more oriented towards the common good mainly prefer cultural ES and perceive nature as suffering from biodiversity loss (Peter *et al.*, 2021).

With data on ES demand/prioritisation and supply, it is possible to calculate the ES multi-functionality of landscapes, i.e., supply relative to human demand (Manning *et al.*, 2018), and to model land-use scenarios that create an 'optimal' landscape with highest distribution equity, i.e., the equitable access of multiple stakeholder groups to ES supply (Neyret *et al.*, 2023). The latter study revealed that the current state of land use (i.e., proportions of different types of grassland, forest, and arable land) in three regions in Germany were almost optimal, potentially because these landscapes have been culturally shaped for centuries and are thus already well adapted to the diverse interests of society. Yet, the identification of scenarios for the equal fulfilment of all interests resulted in a minimal increase in forest area and an extensification of some grasslands leading to a slight improvement towards the optimal distribution

equity compared to the current situation (Neyret *et al.*, 2023). Results from such studies that make use of data on ES supply and demand can help to guide landscape-scale management towards balancing ES (Cong *et al.*, 2014). A landscape being close to the priorities of society does however not mean its composition does not change over time, as land-use decisions are usually taken by few stakeholder groups driven by agricultural policies and markets.

### **Targeted action for balancing ecosystem services: the landscape scale**

As the processes causing ES trade-offs cannot be resolved only at field scale, larger spatial scales such as the farm and landscape are needed to balance competing ES. The landscape is the level of organisation integrating the different aspects and components of ES production, ranging from ecological processes over agricultural practices to social structures and interactions, linking ES (co-)producers and beneficiaries (Vialatte *et al.*, 2019). Indeed, the landscape offers the opportunity to combine different types of grasslands (and further ecosystems types), which all deliver different bundles of ES (Figure 1). Many ES are provided and/or maintained by multiple ecosystems at the same time, due to positive and negative spill-over effects and spatial interrelations between landscapes elements (Le Provost *et al.*, 2023). Thus, only at the landscape scale it is possible to account for the effects of surrounding land uses, driven by spatial arrangement and connectivity of landscape elements (Fahrig *et al.*, 2011; Gebhardt *et al.*, 2023). Balancing ES at the landscape scale is faced by the challenge of variation in space and time, as ES result from processes at multiple spatial and temporal levels.

Several options exist for balancing competing ES on the landscape scale. In heterogeneous landscapes, the biophysical conditions of some areas are usually better suited for a certain type of land use, making spatial targeting a relevant option to improve landscape-scale ES supply and multi-functionality (Franzluebbers and Martin, 2022). Improved spatial targeting of agricultural practices and policies, such as agri-environmental schemes, has the potential to increase the supply of several ES and minimise trade-offs. Therefore, local ES production targets need to be set according to the biophysical conditions best supporting these ES (Assis *et al.*, 2023).

Related to the former, collective contracts and incentives can foster collaborative agri-environmental management through innovative schemes that operate at the landscape scale (Prager, 2015). Shifting restrictions such as the proportion of semi-natural habitat required by greening regulations from the farm to the landscape scale and enhancing cooperation among farms can thus enhance spatial targeting, increase positive spill-overs between ecosystem types, support habitat for higher biodiversity, and ultimately lead to higher landscape multi-functionality (Engel, 2016). Collaborations among farmers should affect the distribution and/or area of land uses across the landscape and also the connectivity between them, leading to a more efficient landscape-scale ES supply. Cooperation between farmers can further enhance circularity and sustainability, which in turn leads to increases in ES at landscape scale (Andersson *et al.*, 2005). Various types of landscape-scale collaboration among farms and farmers are possible, such as the exchange of materials (e.g., hay and manure) and shared investments (Prager, 2015).

Despite the widely-acknowledged relevance of the landscape for ES, policy tools to set management targets and stimulate cooperation on the landscape scale are still widely absent (Cong *et al.*, 2014). Examples of existing landscape-scale multi-stakeholder instruments include the Swiss habitat network areas ('connectivity projects'), a collective agri-environmental scheme in which different land users need to cooperate to create links between fields with existing biodiversity-focussed schemes and/or nature conservation areas (FOEN, 2017). Such collective approaches in implementing but ideally also designing agri-environmental schemes are relevant landscape-scale approaches to balance ES. For example, in the *Dutch model* collectives are intermediaries between governmental decision-makers and farmers and involved in the management of landscapes and habitats, often using specific agri-environmental schemes

(Prager, 2015). To efficiently balance ES, more such instruments are needed to enable landscape-scale decision-making. Yet, approaches that ‘manage the landscape like a big farm’ might also depict a (cultural) challenge for land owners and users.

Due to land competition for different grassland types, balancing ES also translates into increasing the effectiveness of ES production. A higher effectiveness per area can release pressure on land and opens up possibilities to additionally manage for those ES that are in short supply. Therefore, higher effectiveness in producing one ES should not result in increased production of the given ES, but in enhancing another, undersupplied ES. This might, for instance, require conversion of intensive to extensive grassland or vice versa. A higher efficiency can be achieved by, for example, overcoming degradation by weed infestation in intensive grasslands and the ecological restoration of species-poor extensive grasslands, which do not reach their potential for biodiversity conservation and cultural ES (Bullock *et al.*, 2021; Freitag *et al.*, 2021). While the landscape scale offers many opportunities to increase one ES without reductions in another, competing ES, this can also lead to spatial inequality in ES supply. Thus, action also needs to be taken on smaller scales, i.e., farm and field.

### **Targeted action for balancing ecosystem services: the farm scale**

The farm is the key unit of agricultural ES production driven by farming systems and production aims (in social and economic terms). Effectively balancing ES has to involve activities at the farm, where non-provisioning ES must find a balance with farmer’s profits. Because of this, farm-scale intensification threatens several ES not only from extensive but also intensive permanent grassland (Pilgrim *et al.*, 2010). Since the 1980s, in several European countries, maize for silage production and (mixed) grass and leguminous leys have widely replaced permanent grasslands in lowland areas (Lanza *et al.*, 2021). In mountain areas, traditional small-scale farms that once reared locally-adapted ruminant breeds, fed with on-farm forages from permanent pastures, have introduced high-producing dairy breeds and high energy rations based on purchased concentrates (Sturaro *et al.*, 2013). This also led to the loss of ES associated with the abandonment of less suitable mountain pastures, which could be used with the traditional breeds (Pauler *et al.*, 2022).

The farm scale offers interesting options for balancing ES and enhancing ES multi-functionality, for example, by targeting different ES on different fields of the farm (Duru *et al.*, 2014; White *et al.*, 2019; Figure 1). By cultivating different grassland types, some intensively and others extensively managed, it seems possible to better reconcile production and biodiversity conservation objectives on a farm than by applying a uniform management of intermediate intensity. Indeed, the intermediate intensity level over-proportionally reduces both the digestible energy yield (compared with intensive management; Nemecek *et al.*, 2011) and the biodiversity conservation value (compared with extensive management; Gossner *et al.*, 2016). Thus, heterogeneity of grassland management at farm scale, in space and time, can be beneficial for biodiversity and other ES without harming overall productivity (Sabatier *et al.*, 2015). For example, Ravetto Enri *et al.* (2017) show a rotational grazing system that excluded a plot from grazing for two months during the main flowering period, achieving enhanced flower resources for pollinators without penalising farm-scale production. Diversifying grassland types at farm scale can also strengthen the socio-economic resilience of farms (Dumont *et al.*, 2022). Similarly, the importance of a diversity of grassland types on a farm has been suggested for enhanced climatic resilience (Plantureux *et al.*, 2022), because climatic variation differently impacts distinct grassland types and their ES bundles.

While balancing the supply of a range of ES requires grasslands within a farm to be managed in different ways and with different intensity levels, there are limits to farm diversification (Dumont *et al.*, 2022). Biggs *et al.* (2012) suggest that the growing complexity of increasingly diversified farms can lead, after a certain diversification threshold, to the system becoming too complex for adequate management,



thereby reducing its capacity to adapt. As yet, such thresholds remain to be quantified. Further research is therefore necessary to determine what level of diversification of grassland types and farm management is the best solution for increasing farm-scale ES multi-functionality while avoiding the system becoming too complex.

Further farm-scale measures to support ES that are in short supply include digital farming and technical innovations. Examples are fertilisation innovations, which result in both a higher effectiveness per unit nitrogen applied (and related financial inputs) as well as better protection of ES provided by neighbouring semi-natural fields (Morizet-Davis *et al.*, 2023). Moreover, changes in farming systems by, for example, reconsidering breed selection, breeding aims and lifespan of animals can further create opportunities for enhancing specific ES, for example by releasing economic pressure via a more cost-efficient feeding strategy based on self-produced grass (Franzluebbers and Martin, 2022). (Re-)introducing grazing management can not only help to reduce feed-food conflicts but also increases cultural ES (Dumont *et al.*, 2022), and thus overall ES multi-functionality. Yet, depending on the field-scale effects of such measures, it has to be ensured that increasing one ES does not trade-off with another.

### **Targeted action for balancing ecosystem services: the field scale**

Agricultural management practices are key to reduce trade-offs and increase synergies among ES (Power, 2010), and the field is the one place for many such management decisions. Balancing ES can therefore involve a multitude of field-scale management adaptations, usually linked to creating and/or maintaining favourable habitats for important taxa, overcoming degradation, and improving biogeochemical cycles such as the spatial distribution of key resources. Improvements in the field-scale supply of ES have been shown to cascade up to positive effects on larger-scale ES, emphasizing the importance of multi-scale strategies for enhancing ES (Bullock *et al.*, 2021; Figure 2). Measures to enhance ES of a field are often specific for a grassland type, such as (mainly grazed) pastures versus (mainly mown) meadows and fertilised, improved versus unfertilised, extensive grasslands.

In pastures, ES production is strongly affected by trade-offs in ecosystem function driven by stocking rate, such as maximisation of herbage use by animals (carbon uptake) versus carbon returns to soil. Similarly, improved forage quality to reduce emissions of enteric methane conflicts with the decomposability of herbage to increase mean residence time of soil organic carbon (Vertès *et al.*, 2019). Moreover, differences in the spatial distribution of feeding activities and nutrient return (excreta) promote spatial and temporal uncoupling of nutrient cycles in pastures. To improve this, stocking rates and grazing season can be adjusted in line with pedoclimatic conditions, the spatial dispersion of shade and watering points can be improved to encourage more uniform use of the field by the herd, and external dietary supplements that exacerbate plant-soil asynchrony might be restricted (Fontaine *et al.*, 2023).

In the case of mown grasslands, multi-species swards with an optimal abundance of legumes are generally considered to be facilitators of multiple ES. Therefore, the transition from monocultures and simple grass-clover swards to more complex multi-species mixtures is associated with gains in multi-functionality (Suter *et al.*, 2021) and a higher resilience to climatic variability (Lüscher *et al.*, 2022). As for pastures, uncut refuges can support pollinator and general insect diversity.

In extensive grasslands, nature-based solutions can be used to achieve higher supply on the same area, potentially leading to win-win situations (Bullock *et al.*, 2021). For example, the ecological restoration of species-poor unfertilised grasslands, which suffer from a depleted species pool and dispersal limitation, can increase biodiversity conservation and aesthetic quality (e.g., Freitag *et al.*, 2021). In all types of grasslands, rewetting of organic soils during the whole year or at least the winter season, when no management actions are undertaken, helps to sustain remaining peat and improves the carbon balance

of the fields (Renou-Wilson *et al.*, 2016). Agroforestry, precision agriculture, and changing from mineral to organic fertilisation can further help to enhance carbon storage (e.g., Van Vooren *et al.*, 2018). These examples show that several management practices can promote field-scale ES multi-functionality by increasing specific ES without reducing another, competing ES. However, the uptake of such measures is often slow if not stimulated by incentive schemes and other policy measures.

### **Stimulating the production of non-provisioning ES**

At present, concerted actions for increasing and balancing non-provisioning grassland ES are hindered by a number of issues: (i) political prioritisation of food production and security over non-provisioning ES, (ii) lack of understanding of (co-)benefits of ES on human well-being, including agricultural aspects such as farm resilience, (iii) lack of specific ES-targeted policies and incentives, (iv) difficulties to accurately measure, value and monitor many ES with broadly-accepted indicators, (v) missing practical information on how ES-enhancing management can be implemented, and (vi) lack of broad stakeholder involvement and motivation (e.g., Lindborg *et al.*, 2022; Pacual *et al.*, 2023; Stokes *et al.*, 2023; Tindale *et al.*, 2023). As highlighted by the last issue, involving farmers is crucial to increase their motivation for taking enforced efforts to enhance the ES multi-functionality of their land (Mehring *et al.*, 2023).

Participatory approaches to co-design sustainable social-ecological systems together with all relevant stakeholders are promising, but they require a suitable infrastructure for a broad-scale implementation. This infrastructure still needs to be established in most contexts (Berthet *et al.*, 2019). To further facilitate farming for multiple ES, detailed information on how management practices change ES and their trade-offs, and how ES are also beneficial for producers, and must be available and translatable into implementation (Stokes *et al.*, 2023). Thus, exchange and cooperation between all stakeholder groups from 'policy-making to field management' are essential to stimulate balancing competing ES.

The need to address ES production with agricultural policies is strengthened by mismatches between ES producers and beneficiaries. These can operate on local scales, with farmers producing public non-provisioning goods for the whole local society, but also on larger spatial scales when, for example, global climate services are derived from local carbon sequestration (Hein *et al.*, 2006). As farming for multi-functionality can only happen on a robust economic basis, and because market and policy constraints drive grassland farmers towards focussing on production (Lindborg *et al.*, 2022), new and improved policy tools and incentives, such as payments for ES, seem unavoidable to enhance non-provisioning ES (Engel, 2016). Integrating stakeholder priorities in the design of such payment schemes might considerably help to increase both societal and farmer acceptance of the measures (Tindale *et al.*, 2023).

Improving ES assessments holds considerable potential to better understand the full picture of the ES production by different grassland systems, such as organic versus conventional farming and high-input versus low-input systems. Many assessments do not consider that grassland ES are usually co-produced by biotic and abiotic properties and processes as well as anthropogenic inputs such as labour and materials. Yet, these inputs are not considered part of the natural capital that originally produces ES, and they are methodologically difficult to measure (Bethwell *et al.*, 2021). Where such agricultural inputs are overlooked, there is a clear risk of bias. As management intensity is a main driver of most grassland ES, improved ES assessments considering the required agricultural inputs and related externalities are likely to promote extensive, low-input grassland system that exhibit high ES supply at low environmental costs (Schils *et al.*, 2022). Considering agricultural inputs can therefore be seen as an important step towards balancing ES, also in view of economic and environmental costs.

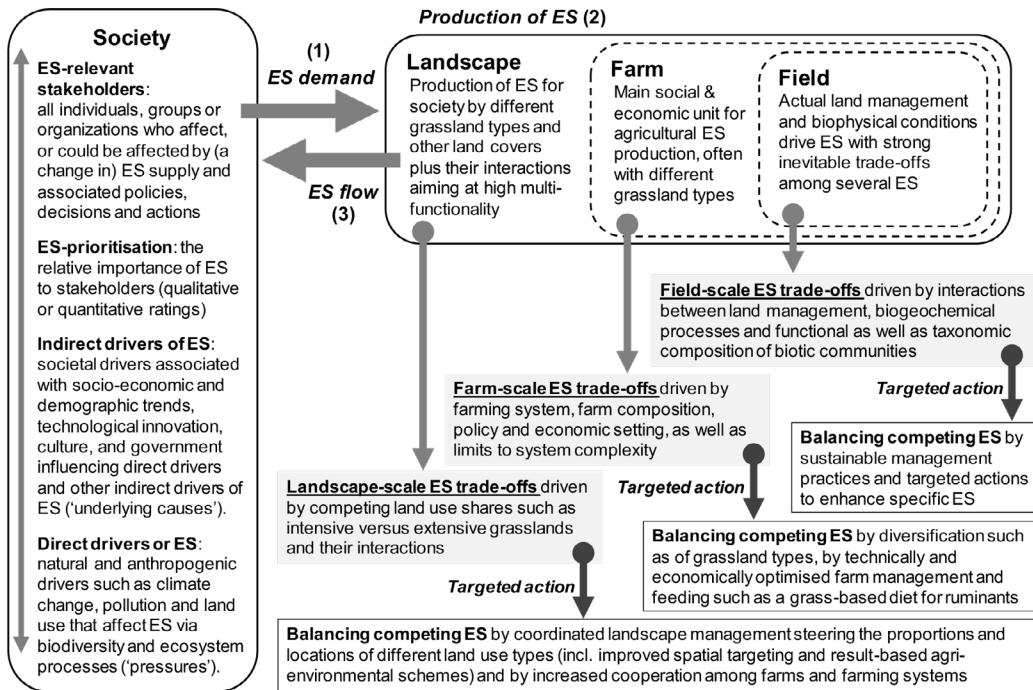


Figure 2. Synthesis figure showing grassland ecosystem services (ES) are (1) demanded by society, with (2) ES production across different spatial scales leading to (3) ES flow to society. The three spatial scales highlighted in this work are *field*, *farm* and *landscape*, which are all relevant for the production of ES due to their agricultural relevance and different mechanisms causing trade-offs among ES. Therefore, targeted action to balance ES can and must be taken on all these scales. Definitions of stakeholder groups, ES prioritisation and indirect as well as direct ES drivers according to the IPBES framework (IPBES, 2019) and Peter *et al.* (2022).

To bridge the gap between ES demand and supply in the future, we suggest focusing on (i) improved policy-making and a co-design of agri-environmental measures by stakeholder involvement, (ii) stimulation of formalised and institutionalised landscape-scale cooperation among farms and among stakeholder groups, (iii) refinement of practical actions and restorative measures across all spatial scales, and (iv) informing farmers about the relevance and the options to adjust farm and field management to enhance ES that are in short supply (Figure 2). Almost all these points require an inter-disciplinary dialogue with stakeholders to set broadly-accepted land-use targets and to co-design respective policies. This involvement is particularly relevant for a system change, as scientific facts alone will not lead to changes in behaviour, while group dialogue and debate including emotions and embracing multiple perspectives may yield much more positive outcomes (Toomey, 2013).

## Conclusions

Our considerations underline that the future of balancing ES is *multi*: multi-functionality can only be achieved if multiple stakeholders are intensely involved and multiple spatial scales are targeted with multiple measures. Although we present only a selection of practical approaches to balance competing ES across field, farm and landscape scales (Figure 2), we highlight that a multitude of options exists to reduce trade-offs between ES and bring ES supply and demand closer together. We suggest that all these actions need to be embedded in an improved policy setting, which enables farmers to farm together for grassland multi-functionality.

## Acknowledgements

V.H.K. and O.H.-E. acknowledge funding by the Agroscope research program *Indicate* (project IndiGras). The authors further thank the anonymous reviewers that commented on the paper.

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# Assessing the economic value of cultural ecosystem services from grasslands using choice experiments

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## Abstract

Although there is growing recognition of the importance of grasslands and their ecosystem services, there is little research on the economic valuation of cultural ecosystem services provided by grasslands. The economic value of cultural ecosystem services from grassland may be estimated by using the choice experiments method which utilizes hypothetical scenarios to understand how people make choices related to these services and assign values to them. This study presents a conceptual framework designed to assess the economic value of cultural ecosystem services from grasslands by engaging different categories of stakeholders, as beneficiaries of grassland use. The approach provides a comprehensive understanding of the benefits grasslands can offer, which can lead to more effective policies, practices, and investments to protect and preserve these valuable landscapes.

**Keywords:** choice experiments, permanent pastures, cultural hay meadows, sustainability, land management

## Introduction

Permanent grasslands are one of the main agricultural land use activities covering almost one-third of total world land resources (Bengtsson *et al.*, 2019). In the EU, grasslands currently represent around 34% of the overall agricultural area. Nevertheless, for the last decades, the permanent grassland area in the EU has decreased due to land-use change and intensification of management practices (Gaitán-Cremaschi *et al.*, 2017; Schils *et al.*, 2022). The abandonment of traditional agricultural practices leads to changes in land use and to habitat loss and degradation resulting in, amongst others, the decline of biodiversity. Many plants and animal species have adapted to specific agricultural landscapes and, therefore, when these landscapes are abandoned or changed it can disrupt the ecological balance and lead to the decline or loss of biodiversity (Prangel, 2023).

Ecosystems offer a broad range of services from which people can benefit directly or indirectly, some being tangible and others intangible. The ecosystem services can be categorized as provisioning services, regulating services, cultural services, and supporting services (MEA, 2005). Grasslands provide many ecosystem services from which people can benefit (Huber *et al.*, 2019; Richter *et al.*, 2021), e.g. production of fodder (provisioning service), carbon sequestration (regulating service), recreation (cultural service) and nutrient cycling (supporting service).

Cultural ecosystem services (CES) are defined as ‘nonmaterial benefits that people obtain from ecosystems through spiritual enrichment, cognitive development, reflection, recreation, and aesthetic experiences’ (MEA, 2005) and include cultural diversity, spiritual and religious values, knowledge systems, educational values, inspiration, aesthetic values, social relations, sense of place, cultural heritage, recreation and ecotourism. According to Huber *et al.* (2019), the most commonly studied CES from grasslands include landscape aesthetics, cultural heritage, biodiversity conservation, and recreation.

Cultural ecosystems provide a wide variety of benefits that are often shared by the public, being inherently accessible to all members of society. Recognizing the importance of CES in land management decisions is essential for making informed and sustainable land management decisions (MEA, 2005) as they contribute to the overall well-being of communities (farmers and non-farmers), create cultural identity, and enhance the quality of life.

The literature recognizes the importance of assessing the economic value of non-market grassland ecosystem services, which may provide important decision support for grassland management (Liu *et al.*, 2022). Lately, efforts have been made on the economic value of pastoral farming (Mazzocchi *et al.*, 2018), economic value of mountain grassland ecosystem services (Faccioni *et al.*, 2019), consumers' willingness to pay for ecosystem services from mountain pastures incorporated in dairy products (Cavalletti *et al.*, 2023), species richness impact on the economic values of grasslands (Hungate *et al.*, 2017) or the overall economic value of grasslands ecosystem services in a global meta-analysis (Liu *et al.*, 2022).

Although the value of grasslands and their ecosystem services is becoming more widely acknowledged (Cheng *et al.*, 2019; Huber *et al.*, 2019; Richter *et al.*, 2021) empirical research on the economic assessment of the CES has lagged behind. This study presents a conceptual framework to assess the economic value of CES from grasslands by engaging different categories of stakeholders, as beneficiaries of grassland use. The model is designed for the use of the choice experiments method and exemplified in a case study, a recognized region at the European level for its high biodiversity, the Eastern Hills of Cluj from Romania.

### **Choice experiments method to assess the economic value of CES from grasslands**

Non-market valuation methods can be employed to assess CES from grasslands (Huber *et al.*, 2019; Rewitzer *et al.*, 2017; Richter *et al.*, 2021). These approaches are often used to capture the economic value of goods and services that are not traded in traditional markets with the aim of informing decision-makers (Johnson *et al.* 2017; Louviere *et al.*, 2000). Huber *et al.* (2019) emphasize, based on a meta-analysis conducted on studies from Europe on the willingness to pay for cultural services, that stated preference methods are more common than the revealed preference methods. While the revealed preference methods (e.g. travel cost method, hedonic pricing method) are used to analyse the actual behaviour of individuals in real situations, the stated preference methods (e.g. contingent valuation method, choice experiments method) are used to analyse the preferred behaviour expressed by individuals in a hypothetical framework created to identify and evaluate the impact of possible quantitative and/or qualitative changes of the goods or services. Stated preference methods are useful when it is difficult to observe actual behaviour, such as when assessing the value of cultural ecosystem services. Since these services often lack observable market prices, it might be challenging to determine their economic value through revealed preference methods. The stated preference methods allow researchers to explore individuals' preferences in a controlled setting and to estimate the value they place on certain attributes or changes.

The choice experiments method is widely utilized in environmental economics for understanding preferences, estimating economic values, and determining individuals' willingness to pay for specific attributes of environmental goods or services (Adamowicz *et al.*, 1994; Bennett *et al.*, 2011; Champ *et al.*, 2003; Hanley *et al.*, 1998). Assigning a monetary value to CES from grasslands is not a straightforward process. It requires special attention to the design and analysis of choice sets (an aspect discussed in the next section). The method is based on Lancaster's theory (Lancaster, 1966), in which consumer utility is defined according to a series of attributes that describe the good or service (Champ *et al.*, 2003). By including a monetary attribute, one can estimate how much individuals are willing to pay for changes in the other attributes. The method involves presenting individuals with hypothetical scenarios and asking them to make choices among different combinations of attributes that describe the good or service, each associated with a certain level (Hanley *et al.*, 1998). The analysis of choices allows researchers to



understand individuals' preferences and estimate the economic value that individuals place on specific characteristics of the good or service.

The literature provides evidence for the utility of measuring economic values of ecosystem services from grasslands for management and policy support (Barkmann *et al.*, 2010, Hafner *et al.*, 2018, Mazzochi *et al.*, 2018, Tienhaara *et al.*, 2021). However, Richter *et al.* (2021) identified a paucity of economic value studies on all types of grassland ecosystem services including cultural ecosystem services.

A conceptual model to assess the economic value of CES from grasslands illustrated by a case study

### **Case study: Eastern Hills of Cluj, Romania**

Eastern Hills of Cluj is a Natura 2000 site (code ROSCI0295) located in the middle of Transylvania, one of the Romanian historical regions that is surrounded by the Carpathians Mountains. The area is recognised for its unique biodiversity - the xero-mesophilic grasslands hold one of the 'world records' in terms of the number of plant species (Wilson *et al.*, 2012). The mosaic of seminatural grasslands parcels farmed using different low intensive techniques at different times of the year host important populations of four endangered *Maculinea* butterfly species (Timus *et al.*, 2016), and other protected species listed in the Habitat Directive: two mammals, seven amphibians and reptiles, five plant species and ten Lepidoptera (Dumitras *et al.*, 2017). The use of low intensive techniques also supports important cultural ecosystem services. Among others, from an aesthetic point of view, such grasslands are unique for present-day rural landscapes because one can find a mosaic of colours and shapes in different grasslands and arable plots (Figure 1). Such areas are farmed using extensive grazing farming techniques or are used to produce hay by manual mowing. Also, specific for the region are mixed farming systems (arable and animal breeding) using local breeds (Baltata romaneasca for cows; Turcana for sheep; or Romanian Buffalo).

The region provides interesting recreation possibilities mainly represented by hiking or cycling tourism, nature and observation facilities but also accommodation and restaurant facilities (Crisan, 2020). The region is specific for particular architectural values that valorise local resources like wood and volcanic tuff rocks in houses and buildings (Racasan *et al.*, 2016). Important educational resources like castles and palaces are also available. They were built by different populations, with different religions and traditions. Nowadays the rural population is represented by a mixed ethnic group formed by Romanians (the vast majority, more than 90%), Hungarians, and Romas with different traditions, cultures, and religions (Dumitras *et al.*, 2017).

#### *Conceptual model*

The proposed conceptual model to assess the economic value of cultural ecosystem services (CES) from grassland is depicted in Figure 2. It uses the choice experiments method. The steps are explained along with illustrating how the choice experiments method may be applied to assess CES from grasslands. CES offer various benefits that can be perceived differently by different individuals. Consequently, the economic value of CES may also differ. Therefore, the proposed model incorporates separate sets of choice sets for the identified categories of stakeholders.

#### *Stakeholders and choice sets*

In the study area, three main categories of stakeholders were identified as key actors (Step 1 in Figure 2): (1) farmers; (2) non-farming residents; (3) tourists and potential tourists.



Figure 1. Aesthetic values of Eastern Hills of Cluj. Source: HNVLink Project, <http://www.hnmlink.eu/>

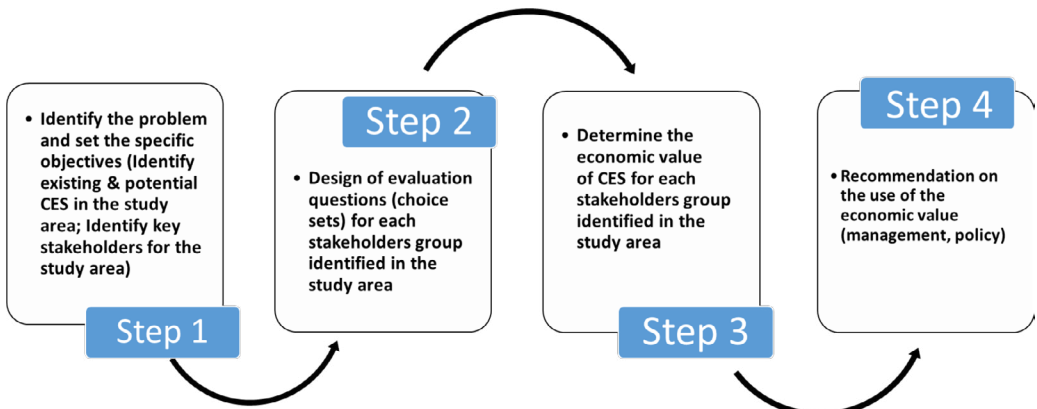


Figure 2. Conceptual model to assess the economic value of CES from grassland using the choice experiments method.

By supporting CES, farmers might aim to develop economic self-sustainable farming businesses, while non-farming residents might want to live in a community that is resilient and offers a better quality of life. These objectives are consistent with research conducted in the study area (e.g. HNV-Link project, Grazing4Agroecology project) and with the goals of public policies/governmental initiatives to prevent migration to urban areas, especially among young people (e.g. Romania’s CAP Strategic Plan 2023–2027, Local Action Group Someș Transilvan strategy). On the other hand, by supporting CES, tourists and potential tourists may seek authentic cultural experiences in rural areas with grazing heritage. This objective is consistent with the Local Action Group strategy/ local development strategies to attract more tourists in the area to support the local economy. Acknowledging and understanding the different benefits and preferences of various stakeholders for ecosystem services may strengthen arguments for decision-making processes that take into account the needs and values of main stakeholders in order to create resilient rural areas (Bennett *et al.*, 2015).

To build the choice sets, which are the evaluation questions specific to the choice experiments method, availability of complete and detailed documentation is recommended regarding the analysed CES along with potential qualitative changes. This results in an inventory of existing and potential CES from the analysed grasslands with a full description of benefits for each identified stakeholder category. It can be

difficult and time-consuming to select the appropriate attributes and levels (Hanley *et al.*, 1998) needed to build the choice sets, but this difficulty can be avoided if they are constructed using recent data.

After establishing the attributes and the levels, a factorial experimental design is used to arrange the levels for each attribute into choice sets. The process of building the choice sets is complex as it is built on combining each level of each attribute with each level of the other attributes, resulting in a rather large number of scenarios that are impossible to be evaluated by individuals (Champ *et al.*, 2003; Louviere *et al.*, 2000). A common approach to reducing the number of choice sets is to use the orthogonal fractional design (Louviere *et al.*, 2000) and block the resulting choice sets into several versions which are randomly assigned to participants. Often a choice set is composed of two alternatives resulting from combining the levels of the attributes and one neutral alternative that is regarded as the status quo and indicates the choice to not prefer the other two. Participants are asked to evaluate multiple choice sets and choose the preferred alternative for each choice set. The attributes and levels along with examples of choice sets for each stakeholder category are depicted in Tables 1–4.

In the case of farmers and non-farming residents, the choice of attributes presented in Table 1 was based on previous research carried out in the case study area using focus groups and informal interviews (Dumitras *et al.*, 2017; HNVLink Project, <http://www.hnmlink.eu/>) and follow-up activities in the field in collaboration with local stakeholders. In the case study area, raising livestock is a vital component of the cultural heritage. Thus, the first two attributes are related to farm management; specifically, the first is the preservation of the traditional landscape, which includes permanent pastures and hay meadows, and the second is the existence of a mosaic system in the study area (Dumitras *et al.*, 2017). The following two attributes refer to the local customs related to grazing (e.g. open farm days, harvest festivals) and to products and facilities that tourists may enjoy while hiking or walking on the permanent pastures and hay meadows. These may include locally produced goods with added value due to the presence of CES at the farm level, food establishments, or lodging. These are CES present in the area that could be threatened if farming and tourism are not conducted in a sustainable manner. Additionally, a monetary attribute was added to capture the willingness of residents to support the presence of CES which benefits everyone. Donations could be used for management purposes by the local authorities (e.g. town hall, Natura 2000 site administrator). Table 2 presents an example of a choice set for farmers and non-farming residents.

In the case of tourists and potential tourists, the choice of attributes is presented in Table 3. The reasons why tourists could choose to visit the area are their particular interest in this area (e.g. due to the distinctive biodiversity) and / or to experience the tourism initiatives implemented in the last years (e.g. educational guided tours, walking/hiking/bicycle paths). These initiatives contribute to the economic development of the area, however, over time, they can also have a negative impact if not controlled by local policies that promote sustainable tourism. Tourist engagement in local CES conservation activities through financial contributions benefits the region while also demonstrating that individuals understand the necessity of sustaining ecosystem services for future generations.

The first two attributes refer to the agricultural heritage of permanent pastures and hay meadows and the aesthetic value of the landscape (free grazing animals), CES that may attract recreation and tourism (Bernues *et al.*, 2016). The following two attributes refer to products and facilities that tourists may enjoy while visiting the area and to environmental information available in addition to trail marks and signs. The monetary attribute refers to the tourists' willingness to contribute to the preservation of CES in the area. The Natura 2000 site administrator or other local authorities may use such donations for management reasons. Table 4 presents an example of a choice set for tourists and potential tourists.

Table 1. Design of choice sets for farmers and non-farming residents

Attribute	Description	Levels
Agricultural heritage	Traditional landscape: permanent pastures and hay meadows	Abandoned pastures and hay meadows A few pastures and hay meadows with grazing animals Majority of pastures and hay meadows with grazing animals
Sense of place	Local cultural landscape (mosaic system)	Monoculture Mosaic system to some extent on the land Mosaic system predominant on the land
Social relations	Local customs related to grazing	Local customs related to grazing are declining Local customs related to grazing are maintained Local customs related to grazing become more popular
Recreational services	Recreational services provided by the community	No recreational services Local products Local products and food facilities Local products and overnight accommodation
Contribution	Contribution to support the conservation of CES in the area*	0 RON person <sup>-1</sup> year <sup>-1</sup> 20 RON person <sup>-1</sup> year <sup>-1</sup> 50 RON person <sup>-1</sup> year <sup>-1</sup> 100 RON person <sup>-1</sup> year <sup>-1</sup>

Average monthly exchange rate for February 2024: 1 EUR=4.9748 Romanian leu (RON). Source: Romanian National Bank, <https://www.bnr.ro/>

Table 2. Example of a choice set for farmers and non-farming residents

Attribute	Option A	Option B	Option C
Agricultural heritage	Abandoned pastures and hay meadows	Majority of pastures and hay meadows with grazing animals	I am not interested in choosing Option A or B
Sense of place	Mosaic system predominant on the land	Mosaic system to some extent on the land	
Social relations	Local customs related to grazing are maintained	Local customs related to grazing are declining	
Recreational services	Local products	Local products	
Contribution to support CES	50 RON person <sup>-1</sup> year <sup>-1</sup>	20 RON person <sup>-1</sup> year <sup>-1</sup>	
The preferred option is:	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Table 3. Design of choice sets for tourists and potential tourists.

Attribute	Description	Levels
Agricultural heritage	Traditional landscape: permanent pastures and hay meadows	Abandoned pastures and hay meadows A few pastures and hay meadows with grazing animals Majority of pastures and hay meadows with grazing animals
Aesthetics	Visibility of livestock grazing on permanent pastures and hay meadows	No animals grazing on permanent pastures and hay meadows A few animals grazing on permanent pastures and hay meadows Many animals grazing on permanent pastures and hay meadows
Recreational services	Recreational services	No recreational services Local products Local products and food facilities Local products and overnight accommodation
Education	Environmental education through information accessible online in addition to trail marks and signs	Marks Marks and map Marks and list with protected species Marks, map and list with protected species
Contribution to support CES	Contribution to support the preservation of CES in the area	0 RON person <sup>-1</sup> year <sup>-1</sup> 10 RON person <sup>-1</sup> year <sup>-1</sup> 20 RON person <sup>-1</sup> year <sup>-1</sup> 50 RON person <sup>-1</sup> year <sup>-1</sup>

Table 4. Example of a choice set for tourists and potential tourists.

Attribute	Option A	Option B	Option C
Agricultural heritage	A few pastures and hay meadows with grazing animals	Majority of pastures and hay meadows with grazing animals	I am not interested in choosing Option A or B
Aesthetics	No animals grazing on permanent pastures and hay meadows	A few animals grazing on permanent pastures and hay meadows	
Recreational services	Local products	Local products and food facilities	
Education	Marks and map	Marks, map and list with protected species	
Contribution to support CES	10 RON person <sup>-1</sup> year <sup>-1</sup>	20 RON person <sup>-1</sup> year <sup>-1</sup>	
The preferred option is:	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

### *Analysis of choice sets*

Following the collection of responses on the preferred alternatives within each choice set, data is analysed using econometric models to study the choices of each stakeholder category (farmers, non-farming residents, tourists) and estimate the preference parameters associated with the attributes. Thus, the marginal willingness to pay for each attribute level may be estimated indicating the additional amount of money an individual is willing to pay/support for a small increase in the quantity or quality of the service. These values provide insights into the relative importance of different attribute levels for each stakeholder category (Step 3 in Figure 2). For instance, the marginal willingness to pay would be the extra money that farmers are ready to pay for keeping the mosaic system predominant on the land or that non-farming residents are ready to pay for maintaining local customs related to grazing in their

community or that tourists are ready to pay to see traditional landscapes if they would rather see plenty of hay meadows and permanent pastures with grazing cows rather than just a few. The application of the proposed conceptual framework may be challenging because it requires the simultaneous implementation for different stakeholder categories, but the type of information that can be obtained by employing the choice experiments method makes it valuable. The next step is to identify effective methods for using the information for management and policy initiatives (e.g. LEADER+ local development strategies) that can be implemented at local level to support the preservation of CES while also benefiting all categories of local stakeholders (Step 4 in Figure 2). The proposed model has also the potential to serve as an informing tool, educating individuals about the CES in the area and raising awareness of their significance for present and future generations.

## Conclusion

The use of techniques such as the choice experiment to elicit people's preferences and values for CES might assist decision-makers in making informed decisions about land use, conservation, and development while also considering the local cultural dimensions of ecosystems. Engaging local stakeholders in the valuation process is critical for ensuring that varied perspectives and cultural values are effectively represented in decision-making. The conceptual framework presented is based on a case study and should be tailored to the specific characteristics of the area. Studies on the economic valuation of cultural ecosystem services from grasslands may help to raise awareness about their importance for the preservation of local culture and the conservation of highly valuable natural regions for future generations.

## Acknowledgements

This paper was supported by European Union's Horizon Europe Research and Innovation Programme under grant number 101059626 – Grazing4AgroEcology ([www.grazing4agroecology.eu](http://www.grazing4agroecology.eu); @Grazing4AgroEcology).

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# Ecosystem services of temperate grasslands under climatic extremes: a literature review

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## Abstract

Ecosystem services (ES) of temperate grasslands are strongly related to plant diversity and influenced by extreme events. Existing studies focused mostly on single ES while syntheses on multiple ES under extreme climatic conditions are still rare. Thus, we conducted a systematic literature review to assess the interplay between plant diversity effects on ES and the impact of extreme events (here: drought and/or heat stress) on ES in temperate grasslands. With a structured literature search, we identified 775 papers from Web of Science and CAB Abstracts databases published between 1940 and 2022. After an eligibility screening, 31 unique studies remained for further evaluation. We found that all studies focused on provisioning services, while only 39% additionally investigated non-provisioning services. Twenty-six studies were performed in biodiversity experiments (sown and weeded), while only five studies were based on grasslands under real-world agricultural conditions. Overall, the positive effects of higher species richness on grassland ES persisted under extreme conditions across different ES. Therefore, maintaining and increasing plant diversity can increase various ES despite increasing climate risks.

**Keywords:** temperate grasslands, ecosystem services, drought and heat stress, plant diversity

## Introduction

Climate change and biodiversity loss are posing significant challenges to grassland ecosystems which cover 40% of the global land surface (excluding Greenland and Antarctica; White *et al.*, 2000). These grassland ecosystems deliver a broad set of ES (Richter *et al.*, 2021), which are strongly affected by extreme events (Gilgen and Buchmann, 2009; Buchmann *et al.*, 2019; Dodd *et al.*, 2023). Higher plant species diversity has been shown to mitigate the impact of climatic extremes on some ES from temperate grasslands (Isbell *et al.*, 2015; Craven *et al.*, 2016; Haughey *et al.*, 2018). However, existing studies frequently focused on single ES while syntheses on multiple ES under extreme climatic conditions are still rare. In this study, we used a systematic review to identify the effect of plant species diversity on temperate grassland ES under extreme conditions.

## Materials and methods

The literature search was conducted within the Web of Science and CAB Abstracts databases in February 2023. We used four keyword groups (about grasslands, ES, plant diversity, extreme events) to form the search strings, with “AND” operators among the four groups (Table 1). For ES, we adopted and modified the ES indicators from Schils *et al.* (2022). For extreme events, we focused on drought and heat stress. We identified 775 papers, which were uploaded to an EndNote library for duplicate removal and initial screening based on titles and abstracts. The full texts of the remaining 82 papers were further screened and additional 25 papers were identified to be included in the study. During the eligibility screening, studies in non-temperate climatic regions, studies including C4 species, and studies that did not consider species richness gradients, were excluded. We selected 31 unique studies that matched our objective for further analysis.



Table 1. Keywords used in the literature search.

Group	Keywords
Grasslands	grass* OR graze* OR grazing* OR hay* OR meadow* OR pastur* OR rangeland*
ES	Modified from Schils <i>et al.</i> (2022)
Plant diversity	"plant diversit*" OR "plant richness*" OR "number of plant*" OR "species composition*" OR "species diversit*" OR "species richness*" OR "vegetation composition*" OR "vegetation richness*" OR biodiversit*
Extreme events	drought* OR "dry spell*" OR "dry phase*" OR heatwave* OR hot* OR "hot spell*" OR "warm spell*" OR extreme* OR "dry condition*" OR "water stress*" OR "heat stress"

For each study, we extracted country, year and duration (in years) of study, study type (i.e., field or pot), grassland type (i.e., sown experimental grasslands or grasslands under real-world agricultural conditions), plant species richness gradient, extreme event (i.e., drought, heat stress, or compound drought and heat), extreme type (i.e., natural events or experimental manipulations), ES indicators, and the effect of higher species richness on ES indicators (i.e., increase, decrease, non-significant change, or not reported) under both normal and extreme conditions.

## Results and discussion

The majority of the studies was conducted in European countries, whereas only three studies from North America and one study from Asia were found (Figure 1a). Some studies also included multi-site experiments across several countries. The duration of studies spanned from one growing season to 24 years. Eighteen studies were carried out in field settings, while 13 were based on pot experiments (Table 2). We found all studies to include provisioning services, while only 39% also investigated non-provisioning services (Figure 1b). The dominance of studies on provisioning services and the lack of studies on cultural services were also consistent with the findings of a previous ES review focusing on European permanent grasslands (Schils *et al.*, 2022).

For sown grasslands (mainly belonging to biodiversity experiments), the species richness gradient ranged from one species to 60 species, while studies from grasslands under real-world agricultural conditions had a minimum species richness of 13 species. Twenty-six studies took place in sown and weeded experimental grasslands, while only five studies employed real-world agricultural grasslands (Table 2).

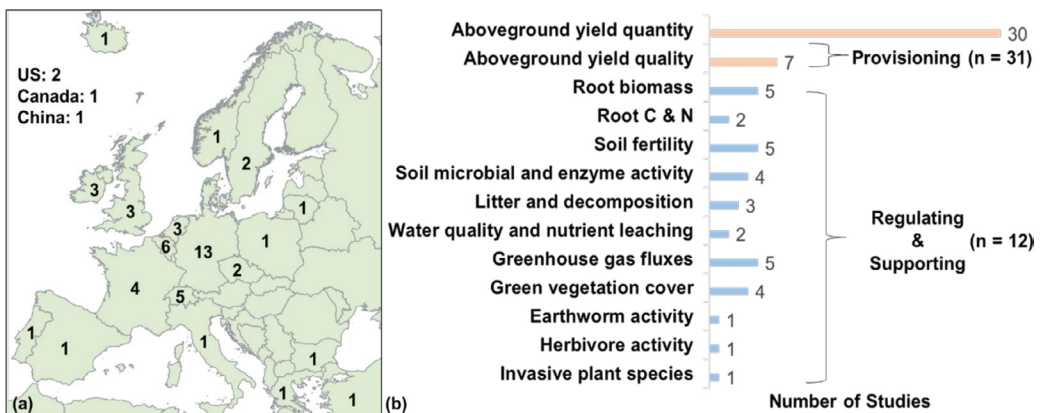


Figure 1. (a) Geographical distribution of studies that were finally included in this review; (b) ES indicators that were explored in the 31 studies. Note that in some studies multiple services were investigated.

Interestingly, none of those five studies investigated naturally occurring extremes but had an experimental drought or heat treatment (Table 2). This makes the assessment of the potentially mitigating effect of plant diversity against climatic extremes in real-world agriculture difficult. Moreover, 77% of the studies showed a positive effect of plant species richness on ES under extreme conditions.

Table 2. Number of studies categorised based on their study types.

Grassland types	Experimental extremes	Naturally occurring extremes
Sown and weeded grasslands	20 (field: 8; pot: 12)	6 (only field)
Real-world agricultural grasslands	5 (field: 4; pot: 1)	0

## Conclusion

The considerable knowledge gaps about the potential of plant diversity in mitigating the impact of extreme events on temperate grasslands, in particular on non-provisioning ES, and on real-world agricultural settings, make it very difficult to develop strategies for sustainable climate-change-adapted grassland management for the future. However, the positive effect of plant diversity on ES even under extreme conditions calls for maintaining and increasing plant diversity in grassland-based farming systems. Such strategies, including various aspects of plant diversity (e.g., species richness, functional groups, genetic diversity), could provide protection and might act as a natural insurance against current and future climate risks.

## Acknowledgement

This work was funded by the SNF project InsuranceGrass (100018L\_200918).

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# The impact of limited grazing on milk production and methane emission in dairy cattle

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## Abstract

Most pasture milk labels in the Benelux stipulate a minimum of 6 hours grazing daily for a minimum of 120 days annually. Despite the potential ecological benefits of pastured grasslands, little is known about the impact of this specific grazing regime on enteric methane emissions. The initial trial compared no grazing with 6-hour grazing while maintaining an ad libitum barn diet, alongside 6-hour grazing coupled with an increased proportion of corn silage in the barn diet. A subsequent trial compared no grazing with 6-hour and 12-hour grazing periods. Both trials utilized Latin squares, encompassing 3 treatments each and involving 24 Holstein cows. In the first trial, CH<sub>4</sub> emissions notably decreased in both grazing treatments (−12.5% and −14%, respectively). However, maintaining the same barn diet during grazing led to a decline in fat and protein-corrected milk (FPCM) production (26.7 kg) compared to no grazing (27.8 kg). Methane yield measured 19.4, 17.9, and 16.9 g CH<sub>4</sub>/kg FPCM respectively. In the second trial, both grazing treatments exhibited reduced CH<sub>4</sub> emissions (−10% and −11%) alongside decreased FPCM production (33 kg vs 35 kg indoors). Methane yields did not display significant differences. The findings suggest that restricted grazing regimes reduce methane emissions but concurrently diminish milk production.

**Keywords:** restricted grazing, methane, mitigation, climate, pasture milk

## Introduction

Milk originating from pastured cows, commonly referred to as pasture milk, has gained importance in Western Europe. Dairies typically stipulate a minimum of 6 hours of daily pasturing for at least 120 days annually as a prerequisite for categorizing milk under the pasture milk label. However, limited research exists regarding the impact of in-barn feeding combined with restricted pasturing on enteric emissions, particularly for cows in mid to late lactation (Dall-Orsoletta *et al.*, 2016). Achieving equivalent milk production levels during pasturing compared to confinement may be challenging. Furthermore, knowledge on the effect of alterations to the barn diet on enteric emissions in pastured dairy cattle is scarce (Beauchemin *et al.*, 2008). Increasing levels of corn silage or starch in the diet has been identified as a potential method for reducing methane production (Monteny *et al.*, 2006, Beauchemin *et al.*, 2008), concurrently providing the necessary energy to uphold high milk production levels. This study's objective was to compare the enteric methane emissions and milk production between cows subject to restricted pasturing and confined cows when both groups received the same partial mixed ration (PMR) in the barn or a PMR with increased corn silage inclusion, or when pasturing for longer time. We hypothesise that combining restricted pasturing with a high starch diet can reduce enteric methane emissions without compromising milk production, thereby presenting a potentially viable strategy for dairy farmers to economically reduce enteric methane production.

## Materials and methods

This study encompassed two experiments. In a first experiment, a cross-over 3×3 Latin square design was employed to evaluate three treatments, utilizing 24 lactating Holstein Friesian dairy cows in mid to late lactation over three successive 4-week periods. The random assignment of cows considered lactation

stage, parity, milk yield, and body weight, resulting in eight cows receiving identical treatment during each period. The treatments were as follows: (A) no grazing, with ad libitum access to a basal PMR; (B) 6 hours of pasturing after the morning milking, accompanied by ad libitum access to the same basal PMR; (C) 6 hours of pasturing and ad libitum access to a PMR containing a higher proportion of corn silage (55.4% vs. 42.8%) within the barn. Cows in treatments B and C were pastured together to prevent discrepancies in dry matter intake (DMI) attributed to variations in grass quality, availability, or taste during pasturing. Importantly, they had no access to the barn or PMR while pasturing. In a second experiment, a similar configuration involving 24 cows in early lactation was implemented. The treatments included: (A) no grazing, with ad libitum access to a basal PMR; (B) 6 hours of pasturing post-morning milking, with ad libitum access to a PMR enriched with a higher proportion of corn silage in the barn; (C) 12 hours of pasturing during the night post-evening milking, coupled with ad libitum access to the same higher corn silage PMR in the barn during the day. Additionally, a treatment D, mirroring treatment A, was introduced. However, these eight additional cows consistently received the same treatment throughout the experiment, exempting them from the Latin square design.

The experiments were conducted in spring and summer 2020 and 2022 respectively at the ILVO research farm in Melle, Belgium. PMR intake was recorded individually using roughage intake control bins (RIC; Insentec, Marknesse, The Netherlands). The protein and balanced concentrate intake was determined based on individual cow requirements for energy (VEM) and protein (DVE/OEB system) (Tamminga *et al.*, 1994) and supplemented through automatic concentrate feeders. Methane measurements were performed using two GreenFeeds (C-lock, Rapid City, SD, USA), strategically positioned in both the barn and pasture. These devices alternated locations midway through each measurement period, specifically during the last three weeks of each four-week treatment cycle. The n-alkane technique described by Mayes *et al.* (1986) was used to determine fresh grass intake on pasture. Statistical analysis was performed using R (4.1.1), using a linear mixed model incorporating the treatment and lactation stage. If statistically significant, the period was also included as a factor. Significance levels were set at  $p < 0.05$ .

## Results and discussion

Table 1 presents the outcomes of Experiment 1. Milk production was significantly lower when cows were subjected to pasturing, but not when a higher corn silage ratio was present in the PMR. DMI and CH<sub>4</sub> emissions were significantly lower when cows were pastured compared to no pasturing. Methane intensity and methane yield were significantly lower when grazing was combined with a PMR with higher corn silage inclusion, but not when the same basal PMR was provided.

Table 1. Results of the first grazing experiment.

	Treatment A (No grazing+basal PMR)		Treatment B (6 h grazing+basal PMR)		Treatment C (6 h grazing+PMR corn)		p value
	LS mean	CI	LS mean	CI	LS mean	CI	
FPCM (kg day <sup>-1</sup> )	27.8 <sup>a</sup>	[25.3; 30.4]	26.7 <sup>b</sup>	[24.1; 29.2]	27.1 <sup>a,b</sup>	[24.5; 29.6]	0.001
DMI (kg day <sup>-1</sup> )	21.6 <sup>a</sup>	[20.6; 22.6]	19.2 <sup>b</sup>	[18.2; 20.2]	19.8 <sup>b</sup>	[18.8; 20.8]	<0.001
CH <sub>4</sub> (g day <sup>-1</sup> )	511 <sup>a</sup>	[484; 538]	447 <sup>b</sup>	[419; 475]	439 <sup>b</sup>	[412; 467]	<0.001
CH <sub>4</sub> /DMI (g kg <sup>-1</sup> )	23.9 <sup>a</sup>	[22.9; 25.0]	23.6 <sup>a,b</sup>	[22.5; 24.7]	22.4 <sup>b</sup>	[21.3; 23.5]	0.014
CH <sub>4</sub> /FPCM (g kg <sup>-1</sup> )	19.4 <sup>a</sup>	[16.9; 20.3]	17.8 <sup>a,b</sup>	[15.3; 18.8]	16.9 <sup>b</sup>	[14.6; 18.0]	0.002

DMI, dry matter intake; FPCM, fat and protein corrected milk. Significantly different group means are indicated by different letters per variable ( $p < 0.05$ ).

In Experiment 2, milk production and total DMI was lower in grazing treatments compared to no grazing, but not compared to treatment D due to the higher standard error in this treatment (8 cows vs. 24 cows) (Table 2). Methane emission was lower in grazing treatments compared to no grazing (treatment A). Methane yield was significantly lower for 12 h grazing compared to no grazing, but not for 6 h grazing vs no grazing. Methane intensity was not different between treatments.

Table 2. Results of the second grazing experiment.

	Treatment A		Treatment B		Treatment C		Treatment D		<i>p</i> value
	(No grazing+basal PMR)		(6 h grazing+PMR corn)		(12 h grazing+PMR corn)		(no grazing+basal PMR)		
	LS mean	CI	LS mean	CI	LS mean	CI	LS mean	CI	
FPCM (kg day <sup>-1</sup> )	35.2 <sup>a</sup>	[33.3; 37.2]	33.3 <sup>b</sup>	[31.4; 35.3]	33.2 <sup>b</sup>	[31.2; 35.1]	36.6 <sup>a,b</sup>	[36.6; 39.9]	0.014
DMI (kg day <sup>-1</sup> )	23.8 <sup>a</sup>	[22.8; 24.7]	22.4 <sup>b</sup>	[21.4; 23.3]	22.3 <sup>b</sup>	[21.4; 23.3]	23.7 <sup>a,b</sup>	[22.1; 25.2]	<0.001
CH <sub>4</sub> (g day <sup>-1</sup> )	523 <sup>a</sup>	[497; 550]	471 <sup>b,c</sup>	[444; 497]	464 <sup>c</sup>	[438; 491]	534 <sup>a,b</sup>	[490; 578]	<0.001
CH <sub>4</sub> /DMI (g kg <sup>-1</sup> )	22.1 <sup>a</sup>	[21.2; 23.1]	21.3 <sup>a,b</sup>	[20.3; 22.3]	21.0 <sup>b</sup>	[23.7; 25.6]	22.1 <sup>a,b</sup>	[20.6; 23.6]	0.039
CH <sub>4</sub> /FPCM (g kg <sup>-1</sup> )	15.1	[14.2; 16.0]	14.6	[13.7; 15.4]	14.3	[13.4; 15.2]	14.2	[12.9; 15.5]	NS

DMI, dry matter intake; FPCM, fat and protein corrected milk. Significantly different group means are indicated by different letters per variable ( $p < 0.05$ ).

## Conclusion

We concluded that absolute methane emissions were lower in restricted grazing regimes compared to fulltime indoor housing, but milk production can be expected to decrease as well. A higher corn silage inclusion in the barn PMR seemed to help minimizing the difference in milk yield. However, methane production per kg FPCM was only lower in mid to late lactation cows, and not different in high yielding cows in early lactation. Longer grazing during the night had no additional effect on methane production compared to six hour grazing during the day.

## Acknowledgements

This research was funded by the government agency Flanders Innovation and Entrepreneurship (VLAIO, Belgium; HBC.2019.005) and was part of the GrASTech project funded by the 2018 ERA-net SUSan, Facce ERA-GAS and ICT-AGRI ERANET joint call. The authors thank the ILVO dairy farm staff and the ILVO Animalab laboratory.

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# The effect of grassland management intensity on earthworms and leatherjackets

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## Abstract

Grasslands play an important role in the provisioning of ecosystem services like biodiversity. Species-rich grasslands are important for aboveground biodiversity; however, it is unclear whether this also results in increased belowground biodiversity. The objective of our study was to assess earthworm (*Lumbricidae*) and leatherjacket (*Tipulidae*) populations in permanent grasslands with a gradient of grassland management intensity. In an on-farm research we compared intensively managed permanent grasslands (INT,  $n=12$ ) with three types of extensively managed grasslands (EXT,  $n=3 \times 12=36$ ) varying in the degree of herb-richness, on sea clay soils in the Province of Friesland, The Netherlands. The intensively managed permanent grasslands had high fertilizer inputs ( $361 \text{ kg N ha}^{-1} \text{ year}^{-1}$ ) and a high mowing frequency ( $4-5 \text{ cuts year}^{-1}$ ). The extensively managed grasslands received only solid farm-yard manure and the mowing date was postponed until the 15<sup>th</sup> of June ( $1-2 \text{ cuts year}^{-1}$ ). In March 2022, soil samples were taken in each field for soil chemical analysis, and to detect earthworms and leatherjackets. The number of adult and anecic earthworm numbers as well as the biomass of earthworms were significantly higher in the case of INT, and were positively correlated with N-fertilization and pH. There were no significant differences in the number of earthworms between the three types of EXT sward. The results of the abundance of leatherjackets showed a similar (non-significant) tendency as for earthworms, however the variation between fields was large. Our results show that there is a positive relationship between management intensity and earthworm abundance and biomass.

**Keywords:** grasslands, management intensity, biodiversity, soil, earthworms, leatherjackets

## Introduction

Biodiversity of agricultural grassland systems is rapidly declining. This decline is due to a combination of several factors, including intensified grassland management associated with high fertilizer inputs and mowing frequencies (Vickery *et al.*, 2001). Extensive grassland management improves plant species richness and related aboveground biodiversity. However, the effects of management intensity on belowground soil biota are relatively unknown and the results of previous studies are sometimes contradictory. Earthworms and leatherjackets are an important food source for several grassland birds. The objective of our study was to assess earthworm and leatherjackets populations in grasslands with a gradient of grassland management intensity.

## Materials and methods

In March 2022 an on-farm research was established on sea clay soil in Friesland (The Netherlands) to compare four grassland management types in twelve replicates, 48 fields in total:

1. Intensively managed permanent grasslands dominated by perennial ryegrass (*Lolium perenne*) (INT);
2. Three types of extensively managed grasslands varying in the degree of species-richness. All extensively managed fields received limited amounts of farmyard manure ( $\text{max. } 20 \text{ tons ha}^{-1} \text{ year}^{-1}$ ), and had their mowing date postponed after the 15<sup>th</sup> of June (Table 1).
  - a. EXT1 were owned by farmers and had a low botanical species diversity;
  - b. EXT2 were owned by farmers and had a high botanical species diversity;
  - c. EXT3 were owned by Nature-organizations and had a high botanical species diversity.

A composite soil sample (30 cores, 0–10 cm depth, 2 cm diameter) was taken in each field and analysed for soil chemical properties (pH<sub>KCl</sub>, Eurofins-agro, Wageningen, The Netherlands). The abundance, biomass, species and functional groups of earthworms and leatherjackets were determined in two sods of 20x20x20 cm in each field. Soil moisture was measured at 15 locations in each field using a handheld soil moisture meter. Penetration resistance was measured using a penetrometer (0–10 cm depth, 30 locations in each field), which was used as an indicator of the difficulty for a meadow bird's bill in probing the soil. Plant species richness was determined in May in a plot of 25 m<sup>2</sup>, and botanical composition was determined in two plots of 1 m<sup>2</sup> using Braun-Blanquet method. Linear Mixed Models (LMMs, using R package nlme) were used to assess the impacts of the four types of grassland. Some variables underwent a square root transformation for normality of residuals. A Poisson GLMM method (using glmmtmb package) examined the impact on total number of anecic earthworms and leatherjackets, while a negative binomial GLMM method assessed the impact on total number of epigeic and endogeic earthworms.

## Results and discussion

Plant species richness ranged from 8 species per 25m<sup>-2</sup> for INT to 22.6 species per 25m<sup>-2</sup> for EXT3 in line with our set-up (Table 1). N-fertilization levels ranged from 10 kg N ha<sup>-1</sup> year<sup>-1</sup> for EXT3 to 361 kg N ha<sup>-1</sup> year<sup>-1</sup> for INT. EXT fields had a lower pH-KCl and penetration resistance, and a higher soil moisture content compared to INT fields. EXT3 also had a significantly lower soil P-Al and K contents compared to INT and EXT1.

The number of adult earthworms and the total biomass were significantly higher in INT compared to EXT3 (Table 1). The number of adult earthworms and earthworm biomass were positively correlated with N-fertilization ( $r=0.35$ ;  $P=0.015$ ) and pH ( $r=0.39$ ;  $P=0.07$ ). This is in line with other studies (e.g. Edwards and Bohlen, 1996). The intensive management also had a positive effect on anecic earthworms. There was a positive correlation between the occurrence of anecic earthworms and N-fertilization ( $r=0.424$ ;  $P=0.004$ ). The results of the abundance of leatherjackets showed a similar (non-significant) tendency for earthworms (INT>EXT1/EXT2>EXT3); however, the variation between fields was large.

Table 1. Management, botanical, soil chemical and soil biological properties of the four grassland types (mean and standard deviation,  $n=12$ ).

Selected properties	INT	EXT1	EXT2	EXT3	P value
N-fertilization (kg N ha <sup>-1</sup> year <sup>-1</sup> )	361 (82)a	104 (35)b	83 (42)b	10 (14)c	***
Fertilizer type	Slurry + mineral	Farmyard manure	Farmyard manure	Farmyard manure	n.a.
Extensive management (no years)	–	10 (4.7)a	10 (5.8)a	33 (6.9)b	***
Grassland age (years) <sup>2</sup>	26 (35)a	82 (32)b	93 (25)b	100 (0)b	***
Plant species richness (no 25 m <sup>-2</sup> )	8.0 (2.4)a	14.8 (2.5)b	19.7 (3.1)c	22.6 (4.4)c	***
Soil moisture content (Vol%, 0–10 cm)	39.3 (9.1)b	46.7 (12.4)a	47.6 (10.4)a	51.8 (9.1)a	**
Soil penetration resistance (MPa 0-10)	0.46 (0.1)a	0.35 (0.1)b	0.39 (0.1)b	0.37 (0.1)b	*
pH <sub>KCl</sub>	5.7 (0.4)c	5.3 (0.4)b	5.4 (0.4)b	4.9 (0.3)a	**
Earthworms (no m <sup>-2</sup> )	1015 (504)	869 (466)	801 (508)	774 (515)	ns
Adult Earthworms (no m <sup>-2</sup> )	332 (230)a	237 (168)ab	209 (186)ab	164 (118)b	*
Earthworm biomass (g m <sup>-2</sup> )	190 (107)a	133 (80)ab	124 (80)ab	106 (60)b	**
Individual biomass (g worm <sup>-1</sup> )	0.20 (0.1)	0.16 (0.1)	0.18 (0.1)	0.16 (0.1)	ns
Epigeic earthworms (no m <sup>-2</sup> )	180 (142)	178 (175)	106 (83)	96 (80)	ns
Endogeic earthworms (no m <sup>-2</sup> )	820 (460)	687 (355)	679 (447)	675 (491)	ns
Anecic earthworms (no m <sup>-2</sup> )	15.3 (24.4)a	4.1 (9.4)ab	5.7 (12.1)ab	2.1 (7.1)b	**
Leatherjackets (no m <sup>-2</sup> )	102 (160)	58 (70)	24 (35)	31 (42)	ns

\* $P<0.05$ ; \*\* $P<0.01$ , \*\*\* $P<0.001$ . Grasslands with an age since last grassland renewal above 100 were set at 100.

Earthworms are an important food source for meadow birds. The average number of worms per square metre was very high, ranging from 774 to 1015 for the four grassland types. Earthworm biomass averaged between 106 and 190 grams per square metre for the four types (Table 1). This exceeds the specified minimum standard of 60 g m<sup>-2</sup> and the critical threshold for the black-tailed godwit (*Limosa limosa*), which is 25–30 g m<sup>-2</sup> (Van der Weijden and Guldemond, 2006). Thus, food abundance for meadow birds seems to be sufficient in all management types, and was highest in INT. However, in intensively managed grasslands, soil moisture was the lowest and soil penetration resistance was the highest (Table 1), which may negatively affect availability of earthworms for meadow birds in dry conditions.

## Conclusion

Our results show that the effects of grassland management intensity and N-fertilization on earthworm abundance and biomass were positive. The results of the abundance of leatherjackets showed a similar (non-significant) tendency as for earthworms. Earthworm and leatherjacket numbers in INT managed permanent grasslands were higher or equal compared to EXT managed grassland, but the availability to grassland birds may be lower, especially in dry conditions.

## Acknowledgement

This research was part of the project ‘Optimalisatie kruidenrijk grasland’ and was funded by de Dutch Ministry of Agriculture, Nature and Food Quality and the Provinces of Friesland, Groningen and Noord-Holland (the Netherlands).

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# Carbon balance in grassland ecosystems: case studies of 35 Portuguese farms

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## Abstract

Grasslands are considered, in general, as a sink for CO<sub>2</sub>. However, pasture management intensity might determine whether the ecosystem acts as sink or source for carbon (C). Grazing systems from the north of Portugal are more productive and intensive than those from the south; however, the two regions could have similar C balance if they have similar carrying capacity (potential yield/stocking rate), irrespective the regional characteristics. We tested this hypothesis by comparing the C balance in 35 farms from two contrasting Portuguese regions, the northern region of Trás-os-Montes (TM), and the southern region of Alentejo (AL). Mean stocking rates of farms were 0.65 (TM) and 0.42 LU ha<sup>-1</sup> (AL); grass represents 47% (TM) and 78% (AL) of total feeding. Results showed higher significant emissions in TM farms, compensated with higher C assimilation in grassland. However, in both regions, the balance suggested a potential for soil C sequestration not significantly different: -2049±602 and -1327±207 kg C ha<sup>-1</sup> year<sup>-1</sup>, respectively for TM and AL. Our work suggests that Portuguese grasslands might be considered as a sink for carbon regardless of regional variation in the management intensity,

**Keywords:** grasslands; carbon balance; grazing systems; extensive grazing; greenhouse gases

## Introduction

Portugal committed to achieve carbon (C) neutrality by 2050 in the UN Climate Change Conference in Marrakech (2016). Since grasslands have the potential to stock C in the soils, they might contribute to this goal. In the National report on greenhouse gases (APA, 2023), the 2021 Portuguese estimations revealed that the land use category “Grassland plus shrubland” sequestered -769.0 Gg C, while liming emitted 2.5 Gg C, grazing animals (cattle, sheep, and goats) emitted 80.8 Gg C and 3.6 Gg C from manure management. These results suggest a potential C sequestration by “Grassland plus shrubland” (-682.1 Gg C) into soil. However, some constraints seem to influence the C balance: at the local level, experimental data using the eddy-covariance technique showed that rainfall (“normal” vs “drought” year) determines if grassland acts as a C sink or source (Jongen *et al.*, 2011); in relation to management, an estimation at the European level showed that, in comparison with extensive grasslands, intensive pastures decrease the GHG balance (global warming potential) but not the net ecosystem exchange of C (Dangal *et al.*, 2020).

Portuguese grassland systems, despite being extensively managed across the country, present significant regional differences in grassland yields and stocking rates. An important question is whether such regional differences are associated with differences in assimilation or emission of C. Specifically, it is currently unclear whether increased emissions due to higher stocking rate are compensated by increased assimilation from higher pasture yield. Here, we test the hypothesis that different regions, with similar carrying capacity (pasture yield/stocking rate), have similar C balances.

## Materials and methods

We estimated CO<sub>2</sub> and CH<sub>4</sub> emissions on 35 farms from 2 contrasting regions in Portugal (Trás-os-Montes (TM) in the north; Alentejo (AL) in the south), for the years 2021–2022. We used the IPCC guidelines (2006) and APA (2023) inventory method. Both regions have a Mediterranean climate: Bragança (TM) at 690 m altitude, 1989–2018 annual rainfall of 784 mm (lowest in July, 14.6 mm), mean temperature of 12.9°C (mean maximal of 29.7°C in July and August; mean minimal of 0.3°C in January); Portalegre (AL) at 597 m altitude, for the same period, annual rainfall of 843 mm (lowest in July 4.6 mm), mean temperature of 15.9°C (mean maximal of 31.2°C in July and August; mean minimal of 5.7°C in January).

Emissions were calculated for the enteric fermentation, respiration (CH<sub>4</sub>, CO<sub>2</sub>), dung (CH<sub>4</sub>) of grazing animals and soil activity (CO<sub>2</sub>); assimilation was calculated based on pasture yield and residues (CO<sub>2</sub>). Emission factors were adopted from IPCC (2006), APA (2023) and Haque *et al.* (2017); soil emissions, according to grassland type (Carneiro *et al.*, 2005). Pasture yield was estimated from Gross Energy (GE) feed balance (pasture GE=intake GE–forages GE–concentrates GE) considering 18.54 MJ kg DM<sup>-1</sup> (IPCC, 2006). Potential soil C sequestration was derived from the net carbon storage approach (Soussana *et al.*, 2010): soil C stock variation=grassland C assimilation–grazing animals C emissions–soil C emissions.

Results are presented as C means ha<sup>-1</sup>±standard errors. Differences between regions were tested by Kruskal–Wallis One-Way Analysis of Variance on Ranks and pairwise comparison by Dunn's method.

## Results and discussion

Farm characterization per region is presented in Table 1. Northern farms (TM) were smaller, more intensive, and had a lower input of commercial feeding (3.2% as compared with 5.2% of the feeding balance in AL).

The ratio between sown pastures and grasslands was greater in TM (14%) than in AL (5%). Leys presented a similar distribution (23.5% in AL and 22.3% in TM). Grassland represented a lower percentage of farm area in TM. Grasslands in both regions are multispecies type (dominated by annual grasses and legumes and, in TM, perennial grasses like *Poa*, *Agrostis*, *Dactylis* and *Lolium*). The majority of farms in AL produced cattle and sheep (78% cattle, 67% sheep and 11% goats) while in TM the majority exploited sheep, for meat production (94%, 13% cattle and 6% goats). Stocking rates were higher in TM (Table 1); the feeding strategy in this region presented a higher proportion of hay/silage in winter, when animals might be kept indoors; in contrast with AL where grazing occurs during the whole year.

The C inventory for the studied farms is presented in Table 2. Grassland C assimilation was significantly higher in TM, reflecting environmental differences (soil and climate). Emissions from soil activity were not significantly different between the two regions, despite previous reports of higher soil activity in sown pastures compared to grassland in Portugal (Carneiro *et al.*, 2005).

Table 1. Farm characterization (means±SE) in Alentejo (AL) and Trás-os-Montes (TM).

Region (number of farms)	Grasslands (ha)	Sown pastures (ha)	Leys (ha)	Grassland in farm area (%)	Stocking rate (LU ha <sup>-1</sup> )	Grass in feed balance (%)
AL (18)	239.0±35.0	11.6±7.3	56.2±24.3	88.8±2.7	0.42±0.05	78.1±5.3
TM (17)	33.2±19.7	4.5±1.3	7.4±1.8	61.0±3.4	0.65±0.09	47.2±6.0

The emissions of grazing animals (enteric fermentation and respiration) were significantly higher in TM than in AL (Table 2), reflecting the higher stocking rates in TM (Table 1).

Table 2. Carbon assimilation and emissions in 35 farms (means±SE) in Alentejo (AL) and Trás-os-Montes (TM). Carbon balance (emissions - assimilation) reflects the potential for soil sequestration.

Component	AL (kg C ha <sup>-1</sup> year <sup>-1</sup> )	TM (kg C ha <sup>-1</sup> year <sup>-1</sup> )	P value
Grassland	-2932.6±118.6	-3720.5±324.0	<0.05
Litter	-327.6±98.5	-658.2±226	ns
Enteric fermentation	71.0±86.1	148.5±20.6	<0.001
Animal respiration	339.6±80.1	710.9±98.4	<0.001
Dung	2.0±7.5	5.1±0.7	<0.001
Soil activity	1520.6±36.0	1464.6±180.0	ns
Carbon balance	-1327.0±207.9	-2049.5±602.4	ns

In total, the C balance did not show a significant difference between regions, suggesting a potential net soil C sequestration in both regions (with a mean of 1327 and 2050 kg C ha<sup>-1</sup> year<sup>-1</sup> in AL and TM, respectively). Importantly, the IPCC (2006) and APA (2023) estimation guidelines do not take into account grassland C assimilation, animal respiration and soil emissions, since it is assumed that their sum would be zero. Our estimations suggest that this assumption may need to be revised for a better estimation of the capacity of the grassland ecosystem to act as a sink for C.

## Conclusion

Despite differences in grassland management in the two regions, grasslands seem to act similarly as a sink for C. The higher emissions from grazing animals in relatively more intensive TM farms were more than compensated by a higher C assimilation in grasslands.

Following our estimations, a long-term field experiment was established (PRR-C05-i03-I-000027 GEEBovMit) to measure carbon assimilation, emissions, soil sequestration and C balance, under different types of grasslands.

## Acknowledgements

We gratefully acknowledge the Farmers, Farmers Associations, Centro de Competências do Pastoreio Extensivo (CCPE) and the researchers group that supported our study.

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# Can we increase grassland biodiversity by means of renewal without a loss of yield and forage quality?

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## Abstract

Grassland renewal is mainly used for increasing forage yield and quality when other methods (fertilisation or over-seeding) do not provide the desired results. This method can also be used to introduce species-rich swards in protected areas. An experiment was established with the aim of finding differences between grassland renewed using a standard forage seed mixture and an autochthonous species-rich mixture at an organic dairy farm. Uncultivated grass strips were used as a control treatment. In harvest years, swards were cut for silage and hay, and aftermaths were grazed. In the 3<sup>rd</sup> harvest year, forage production (6.55 t ha<sup>-1</sup>) and nitrogen export (115.7 kg ha<sup>-1</sup>) were significantly higher in the sward renewed using the standard forage mixture than the original sward (4.66 t ha<sup>-1</sup> and 83.6 kg ha<sup>-1</sup>) and the species-rich mixture (3.90 t ha<sup>-1</sup> and 81.0 kg ha<sup>-1</sup>). The number of vascular plant species (in an area of 25 m<sup>2</sup>) was significantly higher in the species-rich mixture (42.7) than the standard forage mixture (24.0) and the original stand (30.5). The standard forage mixture produced forage of higher fibre (NDF and ADF) content than the other two treatments, with less N, ash and Ca.

**Keywords:** grassland renewal, species-rich mixture, biodiversity, forage quality

## Introduction

Permanent grasslands present an important source of forage for ruminants, but also provide many other important ecosystem services. One of the key services is ensuring biodiversity in the agricultural landscape. However, there is usually an inverse relationship between agricultural productivity and the number of plant species. An experiment was established to test the hypotheses: (1) that autochthonous species-rich mixtures produce the same forage yield and quality as standard forage mixtures, and higher than an old permanent swards due to the complementarity of the species and their adaptation to local conditions; and (2) the number of plant species in a sward established from an autochthonous species-rich mixture will be higher compared with the standard forage mixture and the original sward.

## Materials and methods

The experiment was established in the municipality Popov (49.07° N, 17.95° E, elevation 370 m a.s.l., mean annual precipitation 711 mm, mean annual temperature 8.6°C), at an organic farm in the White Carpathians Protected Landscape Area. The soil is clay loam (33% clay, 40% silt, 27% sand) with pH<sub>CaCl2</sub> 5.44. The nutrient reserves (Mehlich III) for P were sufficient, for K good, for Ca high, and for Mg good. The original, low productive pasture was ploughed in October 2019. Two seed mixtures (a standard mixture and a species-rich one) were sown into silage oats in four replications in April 2020. Original permanent grassland strips were retained as a control. The composition of the standard seed mix was as follows (seeding rate in kg ha<sup>-1</sup>): *Trifolium pratense* (2), *Trifolium repens* (1), *Lotus corniculatus* (1), *Onobrychis viciifolia* (12), *Arrhenatherum elatius* (7), *Festuca pratensis* (3), *Dactylis glomerata* (6), *Phleum pratense* (2), and *Poa pratensis* (2). The total sowing rate was 40 kg ha<sup>-1</sup>. The autochthonous species-rich mixture was prepared by the local branch of the Czech Union for Nature Conservation. The seeds originated from the White Carpathians Protected Landscape Area (grasslands of high natural value) and were harvested by brushing (grasses) and collecting from cultivated legumes and forbs (nurseries). It consisted of the grasses *Festuca rupicola*, *F. rubra*, *Bromus erectus*, *Anthoxanthum odoratum*, *Cynosurus cristatus* and a few other species (17 kg ha<sup>-1</sup>), followed by the legumes *Onobrychis viciifolia* (0.4 kg),

*Medicago lupulina* (0.4 kg), *Dorycnium herbaceum* (0.2) kg, *Astragalus cicer* (0.4 kg), *Vicia cracca* (0.2 kg), and the forbs *Betonica officinalis* (0.2 kg), *Prunella vulgaris* (0.04 kg), *Dianthus carthusianorum* (0.04 kg), *Knautia kitaibelii* (0.02 kg), *Centaurea jacea* (0.2 kg), *Plantago media* (0.04 kg), *Leucanthemum ircutianum* (0.2 kg), *Agrimonia eupatoria* (0.4 kg), *Galium verum* (0.2 kg), *Salvia pratensis* (0.4 kg), *Filipendula vulgaris* (0.06 kg), and *Veronica teucrium* (0.08 kg). The total sowing rate of this species-rich mixture was 20 kg ha<sup>-1</sup>. Each treatment was established in strips measuring 6×100 m in four repetitions. Farmyard manure (30 t ha<sup>-1</sup>) was applied in the renewed swards before soil tillage, but no other fertilisers were used as a standard rule in protected areas. Similar mixtures were used for the re-grassing of arable land in the White Carpathians in 1990s (Prach *et al.*, 2013).

Dry matter yield and forage quality, along with botanical composition, were evaluated in two cuts in 2023 (3<sup>rd</sup> harvest year). For yield and forage quality estimation, four samples from each sward type were harvested from an area of 1 m<sup>2</sup>. Forage quality was evaluated by means of standard methods using wet chemistry. The botanical composition was assessed on an area of 25 m<sup>2</sup> in three repetitions.

## Results and discussion

The parameters of forage quality are shown in Table 1. There were no differences in chemical element composition, except calcium and ash - contents of which were higher in low-productive swards due to the higher presence of dicots. The tendency to a higher N content was associated with a higher proportion of *Trifolium repens* and *Lotus corniculatus* in the original pasture sward. The forage of the most productive standard mixture contained a higher level of fibre (both NDF and ADF), but lignin content (ADL) was equal to the other mixtures.

The sward renewed by sowing a standard mixture consisted mostly of cultivated grasses, which have a high growth rate and produce thin leaves with low DM content. Nevertheless, their high digestibility in early stages of growth decline quickly during the generative stage (Michaud *et al.*, 2012). In this case, the dominant grasses in the renewed sward were in full heading stage, crude protein was too low, and the fibre content was too high for productive dairy cattle. In the original sward, there were more dicot plants and slowly growing grasses (such as *Agrostis capillaris*) and the maturation of the forage was slower, resulting in a lower fibre content.

Grassland renewal using the standard mixture provided a significantly higher DM yield, and despite the low legume proportion exported more N compared to the two other treatments (see Table 2, N content in the 2<sup>nd</sup> cut not shown).

Table 1. Concentrations of chemical elements, ash, NDF, ADF and ADL in the forage (g (kg DM)<sup>-1</sup>, 1<sup>st</sup> cut 27 May 2023)

Treatment	N	P	K	Ca	Mg	Ash	NDF	ADF	ADL
Standard mixture	1.69	0.29	2.81	0.37 <sup>a</sup>	0.13	7.17 <sup>a</sup>	635 <sup>a</sup>	388 <sup>a</sup>	47.9
Species-rich mixture	1.69	0.31	3.08	0.66 <sup>b</sup>	0.15	8.89 <sup>b</sup>	528 <sup>b</sup>	348 <sup>b</sup>	45.6
Old sward	2.02	0.32	3.12	0.73 <sup>b</sup>	0.16	8.89 <sup>b</sup>	483 <sup>b</sup>	309 <sup>b</sup>	39.3
<i>p</i> value	0.078	0.501	0.470	0.000	0.052	0.027	0.001	0.001	0.300

Values with different letters in a column are significantly different ( $p=0.05$ ).

Table 2. Forage DM yield ( $t\ ha^{-1}$ ), N export in forage ( $kg\ ha^{-1}$ ) and the number of plant species and typical species in particular treatments.

Treatment	DM yield	N export	Number of plant species	Dominant species (over 5% ground cover in at least one 25 m <sup>2</sup> sample)
Standard mixture	6.55 <sup>a</sup>	115.7 <sup>a</sup>	24.0 <sup>a</sup>	<i>Dactylis glomerata</i> , <i>Arrhenatherum elatius</i> , <i>Festuca pratensis</i> , <i>Trifolium repens</i> , <i>Taraxacum officinale</i>
Species-rich mixture	4.66 <sup>b</sup>	83.6 <sup>b</sup>	42.7 <sup>b</sup>	<i>Bromus erectus</i> , <i>Bromus hordeaceus</i> , <i>Lolium perenne</i> , <i>Trisetum flavescens</i> , <i>Trifolium repens</i> , <i>Leucanthemum ircutianum</i> , <i>Taraxacum officinale</i> , <i>Rumex obtusifolius</i> , <i>Fragaria viridis</i> , <i>Prunella vulgaris</i>
Old sward	3.90 <sup>b</sup>	81.0 <sup>b</sup>	30.2 <sup>a</sup>	<i>Agrostis capillaris</i> , <i>Lolium perenne</i> , <i>Elytrigia repens</i> , <i>Poa pratensis</i> , <i>Trifolium repens</i> , <i>Lotus corniculatus</i> , <i>Taraxacum officinale</i>
<i>p</i> value	0.004	0.047	0.001	

Values with different letters in a column are significantly different ( $p=0.05$ ).

The species-rich mixture forage contained a higher number of plant species than the two other treatments (Table 2), but many species not included in the seed mixture originated from the seedbank and emerged spontaneously. After grassland tillage a high occurrence of broad-leaved docks from the seedbank was observed (not in the old sward). Due to the low soil fertility, their cover declined from an initial c. 20% in the 1<sup>st</sup> harvest year to less than 2% in the 3<sup>rd</sup> harvest year. Species from the species-rich mixture were almost absent in the 1<sup>st</sup> harvest year, but their ground cover increased in some cases to more than 60% in the 3<sup>rd</sup> harvest year.

## Conclusions

Permanent grassland renewal led to a higher forage production due to the introduction of productive grass and legume species and soil organic matter mineralisation after soil tillage. Nevertheless, when harvested at the same time as the original low-productive sward, the forage from renewed grasslands contained more fibre and less crude protein due to faster maturing of the cultural species. When using the species-rich seed mixture, the biodiversity was higher, but forage production did not increase and the forage quality was comparable to the old sward. It took two to three years to increase the cover of wild grasses and forbs above 50%.

## Acknowledgements

This work was funded by the European Union Horizon 2020 Research and Innovation programme (Developing Sustainable PERmanent Grassland systems and policies, SUPER-G). Special thanks go out to botanist K. Vincencová for assisting me with the botanical assessment of the swards.

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# Permanent grasslands on peat soils managed for dairy production and biodiversity

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## Abstract

The management of permanent, species-rich grasslands is key to dairy grass production, while at the same time it is an important driver of biodiversity and the provision of ecosystem services. An integrated understanding of the effects of current practices along an intensive – extensive management gradient on herbage yield, grass quality and biodiversity on Dutch dairy farms is, however, still lacking. The combined effects of mowing intensity, stocking density and fertilization intensity (Land Use Intensity (LUI)) had a positive effect on dry matter and protein yield and a negative effect on plant species richness in the Alblasserwaard, our case study region. Farming at moderate management intensity reaching moderate plant diversity allows for adequate yield production while protecting biodiversity and enhancing ecosystem services outcomes. Highly species-rich grasslands with a unique plant and soil biodiversity are rare in this region and need to be protected.

**Keywords:** land use intensity, grass yield, grass quality, biodiversity

## Introduction

The majority of agricultural grasslands in the Netherlands are managed at high intensity. This allows high yields of high-quality grass to be produced, but the provision of ecosystem services and biodiversity is reduced. Although farmers are keen to increase biodiversity on their permanent grasslands, the transition remains challenging. An integrated assessment of herbage yield, grass quality and biodiversity along a gradient of management activities from low to high intensity in an agricultural context is lacking (Francksen *et al.*, 2022). In our research we aim to find the relationship between herbage yield, grass quality, plant diversity and soil indicators along a gradient of management intensity on a wide range of permanent grasslands on dairy farms in the Alblasserwaard. In order to make our research comparable, we aim to test the combined effects of mowing intensity, stocking density, and nitrogen (N) fertilization intensity, the so called Land Use Intensity (LUI) (Blüthgen *et al.*, 2012), on yield, grass quality and plant diversity.

## Methods

The grasslands assessed for this study are located in the Alblasserwaard (51°52'14.39" N, 4°48'1.79" E) and Vijfheerenlanden (51°54'15.59" N, 5°5'5.40" E) in the provinces of South Holland and Utrecht in the Netherlands, where the average grass growing season is 310 days with an average temperature of 10°C and an average annual rainfall of 900 mm. The main soil types of the region are peat soils covered with a clay layer of varying thickness (0–50 cm). The altitude of the grasslands ranges from 0 to 1.75 m below sea level.



The mean regional management intensity is high. On average is the biomass mown 3 times and grazed 3 times per year with an average stocking density of 180 Livestock unit days per hectare per year (LU-days ha<sup>-1</sup> year<sup>-1</sup>) and an average nitrogen application rate of 208 kg N ha<sup>-1</sup> year<sup>-1</sup>. For assessment two types of species-rich grasslands were selected in the early spring of 2020–2022 (highly species-rich grasslands (HSRG) and moderately species-rich grasslands (MSRG)). For contrast, four species-poor grasslands (SPG) were included in this research and sampled for 3 years. Herbage yield and grass quality measurements were taken when farmers planned their regular mowing or grazing activities. Before grazing, four enclosure cages (1.2 m×4.2 m) were placed in the field and four samples were taken with a mower (1.2 m×5 m) at 5 cm cutting height. Grass height was double sampled. Subsamples were sent for dry matter analysis and a fresh sample was sent for chemical analysis (Weende analysis, Tilly and Terry). A manure sample was sent for nutrient analysis. Botanical composition was assessed using the Braun Blanquet method (5 m×5 m). Soil samples were taken for chemical analysis and from a selection of grassland sites biological soil quality was analysed. In autumn of the sampling year, farmers provided detailed information on their fertilization and grazing regime, such as the type of livestock and number of hours the animals were in the field. This information, data from manure analysis and excretion values from Bikker *et al.* (2019), were combined to calculate N excretion during grazing. A weighted average was calculated from the grass quality indicators and compared by ANOVA. We checked for individual correlations and tested for linear regression of the combined effect of mowing intensity, N fertilization intensity and stocking density (LUI), a method developed by Blüthgen *et al.* (2012) on yield, grass quality and plant diversity. Each indicator of the LUI has been standardized to the mean of the regional intensity. Data analysis has been done in Jamovi (The Jamovi project, 2023).

## Results

For this study 33 sites were assessed with an average field size of 1.76 ha. The grasslands included in this study were predominantly mown (Table 1). N Fertilizer application and type were highly variable per grassland type and affected N availability for the plant (HSRG: 5% inorganic N from solid manure; MSRG: 11% of inorganic N from slurry and solid manure; SPG: 42% of inorganic N from slurry and artificial fertilizer). Grazing management in HSRG and MSRG was highly variable (Table 1). HSRG were grazed by dry cows and sheep adding on average 20 kg N ha<sup>-1</sup> year<sup>-1</sup> by animal excretion. MSRG were grazed by dairy cows, dry cows, youngstock, beef and sheep, adding on average 37 kg N ha<sup>-1</sup> by animal excreta.

Table 1. Mean and standard deviation (sd) plant diversity, indicators of land use intensity, herbage yield and grass quality per grassland type

	HSRG		MSRG		SPG <sup>3</sup>		ANOVA
	Mean	SD	Mean	SD	Mean	SD	
Nr of grassland sites assessed	8		21		4		
Nr of plant species (25 m <sup>2</sup> )	21.6 <sub>(a)</sub>	1.7	17.2 <sub>(b)</sub>	2.4	10.8 <sub>(c)</sub>	0.1	***
Nr of Mowing/grazing events per year	1.6/1.3		2.1/1.8		4.5/0.5		
Stocking density (LU -day ha <sup>-1</sup> year <sup>-1</sup> )	74.3	83	120	119	29		
N fertilizer (kg N ha <sup>-1</sup> year <sup>-1</sup> )	35	58	87	66	373	55	
Dry matter yield (kg dm <sup>-1</sup> ha <sup>-1</sup> )	5086 <sub>(a)</sub>	1372	8221 <sub>(b)</sub>	2174	11754 <sub>(c)</sub>	2914	***
Net energy for lactation (VEM kg <sup>-1</sup> dm <sup>-1</sup> )	721 <sub>(a)</sub>	66	753 <sub>(a)</sub>	70.4	863 <sub>(b)</sub>	33.4	***
Crude protein content (g kg <sup>-1</sup> dm <sup>-1</sup> )	128 <sub>(a)</sub>	17.8	126 <sub>(a)</sub>	23.2	171 <sub>(b)</sub>	13.9	***
Magnesium (g Mg kg <sup>-1</sup> dm <sup>-1</sup> )	3.27 <sub>(a)</sub>	0.725	2.53 <sub>(b)</sub>	0.54	2.11 <sub>(b)</sub>	0.238	***
Calcium (g kg <sup>-1</sup> dm <sup>-1</sup> )	8.67 <sub>(a)</sub>	1.65	6.47 <sub>(b)</sub>	1.51	4.76 <sub>(c)</sub>	0.552	***
Zinc (mg kg <sup>-1</sup> dm <sup>-1</sup> )	72.2 <sub>(a)</sub>	42.9	66.8 <sub>(a)</sub>	29.4	38.5 <sub>(b)</sub>	10.2	***

HSRG, highly species rich; MSRG, moderate species rich; SPG, species poor grasslands. Results statistical analysis: ANOVA: \*\*\* $P < 0.001$ ; post hoc test: (a), (b), (c),  $P < 0.05$ .

In total 109 vascular plant species were recorded from all grasslands assessed. The number of plant species were significantly different per grassland type (Table 1). HSRG were found on Natura 2000 sites. Botanical composition changes with increasing N fertilization; the percentage of grass cover increases ( $R=0.76$ ,  $P<0.01$ ) while the percentage of herb cover decreases ( $R=-0.79$ ,  $P<0.01$ ). Legume cover is low on species-rich grasslands and has a positive relationship with grazing intensity ( $R=0.35$ ,  $P<0.05$ ). The yield and grass quality (crude protein content and trace elements) were significantly different per grassland type (Table 1), although we found that concentrations of trace elements can vary throughout the season per grassland type. The LUI on the sampled grasslands varied from 0.67 to 4.06. LUI was a significant predictor for yield, protein yield and number of plants species in the grassland (Table 2). With an increase in LUI we found an increase in yield and protein yield, while the number of plant species declined. Further data exploration is required to include the effects of phosphorus in manure, soil and vegetation, as we found in multivariate analysis of a subset of our data that P availability of the soil in grasslands and the abundance of mycorrhizal fungi have a stronger relationship with species composition in HSRG grasslands.

Table 2. Linear regression of Land Use Intensity (LUI), the combined effects of stocking density, grazing and fertilization intensity.

	$R^2$	$F$	df	$P$ -value model	Linear model
Yield	0.545	46.8	1,39	<0.001	$3907+(2210 \times \text{LUI})$
Crude protein yield	0.676	81.4	1,39	<0.001	$173+(507 \times \text{LUI})$
No. of plant species	0.462	33.5	1,39	<0.001	$22+(-2.73 \times \text{LUI})$

## Conclusions

Grassland management of species-rich grasslands for production of feed while supporting biodiversity remains challenging and requires a good understanding of the consequences of such a transition. The use of solid manure for fertilization is an important component of the management. Feed from grasslands with a higher percentage of herbs might lead to lower yield and protein yield but contents of trace elements can be higher. Farming at moderate management intensity, reaching moderate plant diversity, is an interesting option for dairy farming to reconcile production, biodiversity, and ecosystem services outcomes. Highly species-rich permanent grasslands with a unique plant and soil biodiversity are rare in this region and need to be protected.

## Acknowledgement

We thank Wageningen University Knowledge Base programme: Biodiversity in a Nature Inclusive Society (Pr.nr. KB 36 00 50 07) & the Dutch Ministry of Agriculture, Nature and Food Quality.

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# The effect of ribwort plantain (*Plantago lanceolata*) on enteric methane emission during grazing

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## Abstract

Herb-rich grasslands are of increasing interest due to their potential properties for reducing enteric methane (CH<sub>4</sub>) emission and increasing biodiversity. The objective of this study was to compare enteric CH<sub>4</sub> emission of cows grazing on perennial ryegrass (*Lolium perenne*, LP) pastures with and without ribwort plantain (*Plantago lanceolata*, PL). Thirty-two dairy cows were blocked and assigned to one of the treatments: LP and LP with PL (LP-PL) in Duplo on adjacent pastures that received the same grassland management. Cows were continuously grazing for four weeks per period: two weeks of adaptation and two weeks of measurement period. The trial was repeated three times in 2022: May-Jun (period 1), Jul-Aug (period 2) and Sept-Oct (period 3). Enteric CH<sub>4</sub> emission was measured using GreenFeed. A restricted maximum likelihood (REML) analysis was done with pasture and botanical composition (LP / LP-PL) as fixed effects and block as random effect. The CH<sub>4</sub> production and yield was significantly higher for LP-PL compared to LP ( $P < 0.001$  and  $P = 0.046$ , respectively). This study did not show a CH<sub>4</sub> reduction potential of PL, but that may be due to the low proportion of PL in the pasture and the possibility for cows to select while grazing.

**Keywords:** methane emission, dairy cattle, ribwort plantain, *Plantago lanceolata*, grazing

## Introduction

In response to the challenge of reducing both national greenhouse gas emissions and nitrogen excretion within the Dutch dairy sector, grazing strategies could have an important influence. Among these strategies, the integration of herb-rich grasslands is of increasing interest, due to the possible properties of reducing enteric methane (CH<sub>4</sub>) emission, increasing nitrogen efficiency and enhancing biodiversity within the landscape. One of those herbaceous plants is ribwort plantain (*Plantago lanceolata*, PL), which is known to reduce nitrogen excretion in cattle by lowering urinary nitrogen and can reduce forage deficits in ryegrass pastures due to its higher persistence during summer months (Della Rosa *et al.*, 2022). The effect of including PL in the grazing sward of dairy cattle on enteric CH<sub>4</sub> emission is unclear. Therefore, the objective of this study was to compare enteric CH<sub>4</sub> emission of cows grazing on pastures with and without PL. For this trial two pastures were available, both half perennial ryegrass (*Lolium perenne*, LP) and half a combination of LP and PL (LP-PL). It was hypothesized that the CH<sub>4</sub> emission was lower for cows grazing on LP-PL pastures compared to LP pastures.

## Materials and methods

The study was conducted at Dairy Campus (Leeuwarden, the Netherlands) in 2022. Unrestricted (day and night) strip grazing was applied for four weeks per period, with two weeks of adaptation and two weeks of measurement period. Each day, the cows had access to a new strip. The trial was repeated three times in 2022: May–June (period 1), July–August (period 2) and September–October (period 3). For this trial two pastures were available, both half LP and half LP-PL, which received the same grassland management. The proportion of PL varied during the grazing season between 8 and 25%. Thirty-two lactating dairy cows were blocked by parity, lactation stage and milk production characteristics in eight blocks of four cows per block. Within each block each cow was randomly assigned to one of the four treatment groups: 2 LP groups and 2 LP-PL groups, with one LP group and one LP-PL group on each

pasture. Additionally, all cows received 2.0 kg of concentrates in the milking parlour and, depending on the number of voluntary visits, a maximum of 3.5 kg of concentrates via the GreenFeed (divided over 4 portions with at least 3 hours between each portion). Methane production was measured using GreenFeed (C-lock, Rapid City, SD, USA). Daily milk production was recorded and weekly milk samples were collected and analysed using Fourier-transform mid-infrared spectroscopy by Qlip (Zutphen, the Netherlands). Fat-protein corrected milk (FPCM) production was calculated according to CVB (2016). Fresh herbage samples were collected in the morning and afternoon and analysed by Eurofins Agro (Wageningen, the Netherlands). Fresh herbage intake was estimated on individual level using the energy calculations according to the Dutch VEM system (CVB, 2016). Linear mixed model with restricted maximum likelihood (REML) was done in R (version 4.2.1), using the lme4, lmerTest and emmeans packages. Pasture (1, 2), botanical composition (LP, LP-PL) and period (1, 2, 3) were added to the model as fixed effects. Block within period was added to the model as independent (unstructured) random factor with common variances. The REML analysis on feed characteristics had the same fixed effects, but day within period as random effect.

## Results and discussion

In total 4412 CH<sub>4</sub> records were collected during the measurement periods from 96 experimental units (cow per period), which is an average of 46 records per cow. No differentiation was made regarding PL intake in LP-PL pastures. We assumed no selective grazing when interpreting results related to botanical composition. No differences in total fresh herbage intake or total feed intake were found between LP and LP-PL or between pastures (for all  $P > 0.393$ , Table 1). There was a period effect, with a significant higher fresh herbage and total feed intake (kg DM<sup>-1</sup>) in period 1 compared to period 2 and 3. No effect of botanical composition or pasture was found on any of the milk production parameters (for all  $P > 0.266$ ). There was an effect of period on milk yield and composition. The milk, FPCM, fat, protein and lactose yield were significantly higher in period 1 compared to period 2 and 3. This can be explained by the higher feed intake in that period. The urea content was significantly higher in period 3 compared to period 1 and 2, which can possibly be explained by the higher crude protein content in the fresh herbage in that period (217 g kg DM<sup>-1</sup>, versus 140 and 162 g (kg DM)<sup>-1</sup>, respectively,  $P < 0.001$ ). The CH<sub>4</sub> production and yield was significantly higher for LP-PL compared to LP ( $P < 0.001$  and  $P = 0.046$ , respectively). On the CH<sub>4</sub> production there was also an effect of pasture ( $P = 0.002$ ), period ( $P = 0.018$ ) and an interaction effect of pasture and period ( $P < 0.001$ ). In period 2 the CH<sub>4</sub> production was significantly higher on one of the pastures regardless of botanical composition. No differences were found in neutral detergent fibre (NDF) content between the pastures ( $P = 0.349$ ), and none of the other feed characteristics could explain the effect of pasture specifically for period 2. Significant differences were found in acid detergent fibre (ADF) and acid detergent lignin (ADL) content between treatment groups (for ADF 251 versus 272 g (kg DM)<sup>-1</sup> for LP and LP-PL respectively,  $P < 0.001$ , and for ADL 19.1 versus 34.4 g (kg DM)<sup>-1</sup>,  $P < 0.001$ ). These differences in cell wall components could potentially explain the difference in CH<sub>4</sub> production and yield between LP and LP-PL. A higher ADL content is related to a lower digestibility, which might decrease the passage rate through the rumen and therefore increase CH<sub>4</sub> production (Van Gastelen *et al.*, 2019). Compared to what have been found in previous years (Koning *et al.*, 2022), no lower CH<sub>4</sub> yield was found in period 1. The weather might be a cause for this, in 2022 it was extremely warm and dry in the Netherlands, with almost no rainfall and a record of sun hours in spring (period 1).

Table 1. Estimated marginal means including the standard error of the mean (SEM) of gaseous emissions, milk yield and composition, body weight and feed intake of cows grazing on LP and LP-PL.

	Botanical composition			P-value						
	LP	LP-PL	SEM	Bot	Pas	Per	Bot*Pas	Bot*Per	Pas*Per	Bot*Pas*Per
CH <sub>4</sub> production (g day <sup>-1</sup> )	359	400	10.9	<0.001	0.002	0.018	0.934	0.036	<0.001	0.012
CO <sub>2</sub> production (kg day <sup>-1</sup> )	12.2	12.4	0.44	0.303	0.002	0.238	0.459	0.515	0.008	0.709
CH <sub>4</sub> intensity (g kg FPCM <sup>-1</sup> )	14.3	19.5	3.05	0.202	0.324	0.248	0.368	0.627	0.325	0.302
CH <sub>4</sub> yield (g kg DMI <sup>-1</sup> )	18.5	21.0	0.92	0.046	0.111	0.397	0.538	0.733	0.036	0.330
Milk yield (kg day <sup>-1</sup> )	25.0	24.8	0.67	0.874	0.466	0.001	0.742	0.390	0.607	0.110
FPCM yield (kg day <sup>-1</sup> )	26.3	26.0	0.70	0.738	0.266	<0.001	0.904	0.370	0.564	0.172
Fat yield (g day <sup>-1</sup> )	1094	1066	43.4	0.499	0.334	0.003	0.218	0.696	0.680	0.286
Protein yield (g day <sup>-1</sup> )	862	861	22.2	0.957	0.332	<0.001	0.736	0.252	0.602	0.113
Lactose yield (g day <sup>-1</sup> )	1111	1106	30.7	0.914	0.341	<0.001	0.808	0.519	0.647	0.100
Urea (mg (dl milk) <sup>-1</sup> )	21.3	21.1	0.61	0.874	0.586	<0.001	0.477	0.614	0.002	0.902
Body weight (kg)	625	615	30.1	0.441	0.708	0.437	0.328	0.512	0.666	0.950
Fresh herbage intake (kg DM <sup>-1</sup> )	14.7	15.1	0.40	0.393	0.407	<0.001	0.829	0.538	0.831	0.231
Total feed intake (kg DM <sup>-1</sup> )	19.7	19.9	0.37	0.603	0.487	0.002	0.889	0.504	0.658	0.121

LP, *Lolium perenne*; LP-PL, *Lolium perenne* with *Plantago lanceolata*; Bot, botanical composition; Pas, pasture; Per, period.

## Conclusion

Period affected milk yield and composition, with higher values in period 1, which can be explained by a higher feed intake. Urea content was higher in period 3, possibly due to a higher measured crude protein content in the fresh herbage. This study did not show a CH<sub>4</sub> reduction potential of PL; CH<sub>4</sub> production and yield were higher of cows grazing on LP-PL pastures compared to those on LP pastures. The proportion of PL in the pasture was relatively low, with a maximum of 25%, which might explain these results. Additionally, cows could have been selecting while grazing, which makes it difficult to estimate the actual PL intake. It is recommended to measure enteric CH<sub>4</sub> yield with varying proportions of PL in the sward to further explore its CH<sub>4</sub> reduction potential under grazing conditions.

## Acknowledgements

The authors thank Dairy Campus (Leeuwarden, the Netherlands) for executing the trial. This study was financed by the Dutch Ministry of Agriculture, Nature and Food Quality (LNV).

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# Persistency of plantain and chicory in intensively mowed grasslands

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## Abstract

Interest in the addition of herbs to grass-clover mixtures is growing in Belgian agriculture. We compared a grass+clover+plantain+chicory mixture to a grass monoculture and grass+clover under intensive cutting grassland management at several N fertilization levels in two consecutive production years with a wet and a dry summer, respectively. In both years, adding the herbs had no significant effect on dry matter crop yield (DMY) compared to grass+clover. A moderate N fertilization application (75 kg N ha<sup>-1</sup>) resulted in the highest share of herbs compared to lower and higher N fertilization. In spring of the third production year, however, the amount of chicory and plantain plants was too low to continue the experiment. Maintaining the herbs in an intensive cutting management is difficult and shows no production advantage compared to grass+clover.

**Keywords:** DMY, intensive management, persistence, clover, chicory, plantain

## Introduction

Belgium has a temperate maritime climate with good conditions for perennial ryegrass production. Grassland is typically intensively managed with 4 to 6 cuts annually and a total fertilization of 300–375 kg N ha<sup>-1</sup>. Recent droughts have led to reduced biomass production and lower nutrient uptake. The Russia-Ukraine conflict has led to a spectacular fertilizer price increase and consequent increases in production costs. At the same time, incentives were introduced to stimulate use of leguminous plants and herb-rich mixtures. This has stimulated farmers to experiment with herb-rich grass+clover mixtures. However, farmers are doubtful about the reduced need for N fertilization and about the persistence of the herbs. We started a field trial with a herb-rich mixture under typical Belgian cutting conditions to compare DMY and herb persistence in a grass+clover+herb sward versus a grass monoculture. Perennial ryegrass (*Lolium perenne*) was used as the grass component in all mixtures. The combination of white (*Trifolium repens*) and red clover (*Trifolium pratense*) was used for all other mixtures, as the combination of red and white clover gives good results in cutting management. Chicory (*Cichorium intybus*) and plantain (*Plantago lanceolata*) were selected as the herbs for the field trial. The objectives of this study were to determine (i) the DMY in relation to the mixture and N fertilization, and (ii) the persistence of chicory and plantain in the sward.

## Materials and methods

The field trial was carried out in Merelbeke (Belgium) on an arable field (sandy loam; pH<sub>KCl</sub> 6.3; 0.82% organic C) with flax as the preceding crop. The field trial was sown on September 15<sup>th</sup> 2020, following a randomized complete block design with 6 treatments (Table 1) and 4 blocks. The cultivars of perennial ryegrass, white clover, red clover, plantain and chicory were Melonora, Merlyn, Lemmon, Tuatara and Commander, respectively. Seed rate was advised by Louis Bolk Institute. Field plots were 2.5 m × 6 m. The K fertilization was 190 kg K ha<sup>-1</sup> divided over the first 3 cuts (95–63–32) and was identical for every plot and according to fertilizer advice. According to soil analysis, there was no need for P fertilization. The N fertilization is indicated in Table 1. Net field plots of 1.5 m × 6 m were harvested with a Haldrup forage harvester at a cutting height of 5 cm. DMY of each replicate was calculated after drying a subsample in a forced-draft oven at 70°C for 72 h. To determine the botanical composition, a grab subsample in two blocks of treatments G+C+H\_0N, G+C+H\_75N and G+C+H\_375N were separated into grass,

clover, herb and weed components and dried in a forced-draft oven at 70°C for 72 h. In 2021 and 2022, 5 and 4 cuts were harvested, respectively.

## Results and discussion

The years 2021 and 2022 had very different weather conditions. The average summer temperature and precipitation (June–August) in the last 3 decades were 17.9°C and 234 mm, respectively. The summer of 2021 was the wettest ever recorded (17.8°C and 411 mm precipitation). The summer of 2022 was considered as one of the driest, hottest and sunniest ever (19.6°C and 111 mm). In 2021 5 cuts were possible, while in 2022 only 4 were done. Table 1 illustrates the impact of these summer conditions. In 2021 no significant difference in DMY was observed between G\_375N and G+C+H\_0N. Addition of herbs to a grass+clover mixture at the same fertilizer level (G+C\_125N versus G+C+H\_125N) had no significant impact on DMY. Increased N fertilization on the grass+clover+herb mixtures had a positive effect on DMY, but more than 125 kg N ha<sup>-1</sup> appears to have no benefit, as 250 kg N ha<sup>-1</sup> did not lead to any further significant positive effect. In 2022, the dry summer led to significantly lower DMY for grass in monoculture compared to grass+clover and grass+clover+ herbs, regardless of the N fertilization level. The N fertilization in 2022 had no effect on DMY of the grass+clover+herb mixtures. The results indicate that DMY is not increased by adding chicory and plantain to a grass+clover mixture compared to grass+clover at the same N fertilization level. In dry years there is no effect N fertilization on grass+clover+herb mixtures. Under wet conditions 125 kg N ha<sup>-1</sup> can be considered as a maximum dose.

In Figure 1 the evolution in the sward composition is shown in a ternary plot for the grass+clover+herb mixtures at fertilization levels of 0, 125 and 375 kg N ha<sup>-1</sup>, respectively. The numbers indicate the

Table 1. The mean DMY (±SE) per treatment and per year.

Treatment	Mixture	N fertilization (kg N ha <sup>-1</sup> )	DMY 2021 (kg DM ha <sup>-1</sup> )	DMY 2022 (kg DM ha <sup>-1</sup> )
G_375N	perennial ryegrass, 30 kg ha <sup>-1</sup>	375	19 239 ± 441 <sup>abc</sup>	14 032 ± 217 <sup>a</sup>
G+C_125N	perennial ryegrass 30 kg ha <sup>-1</sup> + white clover 3 kg ha <sup>-1</sup> + red clover 8 kg ha <sup>-1</sup>	125	19 600 ± 378 <sup>bc</sup>	18 960 ± 361 <sup>b</sup>
G+C+H_0N	perennial ryegrass 30 kg ha <sup>-1</sup> +	0	17 313 ± 373 <sup>abc</sup>	18 133 ± 236 <sup>b</sup>
G+C+H_75N	white clover 3 kg ha <sup>-1</sup>	75	19 117 ± 207 <sup>abc</sup>	18 266 ± 204 <sup>b</sup>
G+C+H_125N	+ red clover 4 kg ha <sup>-1</sup>	125	20 542 ± 439 <sup>bc</sup>	18 080 ± 289 <sup>b</sup>
G+C+H_375N	+ plantain 1.5 kg ha <sup>-1</sup> + Chicory 1.5 kg ha <sup>-1</sup>	375	21 106 ± 628 <sup>c</sup>	18 018 ± 55 <sup>b</sup>

Statistically different treatments (Tukey's test,  $p < 0.05$ ) are indicated by different letters per year.

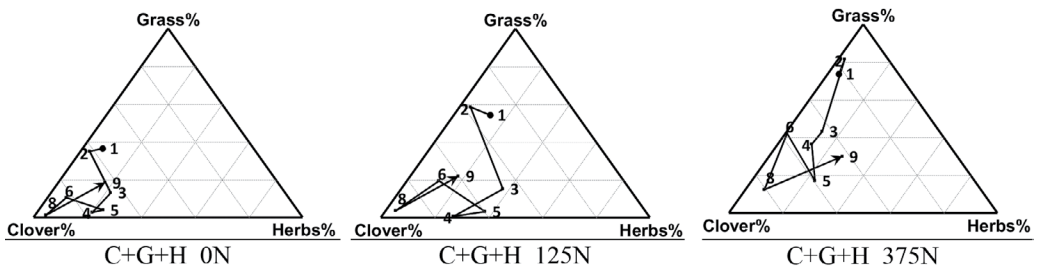


Figure 1. The evolution of the botanical sward composition on dry matter base in 2021-2022.

composition of the sward on the different cutting dates and years. The grassland was cut on (1) 10 May 2021, (2) 16 June 2021, (3) 29 July 2021, (4) 8 September 2021, (5) 18 October 2021, (6) 2 May 2022, (7) 14 June 2022, (8) 26 July 2022 and (9) 24 October 2022. On June 14<sup>th</sup> 2022, no samples were taken. In C+G+H 0N, about one-third of the biomass was grass in the first 2 cuts. However, the clover was immediately very abundant (>50%) and increased over time to >70% at the end of 2021 and even >80% in cut 1 and 3 of 2022. Although the near-absence of plantain and chicory in 2021 in cut 1 and 2, herb presence reached >20% in cuts 3, 4 and 5. In 2022 the percentage of herbs decreased again to <20%. N fertilization had a clear impact on the sward composition. In G+C+H\_375N, the N fertilization suppressed the clover in the sward in the first cuts. However, even at this elevated N fertilization level the grass% gradually decreased over time in 2021 from 70-80% to <20%. Although the first cut in 2022 had a larger grass% (42%), the grass was again suppressed by the clover in the following cuts of 2022. The herb% in 2021 followed the same pattern in G+C+H\_375N as for G+C+H\_0N, but the herbs disappeared almost completely in the first cut of 2022 in G+C+H\_375N. Although plantain and chicory were not separated in the botanical analysis, plantain was clearly more abundant than chicory. The changes over time in G+C+H\_75N followed the same patterns as in G+C+H\_0N, but the herbs were more abundant in all cuts in the fertilized treatments, particularly in 2021. The 375 kg N ha<sup>-1</sup> fertilization in G+C+H\_375N was too high to encourage an abundant share of herbs in the sward. Without N fertilization (G+C+H\_0N), the clover was suppressive for grass as well as for herbs to a lesser extent. In G+C+H\_75N the highest percentage of herbs was reached in the sward. Across all mixtures, grass% was low and clover% was high starting from the 3<sup>rd</sup> cut in 2021. It seems that clover partially pushed out the grass from the sward, as the grass regrowth in autumn 2021 was low and the amount of grass in spring 2022 remained low. This was unexpected as perennial ryegrass is more competitive with clover in a wet summer than a dry summer, and as grass growth is more competitive in spring. In spring 2023 almost all chicory plants had disappeared and too few plantain plants remained to continue the trial.

## Conclusion

In years with wet and dry summer conditions, adding plantain and chicory to a grass-clover mixture at a fertilization level of 125 kg N ha<sup>-1</sup> had no significant effect on DMY compared to grass-clover. Increasing the N fertilization >125 kg N ha<sup>-1</sup> of a grass+clover+herb mixture had no significant effect on DMY. A moderate N fertilization application (75 kg N ha<sup>-1</sup>) led to the highest share of plantain and chicory in the sward.



# Seed hydropriming effects on tall fescue plant growth, water status and chlorophyll content under drought

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## Abstract

Tall fescue (*Festuca arundinacea* Schreb.) is an important forage species that can adapt to various environmental conditions. Improving the yield of tall fescue (cv. Fawn) under stressful conditions might prove to be useful for arid regions. We aimed to determine the effects of seed hydropriming on plant growth under drought stress. Hydroprimed and unprimed seeds of tall fescue were sown on sandy soil and irrigated at 100% field capacity for 30 days. Plants were subsequently subjected to two irrigation regimes: control plants received 100% field capacity and stressed plants received only 40% field capacity. After 17 days of treatment, the plants were harvested, and their growth, water status, and pigment content were studied. Hydropriming enhanced shoot and root dry weights under both control and drought stress conditions. The growth of stressed plants from hydroprimed seeds was similar to that of the control plants. Unlike shoot water content, which showed no significant difference, root water content was reduced by water-deficit conditions in both unprimed and hydroprimed seeds by 21.3 and 25.6%, respectively. Total chlorophyll content and chlorophyll *a*/chlorophyll *b* ratio showed no significant difference. These data demonstrate the importance of hydropriming for tall fescue plants.

**Keywords:** chlorophyll content, *Festuca arundinacea*, growth, hydroprimed seeds, water content

## Introduction

Tall fescue (*Festuca arundinacea* Schreb.) is one of the most important forage species grown extensively in Europe and the United States. Tall fescue is considered a perennial crop, and it can remain in the soil for three to six years and grow under summer heat and high humidity conditions. It can also adapt to various environmental conditions. The tall fescue 'Fawn' is a cool-season cultivar with promising results when cultivated in an arid region (Al-Ghumaiz and Motawei, 2011; Motawei and Al-Ghumaiz, 2012). Improving the yield of this cultivar under stressful conditions in arid and semi-arid regions may be of great importance. Seed priming is a pre-germinative treatment that aims to ensure rapid and uniform seedling emergence and to enhance the ability of seedlings to cope with biotic and abiotic stresses (Ellouzi *et al.*, 2023; Paparella *et al.*, 2015). The aim of the present study was to determine whether seed hydropriming enhances the plant growth, water content, and chlorophyll status of 'Fawn' tall fescue subjected to drought stress at the vegetative stage.

## Materials and methods

Seed hydropriming was performed by immersing the seeds in distilled water for 20 h at room temperature, followed by air-drying. Primed and unprimed seeds were sown in small plastic pots filled with 1 kg of sandy soil each (20 seeds per pot). During the first 30 days after sowing (DAS), all the pots (eight pots sown with hydroprimed seeds and eight pots sown with unprimed seeds) were irrigated with clean water at 100% field capacity. Two weeks after sowing, only 10 seedlings were maintained per pot. At 30 DAS, plants from unprimed and hydroprimed seeds were irrigated at 100% (control) or 40% (drought stress) field capacity. Hence, four treatments were considered: UPC, unprimed seeds control (irrigated at 100%

field capacity); HPC, hydroprimed seeds control (irrigated at 100% field capacity); UPD, unprimed seeds drought (irrigated at 40% field capacity); and HPD, hydroprimed seeds drought (irrigated at 40% field capacity).

Four-pot replicates of 10 plants per pot were used for each treatment. The experiment was conducted using a completely randomised design. After 17 days of treatment (from 30 to 47 DAS), five plants from each pot were harvested, washed with distilled water, cut into shoots and roots, weighed fresh, and oven-dried. Shoot, root, and whole plant dry weights ( $\text{mg plant}^{-1}$ ); root/shoot ratio; and water content ( $\text{ml H}_2\text{O (g DW)}^{-1}$ ) of shoots and roots were measured. The remaining five plants in each pot were used for leaf chlorophyll assay according to Lichtenthaler and Bushmann (2001). All data were subjected to a two-way analysis of variance (ANOVA) followed by a one-way ANOVA using Posthoc Duncan's multiple-range test at  $P \leq 0.05$ .

## Results and discussion

The results are summarised in Table 1. Hydropriming (H) had a significant effect ( $P < 0.01$ ) on shoot ( $P < 0.05$ ), root ( $P < 0.01$ ), and whole plant dry weights, as well as on the root/shoot ratio ( $P < 0.05$ ). Drought stress (D) exhibited a significant effect on shoot ( $P < 0.01$ ) and whole plant ( $P < 0.05$ ) dry weights, and the interaction (H×D) had a significant effect only on shoot dry weight ( $P < 0.01$ ). At 100% field capacity, seed priming (HPC treatment) improved shoot and root growth by 28.1 and 48.3%, respectively. In contrast, a 25% decrease in shoot dry weight was recorded in unprimed seeds under water-deficit conditions (UPD treatment). Compared with unprimed seeds (UPD treatment), seed priming (HPD treatment) improved root dry weight under water-deficit conditions. Interestingly, the whole plant dry weight of the hydroprimed seeds under drought stress (HPD treatment) was not significantly different from that of the control (UPC treatment). The beneficial effect of hydropriming under stress conditions, which increased the root/shoot ratio by 40% compared with that in the control, was more pronounced in roots than in shoots. Tabassum *et al.* (2018) demonstrated that hydropriming and osmopriming alleviated the detrimental effects of drought on wheat, but osmopriming was more effective. Marthandan *et al.* (2020) reported that hydropriming resulted in uniform germination, improved seedling vigour, and improved plant growth and development in several crops.

Hydropriming, drought stress, and their interaction had significant effects on root water content at  $P \leq 0.05$ ,  $P \leq 0.01$  and  $P \leq 0.001$ , respectively, but they showed no significant effects on shoot water content. Root water content increased in plants from hydroprimed seeds under non-stress conditions by 27.7% (HPC treatment) compared with that in the control (UPC treatment). However, root water content was reduced by water-deficit conditions in both unprimed (UPD treatment; 21.3%) and hydroprimed (HPD treatment; 25.6%) seeds, indicating that hydropriming had no beneficial effects under water-deficit conditions. Alzoheiry *et al.* (2023) showed that this species maintained a constant water productivity when subjected to increasing drought stress levels, but that was not the case in Tekapo orchard, in which the root water content decreased with increasing water deficit. The superiority of 'Fawn' tall fescue in coping with drought stress might be explained by the amplification of dehydrin genes in the grass, which had been shown to be homozygous for these genes (Alzoheiry *et al.*, 2023).

Hydropriming and the interaction between hydropriming and drought stress significantly affected chlorophyll content, whereas their individual effects were not significant.

Table 1. Dry weights (mg plant<sup>-1</sup>), water contents (ml H<sub>2</sub>O (g DW)<sup>-1</sup>) and chlorophyll content (mg (g fresh weight)<sup>-1</sup>) in faun-tall fescue plants from unprimed (UP) and hydroprimed (HP) seeds irrigated at 100% field capacity (water sufficiency) or 40% field capacity (drought stress) for 17 days.

	UPC	HPC	UPD	HPD	SE	Two-way ANOVA
Shoot dry weight	105.2 b	134.8 a	76.2 c	93.0 bc	±21.4	H*, D***, H×D***
Root dry weight	46.0 bc	68.2 a	34.8 c	56.6 b	±12.4	H**, Dns; H×Dns
Whole plant dry weight	151.2 b	203.0 a	111.0 c	149.6 b	±32.7	H**, D**, H×Dns
Root/shoot ratio	0.44 b	0.51 ab	0.47 b	0.61 a	±0.06	H*, Dns; H×Dns
Shoot water content	3.96 a	4.05 a	4.19 a	3.82 a	±0.13	H ns; D ns; H×D ns
Root water content	5.43 b	6.94 a	4.28 c	4.04 c	±1.15	H**, D***, H×D***
Chlorophyll content	2.35 ab	2.30 ab	2.67 a	1.96 b	±0.25	H*, D ns; H×D*

Means (n=5) followed by different letters are significantly different according to Duncan's multiple-range test at 5%. UPC, unprimed seeds control (irrigated at 100% field capacity); HPC, hydroprimed seeds control (irrigated at 100% field capacity); UPD, unprimed seeds drought (irrigated at 40% field capacity); HPD, hydroprimed seeds drought (irrigated at 40% field capacity). Data on hydropriming (H), drought (D) and their interaction (H×D) effects are presented as asterisks: significance: \*5%, \*\*1%; \*\*\*0.1%; ns: non-significant.

## Conclusion

The present study showed the beneficial effects of hydropriming on 'Fawn' tall fescue growth under both drought stress and control conditions.

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# Better valorization of dairy products from permanent grasslands to balance ecosystem services: A review

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## Abstract

Grasslands provide many ecosystem services (ES) which depend mainly on farmers through their management, but these do not all benefit the same stakeholders. Farmers mainly benefit from the production of fodder to feed animals, while the whole society benefits from the environmental services and the conservation of biodiversity. Policies such as the Natura 2000 network or natural reserves or parks have been put in place to protect biodiversity but only concern limited areas. For regulating services such as C storage and water quality, payment for environmental services (PES) schemes are developing in Europe. However, these farm-level solutions do not focus on grasslands. A paradigm shift from production to the search for cost-effectiveness could offer an opportunity to balance ES in grasslands. Livestock feeding based on less intensively managed grasslands, leading to lower productivity, reduces energy and fertilizer costs, and can improve biodiversity but also many other ES. Such a system can be encouraged when high quality products, such as cheese, are promoted through labelling and certification. This paper questions the role of the valorization of dairy products in the balance of ES provided by grasslands implied in the dairy production.

**Keywords:** permanent grasslands, biodiversity conservation policies, payment for services, labelled dairy products

## Introduction

Recognised for their biodiversity enhanced by extensive management over centuries, permanent grasslands provide many Ecosystem Services (ES) that are now threatened by land use changes (Henle *et al.*, 2008): intensification, land abandonment, but also their replacement by more productive forages such as temporary grasslands (sown since less than six years) or maize. Dairy farming faces many environmental challenges such as reducing greenhouse gas emissions, improving water quality, and biodiversity conservation, as well as economic and social challenges such as animal welfare (Dumont *et al.*, 2019). These aspects are gaining increasing attention from consumers (Alonso *et al.*, 2020). Some solutions exist to protect permanent grasslands and promote the ES they provide, such as protected areas for biodiversity conservation, payments for environmental services but also certified dairy products that meet the societal demand.

## The preservation of grasslands' ES through protection statuses

In Europe each country has implemented protection statuses such as nature reserves to protect specific grasslands (semi natural grasslands with high heritage value). However, they represent only small areas and their management, which aims to protect threatened species, for example by grazing hardy cattle (heritage breeds), is fairly disconnected from farmers in terms of management and production aims. National and regional nature parks, another protection status, which represent larger areas, act to protect biodiversity in consultation with farmers but often lack the levers for ensuring the other ES provided by grasslands. At the European Union scale, the Natura 2000 network aims to prevent biodiversity loss, but mainly guides grassland management to support ground-nesting birds. Nevertheless, it appears that the conservation of biodiversity can also improve other ES. Grasslands with a favourable conservation status have a higher potential to supply regulating and cultural services (Maes *et al.*, 2012). More specifically, grasslands within the Natura 2000 network contain more organic carbon (C) in their soils than adjacent

grasslands outside the network (Hagyó and Toth, 2018). All these protection statuses are designed to protect biodiversity and the environment, but they do not take into account fodder production, which is essential to support the activities of livestock farmers.

### **Payment schemes for environmental services**

Various payment schemes have been developed in Europe to reward environmental services. They are financial incentives granted by a ‘buyer’ to farmers as ‘service providers’ to implement good management practices leading to the improvement of a well-defined environmental service (Capodaglio and Callegari, 2018). Although these payment schemes provide support for farmers, they only cover a few services, mainly C storage and water quality, and are based on basic indicators of farm management such as grassland area, agricultural inputs and hedge lines (Sénécal *et al.*, 2024).

### **Labelling dairy products for a better balance between ES?**

Other solutions, which would pay farmers directly for their production, could help to preserve SE in grasslands. Less intensive management may reduce productivity, but it can also reduce production costs and improve biodiversity and ES. Encouraging such a system can be achieved through better financial valuation of high-quality products such as cheese through labelling and certification. Growing consumer interest in sustainable dairy production, including the limitation of greenhouse gas emissions, the preservation of biodiversity, and animal welfare, has led to the development of quality labels (McGarr-O’Brien *et al.*, 2023; Mol and Oosterveer, 2015). In France, regional nature parks have created their own certification ‘Valeurs Parc Naturel Régional’, thus contributing to local development while reconciling social and environmental aspects. These products have the advantage to correspond to ‘local’ food but are not available for all consumers. In Europe, a number of dairy labels have been established to improve sustainability in environmental, economic, social or/and animal welfare terms: Arlagården (Denmark/Sweden), Demeter Biodynamic (Germany), Origin Green (Ireland), Pasture for Life Association (UK), Red Tractor (UK) (Mc-Garr-O’Brien *et al.*, 2023). However, to our knowledge there are no studies on the bundles of services (Raudsepp-Hearne *et al.*, 2010) in grasslands involved in dairy labels. Such analyses would represent a relevant approach to integrate the balance between ecosystem services: environmental, agronomic, welfare that these agrosystems provide.

### **Perspectives: a project to evaluate ES provided by grasslands involved in dairy PDOs**

While many labels have emerged in response to consumers’ demand, there have been little research on the ES provided by the grasslands that meet the specifications. The ‘cAnOPée’ project, which has just started, aims at developing multiscale approach to the services provided by a quality labelled dairy chain with a Protected Designation of Origin (PDO) in Normandy. As part of this project, we will assess a set of ES among (i) supporting services with floristic and functional diversity, (ii) provisioning services with forage quality, (iii) regulating services with C storage, water regulation, pollination and (iv) cultural services with landscape heritage value (Figure 1). The cAnOPée project will investigate how the PDO cheese labels of Normandy, linked to management specifications that certify the quality of products, also contributes to balancing the ES between them.

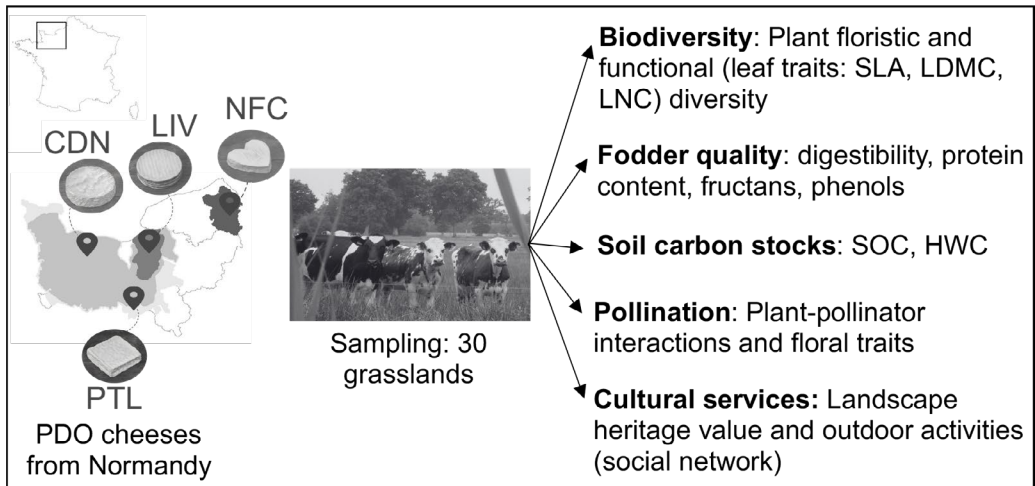


Figure 1. Analysis of ES in grasslands involved in four PDO cheese labels in Normandy. CDN, Camembert de Normandie; LIV, Livarot; NFC, Neufchâtel; PTL, Pont-L'Évêque.

## Acknowledgements

We thank ANR for funding the 'cAnOpée' project and Nathalie Desmasures for the project coordination.

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# The influence of grasses and legumes as a forecrop on the biological activity of the soil

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## Abstract

The paper presents the results of a study on the impact of different forecrops on biological activity of soil. Winter wheat was cultivated in crop rotation after the following crops: red clover, Italian ryegrass, mixtures of these two species and as monoculture. In soil microbiological tests, the following were determined: the total number of microorganisms (bacteria, actinomycetes, fungi) and the physiological groups of microorganisms: active in soil nitrogen and soil carbon metabolism. The soil under wheat cultivation in crop rotation, and especially after the red clover forecrop, was characterized by richer biological life, as compared to the soil under monoculture.

**Keywords:** grass-legume forecrop, wheat, soil microbiota

## Introduction

Crop rotation can have a significant impact on soil microorganisms, including bacteria and fungi, which play a critical role in soil health and plant growth. Studies have shown that long-term fertilization and crop rotation can alter soil microbial community structure (Kracmarova *et al.*, 2022). In addition, crop rotation can influence soil bacterial composition and increase disease suppression capacity. The effects of crop rotation order on soil microbial communities for different food and forage crops are not well understood (Venter *et al.*, 2016). Appropriate crop rotation, with the use of legume-grass mixtures, increases the microbiological and biochemical activity of the soil due to a greater supply of organic matter. High activity of soil microorganisms indicates good soil quality and better conditions for plant growth. Therefore, it is important to consider the impact of crop rotation on soil microorganisms when planning soil management.

## Materials and methods

The experiment was established in 2018, on slightly acid soil, poor in absorbable P and K. Humus content was 2.07% and total nitrogen content was 1.323% (Szkutnik-Sroka *et al.*, 2023). Thus, the following experimental objects in three replicates were obtained (they also mark appropriate objects in tables): Winter wheat monoculture (WW); Red clover (RC); Italian ryegrass (IR); Red clover; and Italian ryegrass mixture 50:50 (M). Soil samples for assessing the number of microorganisms were collected from each site after the wheat harvest in autumn. Soil samples were collected from each plot into sterile plastic containers in accordance with the principles of biological purity. In soil microbiological tests, the following were determined: the total number of microorganisms (bacteria, actinomycetes, fungi) and the physiological groups of microorganisms active in soil nitrogen and carbon metabolism.

The number of microorganisms was determined using the Koch dilution plate method, and the results were given in the form of colony-forming units (CFU), converting the result into 1 g of dry soil mass. For nitrifying and denitrifying bacteria, the titre was determined in diluted soil (Ben-David and Davidson, 2014). The standard selective media were used. The total number of bacteria was assessed on solid medium with soil extract and  $K_2HPO_4$ , and of fungi on Martin's medium. The number of microorganisms involved in nitrogen metabolism was determined on the following media: proteolytic bacteria, Pochon's medium; ammonifying bacteria, Rougieux's medium; *Azotobacter*, Ashby's medium (Pochon and Tardieux, 1962).

The titre values of nitrifying bacteria (Winogradski medium) and denitrifying bacteria (Giltay medium) were also determined (Parkinson *et al.*, 1971). The results were developed using one-factor analysis of variance. To determine the significance of differences, Student's *t*-test was used at  $\alpha=0.05$ . Homogeneous groups of means were determined as a measure of dispersion.

## Results and discussion

In the first year of research, the most numerous populations in the soil were vegetative bacteria, followed by phosphorus bacteria and endospores (Table 1). The first group contained 529 000 CFU (g soil)<sup>-1</sup>, the second 450 000 CFU (g soil)<sup>-1</sup> and the third 377 000 CFU (g soil)<sup>-1</sup>. In turn, actinomycetes and fungi occurred in much smaller quantities, amounting to 54 700 CFU (g soil)<sup>-1</sup> and 57 200 CFU (g soil)<sup>-1</sup> soil. In the last (third) year of the study, compared to the initial state, the number of vegetative bacteria in the soil was clearly higher. In object WW their number was 1.5 times higher, in other objects the difference was 3-fold. The number of endospores in the last year was higher than at the beginning of the research in all objects. In the IR object, this amount was almost 3 times higher, in the RC 2.5 times, and in the M object 2 times higher. However, in the soil of the monoculture (WW), the population of this group of bacteria was only about 25% higher than at the beginning of the research. On average, the number of phosphorus bacteria in the soil in the last year was higher than at the beginning of the experiment. The soil of the IR site contained almost three times more of this group of bacteria, and approximately twice as much in the soil of the RC and M sites. In turn, in the soil of the WW object, the population of phosphorus bacteria increased by only 15%. According to Kracmarova *et al.* (2022) fertilization and crop rotation consequently modified the bacterial and fungal communities. However, the response of prokaryotic and fungal communities to long-term fertilization treatments differed.

The number of actinomycetes in the soil was quite diverse. The smallest amount on average was found in the WW site, and the highest, almost twice as much, was found in sites where wheat forecrops were grass-legume plants. The average number of fungi in the soil in the last year of the study was approximately 20% lower than at the beginning of the study.

Within the population of bacteria active in nitrogen metabolism, five groups were distinguished. These were bacteria of the genus: *Azotobacter*, nitrifying, denitrifying, ammonifying and proteolytic. at the beginning of the research, the most numerous group in the soil were ammonifying bacteria. Their number exceeded 1 700 000 CFU (g soil)<sup>-1</sup> (Table 2). Proteolytic bacteria numbered approximately 157 000 CFU (g soil)<sup>-1</sup>, and the least were bacteria of the genus *Azotobacter*, about 900 CFU (g soil)<sup>-1</sup>. The number of nitrifying and denitrifying bacteria in the soil was generally similar in most sites and at all assessment dates. An exception in this respect was the soil of object WW, which was characterized by lower numbers of the nitrifying bacteria compared to the other objects.

Table 1. The number of soil microorganisms (thousands of CFU g<sup>-1</sup> soil).

Item	Initial state	Experimental objects after 3 <sup>rd</sup> year			
		WW	RC	IR	M
Vegetative forms bacteria	529 <sup>a</sup>	873 <sup>a</sup>	1773 <sup>b</sup>	1674 <sup>b</sup>	1452 <sup>b</sup>
Rest forms bacteria (endospore)	377 <sup>a</sup>	633	1142 <sup>b</sup>	1053 <sup>b</sup>	815 <sup>b</sup>
Phosphorus bacteria	450 <sup>a</sup>	615 <sup>a</sup>	1327 <sup>b</sup>	1272 <sup>b</sup>	1128 <sup>b</sup>
Actinomycetes	54.7 <sup>a</sup>	36.2 <sup>a</sup>	121 <sup>b</sup>	95.4 <sup>b</sup>	102 <sup>b</sup>
Fungi	57.2 <sup>a</sup>	45.3 <sup>a</sup>	41.2 <sup>a</sup>	44.9 <sup>a</sup>	45.3 <sup>a</sup>

The same letter indicates no significant differences.



Table 2. The number of bacteria active in nitrogen metabolism (thousands of CFU (g soil)<sup>-1</sup> or titre)

Item	Initial state	Experimental objects after 3 <sup>rd</sup> year			
		WW	RC	IR	M
Azotobacter	0.90 <sup>b</sup>	0.23 <sup>a</sup>	1.35 <sup>b</sup>	1.05 <sup>b</sup>	0.85 <sup>b</sup>
Nitrifying bacteria	10 <sup>-5b</sup>	10 <sup>-5b</sup>	10 <sup>-6a</sup>	10 <sup>-6a</sup>	10 <sup>-6a</sup>
Denitrifying bacteria	10 <sup>-5b</sup>	10 <sup>-6a</sup>	10 <sup>-6a</sup>	10 <sup>-6a</sup>	10 <sup>-6a</sup>
Ammonifying bacteria	1700 <sup>a</sup>	1675 <sup>a</sup>	3784 <sup>b</sup>	3563 <sup>b</sup>	3636 <sup>b</sup>
Proteolytic bacteria	157 <sup>a</sup>	427 <sup>b</sup>	924 <sup>c</sup>	865 <sup>c</sup>	881 <sup>c</sup>

The same letter indicates no significant differences.

Two types of bacteria involved in carbon transformation were identified: amylolytic bacteria and fibre-degrading bacteria (Table. 3). In the first year of research, their number amounted to 156 000 and 187 000 CFU (g soil)<sup>-1</sup>. In the last year of research, the soil of object WW was the poorest with regard to these groups of bacteria (312 000 and 516 000 CFU (g of soil)<sup>-1</sup>), while the most numerous bacteria were present in the soil of objects RC and IR, where the wheat forecrop was clover and a grass-clover mixture.

Table 3. The number of bacteria active in carbon metabolism (thousands of CFU (g soil)<sup>-1</sup>)

Item	Initial state	Experimental objects after 3 <sup>rd</sup> year			
		WW	RC	IR	M
Amylolytic bacteria	156 <sup>a</sup>	312 <sup>b</sup>	772 <sup>c</sup>	715 <sup>c</sup>	635 <sup>c</sup>
Fibre-degrading bacteria	187 <sup>a</sup>	516 <sup>b</sup>	995 <sup>c</sup>	893 <sup>c</sup>	816 <sup>c</sup>

The same letter indicates no significant differences.

Meta-analysis shows that microbial communities responded to increased plant production of detritus and C substrates associated with higher plant diversity rather than to the diversity itself. In addition, adding legumes to a rotation has been shown to increase bulk soil C pools, supporting a greater abundance of microbiota (Venter *et al.*, 2016).

## Conclusion

When compared to baseline studies after three years, the overall population of microorganisms in the soil repeatedly increased, except the fungal population, which decreased by 15–20%. This may indicate an improvement in the biological condition of the soil in winter wheat grown in crop rotation, especially after red clover, compared to monoculture.

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# Sward species diversity impacts on pasture productivity and botanical composition under grazing

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## Abstract

As part of the management of intensive grazing, the focus on simple and productive forage systems has led to a limited range of plants being used in grazing swards supported by high levels of chemical fertilisers. This study investigated three different combinations of plant diversity and N fertiliser level (perennial ryegrass monoculture (PRG), 250 kg N ha<sup>-1</sup>; two-species perennial ryegrass-white clover (PRG-WC), 125 kg N ha<sup>-1</sup>; eight-species containing grasses, clovers and herbs (MSS), 125 kg N ha<sup>-1</sup>). Each sward type had its own farmlet of 20 paddocks and comprised 50 dairy cows on 20 ha which were rotationally grazed. Over two years, botanical composition of the PRG-WC was composed of 836, 163 and 1 g (kg DM)<sup>-1</sup> of grasses, white clover and unsown species, respectively; MSS had 673, 151, 171 and 5 g (kg DM)<sup>-1</sup> of grasses, clovers, herbs and unsown species respectively. Total net herbage production (13 022 kg ha<sup>-1</sup> year<sup>-1</sup> of DM forage) and nutritive values were unaffected by sward type during the 2 years. These results suggest that increasing sward diversity while reducing the use of chemical N fertiliser can maintain herbage production and nutritive value.

**Keywords:** grass-clover sward, multi-species sward, dry matter yield, botanical composition, chemical composition

## Introduction

As part of the management of intensive grazing, the focus on simple and productive forage systems has led to a limited range of plants being used in grazing swards which are dominated by *Lolium perenne* L. (perennial ryegrass (PRG)) monocultures. Such swards are capable of high levels of productivity and nutritional value over a long growing season (Baker *et al.*, 2023) but are reliant on high levels of mineral fertilizer application and adequate moisture availability (Grange *et al.*, 2020). The inclusion of legumes such as *Trifolium repens* (white clover (WC)) within grazing swards has received much attention in recent years to reduce dependence on chemical nitrogen (N) fertilizer application within such systems (Delaby *et al.*, 2016). More recently, a growing body of scientific evidence has shown that the inclusion of a limited number of additional dicotyledonous complementary species, selected for their agronomic performance, such as chicory (*Cichorium intybus* L.) and plantain (*Plantago lanceolata* L.), can further enhance both productivity and sustainability and improve the overall resilience of grazing systems (Baker *et al.*, 2023; Grange *et al.*, 2020). Much of the evidence has been derived from short-term (3 to 6 month) evaluations, based on grazed or cut plot mechanical defoliation protocols which may not reflect the longer-term performance under grazing conditions. The objective of this study was to evaluate the performance of PRG-WC and multispecies (MSS) swards with intermediate levels of chemical N fertiliser application compared to monocultures of PRG with high levels of chemical fertiliser within a two-year farm systems intensive grazing evaluation.

## Material and methods

The experiment was a randomised block design with three sown swards: a monoculture of PRG, an association of PRG and WC (PRG-WC) and a multispecies sward (MSS) composed of eight species: three grasses (PRG, *Pbleum pratense*, *Festuca pratensis*), three legumes (WC, *Trifolium pratense*, *Trifolium hybridum*) and two herbs (plantain and chicory). Overall, three farmlets of 18.7 ha were created, each divided into 20 paddocks. Paddocks for each treatment were balanced for location, soil type, and soil fertility throughout the farm. During the trial, the PRG sward received 250 kg of chemical N ha<sup>-1</sup> year<sup>-1</sup> while the PRG-WC and MSS received 125 kg. Total net herbage production, botanical composition and chemical composition were measured over the years 2021 and 2022. These data were analysed for sward, season and year effect using linear mixed models (Proc Mixed; SAS Institute, 2006).

## Results

There was no significant difference in annual herbage yield between the three sward systems during the two year study despite large differences in mineral N application (Table 1). The proportional contribution of grasses to herbage DM of the PRG-WC was greatest in spring (966 g (kg DM)<sup>-1</sup>;  $P < 0.001$ ), least in autumn (765 g (kg DM)<sup>-1</sup>,  $P < 0.0001$ ) and intermediate during summer (865 g (kg DM)<sup>-1</sup>;  $P < 0.001$ ). By association, the proportional contribution of legumes to DM yield increased ( $P < 0.001$ ) from spring (32 g (kg DM)<sup>-1</sup>) to summer and autumn (133 and 234 g (kg DM)<sup>-1</sup>, respectively) (Table 2). The same dynamic was observed between grasses and legumes for the MSS sward (Table 2). In contrast, the seasonal contribution of plantain did not differ by season (134 g (kg DM)<sup>-1</sup>; Table 2). There was no significant effects of sward system on sward nutritive parameters (CP, NDF or ADF contents of 220, 403 and 207 g (kg DM)<sup>-1</sup>, respectively), nor was there any significant year effects, or interactions between sward system and either season or year. The effect of sward system on ash content was greater for MSS (114 g (kg DM)<sup>-1</sup>) compared to both PRG and PRG-WC (97 and 102 g kg<sup>-1</sup> DM, respectively). Relatedly, OMD content approached significance ( $P < 0.10$ ) and tended to be lower for MSS (799 g (kg DM)<sup>-1</sup>) compared to both PRG and PRG-WC (812 and 808 g (kg DM)<sup>-1</sup>, respectively).

## Conclusion

This study confirmed the findings of previous component-based evaluations (Baker *et al.*, 2023) and indicated that the inclusion of legumes and herbs within intensively managed grazing swards can yield similar DM production and nutritive characteristics to traditional PRG swards, while substantially reducing requirements for chemical N fertilisation. Further evaluation of such swards within longer-term research is required to evaluate the persistency of the species and to enhance successful adoption of both PRG-WC and MSS systems.

Table 1. The effect of sward type on annual herbage yield as an average of two years

	PRG	PRG-WC	MSS	SEM	Sward type
Annual herbage yield (t DM ha <sup>-1</sup> )	13.3	12.5	13.2	315.7	NS
Chemical N fertiliser (kg N ha <sup>-1</sup> )	243	128	127	3.9	****

Significance: \* =  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , \*\*\*\*  $P < 0.0001$ . <sup>a-b</sup> within rows, means that did not share the same subscript were significantly different ( $P < 0.05$ ). NS, not significant.

Table 2. The proportional contribution of grasses, legumes, and herbs to dry matter production (%) by sward type and by season as an average of two years

	Spring	Summer	Autumn	SEM	Significance <sup>1</sup>		
					S	YR	S*YR
PRG sward (g (kg DM) <sup>-1</sup> )							
Grasses	0.988	0.996	0.997	0.0035	NS	*	NS
Unsovn species	0.012	0.004	0.003	0.0035	NS	*	NS
PRG-WC sward (g (kg DM) <sup>-1</sup> )							
Grasses	0.966 <sup>c</sup>	0.865 <sup>b</sup>	0.765 <sup>a</sup>	0.0101	****	****	***
Legumes	0.032 <sup>a</sup>	0.133 <sup>b</sup>	0.234 <sup>c</sup>	0.0100	****	****	***
Unsovn species	0.002	0.002	0.001	0.0000	NS	NS	NS
MSS sward (g (kg DM) <sup>-1</sup> )							
Grasses	0.763 <sup>c</sup>	0.669 <sup>b</sup>	0.577 <sup>a</sup>	0.0128	****	NS	**
Legumes	0.048 <sup>a</sup>	0.159 <sup>b</sup>	0.225 <sup>c</sup>	0.0113	****	*	NS
<i>Plantago lanceolata</i>	0.146	0.125	0.131	0.0080	NS	NS	NS
<i>Chicorium intybus</i>	0.038 <sup>a</sup>	0.040 <sup>a</sup>	0.062 <sup>b</sup>	0.0046	***	****	NS
Unsovn species	0.005	0.007	0.005	0.0011	NS	NS	NS

Significance: \* =  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , \*\*\*\*  $P < 0.0001$ . <sup>a-b</sup> within rows, means that did not share the same subscript were significantly different ( $P < 0.05$ ). NS, not significant; S, season; YR, year.

## Acknowledgement

The authors would like to acknowledge the support of the Teagasc Walsh Scholarship scheme and University College Dublin.

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# Milk production from grass-white clover and grass-white clover-plantain swards

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## Abstract

The inclusion of forage herb species, such as plantain (*Plantago lanceolata*), to perennial ryegrass (*Lolium perenne* L.)-white clover (*Trifolium repens*) swards may provide increased animal and herbage production gains to pasture based dairy systems. A grazing experiment comparing herbage and milk production from perennial ryegrass-white clover (WC) or perennial ryegrass-white clover-plantain (PI) swards was conducted at Teagasc Moorepark, Ireland. Both swards established successfully with annual cover content of 20% for the WC sward, while the PI sward had 39% plantain and 10% white clover. Seventeen dairy cows rotationally grazed either WC or PI sward from May to October 2023. No difference in herbage yield or milk production was found between swards. As the study continues into the future, the effect of changing sward composition on herbage and milk production will be quantified.

**Keywords:** plantain, white clover, milk production, herbage yield

## Introduction

Irish dairy production systems are characterised by the high utilisation of grazed pasture, which typically consist of perennial ryegrass and white clover. The addition of companion 'herb' forages to these swards is receiving renewed interest, with previous studies citing increased sward and animal production from animals grazing diverse forages over traditional swards (Grace *et al.*, 2018). A disadvantage of such swards is the known lower persistency of certain herb species (Gilliland, 2022), but Hearn *et al.* (2022) did report the continued contribution of plantain in intensively grazed swards up to 4 years of age. The objective of this study was to find/quantify milk production benefits of plantain inclusion in traditional swards by comparing milk production from WC and PI swards.

## Materials and methods

A dairy farm-systems experiment was conducted at Teagasc Moorepark, Ireland from May to October 2023. The experiment had two treatments: a perennial ryegrass-white clover sward (WC) and a perennial ryegrass-white clover-plantain sward (PI). Both treatments were sown at a total rate of 35 kg seed ha<sup>-1</sup>, the breakdown of which was 30 kg ha<sup>-1</sup> perennial ryegrass and 5 kg ha<sup>-1</sup> white clover for the WC treatment, and 27.5 kg ha<sup>-1</sup> perennial ryegrass, 5 kg ha<sup>-1</sup> white clover and 2.5 kg ha<sup>-1</sup> plantain for the PI treatment. In May, 34 spring calving dairy cows (Friesian and Friesian × Jersey) were selected and balanced on mean calving date (28/2/2023), lactation number (2.5), pre-experimental milk yield and pre-experimental milk solids (MS) yield (26.5 kg milk day<sup>-1</sup> and 2.3 kg MS day<sup>-1</sup>), gathered during the 3 weeks prior to the commencement of the study and randomly allocated to one of the two treatment groups (n=17). Both treatments were stocked at 3 cows per hectare in a closed farmlet system with cows remaining in their treatment group for the remainder the experiment. Cows received a daily herbage allowance of 17 kg DM ha<sup>-1</sup> above 4 cm and an individual concentrate allocation of 1 kg per cow per day. Swards were rotationally grazed, typically on a 21-day rotation when a target pre-grazing herbage yield of 1400 kg DM ha<sup>-1</sup> was reached, as is common grazing practice in Ireland. Similar levels of nitrogen fertiliser were applied to both treatments (194 kg N ha<sup>-1</sup> year<sup>-1</sup>). Prior to each paddock grazing event pre-grazing herbage mass was measured by harvesting two strips from each paddock, using an Etesia Motor harvester. Cumulative herbage production was recorded and calculated using PastureBase, an online grass management tool.

Target post-grazing height was 4 cm. Sward species composition was recorded prior to each grazing using a method described by Egan *et al.* (2018). Milk yield was recorded daily (Dairymaster, Causeway, Co. Kerry, Ireland) and milk composition was measured twice weekly using MilkoScan 203 (Foss Electric, Hillerød, Denmark). Data were analysed in SAS using Proc Mixed with variables treatment, rotation and associated interactions. Fixed terms were treatment and rotation, cow and paddock were random factors.

## Results and discussion

The average annual plantain content of the PI sward was  $39 \pm 2.4\%$  while clover content was  $10 \pm 1.4\%$ . The clover content of the WC sward was significantly higher ( $P < 0.001$ ) than the PI at  $20 \pm 1.4\%$ . Weed content for both swards was similar at  $2 \pm 0.9\%$  and the remainder of both swards consisted of perennial ryegrass. Total pasture production was similar for both treatments at 11 065 and 11 578 kg DM ha<sup>-1</sup> for the WC and PI swards, respectively. The similarity in herbage production is interesting especially when considering the difference in sward species composition. It would be expected that increased nitrogen would have been supplied to the WC sward, given the elevated clover content which reached a threshold of 20% annual clover (Egan *et al.*, 2018) and that this would have increased the herbage yield of the WC sward. Plantain-containing swards have also shown increased herbage yields over white clover swards in studies by Jing *et al.* (2017) who cited the deeper rooting ability of plantain as a factor increasing total resource capture of the sward, thereby increasing sward production. Given both swards are relatively young, further data collection is required to robustly assess herbage production. Reseeded swards are known to mineralise higher levels of nitrogen (Hopkins *et al.*, 1990) and therefore nitrogen in this study may not have been limiting to either sward. Should the lower clover percentage in the PI sward continue in future trial years, nitrogen may become limiting for pasture production.

Both sward types supported the same mean daily milk yield and milk solids yield, and fat and protein fractions also did not differ (Table 1). Cumulative milk solids yield was also similar for both treatments at 307 and 302 kg MS cow<sup>-1</sup>. Herath *et al.* (2023) reported a similar result with no difference in milk production in the first year of a farm systems trial investigating the addition of plantain to perennial ryegrass-white clover swards. Milk yield increases of cows grazing plantain-containing swards have been reported by Box *et al.* (2017) and attributed to increased dry matter intake of grazing animals in late lactation. In a meta-analysis by Nguyen *et al.* (2022), increased milk yield in late lactation from plantain-containing swards was attributed to increased digestibility of the sward. Feed intake and sward nutritive quality measurements were obtained during experimentation but have not been analysed to date.

Table 1. Comparison of milk and herbage production from grass-white clover and grass-white clover-plantain swards

	WC	PI	S.E.	P-value
Milk yield (kg cow <sup>-1</sup> day <sup>-1</sup> )	20.9	21.6	0.50	NS
Milk fat (g kg <sup>-1</sup> )	5.38	5.09	0.141	NS
Milk protein (g kg <sup>-1</sup> )	3.77	3.70	0.066	NS
Milk solids yield (kg cow <sup>-1</sup> day <sup>-1</sup> )	1.90	1.87	0.884	NS
Cumulative milk solids yield (kg cow <sup>-1</sup> )	307	302	14.3	NS
Cumulative herbage production (kg DM ha <sup>-1</sup> )	11 065	11 578	350.5	NS

WC, perennial ryegrass-white clover sward; PI, perennial ryegrass-white clover-plantain sward; NS, not significant.

## Conclusion

No differences in herbage or milk production were found between one-year-old WC and PI swards. Additional years of experimentation will be required to assess changes in sward species contribution over time and to link such changes (if any) with differences in herbage and milk production. Future experimentation will also quantify differences in enteric methane production and nitrogen leaching between swards.

## Acknowledgement

This research was funded by the Irish Dairy Levy administered by Dairy Research Ireland.

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# Methane emissions from spring calving dairy cows grazing perennial ryegrass swards with or without white clover

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## Abstract

In recent years there has been increased interest in white clover inclusion in Irish swards as a means to reduce reliance on chemical nitrogen (N) fertilizer. However, there is a lack of knowledge of the impact of white clover on enteric methane emissions. An experiment was established to compare spring calving dairy cows in a conventional perennial ryegrass system receiving 225 kg N ha<sup>-1</sup> year<sup>-1</sup> (grass) to perennial ryegrass with white clover receiving 150 kg N ha<sup>-1</sup> year<sup>-1</sup> (clover). Methane emissions, dry matter intake (DMI) and milk production data were collected from each treatment. Swards were intensively grazed and treatments were managed identically. Clover inclusion had no effect on milk solids yield although it did increase DMI ( $P < 0.01$ ). Clover had higher daily methane emissions compared to grass ( $P < 0.01$ ) but due to the higher DMI on clover, there was no difference in methane yield (g CH<sub>4</sub> (kg DMI)<sup>-1</sup>).

**Keywords:** enteric emissions, legumes, pasture, milk production

## Introduction

Recent trends in environmental legislation have reduced permitted chemical N fertiliser application in European regions such as Ireland (DAFM, 2022). In order to maintain forage supply for a profitable pasture-based milk production systems, alternative N-sources such as biological N fixation by legumes are important. The inclusion of white clover (*Trifolium repens* L.) in perennial ryegrass (*Lolium perenne* L. (PRG)) can achieve similar herbage production to PRG monocultures while receiving 40% less chemical N fertiliser (Egan *et al.*, 2018; Hennessy *et al.*, 2018). As white clover becomes more prevalent on farms, it is important to consider how this will influence greenhouse gas (GHG) emissions. Reductions in fertilizer inputs will reduce carbon dioxide and nitrous oxide emissions associated with fertiliser production and application (Herron *et al.*, 2021; Yan *et al.*, 2013). White clover may reduce enteric methane emissions as it has reduced fibre compared to PRG (Egan *et al.*, 2018). However, current information on the effect of white clover on enteric methane emissions is inconsistent (Lee *et al.*, 2004, Enriquez-Hidalgo *et al.*, 2014). These studies measured enteric methane from white clover swards for short periods but not over an entire grazing season. The aim of the current experiment was to compare enteric methane emissions of cows grazing PRG swards and PRG with clover at a reduced fertiliser application over a grazing season.

## Materials and methods

An experiment was undertaken in conjunction with a full lactation farm systems study at Teagasc, Moorepark, Fermoy, Co. Cork. The experiment consisted of spring calving Holstein-Friesian cows grazing either PRG receiving 225 kg N ha<sup>-1</sup> year<sup>-1</sup> (grass) or PRG with white clover receiving 150 kg N ha<sup>-1</sup> year<sup>-1</sup> (clover). The cows were blocked in February 2022 on calving date, milk yield, milk solids, parity and bodyweight. Methane measurement was carried out using two Greenfeed units (C-Lock, Rapid City, SD, USA). The cows were trained to use the units in March and had constant access during the experimental period. After the training period there were 18 and 16 frequent Greenfeed visitors in grass and clover, respectively. The experimental period started in April and measurements continued until mid-October. The cows were managed in a rotational grazing system with a target post grazing sward height of 4 cm. One kg of concentrate supplementation was provided from the greenfeeds daily and any additional



supplementation, depending on herbage availability, was provided at milking. The cows were milked twice daily at 07:30 and 15:30 with yield measured daily and composition (fat and protein) measured weekly. Dry matter intake was estimated in early-May, mid-July and late-September using the n-alkane technique as described by Dillon and Stakelum (1989). Clover proportion was measured in each paddock prior to grazing as described by Egan *et al.* (2018). All data were averaged over fortnightly periods to ensure adequate methane measurements. Three periods were removed as silage supplementation was required due to drought. Statistical analysis was undertaken using the mixed procedure in SAS with individual cow as a random effect and treatment, parity, calving day of the year, period and period by treatment interaction as fixed effects. A first-order autoregressive covariance structure was applied with period as a repeated measure.

## Results and discussion

The average (SD) clover proportion weighted on total herbage mass was 24% (15.7) ranging from 5% (1.4) in the first rotation to 44% (16.2) in the ninth rotation. Pre-grazing herbage mass was similar, on average, for both treatments (1433 kg DM ha<sup>-1</sup>). Average concentrate supplementation was 2.2 (1.24) kg day<sup>-1</sup> and average daily visits to the greenfeed was 2.2 (0.76). Similar milk yield and milk solids yield were found in both treatments (Table 1) but clover had higher DMI ( $P < 0.01$ ). Clover had higher methane production compared to grass ( $P < 0.01$ ) but methane intensity and methane yield (methane expressed per kg of milk solids and DMI, respectively) were not different ( $P > 0.05$ ). There was an interaction between treatment and period for all animal measurements ( $P < 0.01$ ).

Increased methane from clover swards is likely due to higher DMI of clover as reported by Egan *et al.* (2018). The interaction between treatment and period may be due to fluctuations in clover content across periods, altering the chemical composition of the diet. Based on these results, some of the GHG reductions associated with clover will be negated by an increase in enteric methane. However, clover did not reduce the carbon efficiency of a milk production system in terms of milk solids production. It is also important to consider that fossil fuel emissions are mitigated through reduced fertiliser production associated with the clover system.

Table 1. Effect of sward type on least square means for milk production and methane emissions from cows grazing perennial ryegrass swards receiving 225 kg N ha<sup>-1</sup> (grass) or perennial ryegrass with white clover receiving 150 kg N ha<sup>-1</sup> (clover)

	Sward		SED	P-value
	Grass	Clover		Sward
Milk yield (kg)	20.1	20.6	0.90	NS
Fat (g kg <sup>-1</sup> )	46.9	48.5	1.79	NS
Protein (g kg <sup>-1</sup> )	37.5	38.0	0.83	NS
Milk solids (kg)	1.67	1.74	0.062	NS
DMI	16.4	17.7	0.37	<0.01
Daily CH <sub>4</sub> emissions (g)	311	342	10.3	<0.01
CH <sub>4</sub> intensity (g (kg MS) <sup>-1</sup> )	192	201	6.4	NS
CH <sub>4</sub> yield (g (kg DMI) <sup>-1</sup> )	19.0	19.6	0.68	NS

SED, standard error of the difference of least squares means; DMI, dry matter intake; MS, milk solids; NS, not significant.

## Conclusion

In this study we found that cows grazing white clover swards produced more enteric methane than cows grazing PRG but maintained productivity at a reduced N fertiliser application rate. This highlights the need for balanced consideration when implementing environmental practices on farms.

## Acknowledgement

This research was funded by Science Foundation Ireland under the grant 16/RC/3835 (VistaMilk) and the Irish Dairy Levy administered by Dairy Research Ireland.

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# Milk production and methane emissions of cows fed either grass or red clover-grass silage

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## Abstract

We compared grass silage and red clover-grass silage diets to test if partial substitution of red clover (*Trifolium pratense*) for grass (*Phleum pratense*, *Festuca pratensis*) in the diet increases dry matter intake (DMI) and milk yield but not ruminal CH<sub>4</sub> emissions owing to red clover's lower fibre concentration. The trial was arranged according to switch-back design with three four-week periods. The treatments were grass silage diet and red clover-grass silage diet (50% red clover in silage dry matter). Diets were partial mixed rations fed *ad libitum* together with additional concentrate from the milking robot aiming at proportion of 40% concentrate in the diet dry matter. Methane emissions were measured with GreenFeed system. Rumen fermentation was measured with five rumen-cannulated cows. Inclusion of red clover in the diet increased DMI and milk yield but decreased milk fat and protein concentrations, and increased CH<sub>4</sub> emissions (g day<sup>-1</sup>) and intensity (g (kg DMI)<sup>-1</sup> or g (kg energy corrected milk yield)<sup>-1</sup>) and the molar proportion of acetic acid in the rumen volatile fatty acids. Contrary to our hypothesis, CH<sub>4</sub> emissions were increased on red clover-grass diet compared to grass diet.

**Keywords:** grass silage, red clover, methane, dairy cow

## Introduction

Red clover (*Trifolium pratense*) silage is a suitable and established alternative to grass silage, especially because of red clover's ability to fix atmospheric N<sub>2</sub>. A possible additional benefit could be decreased ruminal CH<sub>4</sub> emissions as red clover has less fibre than grass, but few *in vivo* feeding trials have investigated CH<sub>4</sub> emissions of red clover-containing diets (Vanhatalo and Halmemies-Beauchet-Filleau, 2020). Based on our earlier results (Pitkänen *et al.*, 2023), we hypothesised that partial substitution of red clover silage for grass silage would not affect CH<sub>4</sub> emission per dry unit of matter intake (DMI).

## Materials and methods

The experiment was conducted at the Helsinki University Viikki Research Farm in Finland. The experiment was a change-over design with three 28-day periods. Data for the last 7 days of each period were used in statistical analyses. Treatments were: 1<sup>st</sup> cut grass silage-based diet (GS) in periods 1 and 3 and the same diet with half of silage dry matter replaced with 1<sup>st</sup> cut red clover silage (CGS) in the 2<sup>nd</sup> period. Grass swards were fertilized with 108 kg N ha<sup>-1</sup> and 100 kg K ha<sup>-1</sup>. The red clover sward was fertilized with 50 kg K ha<sup>-1</sup>. Grass silage had more energy (10.7 vs. 9.6 MJ ME (kg dry matter)<sup>-1</sup>), crude protein (177 vs. 136 g (kg dry matter)<sup>-1</sup> dry matter), and neutral detergent fibre (NDF; 523 vs. 445 g kg<sup>-1</sup> dry matter) than red clover silage. Feed was given *ad libitum* as partial mixed ration with concentrate proportion of 27% in dry matter. In addition, cows got concentrates (5.5 kg day<sup>-1</sup> for multiparous and 4.5 kg day<sup>-1</sup> for primiparous) from the milking robot. The concentrates contained oats, barley, rapeseed meal, and minerals. Gas exchange was measured with GreenFeed system and feeds were analysed as described in Halmemies-Beauchet-Filleau *et al.* (2023). Data from cows with at least 10 acceptable CH<sub>4</sub> measurements in sampling week were used (21 cows for the 1<sup>st</sup> and 3<sup>rd</sup> period, and 23 for the 2<sup>nd</sup> period). Five cows with ruminal cannulae were used for collection of ruminal fermentation data during one day at 9:00, 12:00 and 15:00 h. Samples were treated with H<sub>2</sub>SO<sub>4</sub> and analysed for ammonium-N and volatile fatty acids (Lamminen *et al.*, 2017). Data were analysed by ANOVA (SAS 9.4) with period as fixed and

cow as a random variable. Rumen data were analysed as repeated measurements with period, sampling time, and interaction as fixed variables and cow as a random variable. The contrasts were (1) effect of time (1<sup>st</sup> vs. 3<sup>rd</sup> period) and (2) effect of red clover (2<sup>nd</sup> vs. 1<sup>st</sup> and 3<sup>rd</sup> periods).

## Results and discussion

Red clover-grass increased ( $P=0.02$ ; Table 1) DMI and milk yield ( $P=0.01$ ) but did not affect yields of fat or energy corrected milk due to decrease in milk fat concentration ( $P<0.01$ ). In contrast, protein yield was increased by CGS ( $P=0.04$ ) despite minor decrease in protein concentration ( $P=0.05$ ). This increase in DMI and milk yield but decrease in milk fat and, to lesser extent, in milk protein concentrations is typical to red clover (Dewhurst, 2013). Contrary to our hypothesis, both the daily ruminal CH<sub>4</sub> production ( $P<0.01$ ) of cows and CH<sub>4</sub> production per DMI (20.8 vs. 19.3 g kg<sup>-1</sup>;  $P<0.01$ ) were greater in CGS than in GS. This was unexpected as, in previous experiments, red clover has been associated with lower or equal CH<sub>4</sub> production potential in comparison to grass silage (Dewhurst, 2013; Vanhatalo and Halmemies-Beauchet-Filleau, 2020). However, greater ( $P<0.01$ ) H<sub>2</sub> emissions with CGS than in GS were in line with previous experimentation where red clover-containing diet did not increase CH<sub>4</sub> emissions despite increased DMI (Pitkänen *et al.*, 2023). Although red clover silage had less NDF than grass silage, which is typical (Vanhatalo and Halmemies-Beauchet-Filleau, 2020), the difference was rather small. On the other hand, lower crude protein concentration in red clover silage than in grass silage is atypical (Dewhurst, 2013) and in contrast to our previous experiment (Pitkänen *et al.*, 2023). Based on NDF and crude protein concentration grass silage was harvested in early and red clover in late growth stage (Luke, 2023). Less leafy and more mature silage has led to increased ruminal CH<sub>4</sub> emissions (Vanhatalo and Halmemies-Beauchet-Filleau, 2020).

Milk urea concentration was lower ( $P=0.01$ ; Table 1) with CGS than with GS due to difference in crude protein intake. In line with this, rumen ammonium-N concentration was numerically lower in CGS (6.25 mmol l<sup>-1</sup>) than in GS (7.87 mmol l<sup>-1</sup>), although the difference was not significant ( $P=0.23$ ). Of other rumen parameters, molar proportion of acetic acid was greater (+19 mmol mol<sup>-1</sup>,  $P=0.02$ ) in CGS than in GS but differences in other major volatile fatty acids were not significant. Greater acetic acid proportion in rumen fermentation is associated both with more mature silage (Vanhatalo *et al.*, 2009) and with increased CH<sub>4</sub> emissions (Vanhatalo and Halmemies-Beauchet-Filleau, 2020). Thus, ruminal data supports the finding that silage maturity may explain increased CH<sub>4</sub> emissions with CGS in comparison to GS.

## Conclusions

Partial replacement of grass silage with red clover silage increased DMI and milk yield but decreased milk fat and protein concentrations, as expected. However, ruminal CH<sub>4</sub> emissions were also increased with red clover inclusion in contrast to previous results. That implies that red clover maturity and composition affects ruminal CH<sub>4</sub> emissions, and comparisons of silages cultivated and harvested in different conditions are therefore needed.

## Acknowledgement

The experiment was a part of Leg4Life project funded by the Strategic Research Council (SRC) established within the Academy of Finland.

Table 1. Effect of replacing 50% of grass silage with red clover silage on dairy cow performance and gas exchange.

	Period 1 (GS; grass)	Period 2 (CGS; red clover-grass)	Period 3 (GS; grass)	SEM	P-value	
					1 <sup>st</sup> vs. 3 <sup>rd</sup>	2 <sup>nd</sup> vs. 1 <sup>st</sup> and 3 <sup>rd</sup>
n	21	23	21			
DMI (kg day <sup>-1</sup> )	23.8	24.1	23.5	0.444	0.18	0.02
Yield (kg day <sup>-1</sup> )						
Milk	35.6	35.2	32.2	1.28	<0.01	0.01
Energy corrected milk	40.6	39.5	37.5	1.25	<0.01	0.31
Concentration (g kg <sup>-1</sup> )						
Protein	39.1	38.9	39.7	0.61	0.04	0.05
Fat	49.0	47.3	50.7	1.06	0.01	<0.01
Lactose	45.8	46.5	45.7	0.25	0.7.0	<0.01
Urea (mg dl <sup>-1</sup> )	26.6	21.6	28.2	0.78	0.03	<0.01
Gas exchange (g day <sup>-1</sup> )						
CH <sub>4</sub>	461	500	450	13.5	0.23	<0.01
CO <sub>2</sub>	12 400	12 300	11 900	280	0.01	0.21
O <sub>2</sub>	8 870	8 940	8 360	219	<0.01	<0.01
H <sub>2</sub>	1.12	1.21	0.880	0.0735	0.01	0.01

SEM, standard error of mean.

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# Adaptive multi-paddock grazing increases soil organic carbon stocks in temperate Canadian pastures

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## Abstract

Adaptive multi-paddock (AMP) grazing has been promoted as a 'climate-smart' practice due to its potential to mitigate greenhouse gas emissions by increasing soil organic carbon (SOC) stocks, although contrasting results have been observed. The goal of this study was to compare SOC stocks between neighbouring AMP and non-AMP beef farms in southern Ontario while simultaneously evaluating the stability and origin of potential SOC stock differences. Higher SOC and total nitrogen stocks were found in AMP, along with higher mineral-associated carbon stocks in the top 15 cm under AMP, indicating greater SOC stability under AMP. Abundances of soil microbial classes (e.g., bacteria, fungi) measured via phospholipid fatty acid analysis were significantly higher in AMP than non-AMP with no change in community structure or community ratios. These results highlight AMP grazing as an effective management strategy to increase SOC stocks and stability in temperate grasslands.

**Keywords:** soil carbon stocks, grazing management, adaptive multi-paddock grazing, rotational grazing, organic matter quality

## Introduction

Greenhouse gas (GHG) emissions from beef cattle comprise 41% of livestock-sector emissions and 14.5% of anthropogenic emissions globally, making beef production the largest contributor to the livestock sector's GHG emissions in terms of percent of total emissions and in emissions per kg of protein (Gerber *et al.*, 2013). However, a significant portion of the lifespan of beef cattle is often spent grazing agricultural grasslands; thus, there is a need to consider the impacts of grazing cattle on soil carbon sequestration to better account for net GHG emissions. In particular, adaptive multi-paddock (AMP) grazing, a specific type of rotational grazing which is dependent on moving the cattle according to forage growth stage and allowing adequate time for plant recovery between grazing cycles, can increase soil C sequestration compared to continuous grazing (Stanley *et al.*, 2018; Teague *et al.*, 2013). However, results may vary based on climate and soil type (McSherry and Ritchie, 2013). The objective of this study is to provide a regionally-specific SOC stock comparison between AMP grazing and continuous grazing in southern Ontario, as well as to contrast the stability of the SOC, thus improving our understanding of soil C cycling in grazed grasslands.

## Materials and methods

Sites for the study were selected after an initial screening of over 25 beef operations in southern Ontario identified as practicing AMP grazing. Farms were evaluated based on size and number of paddocks, stocking rate, ratio of rest days to graze days, soil amendment application, supplemental feeding, year of pasture establishment, and years of AMP practice. Sites with management closely matching AMP principles consistently for at least 10 years were selected for follow-up in-person site visits and to determine nearby non-AMP operations for possible comparison. Non-AMP pastures were considered suitable for comparison when they were on similar soil types as the AMP field site, had comparable slope patterns, botanical composition, and stocking rates to their AMP comparison, were within a close geographic area (<15 km), and were managed as a considerable feed source for livestock. A total of five pairs of AMP and non-AMP farms were selected with clear differences in grazing management

characteristics. In our study, AMP farms on average kept cattle in one paddock  $\leq 2$  days and maintained a rest to graze days ratio of  $> 17$ . The non-AMP farms ranged in management with some farms never rotating cattle, while other non-AMP farms rotated as frequently as every 9 days. The rest to graze days ratio for non-AMP farms averaged 1.6.

Soil sampling was completed at all sites between July and September 2021. Deep cores were collected to a depth of 60 cm or as deep as possible with a modified post pounder. Ultimately, only the top 45 cm of soil from the cores were included in the analysis due to difficulty in reaching 60 cm consistently at all sites. Soil cores were divided into 15 cm segments and were subsequently air-dried and sieved to 2 mm. Soil organic carbon (SOC) and total nitrogen were determined by dry combustion. Dried and sieved soil from the 0–15 and 15–30 cm depths was separated into particulate and mineral associated organic matter (POM and MAOM, respectively) fractions following the methods of Diochon *et al.* (2016). Surface (0–15 cm) soil samples, taken separately from the deep cores, were frozen within 24 h of sampling and shipped to Ward Laboratories (Kearney, NE, USA) where phospholipid fatty acids (PLFAs) were measured via gas chromatography according to Buyer and Sasser (2012). All statistical tests were conducted in SAS version 9.4 (SAS Institute, Cary, NC, USA) using Proc GLIMMIX (a generalized linear mixed model) with grazing management as the main fixed effect, geographic area as a random effect, and depth as a repeated measure, where applicable.

## Results and discussion

Higher SOC and total nitrogen stocks were found under AMP grazing compared to non-AMP grazing when measured based on an equivalent soil mass ( $P=0.028$  and  $0.024$ , respectively). When analysed by depth, there was a significant interaction of grazing management and depth in SOC stocks, with SOC stocks being higher in AMP than non-AMP pastures in the 0–15 cm depth, but no differences were detected in deeper soil layers (Figure 1a). When SOM fractions were calculated on a stock basis to fixed depth in segments 0–15 cm and 15–30 cm, differences in the mineral associated organic matter fraction were detected. There was a significant interaction of land use and depth for mineral associated organic matter carbon (MAOM-C) stocks with AMP having greater MAOM-C in the surface (0–15 cm) soil depth (Figure 1b), indicating greater SOC stability under AMP grazing. This is consistent with the results of Mosier *et al.* (2021), who also found greater MAOM-C under AMP grazing. Abundances of microbial phospholipid fatty acids (PLFA) classes were significantly higher in AMP soil samples for each microbial class (bacteria, Gram+ and Gram– bacteria, actinobacteria, fungi, arbuscular mycorrhizal fungi, and saprophytic fungi) ( $P<0.05$ ). However, there was no change in community structure or ratios among microbial class ratios.

## Conclusion

This research supports that, in southern Ontario temperate pastures, AMP grazing should be encouraged over continuous grazing to increase SOC stocks. Moreover, through AMP grazing, MAOM-C was also increased, which is linked with an increased microbial contribution to and stabilization of SOC. It is probable that AMP grazing contributes to maintaining a healthy root biomass because overgrazing is avoided and adequate rest periods for forage recovery and provided. Greater root biomass and root exudates would feed the soil microbial population, which is supported by observed greater PLFA abundances under AMP. This project provides critical scientific evidence enabling government policies and programmes relating to grazing management and carbon sequestration to be put forward with greater confidence and impact.

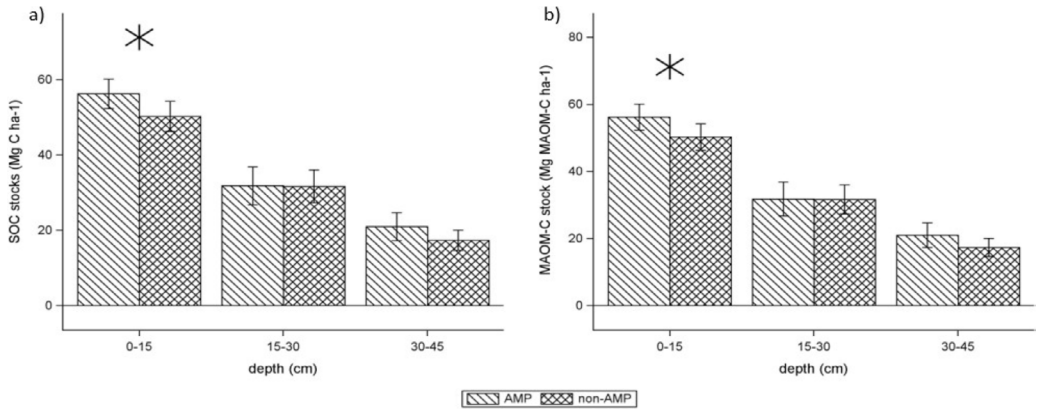


Figure 1. Soil samples from adaptive multi-paddock (AMP) grazing and non-AMP grazing sites in southern Ontario showing (a) mean soil organic carbon (SOC) stocks for soil cores segmented by depth and (b) mineral-associated organic matter (MAOM) carbon stocks by depth. Error bars show the 95% confidence limits ( $n=25$ ), and an asterisk (\*) within a depth represents significant differences between AMP and non-AMP at  $P<0.05$ .

## Acknowledgements

The landowners are thanked for their participation in this research. Funding was provided by the Canadian Cattlemen's Association, Beef Farmers of Ontario, and the National Science and Engineering Research Council (NSERC) Discovery Program.

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# On-farm indicators for surplus-value assessment on alpine mountain farms

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## Abstract

Mountain farming in the Alps provides a multitude of functions, such as ecosystem services (ES) and other surplus-values. The costs of food production (supply services) of mountain farms are remunerated through the market. However, all other ES (regulating, cultural services) or societal values provided by mountain farms are not financially valued by the market and are only partly compensated by public funding. To tackle this market failure, the European Innovation Project (EIP-Agri) ‘Mehrwert Berglandwirtschaft’ (surplus value of mountain farming) was initiated in 2022 with the aim to develop an indicator-based business model that allows private companies to invest in the surplus-value provided by mountain farms in the Austrian national park ‘Kalkalpen’. Two sets of surplus-value indicators were developed within the project: fifty on-farm indicators using Farmlife (FL) in cooperation with twenty-nine sample farms and thirteen positive holistic farm indicators (PH). After qualitative comparison of both sets of indicators the chosen on-farm indicators were found suitable for the description of surplus values and ES. Their database is comparable, but they need to be weighted according to farm situation. Comparison and economic valuation is sensitive.

**Keywords:** ecosystem services, grassland, indicators, mountain farming, surplus value

## Introduction

Mountain farms are farms in ‘less-favoured areas’ (EG No. 1305/2013). Of the total agricultural surface area of Austria (1 294 000 ha) around 49% of the farms are in mountain areas, which is 70% of Austria’s total land surface (Grüner Bericht, 2021). Alpine mountain farming provides multifunctional services (Sinabell, 2003), therefore providing surplus value for society in the form of public goods (Hovorka *et al.*, 2019). In this research, indicators were developed to identify and measure the surplus value of mountain farms on farm level. The indicators were partly built upon the concept of ecosystem services (ES). According to the CICES classification ES are divided into three groups: (I) provisioning services, like food production, (II) regulating services like soil conservation and (III) cultural services, such as recreation (CICES, 2023). The final ES on a regional level (Schwaiger *et al.*, 2011; Staub *et al.*, 2011) set the basis for part of the on-farm indicators. Diverging from Staub *et al.* (2011), who outlined the creation of indicators for final ES, our approach focuses on developing indicators to quantify the societal surplus value of mountain farming. The new approach here is to develop an additional instrument of evaluation on the farm level, which focuses on surplus-values that are not yet fully remunerated. Two sets of surplus-value indicators were developed within the project: Fifty on-farm indicators using Farmlife (FL) by AREC Raumberg-Gumpenstein in cooperation with 29 FL-sample farms and 13 positive holistic (PH) farm indicators by eandp Umweltbüro GmbH. The purpose of this contribution is to present the development of both indicator sets, and to compare them qualitatively in order to make a selection of indicators which have a comparable data basis and are practical to use.

## Data and methods

The study region is the Austrian national park Nationalpark Kalkalpen (NKA) in the region of Upper Austria, with twenty-two municipalities, where 50% of farms have between 0.5–1.5 Livestock Units

(LU) ha<sup>-1</sup> and range with 80–190 difficulty points (in the middle range of the Austrian compensation scheme for mountain farming). Two types of indicators were developed (Figure 1):

(1) Farmlife (FL) indicators: Based on a literature review relations between agricultural practices and ES and other values were described and evaluated with existing INVEKOS (Information System for Agricultural Areas and Environmental Measures in Austria) and on-farm assessed data. On-farm management data were collected with the help of the ‘Farmlife’ (FL) tool for life-cycle assessment (Herndl *et al.*, 2015) for the year 2022. Within FL according to the SALCA (Swiss Agricultural Life Cycle Assessment) model the efficiency of the farms in food production and resource use is calculated. Additional data for the four surplus-value groups of Supply, Regulating, Cultural and Biodiversity (BD) values were assessed via farm-visits and used for the calculation of BD indicators (FL BD) (Fritz, 2022) and newly developed FL ES indicators.

(2) Positive Holistic (PH) indicators: A new approach was explored to describe provisioning, ecological, cultural and holistic surplus values of mountain farming based on existing farming data which are routinely recorded in the INVEKOS database system. The PH-Indicators are based on farming practices known to correlate positively with a variety of ecosystem services defined by CICES (CICES 2023) as well as holistic values such as the option value, legacy value, altruistic value, existence value similar to the model of TEEB (2015) and the biodiversity.

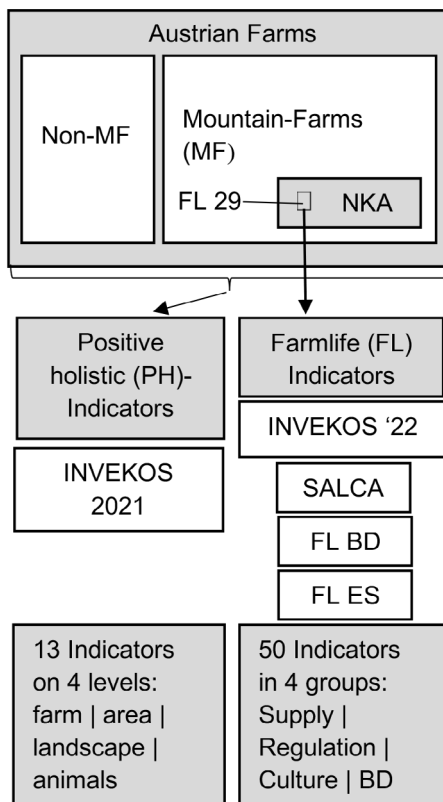


Figure 1. Overview of database, methods and results of the research.

The 29 FL-sample farms were compared to the 1297 NKA-mountain farms sample, based on INVEKOS data of farms that lie within the twenty-two municipalities of NKA. They are compared to 70 709 mountain farms (MF) and 34 425 non-mountain farms (non-MF) in the Austrian INVEKOS Database. For evaluation we use three indicators in this contribution, which have a similar data background and definition. For the indicators ‘extensive grasslands’ and ‘pasture’ the recorded patch areas of each farm according to INVEKOS categories were added and expressed as percentage of the total farm area. In Table 1 the median of the respective basic population is shown. ‘Rare livestock breeds’ are counted with a minimum of two animals per breed and farm, and the share of breeds per farm is shown. In INVEKOS not all breeds are registered.

Table 1. Comparison of Median-values obtained via FL indicators (values for FL-sample) and PH indicators (values in parentheses for FL sample, NKA (National Park Kalkalpen), MF (mountain farms) and Non-MF (non-mountain farms)).

Indicator-group	Indicator	Unit	FL sample (n=29; 2022 (2021))	NKA (n=1297; 2021)	MF (n=70 709; 2021)	Non-MF (n=34 425; 2021)
	Farm size	ha	24.7 (24.6)	15.7	11.3	19.1
	Livestock units (LU)	LU ha <sup>-1</sup>	1.2 (1.1)	1.0	0.9	0.0
Biodiversity (bd) value	Rare livestock breeds	%	24 (10.4)	4.32	5.32	0.54
Culture–landscape value	Extensive grassland	%	7.5 (7.8)	10.9	18.1	0.0
	Pasture	% of farm size	21.6 (23.2)	18	1	0
	Share of pasture	% of total basic feed ration	24	n.a.	n.a.	n.a.

Share of pasture was not available (n.a.) in the INVEKOS database.

## Results and discussion

Two sets of indicators (Table 1) for the FL-sample farms produce comparable values (PH indicator results in parentheses). Differences can be partly explained by the fact that data from different years were used. Values for ‘pasture’ are slightly lower in 2022 FL-results. Numbers in ‘rare livestock breeds’ changed in the two consecutive years. Also, data were partly obtained from different sources. FL indicators based the rare breeds analysis on on-farm-surveys and included races not listed in INVEKOS. Share of pasture is only available for FL-sample farms. Extensive grasslands are directly comparable and pasture differs only in the method of comparison, whereas the share of basic feed matches INVEKOS data.

## Conclusion

The results show, that indicators presented in this contribution are suitable for use on the farm level as well as for regional comparison. The interpretation of datasets for single farms depends as much on the available data as on the situation of the respective year, in which the data are obtained. Moreover the description of surplus-values bears the danger of comparison of farms that have different production conditions. The indicators need to be improved by weighting them according to the farm situation. The research allows a better understanding of the complex relationships between the diverse aspects of farming and their effects on ES and further surplus-values. The selection of indicators for economic valuation has to be sensitive.

## Acknowledgement

We thank all 29 farm-families, for their insights, the project–team and all experts, for advice.

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# ***In vitro* evaluation of grassland adaptation strategies: mitigating potential of herbs and legumes**

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## **Abstract**

Livestock farmers must adapt their grassland management to current and future climate changes. Evaluation of adaptation strategies should not only address climate resilience but also need to consider the impact on enteric methane production (MP). Therefore, five legume species (red clover (*Trifolium pratense*), white clover (*Trifolium repens*), birdsfoot trefoil (*Lotus corniculatus*), sainfoin (*Onobrychis viciifolia*) and crown vetch (*Coronilla varia*) and two grassland herb species (chicory (*Cichorium intybus*) and plantain (*Plantago lanceolata*)) were assessed for their impact on MP in an *in vitro* experiment. Each species was represented by three varieties, except for chicory (two varieties) and crown vetch (one variety). Perennial ryegrass was included as control species, resulting in 19 objects harvested three times over one growing season. Plant material was incubated for 24 hours and MP and production of volatile fatty acids (VFA) were measured. Overall, MP was influenced by herb, harvest moment and the interaction between both, but cultivar had no effect. Compared to perennial ryegrass, absolute MP ( $\mu\text{mol flask}^{-1}$ ) of crown vetch was 31% lower, while relative MP was 33% increased in the first harvest period. In the third harvest period, birdsfoot trefoil reduced (–37%) relative MP and white clover reduced absolute MP (–34%) compared to perennial ryegrass. Some of the tested species showed potential as methane mitigating feed ingredient; however, *in vivo* experiments are needed to explore their potential further.

**Keywords:** enteric methane, mitigation, adaptation, grassland, herbs, legumes

## **Introduction**

Climate change, driven by anthropogenic greenhouse gas emissions, is a global concern. Within the agricultural sector, cattle have been identified as important contributors. In addition to mitigating enteric MP, livestock farmers need to adapt their grassland management to current and future climate changes. Prolonged drought periods have been shown to have detrimental effects on grassland production (Hahn *et al.*, 2021) such as droughts, is assumed to increase and lead to alterations in ecosystem productivity and thus the terrestrial carbon cycle. Although grasslands typically show reduced productivity in response to drought, the effects of drought on grassland productivity have been shown to vary strongly. Here we tested, in a 2-year field experiment, if the resistance and the recovery of grasses to drought varies throughout a growing season and if the timing of the drought influences drought-induced reductions in annual aboveground net primary production (ANPP). To counteract these production losses, there is a growing interest in implementing more drought-tolerant forage species (e.g. legumes and grassland herbs). However, incorporating these plant species into cattle diets may also have implications for enteric MP. Legumes and grassland herbs not only exhibit drought tolerance but are also known for their high nutritive value. Additionally, these plants can contain plant bioactive compounds, such as tannins (Hamacher *et al.*, 2021), which have the potential to reduce enteric MP (Ku-Vera *et al.*, 2020) it is involved in health, nutrient utilization, detoxification, and methane emissions. Methane is a greenhouse gas which is eructated in large volumes by ruminants grazing extensive grasslands in the tropical regions of the world. Enteric methane is the largest contributor to the emissions of greenhouse gases originating from animal agriculture. A large variety of plants containing secondary metabolites [essential oils (terpenoids). The composition and concentration of these bioactive compounds can be influenced by abiotic stress factors, such as drought and may vary over the growing season (Prinsloo and Nogemane,

2018). Moreover, different plant species and cultivars may exhibit unique responses in their primary and secondary metabolism to abiotic stress (Prinsloo and Nogemane, 2018). Hence, this study aims to evaluate the impact of various legumes and grassland herbs on enteric MP and to assess variations resulting from different harvest moments within a single growing season, as well as differences among various cultivars.

## Materials and methods

An *in vitro* batch incubation experiment was conducted at the laboratory for Animal Nutrition and Animal Product Quality (LANUPRO, Ghent University) to assess the methane reducing potential of five legume species (red clover, white clover, birdsfoot trefoil, sainfoin, and crown vetch) and two grassland herbs (chicory and plantain). Each species was represented by three cultivars, except for chicory (two cultivars) and crown vetch (one cultivar). Perennial ryegrass was included as control species. All plants were sown in February 2022 and planted in April 2022 in the field. Plant material was harvested on predefined moments in July, September, and October with at least 6 weeks of regrowth at a height of 7 cm, using an electric hedge trimmer. The harvested material was freeze dried before using it as a substrate. For this *in vitro* experiment, 250 mg with 24 mL of a mixture of rumen fluid and phosphate-bicarbonate buffer at a ratio of 20:80 was incubated over a 24-hour period. After incubation, gas composition ( $\mu\text{mol flask}^{-1}$ ) was measured and a 1 ml sample was taken for analysis of volatile fatty acid (VFA) production and composition ( $\mu\text{mol flask}^{-1}$ , not reported). The incubation was replicated in 3 runs, using different rumen fluids each run. All parameters were analysed using a Linear Mixed-Effects model, with herb, harvest moment, the interaction between herb and harvest moment, and the cultivar as fixed factors and run as a random factor. Pairwise comparison was made using Tukey corrected post-hoc tests. To compare the results with the control species, a Dunnett's post hoc test was used.

## Results and discussion

Table 1 represents the absolute MP, VFA production and relative MP from the different herbs at 3 harvest moments. Perennial ryegrass was harvested only in the first and third moment. Extreme drought limited crown vetch availability in the third harvest, and chicory samples from the third harvest were removed due to issues with freeze-drying. Herb species had a significant influence on absolute MP ( $P < 0.01$ ), total VFA production ( $P < 0.001$ ), and relative MP ( $P < 0.001$ ). Harvest moment had a significant effect on absolute MP ( $P < 0.05$ ), and variations in both absolute MP and VFA production were attributed to different herbs, influenced by the interaction between harvest moment and herb ( $P < 0.01$  and  $P < 0.001$ ). Crown vetch showed a low MP in the first and second harvest moment (203 and 142  $\mu\text{mol flask}^{-1}$ ) along with a low relative MP (0.166 and 0.115  $\mu\text{mol CH}_4$  ( $\mu\text{mol VFA}$ ) $^{-1}$ ). When compared to perennial ryegrass in the first harvest, crown vetch showed a 31% lower absolute MP, but the relative MP did not differ. Compared to perennial ryegrass, red clover, white clover, birdsfoot trefoil and sainfoin had lower absolute MP in the third harvest moment.

The reduction in absolute MP disappears when it is expressed as relative MP. In a study where crown vetch was compared with alfalfa, a legume which is very low in condensed tannins, showed a reduction of 25% in absolute MP (Roca-Fernández *et al.*, 2020). Lotus species are known to reduce enteric MP and an overall reduction in 38% is reported. This is mainly caused by the presence of condensed tannins, which are responsible for a lower VFA production due to impaired ruminal fermentation (Badgery *et al.*, 2023) 71% of agricultural greenhouse gas (GHG). The MP of white clover varied the most over the harvest season, with the highest MP in the first harvest moment (299  $\mu\text{mol flask}^{-1}$ ) but one of the lowest in the third harvest moment (211  $\mu\text{mol flask}^{-1}$ ). During the third harvest period, white clover showed significantly reduced relative MP with 34%, compared to perennial ryegrass. Condensed tannins present in white clover are mainly present in flowers and seeds, and are rather low in the leaves (Roldan *et al.*, 2022). Flowers probably represented a higher proportion of the white clover substrate harvested on the

Table 1. Least square mean values for absolute MP ( $\mu\text{mol flask}^{-1}$ ), total VFA production ( $\mu\text{mol flask}^{-1}$ ) and absolute  $\text{CH}_4$  to total VFA production of the different legumes and grassland herbs and over different harvest moments.

	LP	TP	TR	LC	OV	CV	CI	PL
Absolute $\text{CH}_4$ ( $\mu\text{mol CH}_4 \text{ flask}^{-1}$ )								
Harvest moment 1	293 <sup>ab</sup>	315 <sup>b</sup>	299 <sup>b</sup>	232 <sup>a</sup>	275 <sup>ab</sup>	203 <sup>a*</sup>	287 <sup>ab</sup>	282 <sup>ab</sup>
Harvest moment 2	na	277 <sup>b</sup>	273 <sup>b</sup>	245 <sup>b</sup>	234 <sup>ab</sup>	142 <sup>a</sup>	271 <sup>b</sup>	252 <sup>b</sup>
Harvest moment 3	371 <sup>c</sup>	272 <sup>ab*</sup>	211 <sup>a*</sup>	232 <sup>a*</sup>	262 <sup>ab*</sup>	na	na	309 <sup>bc</sup>
Total VFA ( $\mu\text{mol VFA flask}^{-1}$ )								
Harvest moment 1	1374 <sup>b</sup>	1248 <sup>b</sup>	1253 <sup>b</sup>	1203 <sup>b*</sup>	1148 <sup>b*</sup>	1198 <sup>b</sup>	1204 <sup>b*</sup>	879 <sup>a*</sup>
Harvest moment 2	na	1201 <sup>c</sup>	1236 <sup>c</sup>	1240 <sup>c</sup>	1019 <sup>ab</sup>	1184 <sup>abc</sup>	1156 <sup>bc</sup>	945 <sup>a</sup>
Harvest moment 3	1544 <sup>c</sup>	1215 <sup>ab*</sup>	1349 <sup>bc*</sup>	1277 <sup>ab*</sup>	1163 <sup>ab*</sup>	na	na	1178 <sup>a*</sup>
Relative $\text{CH}_4$ ( $\text{CH}_4/\text{Total VFA}$ )								
Harvest moment 1	0.214 <sup>a</sup>	0.251 <sup>a</sup>	0.240 <sup>a</sup>	0.194 <sup>a</sup>	0.238 <sup>a</sup>	0.166 <sup>a</sup>	0.236 <sup>a</sup>	0.315 <sup>b*</sup>
Harvest moment 2	na	0.229 <sup>bc</sup>	0.222 <sup>bc</sup>	0.204 <sup>b</sup>	0.226 <sup>bc</sup>	0.115 <sup>a</sup>	0.232 <sup>bc</sup>	0.273 <sup>c</sup>
Harvest moment 3	0.236 <sup>abc</sup>	0.221 <sup>bc</sup>	0.156 <sup>a*</sup>	0.182 <sup>ab</sup>	0.228 <sup>abc</sup>	na	na	0.260 <sup>c</sup>

LP, perennial ryegrass; TP, red clover; TR, white clover; LC, birdsfoot trefoil; OV, sainfoin; CV, crown vetch; CI, chicory, PL, plantain; na, values not available. Least square means denoted by a different letter indicate significant differences ( $p < 0.05$ , Tukey corrected post-hoc test).

\*Least square means denoted are significantly different from the control species, LP ( $p < 0.05$ , Dunnett's post-hoc test).

third moment resulting in a higher reduction in MP. Plantain resulted in the highest relative MP (0.315, 0.273 and 0.260  $\mu\text{mol flask}^{-1}$ ) and was 33% higher when compared to perennial ryegrass. Cultivar had a significant effect ( $P < 0.05$ ) on total VFA production but had no effect on the other measured parameters. Our research confirms earlier findings (Verma *et al.*, 2022) that, while differences exist among cultivars, the interspecies variability outweighs the variability between cultivars within a single species.

## Conclusion

This experiment revealed notable variations in absolute MP and VFA production between different herb species and harvest moments. Crown vetch, birdsfoot trefoil and white clover showed potential as a  $\text{CH}_4$  reducing strategy. However, further research is needed to explore the broader impact of these findings on *in vivo* digestibility and methane mitigation.

## Acknowledgement

This experiment is funded by Fonds voor Wetenschappelijk Onderzoek–Vlaanderen (FWO, Research Foundation, Flanders), under project number 1SC6322N.

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# *Lolium perenne* populations effects on nitrogen concentration, use and uptake efficiency when grown on peat

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## Abstract

Grass nitrogen (N) concentrations of dairy grasslands are higher on peat soil than on mineral soils. This can lead to increased N losses from dairy farming systems on peat soils. Our objective was to determine whether perennial ryegrass (*Lolium perenne* L.) populations with different shoot tissue N concentrations, recorded on a sandy soil, would show different shoot tissue N concentrations and N use efficiencies (NUE) or N uptake efficiencies (NUptE) when grown on a peat soil. A pot experiment lasting 62 days was carried out with nine diploid and seven tetraploid populations, followed by a field experiment with two diploid and two tetraploid populations and a control lasting 30 months. In the pot experiment, shoot tissue N concentrations differed among tetraploid populations, the NUE differed among diploid and tetraploid populations and the NUptE differed among diploid populations. In the field experiment, two populations had a 1.4 g kg<sup>-1</sup> lower shoot tissue N concentration compared to a commercial control, after ten harvests and at a N fertilisation level of 25 g m<sup>-2</sup> year<sup>-1</sup>. We conclude that it is possible to alter shoot tissue N concentrations of perennial ryegrass grown on peat soil via the selection of low-N populations.

**Keywords:** crude protein, dairy farming, nitrogen use efficiency, perennial ryegrass, plant breeding

## Introduction

On dairy grassland on peat soil, grass N concentrations often exceed 24–26 g (kg dry matter (DM))<sup>-1</sup>, equal to about 150–169 g crude protein (CP) (kg DM)<sup>-1</sup>, even under limited N fertilisation regimes. This is mainly due to the a high soil N supply (SNS), caused by a high organic matter mineralisation of drained peat soils (Vellinga and André, 1999). At dietary CP concentrations above 150 g CP (kg DM)<sup>-1</sup>, milk and protein yields generally do not increase, while urine urea N losses do increase, which can lead to increased ammonia losses (Edouard *et al.*, 2019). The selection of perennial ryegrass with low shoot tissue N concentrations could therefore be a potential way to reduce environmental impacts of dairy farming on peat soils. A pot experiment and a field experiment were carried out consecutively to compare perennial ryegrass population shoot tissue N concentrations, N use efficiency (NUE) and N uptake efficiency (NUptE). It was hypothesised that, on a peat soil, perennial ryegrass populations selected for a different shoot tissue N concentration observed on a sandy soil, would have a different shoot tissue N concentration and NUE or NUptE, and that populations with a high NUE and populations with a low NUptE would have a lower shoot tissue N concentration.

## Materials and methods

Nine diploid and seven tetraploid perennial ryegrass populations differing in N concentration recorded on sandy soil, were selected from a large database of populations from a commercial breeding programme (for details, see Pijlman *et al.*, 2023). In the pot experiment, the populations were grown at three N fertilisation levels with three replicates per treatment. 144 pots (size 15×15×15 cm) were allocated in a randomised complete block design. At day zero, the pots were filled with a peat-based substrate mix (pH 5.8). The pots were placed in a greenhouse without artificial lighting or heating, and received water through sub-irrigation on a daily basis. Per pot, 38 germinating seeds were sown. Prior to sowing, all pots received P, K and S at a rate of 35, 5 and 10 g m<sup>-2</sup>, respectively. On day 42, N was applied at a rate of 0, 6 or 12 g m<sup>-2</sup>. All fertilisation was done with inorganic fertilisers. On days 22 and 42, aboveground

biomass was harvested in order to stimulate perennial ryegrass tillering, and discarded. On day 62, grass was harvested and collected for DM (70°C for 48 h) and total N analyses (Kjeldahl).

In the field experiment, two diploid and two tetraploid populations were used with either the lowest (2Nlow, 4Nlow) or the highest (2Nhigh, 4Nhigh) mean shoot tissue N concentration in the pot experiment. A commercially available diploid perennial ryegrass mixture was used as control. Four replicates per treatment were allocated according to a randomised block design. The experiment was established on a peat soil that had been in use as a permanent dairy grassland (KTC Zegveld, 52°08' N, 4°50' E) and included 10 harvests in three growing seasons. Each growing season, fields were fertilised with in total 25 g N m<sup>-2</sup>, 10 g K m<sup>-2</sup> and 1.7 g P m<sup>-2</sup> using inorganic fertilisers. Every five to eight weeks herbage was harvested and weighed using a small plot harvester (J. Haldrup, Løgstør, Denmark). Representative herbage samples from each plot were analysed for DM (70°C for 48 h) and total N concentration (Kjeldahl).

The NUE of populations was calculated as the increment of aboveground dry biomass weight between two N fertilisation levels ( $\Delta W$ ) divided by the increment of shoot tissue N uptake between two N fertilisation levels ( $\Delta N_{\text{upt}}$ ). The  $N_{\text{uptE}}$  was calculated as  $\Delta N_{\text{upt}}$  divided by the fertiliser N increment between two N fertilisation levels ( $\Delta N_{\text{supply}}$ ), assuming SNS remains constant at different N fertilisation levels (Gastal *et al.*, 2015). Results were analysed taking the nutritional N status into account by using the N nutrition index as an assessment tool (Sandaña *et al.*, 2021) 200, 300, 400 and 500 kg of N ha<sup>-1</sup>. Analyses for differences were done with an ANOVA, in which population was used as factor and N nutrition index (for NUE and  $N_{\text{uptE}}$ ) or N fertilisation (for all other variables) was used as independent variable. In the field experiment, harvest number was used as a within-subject factor according to a repeated measures design.

## Results and discussion

In the pot experiment, shoot tissue N concentrations differed among tetraploid populations (Table 1). The NUE differed among diploid and tetraploid populations and the  $N_{\text{uptE}}$  differed only among diploid populations. Shoot tissue N concentrations of the tetraploid populations correlated negatively with NUE ( $r = -0.85$  and  $P = 0.014$ ), in line with results of Sandaña *et al.* (2021) 200, 300, 400 and 500 kg of N ha<sup>-1</sup>. Dry matter yields did not differ among populations.

In the field experiment, population 2Nlow and 2Nhigh had a 1.4 g kg<sup>-1</sup> lower shoot tissue N concentration than the control (Table 2). Furthermore, population 2Nlow had a higher DM yield than population 4Nhigh.

Table 1. Pot experiment.

Parameter	Diploid			Tetraploid		
	Mean	SEM	P-value	Mean	SEM	P-value
Tissue N concentration (g (kg DM) <sup>-1</sup> )	37.5	1.3	0.122	35.5	1.4	<0.001
NUE (g DM (g N) <sup>-1</sup> )	17.4	1.1	0.008	17.1	0.9	0.005
$N_{\text{uptE}}$ (g Nupt (g Nsupply) <sup>-1</sup> )	0.47	0.02	0.043	0.50	0.02	0.751
DM yield (g m <sup>-2</sup> )	135	5.2	0.505	139	5.3	0.785

Overall mean, standard error of the mean (SEM) and P-value of shoot tissue N concentration, N use efficiency (NUE), N uptake efficiency ( $N_{\text{uptE}}$ ) and dry matter (DM) yield for nine diploid and seven tetraploid perennial ryegrass populations grown at a N fertilisation level of 0–12 g m<sup>-2</sup>.

Table 2. Field experiment.

Parameter	Control	2Nlow	4Nlow	2Nhigh	4Nhigh	SEM	P-value
Tissue N conc. (g (kg DM) <sup>-1</sup> )	35.0 <sup>a</sup>	33.6 <sup>b</sup>	34.4 <sup>ab</sup>	33.6 <sup>b</sup>	34.7 <sup>ab</sup>	0.29	0.018
DM yield (g m <sup>-2</sup> )	252 <sup>ab</sup>	260 <sup>a</sup>	243 <sup>ab</sup>	243 <sup>ab</sup>	238 <sup>b</sup>	7.3	0.032

Means, standard error of the mean (SEM) and P-value of shoot tissue N concentrations and dry matter (DM) yield for five perennial ryegrass populations grown for ten harvests at a N fertilisation level of 25 g m<sup>-2</sup> year<sup>-1</sup>.

<sup>abc</sup> Values with an unequal superscript differed significantly ( $p < 0.05$ ).

Differences in shoot tissue N concentration among populations were inconsistent between the pot and field experiment, and between harvests within the field experiment. These differences were possibly a result of a higher growth rate of grass in spring compared to later in the growing season, of a lower SNS in spring compared to summer and autumn, and of other environmental variations such as weather variations. Understanding these inconsistencies could be an important aspect of future research.

## Conclusion

It is possible to select perennial ryegrass populations for low N concentrations for dairy grassland on peat soil. Further research is needed on the consistency of population effects on N concentrations, for the use of low-N populations as a reduction option for N losses on dairy farms on peat soil.

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# High yields, low fertiliser requirements and low nitrate leaching: win it all with grass-legume mixtures

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## Abstract

Grass-legume leys combine multiple agronomic benefits, several of which are associated with symbiotic di-nitrogen (N<sub>2</sub>) fixation. However, whether significant symbiotic N inputs could lead to increased nitrate leaching is still debated. In a field experiment, we compared pure grass swards (G), grass-legume mixtures (M) and pure legume swards (L) at a fertiliser level of either 50, 150 or 450 kg N ha<sup>-1</sup> year<sup>-1</sup> (N50, N150 and N450). The leys were frequently mown for four years before being tilled to cultivate winter wheat. The risk of nitrate leaching was determined from monitoring soil mineral N and nitrate concentration in the soil solution. Furthermore, the soil surface N balance was calculated by summing up N applied as fertiliser and N derived from symbiosis (total N input) and N in the harvested biomass (N output). During the period of intact plant cover, an increased risk for nitrate leaching was only observed for G- and M-swards fertilised at N450, and L swards at all three N levels. Despite their large N input from symbiosis, no nitrate leaching risk was revealed for M swards. After tilling, the nitrate leaching risk strongly increased until December; importantly, it was not elevated for M- compared to G-swards.

**Keywords:** grass-clover leys, pure swards, NO<sub>3</sub><sup>-</sup> leaching, soil mineral nitrogen, soil surface balance, suction cup

## Introduction

Productive mown grasslands are generally associated with a low risk for nitrate (NO<sub>3</sub><sup>-</sup>) leaching to the environment. A severe risk has however been identified for high N fertiliser applications and for pure legume stands. Balanced grass-legume mixtures benefit the production of both forage (Nyfelner *et al.*, 2009) and the follow-on crop (Fox *et al.*, 2020) thanks to large N input from symbiotic N<sub>2</sub> fixation and positive mixing effects. However, it is not clear whether these N inputs from symbiosis are associated with an increased NO<sub>3</sub> leaching risk. The aim of the current study was to assess the soil surface N balance and the risk of nitrate leaching of leys as affected by legumes and N fertilisation during the two key periods, namely the ley phase for forage production and the phase after tilling for the follow-on crop.

## Material and methods

The experiment included three types of swards and three levels of N fertiliser application, with a total of 54 plots arranged in a completely randomized design. The sward types were pure grass swards (G: *Lolium perenne* or *Dactylis glomerata*; n=4), pure legume swards (L: *Trifolium pratense* or *Trifolium repens*; n=4) and grass-legume mixtures (M: all four species; n=10). These sward types were fertilized with either 50, 150 or 450 kg N ha<sup>-1</sup> year<sup>-1</sup> (N50, N150 and N450). In August 2002, the field (47°26'N, 8°32' E, 491 m a. s. l.) was ploughed at 20 cm depth and the leys were sown on plots of 3 m x 6 m. Starting in 2003 (year 1), all swards were cut five times annually at 5 cm above ground surface. Annual N fertilisation was distributed equally to each regrowth. In the autumn of 2006 (year 4), leys were eradicated by glyphosate and rotary tiller application at a depth of 10 cm for successional sowing of winter wheat. Climate conditions during the period of the experiment were generally in line with the 20-year averages (1031 mm precipitation with a relatively even distribution across the year), except for an exceptionally warm and dry summer in year 1. Soil mineral nitrogen (SMN) was determined in the soil layer 0–60

cm, and nitrate concentration in the soil solution (NCSS) was measured using suction cups at 60 cm soil depth. In this short paper, the third winter period is shown to illustrate the results. Furthermore, SMN was determined at six sampling events during the autumn-winter period following sward eradication. Soil surface N balance was calculated as the difference between N input and N output from year 1 to the last harvest of year 3 (Table 1). Total N input was defined as the sum of applied fertiliser N and N input from symbiosis. N derived from symbiosis was determined by using  $^{15}\text{N}$ -enriched mineral fertiliser and a calculation following the model of Høgh-Jensen *et al.* (2004). Total N output was defined as the amount of N harvested with forage biomass (i.e. N yield). Data was analysed by two-way ANOVAs with the level of N fertiliser application and the sward type (and their interaction). Based on significant effects in the global analysis, significant differences were revealed following the Tukey range test within each factor level (i.e. among different sward types at the same fertilisation level or vice versa). All data were analysed with the statistics software R (R Core Team, 2023).

## Results and discussion

Total N input of the treatments differed from 59 kg N ha<sup>-1</sup> year<sup>-1</sup> (G-N50) to 632 kg N ha<sup>-1</sup> year<sup>-1</sup> (L-N450) (Table 1). At N50 and N150, symbiotically derived N was the major N input for the L- and M-swards, amounting up to 329 kg N ha<sup>-1</sup> year<sup>-1</sup> (Nyfeler *et al.*, 2024). Symbiotic N depended on legume proportion, which, averaged from year 1 to 3, was 42 and 71% at N50, 32 and 73% at N150, and 21 and 80% at N450 in average across the M- and L-swards, respectively. As a result, total N input was only slightly lower in M-N50 and L-N50 than in G-N450. Increased N fertilisation significantly reduced symbiotic fixation for M- and L-swards, however, still manifesting a remarkably high N input from symbiosis at N450. This indicates that legumes only down-regulate symbiotic activity incompletely at high soil N availability. Total N output differed substantially from 113 to 495 kg N ha<sup>-1</sup> year<sup>-1</sup>. At all N fertiliser levels, N outputs of M- and L-swards did not differ significantly from each other, and both were significantly larger compared to G-swards (except for N450), demonstrating the much higher productivity of swards with legumes compared to swards lacking legumes. As the consequence from these results in terms of N input and output, N balance was strongly negative for G-swards and slightly negative for M-swards fertilized at N50 and N150 (Table 1). For swards fertilized at N450 and L-swards, N balance was strongly positive. As a general pattern, it shifted towards more positive values in the order G-, M- and L-swards, and was virtually identical for M-N50 and M-N150.

During the third winter period of intact plant cover, maximal SMN was found at N450 for the L- and the M-swards (>30 kg N ha<sup>-1</sup>). SMN was never significantly higher for M- than for G-swards at N50 and N150 (never exceeding 16 kg N ha<sup>-1</sup>). Similarly, NCSS remained very low under the M-swards at N50 and N150 (Table 1). NCSS was higher under the L- than under the M-sward even at the lowest N fertilizer application rate. After tilling the leys, SMN strongly increased until a maximum in December of winter 4, before levelling off afterwards (Nyfeler *et al.*, 2024). SMN averaged over all samplings of winter 4 did not differ significantly between G- and M-swards at the same fertilisation level (except at N150), however was significantly elevated for L- compared to M-swards (except at N450).

## Conclusions

Grass-legume leys under cutting posed a very limited risk of nitrate leaching during the period of intact plant cover, as long as a substantial grass fraction was provided and the N balance (N input - N output) did not sizably exceed zero. Moreover, the nitrate leaching risk was not greater after tilling such mixtures than after tilling pure grass swards. We conclude that such grass-legume swards combine high yields, low fertiliser requirements, and low nitrate leaching better than either pure grass or pure legume swards.

Table 1. Total N input and N output in the period from years 1 to 3, nitrate concentration in the soil solution (NCSS) and amount of soil mineral N at 0–60 cm depth (SMN) averaged over all samplings of the third winter period (winter from year 3 to 4).

		Intact plant cover									After tilling					
		N input			N output			NCSS			SMN			SMN (kg N ha <sup>-1</sup> )		
		(kg N ha <sup>-1</sup> year <sup>-1</sup> )			(kg N ha <sup>-1</sup> year <sup>-1</sup> )			(mg NO <sub>3</sub> <sup>-</sup> -N L <sup>-1</sup> )			(kg N ha <sup>-1</sup> )					
N50	G	59.3	a	α	113.0	a	α	0.0	a	α	15.2	a	α	50.4	ab	α
	M	371.9	b	α	383.3	b	α	0.1	a	α	16.0	a	α	43.4	a	α
	L	385.5	b	α	361.6	b	α	8.0	b	α	16.2	a	α	77.3	b	α
N150	G	150.2	a	β	182.5	a	α	0.2	a	α	11.9	a	α	62.5	b	α
	M	383.5	b	α	396.6	b	α	0.1	a	α	12.8	a	α	43.0	a	α
	L	449.1	c	β	387.8	b	α	11.6	b	αβ	23.1	a	αβ	77.4	b	α
N450	G	430.0	a	γ	393.3	a	β	13.1	a	β	19.6	a	α	67.8	a	α
	M	545.1	b	β	495.1	b	β	30.0	a	β	34.6	a	β	72.5	a	β
	L	631.9	c	γ	424.4	ab	α	39.1	a	β	32.0	a	β	95.4	a	α
SE		13.58			15.26			4.04			2.79			5.08		

Means of each treatment are shown for G-, M- and L-swards fertilised at three N levels. Within a column, different letters indicate significance of Tukey range test from significant effects in the global analysis (not shown:  $P \leq 0.05$ ). Latin script is used for comparing sward types within fertilisation levels, and Greek script for comparing fertilisation levels within sward types. SE, average of all group mean SEs.

## Acknowledgement

This work was financially supported by the Swiss State Secretariat for Education and Research (COST action 851: BBW C02.0031).

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# Effects of slurry application method on grassland yield, nitrogen utilisation and silage quality

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## Abstract

The use of low-emission equipment has become mandatory for slurry distribution in a number of countries. However, the effects on yield, nitrogen (N) utilisation and silage quality are still intensively debated. In a 3-year field experiment at two experimental sites in Switzerland, we tested the effects of broadcast equipment (BC) and the low-emission devices band-spread (BS) and trailing-shoe (TS) on dry matter yield, N utilisation by plants and silage quality. The different equipment was tested in combination with two slurry consistencies (unaltered dilution; extra dilution), two timings of application (immediately or delayed after preceding harvest), and two sward types (with legumes; without legumes). BS compared to BC revealed positive effects on dry matter yield and N utilisation at one, but not the other site. Delayed application improved apparent N recovery but not yield, and only at one of the two sites. Extra slurry dilution proved positive for both yield and N recovery. In terms of silage quality, low-emission equipment had no relevant detrimental effects, while early application with extra diluted slurry was advantageous. We conclude that slurry application by low-emission equipment can be advantageous in terms of yield and N utilisation without being detrimental in terms of silage quality.

**Keywords:** apparent N recovery, broadcast, band-spread, trailing-shoe, distribution equipment, forage

## Introduction

The need for reduced ammonia (NH<sub>3</sub>) emissions in agriculture is attended by the mandatory use of low-emission equipment for slurry distribution. Due to reduced NH<sub>3</sub> emissions, plant N availability of slurry N should be improved. Results of previous studies in grassland are however not conclusive, having revealed sometimes positive, sometimes indifferent yield effects (Huguenin-Elie *et al.*, 2018). In addition, slurry consistency and timing of application after the preceding harvest might also affect slurry N utilisation due to their impact on slurry infiltration into the soil and protection by the plant canopy, respectively. Finally, the presence of legumes in a sward might offset effects of the slurry application methods by buffering the amount of N available to the swards. Apart from uncertainties in terms of yield and N utilisation, many farmers refrain from using low-emission equipment, because of concerns regarding slurry residues (band-spreading) or soil particles (trailing-shoes) in the harvested plant material. In a field experiment, we aimed at assessing the effects of different slurry distribution devices, slurry consistency, application timing and sward type on yield, N utilisation and silage quality during several years and for different site conditions.

## Material and methods

A 3-year field experiment was established at two sites on the Swiss Plateau, on intensively managed temporary grassland: (1) Site1 (535 m a.s.l.; average annual temperature 7.9°C; 1124 mm precipitation), 18 m<sup>2</sup>-large plots, fully randomized, and (2) at about 30 km distance, Site2 (470 m a.s.l.; average annual temperature 9.4°C; 956 mm precipitation), 135 m<sup>2</sup>-large plots in a randomized block design. The experiment at Site1 included different types of slurry distribution equipment (BC: broadcast, BS: band-spread, TS: trailing-shoe), slurry consistency (unaltered dilution: 4–5% DM content, extra dilution:

2–3% DM content), timing of application (early: 1–3 days after the preceding cut, late: 7–10 days delayed), and sward type (with and without legumes) in a multifactorial design. At Site2, the number of treatments was reduced (without trailing-shoe and diluted slurry). The plots were harvested five times a year (in year 1 however, with only two harvests remaining after the establishment phase), and slurry was applied to each regrowth at a targeted amount of 30 kg NH<sub>4</sub>-N ha<sup>-1</sup>. Dry matter yield and forage N content was determined at each harvest. Slurry was sampled at each application to be analysed for its NH<sub>4</sub><sup>+</sup>-N content. Apparent slurry N recovery, henceforth denominated as N<sub>rec</sub>, was calculated from the difference in N yield with the unfertilised control-plots and the amount of applied NH<sub>4</sub><sup>+</sup>-N (only plots without legumes). At Site1, plant samples of the first, second and fourth harvest of the third experimental year were ensiled in 1.5 l laboratory silos and analysed before (clostridial bacteria occurrence measured as most probable number g<sup>-1</sup>) and after the fermentation process (butyric acid content) to determine silage quality (only plots with legumes). Data were analysed using generalised linear models or generalised linear mixed-effect models with distribution equipment, slurry consistency, application timing and sward type as fixed effects. All interactions were included in the analysis. Differences between the different treatments were then tested by the Tukey range test. All analyses were performed using the statistical software R (R Core Team, 2023).

## Results and discussion

Treatment effects on DM yield and N<sub>rec</sub> were not consistent across the two sites: whereas the distribution equipment had no effect at Site1, there was a significant increase when slurry was applied by BS compared to BC in terms of DM yield (+9%;  $P \leq 0.001$ ) and N<sub>rec</sub> (+16%;  $P \leq 0.001$ ) at Site2. This inconsistency is in line with literature, where both positive and indifferent yield effects have been reported for grassland experiments (Huguenin-Elie *et al.*, 2018). Such results might be explained by the relatively small NH<sub>3</sub>-N quantities being spared with low-emission equipment in comparison with the total amount of plant available N in intensive grassland systems (Häni *et al.*, 2016). Extra slurry dilution affected DM yield (+6%;  $P \leq 0.01$ ) and N utilisation (+24%;  $P \leq 0.05$ ) positively compared to unaltered slurry dilution, which may be associated with both an enhanced soil infiltration and reduced ammonia emissions (Sommer *et al.*, 2006). Delayed application improved only N<sub>rec</sub> and this only at Site2 (+20%;  $P \leq 0.05$ ). The presence of legumes had no influence on the effects of type of distribution equipment but generally increased yield at both sites (+19% and +21% at Site1 and Site2, respectively;  $P \leq 0.001$ ), confirming the well-documented advantages by symbiotic nitrogen fixation (Nyfeler *et al.*, 2011). Evaluated parameters of silage quality were only slightly affected by the tested experimental factors. At the three harvests, there was no indication for consistent detrimental effects by low-emission equipment on silage quality (three inconsistent significant differences for clostridial bacteria occurrence or butyric acid content;  $P \leq 0.05$ ), but rather an indication for early application and extra diluted slurry being advantageous (each one time;  $P \leq 0.05$  at harvest 2 and 1, respectively).

## Conclusions

Slurry application with low-emission equipment compared to broadcast distribution can be favourable in terms of yield and N utilisation. The positive effect of extra slurry dilution on yield and N utilisation was in the same order of magnitude as the one of low-emission equipment. In terms of silage quality, we did not find any consistent detrimental effect of distribution by low-emission equipment. Variability of silage quality across harvests indicates that forage preparation for ensiling (e.g. ideal degree of wilting, avoiding soil residues in the forage) might have a stronger impact on bacteria-related quality parameters than distribution equipment.



Table 1. Dry matter yield ( $\text{Mg ha}^{-1}$ ) and proportion of N recovered from slurry-N averaged for each experimental factor level (both sites) as well as silage quality parameters clostridial bacteria occurrence (CL: most probable number  $\text{g}^{-1}$ ) and butyric acid content ( $\text{g BA kg}^{-1}$ ) (only Site1).

	DM yield and $N_{\text{rec}}$				Bacteria-related silage quality parameters					
	Site1		Site2		Harvest 1		Harvest 2		Harvest 4	
	DM	$N_{\text{rec}}$	DM	$N_{\text{rec}}$	CL	BA	CL	BA	CL	BA
Distribution equipment										
Broadcast (BC)	20.6	0.42	23.9 <sup>a</sup>	0.67 <sup>a</sup>	90	1.6 <sup>a</sup>	163 <sup>ab</sup>	24.6	5.0 <sup>a</sup>	26.4
Band-spread (BS)	20.3	0.36	26.0 <sup>b</sup>	0.78 <sup>b</sup>	60	2.1 <sup>ab</sup>	64 <sup>a</sup>	23.2	6.7 <sup>b</sup>	23.6
Trailing-shoe (TS)	21.3	0.46	-	-	123	4.8 <sup>b</sup>	138 <sup>b</sup>	24.2	5.8 <sup>ab</sup>	29.2
Slurry consistency										
Unaltered dilution	20.2 <sup>a</sup>	0.37 <sup>a</sup>	-	-	113 <sup>b</sup>	2.7	104	24.1	5.8	27.3
Extra dilution	21.3 <sup>b</sup>	0.46 <sup>b</sup>	-	-	45 <sup>a</sup>	2.1	140	23.8	5.8	23.7
Application timing										
Early	21.0	0.40	25.0	0.66 <sup>a</sup>	75	2.8	74 <sup>a</sup>	22.8	5.7	24.4
Late	20.5	0.42	25.0	0.79 <sup>b</sup>	96	2.1	164 <sup>b</sup>	25.0	6.0	27.3
Averaged SEM	0.46	0.033	0.86	0.048	25.9	0.74	40.1	1.31	0.51	1.53

Letters indicating significant differences are only given for factors being significant in the model of the statistical analysis ( $P \leq 0.05$ ). Dry matter yield is shown as the sum over the entire period of the experiment and  $N_{\text{rec}}$  as the proportion of N in the harvested plant material (only swards without legumes) apparently recovered from slurry  $\text{NH}_4\text{-N}$  (as weighted averages from all harvests). Silage quality was only determined at Site1 and for harvest 1, 2 and 4 in year 3. SEM is only given as average over the means of each treatment level.

## Acknowledgements

This work was financially supported by the Swiss federal office for agriculture and the office for agriculture of canton Thurgau. The authors are grateful to all scientific, technical and laboratory staff involved in this project.

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# Maize in permanent grassland: effects of strip tillage and mechanical weeding on soil properties and yields

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## Abstract

Silage maize is a valued crop in grassland-based dairy farming due to its high yield and feeding value. However, the delivery of ecosystem services (biodiversity, soil quality, carbon sequestration etc.) is greatly reduced in maize, compared to permanent grassland. To combine the ecosystem services of maize and grassland, a cropping system was developed for silage maize sown in living permanent grassland. In a field experiment, grass was superficially mulched, either full-field or in strips, maize was sown and weed control was carried out mechanically, both full-field and in strips. The control included chemical full-field grass killing and chemical weed control, without soil tillage. Measurements at the end of the growing season show no significant treatment effects in maize yield and soil quality, but clear negative effects of superficial tillage on earthworm biomass. We conclude that in a fertile soil, silage maize can be grown without use of herbicides and that a strip of permanent grassland reduces the negative impact of superficial tillage for the earthworm population.

**Keywords:** minimal tillage, herbicide-free maize, grassland, soil quality, earthworms

## Introduction

Minimal tillage can be used to reduce the loss of soil quality in dairy farming when silage maize is grown after grassland. Reduced tillage, combined with herbicides to control grass and weed growth, shows similar maize yields compared to ploughing, but higher numbers of earthworms and faster water infiltration (Deru *et al.*, 2015; Sleiderink *et al.*, submitted; Van Agtmaal *et al.*, 2020). By leaving out herbicides, such cropping systems could further gain in biodiversity, nutrient retention and water infiltration due to the permanent living soil cover, as shown by Struyk *et al.* (2021). However, loss of maize yield due to water and nutrient uptake by the grass needs to be minimized, and should be in balance with gains in ecosystem services. As a next step, (strip-) mulching and mechanical weeding techniques were used as a new combination in a field experiment. Our objective was to compare maize yield and soil biological, chemical and physical properties of chemical (full-field or strip) versus mechanical (full-field or strip) methods of grass and weed control in maize sown with minimal tillage in permanent grassland.

## Materials and methods

A randomised block experiment with five treatments (Table 1) in four replicates was carried out in 2023 on a permanent grassland of a dairy farm on sandy soil in Ruinerwold, Drenthe. The grassland had been fertilized in spring with 20 m<sup>3</sup> ha<sup>-1</sup> cattle slurry and was mown (1<sup>st</sup> of May, 1.7 t DM ha<sup>-1</sup>, 53 kg N ha<sup>-1</sup>) prior to the experiment. Before sowing, the grassland was treated either with glyphosate, with a full field superficial mulch machine mixing 3–4 cm of topsoil with the grass sod, or with an adapted version of this machine leaving a strip of 15 cm permanent grassland between the maize rows (Table 1). Maize (Exelon, KWS), was sown (8<sup>th</sup> of May; 75 cm row distance) in all treatments by minimal tillage with a combined strip-cutter and subsoiler (20 cm deep, 10 cm broad). Artificial N fertilizer at 30 kg N ha<sup>-1</sup> was provided in the row. Weed control (May–June) was carried out either with herbicides (8<sup>th</sup> of June, full-field for ‘chem+chem’ and ‘mulch+chem’ and in the maize row for ‘strip+chem’) or with a combination of harrowing (full-field) and hilling (strips).

Table 1. Systems of grass and weed control used per treatment with minimal tillage maize

Treatment	Grass control at start (May)	Weed control after sowing (May–June)
Chem+chem	Chemical (glyphosate)	Chemical (1×cocktail; full-field)
Mulch+chem	Full field mulching	Chemical (1×cocktail; full-field)
Mulch+mech	Full field mulching	Mechanical (5×harrowing, then 2×hilling)
Strip+chem	60 cm mulching, 15 cm grass left	Chemical (1×cocktail; in maize row)
Strip+mech	60 cm mulching, 15 cm grass left	Mechanical (5×harrowing, then 2×hilling)

N mineral content of the top 30 cm soil was measured during the growing season in May, June, August and at harvest (September). At harvest, N mineral was also measured in 30–60 cm and 60–90 cm soil depth. Other soil measurements were carried out at the end of the growing season (28<sup>th</sup> of August): penetration resistance, soil structure, water infiltration and earthworm biomass. The central two maize rows were harvested (21<sup>st</sup> of September), chopped, weighed and dry matter content and feeding value were determined in a fresh sub sample by NIRS (Eurofins, Wageningen). Genstat (v. 23) was used for data analysis.

## Results and discussion

Maize yield in ‘strip+mech’ was 12% lower than in the control (‘chem+chem’), although the overall treatment effect was not significant ( $P=0.16$ , Figure 1) due to large variation between replicates. Protein content was lowest in the treatments with a strip of grass left between the rows (Table 2), possibly indicating N uptake competition by the grass. There were no treatment differences in either soil mineral N content throughout the season or in N uptake. Soil mineral N was highest in June, and the residual amount at harvest in September in the 0–90 cm layer was still high, between 79 and 109 kg ha<sup>-1</sup>. A rough N balance indicated that the soil N supply must have been high, ca. 150 kg N ha<sup>-1</sup>, even without soil tillage. Local variation across the experiment in the soil N supply and N uptake by regrowing grass under the maize may have contributed to the variation in maize yields between replicates. This calls for further development of mulching and weeding techniques.

Soil structure, rooting density, penetration resistance and water infiltration rate were not affected by the treatments (Table 2) but earthworm biomass was (Figure 1).

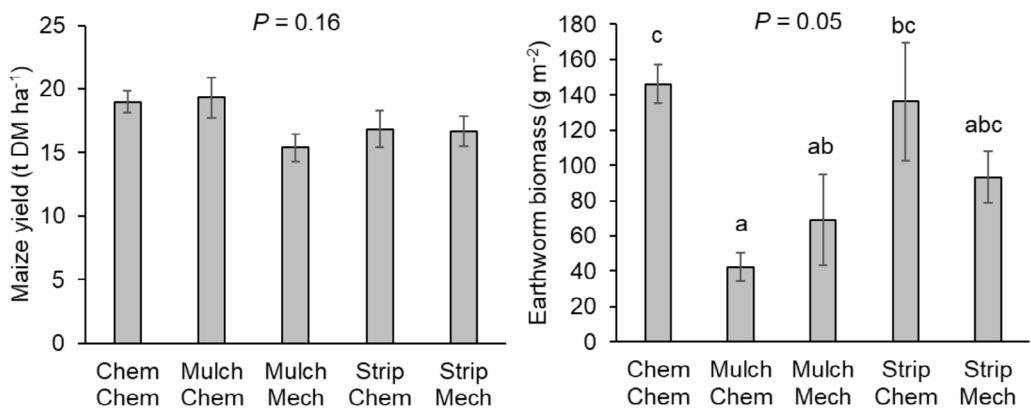


Figure 1. Maize yield and earthworm biomass ( $\pm$  standard error) in different grass and weed control systems. Different letters indicate a significant difference ( $\alpha=5\%$ ).

Table 2. Maize and soil parameters in different grass and weed control systems.

Parameter	Unit	Chem+chem	Mulch+chem	Mulch+mehc	Strip+chem	Strip+mehc	P-value
Maize yield	t DM ha <sup>-1</sup>	19.0	19.3	15.4	16.8	16.7	0.16
Protein content	g kg <sup>-1</sup>	68.3 ab	70.0 bc	71.0 c	66.5 a	67.6 ab	0.04*
Starch content	g kg <sup>-1</sup>	436.8	429.2	424.5	451.0	436.4	0.35
N uptake	kg N ha <sup>-1</sup>	207.2	216.6	174.6	179.6	180.7	0.19
N-min 0–30 cm May	kg N ha <sup>-1</sup>	59.0	n.d.	71.0	78.5	42.8	0.14
N-min 0–30 cm Jun	kg N ha <sup>-1</sup>	180.0	n.d.	145.0	132.0	133.0	0.39
N-min 0–30 cm Aug	kg N ha <sup>-1</sup>	23.8	n.d.	23.8	28.8	20.0	0.66
N-min 0–30 cm Sep	kg N ha <sup>-1</sup>	62.8	n.d.	55.2	70.5	51.0	0.57
N-min 0–90 cm Sept	kg N ha <sup>-1</sup>	108.8	n.d.	79.2	104.7	85.0	0.47
Soil crumbs 0–20 cm	%	32.5	25.0	30.8	27.5	32.5	0.42
Root density 0–20 cm	Score 1-10	5.4	5.2	6.3	5.9	4.9	0.23
Pen.res. 0–30 cm	kPa	2.00	1.99	2.02	2.09	2.20	0.81
Pen.res. 0–10 cm	kPa	1.07	1.18	1.05	1.06	1.18	0.33
Water infiltration	mm min <sup>-1</sup>	4.13	7.00	5.08	4.83	3.25	0.40

\*Significant value ( $P < 0.05$ ).

There was a clear negative effect on earthworms of mulching, compared with chemical grass killing. Biomasses of the full chemical versus full field mulching correspond to those in Van Agtmaal *et al.* (2020) who compared a full chemical minimal-till system with full field spading in a similar grassland. Thus, superficial mulching had the same negative effect as spading to 20 cm. However, there was a higher biomass where the sod was partly left intact (strip versus full field mulch). After maize sowing, whether the weeds were controlled mechanically or with herbicides had no effect on earthworm biomass.

The experiment of Struyk *et al.* (2021) found clearer positive effects of leaving out herbicides for soil mineral N and earthworms than in the present study, but the yields of the herbicide-free maize were lower. We explain these differences by the more effective weeding in 2023, resulting in less difference in grass cover between chemical and mechanical treatments. In both years, minimal overseeding (without tillage) after the maize harvest in all treatments was enough to restore the grassland with good quality grasses.

## Conclusion

We conclude that in a fertile sandy soil, silage maize can be grown in permanent grassland without use of herbicides with only moderate yield loss. A living strip of permanent grassland reduces the negative impact for the earthworm population of superficial tillage. With a novel combination of techniques in a grass-maize rotation, the yield potential of silage maize can be retained with less loss of the ecosystem services delivered by grassland.

## Acknowledgements

The experiment was part of the PPS-project *Innovatieve maisteelt – op weg naar chemievrij, minder uitspoeling, biodiverser*; all partners are acknowledged for their contributions.

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# Subsoiling to remediate compacted grassland soils

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## Abstract

Soil compaction has become a serious threat to the well-functioning of intensively cultivated grasslands. Finding sustainable remediation strategies has therefore become a pressing concern to ensure yield stability. We assessed the effectiveness of a subsoiler, to alleviate moderately compacted grassland loamy sand and silt loam soils in Flanders. We also studied the ideal timing of subsoiling (spring vs. autumn) and the combination with oversowing. Overall, the effect of subsoiling on grassland yield proved negligible. On the silt loam soil, subsoiling even led to a significant reduction in yield in the subsequent growing season. The long-term penetration resistance (PR) reducing effect of subsoiling was only observed in the upper-subsoil (20–40 cm), and only if the subsoiling was done at optimal soil conditions. Especially in silt-rich soils, it is important to avoid wet soil conditions during subsoiling. Oversowing marginally improved the long-term effect on PR, but this was only observed in the loamy sand soil.

**Keywords:** soil compaction, subsoiling, timing, soil water content, oversowing

## Introduction

Soil compaction has become a serious threat to the well-functioning of intensively cultivated grasslands. The main causes of this form of structural degradation are livestock treading and traffic with heavy machinery, all through the year when the soil is moist or wet and thus more susceptible to compaction. By negatively affecting root growth, through both physical impedance and oxygen deprivation, soil compaction can have a serious negative impact on grassland yields. Data on the extent of the problem is quite rare for most countries. In the UK a survey did point out that 16% of grassland fields were badly compacted, while 70% could be described as at least moderately affected (Webster and Oliver, 2007). Finding sustainable remediation strategies has thus become a pressing concern to ensure yield stability. Subsoiling, also referred to as sward lifting in grasslands, has been shown to sustainably alleviate compacted grassland soils (e.g. De Boer *et al.*, 2018). The objective of this study was to assess the impact of subsoiling at different times of the year (spring vs. autumn), with and without oversowing, on the yield and soil structural quality of two moderately compacted grassland fields with distinct soil textures (silt loam and loamy sand).

## Materials and methods

The experiments were conducted on two permanent grasslands in Herzele (silt loam) and Zele (loamy sand) in Flanders, Belgium. These grasslands were selected after an exploratory survey for soil compaction with a penetrometer. At both locations we tested three distinct subsoiling treatments, one spring and two autumn applications (i.e. with and without oversowing of Italian ryegrass), and an uncultivated control. The silt loam experiment was started on 20 May (spring) 2019 (topsoil (0–30 cm) gravimetric water content ( $\theta_g$ ) 0.20 g g<sup>-1</sup>), with the autumn application following on 22 November 2019 ( $\theta_g$  0.24 g g<sup>-1</sup>). The autumn subsoiling in combination with oversowing treatment was performed on 27 October 2020 ( $\theta_g$  0.24 g g<sup>-1</sup>). The loamy sand experiment was started on 14 October (autumn) 2020 (both with and without oversowing,  $\theta_g$  0.18 g g<sup>-1</sup>), with the spring application following on 3 May 2021 ( $\theta_g$  0.26 g g<sup>-1</sup>). In the silt loam experiment the subsoiling depth was 40 cm and in the loamy sand experiment 50 cm. In the years after subsoiling the dry matter (DM) yield was determined by means of a Haldrup grass harvester at three to six cuts per year. The impact on the soil structure was assessed with a hand-held

penetrometer down to 80 cm depth. The statistical analyses were performed with SPSS Statistics 28 as a one-way ANOVA with subsoiling treatment as fixed factor and block as random factor. If a significant effect was observed, a Tukey post-hoc test was performed to show significant differences ( $P < 0.05$ ) between the experimental treatments. For DM yield the statistical analyses were done separately for each experimental location and year. For PR the statistical analyses were done separately for each experimental location and depth interval (10 cm).

## Results and discussion

In the silt loam experiment (Figure 1a) all the subsoiling treatments caused a significant long-term reduction in PR at 20–30 cm depth, while at 30–40 cm depth only spring subsoiling still had a significantly ( $P = 0.02$ ) lower PR compared to the control, but not compared to the other subsoiling treatments. The higher sustainability of spring subsoiling at 30–40 cm depth might be explained by the drier soil conditions (spring:  $0.20 \text{ g g}^{-1}$  vs. autumn:  $0.24 \text{ g g}^{-1}$ ) at the time of subsoiling. At higher soil water content, subsoiling has the added risk of plastic soil deformation instead of the coveted loosening effect through lifting (Spoor, 2006). This is certainly the case for silt-rich soils (Schneider *et al.*, 2017). In the loamy sand experiment (Figure 1b) the only significant differences were observed between the control and spring subsoiling and autumn subsoiling in combination with oversowing. Deeper in the soil profile (40–50 cm), only the latter subsoiling treatment remained; lower, but insignificantly ( $P = 0.05$ ), than the control. The reinvigorated sward might have enhanced root growth to a point that it helped to stabilize the loosened upper-subsoil. Perennial crops, like lucerne have been previously shown to prolong the loosening effect of subsoiling on the upper-subsoil (Löfkvist, 2005).

None of the subsoiling treatments significantly increased the DM yield compared to the control (Figure 2). In the loamy sand experiment the effects of subsoiling were negligible (Figure 2b), while in the silt loam experiment both spring and autumn subsoiling resulted in a significant reduction in the growing season immediately after subsoiling (Figure 2a). Carter and Kunelius (1998) previously observed that subsoiling has the potential to negatively affect grassland yields by damaging sward and roots. In our study this effect was only observed for the silt loam soil, which is more susceptible to negative plastic deformation, like smearing, during subsoiling (Schneider *et al.*, 2017). The negative effect was most noticeable after spring subsoiling, which was followed by a prolonged dry spell in 2019.

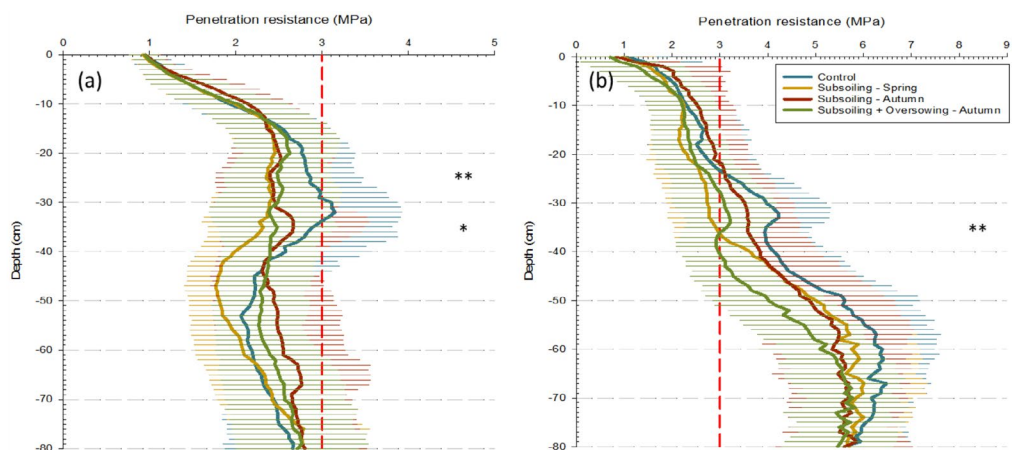


Figure 1. PR (MPa)  $\pm$  standard deviation until 80 cm depth, measured in January 2022 for the silt loam experiment (a) and in December 2022 for the loamy sand experiment (b). Significant (one-way ANOVA) 10 cm intervals are indicated with asterisks (\* $P < 0.05$ ; \*\* $P < 0.01$ ). The vertical red line indicates the standard threshold value of 3 MPa for root impedance.

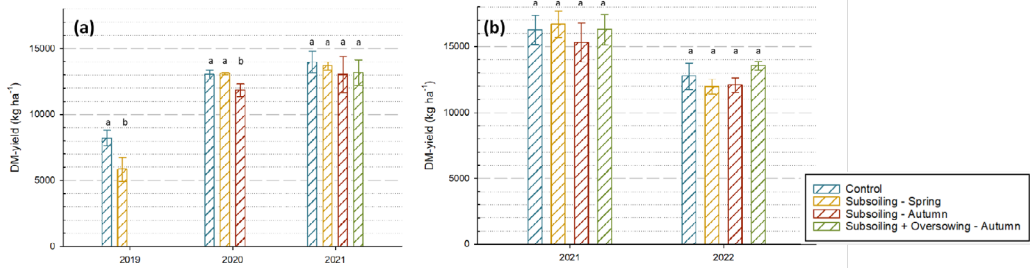


Figure 2. DM yield ( $\text{kg ha}^{-1}$ )  $\pm$  standard deviation per year for all experimental treatments in silt loam (a) and loamy sand (b) experiments. Different letters indicate significant differences ( $P < 0.05$ ) for the Tukey post-hoc test per experiment and per year.

## Conclusion

The long-term PR reducing effect of subsoiling was only observed in the upper-subsoil (20–40 cm), and only if subsoiling was done at optimal soil conditions. Especially in silt-rich soils, it is important to avoid wet soil conditions during subsoiling. Oversowing marginally improved the long-term effect on PR, but this was only observed in the loamy sand soil. Despite the long-term loosening effect, none of the subsoiling treatments significantly increased the DM yield of the experimental grassland fields. In the silt loam experiment subsoiling even had a marked negative effect on the DM yield in the growing season immediately following the operation.

## Acknowledgement

The study was supported by the VLAIO LA project “Prevention and remediation of soil compaction” (HBC.2017.0834).

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# Analysis of the role of agroecology in the sustainability of the Dutch dairy system using the Business Model Canvas approach

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## Abstract

The high-intensity dairy production in the Netherlands comes at a high cost to circularity and biodiversity. The objective of this study was to determine how the application of herb-rich grasslands and recognition of soil microbial diversity embraced in agroecological practices can improve milk production and the income streams of dairy farmers. This study was carried out in the Friesland region (north of the Netherlands) and was divided into (i) a case study, in which 3 farm models (conventional, semi-extensive, and organic) were compared; (ii) an online quantitative survey with 20 dairy farmers; and (iii) in-depth and in-person interviews with 8 dairy farmers and 9 relevant stakeholders of the Dutch dairy chain. Results demonstrated that agroecological practices lead to milk richer in omega-3 and conjugated linoleic acids. The Business Model Canvas was applied, and it demonstrated that these practices contribute to reduced financial costs related to synthetic fertilizers, feeds, and manure disposal, as well as decreasing economic failure risks and providing long-term stability for the farm. Additionally, gains in biodiversity and soil microbiota (natural capital), and in well-being and happiness (both social and emotional capital) can be provided by the adoption of agroecological practices in the dairy chain.

**Keywords:** circular production; biodiversity; milk production; agroecological practices

## Introduction

The Netherlands has more than four times the average European livestock density and is the EU's fourth-largest milk producer by volume (European Commission, 2020). This intense production system, however, comes at a cost for biodiversity, resulting in increased mineral and microbial losses and greenhouse gas and ammonia emissions to the soil, water and air. Consequently, this process is leading to damage to soil health and functions, such as carbon sequestration, nitrogen fixation, and limited nutrient uptake by the plant root system (Cooledge *et al.*, 2022). Despite the many efforts and studies on grassland ecology, several research studies show that little to no attention has been given to the interaction between the soil, rhizosphere, plant, and soil microbiomes as a medium of exchange for nutrients between the soil and the plant root system. This study aimed to determine how the application of herb-rich grasslands and recognition of soil microbial diversity embraced in agroecological practices can improve dairy farms' biodiversity, milk production, and the income streams of dairy farmers.

## Materials and methods

The present explorative research was carried out in the Friesland region (north of the Netherlands) in two stages. The first stage consisted of the determination of fatty acids in milk samples ( $n=4$ ) produced according to conventional and organic systems. Samples were analysed according to the protocol ISO 12966-2/4 (ISO, 2015), based on chromatographic gas separation followed by detection based on flame ionization (GC-FID). The second stage was composed of hybrid research, and it encompassed 3 steps; (i) a case study combined with observations, in which 3 farm models (conventional, semi-extensive, and organic) were compared; (ii) an online quantitative survey distributed among 20 dairy farmers; and (iii) in-depth and in-person interviews with 8 dairy farmers and 9 relevant stakeholders of the Dutch dairy chain. Data regarding milk composition were analysed by applying basic descriptive statistics tools, due to



the limited dataset. Results from the survey were analysed according to the Mann–Whitney test, at a 95% confidence level. Qualitative data were transcribed into MS Word documents and processed using the Ground Theory Method (Strauss, 1997). A Business Canvas Model (BMC) was developed considering a scenario of organic milk production (Osterwalder and Pigneur, 2010); hence, highlighting opportunities for the application of agroecological practices on the income streams of dairy farmers.

## Results and discussion

Results of milk samples from conventional and organic farming (Table 1) demonstrated a comparable content for total saturated, monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids. Conjugated Linoleic Acids (CLA) and omega-3 contents were higher in organic samples, according to results previously reported in the literature (Timlin *et al.*, 2023).

Feeding systems based mainly on (herb-rich) grass, such as organic practices, have been scrutinized over the years due to their positive effects on the fatty acid profile in milk samples, resulting in higher concentrations and greater diversity in the PUFA profile (Timlin *et al.*, 2023). In addition, herb-rich grass feeding promotes circularity in the production system due to the increased diversity of soil microbiota and the availability of nutrients for plant uptake (Verstand, 2022).

The results of the survey show that in the Netherlands extensive farming with an average of 220 cows predominates over organic farming with 120 cows. According to the Mann–Whitney test (at a 95% confidence level), there are significant differences between conventional and organic production systems in terms of frequency of antibiotic use, maintenance of soil quality on the farm, type of grassland, biodiversity on the farm, grazing milking cows (between May and October), acceptance of weed/pest control and the number of cows on the farm. These results of the survey and case study (including field observations) are also consistent with the chemical markers (fatty acids) observed in the milk samples analysed.

After analysing the data collected in research stages 1 and 2, a BMC was developed to promote the adoption of agroecological approaches that embrace the concept of One Health (i.e., soil and herb-rich grasslands) in Dutch dairy farming, as shown in Figure 1.

## Conclusion

Assuming CLA and omega-3 fatty acids were positively affected by the farming practice, organic farming practices can improve the chemical quality of the milk and also can affect soil quality and the microbial diversity, when compared to conventional farming. Adopting agroecological practices can, therefore, generate income sources for farmers in the form of food claims, according to the Business Model Canvas. It also reduces economic failure risks, provides long-term stability for the farm, and contributes to

Table 1. Fatty acids composition in milk produced according to conventional (C) and organic (O) production systems.

Fatty acids	C1	C2	C3	O1
Total saturated (% m m <sup>-1</sup> )	70.3±3.5	67.1±3.4	70.2±3.5	67.1±3.4
Total MUFA (% m m <sup>-1</sup> )	24.9±1.2	28.4±1.4	26.4±1.3	28.4±1.4
Total PUFA (% m m <sup>-1</sup> )	4.8±0.2	4.6±0.2	3.4±0.2	4.6±0.2
Total CLA (% m m <sup>-1</sup> )	0.4±0.02	0.5±0.03	0.5±0.03	1.1±0.1
Total omega-3 (% m m <sup>-1</sup> )	0.9±0.05	0.9±0.05	0.7±0.04	1.2±0.1

Results follow estimated deviations. For matters of comparison, typical deviations for the analytical technique GC-FID (i.e., 5%) were considered, once the protocol only took into consideration one single measurement.

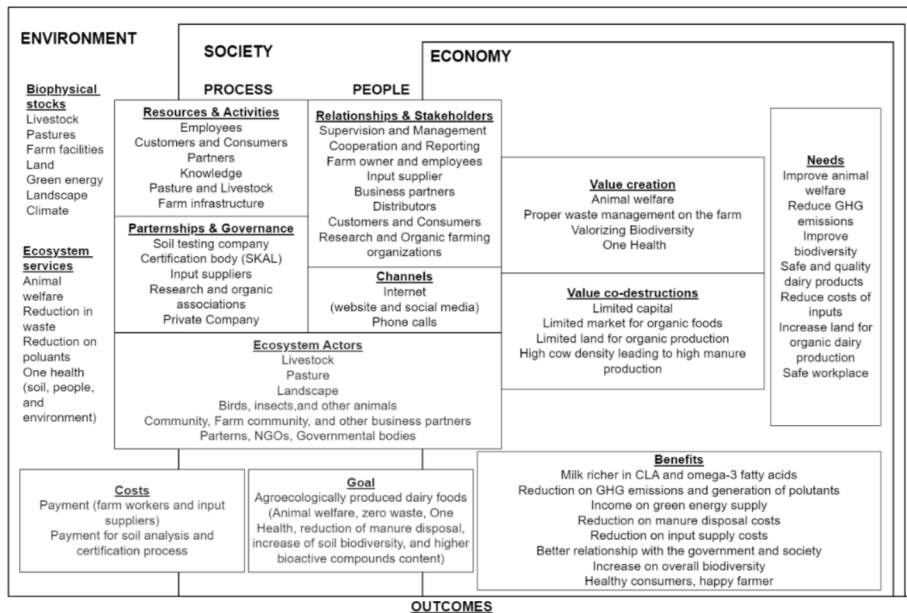


Figure 1. BMC for organic farms, highlighting the 3 principles of sustainability, i.e., environmental, societal, and economic aspects.

the generate well-being and happiness (both social and emotional capital) through the landscape and increased biodiversity.

## Acknowledgements

This work was financially supported by Biosintrum and was done in collaboration with Leer en Kennis Centrum Bodem and Ecostyle BV.

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# The Farming Tree as an assessment tool for ecological and social sustainability

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## Abstract

Grassland management connects to policy objectives like Net-Zero Agriculture and biodiversity conservation and forms an integral part of total farm management, affecting the sustainability level of the farm in relation to its social and ecological environment. An integral assessment tool, Farming Tree, has been designed that allows for an integral assessment of the socio-economic and ecological impact of farming operations using a farm location-based perspective. The tool supports farmers in navigating and communicating the integral complexity of their farms in relation to socially and environmentally sustainable practices. Inspired by Doughnut Economics thinking, the Farming Tree has 16 themes covering all relevant socio-economic and ecological aspects, allowing for a comprehensive overview and an assessment for a specific theme. Each of the 16 themes has either a socio-economic 'floor' or an ecological 'ceiling', meaning that minimum levels for social and ecological well-being are crucial. The model supports increased awareness of the farm in all its aspects and allows communications about farm strategy and activities in an integral manner. The model primarily employs a qualitative approach based on farmers' choices and actions, with the potential for quantitative enhancements.

**Keywords:** ecology, socio-economic, sustainability, farming, self-assessment, grassland

## Introduction

Grasslands are a critical component of agricultural production systems affecting biodiversity, and the efforts needed to restore biodiversity loss translate to adaptations in farm production systems. This is in line with a broader paradigm shift on the development of agricultural practices in relation to the social and ecological environment. A renewed focus on the relation with the local social environment and agricultural ecosystems needs to be part of the strategic choices in farm management (Methorst, 2013). Besides producing food, farms also create ecological and socio-economic value for the environment and society as a whole. While there are many tools to assess specific (negative) environmental aspects, there is lack of effective tools for an integrated assessment of the combined farm activities using a socio-economic and ecological view. An integrated farm assessment tool supports the alignment of farm strategy development with the needs and concerns of the social and ecological environment. Grassland management is intrinsically tied to these needs and concerns of ecology and regional landscape with the potential to significantly influence greenhouse gas emissions, water management, and biodiversity conservation (Hou *et al.*, 2022).

It is within this context that at AUAS the Farming Tree tool was developed, a practical model rooted in the principles of Doughnut Economics which advocates the need for economic activities to have a social floor and an ecological ceiling (Raworth, 2018). The Farming Tree tool aims to support the strategic evaluation of an individual farm where farmers' choices affect the ecological sustainability and also relate to the socio-economic context. A tool that allows for an independent assessment also supports a common understanding between stakeholders.

The Farming Tree model incorporates 6 socio-economic themes and 10 ecological themes (farm income, food with identity, animal welfare, farmer-citizen, farmer-value chain, job satisfaction, soil, plant health, food production, energy, water, nutrients cycle, material recycling, air quality, biodiversity, regional

landscape). The depiction of a tree was selected because a tree, like a farm, is location-based and on this location it is (socio-economically) rooted and the (ecological) branches reach out in its environment. A theme has a core sentence demarcating the focus of each theme and a short sentence describing the sustainability level that is looked for in this theme. Each theme has a list on a 1–10 scale describing the development of farm activities towards this high sustainability level. A higher score is depicted in the Farming Tree in a way that it signals longer roots or branches. The Farming Tree tool is designed to be independent of a specific sector or region.

A location-based approach differs from a product-based approach as found in most sustainability assessment schemes as it is highly relevant when comparing the sustainability level of a specific product (e.g. for a consumer in a supermarket). A location-based approach is, however, of added value for a farmer in assessing the socio-economic and ecological effects of the farm on the local situation, which is relevant in communication with stakeholders with a specific interest in the farm within its local context. In this situation the Farming Tree allows for an informed communication between stakeholders based on an independent assessment. While developing the Farming Tree tool, a range of tests with farmers and stakeholders in farm development on the effectiveness and usefulness of the tool was conducted. As the tool was in development not all themes and assessment schemes were in its final phase; it was however possible to test the tool on the suitability of the themes and the assessment approach with farming practice and the needs of (local) stakeholders with an interest in the socio-economic and ecological effects of farm management and strategy.

## Materials and methods

The chosen research design is exploratory, focusing on generating insights and ideas for theory or hypothesis development (Creswell *et al.*, 2017). This approach is useful when exploring questions about social processes (Swanborn, 2013), providing an opportunity to delve into insiders' perspectives and the approach fits the inductive nature of this research. For the purpose of this study individual farms were selected with a focus on a locally embedded farming strategy, either in the context of local food systems or local ecological benefits for biodiversity or regional landscape or a close relation to citizens in the region. Farmers were both tenant farmers on an estate and privately owned farms. The farmers were positive about the aims of the tool and willingly cooperated enriching the sample, yet were not involved in the development and future use of the tool, ensuring their independence. The research setting involved an explanation on the aim of the tool, a test assessment and an evaluation of the usefulness of the tool and the adjustments needed to improve the tool. This approach ensured the validity and reliability of the research design (Campbell *et al.*, 2013).

Semi-structured in-depth interviews were conducted with the farmers. The selected tenant farms were either part of an estate or tenants of a foundation managing in total ca 4500 ha of farming and nature land. In both cases the lessor of the tenant farms has an interest in the management of grasslands as it forms a vital part of the local ecology and history of the landscape. The individual farms were all to some extent interested in adapting the strategy in order to restore the ecological value of the grasslands and the quality of the social relations with clients and citizens in the region. The interviews were done using an interview guideline with open-ended questions (Braster, 2000). Besides a test with farmers, it was as well tested with students to assess a farm on the sustainability strategy.

## Results and discussion

The results are linked to strengths and limitations of the Farming Tree tool. Regarding the strengths the completeness of the tool in covering all socio-economic and ecological aspects of the farm was mentioned as well as the farm activities based qualitative assessment system. For some farmers it felt like a welcome escape from quantitative methods derived from calculations based on accounting systems, though

acknowledging the value of a quantitative approach. This combination allows for a complete view on the farm. A farmer specifically mentioned the usefulness in farm strategy development due to the overview of 16 themes with the connected activities. The Farming Tree allowed comparison of the sustainability levels of different farms. Often mentioned was the importance of socio-economic factors with an emphasis on the themes of Farm income and Job satisfaction.

On the limitations it was noted that a quite thorough understanding of farm practices is needed for the assessment of a farm using the Farm Tree tool. The study also revealed varying levels of interest and engagement with the Farming Tree as a tool for self-reflection and personal growth. While some participants found it invaluable as a 'mirror' to assess and improve themselves, others deemed it less relevant or too personal. It was clear that the tool could encourage self-reflection and intrinsic motivation.

## Conclusion

The Farming Tree tool received a positive feedback on its potential and usefulness in practice. Of specific added value is the completeness of the assessment tool in covering all aspects of the farm, including the economic results and job satisfaction of the farmer and employees. To be able to use the tool for a specific farm, a good knowledge of farm practices is needed. The Farming Tree tool offers a hands-on approach to evaluate and develop the farm strategy in all the relevant relations with the local context. The Farming Tree encourages self-reflection and intrinsic motivation with a specific niche in which it is of use. The tool allows for an assessment of the effects of changes in grassland management from a socio-economic and ecological perspective.

## Acknowledgement

The Farming Tree tool is developed in projects partly funded by Regieorgaan SIA, part of Dutch Research Council (NOW).

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# Carbon sequestration potential of permanent grasslands compared to grass-clover leys

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## Abstract

Grasslands are widely acknowledged for their ability to significantly increase soil organic carbon (SOC) stocks. However, studies on the long-term impact of permanent grassland on SOC levels compared to grass-clover leys are rare. This study aimed to quantify the SOC content and stock of permanent grassland (since 1993) and different proportions of grass-clover in the crop rotation (with a grassland proportion of 1/3 since 1987 and 2/3 since 2006). To achieve this objective, we exploited a long-term field experiment initiated in 1987, particularly focusing on SOC values of samples collected in 2022. The findings revealed a significant increase in SOC levels across all treatments compared to the initial SOC value (1.61%). Notably, the highest SOC was observed in the permanent grassland (2.04%) compared to 2/3 of grass-clover (1.87%) and 1/3 of grass-clover (1.72%). The SOC stock based on equivalent soil mass (SOC stock<sub>FM</sub>) was highest for the permanent grassland with 57.0 Mg C ha<sup>-1</sup>, moderate for 2/3 of grass-clover with 53.9 Mg C ha<sup>-1</sup> and the lowest for 1/3 of grass-clover with 49.8 Mg C ha<sup>-1</sup>. Our results may contribute to the saturation concept and suggest that permanent grassland may be used as the natural upper limit for SOC.

**Keywords:** grass-clover ley, permanent grass, soil carbon sequestration

## Introduction

Grasslands, as fundamental and integral components of mixed farming systems, stand out for their remarkable carbon storage capacity. The soil organic carbon (SOC) sequestration potential of increasing grassland proportion in the crop rotation is highly necessary for carbon accounting, validation of C models (Smith *et al.*, 2020) and advancing life cycle assessment (Knudsen *et al.*, 2019), thereby contributing to sustainable measures of agriculture. Hence, it is essential to explore the potential of crop rotations with varying grassland proportions and permanent grassland for carbon sequestration in the topsoil layer.

## Materials and methods

The grassland plots were part of the organic dairy crop rotation experiment which was initiated in 1987 at Foulumgaard Experimental Station (56°29' N, 09°34' E; elevation 53 m a.s.l.), Aarhus University, Denmark. The soil type is classified as Typic Hapludult with 7.7% clay, 9.9% silt and 82.1% sand in the topsoil (0–20 cm). The experiment has a pre-history of crop rotation dominated by cereal crops from 1978 to 1986. An initial soil sampling was done in 1986 prior to setting up the experiment. In 1987, a six-year rotation with two years of grass-clover was introduced. From 2006, the rotation was split into two crop rotations. Rotation 1 continued with 1/3 grass-clover, while Rotation 2 included four years of grass-clover in the six-year rotation (2/3 grass-clover). Each of the six fields in the two rotations was further subdivided into two blocks, where the selected plots were kept unfertilized. Each experimental plot had a neighbouring plot where unfertilized permanent grassland has been grown since 1993. More details about the experiments can be seen in Jensen *et al.* (2022).

In 2022, the soil was sampled in the 0–20 cm soil layer from the unfertilized plots in the two crop rotations and their neighbouring permanent grassland plots. The soil was air-dried, crushed, and sieved (<2 mm). The samples were analysed for SOC by dry combustion at 950°C using a Vario Max Cube. Additionally, three undisturbed soil cores with a volume of 100 cm<sup>3</sup> were extracted from the 6–10 cm soil layer in

the permanent grassland plots in 2022. The soil cores were oven-dried at 105°C until constant mass was reached. The bulk density (BD) was corrected for mass and volume of >2 mm particles. The SOC stock based on a fixed depth (SOC stock<sub>FD</sub>) and on a fixed mass (SOC stock<sub>FM</sub>) were calculated as described in Johnston *et al.* (2017). Linear mixed effect models were used to test the significance of treatments (1/3 and 2/3 of grass-clover and permanent grass) on SOC, SOC stock<sub>FD</sub> and SOC stock<sub>FM</sub> using the *lmer* function of the *lme4* package and the R-project software package Version 4.3.0 (R Foundation for Statistical Computing). Treatment and block were considered fixed effects, while Plot ID was considered a random effect. Post hoc comparisons were performed by use of Tukey's HSD test using the estimated marginal means (*emmeans*) function implemented in the *emmeans* package in R.

## Results and discussion

Changing from a cereal-dominated crop rotation to grassland proportions of 1/3, 2/3 and permanent grassland resulted in an increase in SOC (Figure 1). The average SOC content in 1986 was 1.61%. In 2022, the crop rotation with 1/3 grass-clover (since 1987), the crop rotation with 2/3 grass-clover (since 2006) and the permanent grassland (since 1993) had average SOC contents of 1.72, 1.87 and 2.04%, respectively.

The average BD in 1986 was 1.43 g cm<sup>-3</sup>. Bulk density was on average 1.45 g cm<sup>-3</sup> for grass-clover leys and 1.32 g cm<sup>-3</sup> for annual crops (Jensen *et al.*, 2022). Permanent grassland had the lowest BD with an average of 1.20 g cm<sup>-3</sup>. For robustness, the average BD values were used for calculating SOC stock<sub>FD</sub> and SOC stock<sub>FM</sub>. A significant effect of treatment on the SOC stock<sub>FD</sub> was found ( $F_{(2,30)} = 4.89, P < 0.001$ ). As illustrated in Figure 2a, the highest SOC stock<sub>FD</sub> was found for 2/3 grass-clover with 52.5 Mg C ha<sup>-1</sup>, a moderate level for permanent grass with 48.9 Mg C ha<sup>-1</sup> and the lowest for 1/3 grass-clover with 47.0 Mg C ha<sup>-1</sup>. As BD has changed since the initiation of the experiment, mainly due to tillage, and the soil has been sampled to the same depth, different weights of soils are compared, making the comparisons erroneous. Consequently, we calculated the SOC stock<sub>FM</sub> and found a significant effect of treatment ( $F_{(2,30)} = 16.6, P < 0.01$ ). However, the treatment differences were different when expressed on a fixed mass basis as compared to fixed depth (Figure 2). The SOC stock<sub>FM</sub> for 2/3 grass-clover (53.9 Mg C ha<sup>-1</sup>)

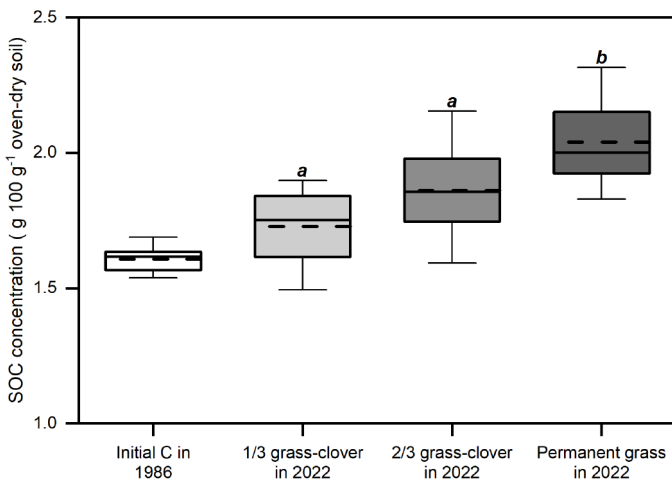


Figure 1. Soil organic carbon (SOC) concentration in 2022 for 1/3 and 2/3 grass-clover in a six-year rotation (since 1987 and 2006, respectively) being unfertilized ( $n = 12$ ) and permanent grassland since 1993 ( $n = 24$ ). The initial SOC in 1986 is indicated ( $n = 24$ ). Dash lines indicate mean values. Lines within the boxes represent median values, box boundaries include the 25<sup>th</sup> and 75<sup>th</sup> percentiles, and the whiskers extend from the box boundary to the 10<sup>th</sup> and 90<sup>th</sup> percentiles. Letters denote statistical significance between crop rotations and permanent grassland at  $P < 0.05$ .

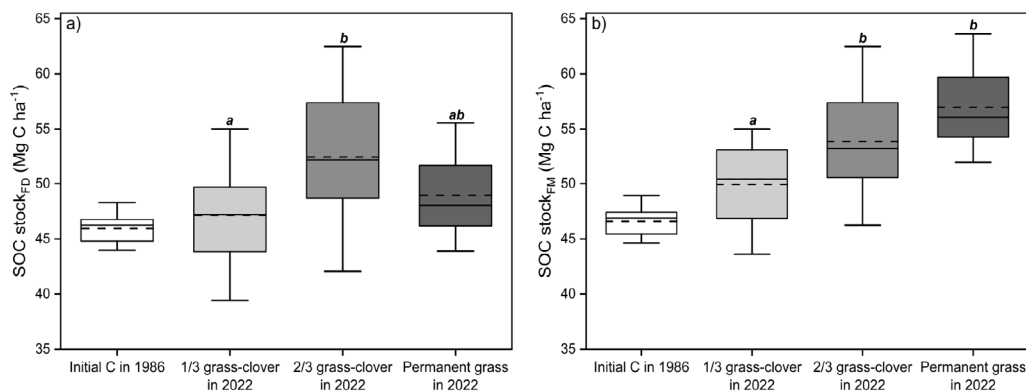


Figure 2. Soil organic carbon stock at the fixed depth (a) and at fixed mass (b) for two crop rotations from unfertilized plots ( $n = 12$  for 1/3 and 2/3 grass-clover) and permanent grassland ( $n = 24$ ) in 2022. Dash lines indicate mean values. Lines within the boxes represent median values, box boundaries include the 25<sup>th</sup> and 75<sup>th</sup> percentiles, and the whiskers extend from the 10<sup>th</sup> and 90<sup>th</sup> percentiles. Letters denote statistical significance between crop rotations and permanent grassland at  $P < 0.05$ .

and permanent grassland ( $57.0 \text{ Mg C ha}^{-1}$ ) was significantly larger than for 1/3 grass-clover ( $49.8 \text{ Mg C ha}^{-1}$ ), and permanent grassland represented the largest average SOC stock<sub>FM</sub> (Figure 2b).

## Conclusion

Changing the cropping system from a cereal-dominated to a crop rotation with 1/3 grass-clover, 2/3 grass-clover or permanent grassland, all left unfertilized, resulted in increases of 3.3, 7.2 and  $10.4 \text{ Mg C ha}^{-1}$  in the SOC stock based on equivalent soil mass (SOC stock<sub>FM</sub>). Including grass-clover in crop rotations or converting arable soil to grassland thus leads to SOC accrual and potentially contributes to climate change mitigation.

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# The present situation of grazing in Flanders

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## Abstract

Grazing in Flanders is under pressure. According to a voluntary survey among 127 dairy farms in 2022, 25% of dairy farms employ a zero-grazing system. Zero-grazing is more prevalent on larger farms with high production levels. According to the farmers, the main reason for grazing was the health and welfare of the cows. The survey revealed that the main reasons for using zero-grazing systems are: (i) fluctuations in rations, (ii) difficulties to combine with automated milking systems, (iii) limited size of the grazing area around the farm and (iv) a suboptimal use of the nutrients out of the animal manure. The argument for a limited amount of pasture per cow close to the barn is reflected in the key figures in the survey. Grazing farms had 7.9 cows ha<sup>-1</sup> versus 15.1 for the zero-grazing farms. If 10 dairy cows<sup>-1</sup> ha is considered to be the upper limit for 'meadow milk', the livestock density on the available grazing area on Flemish dairy farms emerges as an important bottleneck.

**Keywords:** survey, dairy, livestock density, pasture

## Introduction

Intensive grazing dairy systems have a higher sustainability performance than zero-grazing dairy systems (Meul *et al.*, 2012). Moreover pasture-based production systems are considered more environmentally friendly and have a higher animal welfare. Milk and dairy products from cows on pasture-based farms show a different nutrient profile compared to products from cows in zero-grazing systems. (Moscovici Joubran *et al.*, 2021).

During the past 20 years, the number of specialized dairy farms in Flanders has halved. Over the same period the average number of cows per farm has increased from 43 to 106, a 2.5 times increase in two decades (Departement Landbouw & Visserij, 2023). Flanders has one of the highest dairy cattle densities in Europe. Only in the Netherlands (1 ha<sup>-1</sup>) and Ireland (0.94 ha<sup>-1</sup>) are there more cattle per ha than in Flanders (0.92 ha<sup>-1</sup>) (Eurostat, 2023). For grazing, it is not the total area but the area adjacent to the dairy barn that is of importance. In Belgium there are no official statistics for pasture-based dairy production and the underlying reasons for grazing or confinement. Data were gathered to fill this gap in knowledge.

## Materials and methods

A survey was sent by mail to Flemish dairy farms. The on-line survey was developed using Google Forms and was conducted during spring 2022. On a voluntary base, 127 dairy farmers responded. They represent 2% of the dairy farmers in Flanders.

## Results and discussion

The survey revealed that 75% of the farms allowed their lactating cows to graze at pasture. This corresponds to a 62% share of dairy cows (Table 1. Characteristics for farms with and without grazing system (n=127)). Grazing is particularly prevalent on smaller farms at lower milk production levels (Table 1. Characteristics for farms with and without grazing system (n=127)), Figure 1).

Table 1. Characteristics for farms with and without grazing system ( $n=127$ ).

	Grazing farms	% or (range)	Zero-grazing farms	% or (range)
Number of farms	95	75%	32	25%
Mean farm size (number of dairy cows)	77.4	(22–450)	140.7	(60–330)
Total number of dairy cows	7357	62.0%	4502	38.0%
Total forage area (ha)	54.7	(2–150)	74.8	(10–180)
Stocking rate (cows ha <sup>-1</sup> )	1.94		2.12	

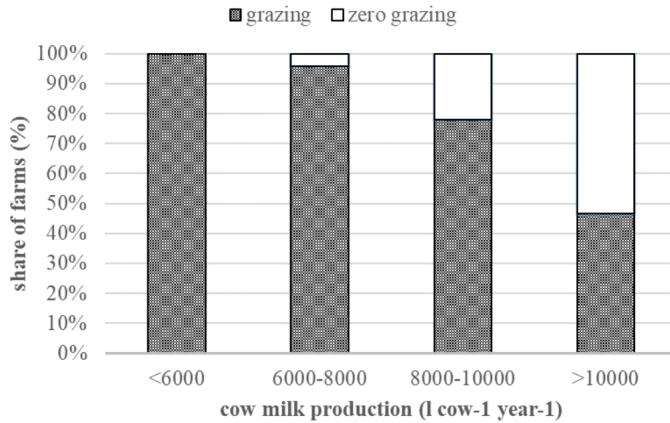


Figure 1. Distribution according to category of milk production cow<sup>-1</sup> year<sup>-1</sup> for farms with grazing and without grazing.

Earlier surveys in Flanders had indicated a decline in the proportion of dairy farms conducting grazing from 93.1% in 2006 to 84% in 2014 (Table 2. Figures on grazing in Flanders according to surveys over time).

Table 2. Figures on grazing in Flanders according to surveys over time

	2006 <sup>1</sup>	2007 <sup>2</sup>	2014 <sup>3</sup>		2016 <sup>4</sup>		2022 <sup>5</sup>	
			Grazing	Zero grazing	Grazing	Zero grazing	Grazing	Zero grazing
Number of survey respondents	600	187	145		58		127	
Grazing (% of dairy farms)	94%	93%	84%		60%		75%	
Area adjacent to the dairy barn (ha)	15.8	11.2	10	4.5	16.8	8.7	13.8	15.7
Mean number dairy cows per farm	55.1	62.7	63	122	70.5	113.4	77.4	140.7
Mean number dairy cows per ha area adjacent to the dairy barn	3.5	5.6	8.1	14.3	5.9	14.8	7.9	15.1

<sup>1</sup> Ryckaert I., *Enquête beweiding in Vlaanderen*, unpublished report (2017).

<sup>2</sup> Schellekens *et al.* (2008).

<sup>3</sup> Landbouwcentrum voor Voedergewassen, unpublished report (2014).

<sup>4</sup> Landbouwcentrum voor Voedergewassen, unpublished report (2016).

Flemish dairy cows have considerably less access to pasture compared to the situation in the Netherlands where cows are allowed to graze on 83.9% of dairy farms (CBS, 2022). However, Flanders surpasses Germany, where only 15-40% of dairy farms operate pasture-based systems (Van de Pol-Van Dasselaar *et al.*, 2020).

The main reasons that farmers choose grazing are: (i) health and welfare of the cows (ii) fitting into the farm management (iii) good for the farm's image and (iv) lower feed costs. The main reason for using zero-grazing systems are: (i) fluctuations in feed rations, (ii) difficulties to combine with automated milking systems, (iii) limited size of the grazing area around the farm, and (iv) a suboptimal use of the nutrients of animal manure.

The argument of a limited amount of pasture per cow close to the barn is reflected in the key figures in the survey. Grazing farms maintained 7.9 dairy cows ha<sup>-1</sup>, while zero-grazing farms had a higher density with 15.1 dairy cows ha<sup>-1</sup> of potential grazing area (Table 2. Figures on grazing in Flanders according to surveys over time), a significant difference (Figure 2).

Notably, if 10 dairy cows ha<sup>-1</sup> is considered the upper limit for 'pasture milk'-label (Stichting Weidegang, 2019), the available grazing area on Flemish dairy farms emerges as a significant bottleneck.

## Conclusion

The survey sheds light on grazing practices in Flanders, with a growing prevalence of zero-grazing systems. While health and welfare of the cows is a priority, confinement is chosen for its efficiency and convenience. To maintain grazing on expanding dairy farms, efforts should be made to increase the potential grazing area through means such as land rearrangement.

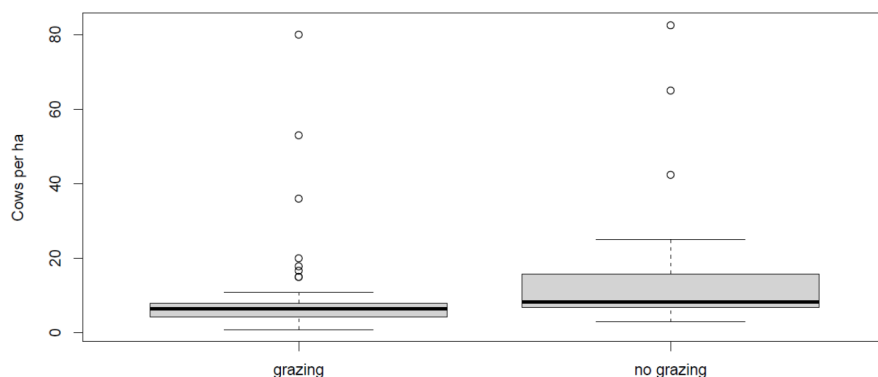


Figure 2. Box plot of cows per hectare (area adjacent to the dairy barn) for farms with grazing and farms without grazing. Pairwise Wilcoxon test:  $p=0.00072$  ( $<0.5$ ).

## Acknowledgments

The survey was part of the project ‘WeideWijjs’ a project of the EIP-AGRI Network that has become part of the EU CAP (Common Agricultural Policy) Network.

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# Effect of twice-daily Bovaer supplementation on enteric methane of grazing dairy cows

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## Abstract

Although 3-nitroxypropanol (3-NOP; DSM-firmenich, Kaiseraugst, Switzerland) has been proven to reduce enteric methane ( $\text{CH}_4$ ) by approx. 30% in total mixed ration (TMR) feeding systems, there has been limited research in grazing systems in which the predominant opportunity for supplementation is during milking. To investigate the effect of 3-NOP on  $\text{CH}_4$  of grazing dairy cows, a study was undertaken in which treatment (TRT) cows received 3-NOP twice daily during milking, while control (CON) cows received no additive. Methane was measured using GreenFeed units, and milk production, body weight, body condition score and dry matter intake were monitored. With the exception of  $\text{CH}_4$ , there was no effect of 3-NOP on any of the aforementioned parameters. Cows supplemented with 3-NOP produced 28.5% less  $\text{CH}_4$  in the 2.5 h after additive consumption but  $\text{CH}_4$  levels returned to that of the control thereafter. Future research should focus on extending 3-NOP efficacy using a slow release format or an out-of-parlour feeding system that allows animals to consume the product across the day.

**Keywords:** methane, dairy, grazing, feed additives

## Introduction

The conversion of human inedible protein, i.e., grass, into human edible protein, i.e., milk is the main competitive advantage of pasture-based systems (Hennessy *et al.*, 2021). Nonetheless,  $\text{CH}_4$  is released as a by-product of feed digestion (Danielsson *et al.*, 2017) and contributes to global warming. Anti-methanogenic additives may provide the solution to Ireland achieving emissions reductions targets of 25% by the year 2030, relative to 2018 levels (DAFM, 2022). 3-NOP has been proven to reduce  $\text{CH}_4$  by approx. 30% in TMR feeding systems (Melgar *et al.*, 2021), but its efficacy has not been tested in grazing systems where the main opportunities for supplementation are twice daily during milking. Further challenges associated with 3-NOP are that it is transient in nature (Reynolds *et al.*, 2014) and its efficacy is conditional on dietary fibre (Dijkstra *et al.*, 2018). The objective of the study was therefore to determine if 3-NOP, fed to grazing dairy cows twice daily during milking, was effective at reducing enteric  $\text{CH}_4$ .

## Materials and methods

Fifty-two spring-calving grazing dairy cows were assigned to a 10-week study evaluating 3-NOP efficacy. Cows were rotationally grazed day and night. Cows were blocked to TRT ( $n=26$ ) and CON ( $n=26$ ) groups using pre-experimental data which was collected for two weeks prior to the experiment. CON cows did not receive any additive and TRT cows received 3-NOP ( $80 \text{ mg (kg dry matter intake)}^{-1}$ ) at a rate of  $13.6 \text{ g cow}^{-1} \text{ day}^{-1}$ ;  $6.8 \text{ g}$  at morning and evening milking, respectively. Cows grazed in two groups, with an equal amount of TRT and CON cows in each group. Each grazing group had access to a GreenFeed (C-Lock; Rapid City, South Dakota, USA) which were swapped weekly to eliminate machine bias. CON cows received  $1 \text{ kg cow}^{-1} \text{ day}^{-1}$  concentrate ( $500 \text{ g}$  at AM and PM milking, respectively) through in-parlour feeders. To facilitate feeding 3-NOP, TRT cows were not offered any concentrate through the in-parlour feeders, and were instead offered  $500 \text{ g}$  of concentrate, formulated as a coarse ration for ease of mixing with 3-NOP, in the bailing unit on exit from the parlour at morning and evening milking, respectively. TRT and CON cows received up to  $1 \text{ kg cow}^{-1} \text{ day}^{-1}$  of concentrate in the GreenFeed. Cows were weighed and body condition scored weekly. Milk yield was measured daily,

and milk solids were measured weekly. Grass dry matter intake was estimated once using the *n*-alkane technique. Statistical analyses were conducted using SAS (version 9.4; SAS Institute, Cary, NC, USA). Cow was included as a random effect, while week was included as a repeated effect. Fixed effects included in the models were treatment, breed, parity and week. The corresponding pre-experimental values centred within breed and parity, and calving date were included as covariates. Spot measurements of CH<sub>4</sub> during the 3 h after additive feeding were extracted for analysis. In this model, interval since feeding nested within cow was a random effect, while the interaction between interval since feeding and week was a repeated effect. Fixed effects included in the models were treatment, breed, parity, interval since feeding and the interaction interval since feeding and treatment. Calving date and pre-experimental CH<sub>4</sub> centred within breed and parity were included in the models as covariates.

## Results and discussion

There was no effect of 3-NOP on milk production, dry matter intake, body weight or body condition score. For 2.5 h after consumption cows supplemented with 3-NOP produced 28.5% less enteric CH<sub>4</sub> than the control (Figure 1). When emissions across the 24 h period were considered, the reductions in daily CH<sub>4</sub> were 5.3%. Reductions in CH<sub>4</sub> were recorded within 30 minutes of additive consumption; these findings are consistent with research undertaken by Hristov and Melgar (2020) whereby 3-NOP acts promptly once introduced into the diet. As can be seen from Figure 1, CH<sub>4</sub> levels returned to that of the control after ~3 h, which was also reported by Reynolds *et al.* (2014) in a study in which 3-NOP was infused into the rumen twice daily.

It is speculated that the short-lived effect of 3-NOP is due to metabolism of the substance itself or the outflow of the additive in rumen fluid (Reynolds *et al.*, 2014). Although rumen passage rates were not measured in the present study, outflow rates of approx. 5 h are common in grazing cows (Dineen *et al.*, 2020), which exceed the approx. 3 h window of efficacy in the present study; the transient effect of 3-NOP is likely due to the rapid metabolism of substance itself. Findings presented indicate that while 3-NOP is effective in grazing dairy cows, technologies such as encapsulation (Beauchemin, 2009) or feeding 3-NOP at additional time-points during the day in out-of-parlour feeders (Van Wesemael *et al.*, 2019) may help to extend its efficacy.

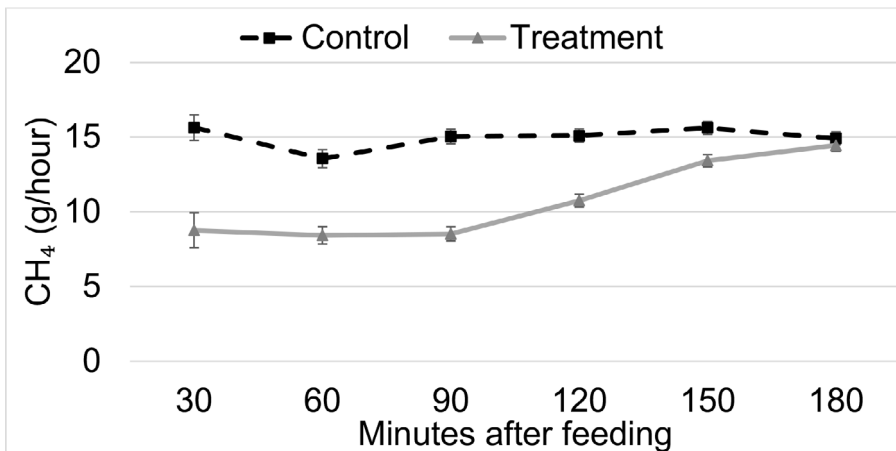


Figure 1. Least square means (standard error bar represents  $\pm 1$  SE unit) of the enteric methane emissions (CH<sub>4</sub>) in grazing dairy cows both with (Treatment) and without (Control) 3-NOP Bovaer supplementation in the three-hour period after additive feeding.

## Conclusion

The efficacy of 3-NOP in the 2.5 h after consumption was similar to that of previous studies in which the additive was present in the rumen throughout the day. However, 3 h after 3-NOP consumption, methane emissions had returned to the level of the control cows. Technologies such as encapsulation may enhance the efficacy of 3-NOP.

## Acknowledgement

The authors would like to acknowledge the support of Science Foundation Ireland (FarmZeroC project (18/FIP/ZE/7558P)) for their funding.

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# Soil nutrient concentrations reveal nutrient transfer by wild red deer from grassland to forest

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## Abstract

Maintaining plant diversity in grasslands often depends on nutrient-poor soil conditions. Grazing by large herbivores can lead to a net nutrient removal, but spatially clumped deposition of excreta involves net nutrient input. We hypothesized that free-ranging red deer (*Cervus elaphus*) foraging in grasslands transfer nutrients concentrated in excreta (phosphorus (P) and potassium (K)) to resting sites in forests. Therefore, differences in soil nutrient concentrations between habitats should become more pronounced with increasing red deer habitat use intensity. We collected 200 soil samples from a military training area in Germany, where red deer abound. Samples were taken in grasslands and forests on 32 linear transects. The sampling area comprised three zones differing in red deer use intensity. Linear mixed effects models affirmed the expected positive interaction between habitat type and red deer use intensity. In the zone most intensively used by deer, soil P concentration (resin-extracted) was significantly higher in forests than grasslands. Soil K concentration was significantly higher in forests for both intermediate and high red deer use intensity. The spatial behaviour of wild red deer thus contributes to maintaining low-nutrient conditions favourable to plant diversity in grasslands, while the nutrient transfer to forests enhances forest soil fertility.

**Keywords:** open habitat conservation, phosphorus, potassium, wildlife management, zoogeochemistry

## Introduction

Biomass accumulation and successive changes in plant community composition caused by eutrophication related to anthropogenic activities are among the main threats to plant diversity in semi-natural grasslands (EEA, 2020). Extensive livestock grazing is a successful approach to maintain nutrient-poor soil conditions favourable to plant diversity in open habitats (Uytvanck *et al.*, 2010). Where conservation management is difficult, e.g. due to restricted access, wild herbivores, particularly red deer (*Cervus elaphus*), can surrogate livestock and support open habitat conservation and grassland plant diversity (Riesch *et al.*, 2020). Previous research has shown that red deer grazing can lead to a net export of nitrogen (N) and phosphorus (P) from grasslands and heathlands because the animals remove higher amounts of nutrients by grazing than deposit with excreta (Riesch *et al.*, 2022). From livestock pastures, it is known that nutrients can accumulate in areas where animals rest (Koch *et al.*, 2018). We deduce that free-ranging red deer might transport nutrients from open habitats to forest and shrubs because they presumably prefer covered habitats for resting. This should manifest in a positive interaction between habitat type and red deer habitat use intensity, i.e. the concentration of nutrients returned via dung and urine (especially P and potassium (K)) in forest soil should increase compared to grassland with increasing red deer use intensity.

## Materials and methods

We collected soil samples to compare grassland and forest nutrient concentrations along a gradient of red deer habitat use intensity on the Grafenwöhr military training area (GTA), Bavaria, Germany (Natura 2000 site DE6336301, 230 km<sup>2</sup>). In the GTA, the targeted wildlife management by the Federal Forests Administration allows abundant red deer to forage in open habitats (Richter *et al.*, 2020). In an area of



ca. 800 ha within the GTA, we collected 200 soil samples on 32 linear transects running from grasslands to forest patches (100 m minimum distance between transects) in November 2021. The samples (10 cm depth) were taken at 4 m, 20 m and 50 m from the forest edge in both grasslands and forests. In 14 cases, grasslands were large enough to collect an additional sample at 100 m distance to the forest edge.

The sampling area was unevenly frequented by red deer, so that for analyses, we divided it into three zones of red deer use intensity (low, intermediate, high) based on information from local experts and telemetry data. For both soil P (resin-extraction) and K, we tested the effects of habitat type (open/covered) and distance to the forest edge in interaction with red deer use intensity, respectively, in a linear mixed effects model including transect ID as a random factor. The response variable was log-transformed. Nested models were compared using likelihood ratio tests to select the minimum adequate model (MAM).

## Results and discussion

For both soil P and K (Figure 1), the MAM contained habitat type and red deer use intensity and their interaction as fixed effects. Soil P concentration ( $R^2_{(m)}=0.24$ ,  $R^2_{(c)}=0.59$ ) was significantly higher in forest than grassland only in the zone where red deer habitat use was high (estimated marginal means forest: 29.2 mg P kg<sup>-1</sup>, grassland: 13.9 mg P kg<sup>-1</sup>,  $P<0.001$ ). Soil K concentration ( $R^2_{(m)}=0.36$ ,  $R^2_{(c)}=0.61$ ) was significantly higher in forest than grassland at intermediate (forest: 246.3 mg K kg<sup>-1</sup>, grassland: 101.9 mg K kg<sup>-1</sup>,  $P<0.001$ ) and high red deer use intensity (forest: 371.3 mg K kg<sup>-1</sup>, grassland: 118.2 mg K kg<sup>-1</sup>,  $P<0.001$ ).

If the differences in P and K concentrations between forest and grassland were similar across zones of differing red deer use intensity, our results would have only provided evidence for habitat-specific differences in soil nutrient availability. However, confirming a positive interaction between habitat type and red deer use intensity for both P and K, our results provide strong support to our hypothesis

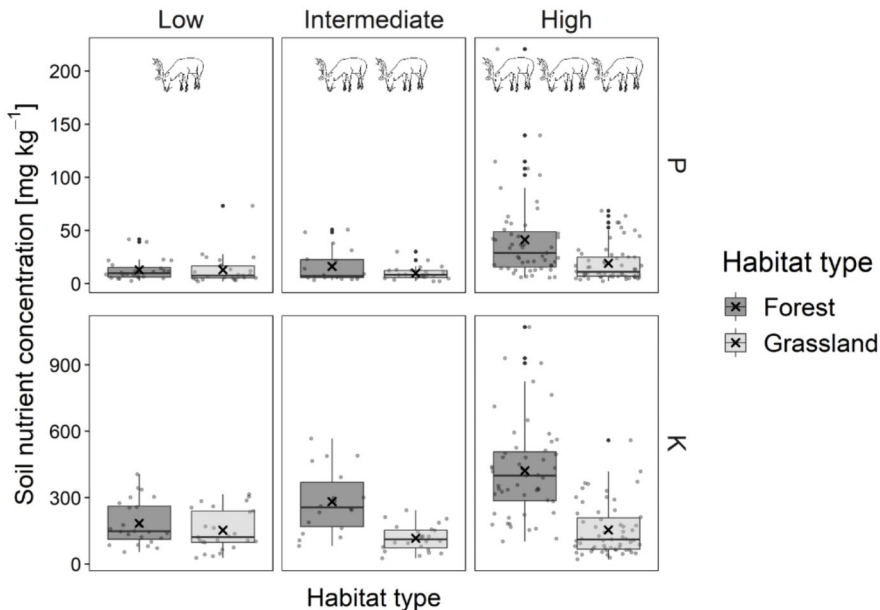


Figure 1. Concentrations (mg kg<sup>-1</sup>) of phosphorus (P) and potassium (K) in forest and grassland soil at low ( $n=49$ ), intermediate ( $n=42$ ) and high red deer use intensity ( $n=109$ ) in the Grafenwöhr military training area, Germany.

that the differences in soil nutrient concentrations are mediated by red deer. In addition, accelerometer data at 5-minute resolution from collared red deer in our study area (17 females and eight males during 2015–2022) confirmed that the animals spent more time resting and less time active in the forest (mean±SE proportion of active time  $0.33\pm 0.002$ ) than in open grassland (mean±SE proportion of active time  $0.56\pm 0.003$ ), which is likely to be associated with more excreta deposition in the forested habitat. Similarly, roe deer in an agricultural landscape in south-western France have been found to transfer significant amounts of N and P to forest patches because they prefer nutrient-rich crop fields for feeding but defecate and urinate preferably in the forest (Abbas *et al.*, 2012). Such herbivore-mediated nutrient subsidies can locally increase the concentration of plant-available nutrients in forest soils, which can benefit mature tree growth (Lucas *et al.*, 2013) and hence be potentially beneficial from a forestry perspective (Abbas *et al.*, 2012). For future studies, it will be interesting to address how the return of natural predators, such as the wolf in Europe, affect zoogeochemical processes related to wild herbivores (Monk and Schmitz, 2022).

## Conclusions

Extensive soil testing corroborated our hypothesis that wild red deer transfer nutrients from open to covered habitats, leading to significantly increased forest soil nutrient levels in zones with intensive red deer use. Consequently, in areas with focus on open habitat conservation, there might be a win-win situation: protected open habitat types could benefit from net nutrient removal by red deer while nutrient subsidies could raise soil fertility in forests.

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# Effects of ribwort plantain on agronomy, biodiversity, animal health and behaviour — a grazing study

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## Abstract

This exploratory study investigates the effects of including ribwort plantain (*Plantago lanceolata*, PL) on mineral content of the sward, soil structure, earthworms, number of flying insects, animal health, burden of intestinal parasites and cow behaviour during grazing. The experiment was carried out in two periods in 2021, namely in June/July and September/October. In both periods, the study involved four randomized groups consisting of eight pregnant heifers in a full-time grazing system. Two groups grazed on a mixture of perennial ryegrass (*Lolium perenne*, LP) with approximately 20% PL (LP-PL), while the other two groups grazed on a monoculture of LP. Over the study period (between June and October) the share of PL decreased from 21% to 15%. Herbage calcium, zinc, and copper concentrations were significantly ( $P < 0.05$ ) higher on LP-PL than on LP. The proportion of young roots and the earthworm biomass were significantly ( $P < 0.05$ ) higher for LP-PL than LP. There was no significant treatment effect on the abundance and diversity of aerial insects. Heifers showed statistically insignificant increased grazing time on LP-PL. Compared to LP, heifers grazing on LP-PL recorded significantly shorter rumination time ( $P < 0.01$ ). In September/October, the percentage of nematode egg shedding heifers was lower for LP-PL (12.5%) compared to LP (50%). Overall, while challenges in maintaining PL share were evident, this explanatory study suggests potential agronomic, nutritional and animal health benefits associated with LP-PL grazing, emphasizing the complexity of factors influencing pasture dynamics and animal behaviour.

**Keywords:** ribwort plantain, grazing, biodiversity, animal health, behaviour

## Introduction

There is growing interest in incorporating ribwort plantain (*Plantago lanceolata* (PL)) into productive grasslands due to its potential positive effects. These include enhanced nutritional value in terms of organic matter digestibility and mineral content and higher drought resistance (Pol *et al.*, 2021). Moreover, there are potential health benefits for cows, such as improved metabolism, diarrhoea inhibition, and antibacterial effects (Pol *et al.*, 2021). However, the bitter taste may negatively impact grazing behaviour and herbage uptake. Despite the potential positive effects, there is limited knowledge about the combined effects of perennial ryegrass — PL grazing on sward and soil properties, biodiversity indicators, cow health, and cow behaviour. Therefore, an exploratory grazing study with pregnant heifers was conducted in 2021 to assess the effect of LP-PL mixture compared to LP monoculture on mineral content of the sward, soil structure, earthworms, number of flying insects, animal health, burden of intestinal parasites and cow behaviour during grazing.

## Material and methods

The experiment was carried out at Dairy Campus in Leeuwarden, the Netherlands in two periods of six weeks in 2021, namely in June/July and September/October. In both periods, the study consisted of four randomized groups of eight pregnant heifers each, in a full-time grazing system with fresh grass only. Two groups grazed on a mixture of perennial ryegrass (*Lolium perenne* (LP)) with addition of approximately 20% PL (LP-PL), while the other two groups grazed on a LP monoculture.

To quantify the presence of plantain in the sward, visual observations were conducted, assessing the percentage of the soil surface covered with plantain and other species. Herbage samples were taken twice a week per treatment per replicate and were analysed for macro- and micronutrients (wet chemistry, EurofinsAgro, Wageningen, the Netherlands). In October 2021, visual assessments of soil structure and rooting were carried out on soil cubes measuring 20×20×25 cm (four per replicate treatment). Soil structure was assessed by estimating the proportion of soil crumbs, sub-angular block elements and angular blocky elements in the cubes. Rooting score was assessed by scoring visible root density (score 1–10; 1 for no roots and 10 for high root density), with an estimation of the proportion of young roots relative to the total. Additionally, earthworms were sampled in soil blocks measuring 20×20×20 cm, counted, weighed and classified according to functional groups. In July and September 2021, yellow sticky traps were placed in the field for 48 hours to assess the number and diversity of aerial insects in both systems.

Animal behaviour data were collected for each individual cow using the Cowmanager SensOor sensor and weekly observations. Parameters included grazing, rumination, activity, in-activity, high-activity and selective grazing behaviour. Animal health data was collected for each individual cow using general health and clinical observations and macroscopic cowpat observations and faecal sample collection for the determination of the faecal egg count.

All data were analysed using ANOVA to assess the effect of PL in the grazing sward. The model consisted of 2 treatments×2 replicates×2 rounds (not for soil measurements) and a random structure to account for (pseudo) replication within experimental units (8 heifers, 4 soil blocks) and repeated measures.

## Results and discussion

The share of PL decreased on average from 21 to 15% between June and October. It proved to be a challenge to keep the plantain in the sward during day and night grazing with heifers. Herbage calcium, zinc, and copper concentrations were significantly ( $P<0.05$ ) higher in the LP-PL mixture compared to the LP monoculture (Table 1). These higher mineral components of the LP-PL mixture might have potential benefits for cow health and milk composition.

There was no significant effect of sward type on soil structure. However, the proportion of young roots was significantly ( $P<0.05$ ) higher for LP-PL compared to LP (Table 1). Also, earthworm biomass was significantly ( $P<0.05$ ) higher for LP-PL compared to LP, but there were no significant differences in the earthworm abundance, or abundance of the different functional groups (data not shown). The increased proportion of young roots is in line with previous observations that PL generally shows faster and deeper rooting than LP (Pol *et al.*, 2021). However, within the short time span of the current experiment, this

Table 1. The effects of sward type (LP=*Lolium perenne*, PL=*Plantago lanceolata*) on selected herbage parameters, soil, biodiversity and animal behaviour including standard error (SE).

Parameter	PL-LP	SE	LP	SE	P value
Herbage calcium concentration (g (kg DM) <sup>-1</sup> )	11.9	1.2	6.0	1.2	0.009
Herbage zinc concentration (g (kg DM) <sup>-1</sup> )	39.0	1.6	31.1	1.6	0.016
Herbage copper concentration (g (kg DM) <sup>-1</sup> )	11.3	0.5	9.0	0.5	0.016
Proportion young roots (%)	35.6	6.8	20	4.7	0.033
Earthworm biomass (g m <sup>-2</sup> )	144	14.1	106	27.6	0.032
Rumination time (min)	311.7	4.78	346.2	4.78	0.007

had no significant impact on soil structure. The average number of insects per sticky trap in the first and second measurement rounds was 380 and 261, respectively. The presence of (flowering) PL had no significant effect on the number and type of aerial insects captured on sticky traps during the two measurement rounds (data not shown).

There was a statistically non-significant increase ( $P=0.137$ ) in the grazing time of pregnant heifers on LP-PL. Compared to LP, pregnant heifers grazing on LP-PL recorded significantly ( $P=0.007$ ) shorter rumination time (Table 1). The shorter rumination time on LP-PL can potentially be explained by the higher organic matter digestibility of LP-PL mixtures (Pol *et al.*, 2021). Observations indicated that the heifers tended to graze around PL in swards with flowering plants. In contrast, in swards with young short leaves, PL and LP were grazed simultaneously. The reasons for this behaviour, whether influenced by the smell and taste of young PL or the challenge of selection in shorter swards, remain uncertain. It is known that selective behaviour increases as grazing pressure decreases (Orr *et al.*, 2013).

In June/July no significant differences were observed in the faecal egg counts, whereas in September/October, significantly fewer ( $P=0.009$ ) animals (2 out of 16) grazing on LP-PL were detected with nematode eggs compared with animals grazing on LP (8 out of 16). The lower percentage (12.5%) of infected animals on LP-PL in September/October hints at potential positive health benefits. Overall, there was a low incidence of gastrointestinal worm infection, most likely due to the history of the fields combined with suboptimal weather conditions for infections to manifest.

## Conclusion

Overall, while challenges in maintaining PL share were evident, this exploratory study suggests potential agronomic, nutritional and animal health benefits associated with LP-PL grazing, emphasizing the complexity of factors influencing pasture dynamics and animal behaviour.

## Acknowledgements

We acknowledge the contribution of Bert Philipsen and Nick van Eekeren in the design of the trial, Gertjan Holshof in grassland management and Agnes van den Pol in connecting to education. We thank the interns Jente McGarvey, Madelon van der Have and Kevin Oldenwening for their work in sample collection and analyses, the Dairy Campus staff for their help in the execution of the trial and Tamara Wind and Joop van der Werf for contribution to the report. Johan van Riel and Waldo de Boer for performing the statistical analyses.

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# Mineral N fertilization rather than timing optimises P or N efficiency of slurry in grasslands

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## Abstract

Manures are potentially both a source of nutrients for plants and a source of pollution. Manure produced depends on animal densities and type rather than plants need. Over time, this has enriched soils with P and organic N. The challenge is maximal nutrient recycling and minimal pollution from the manure used for plant production. To investigate the optimal seasonal distribution of manure, field experiments were carried out in 2022 and 2023 on grassland in three agricultural regions in Norway. Three distributions of cattle slurry at 30 kg P ha<sup>-1</sup> were tested, with or without additional N fertilizer. These were compared with control treatments without slurry: no fertilizer, and compound NPK and NK fertilizers. Different distributions had little effect on grass yield and uptake of P and N. Applying a larger proportion of manure in spring increased grass yield, while additional mineral N fertilizer significantly increased yield but reduced N use efficiency. Slurry alone gave a P surplus, while added mineral N fertilizer allowed a net mining of P. There seems therefore to be a trade-off regarding whether the efficient use of N or P is to be prioritized. The decision should likely depend on required yields as well as local pollution risks.

**Keywords:** dry matter yield, N balance, P balance, field experiment, Norway

## Introduction

The amount of manure produced on farms depends largely on animal density, but also on production system and animal size. Over time, import of feed and fertiliser causes a net import of nutrients to the farm. Long-term application of manure has caused a build-up of soil P in areas dominated by animal husbandry (Øgaard, 2020). The national N surplus of agricultural areas in Norway, from all sources, is estimated to be 100 kg ha<sup>-1</sup> year<sup>-1</sup> (Hellsten *et al.*, 2019). Richardson *et al.* (2023) identifies the loss of both P and N as having high risk of negative long-term effects on the Earth system.

Timing of application is expected to impact N use efficiency of the slurry. Part of the mineral N in applied slurry will be lost as NH<sub>3</sub>, increasing with warmer and drier conditions (Sommer & Hutchings 2001). Mineralisation of organic N, both from manure and from soil, also increases with temperature.

Loss of P increases with P-AL, and manure P is more easily leached than mineral fertiliser P (Liu *et al.*, unpublished). Manure P is predominantly applied during the growing season, while the loss of dissolved P occurs mostly after the end of the growing season, when the runoff is greatest (Liu *et al.*, 2023). Soil P tends to become less susceptible to leaching with time (Amery *et al.*, 2021), and application earlier in the growing season may reduce P losses (Aronsson *et al.*, 2014).

An early application of manure may thus increase N use efficiency while reducing the risk of P losses. To investigate the optimal use of nutrients from cattle slurry, we established field trials investigating the nutrient use efficiency of different slurry distributions, with or without additional N fertilisation.

## Materials and methods

Field trials were carried out in 2022–2023 in established grasslands at three different sites representing different agricultural regions in Norway: conventional farms in Steinkjer in Trøndelag (63°56' N, 11°26' E) and Særheim in Jæren (58°45' N, 5°38' E) and on the Fureneset experimental farm on the West Coast (61°17' N, 5°2' E). At all sites, grasslands were cut three times a year.

There were three different distributions of slurry, all totalling 30 kg P ha<sup>-1</sup>. The distributions were: 2/3 in spring and 1/3 after 1<sup>st</sup> cut, 1/2 in spring and 1/2 after 1<sup>st</sup> cut, or 1/3 in spring, 1/3 after 1<sup>st</sup> cut and 1/3 after 2<sup>nd</sup> cut. The expected N effect of slurry for the grass was estimated to be 0.85 NH<sub>4</sub>-N in slurry+10 kg N ha<sup>-1</sup> for the subsequent cut, following recommendations of the local extension service. All distributions were applied both as slurry alone, and as slurry complemented with additional mineral N fertilizer (YARA Opti-NS 27-0-0) giving a total of 300 kg N ha<sup>-1</sup>. These treatments were compared to 300 kg N ha<sup>-1</sup> and 30 kg P ha<sup>-1</sup> in compound fertilizer (YARA Mila 18-3-15 and 22-2-12), 300 kg N ha<sup>-1</sup> without P ha<sup>-1</sup> in compound fertilizer (KCl, YARA Opti-NS and YARA Opti-NK 22-0-12), and unfertilized plots.

We sampled the soil of each plot at the start of the trial, and sampled manure each year. After each cut, plant samples were taken per plot and dried at 60 °C for 48 hours for determination of dry matter yield. A subsample from each plot was analysed by NIR for content of N (calculated as crude protein divided by 6.25) and P (Fystro & Lunnan, 2006). N use efficiency was calculated as N harvested N applied<sup>-1</sup>, using either total N applied or expected slurry N plus mineral fertilizer N. The P balance was calculated as P fertilized minus P harvested.

Statistical analyses were done in R (R Core Team, 2023), using generalised linear models to explain yield, N use efficiency and P balance, respectively as a function of treatment, location and year. Differences between treatments were tested using Tukey-tests in the emmeans package with a confidence level of 0.95 (Lenth, 2023).

## Results and discussion

Yield data, P balance and N use efficiency are presented in Table 1.

Table 1. Effect of different fertiliser treatments on dry matter yield, P balance and N use efficiency

Treatment	Dry matter yield (t ha <sup>-1</sup> )	P balance (kg ha <sup>-1</sup> )	N use efficiency	
			Harvested N total N <sup>-1</sup>	Harvested N expected N <sup>-1</sup>
Unfertilized	0.63 d	-15.8 b		
NPK	1.23 a	-2.4 c	0.89 a	0.89 b
NK	1.20 ab	-34.3 a	0.96 a	0.95 b
2/3–1/3–0	0.89 c	7.1 d	0.61 b	1.39 a
+mineral N	1.19 ab	-3.2 c	0.57 b	0.84 b
1/2–1/2–0	0.90 c	6.9 d	0.63 b	1.44 a
+mineral N	1.14 b	-1.9 c	0.58 b	0.86 b
1/3–1/3–1/3	0.87 c	7.6 d	0.61 b	1.38 a
+ mineral N	1.13 b	-1.7 c	0.57 b	0.83 b
Standard error	0.02	0.6	0.036	0.021

Slurry treatments are given in fraction of slurry applied for 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> cut respectively. +mineral N signifies the same slurry distribution as in the treatment above, but with additional mineral N fertilizer. Values sharing a letter are not significantly different ( $p=0.05$ ).

The different distributions of slurry were not significantly different from each other, and we did not find the expected benefits of application earlier in the season. Calculating the N balance from total slurry and mineral fertilizer N, will assume complete in-season mineralisation, resulting in no difference between slurry treatments with or without additional mineral N fertilizer. Using expected N effect assumes much slower mineralisation of organic N, which splits slurry treatments into two groups, where additional mineral N fertilizer lowers N use efficiency. Some of the unfertilised plots had a high clover content, as determined by NIR, hampering the use of the unfertilised plots as a measure of N mineralisation. More N applied increased dry matter yields, and therefore nutrient offtake of both N and P.

Soil P supply was apparently sufficient for plant growth, as the yields of NPK and NK were similar. P-AL ranged from 47 to 230 mg (kg dry soil)<sup>-1</sup>. The greatest net offtake of P was a result of omitting P application. Omission of P will be unrealistic for farm-wide on animal-producing farms, but may be applicable on certain fields with high P-AL. High yields, caused by high N applications, also allowed net offtake of P, though it was significantly lower. This allows both the continued utilization of manure as a fertilizer and a modest mining of P.

## Conclusions

Application of extra mineral N had a significant effect on the nutrient use efficiencies, while the different slurry distributions had not. High yields seem to allow reducing P content of soils while still allowing the application of manure. This required large amounts of N fertilizer, resulting in a lower N use efficiency. Whether the efficient use of P or N is to be prioritized, will likely depend on which nutrient will be the riskier pollutant locally, as well as the required yields.

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# Short-term biochar effects in cultivated forages: ecosystems services, soil characteristics, herbage yields, and nutritive value

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## Abstract

Via a series of laboratory, greenhouse and field trials in north-central Texas (latitude 32° north) we studied greenhouse or first-season, short-term effects of biochar type, dairy manure application, incorporation, and forage species on grass forage ecosystems services, soil characteristics, yields and nutritive values. Biochars were derived from wood, forage residues, dairy-manure and dairy-manure modified with calcium. Cool- and warm-season grass and legume forage species greenhouse studies indicate benefits to mitigating manure-derived pathogens and antibiotic residues. However, forage yields and nutritive values were sometimes negative (legumes) or neutral (grasses), especially at high biochar rates. Field forages included sub-tropical perennial hay, silage maize, and annual sorghum-Sudan hay. These indicated greater effect of incorporation and dairy manure than biochar type or loading rate, the latter having few short-term (first and second year) effects on either forage yields or nutritive values. Changes in some soil micro-nutrients and microbiome fractions were detected.

**Keywords:** engineered biochar, pastures, soil, herbage, tillage, manure

## Introduction

Biochar as a soil amendment to improve soil fertility and row-crop yields has been well documented as it improves soil moisture and nutrient retention as well as soil microbiome health (Kapoor *et al.*, 2022). Short-term (first growing season) biochar amendment efficacy for ameliorating pasture soil characteristics and increasing forage yields and nutritive values in warm climates, however, has not been widely tested and remains controversial. In temperate climates, research results indicate that initial soil fertility effects can be negative due to binding nutrients that then become unavailable to forage root uptake. Its eventual effects on subtropical herbage characteristics are particularly lacking. Environmental benefits, such as sequestering C or excess P (from manure), binding antibiotic residues and reducing the dangers of pathogens are even more poorly understood.

Our research group focused on engineered biochar produced from dairy concentrated animal feeding operation manure specifically for forage production. The objectives of a series of laboratory, greenhouse and field microplot studies, were to examine the short-term (first year following application) effects of biochar parent material, loading rate, soil tillage (tilled or surface application), manure addition, soil texture, and forage species responses benefits.

## Materials and methods

Soil nutrients were measured using Mehlich III (Mehlich, 1984), which generally reflects plant-available nutrients. Forage responses included routine mineral analyses (totals from combustion or inductively coupled plasma assays), fibre contents and *in vitro* rumen digestibility. Laboratory, greenhouse, and field microplot studies compared various factors related to biochar in south-central North America (Table 1). Pot studies looked at above-ground herbage and below-ground root dry matter yields and nutrient contents (Taggart *et al.*, 2023).

Table 1. Treatment summaries for various biochar and forage trials.

Trial	Species	Soil type	Biochar	Amendments
Greenhouse winter	<i>T. incarnatum</i> ; <i>L. multiflorum</i>	Sandy loam	Wood/manure (0, 2, 5 and 10%)	Dairy effluent
Greenhouse summer I	<i>C. dactylon</i>	Sandy and clay loam	Wood/manure (0 and 2%)	Dairy manure (0 and 2%)
Greenhouse summer II	<i>V. unguiculata</i> ; <i>C. dactylon</i>	Sandy loam	Wood/manure (0 and 2%)	Dairy effluent
Field microplots	<i>C. dactylon</i> ; <i>Z. mays</i> ; <i>S. bicolor</i> × <i>S. drummondii</i>	Clay and sandy loam	Wood (0, 5 and 10 Mg/ha)	Dairy manure (0 and 10 Mg/ha); NPK fertilizer
Field runoff	<i>Z. mays</i>	Sandy loam	Wood and calcium (0 and 10 Mg ha <sup>-1</sup> )	Dairy manure

All greenhouse and field experiments were multi-factorial and arranged in completely randomized block designs with at least three replications per treatment combination. Statistics included analyses of variance and least-significant difference multiple-mean separations using  $p \leq 0.05$  for significance.

## Results and discussion

Laboratory water and soil-column trials indicated that engineered biochar derived from either forages or manure partially neutralized residual manure antibiotics, *E. coli*, and diminished antibiotic resistant bacteria presence (Jan and Kan, 2019, 2022; Zeng and Kan, 2023). We are currently testing the hypothesis that these environmentally beneficial effects will also occur in field soils.

Feedstock had a strong effect on biochar effect on soil and herbage characteristics. When produced from manure (greater ash content) or saturated with manure effluent, biochar had a more positive or neutral effect on forage growth and nutritive values (Taggart *et al.*, 2023). Although we also found a negative effect on forage growth when applied to the warm-season legume *V. unguiculata*, regardless of manure BC was saturated or not. Wood-derived biochar, by contrast, contained greater amounts of C which raised soil cation exchange capacity and organic C content but tended to affect cool-season legume negatively when applied at high rates (10%).

In the greenhouse, cool-season legume (*T. incarnatum*), adding biochar at 10% decreased herbage and root mass accumulation while moderate rates (2%) had neutral effects, whereas in case of the warm-season legume (*V. unguiculata*) adding manure biochar or any biochar at 2% decreased herbage and root mass accumulation, respectively (Table 2). By contrast, the cool-season grass (*L. multiflorum*) responded positively to biochar addition up to 10%, as long as key soil nutrients such as N or P were not limiting, while the warm-season grass (*C. dactylon*) had a neutral response up to the highest load (2%).

Unlike manure application and soil texture (Hays *et al.* 2023), no effect, negative or positive, was detected in field studies for biochar at low (2% or 10 Mg ha<sup>-1</sup>) rates on forage nutritive values (macronutrients, micronutrients, fibre composition, and *in vitro* digestibility) or dry matter herbage yields (Cooper, 2023). This held true in greenhouse as well as field studies. Exceptions included decrease in herbage Fe uptake and an increase in Mg uptake when biochar was incorporated into the soil.

Biochar application increased soil microbial diversity, abundance, and functions (Obayomi, 2023). Microbial diversity, abundance and functions differed between wood and manure biochars. Manure biochar was associated with a more diverse microbial community and this correlated with its higher nutrient content. Increased biochar loading rate resulted in a microbial community shift towards alkaliphiles and copiotrophs. Biochar increased nitrogen and phosphorus metabolism genes but had

mixed effects on carbon and methane metabolism genes. Crop type did not affect microbiome diversity, composition, and functions.

## Conclusions

Our summary focused on short-term biochar effects on warm- and cool-season forage legume and grass responses to biochar from multiple trials. Pot studies and first-year field studies, however, do not necessarily predict medium or long-term effects. In short:

1. Warm and cold-season forage legume yields declined at high biochar rates.
2. At 10 Mg ha<sup>-1</sup> rates, biochar did not change forage grass yields or nutrient content.
3. Course soil characteristics changed more with biochar than finer soils.

Table 2. Summary of greenhouse trial results.

Parameter	Clay loam			Sandy loam	
	<i>C. dactylon</i>	<i>C. dactylon</i>	<i>V. unguiculata</i>	<i>L. multiflorum</i>	<i>T. incarnatum</i>
Forage yield	No effect	No effect	-1/2% manure BC (-44%)* -1/2% wood BC (No effect)	-10% blend or wood BC (+88%) -2/5/10% manure BC (+110%)	-5/10% blend or manure BC (-100%) -2/5/10% wood BC (No effect)
Soil NO <sub>3</sub> N	No effect	No effect	No effect	No effect	No effect
Soil total N	No effect	No effect	No effect	N/A	N/A
Soil P	No effect	-2% manure BC (+76%) -2% wood BC (No effect)	-1/2% manure or blend BC (+150%) -1/2% wood BC (No effect)	-2/5/10% blend or wood BC (+1702%) -2/5/10% wood BC (No effect)	-5/10% blend or manure BC (+1869%) -2/5/10% wood BC (No effect)
Soil oxidizable C	No effect	No effect	No effect	No effect	No effect
Soil total C	-2% BC (+24%)	-2% BC (+33%)	-2% BC (+39%)	N/A	N/A

\*Percentage changes compared to control with no biochar.

## Acknowledgements

Funding for this work: National Institute of Food and Agriculture, USDA grant numbers 2020-70001-31552, NR213A750013G032 and NR213A750023C001.

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# The effect of slurry-based intensive N fertilisation on yield and N leaching in a four-year-old ley

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## Abstract

On Finnish cattle farms, a common practice involves a four-year rotation (including the establishment year) of temperate grasslands and the use of slurry combined with mineral nitrogen (N) fertiliser. More knowledge is needed about the N fertiliser application response and N leaching risks in longer rotations with intensive slurry use. The yield response of a four-year-old timothy sward on mineral soil was studied in Central Finland using three slurry application strategies: no slurry; slurry for the second harvest; and slurry for the first and second harvest. Each of these strategies was supplemented with mineral N to achieve five soluble N levels ranging from 0 (or slurry N only) to 450 kg N ha<sup>-1</sup> year<sup>-1</sup>. Herbage DM yield and N balance were determined for each treatment. After autumn ploughing, a leaching experiment was conducted in a climatic chamber (SIMU) using soil monoliths from six of the treatments. The yield response to N fertilisers was slightly lower in the fourth year than in the earlier years. The amount of soluble N in fertilisation significantly affected yield and leachate N content, while the effect of slurry was minor on both.

**Keywords:** cattle slurry, grass, nitrogen, N leaching, N balance, silage

## Introduction

On Finnish cattle farms, it is a prevalent practice to implement a four-year rotation, including the establishment year, on intensively cultivated temperate grasslands, accompanied by the combined use of slurry and mineral N fertiliser. The risk for winter damage has decreased (e.g. Termonen *et al.*, 2020) allowing the elongated rotation to be used as a mitigation tool of greenhouse gas emissions. However, more knowledge is needed about the N fertiliser application response and N leaching risks in longer rotations that involve intensive slurry use.

## Materials and methods

The response of a grass sward to N application in four-year grass rotation was studied in Central Finland in 2022 in an experiment described by Termonen *et al.* (2022) (Site 1). The studied plant was timothy (*Phleum pratense* L.) cultivated on mineral soil (loam, SOM 3.4%). The experimental design was a split plot design with four replicates. The main plot consisted of three N fertilisation strategies: mineral fertilisation ('no slurry'); slurry (on average 29 Mg ha<sup>-1</sup> cut<sup>-1</sup>, 43 kg soluble N (Sol-N) and 93 kg total N (Tot-N) ha<sup>-1</sup>) for the 2<sup>nd</sup> harvest ('slurry'); and both for the 1<sup>st</sup> and 2<sup>nd</sup> harvests ('slurry+slurry'). The subplot consisted of five adjusted Sol-N (NH<sub>4</sub>-N+NO<sub>3</sub>-N) levels, which were used to produce annual Sol-N levels 0 (or slurry N only), 150, 250, 350, and 450 kg N ha<sup>-1</sup> year<sup>-1</sup>. Applied Sol-N was divided for three harvests using the ratios 0.44:0.36:0.20. DM yield, Tot-N balance and Sol-N balance were determined for each treatment. In October 2022, after ploughing, soil monoliths (Ø 15 cm, depth 40 cm) were lifted from the plots. In the experiment, the diurnal temperature pattern in the SIMU chamber was adjusted to -2°C at night and +10°C during the day. The amount of rain applied to monoliths was 120 mm during the 14-day test period. Tot-N, NO<sub>3</sub>-N and NH<sub>4</sub>-N were analysed from runoff water. The amount of nutrient loading (kg ha<sup>-1</sup>) was calculated to correspond to the average annual lysimeter runoff (244 mm) on the study site. Statistical analysis was done using the GLIMMIX procedure of SAS

9.4. Sol-N fertilisation (considered as a continuous variable), the main plot, and their interaction were fixed variables and main plot×replicate was a random variable. Depending on the variable, either a linear or quadratic model was used. Pairwise comparisons were calculated, depending on the variable, in Sol-N levels of 90, 150, 250, 300 and 400 kg ha<sup>-1</sup>. This part of the experiment was funded by the European Agricultural Fund for Rural Development ('Sustainability from grass' project).

## Results and discussion

In the fourth production year the maximum yield level of the 'no slurry' fertilisation strategy (9200 kg DM ha<sup>-1</sup> year<sup>-1</sup>, achieved at the Sol-N level of 347 kg ha<sup>-1</sup>) was lower than the corresponding yields in the same Sol-N level in previous three production years (10 350–10 440 kg DM ha<sup>-1</sup> year<sup>-1</sup>; data based on Termonen *et al.*, 2022). The yield level of timothy sward decreases as it ages, which is mainly due to winter damage (Virkakjärvi *et al.*, 2015). However, compared to the average yield level (5500 kg DM ha<sup>-1</sup> year<sup>-1</sup>; Virkakjärvi *et al.*, 2015) of dairy farms in Finland, the yield level can still be considered relatively high. The yield response of N fertilisation was strong, but the difference between fertilisation strategies on total yield was negligible at Sol-N levels of 150, 250 and 400 kg ha<sup>-1</sup> (Figure 1a).

However, the slurry application in the 2<sup>nd</sup> cut decreased yield by 13–22% compared to a 'no slurry' strategy ( $P < 0.05$ , Table 1). This DM yield decrease was compensated for in other cuts by the residual effect of slurry. Slurry application consistently increased the Tot-N balance (Figure 2a, Table 1). However, the Sol-N balance was moderate without relevant differences in practice between fertilisation strategies (Fig. 2b, Table 1). Balances were not higher than in the previous grass years (Termonen *et al.*, 2022). In the leachate, increasing Sol-N had a significant effect on the Tot-N (ca. 82% NO<sub>3</sub>-N) content (Figure 1b). The average concentration of Tot-N in the different fertilisation strategies at a Sol-N level of 300 kg ha<sup>-1</sup> was 10.5 mg N l<sup>-1</sup>, while in the Sol-N level of 90 kg ha<sup>-1</sup>, the concentration was only 4.5 mg N l<sup>-1</sup>. Calculated for 244 mm annual runoff, this would lead to approx. 11 kg leaching from the Sol-N level of 90, approx. 15 kg from the Sol-N level of 150, and approx. 26 kg Tot-N ha<sup>-1</sup> from the Sol-N level of 300 kg ha<sup>-1</sup>. In this study, N leaching was moderate compared to previous field studies (Valkama *et al.*, 2016), probably due to the monolith method with short experimental period, which does not provide a straightforward comparison with annual leaching. The use of cattle slurry had little effect on leaching, but the explanatory factor was the amount of Sol-N fertilisation.

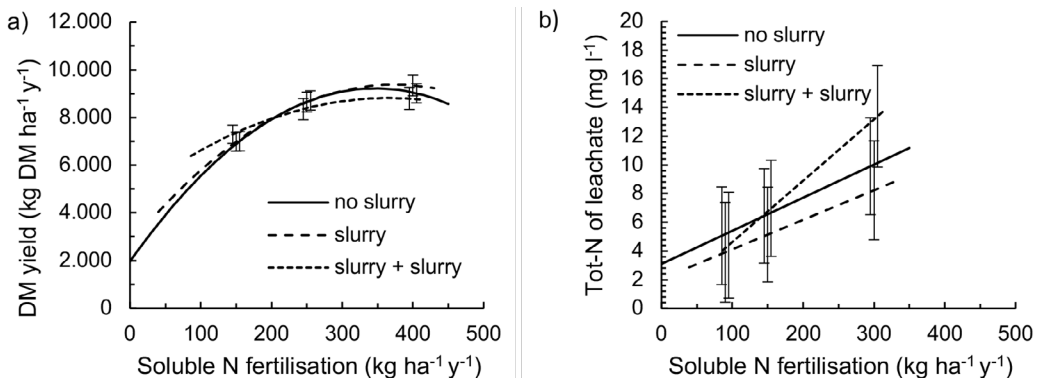


Figure 1. (a) Total yields (kg DM ha<sup>-1</sup> year<sup>-1</sup>) in 2022. (b) Tot-N content of leachate mg l<sup>-1</sup>. No slurry=mineral fertilisation, slurry=slurry for the second harvest. Slurry+slurry=slurry for the first and second harvest. Soluble N=NH<sub>4</sub><sup>+</sup>-N+NO<sub>3</sub><sup>-</sup>-N. Error bars are 95% confidence intervals for soluble N fertilisation levels: (a) 150, 250 and 400 kg ha<sup>-1</sup> year<sup>-1</sup> and (b) 90, 150 and 300 kg ha<sup>-1</sup> year<sup>-1</sup>.

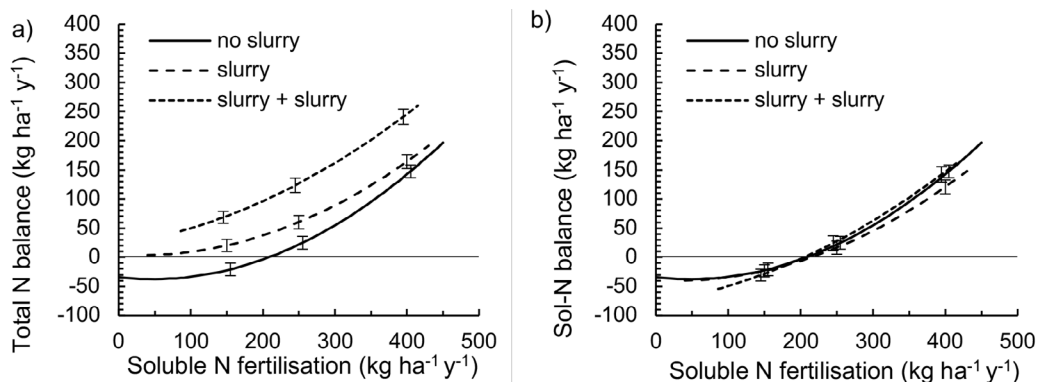


Figure 2. (a) Total N balance and (b) soluble N (Sol-N) balance ( $\text{kg ha}^{-1} \text{ year}^{-1}$ ) in 2022. No slurry=mineral fertilisation, slurry=slurry for the 2<sup>nd</sup> harvest. Slurry+slurry=slurry for the 1<sup>st</sup> and 2<sup>nd</sup> harvest.  $\text{Sol-N}=\text{NH}_4^+\text{-N}+\text{NO}_3^-\text{-N}$ . Error bars are 95% confidence intervals for Sol-N fertilisation levels 150, 250 and  $400 \text{ kg ha}^{-1} \text{ year}^{-1}$ .

Table 1. DM yield, N balances and Tot-N content of leachate at Sol-N levels 150 and  $250 \text{ kg ha}^{-1} \text{ year}^{-1}$  ( $54$  and  $90 \text{ kg ha}^{-1}$  for 2<sup>nd</sup> cut, respectively).

Sol-N	Unit	Sol-N $150 \text{ kg ha}^{-1} \text{ year}^{-1}$			Sol-N $250 \text{ kg ha}^{-1} \text{ year}^{-1}$		
		no slurry	slurry	slurry+slurry	no slurry	slurry	slurry+slurry
DM yield, total	$\text{kg DM ha}^{-1}$	6890 a	6980 a	7360 a	8660 a	8650 a	8390 a
DM yield, 2 <sup>nd</sup> cut	$\text{kg DM ha}^{-1}$	1600 b	1260 a	1380 a	2080 b	1780 a	1810 a
Tot-N balance	$\text{kg ha}^{-1}$	-22 a	20 b	71 c	22 a	60 b	127 c
Sol-N balance	$\text{kg ha}^{-1}$	-22 a	-23 a	-28 a	22 a	17 a	28 a

Means ( $n=4$ ) of each Sol-N level with the same letter are not significantly different at  $P \leq 0.05$ .

## Conclusion

The elongation of the rotation slightly decreased total DM yield in the 4<sup>th</sup> year but did not increase N balance. The leaching of N increased with the application of Sol-N fertilisation but the N concentration of leachate remained below the EU threshold for drinking water ( $11.3 \text{ mg NO}_3\text{-N l}^{-1}$ ) even when fertilised  $300 \text{ kg Sol-N ha}^{-1}$ . This indicates low N leaching risk with extended rotation when fertilisation is applied within currently allowed limits (max  $250 \text{ kg Sol-N ha}^{-1}$ ), even when using cattle slurry. The timing and method of slurry application and weather conditions (e.g. the timing of rains), can affect considerably the risk of N leaching.

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# Reed (*Phragmites communis* Trin.) hay as a potential feed source in drought

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## Abstract

A comparison of the yield and nutritional value of the main crops of common reed grass (*Phragmites communis*) and false sheep's fescue (*Festuca pseudovina*) was carried out in 2022 in Karcag, in a grassland with solonyec soil. In terms of yield indicators, the values for the reed were demonstrably higher than those for the simultaneously mown fescue at flowering. The results of the nutrient analysis showed higher N, P, K, Zn and Mn contents in the reed than in the fescue samples. However, the Ca, Mg, Na, Cu and Fe contents were higher in the fescue samples than in the reed samples. Our results can only be seen as a first step in refining of the value metrics for potential alternative fibre feeds.

**Keywords:** *Phragmites communis*, *Festuca pseudovina*, yield, nutritional value

## Introduction

The role of Hungarian grasslands as a fodder base is gradually declining due to the effects of climate change and the season-dependent forage mass yields of grasslands participating in environmental programmes and which are therefore unfertilized. Paradoxically, according to estimates by Tasi *et al.* (2014) 20% of domestic grassland is fallow, and a drought year can lead to dramatic shortages of dried roughage, as in the winter of 2022–2023. A temporary solution could be to collect low energy high fibre plants to meet the basic fibre needs of animals.

For centuries reeds have been valued as emergency fodder in years of drought and are considered a famine fodder for livestock production (Havel *et al.*, 2016). Instead of burning it, ancient farmers used reed beds in the saline lakes between the Danube and the Tisza, cutting reed above the water level and offering it to cattle. Ethnographic research in the plains also confirms that young reed-shoots, also known as *cillas*, helped a large number of livestock to survive the summer depression in grassland production and the winter fodder shortage (Györffy, 1922, 1941). Molnár and Csizi (2015) also refer to reedbeds growing on the flats of saline grasslands as “hidden fodder bases” in case of drought. They produce a very large amount of phytomass per unit area; therefore a considerable amount of carbon dioxide is also removed from the atmosphere (Clevering and Lissner, 1999). As *Phragmites* sp. belongs botanically to the Gramineae family, the possibility of alternative use as fodder has already been studied (Marks *et al.*, 1994; Nikolajevsky, 1971). Haslam (1973) points out that one of the great advantages of reed is that the nutrients stored in its rhizomes in spring give it an advantage over other grasses. Another great advantage is that, if no tussocks develop, a large mass of forage mass can be recovered from areas that are periodically waterlogged. However, Herodek *et al.* (2005) and Lukács (2009) draw attention to the deterioration of European reedbeds, the so-called clumped growth, that may arise from insufficient maintenance or sludge accumulation. Goman and Wells (2000) emphasise the role of reed clones adapted to a particular habitat to counteract climate change.



## Materials and methods

The study was carried out at the Hungarian University of Agriculture and Life Sciences Research Institute in Karcag on the 25<sup>th</sup> of May 2022 on the grassland (47°17'03.8" N 20°54'35.8" E). The altitude of the experimental area is 92-93 m a.s.l.. The 50-year average precipitation is 503 mm. The soil type of the study area consists of solonchec soils, and is used as a meadow, maintained by annual mowing, at the time of flowering. This is the usual field management. After mowing the main (first) crop the grass is grazed by sheep. It is a mix of grass with fescue as a dominant grass (*Achilleo setaceae–Festucetum pseudovinae* Soó (1933) 1947 corr. Borhidi 1996). Soil samples taken and analysed in the laboratory of the Research Institute show values of pH 4.6, humus content 5.7%, soil plasticity of Arany 56.1, nitrogen content 2.9 mg kg<sup>-1</sup>, phosphorus pentoxide 202.2 mg kg<sup>-1</sup>, potassium oxide 577 mg kg<sup>-1</sup>. Eight representative sampling points were used and the first growth of common reed (*Phragmites australis*) and of fescue (*Festuca pseudovina*) were cut at 5 cm stubble height (1 m<sup>2</sup> sampled). Samples were height on the spot (for green yield), natural dried (the samples were dried in a closed room for 1 week) for calculate hay yield and further analysed for dry matter, and crude protein. The plant tests were carried out in the accredited laboratory of the Research Institute of Karcag in 2022 according to MSZ-08-1783. The laboratory tested for nitrogen, phosphorus, calcium, potassium, zinc, sodium, magnesium, copper, iron and manganese in the plant samples. The data collected in the experiments were recorded and the results were processed using Microsoft® Office Excel (version: LTSC Professional Plus 2021). One-way analysis of variance (ANOVA) was used for statistical analysis of the data. Analysis of variance is used to determine whether there is a significant difference between the means of two groups. It is important to note, however, that this statistical analysis does not show where the difference between the means of the two groups lies. For the statistical evaluation were used at 5% significance level with the *P*-value.

## Results and discussion

The comparison of nutritional composition values in reed and fescue are presented in Table 1. Four yield indicators were tested in our experiment: fresh matter yield, hay yield, crude protein yield, dry matter yield. The fresh reed sample had a higher fresh matter yield (91.3% on average), hay matter yield (121.3% on average), crude protein yield (152.3% on average) and dry matter yield (120.3% on average) than the control grassland sample. Statistical analysis of variance showed differences in all 4 measures of yield. The dry matter content of the reed sample was on average 0.4 (m/m)%, nitrogen content on average 1.6 (m/m)%, potassium content on average 0.7 (m/m)% and manganese content on average 11 mg/kg higher than the control area sample. Statistical analysis showed a significant difference in the analysis of variance. Phosphorus content of the reed sample was on average 0.01 (m/m)% higher than the control area sample by an average of 0.4 mg (kg zinc)<sup>-1</sup>. Statistical analysis showed no significant difference in the analysis of variance. The calcium content of the reed sample was on average 0.2 (m m<sup>-1</sup>)% lower than the control area sample by an average of 0,075 (m m<sup>-1</sup>)% magnesium content by an average of 0.08 (m m<sup>-1</sup>)% sodium content by an average of 0.4 (m m<sup>-1</sup>)% copper content by an average of 62.8 mg kg<sup>-1</sup> iron content by an average of 45 mg/kg. Statistical analysis showed a significant difference in the analysis of variance.

## Conclusion

Our preliminary results indicate that reed hay is potential of interest as a source of feed. The regularly mown reedbeds that grow in the low-lying, waterlogged parts of our pastures can thus play an important role as a supplementary source of fodder in drought years, as they have done many times in the past. Nonetheless, further studies are necessary, namely intake studies, to determine the acceptability of reed hay by animals.

Table 1. Comparison of yield and nutritional composition.

	Reed	Control (Fescue)	P-value
Average fresh matter yield (kg ha <sup>-1</sup> )	17313	10148	0.0009
Average dry matter yield (kg ha <sup>-1</sup> )	7025	3502	2.3E <sup>-05</sup>
Average crude protein yield (kg ha <sup>-1</sup> )	20	9	3.7E <sup>-08</sup>
Average dry matter yield (kg ha <sup>-1</sup> )	7520	3766	2.5E <sup>-05</sup>
Average dry matter content ((m m <sup>-1</sup> )%)	93.4	92.9	0.0008
Nitrogen ((m m <sup>-1</sup> )%)	3.2	1.6	1.3E <sup>-11</sup>
Phosphorus ((m m <sup>-1</sup> )%)	0.3	0.3	0.4
Potassium ((m m <sup>-1</sup> )%)	2.5	1.8	1.9E <sup>-06</sup>
Calcium ((m m <sup>-1</sup> )%)	0.3	0.5	0.008
Magnesium ((m m <sup>-1</sup> )%)	0.1	0.2	0.01
Sodium ((m m <sup>-1</sup> )%)	0.1	0.4	1.7E <sup>-05</sup>
Zinc (mg kg <sup>-1</sup> )	12.5	12.1	0.4
Copper (mg kg <sup>-1</sup> )	28.8	91.5	0.0007
Iron (mg kg <sup>-1</sup> )	68.8	113.8	0.008
Manganese (mg kg <sup>-1</sup> )	35.6	24.6	0.03

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# How can algal extracts be used to help grasslands cope with climate change?

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## Abstract

In the context of climate change, the productivity and long-term persistence of grasslands depend on the resistance of grassland species to abiotic stresses. Applying biostimulants is one possible strategy for improving plant productivity and resistance to abiotic stresses. Our aim is to assess the ability of algal extracts to improve the drought resistance of perennial ryegrass (*Lolium perenne*), a major species in temperate European grasslands. Plants were grown under controlled conditions in a growth chamber. Three *Laminaria digitata* extracts, differing in their proportion of alginates, fucoidans, laminarans and mannitol, were sprayed on the leaves at three doses, seven days before irrigation was stopped. Drought resistance indicators were measured at the end of a 14-day drought period (water content, cell membrane stability, leaf osmotic adjustment, sucrose and fructan contents). Algal extracts improved the cell membrane stability and water content of leaf meristems, with a dose-dependent effect. Fucoidans and laminarans appeared as the most active compounds and high levels of alginates tended to reduce their activity. Further studies are needed to elucidate the underlying mechanisms. Field trials will be carried out to assess their biostimulant potential for improving the sustainability of grasslands in the face of climate change.

**Keywords:** perennial ryegrass, climate change, adaptation, drought, biostimulant, algae

## Introduction

The sustainability of grasslands in the face of climate change depends, among others, on the resistance of plant species to abiotic stresses. The use of biostimulants is one way of improving plant acclimation to stresses such as drought (Del Buono, 2021). Among biostimulants, brown seaweed extracts have been shown to improve the drought resistance of various species (Ali *et al.*, 2021), but studies on grasses are rare (Elansary *et al.*, 2017). Although alginates, fucoidans, laminarans and mannitol are known as bioactive compounds capable of triggering plant responses (Goñi *et al.*, 2018), the underlying mechanisms of action have not been fully elucidated, and this knowledge is needed to develop appropriate preparations. Our aim was to compare the ability of three extracts of the brown seaweed *Laminaria digitata* to improve the protection of leaf meristems during drought in *Lolium perenne*, a major species of temperate European grasslands.

## Materials and methods

Extracts of *L. digitata* containing contrasting proportions of alginates, fucoidans, laminarans and mannitol (Table 1) were obtained by successive filtrations on membranes. Plants were grown in a growth chamber in 11×11×20 cm pots filled with sand and perlite (50:50). Each pot was divided into four compartments (i.e. four replicates each containing 25 plants). Seven days before irrigation was stopped, the three extracts were sprayed on the leaves in three doses. Irrigation was stopped during 14 days and the 0-3cm of the shoot base containing leaf meristems was sampled to measure water content (WC), cell membrane stability (CMS) and the contents of fructans with degree of polymerisation (DP)<sub>≥3</sub> (fructans), DP<sub>3</sub> fructans (DP<sub>3</sub>), sucrose, glucose and fructose (Voltaire *et al.*, 2020). Leaf osmotic adjustment was estimated by the relative water content (RWC) of fully developed leaf blades.

Table 1. Quantities of alginates, fucoidans, laminarans and mannitol applied by foliar spray (in equivalent  $\text{g ha}^{-1}$ ) for each extract (A, B, C) and each dose (1, 2, 3).

Extract	Doses	Alginates ( $\text{g ha}^{-1}$ )	Fucoidans ( $\text{g ha}^{-1}$ )	Laminarans ( $\text{g ha}^{-1}$ )	Mannitol ( $\text{g ha}^{-1}$ )
A	1	10.1	0.78	0.070	2.61
	2	40.5	3.10	0.280	10.4
	3	101.1	7.75	0.700	26.1
B	1	10.1	0.20	0.040	0.53
	2	40.5	0.80	0.161	2.12
	3	101.1	2.01	0.402	5.30
C	1	1.47	0.78	0.016	0.00
	2	5.88	3.10	0.065	0.00
	3	14.7	7.75	0.161	0.00

## Results and discussion

Foliar spraying with algal extracts tended to limit the reduction in leaf meristem CMS and WC during drought (Figure 1A, B). The level of response varied from one extract to another and was dose-dependent. The greatest effects were observed with treatments A2, B3, C1 and C2. This result was confirmed with the principal component analysis (PCA) performed with all the parameters (Figure 2). The graph of individuals shows that these four treatments are separated from the control and from the other treatments (Figure 2B). The best protective effects (A2, B3, C1, C2) correspond either to high quantity of alginates ( $40\text{--}100 \text{ g ha}^{-1}$ ) combined with intermediate quantity of fucoidans ( $2.0\text{--}3.1 \text{ g ha}^{-1}$ ) (A2, B3) or to low quantity of alginates ( $1.5\text{--}5.9 \text{ g ha}^{-1}$ ) combined to low quantity of fucoidans ( $0.7\text{--}3.1 \text{ g ha}^{-1}$ ) (C1, C2). The active treatments correspond to low (C1, C2) or intermediate (A2, B3) quantities of laminarans ( $0.16\text{--}0.4 \text{ g ha}^{-1}$ ) and are independent of the presence of mannitol. The graph of variables (Figure 2A) shows that the indicators of drought resistance (CMS, WC, RWC) are not correlated with any of the quantities of the compounds studied taken independently, indicating complex interactions between the effects of each compound. Overall, fucoidans and laminarans appeared to be the most active compounds and high levels of alginates tended to reduce their activity. The graph of variables (Figure 2A) shows that CMS correlates strongly with the DP3:sucrose ratio (DP3:Suc), as previously observed in other grass species (Volaire *et al.*, 2020). This suggests that fine-tuning of soluble carbohydrate metabolism is necessary for membrane protection under dehydration in temperate grasses and that the DP3:suc ratio measured under drought may be an suitable indicator of drought resistance.

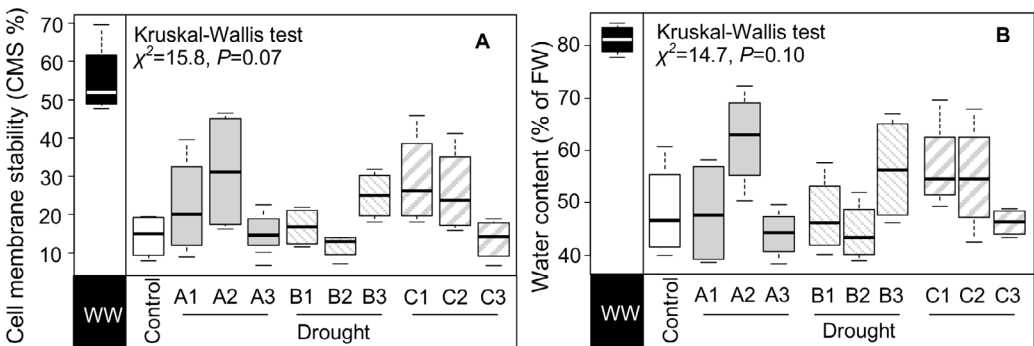


Figure 1. Cell membrane stability (CMS, A) and water content (WC, B) of leaf meristems in well-watered plants (WW) and in plants subjected to drought and sprayed with water (Control) or with the algal extracts (A, B, C) at three doses (1, 2, 3) ( $n=4$ ).

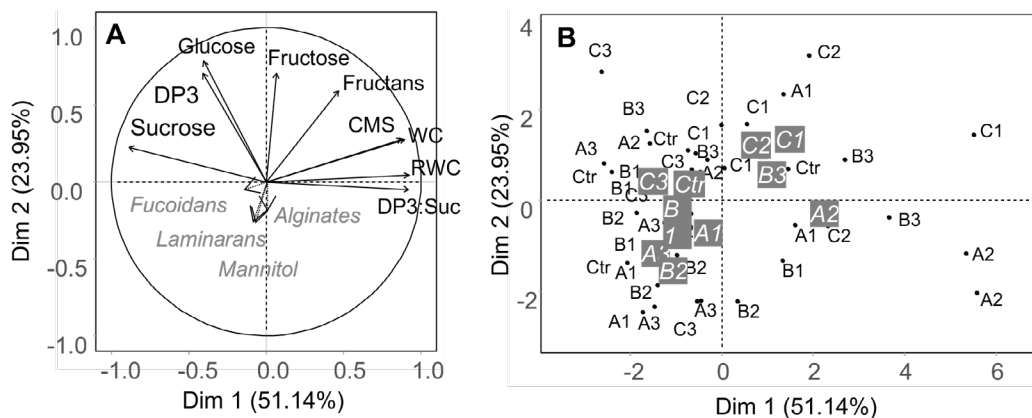


Figure 2. Principal component analysis with the parameters measured in plants subjected to drought sprayed with water (control Ctr) or sprayed with extracts A, B or C at three doses (1, 2, 3) ( $n=40$ ). (A) Graph of variables, (B) Graph of individuals. Quantities of alginates, fucoidans, laminarans and mannitol are used as quantitative explicative variables (dotted arrows in A) and treatments as qualitative explicative variables (grey squares in B).

Our results showed the potential of *L. digitata* extracts as a biostimulant to improve drought resistance in *L. perenne*. The most promising extracts and doses will be used to decipher the underlying mechanisms by transcriptomic analysis and for field trials to assess their potential for improving the sustainability of grasslands in the face of climate change.

## Acknowledgements

This work was funded by the University of Caen Normandy, the Regional Council of Normandy, the U.E. (EAFRD 2014-2020; BIOSTIM-ALMAP project) and the French National Technology Research Association (ANRT, CIFRE funding).

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# Carbon footprint of grassland systems on non-equilibrium soils in Uruguay

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## Abstract

Extensive livestock production systems are supported by grasslands, which play an important role in converting macronutrients, non-digestible to humans, into digestible protein of higher biological value. Non-equilibrated soils with managed grassland constitute a carbon sink commonly considered a parallel process outside the whole livestock production system. A soil that is not in equilibrium is when the carbon dynamics within the soil are still in a state of flux, and the system has not stabilized in terms of organic carbon content. Our aim in this exploratory study was to estimate carbon balance by non-equilibrated grassland soils, simulating different soil type scenarios in Uruguay where the animal is the main output of the system. Net greenhouse gas emissions (GHG) calculation follows a Life Cycle Analysis (LCA) methodology from cradle to the farm gate. The results allow us to demonstrate the value of the LCA approach to assess soil organic carbon (SOC) removals when an integrated system is properly defined. Additionally, the results indicate that the soil type is related to GHG balance and must be studied as a determinant factor of the carbon neutrality property of the whole system. They provide new starting points for advancing studies oriented to assess sustainable practices in livestock production.

**Keywords:** livestock, SOC, LCA, GHG removals, grassland

## Introduction

Grasslands are crucial in promoting sustainability. In Uruguay's historical context, grasslands have been abundant (Modernel *et al.*, 2016). FAO (Food and Agriculture Organization) reports (Opio and Oyhantçabal, 2017) indicate that around 75% of Uruguay's primary meat production takes place on grasslands. It is, therefore, imperative to assess the potential for environmental benefits of these systems, with a particular focus on greenhouse gas (GHG) balances. The potential carbon removal from soils, that are not in equilibrium, is intricately linked to the life cycle of animals grazing in such environments. Therefore, the aim of this work is to carry out an exploratory and hypothetical study using a partial Life Cycle Assessment (LCA) approach for livestock production in Uruguay based on the possible carbon footprint of livestock production if it were to be carried out on non-equilibrated soils. In soil that is not in equilibrium, the input, output, and transformation processes related to organic carbon are not yet balanced, leading to changes in carbon concentrations over time allowing carbon sequestration potential.

## Materials and methods

For the theoretical study, data were collected from a local facility and validated with the relevant bibliography (Picasso *et al.*, 2014), thus obtaining the consumption of materials associated with grazing in fields, such as fertilizers and pesticides. The emission factors related to emissions by the animal, such as enteric fermentation and manure management, were obtained from national data (Ministerio de Ambiente, 2021). For the full analysis, Picasso *et al.* (2014) provided the corresponding footprint of inputs. Livestock productivity values for typical grazing systems in the different regions of Uruguay were taken from Aguirre (2018). Pasture productivity data were taken from INIA (2022), in which each region of Uruguay was characterized according to the soil taxonomy described in Opio and Oyhantçabal (2017). In the removal quantification model, the soil was considered to be out of equilibrium due to a carbon deficit (i.e. in the removal phase). Therefore, it is in the removal phase. The quantification of

the removals is presented in Viglizzo *et al.* (2019), which represents the growth of the pasture and the consequent degradation of the root. A hypothetical case of a surface per head ratio of 1.4, considered a purely extensive system, was proposed. The functional unit was 1 kg of live weight (LW), and Fifth Assessment Report (AR 5) of the Intergovernmental Panel for Climate Change (IPCC) was used for the conversion factors of the Global Warming Potential (GWP).

## Results and discussion

The heat map for emissions from livestock production in different regions of Uruguay is presented in Figure 1, and on the right side is the corresponding boxplot for the country. There are variations of almost 20% around the average. The variations could be explained by the differences in livestock productivity between the different regions of Uruguay.

Figure 2 shows the quantities of emissions obtained based on the different types of soils found in Uruguay. The lowest values are present in crystalline and deep soils, related to those with higher productivity. Figure 3 shows the heat map for Uruguay of the GHG balance if grazing is carried out on non-equilibrated soils. It indicates the possibility of having regions with negative amounts if they are on this working hypothesis. Therefore, the potential of having systems with removals is evident if carried out on soils that have not reached their carbon stock balance, which is the theoretical case proposed in this work. An average value is seen around negative amounts. However, there are regions that, even if carried out on non-equilibrated soils, would not reach the possibility of negative GHG balances in extensive systems with a ratio of 1.4 ha head<sup>-1</sup>.

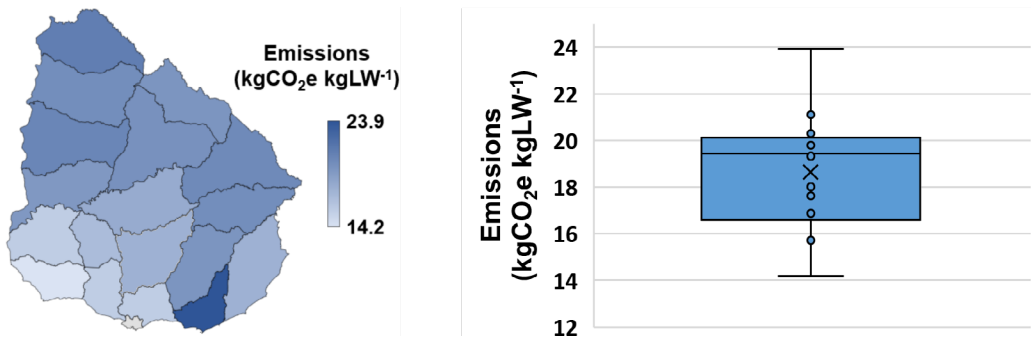


Figure 1. Heat map of livestock production GHG emissions by administrative region (left); boxplot of the country average GHG emissions for all evaluated regions (right).

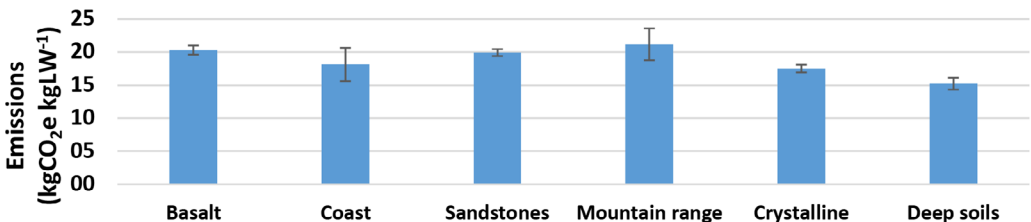


Figure 2. Emission values for different soil types in Uruguay.

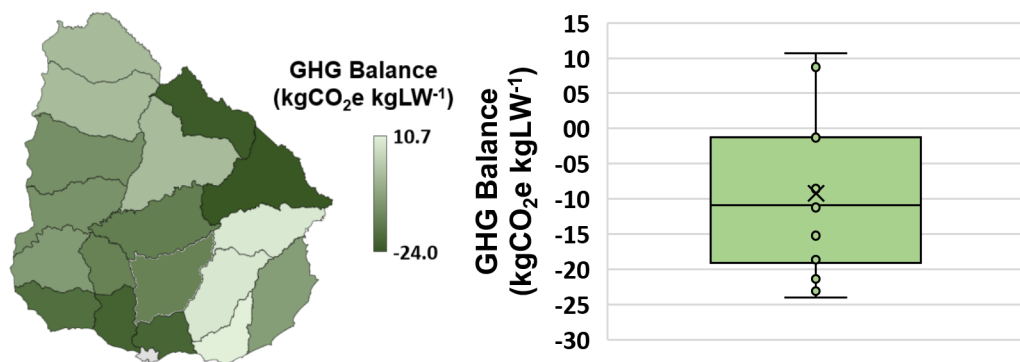


Figure 3. GHG Balance heat map for the hypothetical case of non-equilibrated soils by administrative region (left) and the corresponding balance boxplot of the country average GHG balance (right).

## Conclusion

Using the methodology described, it was possible to determine the impact of climate change, considering GHG removals from a pastoral system in a field using a partial LCA. The results show a potential negative GHG balance under the hypotheses of non-equilibrated soils and the soil types present in Uruguay. Under the same hypotheses, there are regions where it is possible to affirm that carbon neutrality cannot be achieved for the grazing system described. It is therefore highlighted that the analysis of soils in the different livestock production systems is relevant in a study of the impact of climate change, as this is a sensitive variable in the analysis. At the same time, due to the hypothesis adopted, it is essential to determine the conditions in the soil so that it is not in equilibrium, leaving this last point for future work.

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# Grazing system performance and environmental quality in relation to long-term stocking intensity

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## Abstract

The stocking intensity on grassland affects livestock performance, the greenhouse gas (GHG) emission and the soil organic carbon sequestration. For the present study, we used long-term data of the 'Forbioben' grazing experiment which compares three stocking intensity treatments. Grassland performance was calculated in terms of livestock unit grazing days (LUGD) and environmental quality in terms of soil organic carbon stock (SOC) changes and predicted area-related methane (CH<sub>4</sub>) emission in order to evaluate trade-offs from stocking intensity. Mean values of 411, 235 and 172 LUGD ha<sup>-1</sup> year<sup>-1</sup> resulted for the moderate, lenient and very lenient stocking intensities. The SOC stocks increased at an annual rate corresponding to 3.7 t CO<sub>2eq</sub> ha<sup>-1</sup> year<sup>-1</sup> irrespective of stocking intensity. Area-related methane emission increased with stocking intensity (between 1.54 and 2.65 t CO<sub>2eq</sub> ha<sup>-1</sup> year<sup>-1</sup>) which were obviously compensated for by carbon sequestration of >3 t CO<sub>2eq</sub> ha<sup>-1</sup> year<sup>-1</sup> under these conditions. More intensive low-input grazing does not compromise environmental quality in terms of climate protection goals.

**Keywords:** carbon sequestration, methane emission, herbage quality

## Introduction

Ruminants are considered to be a major contributor to greenhouse gas (GHG) emission through enteric methane (CH<sub>4</sub>) production. The sequestration of carbon dioxide (CO<sub>2</sub>) in the soil organic carbon stock (SOC) compensates emissions. The intensity of grazing is a key control variable for the SOC sequestration (McSherry and Ritchie, 2013), the CH<sub>4</sub> emission and also the grassland productivity (Grinnell *et al.*, 2023). Long-term data are needed to find the most suitable environmental-friendly grazing management for reducing trade-offs with productivity. The hypothesis is that grazing intensity supports grassland performance without trade-off for environmental quality in terms of SOC sequestration. For this, long-term real-world data are needed for evaluation of SOC trends.

## Materials and methods

The 'Forbioben' grazing experiment, located under temperate climate in Central Germany at the experimental farm of the University of Göttingen, in Relliehausen (51°46'56.3" N 9°42'11.6" E; 265–340 m above sea-level) was used for experimentation. The experimental site was established in 2002 and has been maintained in its current form since 2005. No fertilizer or lime was added to the experimental area for at least 30 years. The experimental setup represents a one-factorial randomized block design with three replicates comparing three stocking intensity treatments, i.e. moderate (M), lenient (L) and very lenient (VL) stocking, on nine 1-ha paddocks. The grazing management is based on continuous stocking. The treatment-specific stocking intensity is defined by a target compressed sward height (for details, see e.g. Grinnell *et al.*, 2023). Prior to and including 2004, VL was grazed with German Angus steers to a target compressed sward height of 12 cm. In the M and L treatments growing Fleckvieh steers were used. From 2005 onwards, paddocks were grazed by pregnant, non-lactating Fleckvieh beef cows. Data of live weight (LW) gain collected annually between 2002 and 2022 were used to calculate grazing system performance in terms of net grassland productivity (Grinnell *et al.*, 2023). The treatment

average daily LW gain per individual animal and the average annually accumulated area-related LW gain were calculated for each treatment from LW measurements using linear interpolation. Values were calculated per paddock and year as the cumulative number of days of grazing for all individual animals in a paddock adjusted to livestock units (LU, where 1 LU=500 kg LW) based on calculated LW and daily LW gain. The treatment-specific livestock performance is then expressed as annual stocking intensity in livestock unit grazing days (LUGD ha<sup>-1</sup>). Environmental quality was determined using in total 477 SOC stock data points collected occasionally between 2006 and 2022, sampled to depths of 10 and 15 cm, usually from pooled samples of multiple subsamples using a soil corer (2 cm diameter). A cumulative log-log model was used to calculate SOC stocks to an equivalent soil depth of 30 cm (Jobbágy and Jackson, 2000). Soil samples were dried, milled and analysed using elemental analysis of SOC. Soil bulk density was measured on several occasions. The herbage dry matter intake (DMI) was assumed constant at 7.1 kg day<sup>-1</sup> (Grinnell *et al.*, 2023). The DMI is required to estimate daily individual enteric methane emission (daily CH<sub>4</sub>) (van Lingen *et al.*, 2019) using mean values of the Neutral Detergent Fibre (NDF) concentration per stocking intensity treatment. The NDF concentration in organic matter was determined on manually sampled standing aboveground herbage biomass as obtained on 27 dates between 2019 and 2021. Samples were dried (60°C, 48 h), then milled (1 mm) and subsequently analysed using Near Infrared Reflectance Spectroscopy (NIRS) on a Phoenix 5000 SL (BlueSun Scientific, Jessup, MD, USA) by double-scanning each sample. The area-related CH<sub>4</sub> emissions were then calculated as a function of the daily individual CH<sub>4</sub> emission multiplied with the LUGD. Statistical analyses were done using linear-mixed effects models in R Studio with the stocking intensity, the year, and their interaction as fixed and interaction effects for the grazing system performance. The paddock nested in block was used as a random effect. A quadratic model was used to analyse the interaction of stocking intensity and year for the SOC sequestration. Year was treated as a numerical variable in this analysis. The resulting rate of increase in SOC per year was calculated and then multiplied with 44/12 (molar mass of CO<sub>2</sub>) in order to determine CO<sub>2</sub>-equivalents (CO<sub>2eq</sub>). The CH<sub>4</sub> emission was converted into CO<sub>2eq</sub> assuming a global warming potential of 28 over a 100-year period.

## Results and discussion

Grazing system performance was affected by the interaction of stocking intensity x year ( $F_{40,120}=8.1$ ,  $P<0.001$ ). Mean values of 410.9, 234.7 and 172.2 LUGD ha<sup>-1</sup> year<sup>-1</sup> resulted for the moderate, lenient and very lenient stocking intensities, respectively. Differences within year between the moderate and very lenient stocking intensities were always significant, while the difference between the lenient and very lenient stocking intensity was significant in 24% of the comparisons. The moderate and the lenient stocking intensities differed significantly in 71% of comparisons. The SOC stock increased between 2006 and 2022 at a rate of 0.70 kg SOC m<sup>-2</sup> year<sup>-1</sup>. The rate of increase corresponds on average to 0.37 kg CO<sub>2</sub> m<sup>-2</sup> year<sup>-1</sup> or 3.7 t CO<sub>2</sub> ha<sup>-1</sup> year<sup>-1</sup>. The linear and quadratic term for the year had significant effects on SOC whereas the stocking intensity or interactions with the stocking intensity had no significant effects (Figure 1). So far, it remains an open question whether the stagnation in SOC increase in recent years (Figure 1) was a result of drier climatic conditions or a sign of reaching an equilibrium in the soil. In total, between 1.61 and 2.65 t CO<sub>2eq</sub> ha<sup>-1</sup> year<sup>-1</sup> were released from CH<sub>4</sub> emission with greater values under more intensive stocking due to a larger cow number and longer stocking duration.

## Conclusion

Moderate stocking with on average 1.1 LU ha<sup>-1</sup> is beneficial for agronomic performance without consequence for environmental quality in terms of carbon sequestration. This intensity causes greater methane emission which may pose problems in cases where soil carbon stocks reach an equilibrium state.

## Acknowledgement

This study was financially supported by the DFG (FKZ 467394361).

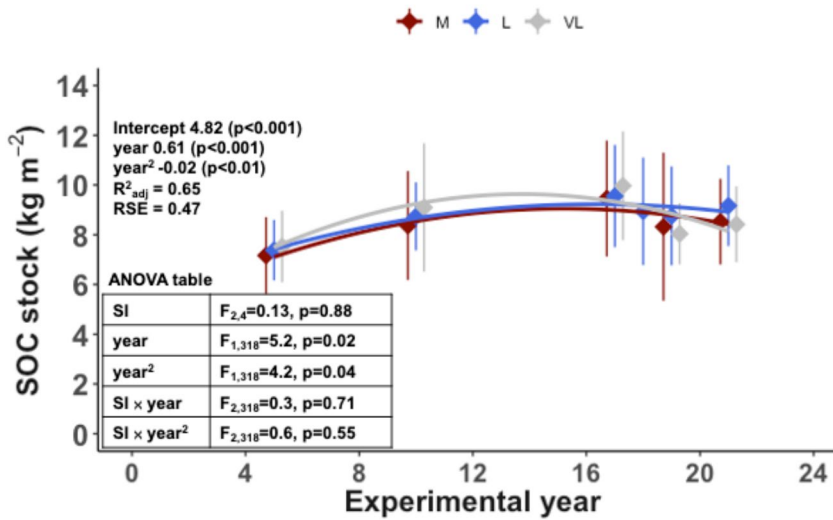


Figure 1. Mean temporal development of soil organic carbon stock (SOC) to 30 cm soil depth separated between stocking intensities as affected by the experimental duration (in years after start). Dots represent arithmetic means  $\pm$  SD. Continuous lines represent the fitted quadratic regression. The table in the lower part shows the ANOVA output of the linear mixed effects model with degrees of freedom,  $F$ - and  $P$ -value. M, moderate; L, lenient, VL, very lenient; RSE, residual standard error.

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# The Ossekampen Long Term Grassland Experiment; yield responses to temperature and precipitation surplus

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## Abstract

The Ossekampen Long Term Grassland Experiment was established in 1958 and consists of eight fertilizer treatments. Since its establishment the management has remained unchanged, with two cuts per year. We used the Ossekampen experiment dataset to assess the effects of temperature and precipitation surplus on annual yields and botanical composition. Yield data of the complete sward were available for all 63 years, while data of the functional groups were available for 40 years. Using a linear mixed model, we found that the dry matter (DM) yield increased with increasing temperature and increasing precipitation surplus. We found that grasses and herbs responded differently to temperature and precipitation surplus. Temperature did not affect the grasses, but only affected the herbs. Also, the yields of the herbs were less affected by drought than those of the grasses.

**Keywords:** long term experiment, nutrients, species-rich grassland, weather

## Introduction

The Ossekampen Long Term Grassland Experiment was established in 1958 on a species-rich old natural grassland on heavy clay soil near Wageningen in the Netherlands. The aim was to track changes in productivity and plant species shifts under long-term application of inorganic fertilizers and lime combined with an extensive mowing regime with a late first cut. The Ossekampen Experiment provides us with a unique opportunity to assess the long-term effects of different fertilization levels on species composition and forage yield. Previous analyses have demonstrated the effects of nutrient application on productivity and species richness (e.g. Korevaar and Geerts, 2015). We know that meteorological conditions influence grass growth, but most grassland experiments are too short to conduct a sound empirical study of this aspect. Therefore, we used the dataset of the Ossekampen experiment to assess the effects of temperature and precipitation surplus on annual yields and the contributions of functional groups.

## Materials and methods

The Ossekampen experiment consists of an unfertilized control and seven fertilizer treatments in two replicates on plots of 40 m<sup>2</sup>. Fertilizers were applied annually as a single nutrient: nitrogen (N), phosphorus (P), potassium (K) and lime (Ca) or in the combinations of PK, NPK1 and NPK2. The application rates (kg ha<sup>-1</sup> year<sup>-1</sup>) are 160 N, 22–33 P, 108–311 K and 357 Ca. The application rates of P and K are adapted to the expected offtake of a particular treatment, and thus vary per treatment. In the NPK1 treatment, 60% of the annual N rate is applied for the first cut, while in the NPK2 treatment all N is applied for the second cut. Since its establishment in 1958 the management has remained unchanged with two cuts a year, the first cut in July and the second cut in October. Botanical composition was assessed using the frequency analysis developed by de Vries (Korevaar and Geerts, 2005). We collected the following data from the period between 1958 and 2021: DM-yield (kg ha<sup>-1</sup> year<sup>-1</sup>) of total harvested sward herbage and the contribution of the functional groups grasses and herbs. The latter group also includes the legumes. More details on species and their development over time were published by Korevaar and Geerts (2015). Furthermore we recorded the average temperature (°C) and the accumulated precipitation surplus

(mm) between March 1 and September 30. The precipitation surplus was calculated as precipitation minus Makkink evaporation (Nagai, 1993). Yield data were available for all 63 years, while botanical composition data were available for 40 years. The responses of DM yields of the complete sward and its grass and herb components were analysed using a linear mixed model. The fixed effects included the factor fertilization and the variables temperature and precipitation surplus. The random effects took into account the experimental design and included a plot nested in a block effect. The analysis was carried out in R version 4.0.3. mainly using lme4, lmerTest and emmeans packages in addition to the base packages.

## Results and discussion

The average yield of both NPK treatments was  $8.9 \text{ t ha}^{-1} \text{ year}^{-1}$ , while the control, K and N treatments yielded in the range of  $4.6$  to  $4.9 \text{ t ha}^{-1} \text{ year}^{-1}$ . Intermediate yields in the range of  $5.6$  to  $6.1 \text{ t ha}^{-1} \text{ year}^{-1}$  were recorded for the Ca, P and PK treatments. The ratio of grass to herb content ranged from around 3.9 for the NPK treatments to around 1.5 for the other treatments. The DM yield of grasses ranged from  $2.4$  (control) to  $6.8$  (NPK)  $\text{t ha}^{-1} \text{ year}^{-1}$ , while the DM yield of herbs ranged from  $0.8$  (NPK) to  $2.2$  (Ca and PK)  $\text{t ha}^{-1} \text{ year}^{-1}$ .

The average temperature during the growing season varied from  $10.9$  to  $15.0^\circ\text{C}$  and showed an annual increase of  $0.03^\circ\text{C}$ , which in total gave an increase of around  $2^\circ\text{C}$  during the experimental period. The average accumulated precipitation surplus during the growing season varied from  $-211$  to  $273 \text{ mm}$ , but without a noticeable trend.

Besides the effect of fertilizer treatment ( $P < 0.001$ ), both temperature ( $P < 0.001$ ) and precipitation surplus ( $P < 0.001$ ) affected the total DM yield of the sward (Figure 1). The DM yield of the sward increased by  $353 \text{ kg } ^\circ\text{C}^{-1}$  and by  $5 \text{ kg mm}^{-1}$ . The DM yield of the grasses was affected by fertilizer treatment ( $P < 0.001$ ) and precipitation surplus ( $P < 0.001$ ), while temperature had no effect. The DM yield of the herbs was affected by the fertilizer treatment ( $P < 0.001$ ), temperature ( $P < 0.001$ ) and precipitation surplus ( $P < 0.001$ ). The response of the DM yield of grasses to drought was  $3.3 \text{ kg mm}^{-1}$  compared to  $1.6 \text{ kg mm}^{-1}$  for herbs. The grasses and herbs also responded differently to temperature. The DM yield of herbs increased by  $211 \text{ kg } ^\circ\text{C}^{-1}$ , while the yield of grasses did not respond to temperature.

For all response variables, we found no two-way or three-way interactions between fertilizer treatment, temperature or precipitation surplus.

Our results support other findings on the beneficial contribution of herbs to gain advantage from increasing temperatures or to mitigate the effects of increasing droughts (e.g. Grange *et al.*, 2021). The results are based on the first basic analysis of weather parameters on the yield of species-rich grassland. The analysis was limited to temperature and precipitation surplus and two large functional groups. Further work will address other weather parameters and also move from functional groups to the species level. We will also implement other statistical model that allow yield variance to be determined independently of yield level (Machtold *et al.*, 2023).

## Conclusions

In a long-term grassland experiment, we found that the total DM yields increased with increasing temperature and increasing precipitation surplus. Temperature only affected the yield of herbs. The DM yields of herbs were less affected by drought than those of grasses.

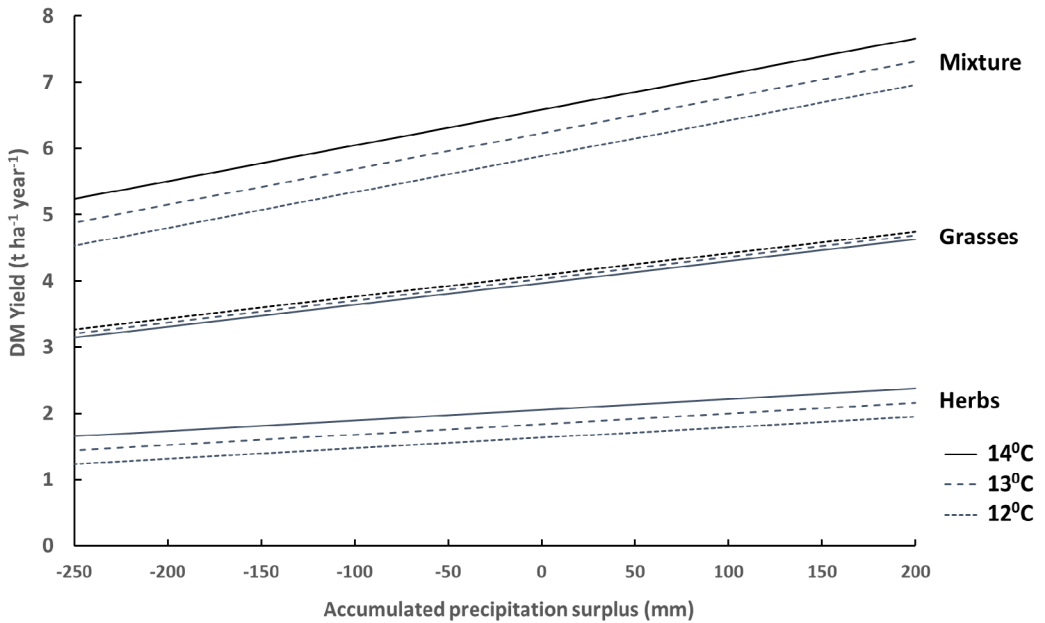


Figure 1. Predicted DM yields ( $\text{t ha}^{-1} \text{ year}^{-1}$ ) of the whole sward, and grasses and herbs in relation to precipitation surplus and temperature. The effects of precipitation surplus are significant for all components. The effects of temperature are significant for the sward and herbs, but not for grasses. Yields are the average results of all fertilizer treatments.

## Acknowledgement

This work was funded by the Dairy Systems IF program of Agrosystems Research and the European Union Horizon 2020 Research and Innovation programme (Grant Agreement 774124, 'Developing SUsustainable PERmanent Grassland systems and policies, SUPER-G').

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# Use of digestate from anaerobic digestion of dairy cattle slurry as fertiliser in maize crop

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## Abstract

There is an EU target to reduce the use of mineral fertilisers in agriculture by promoting precision farming and the use of organic fertilisers. Anaerobic digestion and mechanical separation of dairy slurry produces a solid digestate by-product that can be used as organic fertiliser on pastures and crops for animal feed, moving towards a circular economy. The aim of this paper was to evaluate the fertilising capacity of the solid fraction of fresh digestate (FD), and composted digestate (CD), and composted sewage sludge (CA) in comparison with inorganic fertiliser (C). These treatments were evaluated in a randomised complete block design with three replications. The trials started in the spring of 2023 with the sowing of forage maize (*Zea mays* L.) variety FAO 350. At harvest time (late September 2024), some maize plant characteristics differed between treatments, showing more advanced phenology, greater height, and higher cob production in digestate treatments, resulting in different yields and nutritional values between treatments. The results show that the use of digestate has no detrimental effect on yield, plant characteristics or nutritional value. Consequently, these results seem to indicate that anaerobic digestion of dairy slurry may be a viable technique to transform conventional farming.

**Keywords:** anaerobic fermentation, digestate, organic fertiliser, crop yield, circular economy

## Introduction

The goal of this work is aligned with the CAP policy to improve the circular economy in the agrifood chain through research, experimental development, and the transfer of acquired knowledge to farmers and the agro-industry. In this sense, anaerobic digestion and mechanical separation of slurry from dairy farms produces a solid digestate, a liquid fraction and biogas (Mao *et al.*, 2015). The solid digestate and the liquid fraction have a high fertiliser value and can be used as organic fertiliser or biofertiliser (Möller and Müller, 2012). Therefore, the use of these fractions can promote nutrient recycling and can be considered as a suitable option to replace the use of synthetic fertilisers, reducing their use, and avoiding the contamination problems due to concentration (Diéguez Santana *et al.*, 2022) heating, or agriculture through biogas or fertilizer production. However, attention must be paid to cheap fertilizers such as digestates because these digested fractions contain heavy metals, (nano. However, before expanding its use, the associated environmental risk due to excessive nitrogen fertilization into the soil and watercourses should be assessed (Nkoa, 2014). To achieve this objective, a maize crop fertilisation experiment was carried out comparing the use of a chemical fertiliser with various fertilisers of organic origin.

## Materials and methods

The study was carried out at the SERIDA Experimental Farm at Grado, Asturias, Spain (43°22'34.597" N, 6°4'10.261" W; altitude 65 m a.s.l.) from April to the end of September 2023. Based on the physico-chemical parameters of the soil analysed before beginning the experiment, it was defined as a non-saline soil with a clay loam texture, with high content of organic matter, nitrogen, and phosphorus, and low pH and potassium content. Three replicates of four adjacent plots of 16 m<sup>2</sup> each were used to test the different fertilisation treatments: conventional inorganic fertilisation (C), organic fertilisation with: fresh solid digestate (FD), composted solid digestate (CD), and composted sewage sludge (CA). The solid fraction of the digestate was obtained at the BioGAS<sub>Stur</sub> treatment plant (Navia, Spain), after an

anaerobic digestion process in a biodigester and a mechanical separation of the solid and liquid fractions using a screw system. Each plot was sown with forage maize variety FAO 350, at a seeding rate of 90 000 plants ha<sup>-1</sup>. A basal dressing at a dose of 150 kg N ha<sup>-1</sup> and 260 kg K<sub>2</sub>O ha<sup>-1</sup> was applied before sowing the maize with inorganic or organic fertilisers taking into account the content of nitrogen, phosphorus, and potassium, the soil fertility value and the fertiliser needs of the crop. There was no need to add P<sub>2</sub>O<sub>5</sub> because the soil was rich in phosphorus. The NPK content of all organic fertilisers is shown in Table 1. To cover the total N requirement, 41.9 kg per plot of FD, 13.8 kg per plot of CD, and 18.7 kg per plot of CA were applied. This also partially covered the K<sub>2</sub>O requirement. To complete the K<sub>2</sub>O requirement the plots were then supplemented with K<sub>2</sub>SO<sub>4</sub> until the requirement was met.

The crop was treated with a selective pre- and post-emergence herbicide (Wing P, BASF Española, Barcelona, Spain) for weed control, and a broad-spectrum insecticide (KARATE ZEON + 1.5 CS, Syngenta España, Madrid, Spain) for pest control. At harvest, forage yield data were collected from the middle two rows of maize in each plot, discarding the first two and last two plants in each row. The following variables were measured: total plant height, main insertion height of cobs, number of plants per hectare, and fresh and dry weights of cobs and foliage to determine dry matter (DM) yield per hectare. The greenery index, an index phenological status of the plant, was estimated on the basis of the DM content of the cobs and the percentage of foliage (Pedrol *et al.*, 2005). Samples were analysed to determine nutrient composition, digestibility and energy supply using NIRS. R Studio 2023.09.1+494 software was used to perform one-way ANOVAs and Tukey tests to know whether there were differences between the average variables of the plants in the four experimental treatments. Significance was set at  $P < 0.05$ .

## Results and discussion

Table 2 shows the results of the crop variables. Plant height showed a significant difference between treatments ( $P < 0.001$ ), with CA having the tallest plants. The trend of cob insertion height is higher in the CA than the C treatment ( $P < 0.1$ ). This means that the CA treatment promotes the overall height of the maize plant, which could affect its yield. There were no significant differences in forage yield, but the CA treatment had 5 Mg DM ha<sup>-1</sup> more than the other treatments. This contrasts with evidence of increased forage production with the use of organic fertiliser (Jiménez-Calderón *et al.*, 2018). Nutritional value was similar between treatments.

## Conclusions

The use of solid digestate, both fresh and composted, as fertiliser is a potential alternative to chemical fertilisers, because it has no adverse effects on yield, plant characteristics or nutritional value compared to inorganic fertilisers. Conversely, the use of composted sewage sludge has shown some positive effects on the physical characteristics of maize plants, and therefore its use as fertiliser should be further investigated.

Table 1. NPK content (nitrogen, phosphorus, and potassium, in (g (kg DM)<sup>-1</sup>), of fresh solid digestate (FD), composted solid digestate (CD), and composted sewage sludge (CA).

	FD	CD	CA
Nitrogen	25.3	31.5	23.1
Phosphorus	21.4	22.4	9.6
Potassium	13.0	9.0	16.6



Table 2. Mean values of different crop variables measured for each treatment: conventional inorganic fertilisation (C), organic fertilisations with fresh solid digestate (FD), composted solid digestate (CD), and composted sewage sludge (CA).

	C	FD	CD	CA	SD	P value
Plant height (m)	2.64 <sup>a</sup>	2.76 <sup>a</sup>	2.65 <sup>a</sup>	2.98 <sup>b</sup>	0.210	<0.001
Cob insertion height (m)	1.05 <sup>a</sup>	1.13 <sup>ab</sup>	1.12 <sup>ab</sup>	1.21 <sup>b</sup>	0.110	0.051
Plant weight (g plant <sup>-1</sup> )	166	167	172	238	35.5	0.132
Yield (Mg DM ha <sup>-1</sup> )	14.09	14.46	14.00	19.75	3.554	0.115
Foliage (%)	46.35	50.21	49.43	44.15	6.291	0.359
Cob (%)	53.65	49.79	50.57	55.85	6.291	0.359
Dry matter at harvest (g kg <sup>-1</sup> )	328.9	360.0	343.5	371.9	28.76	0.302
Greenery index	36	39	39	39	2.5	0.131
Organic matter digestibility (%)	77.05	73.45	75.27	75.02	2.165	0.259
Crude protein (Mg ha <sup>-1</sup> )	1.10	1.04	0.98	1.32	0.219	0.260
Starch (Mg ha <sup>-1</sup> )	5.13	4.89	4.67	7.17	1.584	0.186
Metabolizable energy (GJ ha <sup>-1</sup> )	169	163	162	229	43.7	0.177

SD, standard deviation; a, b, different letters in the same row indicate significant differences among treatments.

## Acknowledgements

Work funded by grants CPP2021-008651 (CERES Project) funded by MCIN/AEI/10.13039/501100011033 and by the “European Union NextGenerationEU/PRTR”, IDI/2021/000102 (NySA Group) funded by FICYT /AYUD/2021/51906 and “ERDF A way of making Europe”, and IDI/2021/000200 (Open Lab La Granja) funded by FICYT/AYUD/2021/57185 and “ERDF A way of making Europe”. María Campo-Celada is the recipient of grant PRE2021-100447 funded by MCIN/AEI/10.13039/501100011033 and by “ESF+”.

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# Is there a future for grassland on peat soils in the Netherlands?

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## Abstract

The Dutch peat meadow area covers about 185 000 ha. To meet the needs for modern dairy farming, ditch water levels were lowered in the 1960s and 1970s by up to 60 cm below the soil surface in the western peat meadow areas and by up to 120 cm below surface in Friesland. This resulted in accelerated peat oxidation and an emission by the 220 000 ha peatlands in agricultural use of 4.2 Tg CO<sub>2</sub> year<sup>-1</sup>. According to the Dutch Climate Agreement the annual CO<sub>2</sub>-emission must be reduced, with 1 Tg CO<sub>2</sub>-eq year<sup>-1</sup> in 2030. To reduce CO<sub>2</sub>-emissions, groundwater levels in summer must be raised considerably. This can be done by land-use change to wetlands or paludiculture or maintaining grasslands with high groundwater levels by irrigation or infiltration via submerged drains or other techniques. In our contribution we will compare the GHG reductions of the wet systems versus grasslands with tightly managed high groundwater levels to show that grasslands may be the best option to reduce GHG emissions before 2050.

**Keywords:** peat, meadow, rewetting, GHG, CO<sub>2</sub>, CH<sub>4</sub>

## Introduction

In November 2023 the European Commission, the European Parliament and the EU Council agreed on the Nature Restoration Law (NRL) (European Council, 2023) with targets to implement restoration measures for peatlands in agricultural use on at least 30% of peatlands by 2030 (with a quarter rewetted), 40% by 2040 (with at least a third rewetted), and 50% by 2050 (with at least a third rewetted). It is assumed that restoration and rewetting of organic soils in agricultural use will result in an important reduction of GHG emissions. Rewetting can range from reducing drainage, to full rewetting with the opportunity of paludicultural use, or the establishment of peat-forming vegetation. The extent of peatlands to be rewetted may be set lower by Member States only if duly justified, and if there are considerable negative impacts on public interests.

In our contribution we will also use first results of the Netherlands Research Programme on GHG dynamics in Peatlands and organic soils (NOBV: <https://www.nobveenweiden.nl/en/>). Papers and more in-depth analysis of the results of this research can be found on the website. The aim of our paper is to show that in the next decades GHG emissions of grasslands with managed high groundwater levels are likely to be lower than wet systems with inundation. Grasslands on peat soils therefore do have a future.

## Wet systems with inundation

Wetland restoration is often mentioned as a straightforward way to turn over CO<sub>2</sub>-producing grasslands into carbon sequestering fens and bogs. In the long run the CH<sub>4</sub> production of the wet systems will be fully compensated by their potential carbon sequestration. However, rewetting agricultural peat soils, including partly inundation, results in a boost of CH<sub>4</sub> and maybe also N<sub>2</sub>O. Antonijević *et al.* (2023) reported that the average CH<sub>4</sub> emission of an inundated grassland on a fen peat soil in North-East Germany during 14 years was 30 Mg ha<sup>-1</sup> year<sup>-1</sup> CO<sub>2</sub>-eq with a maximum of 83 Mg ha<sup>-1</sup> year<sup>-1</sup> considering a Global Warming Potential of 100 years (GWP100). With a GWP20, which is in better agreement with the target years 2030, 2040 and 2050, this is on average 91 and maximum 248 Mg

CO<sub>2</sub>-eq ha<sup>-1</sup> year<sup>-1</sup>. This is by far more than the average of 17.5 Mg CO<sub>2</sub> ha<sup>-1</sup> year<sup>-1</sup> of Dutch peat soils in agricultural use (Ruysenaars *et al.*, 2022). First NOBV measurements of CH<sub>4</sub>-emissions in natural vegetation sites ranged from 8 to 15 Mg CO<sub>2</sub>-eq ha<sup>-1</sup> y<sup>-1</sup> (GWP100) or 24 to 45 Mg CO<sub>2</sub>-eq ha<sup>-1</sup> year<sup>-1</sup> (GWP20). Uptake of CO<sub>2</sub> by the vegetation ranged from 3 to 15 Mg ha<sup>-1</sup> year<sup>-1</sup>, so in the long run there can be an equilibrium in GHG emissions; however, a newly created wetland with natural vegetation will have the first 20 years even without the boost-effect a net emission in the range of 9 to 42 Mg CO<sub>2</sub>-eq ha<sup>-1</sup> year<sup>-1</sup>. Therefore, Zak and McInnes (2022) advocate a more controlled and progressive ‘slow rewetting’ strategy as an alternative to spontaneous inundation of long-term drained peatlands or costly topsoil removal. Topsoil removal may reduce CH<sub>4</sub> and N<sub>2</sub>O-emissions, however, it is costly in money and CO<sub>2</sub>-emissions, and presents a problem of what to do with the nutrient rich 30 cm topsoil which represents a potential CO<sub>2</sub>-emission of about 2 Gg ha<sup>-1</sup> and has a high risk of nutrient leaching.

Experiments with wet agriculture (‘paludiculture’) in the Netherlands concern mainly the growth of typha (cattail). In most cases the topsoil is removed to limit CH<sub>4</sub> and N<sub>2</sub>O emissions and nutrient leaching. Up to now farmers do not consider it as an economically viable alternative for dairy farming. In the NOBV project first results of the GHG emissions of the two sites proved to be in the same range as the natural sites. At both sites the uptake of CO<sub>2</sub> was 11 Mg CO<sub>2</sub>-eq ha<sup>-1</sup> year<sup>-1</sup> without harvest taken into account. If GWP20 is considered then emissions will be 10 to 34 Mg CO<sub>2</sub>-eq ha<sup>-1</sup> year<sup>-1</sup>.

### Grassland with managed high groundwater levels

To derive high groundwater levels one can use irrigation or infiltration via high ditch water levels or narrow spaced trenches. In the NOBV project we used a water infiltration system (WIS) consisting of drain tubes every 4 to 6 metres at a depth of 70 cm below surface. CO<sub>2</sub>-emissions are continuously measured with closed chambers on 4 reference parcels and 6 parcels with a WIS. Three of the parcels have an Active WIS (AWIS) in which the infiltration drains are connected via a collector tube to a reservoir. The water level in the reservoir is managed to control the groundwater level. The target groundwater levels are respectively 20, 25 and 50 cm below soil surface. Measurements show that in very dry periods the deepest groundwater levels can become 20 to 25 cm lower than this target groundwater level. In 3 parcels the infiltration drains are connected to the ditch, so groundwater level is passively managed via the ditch water level (PWIS). Ditch water levels are 40, 45 and 50 cm below soil surface and the deepest groundwater levels in dry periods ranged from 50 to 76 cm below soil surface. The ditch water levels of the reference parcels are 45, 50, 45 and 75 cm below soil surface and the deepest groundwater levels in dry periods ranged from 49 to 114 cm below soil surface. Results are presented in Figure 1. The first measurement sites started in 2020, and in total we have 22 measurement years. We excluded 3 obvious outliers of which one with a negative NECB, so not visible in the figure. Results in Figure 1 show that a reduction to 1/3 of the average 17.5 Mg CO<sub>2</sub> ha<sup>-1</sup> year<sup>-1</sup> (Ruysenaars *et al.*, 2022) of Dutch peat soils is possible with AWIS. With PWIS the ditch water level has to be raised up to at least 20 cm or 30 cm below soil surface. In dry periods in summer this is possible without problems concerning trafficability and trampling. The net effect on grass production under fertilized conditions is relatively limited (Hoving *et al.*, 2024). CH<sub>4</sub> emissions proved to be negligible.

### Conclusion

Rigorous rewetting of grasslands on peatlands into wetlands may be quick and dirty and has a high risk of a boost of CH<sub>4</sub>-emissions, which may last for more than a decade. After that period CH<sub>4</sub>-emissions may still be high (a GWP20 of 9 to 42 Mg CO<sub>2</sub>-eq ha<sup>-1</sup> year<sup>-1</sup>). Therefore, for the short term, so before 2050, this may not be a good solution.

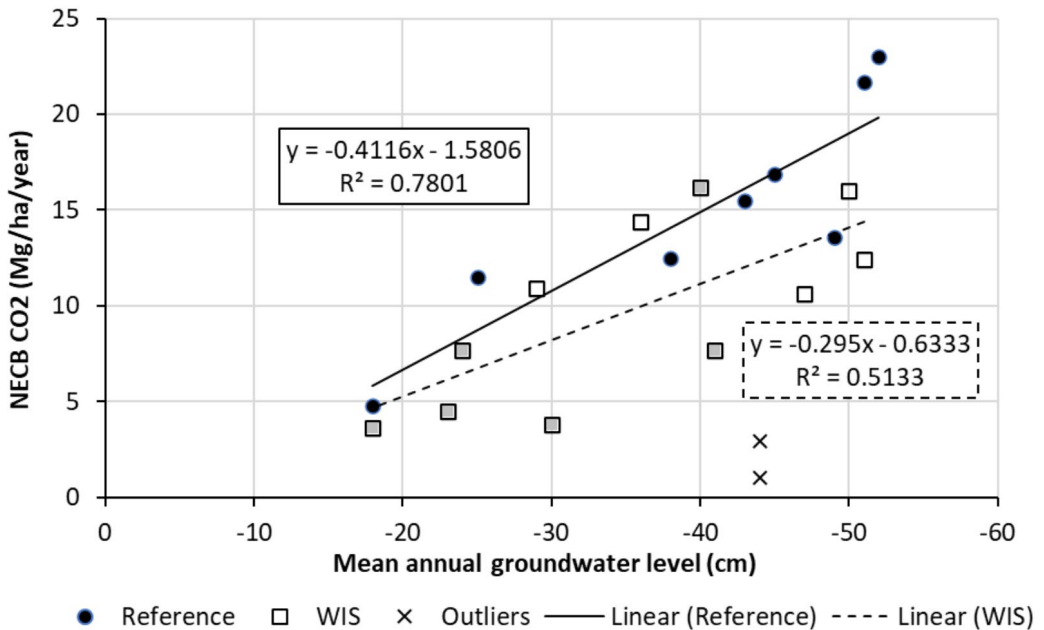


Figure 1. The net ecosystem carbon balance (NECB) versus the mean annual groundwater level. The AWIS are indicated with a grey square, the PWIS with a white square.

Paludiculture has more or less the same problem as inundation to create wetlands. The  $\text{CH}_4$  emission expressed in  $\text{CO}_2$ -eq (GWP20) is much higher than the uptake of  $\text{CO}_2$ . Therefore, paludiculture is also not the solution in the short term.

Raising groundwater levels in peat meadows with, among others, WIS may reduce  $\text{CO}_2$  emissions to about 1/3 of the  $\text{CO}_2$  emissions at this moment. This seems the best option up to now to realize a strong reduction of GHG emissions before 2050.

So at least for the next decade grasslands on peat soils do have a future in the Netherlands.

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# Trade-offs and synergies among ecosystem services in mountain pastures

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## Abstract

Mountain pastures cover one third of Swiss agricultural land. They provide forage for grazing livestock (provisioning ecosystem service (ES)), serve as carbon sink (regulating ES), offer a habitat for an outstanding biodiversity of plant and animal species including pollinators (supporting ES) and are important places of recreation, tourism, and identity (cultural ES). However, normally not each ES is provided at the same place to the same extent. Increasing a single ES sometimes leads to a decrease of others. Thus, we aimed at disentangling trade-offs and synergies among a bundle of ES relevant for mountain pasture ecosystems. Therefore, we measured six ES indicators: (1) forage quantity, (2) forage quality, (3) soil carbon stocks, (4) resources for pollinators, (5) flower colour abundance and (6) plant species richness in 66 plots at six mountain summer farms in the Swiss Alps. We found strong synergies among forage quality and quantity on the one hand, and pollinator resources, colour abundance and species richness on the other. However, there were clear trade-offs among these two groups. We conclude that there is no one-size-fits-all strategy to realise all ES at the same place, but the large variability of mountain pastures allows many ES to be realised at the farm level.

**Keywords:** conservation, ecosystem services, livestock management, mountain pastures, trade-offs, synergies

## Introduction

Mountains cover one third of the European surface area (Price, 2010). Often, grazing is the only agricultural option in these regions. In Switzerland, where our study was conducted, one third of agriculturally used lands are mountain summer pastures. Thus, these grasslands contribute substantially to food production (provisioning ecosystem service (ES)). However, they provide many other ES: for instance, in Switzerland, mountain pastures host 75% of all protected fens which are important sources of carbon storage (regulating ES). More than three quarters of all Swiss protected dry grasslands are placed within mountain pastures. They are rich food resources for pollinators (supporting ES). Moreover, Swiss mountain pastures are crossed by 14 000 km of hiking trails, which makes them essential places of recreation, tourism, and Swiss identity (cultural ES). Finally, mountain pastures are the habitat of 64% of all endangered plant species and 66% of all endemic plant species in Switzerland (all data based on Lauber *et al.*, 2013).

However, mountain pastures are highly heterogeneous. An ES provided at a certain place may be almost non-existent in a pasture nearby. It is also largely known that increasing a single ES can impair other ES. However, there is little systematic knowledge about trade-offs and synergies among specific ES and the strength of their relationship in mountain pastures. Thus, we aimed at (a) quantifying ES that are relevant for mountain pasture ecosystems (i.e., forage for ruminants, resources for pollinators, carbon storage, aesthetic landscape for recreation, and biodiversity conservation) and at (b) disentangling trade-offs among them.

## Materials and methods

A field survey was conducted at six (sub-)alpine, mountain farms in the Swiss Alps: three in the Northern Alpine foothills and three in the Central Alps, to represent the two most relevant areas of Swiss mountain livestock farming. At each farm, we observed 11 plots of 25 m<sup>2</sup>: nine plots were distributed along two gradients: remoteness (close to the farmhouse; medium distance; edge of the farm) × slope (flat; medium; steep). Two additional plots were placed in the most and the least frequently grazed area. The sampling did not consider shrub-encroached pastures. In each plot, six ES indicators were quantified (selection based on Richter *et al.*, 2021): forage quantity (measured as dry matter biomass cut twice a year), forage quality (percentage digestible organic matter), soil carbon stock (soil organic C content), pollinator resources (floral reward indicator of recorded plant species; derived from trait database), flower colour abundance (percentage abundance of coloured plant species in vegetation surveys) and species richness (number of vascular plant species recorded by vegetation survey). To statistically analyse the relationship among the ES indicators we used allometric line fitting (Warton *et al.*, 2006).

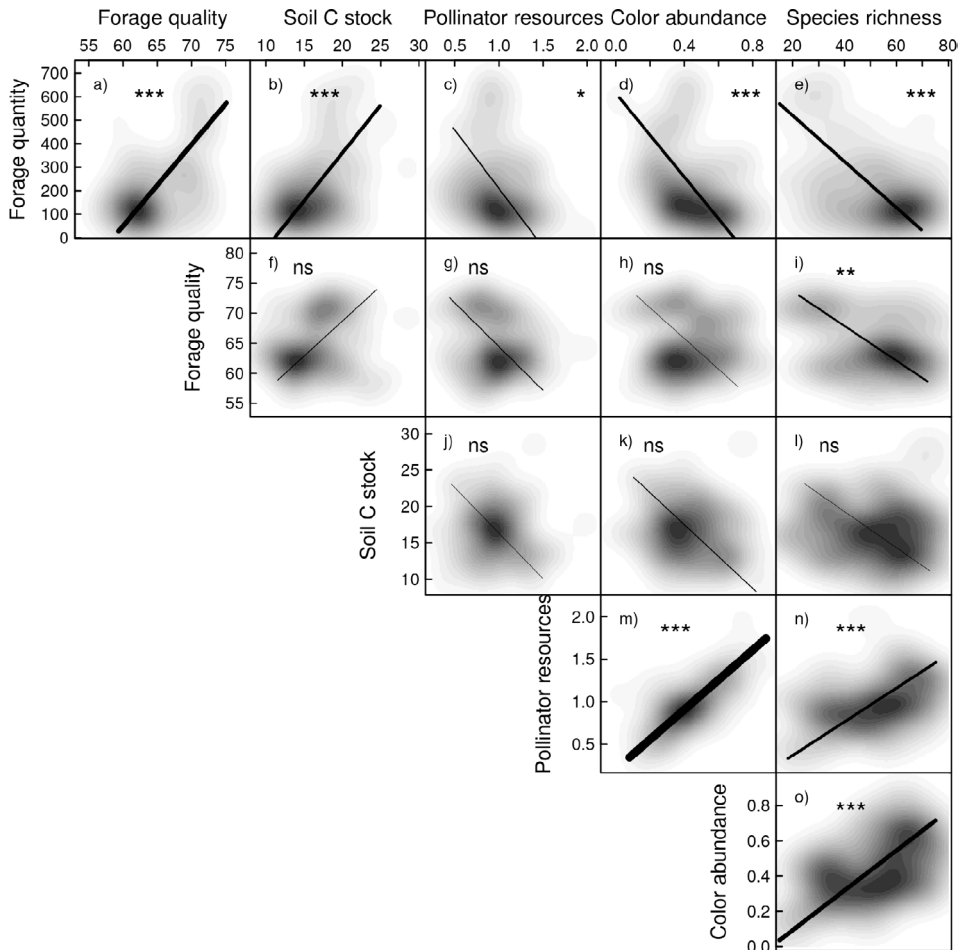


Figure 1. Allometric relations among six ecosystem service indicators of mountain pastures. Widths of allometric lines are scaled according to coefficients of determination ( $R^2$ ); their significance is provided as \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$ ; ns,  $P \geq 0.05$ . Background shading indicates the Normal Kernel density of observations.

## Results and discussion

Many ES were significantly related to other ES and, thus, there were clear synergies and trade-offs. A strong positive relationship was found between the two provisioning ES indicators forage quantity and forage quality (Figure 1a), indicating that mountain pasture which offer high amount of forage are also likely to provide highly digestible forage. In practical application, measures to increase forage amount likely also enhance forage quality, because species of high digestibility are promoted.

A second bundle of synergies was found among the three ES indicators pollinator resources, colour abundance and species richness (Figure 1m–o), indicating that a rich biodiversity comes along with supportive conditions for insects and offers an attractive sight for humans. Thus, by supporting biodiversity, farms likely also enhance pollinator abundance and public appreciation of their farmland, which can be an important factor in landscape attractiveness and therefore in direct marketing.

Remarkably, we found significant trade-offs between these two ES groups, i.e., forage quality and quantity on the one hand, and pollinator resources, colour abundance and species richness on the other (Figure 1c–e, i): Low-productive areas are more valuable for supporting and cultural ES than highly productive mountain pastures. Finally, the soil carbon storage potential of mountain pastures was largest in areas with high forage quantity (Figure 1b), probably because these are the places with deepest soil layer and high inputs of organic material. Other ES indicators were not related to soil carbon stocks (Figure 1j–l).

## Conclusion

There are not only synergies, but also trade-offs among ES in mountain pastures. Thus, there is no one-size-fits-all strategy (Dumont *et al.*, 2022) and it is not possible to realise all types of ES at the same place. However, in the large variability of mountain pastures also lies an opportunity: the huge heterogeneity in environmental conditions and management strategies allows high quantity and quality forage production in close proximity to biodiversity support and recreational values in less intensively managed areas within the same farm.

## Acknowledgements

We thank all farmers, herdsman and herdswomen who provided access to their pastures, and Fondation Sur-la-Croix and Canton of Grisons for funding within the AlpFutur project.

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# Grassland albedo: a climate change mitigation lever complementary to carbon sequestration

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## Abstract

Grasslands capture atmospheric CO<sub>2</sub> through photosynthesis and often sequester more carbon in the soil as organic matter, than cropland. In addition, they have a higher surface albedo ( $\alpha$ ). Maintaining, expanding, and optimizing grassland management can therefore contribute to climate mitigation. In this study, albedo dynamics were measured for 3 years in 7 meadows. We perform the first estimate of the albedo-induced radiative forcing (RF) due to management changes and converted it to kg CO<sub>2</sub>-equivalent sequestration. Compared to winter wheat, grassland has a cooling effect (RF=-6.7 W m<sup>-2</sup>), while drought, intensive grazing and mowing induce warming effects.

**Keywords:** albedo, radiative forcing, grassland

## Introduction

Livestock farming contributes to climate change (CC) through greenhouse gases emissions. In France, agriculture and forestry account for 18.4% of total emissions (Citepa, 2023). Therefore, converting cropland to grassland for animal farming or adapting grassland management can contribute to CC mitigation both through biogeochemical (e.g. carbon (C) storage) and biogeophysical, e.g. increased albedo ( $\alpha$ ) effects. For example, the 2019 INRAE's collective expertise (Launay *et al.*, 2021) estimated that crop/temporary grassland rotation stores +370 kgC ha<sup>-1</sup> year<sup>-1</sup>, whereas rotations with only crops destock on average -59 kgC ha<sup>-1</sup> year<sup>-1</sup>. On the other hand, the albedo mitigating effect of grassland has been poorly documented. The aim of this study was to monitor and quantify how the albedos dynamics of grasslands were affected by various management or meteorological events, to find out to what extent they can contribute to CC mitigation, based on biogeophysical processes.

## Materials and methods

The dynamic of daily albedo of grazed and mown meadows was studied at 7 experimental farms belonging to INRAE's, Chambers of Agriculture and an Agricultural School, located from the North to the South of France under different weather, grazing and mowing conditions. They are equipped with meteorological stations and albedometers, recording data every 10 minutes. The presented analysis concerns 2 meteorologically contrasting years: 2021 and 2022. The effect of management practices such as grazing and mowing as well as the effect of climatic events such as rainfall on surface albedo, were assessed. The daily radiative forcing (RF) caused by changes in surface albedo was calculated as the product of the daily mean surface albedo difference between the measured surface albedo of that day and the arbitrary reference albedo (i.e.  $\alpha_{\text{ref}}$  see below), the daily global incident solar radiation (in W m<sup>-2</sup>) was measured at the surface of the plot and daily atmospheric transmittance was calculated as the ratio between the daily incident solar radiation and the daily solar radiation at the top of the atmosphere, according to the Fu-Liou radiative transfer model (Schimel *et al.*, 1995; Ferlicoq, 2016; Ceschia *et al.*, 2017). The daily RF (in W m<sup>-2</sup>) represents the energy added to or subtracted from the Earth system. A positive RF corresponds to a warming effect, while a negative RF corresponds to a cooling effect. Of course, considering bare soil or wheat field albedo, as reference point is arbitrary and other studies have compared albedo between treatments (Ceschia *et al.* 2017) or between and anthropogenized surface area versus a natural one (e.g. Rottenberg & Yakir, 2010). In our study, the albedo reference point ( $\alpha_{\text{ref}}$ ) was



defined as follows: (1) to calculate the annual RF of the 7 grasslands, the  $\alpha_{\text{ref}}$  was either the mean albedo of a bare soil ( $\alpha_{\text{ref}}=0.150$ ), or of a winter wheat crop ( $\alpha_{\text{ref}}=0.168$ ); (2) for the assessment of the RF caused by management practices, the  $\alpha_{\text{ref}}$  was an estimate of the mean albedo of the plot over that year, in the absence of any management practices. In that case,  $\alpha_{\text{ref}}$  was calculated as follows:

$$\alpha_{\text{ref}} = \alpha \text{ estimated without events} = ((\alpha - 5 \text{ days})n) + ((\sum \alpha_{\text{daily}})(365 - n)) / 365$$

Where:  $\alpha - 5$  days is the mean albedo during the 5 days preceding the event,  $n$  is the number of days of decrease in surface  $\alpha$  following the event, and  $\sum \alpha_{\text{daily}}(365 - n)$  is the daily mean  $\alpha$  calculated for the rest of the year (365 days -  $n$ ), i.e. for the days without events. Finally, the RF expressed in  $\text{W m}^{-2}$  was converted to  $\text{kg CO}_2\text{-equivalent (eq) per hectare (ha) and per year (yr)}$  as reported by Bright *et al.* (2016).

## Results and discussion

The RF of the 7 grasslands based on the mean albedo value of the grasslands in 2021 and 2022 ( $\alpha=0.228$ ), is  $-8.5 \text{ W m}^{-2}$  considering the bare soil albedo as the reference point (Mischler *et al.*, 2022a) and  $-6.7 \text{ W m}^{-2}$  (winter wheat as the reference). Therefore, grassland has a cooling effect on the climate equivalent to  $-1800 \text{ kg CO}_2\text{-eq ha}^{-1} \text{ year}^{-1}$  compared to bare soil and  $-1400 \text{ kg CO}_2\text{-eq ha}^{-1} \text{ year}^{-1}$  if compared to wheat. These values are comparable to those observed in Sweden on temporary grassland (Sieber *et al.*, 2023).

In 2021, the meteorological conditions of all experimental sites were consistent with the situation encountered in France that year. The temperatures were slightly above the 1981-2010 normal ( $+0.4^\circ\text{C}$ ), while precipitations were close to those in the Météo-France report (2022). In 2022, temperatures were  $+1.6^\circ\text{C}$  above normal, with a rain deficit of 25% (Météo-France, 2023). The average annual albedo in 2021 (0.232) was slightly higher than in 2022 (0.225), as more soil area was visible because of the drought.

On annual average, the 32 grazing and 3 mowing events observed in 2021 slightly decreased albedo (by  $-0.004$ ) inducing a positive RF ( $+0.42 \text{ W m}^{-2}$ , i.e.  $+89 \text{ kg CO}_2\text{-eq ha}^{-1} \text{ year}^{-1}$ ). This albedo decrease was significantly stronger at the Trévarez (RF= $+0.95 \text{ W m}^{-2}$ ,  $+202 \text{ kg CO}_2\text{-eq ha}^{-1} \text{ year}^{-1}$ ) and Mourier (RF= $+0.83 \text{ W m}^{-2}$ ,  $+176 \text{ kg CO}_2\text{-eq ha}^{-1} \text{ year}^{-1}$ ) farms because of overgrazing caused by a high animal

Table 1. grazing and mowing, soil humectation effect on meadow albedo and RF

Average of 7 meadows	Effect of grazing and mowing on $\alpha$	Effect of rain after dry period on $\alpha$	Weather (March–September)
<b>2021</b>			
Number of events	32 grazing + 3 mowing	12 soil moistening	Irradiance: $211 \text{ W m}^{-2}$ .
$\alpha_{\text{ref}}$ /mean annual albedo ( $\alpha_{\text{mean}}$ )	0.236/0.232	0.233/0.233	Soil humidity: 26 %
$\alpha_{\text{ref}} - \alpha_{\text{mean}}$ (yearly)	+0.004 (5ns/2s)	+0.001 (NS)	Precipitation: 409 mm
RF ( $\text{W m}^{-2}/\text{kg CO}_2\text{-eq ha}^{-1} \text{ year}^{-1}$ )	+0.42/+89	+0.06/+14	Temperature: $15.5^\circ\text{C}$
<b>2022</b>			
Number of events	16 grazing + 1 mowing	20 soil moistening	Irradiance: $234 \text{ W m}^{-2}$
$\alpha_{\text{ref}}$ /mean annual albedo ( $\alpha_{\text{mean}}$ )	0.227/0.225	0.234 /0.225	Soil humidity: 16 %
$\alpha_{\text{ref}} - \alpha_{\text{mean}}$ (yearly)	+0.002 (NS)	+0.009 (S)	Precipitation: 247 mm
RF ( $\text{W m}^{-2}/\text{kg CO}_2\text{-eq ha}^{-1} \text{ year}^{-1}$ )	+0.25/+53	+0.93/+195	Temperature: $17.2^\circ\text{C}$

$\alpha_{\text{ref}}$ =albedo estimated without event,  $\alpha_{\text{measured}}$ =albedo measured with the events. Statistical analysis comparing  $\alpha_{\text{ref}}$  and  $\alpha_{\text{mean}}$  Student's t-test ( $P<0.05$ ). S, significant; NS, not significant for all 7 meadows; 5ns/2s, not significant for 5 and significant for 2 meadows ( $P<0.1$ ).

load (Mischler *et al.*, 2022b). In 2022, there was less mowing and grazing because of the drought which reduced grass growth: the mean RF caused by grazing and mowing events was only  $+0.25 \text{ W m}^{-2}$ , and the albedo decrease ( $-0.002$ ) was not significant (Table 1). This suggests that intensive grazing and mowing may worsen the cooling effect of grasslands compared to less intensive practices.

In 2021, 12 rain events occurred during periods of dry soil. The albedo decreases on average by only  $-0.001$  (not significant) and generated a RF close to 0. In 2022 because of the drought, the albedo decreased strongly after the first rain event in August after several drought weeks. This decrease lasted from 29 to 78 days after the rain, depending on the farm. The decrease was to  $-0.009$  on annual average (significant for each meadow), and the induced RF was positive:  $+0.93 \text{ W m}^{-2}$ . Weather had a significant impact on surface albedo: the drought in 2022 had greater RF effect on albedo, than mowing and grazing, except for the 2 meadows at the Trévarez and Mourier farms.

## Conclusion

For the first time in France, the albedo dynamics of grasslands with contrasted pedoclimatic conditions and management methods were assessed. The results are encouraging: grassland has a cooling effect compared to winter wheat. At the opposite, intensive mowing and grazing may have resulted in a significant decrease in the surface albedo, while drought significantly affected the albedo of grasslands. The next step will be to compare the albedo effect with soil C sequestration, the methodology for which has not yet been determined, but will be continued in a new project, which analyses the energy budget of grassland and forage cropping systems.

## Acknowledgement

This Albedo-Prairies project (2020-23) was financed by the Compte d'Affectation Spécial Développement Agricole et Rural (Casdar).

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# Mixed effects of sward biodiversity and management regime on ecosystem services

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## Abstract

Species-rich grasslands provide ecosystem services such as flora and fauna diversity, forage for livestock, carbon sequestration and water regulation. These ecosystem services can be affected by sward botanical composition and management intensity. However, the effects and interactions of these factors to optimise ecosystem services are not fully known. To address this, we established experimental plots with three types of sward with varying levels of diversity: productive monoculture (PM; perennial ryegrass (*Lolium perenne*)), biodiverse (BD) and productive biodiverse (PBD), which were subjected to four management regimes ranging from extensive (i.e., low input, late mowing) to intensive (i.e., high input, early mowing). After three years, we found successful establishment of biodiverse swards with high forb cover, particularly under extensive management. Forage dry matter yield was the highest in BD and intensively managed swards. Forage N concentration was the highest in PBD swards and digestible organic matter was the highest in PM and PBD swards. Treatment effects on carbon sequestration and water regulation were minimal. Collectively, diverse swards, different management regimes and their interactions benefit some, but not all, ecosystem services, and highlight the need for careful consideration of sward species composition and long-term management of biodiverse grasslands to achieve site-specific goals.

**Keywords:** biodiversity, diverse grasslands, ecosystem services, forage

## Introduction

Species-rich grasslands provide ecosystem services such as flora and fauna diversity, forage for livestock, carbon sequestration and water regulation. However, the effects of sward diversity and management intensity (and their interactions) to achieve the above-mentioned ecosystem services are not fully known. To address this, we established experimental plots with three types of sward with different levels of diversity, which were subjected to four management regimes, from extensive to intensive. We hypothesised that sward diversity and management regime differentially affect different ecosystem services.

## Materials and methods

The experiment was established in August 2017 on a clay soil at the Dairy Campus Research Facility (WUR) in Leeuwarden, The Netherlands. A detailed description of the experimental design can be found in Hoekstra *et al.* (2023). We established experimental plots (6 m × 10 m) with three types of sward with varying levels of diversity: (1) productive monoculture (PM; perennial ryegrass (*Lolium perenne*)), (2) biodiverse (BD; a species-rich mixture from natural sources aimed at meadow bird conservation, [www.biodivers.nl](http://www.biodivers.nl)) and (3) productive biodiverse (PBD; i.e., biodiverse mixture with additional species selected to increase the quantity and quality of forage, including *L. perenne*, *Trifolium repens*, *Cichorium intybus* and *C. carvi*). These plots were subjected to four management regimes differing in the date of the first harvest and the timing, type and amount of fertiliser applied (Table 1). The experiment was conducted in four replicate blocks, resulting in a total of 48 plots.

Table 1. Overview of the management regimes

Code	Management regime			Fertilisation				Applied before first cut?
	N fertilisation level	Timing of first cut <sup>1</sup>	cuts/year	FYM (t ha <sup>-1</sup> )	CS (m <sup>3</sup> ha <sup>-1</sup> )	CAN (kg N ha <sup>-1</sup> )	N (kg ha <sup>-1</sup> )	
LL	Low	Late	3	18	–	–	115	Yes
ML	Medium	Late	4	–	18	90	162	No
ME	Medium	Early	4	–	18	90	170	No
HE	High	Early	4	–	42	180	378	Yes

CAN, calcium ammonium nitrate; CS, cattle slurry; FYM, Farmyard manure; Late, after the 1<sup>st</sup> week of June, in line with meadow bird conservation guidelines.

The proportion cover of individual plant species was determined in two 1×1 m quadrats per plot in August 2020. Herbage dry matter yield (DMY) was determined for each harvest using a Haldrup plot harvester and in 2019 all cuts were analysed for total nitrogen and in-vitro digestibility of organic matter. In autumn 2019, soil samples (0–10 cm) were taken in all plots, and analysed for soil organic matter content (EurofinsAgro, Wageningen, the Netherlands). Soil penetration resistance was measured with a penetrometer at a depth of 0–30 cm. The effects of sward type and management and their interaction on ecosystem services were determined using general linear mixed effects models, taking into account the block structure.

## Results and discussion

In 2020 the proportion of forb cover (Figure 1a) was the highest for the biodiverse mixture and the lowest for the perennial ryegrass monoculture. In the PBD mixture, the proportion of forbs was lower due to the competitive advantage of perennial ryegrass, particularly at higher levels of N application (significant management (MR)×sward type (ST) interaction  $P<0.01$ ). The proportion of legumes was very low and ranged from 0% in PM to 3% in the PBD and BD mixture under LL management (significant MR×ST interaction,  $P<0.05$ ).

Grasses showed the opposite pattern to forbs. For PM, the main grass was *Lolium perenne*, whereas for BD the main grass species were *Festuca rubra* and *Festuca pratensis*.

Herbage DMY was significantly ( $P<0.001$ ) higher for the BD sward compared to PM and PBD (Fig. 1b). This may be related to the strong growth of grasses such as *Festuca rubra* and *Festuca pratensis* and the relatively strong growth of forbs in the dry conditions prevailing in 2018–2020. There was a strong effect ( $P<0.001$ ) of management regime, and herbage DMY ranged from 10 000 kg ha<sup>-1</sup> year<sup>-1</sup> for LL to 15 000 kg ha<sup>-1</sup> year<sup>-1</sup> for HE. Late mowing of the first cut had little or no effect on total herbage DMY (ME vs ML). Intensively managed swards (HE) had higher dry matter yield regardless of the type of sward (MR×ST,  $P<0.05$ ).

Forage crude protein (CP) concentration was highest in the PBD swards (135±18 g kg<sup>-1</sup> vs 124±15 g kg<sup>-1</sup> for PM and BD) and organic matter digestibility (OMD) was higher in PM (73±8.3%) and PBD (74±4.8%) swards compared to BD (64±7.4%) ( $P<0.001$ ), indicating that productive plant species (mainly *L. perenne*) contributed to the improvement of forage quality in PBD swards. Due to the poor establishment of legumes, they had little or no effect on the herbage N concentrations. Both CP and OMD were significantly ( $P<0.001$ ) higher for the HE compared to the LL management. For CP this could be related to the increased N application rate but not to the mowing date (ME=ML), while for OMD this appeared to be mainly related to the first cut date (ME<ML); data not shown.

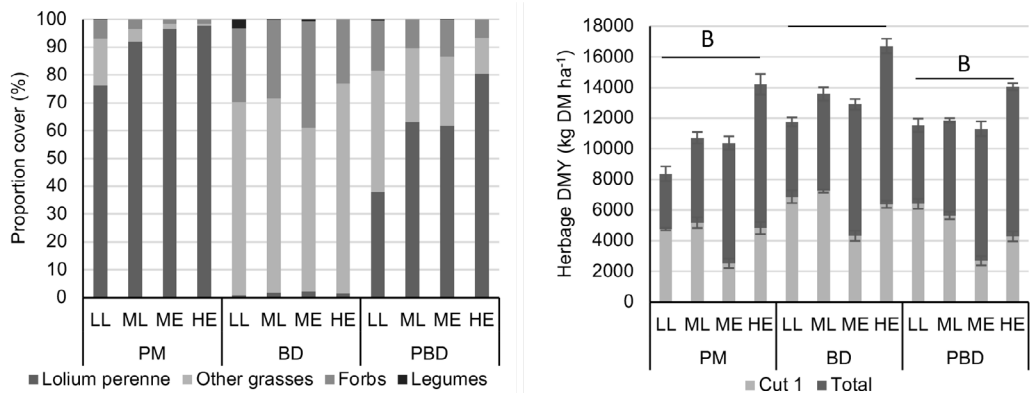


Figure 1. The effect of sward type (PM, productive monoculture; BD, biodiverse; PBD, productive + biodiverse) and management regime (abbreviations see Table 1) on (a) the proportion of grasses, forbs and legumes in autumn 2020, and (b) herbage dry matter yield of the first cut and cumulative cuts in 2020 ( $n=4$ ; error bars, standard error).

Effects of management and sward type on carbon sequestration and water regulation (as indicated by soil penetration resistance) were minimal (data not shown), which may be related to the relatively short interval between sowing (autumn 2017) and measurement (autumn 2019).

## Conclusion

The biodiverse mixtures have resulted in a strong increase in the proportion of forbs, particularly in more extensive management regimes. Herbage productivity was the highest in the case of BD swards and intensive management, whereas herbage quality was the highest in the case of PM and PBD swards and intensive management.

Collectively, diverse swards, different management regimes and their interactions benefit some ecosystem services, but may negatively affect others. Taken together, these findings focus attention on the need to carefully consider sward species composition and long-term management of biodiverse grasslands in order to maximise specific ecosystem services depending on the site-specific goals.

## Acknowledgements

This research was part of the project ‘Koeien en Kruiden’, which was funded by the Ministry of Agriculture, Nature and Food Quality, the province Friesland, the Dairy Campus Innovatiefonds, the Centre of Expertise Agrodier and the University of Applied Research Van Hall Larenstein. This research was partly funded by the public private cooperation program “Raw forage, soil and circular agriculture” via the Top Sector Agri & Food TKI-AF-15102/15284 LWV190195.

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# Trade-offs between milk production potential, tannin content and plant diversity depend on grassland mixtures

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## Abstract

Temporary grasslands are crucial for addressing dairy cow sector challenges, including milk production, methane emissions mitigation, and biodiversity enhancement. In this study conducted in Frick, Switzerland, we investigated four distinct temporary grassland mixtures, replicated across 16 plots. These mixtures comprised: (1) pure grasses, (2) a combination of grasses and legumes, (3) a combination of grasses and tannin-rich plants, and (4) a combination of grasses and plants rich in essential oils. Over the course of six grazing rotations in 2022, our research encompassed botanical surveys and vegetation chemical analyses for each mixture. We calculated three pivotal variables: the potential milk production for a hypothetical dairy cow, the plant species diversity, and the condensed tannin content. Principal component analyses (PCA) were employed for each mixture to highlight the trade-offs among these variables. Grass mixture correlated potential milk production with species diversity. Essential-oil mixture correlated milk production to tannin content. Grass-legume and tannin mixtures traded species diversity for tannin content. These findings underscore the importance of designing specific temporary grassland mixtures at the plot and farm scale to effectively address the predominant challenges facing the dairy cow sector.

**Keywords:** multi-species grassland, plant secondary metabolites, temporary grassland, ecosystem services, organic agriculture

## Introduction

Temporary grasslands play a pivotal role in addressing challenges in the dairy cow sector, such as achieving optimal milk production, mitigating methane emissions, and promoting biodiversity. Yet, our understanding of the relationships among these characteristics remains limited, particularly in the context of organic grassland systems. The trade-off between grassland productivity and species diversity has been strongly debated. In sown grasslands, this relation is mostly positive: multi-species grasslands tend to yield higher biomass (Baker *et al.*, 2023) sown species and unsown species contribution to herbage DM under an intensive dairy-calf to beef system. Three sward types were investigated in a farmlet experiment: *Lolium perenne* (LP; 205 kg N ha<sup>-1</sup> a<sup>-1</sup>, and have the potential to result in higher milk yields (Schaub *et al.*, 2020) plant diversity is often associated with low biomass yield and forage quality, while biodiversity experiments typically find the opposite. We address this controversy by assessing, over 1 year, plant diversity effects on biomass yield, forage quality (i.e. nutritive values. Diversity in species and functional groups (grasses, legumes, forbs) influences these outcomes, while condensed tannins (CT) offer promise in methane mitigation while having a negligible impact on milk production (Herremans *et al.*, 2020) composition and nitrogen metabolism of lactating dairy cows fed diets with or without tannins. The meta-analysis shows that tannins have no impact on corrected milk yield, fat and protein content or NUE ( $p > .05$ ). However, the connection between plant diversity and CT content remains understudied. It is reasonable to expect that this relationship largely depends on the composition of sown species and the presence of weed species. If sown species are tannin-rich, weeds may boost species diversity but dilute tannin content; conversely, in tannin-poor grasslands, weeds may increase CT content. Therefore, we propose that the correlations between milk production potential, species diversity, and CT content depend on the sown grassland mixtures.

## Materials and methods

We conducted this study from April to October 2022 in Frick, Switzerland (47°30'51" N 8°1'26" E), investigating four distinct temporary grassland mixtures, replicated in four plots each (16 plots total). The swards were established in 2021 with seed mixtures encompassing: (1) pure grasses (mixture G), (2) a combination of grasses and legumes (L), (3) a combination of grasses and tannin-rich forbs and legumes (T), and (4) a combination of grasses and forbs rich in essential oils (EO) (Table 1). Across six rotations in 2022, botanical surveys and chemical analyses of vegetation were performed for each plot. For every rotation, we calculated three key variables: the potential milk production of a theoretical dairy cow ( $\text{kg ha}^{-1}$ ), based on INRA (2010) equations; the plant species diversity; and the condensed tannin content ( $\text{g (kg DM)}^{-1}$ ). Finally, we conducted a principal component analysis (PCA) for each grassland mixture to investigate the correlations between potential milk production, plant species diversity, and condensed tannin content.

## Results and discussion

In the four PCA, the two first dimensions explained more than 70% of the variability, so we focused our analysis on these dimensions only. The results showed three distinct trade-off profiles dependent on grassland mixtures (Figure 1).

Mixture G exhibited a positive correlation between milk production and species diversity, while CT content was uncorrelated to these variables. This correlation could be due to the inclusion of dicotylous weed species in the grassland like white clover or dandelion (up to 67% of biomass), which increased the diversity and exhibits greater drought resistance compared to the sown grass species (Haughey *et al.*, 2018). The lack of correlation with CT content may be due to the generally low presence of tannin-rich species in this mixture.

In the mixture EO, milk production positively correlates with CT content, while species diversity was uncorrelated to these variables. A closer examination revealed that higher CT contents were associated with high milk production (values above  $7 \text{ g kg}^{-1}$  CT linked to yields exceeding  $1000 \text{ kg ha}^{-1}$ ), but lower CT contents were associated with both low and high milk production. Samples with the highest CT content in mixture EO had a lower grass proportion, potentially explaining their elevated CT content. However, our calculation for milk production did not counter for the potential effect of CT on protein digestibility nor on palatability, which could lower milk production (Herremans *et al.*, 2020).

In mixtures L and T, species diversity showed a negative correlation with CT content, weakly linked to potential milk production. In mixture T, plots with fewer than ten species had an average CT content of  $9 \text{ g kg}^{-1}$ , compared to  $6 \text{ g kg}^{-1}$  in plots with over ten species. This indicates that weeds with lower CT contents diluted the high CT content of sown species. Furthermore, in both mixtures, summer drought reduced species diversity but increased CT content in plants, confirming results from other studies (Anuraga *et al.*, 1993).

Table 1. Functional composition of the four grassland mixtures (grasses; legumes; forbs).

	G	L	T	EO
Number of species	(4; 0; 0)	(4; 3; 0)	(4; 1; 3)	(4; 0; 6)
Proportion (%)	(100; 0; 0)	(50; 50; 0)	(50; 12; 38)	(60; 0; 40)

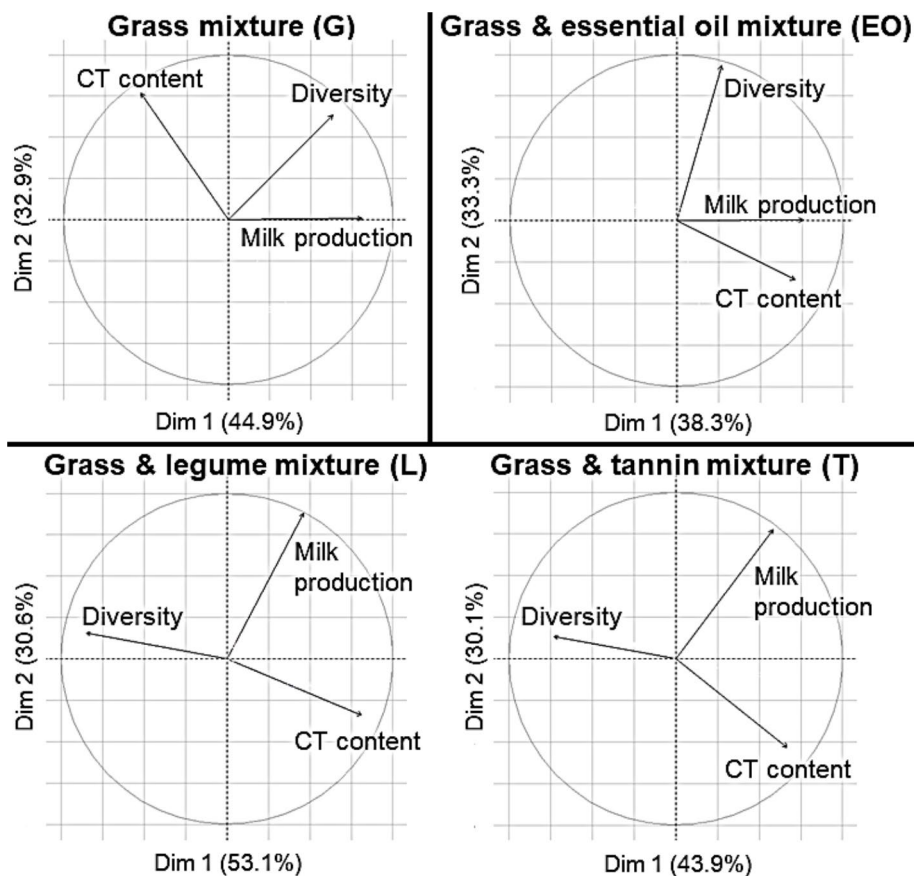


Figure 1. Trade-offs between potential milk production, species diversity and condensed tannin content for each mixture.

## Conclusion

Species composition and functional group ratios, rather than species diversity, are crucial in determining trade-offs between milk production, plant diversity, and CT content in grassland mixtures. Thus, temporary grasslands composition should be considered before assessing ecosystem goods and services.

## Acknowledgements

We warmly thank Samuel Imboden for his help in conducting preparative work in the lab, and all colleagues who promptly helped us in the field. This project received support from the Stiftung Edith Maryon.

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# Multispecies for multifunctional grassland: evaluating novel for species for temperate climates

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## Abstract

Grassland provides a wide range of ecosystem services that are threatened by various challenges such as climate change. To tackle these challenges minor and underutilised plant species local to temperate grasslands and, so far, novel or exotic plant species usually grown under warmer conditions (e.g. from the Mediterranean region) could become more popular in future as these are expected to better cope with drought and heat. We evaluated a set of 21 dicotyledonous plant species, of which ten were legumes, including four exotic species, over several weeks under controlled conditions in a greenhouse experiment in order to evaluate plant functional traits, herbage accumulation and herbage quality. Data analyses were done with Analysis of Variance to assess plant species effects. The factor ‘species’ had a significant influence ( $p < 0.001$ ) on all target variables. All studied species showed relatively high protein concentrations. Legumes accumulated a higher herbage mass than non-legumes, whereby the exotic legumes reached higher values than the minor local legumes. The results show the potential to better cope with future challenges and to enhance phytodiversity.

**Keywords:** dicotyledons, climate change, phytodiversity, multifunctionality

## Introduction

Biodiverse grassland delivers multiple ecosystem services. In mixture with legumes, dicotyledonous non-legumes improve N acquisition from the soil (Dhamala *et al.*, 2016) and are more resistant to drought through deep-rooting (Hoekstra *et al.*, 2015). Dicotyledonous species also promote the provision of a floral supply for pollinators (Cong *et al.*, 2020). Alternative drought- and heat-tolerant species are necessary in future under ongoing climate change in order to preserve the grassland functions. We evaluated several minor dicotyledonous and ‘exotic’ species in a pilot study to make specific information on their herbage production, herbage quality and functional traits available, which are scarce, so far.

## Material and methods

Based on a literature survey and own experiences we chose one to three accessions of 21 dicotyledonous species, including four exotic legumes, which usually grow under warmer conditions (Mediterranean dry climate) but do not occur in Central European grasslands (Table 1). Due to low growth and thus insufficient sample biomass quantity the following species had to be removed from herbage quality analyses: *Carum carvi*, *Cichorium intybus*, *Lotus pedunculatus*, *Pimpinella saxifraga*, *Sanguisorba minor*, *Thymus pulegioides* and *Trifolium tumens* (exotic legume). These were consequently not statistically analysed further.

The experimental design was a randomised block with four replications. Plants were grown in mesocosms in a roofed open-sided greenhouse with natural light but increased temperature conditions. They were sown on the same day and had the same growth period. Each mesocosm comprised one individual plant without competition between the individuals in neighbouring mesocosms. The data collection took place between April–July 2022. There were no applications of fertilisers, herbicides or pesticides. During the study period, a temperature measurement was implemented and day length was always  $> 14$

h. The following target values were evaluated: herbage dry matter accumulation (HDM), leaf dry matter content (LDMC) (Harzé *et al.*, 2016), days until flowering, contents of neutral detergent fibre (NDF) determined with an ANKOM fibre tec, A200 Fiber Analyser, and crude protein (CP) converted by multiplying the total nitrogen (N) concentration with 6.25. The N concentrations were determined using elemental analysis (vario EL cube, Elementar, Langensfeld, Germany). Statistical analyses were performed in R version 4.2.1 (R Core Team, 2022), using an Analysis of Variance (ANOVA) to test the influence of the factor species on all target variables.

## Results and discussion

The effect of species was highly significant ( $p < 0.001$ ) on all target values under consideration. Especially the exotic legumes, except *T. tumens*, attained a good performance under the given conditions. The temperature sum within the greenhouse was 32% higher compared to ambient conditions. Thus, the exotic legumes were potentially better adapted to occurring heat periods and could become more important for higher future temperatures (European Environment Agency, 2012).

The exotic legumes accumulated on average the highest amount of herbage biomass of up to 2.7 g (DM mesocosm)<sup>-1</sup>. The minor legumes produced on average 22% less. Non-legumes accumulated on average 29% less herbage than legumes. A high LDMC indicates more resistance to physical stress than species with a low LDMC (Guo *et al.*, 2022). In our study, *S. minor* reached the greatest LDMC and *P. lanceolata* the lowest. The fewest number of days until flowering is presented by *P. lanceolata*, *P. anisum* and *T. michelianum*. These species are potentially beneficial for early floral resources for pollinating invertebrates. For a continuous provision of floral supply throughout the growing season, species across a wide range of days until flowering could be combined, at best consisting of complementary functional traits in order to enhance multifunctionality (Blesh, 2018). For this purpose, *P. lanceolata*, *L. corniculatus* and *T. michelianum* are potentially suitable partners. For evaluation as forage for ruminants, specific requirements are desired. In the present study, the minor non-legumes achieved a CP concentration of 112.7 g (kg DM)<sup>-1</sup>. The minor legumes achieved an average value of 130 g (kg DM)<sup>-1</sup> and the exotic

Table 1. Arithmetic means and standard errors of means (in brackets) of herbage dry matter accumulation (g (DM mesocosm)<sup>-1</sup>), leaf dry matter content (%), days until flowering, the NDF concentration (g (kg organic matter)<sup>-1</sup>) and crude protein (CP) (g (kg DM)<sup>-1</sup>).

Functional group	Study species	HDM	LDMC	Days until flowering	NDF	CP
Minor legumes	<i>Astragalus cicer</i> L.	3.6 (0.56)	27.3 (0.56)	59.5 (0.01)	322.0 (12.0)	93.9 (4.8)
	<i>Lotus corniculatus</i> L.	2.7 (0.74)	26.5 (0.74)	42.3 (0.02)	368.8 (15.9)	150.2 (12.2)
	<i>Trifolium ambiguum</i> M. Bieb.	1.2 (0.22)	27.5 (0.22)	n.f.	358.8 (4.6)	94.2 (8.9)
	<i>Trifolium subterraneum</i> L.	2.4 (0.34)	30.8 (0.34)	38.0 (0.01)	371.5 (7.7)	117.8 (6)
Minor non-legumes	<i>Pimpinella anisum</i> L.	5.9 (1.70)	25.8 (1.7)	26.8 (0.02)	350.1 (8.3)	121.9 (10)
	<i>Plantago lanceolata</i> L.	2.4 (0.69)	19.2 (0.69)	21.6 (0.02)	381.5 (12.0)	175.0 (14.4)
	<i>Rumex acetosa</i> L.	1.2 (0.329)	20.0 (0.32)	n.f.	353.7 (5.6)	103.1 (11.8)
	<i>Sanguisorba officinalis</i> L.	2.6 (0.75)	35.2 (0.75)	n.f.	356.5 (4.6)	104.1 (8.5)
Exotic legumes	<i>Bituminaria bituminosa</i> (L.) C.H. Stirt	5.0 (0.90)	34.6 (0.9)	56.8 (0.02)	475.3 (20.1)	58.8 (4)
	<i>Hedysarum coronarium</i> L.	3.6 (0.58)	21.2 (0.58)	n.f.	325.7 (7.1)	89.2 (4.7)
	<i>Trifolium michelianum</i> Savi	1.7 (0.33)	21.3 (0.33)	26.9 (0.01)	377.3 (11.0)	172.1 (10.2)

legumes of 106.7 g (kg DM)<sup>-1</sup>. The basis of comparison is grounded on harvests at the time of flowering across species. The time of flowering, however, varied due to different phenological development speed. We compare the values of species when potentially harvested at flowering. The NDF concentrations were generally low, although the harvest took place around flowering. Our data show that especially legume-based forages can supply large quantities of protein. High-merit dairy cows require at least 140 g CP (kg DM)<sup>-1</sup> in their diets (Kalscheur *et al.*, 1999). These requirements are met by *L. corniculatus*, *P. lanceolata* and *T. michelianum* (Table 1).

## Conclusion

To find most suitable plant species to create a diverse sward composition for multifunctionality of grassland, the evaluation of minor and exotic plant species pinpoints towards potential for plant breeding. With respect to future climatic conditions, Mediterranean plant species potentially offer enhanced sward phytodiversity under humid temperate climate.

## Acknowledgements

We are especially grateful to Barbara Hohlmann for establishment of the plant species and lab analyses. The Technical Team of the Department of Crop Sciences is gratefully acknowledged for maintaining the experiment and regular watering. Gabriele Kolle is gratefully acknowledged for performance of all CN analyses. The research was supported by a fund of the Federal Ministry of Food and Agriculture through project manager Federal Office for Agriculture and Food on the basis of a resolution of the German Bundestag (FKZ: 281C702A21).

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# Management and vegetation affect grassland soil organic carbon stocks

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## Abstract

Permanent grasslands (PG) are touted as a promising land use-based solution for countering the causes and effects of climate change. How different management strategies, management intensities, and sward compositions affect soil organic carbon (SOC) stocks in grassland ecosystems has been comprehensively studied. However, under real field conditions, the changes occur concurrently and are difficult to disentangle. To better understand how grassland management and plant communities affect SOC stocks, we sampled 18 PG in an on-farm survey in Piedmont region (NW Italy), in the 0–30 cm soil layer. Results showed that stocks in old PG (>20 years.) were 31% higher than in the younger (<20 years). Mineral nitrogen supply in addition to organic fertilisers (manure or slurry) significantly increased stocks by 31%, compared to only manures. Irrigation also played an important role in shaping SOC stocks: rainfed PG had 26% lower stocks than the irrigated PG. Lastly, mown PG had stocks 9% higher than grazed-only PG and 22% higher than mown+grazed PG. Concerning the effect of vegetation, PG dominated by *Lolium multiflorum* showed higher stocks than the other PG dominated by less intensively managed swards. These results are important for identifying good management practices to be promoted by specific policies.

**Keywords:** grazing, irrigation, mowing, permanent grassland, species composition, pasture

## Introduction

Permanent grassland (PG) provides habitats for biodiversity, contributes to food production, and delivers many other ecosystem services (Schils *et al.*, 2022). The potential of PG to affect soil organic carbon (SOC) depends greatly on their management, environmental conditions (e.g., soil texture, soil compaction, moisture, and temperature) and by plant community composition and therefore litter and root inputs (Schils *et al.*, 2022). Permanent grasslands are highly vulnerable to human disturbance, which leads to reductions in soil organic carbon (SOC) storage (Bai and Cotrufo, 2022). The effect of different PG management strategies on SOC stocks in grassland ecosystems has been extensively studied (Schils *et al.*, 2022). However, under ‘real field’ conditions, changes occur concurrently and are difficult to disentangle. Thus, a farm survey was carried out to better understand how grassland management and plant communities affect topsoil SOC stocks in Piedmont region (NW Italy).

## Materials and methods

In the framework of H2020 SUPER-G project, a farm network was established in 2021, by selecting eight farms located in the Po river plain of Piedmont Region, northwest Italy. The climate was sub-continental, with mean annual temperatures between 11.8 and 12.9°C and total annual rainfalls between 702 and 760 mm. At each farm, two or three PG were selected considering different management conditions as categorical variables PG age (old, >20 years or young), defoliation (grazing, mowing or both), irrigation (yes/no), and fertilisation (manure or manure+mineral), aiming to encompass a wide range of contrasting conditions. This information was collected by interviewing the farmer. Soil and plant species composition were sampled in spring 2021, before the first defoliation, with three pseudoreplicates

per PG, treated as replicates. Plant species composition was assessed with a vertical point-quadrat method (Daget and Poissonet, 1971) along 5-m linear transects, on 25 points. PG vegetation types were identified by a cluster analysis based on species relative abundance. Close to each vegetation transect, soil samples at 0-30 cm depth were collected for SOC analysis (dry combustion with an elemental analyser), in addition to three undisturbed soil cores to measure soil bulk density.

A linear mixed model was used to test the differences among management practices on SOC stock. Management practices were considered as fixed effects, while PG within farm was included as random factor.

## Results and discussion

SOC stock was significantly influenced by grassland management practices and vegetation type. Statistical analysis of all PG evidenced ( $P < 0.001$ ) that old, mown, manured, and *Lolium multiflorum*-dominated PG had the highest SOC stock ( $127.7 \text{ Mg C ha}^{-1}$ ), while the lowest SOC stock was observed in young, mown and grazed, irrigated, and *Festuca arundinacea*-dominated PG ( $45.12 \text{ Mg C ha}^{-1}$ ).

In old PG (>20 years old) stocks were 31% higher than in the younger PG (Figure 1a). Surprisingly, further mineral N addition to manures significantly increased SOC stocks by 31% compared to only manures (Figure 1b); this was likely due to increased grassland productivity that led to higher C inputs into the soil, as confirmed by Poeplau (2021). Irrigation also played an important role in shaping SOC stocks, as rainfed PG had 26% lower stocks than the irrigated PG (Figure 1c), as also pointed out by Conant *et al.* (2017). This again was also probably due to an increase in grassland productivity and therefore higher C inputs. Lastly, the effect of grazing and mowing was not disentangled by the models, since SOC stock in mown PG were slightly, but not significantly (9%), higher than only-grazed PG (Figure 1d) and 22% higher than in mown and grazed PG ( $p < 0.001$ ). This was also unexpected, since literature suggests that grazing management has the highest potential in increasing SOC, even if recent studies found that it is a context-dependent factor (Bai and Cotrufo, 2022). Grazed PGs receive inputs

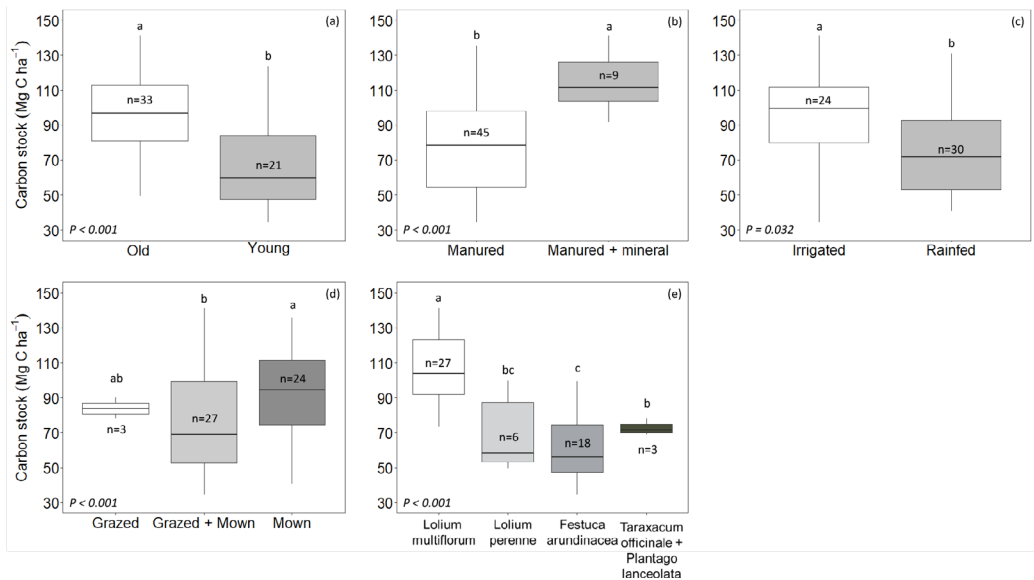


Figure 1. Boxplot showing SOC stocks as a function of (a) age, (b) fertilisation, (c) irrigation, (d) defoliation and (e) vegetation type (identified by the dominant species). Letters highlight significant differences among PG conditions at  $p < 0.05$ .

from animal excreta, which stimulates soil organic matter formation and turnover, while in mown systems the main input is from plant litter residues, beside the regular fertilisation (Gilmullina *et al.*, 2020).

Concerning the effect of vegetation, PG dominated by *L. multiflorum* showed higher stocks than the other PG. This confirms the findings obtained by the evaluation of the different management strategies, as *L. multiflorum* generally dominates in intensively fertilised, irrigated swards on fertile soils. Conversely, in PG dominated by *F. arundinacea* (which identified a less demanding vegetation type) SOC stocks were 43% lower than in *L. multiflorum*-dominated PG.

Future analysis will include multivariate approaches, in order to study correlations between different management practices, plant species composition and environmental variables.

## Conclusion

Our study found that the age of sward, the mineral fertilisation in addition to manure, and the irrigation can lead to an increase in SOC stocks, possibly through increased additions. The presence of highly competitive and resource-demanding plants such as *L. multiflorum* can serve as an indicator of this positive outcome. Therefore, PG management options play a key role in the ecosystem ability of stocking carbon in the soil. Further comprehensive studies on this issue, including additional measurements such as greenhouse gases emissions, are essential to identify effective agricultural practices that should be promoted by specific policies aimed at mitigating climate change.

## Acknowledgements

This research was funded by European Union Horizon 2020 research and innovation programme, under grant agreement 774124, project SUPER-G (Developing Sustainable Permanent Grassland Systems and Policies). The authors also thank the generous collaboration of the farmers.

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# Effects of potential evapotranspiration on condensed tannin and milk production potential in four grassland mixtures

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## Abstract

Dairy farming faces challenges of summer fodder scarcity and methane emissions. To address these issues, one may incorporate plants rich in condensed tannins (CT) into temporary grassland mixtures. However, knowledge regarding the link between CT, milk production and climate remains limited in a field context. We conducted an experiment with four grassland mixtures (grass, grass and legumes, grass and plants rich in essential oils, grass and plants rich in tannins) replicated four times and grazed over six rotations. CT content, dry matter yields, and botanical composition were analysed. We calculated milk production potential (MPP) based on nutrient analysis of the plant material and obtained potential evapotranspiration (PET) data. No differences between mixtures were observed during rotation where PET was low whereas CT content in the tannin mixture was higher in high PET rotation. MPP remained stable over time for the legume, the essential oil and the tannin mixtures but decreased significantly for pure grass. CT content in the tannin mixture was strongly correlated with *Lotus corniculatus* abundance. Our study suggests that plants produce most CT during peak PET in summer, but forage production is highest during lower PET in spring. Thus, tannin-rich mixtures may primarily mitigate methane emissions in summer.

**Keywords:** multi-species grassland, condensed tannins, evapotranspiration, *Lotus corniculatus*

## Introduction

Pastures characterized by a higher diversity of plant species have been proposed to facilitate increased nutrient absorption by ruminants and promote better cattle health (Distel *et al.*, 2020). This effect is due in part to the absorption of plant secondary metabolites such as condensed tannins (CT). These tannins, or proanthocyanidines, are polyphenolic compounds found in various plant species, in which they act as defence mechanisms against biotic (Barbehenn and Constabel, 2011) and abiotic stress such as drought (Gourlay *et al.*, 2022). CT could potentially reduce methane emissions from cattle during digestion by inhibiting certain microorganisms involved in methane production in the rumen (Wang *et al.*, 2015). On-field studies on this topic remain scarce, highlighting the importance of comparing forage production and quality in grassland mixtures with tannin-rich plants versus conventional blends. We anticipated that a grassland mix enriched with tannin-rich plants would consistently display higher CT levels throughout the vegetation season, given the intentional selection of tannin-rich plant species. Concurrently, we expected these CT levels to correlate with potential evapotranspiration (PET). In addition, traditional blends, selected for their productivity, were predicted to sustain elevated productivity across the entire vegetation period.

## Materials and methods

The study was conducted during a single growing season from the end of March to October 2022 in Frick, Switzerland (47°30'51" N 8°1'26" E). The experimental field, covering approximately 1.3 hectares, was divided into 16 plots. In the autumn of 2021, four grassland mixtures were randomly allocated and sown on four plots each: grass, grass and legumes, grass and plants rich in essential oils, grass and tannin-rich plants (in the following referred to as Grass; Legume; Tannin and Oil mixtures). Throughout six grazing rotations by a herd of 23 dairy cows, we closely monitored the botanical composition, the dry matter yield (DM in kg ha<sup>-1</sup>) and the CT content (in g (kg DM)<sup>-1</sup>) of each plot. Furthermore, we calculated the milk



production potential (MPP in kg ha<sup>-1</sup>) of a hypothetical dairy cow for each rotation using the INRA equation (INRA, 2010). PET was assessed (mm/day average over each rotation period, Turc method, [agrometeo.ch](http://agrometeo.ch)). All statistical analyses were conducted using R. Given the non-normal distribution of our data, we employed Spearman tests to assess the correlation between CT levels and PET, between MPP and PET (for each mix) and between the relative abundance of supposedly tannin rich species (only in Tannin mix) and CT levels. Wilcoxon pairwise comparison tests were executed to discern variations in CT and MPP between the mixtures throughout the six rotations.

## Results and discussion

The PET exhibited an increase during the first four rotations, spanning from the end of March to the end of June. Rotations 4 and 5 were conducted during the period when PET reached its peak. Subsequently, there was a decline observed from rotation 5 (August) to rotation 6 (October) (Figure 1). There were significant positive correlations between CT and PET in each of the four mixtures (Table 1). The strongest correlation was observed in the Tannin mix.

Significant differences in CT levels among the four mixtures were observed only at rotation 5, where the Legume mixture exhibited significantly higher CT levels than the Grass mixture, and the Tannin mixture demonstrated significantly higher CT levels than each of the other three (Figure 2a). In the Grass mixture, there was a significant negative correlation between MPP and PET ( $\rho=-0.47$ ,  $S=3370$ ,  $P=0.02197$ ). Significant differences in MPP among the four mixtures were observed only at rotation 1, where the Grass and the Legume mixtures exhibited significantly higher MPP than the Tannin and the Oil mixtures (Figure 2b). These findings suggest that the initial highest yields of the Grass mixture were later negatively impacted by the PET. It also appeared that the MPP of the Grass mixture was lower in

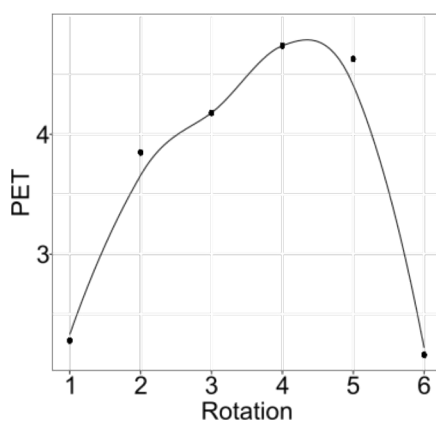


Figure 1. Variation of the PET along the 6 rotations.

Table 1. Results of the Spearman correlation tests between CT and PET in each of the mixtures.

Mix	Spearman correlation coefficient ( $\rho$ )	S	P-value
Grass	0.46	1237.8	0.023*
Legume	0.55	1043.5	0.006**
Tannin	0.84	362.56	<0.001***
Oil	0.41	1359.7	0.047*

\* $P \leq 0.05$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.001$ .

the rotation 5 than for the other mixtures but the correlation was not more than marginally significant. Within the potentially tannin-rich plants of our Tannin mixture, only the relative abundance of *Lotus corniculatus* and the CT levels correlated significantly ( $\rho=0.82$ ,  $S=419.59$ ,  $P=1.074e-06$ ).

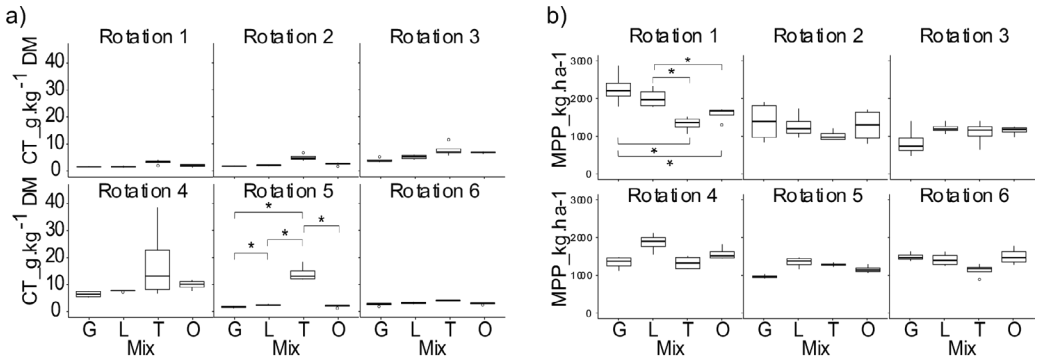


Figure 2. CT levels in  $\text{g (kg DM)}^{-1}$  (a) and MPP (b) in each of the mixtures and rotations. Significant P-values from the Wilcoxon pairwise comparison tests are depicted by the corresponding asterisk, with  $*P \leq 0.05$ ,  $**P \leq 0.01$ ,  $***P \leq 0.001$ .

## Conclusion

Conforming to our hypothesis, CT were correlated with PET in all mixtures. However, in contradiction with what we expected, the Tannin mixture showed higher CT levels than the other mixture only in rotation 5. This result could be explained by the higher *Lotus corniculatus* abundance in rotation 5 as this species has the ability to sustain higher PET. This implies that using tannin-rich species to modulate digestive processes in the rumen, i.e. reduce methanogenesis or improve protein digestibility, may only be effective during periods with high PET. Surprisingly, the Grass and Legume mixtures displayed higher MPP than the other mixtures only at rotation 1. This could be partially explained by the adverse impact of PET on Grass, along with the Tannin and Oil mixtures' ability to maintain yields despite higher PET. In conclusion, our results underscore the significance of climate and timing in designing grassland systems that offer multiple services.

## Acknowledgements

We express gratitude to Samuel Imboden for his work in the lab and colleagues for their precious work in the field. This project is supported by the Stiftung Edith Maryon.

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# Herbage mass and herbage N yield in grass and grass-clover swards receiving zero N

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## Abstract

There are a number of sources of nitrogen (N) in grazing systems including the soil organic N pool, chemical fertilisers, organic manures, deposition, biological N fixation (BNF) and dung and urine recycled by the grazing animal. Legumes, including white clover, play an important role in contributing N for plant growth via BNF. An experiment was established in early 2021 to examine the herbage production and herbage N yield in grass-only and grass-white clover swards. The swards had previously been grazed by lactating dairy cows. The sward areas were fenced off within paddocks, and measurements were undertaken at the same time the surrounding paddock was grazed. No N was applied to the swards in 2021 and they were harvested 8 times during the year between March and October. The average annual sward clover content was 30% in the grass-clover swards. Herbage mass, herbage N content and herbage N yield were greater on the grass-white clover compared to the grass-only swards (+1762 kg DM ha<sup>-1</sup>, +5.1 g (kg DM)<sup>-1</sup> and +75 kg N ha<sup>-1</sup>, respectively).

**Keywords:** white clover, nitrogen yield, herbage production, crude protein

## Introduction

In pasture-based ruminant production systems there are a number of sources of nitrogen (N) for plant growth including the soil organic N pool, chemical fertilisers, organic manures, atmospheric deposition, biological N fixation (BNF) and dung and urine recycled by the grazing animal. Legumes, including white clover (*Trifolium repens* L.), play an important role in contributing N for plant growth via BNF. Pasture-based ruminant production systems in many European countries are required to reduce chemical N fertiliser application to help agriculture achieve a range of climate targets, including reducing greenhouse gas and ammonia emissions, as well as improving water quality, and supporting and enhancing biodiversity. The supply of N within pasture-based systems is crucial for plant growth, and in a scenario of reduced chemical N fertiliser allowances, better use of soil N and alternative N sources are important for herbage production. Knowledge of soil plant available N is important for developing appropriate N fertiliser strategies and can enhance grassland management decisions support tools such as the MoSt Grass Growth Model (Ruelle *et al.*, 2018). The objective of this study was to examine the herbage production and herbage N yield from long-term grass-only and grass-white clover swards where no chemical N fertiliser or organic N fertiliser was applied.

## Materials and methods

This experiment was undertaken at the Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland (52°9' N; 8°15' W) in 2021. Four 5 m × 5 m plots were established in each of existing grass-only and grass-white clover paddocks. The paddocks were sown in July 2012, as described by Egan *et al.* (2018). The grass-only paddocks received 250 kg N ha<sup>-1</sup> annually and the grass-white clover paddocks received 150 kg N ha<sup>-1</sup> annually from 2013 to 2020 inclusive. Paddocks were grazed 8–10 times per year by lactating dairy cows in that period. The plots were fenced so grazing animals were excluded and received no chemical or organic N fertiliser during 2021.

Herbage mass was estimated on the plots at the same time as the surrounding paddock was grazed on 8 occasions between March and October. Herbage mass was estimated with an Etesia mower (Etesia UK, Warwick, UK) by cutting one strip in each plot. The harvested herbage was weighed and a 100 g subsample taken for dry matter (DM) determination and this value used to calculate herbage mass (kg DM ha<sup>-1</sup>). A further subsample was dried at 60°C for 48 hours and milled through a 1 mm sieve and stored for analysis. Sward clover content was measured in the grass-white clover plots at the same time as herbage estimation as described by Egan *et al.* (2018). Herbage crude protein content and herbage N content was estimated using NIRS. Herbage N yield was calculated by multiplying the herbage DM yield by the herbage N content. Data were analysed using PROC MIXED in SAS with terms for treatment, rotation and associated interactions. Fixed terms were treatment and rotation, and the random term was plot.

## Results and discussion

Total annual herbage production and herbage N yield, and annual herbage N content was greater ( $P < 0.01$ ) on grass-clover compared to grass-only (Table 1), similar to Enriquez-Hidalgo *et al.* (2018). Herbage mass was significantly greater ( $P < 0.05$ ) in June, July and October (Figure 1) on the grass-white clover compared to the grass-only sward. Annual average grass-white clover sward white clover content was 30.4%, a percentage considered desirable for biological N fixation (e.g. Andrews *et al.*, 2007). The herbage N content and herbage N yield were also greater on grass-clover compared to grass-only; likely a reflection of the sward clover content (Enriquez-Hidalgo *et al.*, 2018) but potentially also a legacy of the long-term presence of white clover in the grass-clover treatment.

Herbage N yield was similar in the first rotation for each treatment (Figure 2) and was significantly greater ( $P < 0.05$ ) from May to October on the grass-clover treatment compared to the grass-only treatment. While herbage N yield is a product of the quantity of plant available N in the soil, this research indicates that in established grass-white clover swards, the plant available N in the soil is significantly greater than in grass-only swards, which provides opportunities for strategic reductions in chemical (and organic) N fertiliser applications from May onwards.

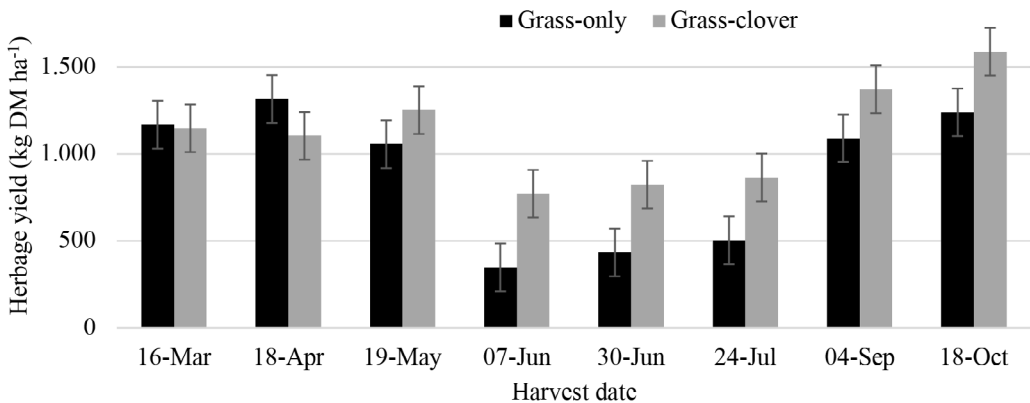


Figure 1. Herbage mass (>4 cm; kg DM ha<sup>-1</sup>) at each harvest on grass-only and grass-clover swards.

Table 1. Total annual herbage production and herbage N yield, and average herbage N content on grass-only and grass-clover swards.

	Grass-only	Grass-white clover	SE	P-value
Herbage production (kg DM ha <sup>-1</sup> )	7150	8912	166.6	<0.001
Herbage N content (g (kg DM) <sup>-1</sup> )	25.7	30.8	0.11	<0.001
Herbage N yield (kg ha <sup>-1</sup> )	191	266	4.3	<0.001

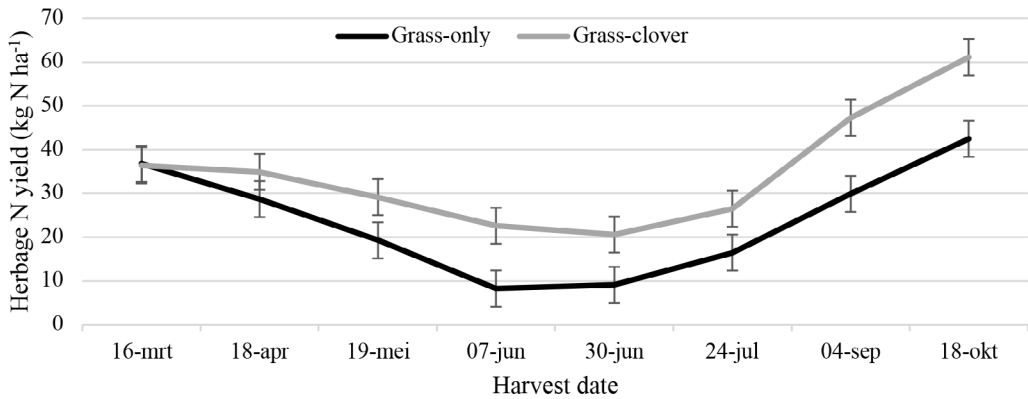


Figure 2. Herbage (>4 cm) N yield (kg N ha<sup>-1</sup>) at each harvest on grass-only and grass-clover swards.

## Conclusions

Herbage production on grass-white clover swards receiving zero chemical fertiliser N is significantly greater than on grass-only swards which offers opportunities to reduce chemical N fertiliser input.

## Acknowledgements

This research was funded by the Irish Dairy Levy administered by Dairy Research Ireland, the Teagasc Walsh Scholarship and the SFI DAFM VistaMilk Research Centre (Grant no. 16/RC/3835).

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# Yield and qualitative evaluation of fodder crops in Mediterranean conditions, under a conservation agriculture technique

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## Abstract

Extensive ruminant production systems represent an important sector of the Portuguese land management and economy. In this study, an assessment was made on the effect of 'cleaning cut: early fodder harvest for weed control' on dry matter production and the quality at different stages of growth of Italian ryegrass forage and a forage mixture, based on Italian ryegrass and clovers. Hence, in 2022–2023, 5 ha of each forage type were sown in the experimental fields of INIAV-Elvas-Portugal. Four biomass cuts were made, in plots with and without cleaning cut. Therefore, the factorial scheme implemented was to combine in each forage, the 'cleaning cut' (with and without) with 4 cutting dates in each of the five blocks. Qualitative characteristics, including protein percentage and dry matter digestibility were measured at each cut. The dry matter production was not affected by the practice of a 'cleaning cut' in any of the forages studied, averaging  $3652 \pm 1143.2$  kg ha<sup>-1</sup>. The effect of time on dry matter production followed a quadratic pattern, with a linear coefficient of  $73.3 \pm 12.30$  kg ha<sup>-1</sup> day<sup>-1</sup> and a quadratic coefficient of  $-0.88 \pm 0.157$  kg ha<sup>-1</sup>. Both the total protein production and evolution of the protein percentage were affected by fodder crop.

**Keywords:** fodder crops, dry matter, fodder quality, extensive livestock

## Introduction

Livestock is a major component of the global agricultural production systems (Manoj *et al.*, 2021). Extensive livestock production is a low-input production system, mostly relying on permanent grasslands (Jenet *et al.*, 2016), representing an important sector of land management to the Portuguese economy. Extensive livestock production provides ecosystem services, such as the conservation of plant biodiversity in pastures. It also contributes to the creation of a high-quality landscape and can provide humans with quality products. The current need in global agriculture is the search for highly productive, sustainable and ecological agricultural production systems. The efficient use of farm resources, such as home-grown forage, is one of the keys to a sustainable and successful farm operation for grassland farmers (Iqbal *et al.*, 2018). The production and conservation of quality forage, i.e., forage based on different botanical families/species, is one of the systems practised on many livestock farms to guarantee the stability of ruminant production. This is all the more important the more arid the production regions are. Direct seeding is also a good strategy for improving the global sustainability of this production system, because it reduces operating costs with significant gains resulting from lower fuel consumption per hectare, and in the medium and long term, improves fertility conditions and water management in the soil, resulting from less exposure to erosion factors and less compaction resulting from fewer passes by machinery.

The aim of this study was to assess the effect of the cleaning cut (early fodder harvest) for weed control on dry matter production and quality at different stages of growth of Italian ryegrass forage and a biodiverse forage mixture based on Italian ryegrass and annual clovers. Carrying out an early cut of the fodder crop contributes to the elimination of winter weeds and, thus, it avoids future competition between

these plants and the fodder crop. This work was carried out as part of the GEEBovMit project - LA 3.3- Mitigation of GHG emissions in beef cattle production - pastures, fodder and natural additives, whose main objective is to optimize forage production by reducing the application of chemical nitrogen fertilizers and contributing to soil conservation.

## Materials and methods

It was decided to sow using a direct seed drill because this is a conservation agriculture technique. It was therefore necessary to apply herbicide (glyphosate: 3 l ha<sup>-1</sup> of commercial product) to eliminate the existing weeds. On November 8, 2022, 5 hectares of Italian ryegrass (*Lolium multiflorum* Lam.) and 5 hectares of a biodiverse mixture based on Italian ryegrass and annual clovers (*Trifolium* sp.) were sown in the experimental fields of INIAV-Elvas-Portugal, under Lixisols. Both fodder crops can be used for multiple cuttings. The sowing density of both forages was 50 kg ha<sup>-1</sup>. Portugal is mainly characterized by a Mediterranean climate (warm to hot dry summers and mild to cool wet winter; rainfall in this area has a strongly seasonal pattern). Relief irrigation was necessary throughout the growing season, using pivot irrigation. The cleaning cut took place on 3 March 2023. After this cut, four biomass cuts were made (Cut 1: 30 March=day 1; Cut 2: 4 May=day 35; Cut 3: 18 May=day 49 and Cut 4: 16 June=day 75), in plots with and without cleaning cut. Therefore, the factorial scheme implemented was to combine in each forage, the treatment: 'cleaning cut' (with and without) with 4 cutting dates in each of the five blocks. The plant samples were taken from a randomly selected 1 m<sup>2</sup> area of each plot. Plant sub-samples were taken from each plot, dried in a forced-air oven at 65°C (48 hours) and weighed to obtain dry matter yield (DM). Qualitative characteristics, including protein percentage (Pro) and dry matter digestibility (DMD) were measured at each cut. The crude protein percentage was measured by Kjeldahl method and the conventional factor of N ×6.25 was used. The in vitro digestibility was estimated according to the determination in vitro of the dry matter digestibility of samples that was performed by the two-stage pepsin-cellulase enzymatic method described by Jones and Hayward (1975). The data were analysed using Proc Mixed in SAS. The heterogeneity of variances was tested and when it was significant ( $P<0.01$ ) it was accommodated in the model using the Group option within the repeated statement of the Proc MIXED. For total DM production, total protein production and protein percentage in DM, the model used included cleaning cut, forage type and their interaction as the fixed effects and also the block as random effect. The day and the day squared were included in the model as continuous variables and the solution option of SAS was used to obtain the regression coefficients. For DMD, data only include the results of cuts 1 and 2, which are the only ones available at the moment. The model used included the effects of cleaning cut, forage type, day and their interactions as fixed effects and Block as Random effect. Data presented were least-square means (LSMeans) for fixed effects and interactions when significant. Statistical significance was set at  $P<0.05$ .

## Results and discussion

The autumn of 2022 was classified as very warm and rainy. The total amount of rainfall in the months of November to December, 322.8 mm, corresponds to around 56% of the total annual rainfall for 2022/23 in Elvas, which negatively affected the establishment of the forage crops under study. By contrast, spring was classified as extremely hot and extremely dry. These climatic conditions were severe enough to affect normal fodder crops growth.

The dry matter production was not affected by the practice of cleaning cut in any of the forages studied, averaging  $3652 \pm 1143.2$  kg ha<sup>-1</sup>. The effect of time on dry matter production followed a quadratic pattern, with a linear coefficient of  $73.3 \pm 12.30$  kg ha<sup>-1</sup> day<sup>-1</sup> and a quadratic coefficient of  $-0.88 \pm 0.157$  kg ha<sup>-1</sup>. No significant differences were recorded between treatment (with or without cleaning cut) ( $P=0.125$ ) and between fodder crop ( $P=0.236$ ). There was no interaction between fodder crops and treatment (with or without cleaning cut) ( $P=0.669$ ). The difference between days ( $P<0.001$ ) had a significant influence

on DM yield. Total protein production varied significantly between the two treatments and between the two crops (all  $P < 0.001$ ). Both the total protein production and evolution of the protein percentage were affected by fodder crop and, by the interaction between cutting day and fodder crop (all  $P < 0.001$ ). The most important factors affecting the forage nutritional value are forage species and growth stage in the moment of harvest (Carita *et al.*, 2016).

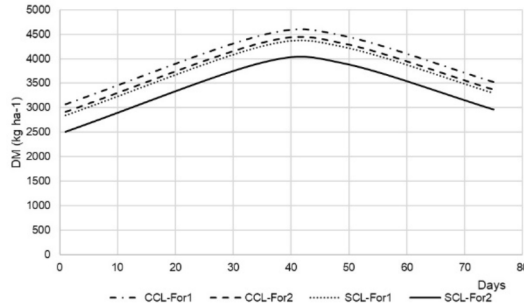


Figure 1. Total dry matter (DM) yield (in  $\text{kg ha}^{-1}$ ) of two treatments (with and without cleaning cut: CCL and SCL) on both fodder crops (For 1, Italian ryegrass; For 2, Italian ryegrass+annual clovers).

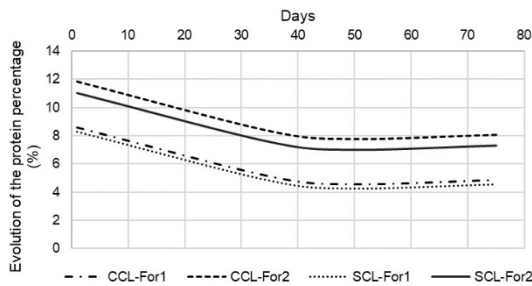


Figure 2. Evolution of the protein percentage of two treatments (with and without cleaning cut: CCL and SCL) on both fodder crops (For 1, Italian ryegrass; For 2, Italian ryegrass+annual clovers).

## Conclusion

This preliminary study provides information on the influence of cutting date and the presence of leguminous species on fodder quality: early cuts and forage mixtures with legumes have a higher nutritional value.

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# European expert opinions on implementation, viability and relevance of innovative grazing practices

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## Abstract

Grazed grasslands deliver many ecosystem services and are therefore worth preservation. Applying the concept of agroecology to grazing systems could help develop grazing systems that are both ecologically and economically viable. Agroecological transformation aims at reducing production inputs and pollution, enhancing the diversity of production to strengthen the resilience of farms, improving animal health and welfare, and preserving biodiversity. To reach these goals, grazing management innovations are needed. Therefore, we conducted a workshop with 27 grazing experts from eight European countries, working in science and/or practice, to identify the most important innovative grazing practices. In an online survey, the experts were first asked to name the three grazing management innovations they considered most relevant in their country and a synoptic list of innovations was compiled. Then, we asked the experts to assess the current rate of implementation of each innovative practice in their country, their feasibility and their relevance to reach the agroecological goals on a 5-point scale. Six innovations (botanical composition for resilience against drought, decision support systems, local/adapted livestock breeds, rotational grazing, trees and shrubs on pastures) were found to deserve special attention, as they combine a low implementation rate, a high feasibility and a high agroecological relevance.

**Keywords:** adaption, agroecology, grassland, grazing management, innovation

## Introduction

Grazed grasslands with optimised management deliver many ecosystem services. The benefits of grasslands for farmers, the environment and the whole society depend on the specific management and differ between countries and regions (Bernués *et al.*, 2022). Applying the concept of agroecology to grazing systems is expected to improve their ecological and economic viability (Hatt *et al.*, 2016). The main aims of agroecological transformation are (I) to reduce production inputs, (II) to reduce pollution, (III) to enhance the diversity of production systems to strengthen the resilience of farms, (IV) to promote management practices to improve animal health and welfare and (V) to preserve biodiversity (Dumont *et al.*, 2013). To reach these goals, it is necessary to identify innovative grazing management practices and to evaluate their current rate of implementation, their feasibility and their agroecological relevance. The present study took advantage of a European network of experts from practice and science to identify and assess innovations currently considered to be relevant to agroecological transformation. We aimed to identify innovations with a low rate of implementation, a high feasibility, and a high agroecological relevance at the same time.

## Material and methods

We conducted a workshop with 27 grazing experts of eight European countries participating in the Horizon Europe project Grazing4AgroEcology (<https://grazing4agroecology.eu/>). These experts are working in agricultural science and/or practice in the following countries: Germany, France, Ireland, Italy, the Netherlands, Portugal, Romania and Sweden. Since September 2022, sixteen facilitator agents have been exploring grazing innovations in the respective countries within a network of 120 partner farms.

In a first step, we used the interactive survey software *mentimeter* ([menti.com](https://www.menti.com)) to gather information about grazing management innovations. Each expert was asked to name the three innovations that he or she considered most relevant in his/her country or region. In the following step, we compiled a synoptic list of the gathered innovations. These innovations were then evaluated by the experts regarding three different aspects: the current rate of implementation in the respondent's country (1, <2%; 2, 2–5%; 3, 5–10%, 4, 10–20%; 5, >20%), their feasibility in terms of implementation in practice (5-point Likert-type scale from 1: very hard to 5: very easy) and their relevance to reach the agroecological goals (5-point Likert-type scale from 1, highly irrelevant to 5, highly relevant). The responses were first averaged at country level to remove the bias of countries having more participants than others. Then, these values were analysed by means of descriptive statistics.

## Results and discussion

In total fourteen innovations were developed. The evaluations of European grazing experts showed that few innovations were considered to have a low rate of implementation (mean value <2). These are decision support systems, society involvement, solar grazing and virtual fencing. A high feasibility (mean value >3) was assessed for the innovations of botanical composition for resilience against drought, legumes and herbs for nutrient supply and biodiversity, rotational grazing (long and short interval of stocking density), seasonal calving and timely start of grazing. A high agroecological relevance was attributed to the following innovations (mean value >4): botanical composition for resilience against drought, legumes and herbs for nutrient supply and diversity, local/adapted livestock breeds, rotational grazing (short interval of stocking density) and seasonal calving (Figure 1).

Combining a low implementation, a high feasibility and a high agroecological relevance the following innovations deserve special attention: botanical composition for resilience against drought, decision support systems, local/adapted livestock breeds, rotational grazing, trees and shrubs on pastures (against erosion, for shade, water and nutrient retention) (Figure 1). Virtual fencing is considered to be a promising future opportunity, but it is not allowed in Europe so far (Aaser *et al.*, 2022).

As our workshop was conducted with grazing experts working in science and practice, the suggested innovations generally have potential of being adopted in the future. However, it must be kept in mind that the willingness to adopt agroecological innovations depends on various factors (Blazy *et al.*, 2011). During

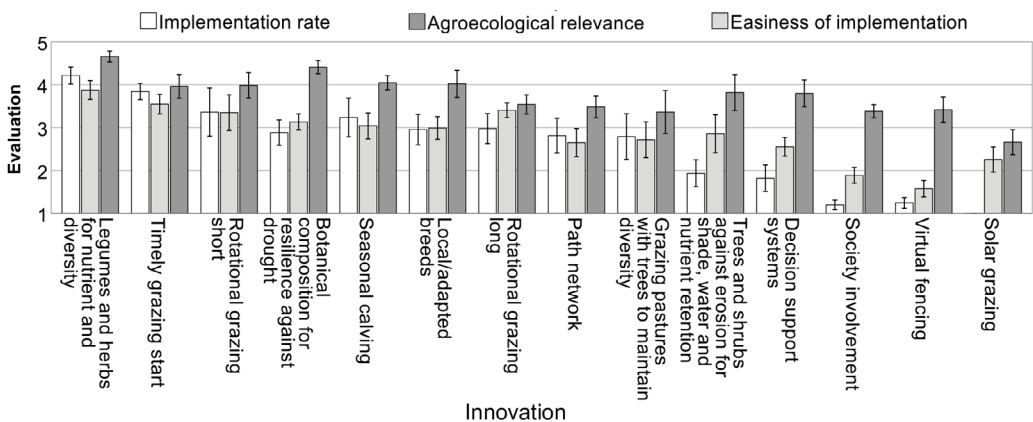


Figure 1. Mean and standard error of the evaluation of 27 European grazing experts on the current rate of implementation, the feasibility, and relevance to agroecological goals of fourteen different innovations in grazing management.

our workshop, we learned that the rate of implementation as well as the feasibility and the agroecological relevance of innovations are evaluated differently when comparing countries or agroclimatic zones. This opens up the possibility to learn from each other across countries and agroclimatic zones and is one aim of the networking within the project Grazing4AgroEcology.

## Conclusion

With our workshop we created an overview of grazing management innovations and gained an insight into their rate of implementation, their feasibility and their agroecological relevance in different European countries. The next step should be to investigate which obstacles and advantages farmers see in adopting these innovations. A further step could be to create a roadmap to support farmers in adopting such innovations to enhance an agroecological transformation. In this context, a network of farmers and stakeholders across Europe is helpful to learn from each other beyond national borders.

## Acknowledgements

A great acknowledgement goes to the grassland experts of the project Grazing4AgroEcology for participating in our workshop and their evaluation of innovative grazing practices. The project leading to these results (Grazing4AgroEcology) has received funding from the European Union's Horizon Europe research and innovation programme under grant agreement No 101059626.

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# Effect of botanical composition and fertilisation on yield and quality of legume-grass mixtures

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## Abstract

The objective of this research was to determine the effect of legumes and nitrogen (N) fertiliser application on the herbage quality and botanical composition of species-rich mixture (SRM). Species in grass mixture (G) were perennial ryegrass (*Lolium perenne* L.), tall fescue (*Festuca arundinacea* Schreb.), meadow fescue (*Festuca pratensis* L.) and timothy (*Phleum pratense* L.). Legumes-grass mixture (LG) consisted of red clover (*Trifolium pratense* L.), white clover (*Trifolium repens* L.) tall fescue and meadow fescue. SRM included six legumes, seven grass species and chicory (*Cichorium intybus* L.). The effects of two N application rates (0 (N0) and 200 kg ha<sup>-1</sup> (N200)) were studied. Dry matter (DM) yield and feeding value parameters such as crude protein, neutral detergent fibre, acid detergent fibre and digestible DM were determined. The average DM yield in the unfertilised grass mixture was 9.99 Mg ha<sup>-1</sup> and in the fertilised SRM 15.86 Mg ha<sup>-1</sup>. Crude protein content (159–197 g (kg DM)<sup>-1</sup>) was closely related to the proportion of legumes in the sward. SRM exceeded the yields of G and LG in all trial years.

**Keywords:** grasses, legumes-grass, species-rich mixture, nutritive value, nitrogen

## Introduction

In fertile agrosystems multi-species swards can reduce energy consumption by replacing highly energy demanding nitrogen (N) fertiliser by natural nitrogen fixation, whilst maintaining biomass production (Lüscher *et al.*, 2014). An optimal combination of suitable grasses and legumes companion species are needed to obtain high N-use efficiency, high herbage yield and high contents of nutritive compounds in grass-legumes mixtures (Elgersma and Søegaard, 2015; Suter *et al.*, 2023). When choosing legumes for grass-legumes mixtures, the rate of phenological development of the species, persistency and nutritive value should be considered (Tamm *et al.*, 2018). The nutritive value is highest when the first cut is harvested at a shooting stage of grasses and budding for legumes. The aim of this study was to determine the effect of nitrogen fertilisation on the performance of species-rich mixture (SRM).

## Materials and methods

This study was initiated in 2019 and carried out in Saku, Estonia (59°28' N, 24°65' E) with SRM, legumes-grass (LG) and grass (G) mixtures (Table 1). The study included data from three years (2020–2022). The trial plots were seeded in early summer on a typical soddy-calcareous soil. These trials had four replicates with a split-plot design, sward type as a main plot and two nitrogen rates of N0 and N200 as subplots. Total N application of 200 kg ha<sup>-1</sup> was divided into three applications (80+60+60 kg ha<sup>-1</sup>). Autumn fertiliser (7-20-28) was also applied at 300 kg ha<sup>-1</sup> in all treatments. A three-cut system was used with the first cut on May 26 to June 9, second on June 26 to July 18 and third cut on September 21 to 28. The forage was harvested, weighed and samples were taken for laboratory analyses and for estimation of botanical composition. The following data were determined: dry matter (DM) yield, the contents of crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), metabolisable energy (ME) and digestible dry matter (DDM). The sum of effective temperatures (over 5°C) for the growing season (April–September) was 1394°C in 2020, 1523°C in 2021 and 1431°C in 2022. Total amount of rainfall during the growing season was 459 mm in 2020, 293 mm in 2021, and 230 mm in 2022 (long-term average 353 mm). Statistical analyses (ANOVA and Fisher's LSD) were carried out with Agrobase 20™.

Table 1. Seed rates (kg ha<sup>-1</sup>) of legumes-grass and pure grass mixtures.

Species/cultivar	Species-rich mixture (SRM)	Legumes-grass (LG)	Grass (G)
Lucerne ( <i>Medicago sativa</i> L.) cv. Juurlu	2.5		
Red clover ( <i>Trifolium pratense</i> L.) cv. Jõgeva 433	3	2.5	
White clover ( <i>Trifolium repens</i> L.) cv. Tooma	1		
White clover ( <i>Trifolium repens</i> L.) cv. Jõgeva 4	1	0.8	
Alsike clover ( <i>Trifolium hybridum</i> L.) cv. Jõgeva 2	1		
Bird's foot trefoil ( <i>Lotus corniculatus</i> L.) cv. Leo	0.5		
Timothy ( <i>Phleum pratense</i> L.) cv. Tika	5	7.5	4
Meadow fescue ( <i>Festuca pratensis</i> L.) cv. Arni	4	3	
Kentucky bluegrass ( <i>Poa pratensis</i> L.) cv. Esto	2	2	
Red fescue ( <i>Festuca rubra</i> L.) cv. Kauni	1		1
Perennial ryegrass ( <i>Lolium perenne</i> L.) cv. Raite	2	2.5	10
Tall fescue ( <i>Festuca arundinacea</i> Schreb.) cv. Kora		4.2	
Tall fescue ( <i>Festuca arundinacea</i> Schreb.) cv. Barelite	4		5
Tall fescue ( <i>Festuca arundinacea</i> Schreb.) cv. Barolex			5
Italian ryegrass ( <i>Lolium multiflorum</i> Lam.) cv. Talvike	3	2.5	
Chicory ( <i>Cichorium intybus</i> L.) cv. Spadona	0.3		
Total	30.3	25	25

## Results and discussion

SRM exceeded the yields of G and LG in all trial years whereas LG overyielded the G, except for 2022. The N rate of 200 kg ha<sup>-1</sup> contributed to a significant DM yield increase in all harvest years for all mixture types. The highest DM yield was obtained from SRM and LG supplied with N fertiliser, with the three-year average yields of 15.86 and 13.61 Mg ha<sup>-1</sup>, respectively. The three-year average DM yield at N0 was 12.60 Mg ha<sup>-1</sup> for SRM and 10.52 Mg ha<sup>-1</sup> for LG (Table 2). N200 increased the forage yield of G by 7.03 Mg ha<sup>-1</sup> and its crude protein content. The highest yield of 13.97 Mg ha<sup>-1</sup> was obtained in the first year. The DM yield of G was highly dependent ( $p < 0.001$ ) on fertilisation. The three-year average DM yields of the G at N0 and N200 were 2.96 and 9.99 Mg ha<sup>-1</sup>, respectively. Lucerne and red clover in the mixtures increase the CP concentration compared to pure grass swards (Meripõld *et al.*, 2022). The growth and development of legumes and perennial ryegrass are slow in spring as they require higher temperatures. The high air temperatures and drought in summer of 2022 reduced the yields of mixtures. The effect of the level of nitrogen fertilisation on the increase in DM yield was closely connected with the botanical composition of swards (Gutmane *et al.*, 2018). In the LG at N0 level in 2020, leguminous plants' content was 38% in the first cut and 52% in the second cut in 2022 (Figure 1). The fertilisation rate of N200 reduced the proportion of legumes in the mixtures. The forage CP content in the first cut at N200 was 164 g (kg DM)<sup>-1</sup> in LG, 159 in SRM, and 135 g (kg DM)<sup>-1</sup> in the G. The CP contents in the SRM and LG were higher in the first cut than in the second cut. The physiological development of lucerne during harvest has a significant impact on the CP content (Tamm *et al.*, 2020). On average, N200 increased DM yield of all mixtures but improved the forage nutritional value only in G. The legumes ensured forage with high ME and DDM content in the mixtures.

In first cuts, the NDF values of the LG and SRM at N0 were lower than those of the pure grass variants because of higher fibre content in grasses compared to lucerne and red clover. At N200, the values of DDM in the first cut for the SRM and LG were 676 and 684 g (kg DM)<sup>-1</sup>, respectively. The same N application rate increased the forage nutritive value of G in the first cut, up to 686 g (kg DDM)<sup>-1</sup> and

10.6 MJ kg<sup>-1</sup> ME. At N0, the SRM and LG had equally high concentrations of DDM (696 g (kg DM)<sup>-1</sup>) and ME (10.8 MJ (kg DM)<sup>-1</sup>).

Table 2. The DM yield (Mg ha<sup>-1</sup>) of mixtures in 2020–2022

Mixture	2020		2021		2022		Average 2020–2022	
	N0	N200	N0	N200	N0	N200	N0	N200
Grass (G)	6.45 <sup>e</sup>	13.97 <sup>d</sup>	1.10 <sup>b</sup>	6.44 <sup>d</sup>	1.33 <sup>c</sup>	9.57 <sup>b</sup>	2.96 <sup>d</sup>	9.99 <sup>c</sup>
Legumes-grass (LG)	12.83 <sup>d</sup>	17.22 <sup>b</sup>	11.23 <sup>c</sup>	14.98 <sup>b</sup>	7.51 <sup>d</sup>	8.64 <sup>c</sup>	10.52 <sup>c</sup>	13.61 <sup>b</sup>
Species-rich mixture (SRM)	15.31 <sup>c</sup>	18.81 <sup>a</sup>	11.78 <sup>c</sup>	18.71 <sup>a</sup>	10.71 <sup>a</sup>	10.05 <sup>ab</sup>	12.60 <sup>b</sup>	15.86 <sup>a</sup>

Different lowercase letters within years are statistically different ( $p < 0.05$ , Fisher LSD test).

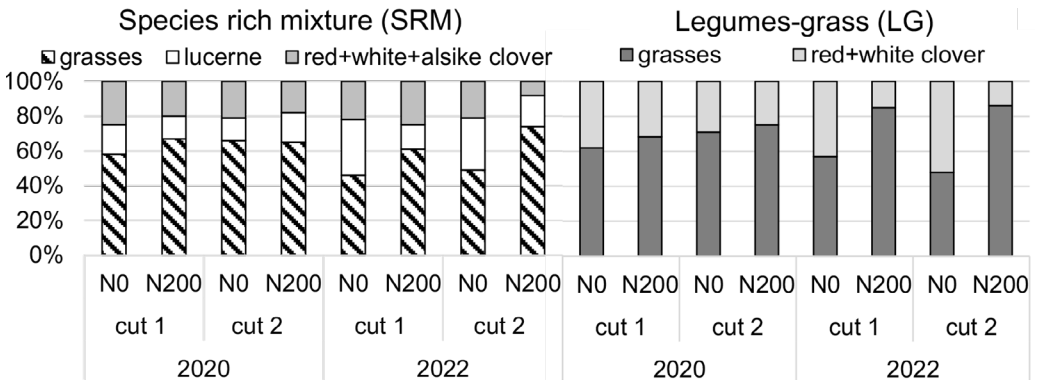


Figure 1. Botanical composition of mixtures in 2020 and 2022.

## Conclusions

SRM exceeded the yields of G and LG in all trial years whereas LG overyielded the G, except the 2022. The addition of legumes species enhanced forage yield and nutritional value. The positive effect of N fertilisation on DM yields is closely related to the botanical composition of swards. The N200 fertilisation rate increased the forage DM yield of G and improved its nutritional value, but reduced the proportion of legumes in the mixtures. Without N fertiliser, legumes ensured high ME and DDM content of the forage in the first cut.

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# The effect of nitrogen and phosphorus chemical fertiliser and slurry application on white clover establishment in grazed swards

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## Abstract

Ensuring an adequate establishment of white clover content in intensively managed grazing swards can be challenging. The objective of the current study was to assess the impact of nitrogen (N) and phosphorus (P) chemical fertiliser and organic manure on clover establishment. A 4 by 2 by 2 factorial design study was established on a grass-clover sward with 4 rates of P fertiliser (0, 15, 30 and 45 kg P ha<sup>-1</sup>) and 2 N strategies, for the first (at sowing), second (6 weeks post-sowing) and third application (after the 2<sup>nd</sup> defoliation) as follows: (0:0:0, Zero; 30:30:30, High, kg N ha<sup>-1</sup>) and 2 slurry treatments, (Slurry/No slurry) at sowing. Plots were defoliated on 3 occasions, herbage mass and clover content were determined at each defoliation. There was no effect ( $P>0.05$ ) of P fertiliser on herbage mass, however, the high N treatment had the greatest while the zero N treatment had the lowest, 1886 and 1468, kg DM ha<sup>-1</sup>, respectively. The zero N treatment had ( $P<0.001$ ) the greatest content (29.1%) with the high N treatment at 19.3%. Phosphorus had no effect on clover content. The application of slurry had an effect ( $P<0.05$ ) on both the herbage mass and clover content. These findings highlight the importance of strategic nitrogen and slurry application during the establishment phase of a sward while phosphorus had no notable influence.

**Keywords:** white clover, nitrogen fertiliser, phosphorus fertiliser, persistency

## Introduction

The Ag Climatise Roadmap has set an ambitious target to reduce chemical N from 408,000 t to under 325 000 t by 2030. The importance of establishing white clover (*Trifolium repens* L.) consistently in reseeded swards while achieving the required proportion (>20%) of clover in the sward is to improve animal and DM production. It has been previously reported that clover can supply up to 230 kg N ha<sup>-1</sup>, through N fixation. Soil fertility is one of the most important factors affecting clover growth because it directly impact the plant's ability to carry out essential physiological processes such as photosynthesis and structural plant formation, which can negatively impact plant metabolism, growth, and reproduction (Caradus *et al.* 1995). Nitrogen fixation in the initial 12–18 months post-sowing, is low and the release of this fixed N to the soil and companion species is negligible (Frame & Paterson, 1987). The application of chemical N post-sowing is potentially vital to encourage growth and development of grass and clover plants. When nutrients are applied to newly established leys they can potentially result in grass outcompeting the clover, resulting in shading, limiting the potential for photosynthesis of the clover plant compared to the grass plant (Frame and Paterson, 1987). The objective of the current study was to investigate the impact of nutrient application on grass-clover swards in the establishment period.

## Materials and methods

The experiment was carried out at the Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Ireland from July 2023 to November 2023. Soil samples were collected at a depth of 10 cm, and their phosphorus (P) and potassium (K) indices were both determined to be 4, on a scale ranging from 1 to 4 (where 1 indicates deficiency and 4 indicates sufficiency; Alexander *et al.*, 2008). A 4×2×2 factorial design with 4 rates of P fertiliser (0, 15, 30 and 45 kg P ha<sup>-1</sup>) and 2 N strategies (Zero



and High) for the first (at establishing), second (6 weeks post-sowing) and third application (after the 2<sup>nd</sup> defoliation) was as follows (kg N ha<sup>-1</sup>): 0:0:0, Zero; 30:30:30, High, and the application of slurry or no slurry was applied at establishment at a rate of 14 kg N ha<sup>-1</sup> and 10 kg P ha<sup>-1</sup>, resulting in 48 plots measuring 1.5×10 m across three replicates. All plots were defoliated on three occasions by lactating dairy cows. Prior to each grazing, herbage mass (>3.5 cm; DM yield), was measured by harvesting a proportion of each plot using an Etesia mower, the sample was weighed and a random grab sample of 100 g was taken and dried at 90°C for 16 hours to determine DM content. Sward clover content was measured prior to each defoliation by taking three random grab samples across the plot; a 70 g sub-sample was then separated into grass and clover fractions and dried at 90°C for 16 hours to determine DM proportions. Post-grazing sward height (cm) was measured on each plot after each defoliation. All data were analysed using PROC MIXED in SAS 9.4 (SAS Institute, Cary, NC, USA, 2002). Nitrogen, P, slurry, and associated interactions were included as fixed effects, repetition was included as a random effect, and rotation was included as a repeated effect and plot as the subject. All insignificant interactions were removed from the model.

## Results and discussion

Nitrogen treatment had a significant effect on the average DM yield ( $P<0.001$ ; Figure 1); the zero N treatment had a lower average pre-grazing DM yield than the high N treatment, 1468 and 1886 ± 28.5 kg DM ha<sup>-1</sup>, respectively, resulting in an increase of 1255 kg DM ha<sup>-1</sup> in total DMY for the high N treatment. Ledgard *et al.* (1995) reported a similar impact with the application of N fertiliser as it can increase total DM by up to 25%, similar to the 23% increase in the current study, and a total N application of 90 kg N ha<sup>-1</sup>. The application of P fertiliser in the current study had no significant effect on herbage mass across all 4 treatments with the average at 1678 kg DM ha<sup>-1</sup>. This is in contrast to the results of Schils and Snijders (2004) which concluded that the application of P fertiliser, had a positive impact on the DM yield on a comparable soil P index site (Alexander *et al.*, 2008). The application of P fertiliser has been reported to increase tillering, root growth, and nodulation in establishing swards, even when soil P index are of a high status (Sheils, 2014). Previous studies by Thers *et al.* (2022), have reported a greater herbage production with the application of organic manure (e.g. slurry) on established swards. Conversely, there has been little investigation on the impact of slurry on establishing swards. The application of slurry in the current study resulted in a significantly lower DM yield compared to no slurry, with values of 1638 and 1717 ± 28.53 kg DM ha<sup>-1</sup>, respectively.

The use of N contributes to substantial increases in DM yield; however, the application of N fertiliser in the current study also reduced sward clover content (Figure 1). The zero N treatment had a greater ( $P<0.001$ ) average sward clover content compared to the high N treatment (29.1 and 19.3±1.25%, respectively), with no difference in rotation 1 between treatments; however, in rotation 2 and 3 the zero N had a higher clover content (Figure 1). Similar to Ledgard *et al.* (1995), Egan *et al.* (2018) reported that N fertiliser application decreased clover in the sward; however, much of this work was carried out on established swards, not establishing swards as in the current study, which could have longer term implications on clover persistence (Frame and Paterson, 1987). Similar to the DM yield, the P application of fertiliser had no significant effect on clover content of the sward (24±1.77%). Contrasting results were found previously that reported increasing amounts of P resulted in a reduction of clover content, primarily attributed to greater competition from grass. Conversely, when soil P indexes are limiting, it has resulted in an increase sward clover content (Sinclair *et al.*, 1996). Nesheim *et al.* (1990) reported that organic manure can result in greater clover content, similar to the current study where the slurry treatment increased sward clover content ( $P<0.01$ ) (27.09 and 21.25±1.25%).

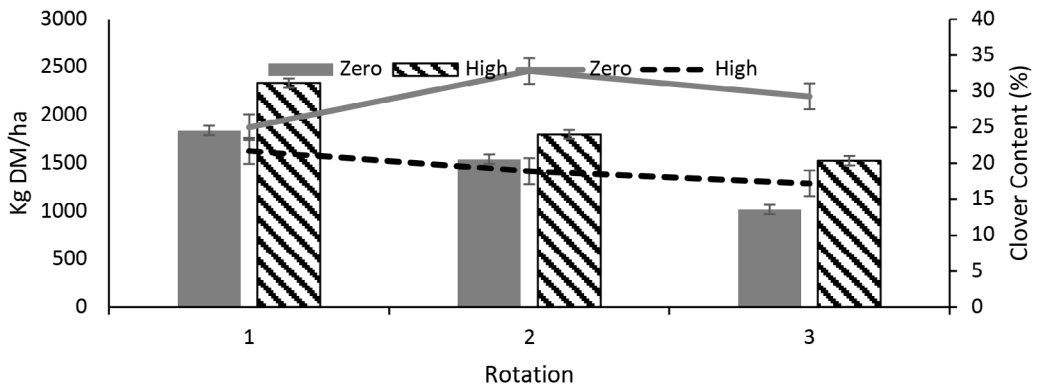


Figure 1. Mean DM yields (bars) and clover content (lines) across 3 rotations, for Zero N (solid grey bars and grey line) and High N treatment (broken black bars and broken black line). Error bars show standard deviation.

## Conclusions

The application of P fertiliser when establishing grass-clover swards did not impact the subsequent DM yield or sward clover content. Increasing the application of N fertiliser in establishing grass-clover swards results in increased DM yield; however, clover content was adversely affected by N rate. The application of slurry during sward establishment increased sward clover content but resulted in a reduction in DM yield. The application of N fertiliser to increase DM yield in establishing swards is required; however, careful consideration should be given to ensure an adequate level of clover content is established and maintained to further reduce the longer term requirement for chemical N fertiliser.

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# Effects of water infiltration on grass yields and soil nitrogen supply of Dutch peat soils

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## Abstract

Despite the large proportion of surface water and high ditch water levels, Dutch peat soils have a high nitrogen supply due to peat oxidation. To reduce land subsidence and GHG emissions, raising groundwater levels can reduce oxygen entry and so peat oxidation. To this end, field experiments with submerged drains for water infiltration were carried out from 2003 to 2021. Grass yields were determined up to and including 2014 with and without nitrogen (N) fertilization. N yields of the unfertilized fields represent the soil N supply (SNS). Under fertilized conditions grass yields were not influenced, or hardly influenced, by submerged drains. Where submerged drains provided extra water infiltration during summer, the SNS was reduced. This yield-reducing effect was compensated by a higher N utilization.

**Keywords:** grass, yield, nitrogen, peat, water, oxidation

## Introduction

A characteristic of the western peat meadow areas in the Netherlands is the large proportion of surface water and high ditch water levels, which makes the soil too wet for arable farming. Grassland can withstand wet conditions and is productive because of the high N supply of the soil due to peat oxidation. According to Schothorst (1977), extra drainage increases the soil N supply (SNS). SNS stabilizes after years (Vellinga and André, 1999). To prevent declining groundwater tables during summer and oxygen entering the soil, field experiments have been carried out with water infiltration via submerged drains. Submerged drains are drainpipes that lie below ditch water level and infiltrate in dry periods and drain in wet periods and so provide a flatter groundwater course. In 2003, research was started at experimental farm KTC Zegveld with the aim to determine the effect of submerged drains on the groundwater level, grass yield and SNS. Since then, several field experiments and pilots have been carried out up to and including 2021 (Hoving *et al.*, 2023). An overall analysis of all those experiments showed that where submerged drains had a significant effect on the groundwater level, the groundwater level course was 23% flatter compared to the reference situation without water infiltration. Here the long-term average groundwater level of the reference treatment was taken as a reference. The effect on grass yield and SNS has only been investigated from 2004 to 2014. In this article the focus is on N yield and SNS.

## Material and methods

The first field trial with submerged drains was started at the experimental farm Zegveld (2004–2007) at ditch water levels of 20 and 55 cm below field level with drain distances of 4, 8 and 12 m. The experiment was continued on the same plots in 2011–2012. Furthermore, experiments with submerged drains were carried out at Hobrede and Kwadijk (2007–2010) and Warder (2012–2014). The ditch water levels at Hobrede, Kwadijk and Warder were respectively 60, 60 and 40 cm below field level (stabilized situations) and the drain distances were 6, 6 and 4 m, respectively. On the plots, strips were mown (1.5×6–9 m) in duplicate per plot on the treatment sections without (C) and with (D) submerged drains and without (N0) and with (N1) N fertilizer. The N fertilization amount was 225 kg N ha<sup>-1</sup> year<sup>-1</sup> in five dressings. A statistical analysis on the effect of the treatment submerged drains at different locations with two N

fertilizer levels on DM yield and N yield (on logscale) has been carried out with a mixed model in Genstat 19<sup>th</sup> edition (VSN, 2018). The main effect of submerged drains, N fertilizer, ditch water level (locations) were estimated and tested in the fixed model (including the interaction with location). With Microsoft Excel linear regressions were specified (equation and  $R^2$ ) to determine how water levels relates to N0 yield and how DM yield relates to the total N availability.

## Results and discussion

The differences in N yield between the treatments with and without submerged drains differed significantly per combination location and ditch water level, but not for nitrogen fertilizer level. The relative change in N yield for submerged drains compared to the reference per location and ditch water level (cm) are shown in Figure 1.

At locations with relatively low ditch water levels submerged drains had an extra draining effect which caused a higher N yield (>100%) and at locations with relative high ditch water levels submerged drains had an extra infiltrating effect which caused a lower yield (<100%) than the reference treatment (except for Zegveld 2011–2012). For both situations the effect for N0 was statistically equal to the N1 treatment. The N yield of the N0 treatment represents SNS and was only dependent on mineralization. In particular, the extent to which submerged drains changed the summer groundwater level influenced SNS and the N uptake by grass.

In the left graph of Figure 2 ditch water levels are plotted against N0 yield for the treatment with and without submerged drains. This resulted in different linear relationships, and although these have a relatively low  $R^2$  (probably due to difference in clay cover), they confirm that rewetting reduces soil mineralization and releases less nitrogen. For submerged drains this effect was greater than without submerged drains. The relations intersect at a ditch water level of 40 cm below surface level. Submerged drains at ditch water levels <40 cm had a lower SNS and ditch water levels > 40 cm below field level had a higher SNS. To determine how dry matter yield relates to the total N availability, N yield with (N1) and without (N0) N fertilization is plotted against dry matter yield in the right graph of Figure 2.

This resulted in slightly different linear relationships for the treatment with and without submerged drains, which means that N utilization differed per situation. The relationship for N fertilization, or not, was the same for drains and no drains. For the conversion of N into biomass, it therefore did not matter whether the N was supplied by mineralization or by fertilization. The relationships differ in slope

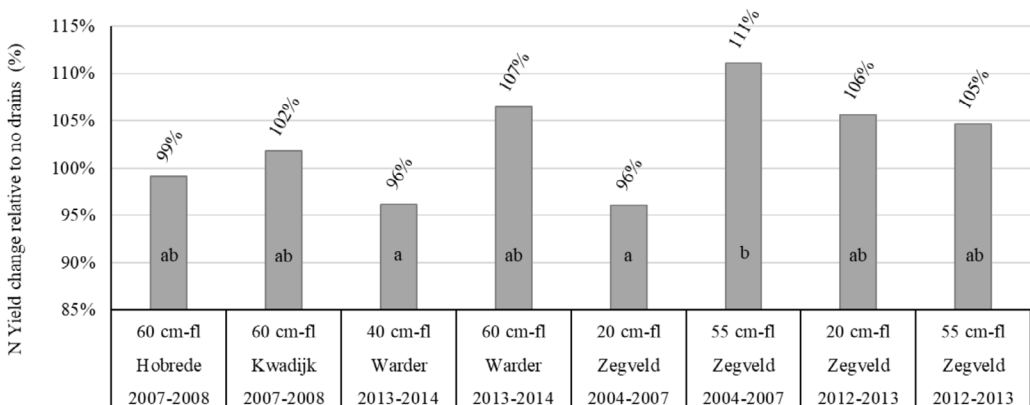


Figure 1. Relative change in N yield of submerged drains versus no submerged drains (%) per location and ditch water level in cm below field level (fl). Statistical effects are indicated with different letters.

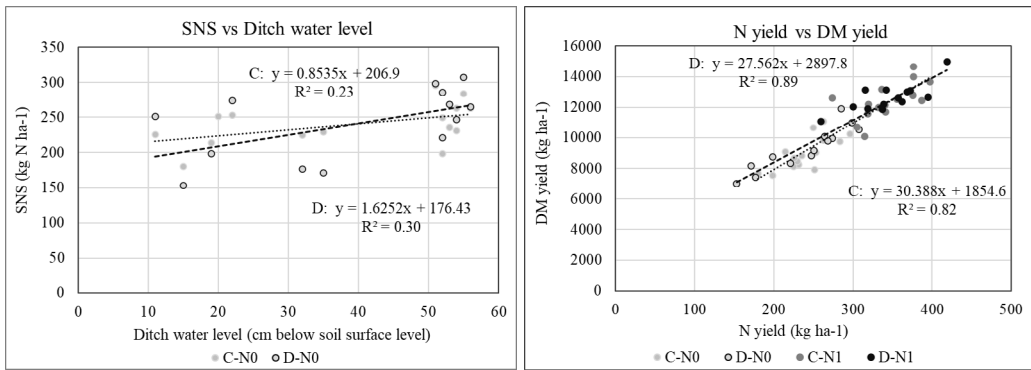


Figure 2. Left: Ditch water level versus SNS for the treatments with (D) and without (C) submerged drains. Right: N yield versus dry matter yield with (N1) and without (N0) N fertilization and with (D) and without (C) submerged drains.

and intercept for the situation with and without submerged drains. Submerged drains gave a higher dry matter yield at a relatively low SNS due to higher N utilization. A relatively high total N supply (soil+fertilization) resulted in similar dry matter yields with and without submerged drains. This means that N fertilization compensates for a lower SNS by a higher utilization.

## Conclusions

It is concluded that under fertilized conditions grass yields were not influenced, or hardly influenced, by submerged drains. Where submerged drains provided extra water infiltration in the summer half-year, SNS decreased and N uptake increased (conversion of available nitrogen from the soil and fertilization into biomass). This higher N utilization compensated for the reduction in SNS. Ultimately, the net effect on grass production under fertilized conditions was relatively limited.

## Acknowledgements

The statistic overall analysis was funded by the WUR internal program KB34 Towards a Circular and Climate Neutral Society (2019-2022), project KB34-KB-34-005-001 1-2A-1 Peatlands in the new circular and climate positive productions systems (GREENDEAL) 2022.

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# Conservation and promotion of genetic resources of native plants in Swiss grasslands — the RegioFlora project

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## Abstract

The decline in genetic diversity is a well-known problem. With the massive increase of newly established extensive grassland in agriculture across Europe the use of seeding material from native plants (not varieties) has also increased. The provenance of seed material, i.e. the origin of the collected and subsequently propagated plant species, has long been neglected. The use of native plants from non-regional origin can alter the genetic composition of populations, even leading to a loss of ecotypes typical of the region, or site in the worst case. Today, increasing attention is being paid to the provenance of plant seeds, as shown by the European directive 2010/60. In Switzerland, the RegioFlora project is dedicated to improving the consistent implementation of regional seed provenance in semi-natural habitats.

**Keywords:** genetic diversity, grassland restoration, native seed, seed origin

## Introduction

Genetic diversity stands as a cornerstone of ecological resilience and agricultural sustainability. The intensification along with abandonment of grassland has heavily contributed to a decline in genetic diversity in European agriculture (Boch *et al.*, 2020; Stoate *et al.*, 2009; Török *et al.*, 2018). At the same time, the restoration of extensively managed grasslands has increased in importance — especially with the UN Decade of Ecosystem Restoration (2021–2030) ongoing but inadvertently poses a further threat for genetic diversity of grasslands. While grassland restoration practice gained traction, the oversight of seed material provenance — the origin of collected and propagated plant species — has persisted (Mainz and Wieden, 2019; Vogel, 2002). Neglecting the regional origins of native plants used in seeding material can result in significant genetic alterations within populations (Hufford and Mazer, 2003; Krauss *et al.*, 2013). The genetic composition of a plant population is closely linked to its adaptive capacity and resilience to environmental stressors (Chung *et al.*, 2023). When seeds sourced from foreign origins are introduced, they can disrupt the complex balance that has evolved over time between the plant species and their environment. This disruption might manifest as a loss of unique ecotypes that are finely attuned to the specific regional conditions (Hufford and Mazer, 2003). Using seed from native plants from the same region (native seeds) to restore grassland is therefore recognised as a good practice, reducing potential risks to native populations (Baasch *et al.*, 2016, Kirmer *et al.*, 2018). This also applies to all other types of semi-natural habitat restorations (Pedrini and Dixon, 2020).

The European Directive 2010/60 (European Commission 2010) on preservation mixtures is an example of first efforts to help address the needs of native seeds. This directive has partially eased the regulations of other directives that apply to certain species in preservation mixtures and has facilitated the production and use of regional seed material. In the context of the European Green Deal (European Commission, 2019) and the corresponding political objectives, EU legislation on plants and reproductive material is currently being revised and directives are being merged into a single legal framework (European Commission, 2023). For preservation mixtures and native seed, the revision is a critical step in creating a simple and workable framework that defines terms, emphasises the need for traceability of seed origin and promotes the quality of native seed without interfering with the needs of agricultural seed regulation.

While the importance of regional seed provenance is being recognised, challenges still exist. Implementation on a broader scale requires further education and advocacy among agricultural stakeholders. Furthermore, ensuring the availability of regionally sourced seed material poses logistical and economic challenges. And lastly, different methods of grassland restoration must be considered and coordinated to ensure that appropriate methods are available and used according to restoration objectives.

Overcoming these challenges will require the combined efforts of policymakers, conservationists and agricultural practitioners alike. Mediating between these groups is therefore an essential task.

## **Implementing the conservation and promotion of genetic diversity in grasslands — Case study Switzerland**

In order to guide and coordinate the efforts to safeguard the genetic diversity of grasslands in Switzerland, the Swiss Federal Office for Agriculture (FOAG) is funding a national advisory and coordination office for the conservation and promotion of genetic resources of native plants in agriculture: RegioFlora. This initiative aims to secure genetic diversity of native plants and to promote the consistent implementation of regional origin of seeding material. The office is broadly supported by the Bern University of Applied Sciences — School of Agricultural, Forest and Food Sciences (BFH-HAFL), the Swiss Grassland Federation (AGFF) and the National Data and Information Center on the Swiss Flora (InfoFlora).

RegioFlora has two main objectives. One is to raise awareness for seed origin among major disseminators — policymakers, agricultural administration, consulting services and seed companies. The second is to provide and spread information about the responsible use of native plants and best practices in grassland restoration.

As a neutral and independent organisation, RegioFlora can advocate the conservation and promotion of plant genetic resources of native plants and represent the subject in specialist groups and committees. It can provide information for all methods of near-natural restoration and consolidate best practices. With that, the organisation is also well-suited to oversee the establishment of quality standards and certifications for the production of native seed and other near-natural restoration methods.

Restoration methods are manifold. RegioFlora promotes two primary grassland restoration concepts with all their variations:

1. **Direct seeding:** Direct seeding refers to a restoration method involving the direct transfer of seeds from a donor site to a recipient site, typically with comparable ecological conditions and geographic proximity, often within the same biogeographical region. This technique skips intermediate steps in seed propagation and may involve transferring seed-containing cuttings or seeds alone, depending on the approach.
2. **Native propagation seeds:** Native propagation seeds refer to a restoration method employing agriculturally propagated seeds sourced from indigenous plants. These seeds originate from natural, native populations within a specific biogeographical region or seed zone where the seed is intended for use. The multiplication of these seeds typically occurs within the same region.

## **Conclusion**

Moving forward, integrating seed provenance considerations for the restoration of extensively managed grasslands into agricultural policies and practices is imperative. Initiatives like RegioFlora play a central role in translating established scientific knowledge about the risks associated with the use of native plants into tangible changes in practice. There is an urgent need to link scientific knowledge with practical application. In agricultural grassland management, collaborative groups and federations across Europe have emerged to forge this connection and have since drastically improved management practices (Prins,

2004). Analogously, establishing such a link is now necessary to maintain plant genetic diversity in grasslands. It is an opportunity to strengthen one important ecosystem service of grassland systems by taking better account of genetic diversity — a neglected component of biodiversity. Grassland federations can play a major role here and can create added value to extensively managed grassland systems by increasing the overall ecosystem services. Their support for initiatives like RegioFlora is decisive for the improvement of best practices in extensive grassland restoration and management.

## Acknowledgement

We thank the Swiss Federal Office for Agriculture (FOAG) to fund the RegioFlora project.

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# Overseeding clovers and forbs in permanent grassland on peaty soils

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## Abstract

There is a growing interest for multi-species swards because of their benefits of nitrogen fixation and increased biodiversity. A challenge for these multi-species grasslands is persistency of clovers and forbs, especially in nutrient-rich peat soils. Attempts to overseed multi-species swards have yielded mixed results, prompting an investigation into two overseeding machines and pre-seeding management strategies to introduce clovers and forbs into permanent grasslands. Given organic matter losses on peaty soils, reseeding is not preferred, necessitating methods with minimal soil disturbance for forb introduction. An experiment initiated in August 2019 on a permanent grass sward on peaty soil, utilized a tine harrow in short grass (<3 cm) and longer grass (10 cm), along with a strip-till cultivator in short grass, compared to complete reseeding. Two years of measurements (2020 and 2021) revealed the strip-till cultivator had the highest clover and forb content (39%) of the overseeding methods, followed by the tine harrow in short grass (19%) and long grass (6%) whereas reseeding control resulted in 48% clovers and forbs. All four treatments resulted in similar total dry matter yields. In conclusion, strip-till showed the highest clover and forb content of the overseeding methods, while reseeding showed even higher contents.

**Keywords:** multi-species swards, clover, persistence, legumes

## Introduction

Multi-species swards have gained increasing attention in European grasslands due to their associated benefits, including nitrogen fixation, drought resistance, and increased biodiversity. However, the limited persistence of these species poses challenges in permanent productive grasslands (Baker *et al.*, 2023). Reseeding grasslands encompasses disturbance of soil which results in loss of soil organic matter (Iepema *et al.*, 2022), and is particularly undesirable on peat soils where soil subsidence and climate impacts are major concerns. Introducing clovers and forbs in existing pastures through overseeding has yielded mixed results, particularly in the context of competition with existing grasses (Skinner and Dell, 2010). This study aims to evaluate the efficacy of two overseeding machines and pre-seeding management practices for the introduction of clovers and forbs into permanent grassland in comparison with reseeding.

## Materials and methods

The experiment was established in August 2019 on a permanent grassland sward on a peaty soil in Stolwijk, the Netherlands. Soil pH<sub>KCl</sub> was 5.6, organic matter content was 51.9% and soil texture was 24% sand, 4% clay and 17% silt. The experiment was subjected to four treatments: (A) long mowing (10 cm) followed by overseeding with a tine harrow and pressing with a Cambridge roller; (B) short mowing (<3 cm) followed by overseeding as in (A); (C) mowing to <3 cm followed by overseeding using a strip-till cultivator that seeded into 8 cm wide strips at 30 cm intervals; and (D) reseeding using a pneumatic reseeding machine after the sward was destroyed using a rotary tiller followed by a power harrow. Plot size was 6m by 10m with five replicate blocks. No fertilizer was applied in the months preceding the over/reseeding. In 2020 all treatments received 60 kg nitrogen in the form of dairy cattle slurry. All sown mixtures consisted of 0.7 kg of *Cichorium intybus*, 1.3 kg of *Plantago lanceolata*, 3.0 kg of *Trifolium pratense*, 2.0 kg of *Trifolium repens*, 1.0 kg of *Carum carvi*, 0.6 kg of *Lotus corniculatus*, 0.3 kg of *Achillea millefolium*, and 0.3 kg of *Scorzoneroideis autumnalis* per ha. Additionally, the reseeding treatment included 30 kg *Lolium perenne* per hectare to compensate for the destroyed grassland sward.

Dry matter yield (DMY) was determined for four cuts in 2020, and the first cut of 2021 using a Haldrup plot harvester. The proportion of grass, red clover, white clover, chicory and plantain in the sward on DM basis was determined for each harvest by sorting grab-samples. Statistical analysis (ANOVA and Tukey HSD,  $P < 0.05$ ) was carried out in Rstudio.

## Results and discussion

The DMY was on average 12 502 kg DM ha<sup>-1</sup> in 2020 and 6154 kg DM ha<sup>-1</sup> in 2021 (first cut only), and there was no significant effect of treatment in either year (Table 1). The proportion of white clover did not show any significant effect of treatment in 2020 (Table 1). The proportions of the other species were lowest for treatment A and B. The proportion of red clover was highest in the reseeded treatment while the proportion of chicory and plantain were similar for treatment C and D. The total proportion of sown clovers and forbs was significantly ( $P < 0.05$ ) lower for the two-tine harrow overseeding treatments (A and B) compared to the strip-till overseeding and reseeding treatments (C and D) in 2020 (Figure 1). Additionally, in 2021, the total proportion of clovers and forbs of the reseeding treatment was significantly higher than treatment C. Similarly, the yield of sown clovers and forbs increased in the order A = B < C < D in both years (Figure 1).

Overall, strip-till appears to be the overseeding method that achieves the highest proportion and yield of sown clovers and forbs while having no negative consequence on total yield compared to other treatments. It shows the importance of setting back the existing grass population for the successful establishment of clovers and forbs. However, it does not achieve the same yield in sown clovers and forbs compared to complete reseeding.

Strip-till offers an opportunity for overseeding multi-species clovers and forbs into permanent grasslands, without the negative consequences of complete reseeding. This is especially important for peaty soils. It should be noted that the selected soil had a relative high pH for a peaty soil (5.6), the establishment of clovers and forbs might be less successful on peat soils with a lower (more typical) pH.

## Conclusion

We conclude that overseeding using a strip-till method is an effective way of introducing clovers and forbs into existing grass swards because it creates a more suitable environment for establishment of the newly germinated seeds. However, complete reseeding showed to have the highest proportion of clovers and forbs.

Table 1. Effect of (A) overseeding after tine harrowing in long grass, (B) overseeding after tine harrowing in short grass, (C) strip-till overseeding and (D) reseeding on total DM yield in 2020 (total of four cuts), 2021 (first cut only), the proportion (DM-basis) of white clover, red clover, chicory and plantain over the whole yield of 2020.

Treatment	Yield (kg DM ha <sup>-1</sup> )		White clover 2020 (%)	Red clover 2020 (%)	Chicory (2020)	Plantain 2020 (%)
	2020	2021				
A	12 150 (899)	6107 (531)	1.5 (1.2)	0.5 <sup>a</sup> (0.9)	1.4 <sup>a</sup> (2.0)	0.1 <sup>a</sup> (0.2)
B	12 257 (937)	6329 (907)	5.0 (2.3)	3.2 <sup>a</sup> (1.4)	1.1 <sup>a</sup> (0.9)	2.4 <sup>a</sup> (2.1)
C	12 202 (521)	5770 (655)	4.3 (2.3)	9.1 <sup>b</sup> (3.0)	7.3 <sup>b</sup> (2.5)	14.3 <sup>b</sup> (5.3)
D	13 423 (951)	6173 (141)	3.6 (2.6)	15.0 <sup>c</sup> (3.6)	10.2 <sup>b</sup> (4.1)	15.9 <sup>b</sup> (1.5)
Average	12 502	6154	3.7	6.8	4.5	7.5
P	ns	ns	ns	***	***	***

Superscript letters indicate significant differences between treatments at  $P < 0.05$  ( $N = 5$ ). Values in parentheses are standard errors.

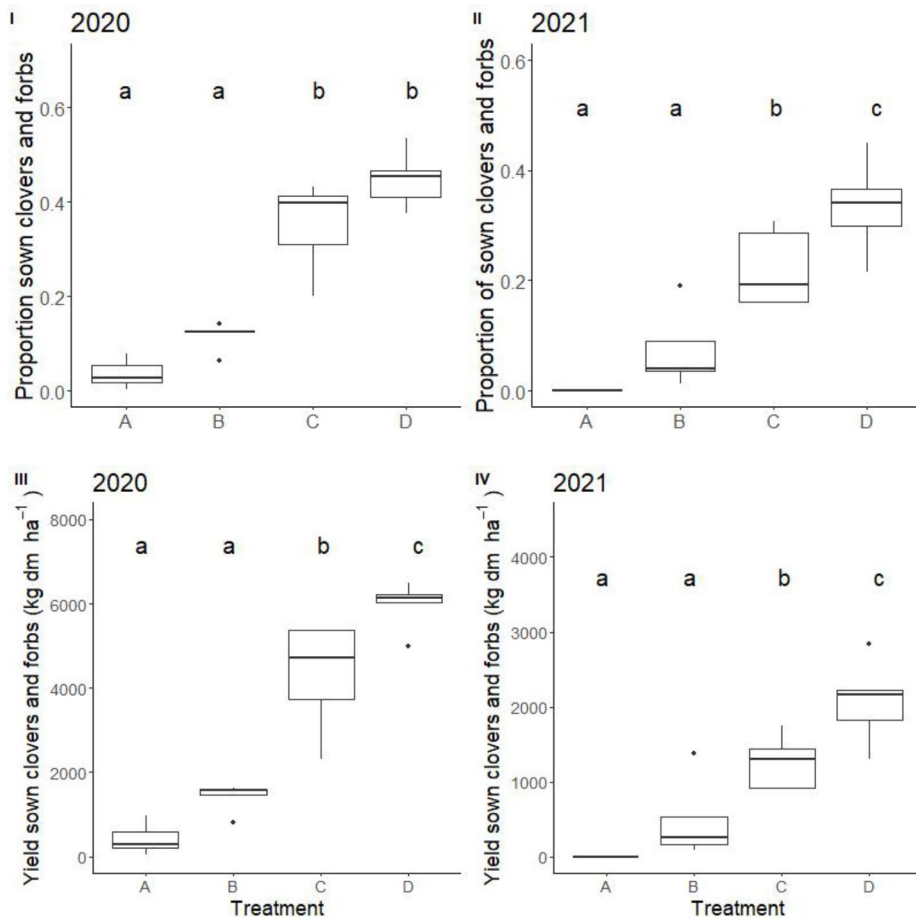


Figure 1. Effect of (A) tine harrow in long grass, (B) tine harrow in short grass, (C) strip-till overseeding and (D) reseeding on fraction of sown clovers and forbs in 2020 (I,  $P < 0.001$ ), 2021 (II,  $P < 0.001$ ), total yield of sown clovers and forbs in 2020 (III,  $P < 0.001$ ), 2021 (IV,  $P < 0.001$ ). Lower case letters show significant ( $P < 0.05$ ) difference between treatments ( $N = 5$ ).

## Acknowledgements

We want to thank Jan de Pater for using his field. Furthermore, we would like to thank Karel van Houwelingen and Loes van Ederveen for measurements.

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# Long-term effect of increasing nitrogen level on plant species composition in an alluvial meadow

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## Abstract

To understand the dynamic of grassland productivity and ecosystem services, long-term grassland experiments are essential. A fertilization experiment with constant P (41.90 kg ha<sup>-1</sup> year<sup>-1</sup>) and K (99.62 kg ha<sup>-1</sup> year<sup>-1</sup>) and an increasing N level (0, 40, 80, 120 kg N ha<sup>-1</sup> year<sup>-1</sup>) was set up in an alluvial wet meadow in Admont (Austria) in 1944. A non-fertilized treatment from an experiment established in the immediate vicinity in 1946 was used as a control. Detailed botanical evaluation was conducted for all treatments in June 2015: (1) unfertilized control, (2) PK, (3) N<sub>40</sub>PK, (4) N<sub>80</sub>PK and (5) N<sub>120</sub>PK. A significant effect of treatment on plant species composition, species richness and sward height was found. For example, *Poa trivialis*, *Glechoma hederacea* and *Aegopodium podagraria* occurred predominantly on the two most N fertilized treatments; *Alopecurus pratensis*, *Arrhenatherum elatius*, *Trisetum flavescens* and *Plantago lanceolata* preferred moderate N fertilization. PK fertilization promoted legumes *Trifolium pratense* and *Vicia cracca* as well as *Leontodon hispidus*. Species with low nutrient demand, *Anthoxanthum odoratum* and *Luzula campestris*, were promoted by non-fertilization. The moderately fertilized sward with the 3-cut regime supported plant species richness.

**Keywords:** compressed sward height, fertilization, grassland vegetation, species richness, three-cut management

## Introduction

The negative effect of high nitrogen fertilization on grassland vegetation has been studied in many experiments; however, due to different climatic and soil conditions as well as different management and plant community the results are not straightforward. Generally, nitrogen fertilization increases the production of aboveground biomass, but it is often responsible for reducing plant species richness and promoting species that are better competitors for light. According to Humbert *et al.* (2016) sustained application of low to moderate levels of N over time has negative effects on plant species richness similar to short-term application of high N doses. To determine how different rates of N fertilizer (along with a constant rate of PK fertilizer) affect plant species richness and sward height we analysed data from the Admont (Austria) long-term experiment.

## Materials and methods

The long-term fertilization experiment was set up in 1944 in an alluvial wet meadow in Admont, province of Styria (Austria), (47°34'52" N, 14°27'4" E; altitude of 635 m a.s.l.). The soil type is a Gleyic Fluvic Dystric Cambisol. Mean annual air temperature is 6.8°C and the average annual precipitation is 1227 mm. All treatments (apart from control treatment) were fertilized with constant P (41.90 kg ha<sup>-1</sup> year<sup>-1</sup>) and K (99.62 kg year<sup>-1</sup>) and an increasing N level (0, 40, 80, 120 kg N ha<sup>-1</sup> year<sup>-1</sup>). A control treatment was established in the immediate vicinity in 1946 and has not been fertilized at all. The treatments applied were: (1) unfertilized control (Co), (2) PK, (3) N<sub>40</sub>PK, (4) N<sub>80</sub>PK and (5) N<sub>120</sub>PK. All treatments were cut regularly three times a year (around 25 May, 20 June and 30 September). The experiment was established in four blocks, using rectangular plots of 4.1 m×5.0 m. The percentage cover of all vascular plant species was recorded visually in each experimental plot in June 2015. The nomenclature of the plant

species follows Fischer *et al.* (2008). The species richness was defined by the total number of vascular plant species in each plot. Compressed sward height was measured with a rising plate meter (Corell *et al.*, 2003) before the first cut. Redundancy analysis (RDA) in the CANOCO 4.56 program was used to evaluate multivariate vegetation and ANOVA for univariate data.

## Results and discussion

Different levels of fertilization significantly influenced plant species richness (Figure 1a). The lowest total number of plant species was recorded in N<sub>120</sub>PK and Co treatments with mean values of 26.3 and 28.8, respectively. In contrast, the highest mean number of species was in PK, N<sub>40</sub>PK and N<sub>80</sub>PK treatments with mean values of 37.8, 37.0 and 36.0 respectively. Sward height before the first cut was significantly influenced by treatment (Figure 1b). The lowest mean sward height was measured in Co treatment (7.4 cm), whereas the highest mean height was in N<sub>120</sub>PK (31.1 cm) and N<sub>80</sub>PK (29.0 cm) treatments.

The lowest mean number of plant species and the shortest sward height in the Co treatment were likely influenced by nutrient depletion in the soil after 71 years of three-cut management with the removal of cut biomass. In the most fertilized treatment (N<sub>120</sub>PK), despite being cut three times a year, the sward attained such height that it hindered light penetration, suppressing the growth of shorter species (Francksen *et al.*, 2022). This result is in agreement with the results of most of the studies dealing with fertilization, yield and diversity issues.

In the RDA based on the vegetation data the effect of the treatments on plant species composition explained 68.9% of the variability ( $F=6.7$ ,  $P=0.001$ ) on all constrained axes. Four groups of treatments with similar plant species composition were recognised on the ordination diagram (Figure 2). The first group (N<sub>120</sub>PK and N<sub>80</sub>PK treatments) included mainly species requiring nutrient-rich soils, for example *Poa trivialis*, *Glechoma hederacea* and *Aegopodium podagraria*. The second group (N<sub>40</sub>PK treatment) included species that prefer moderately nutrient-rich soils, *Trisetum flavescens*, *Arrhenatherum elatius*, *Alopecurus pratensis* and *Plantago lanceolata*. The third group (PK treatment) was especially associated with legumes (around 30%) *Trifolium pratense* and *Vicia cracca*, and also to *Leontodon hispidus* and *Achillea millefolium*. The fourth group (Co treatment) included species occurring predominantly on nutrient-poor soils, such as *Anthoxanthum odoratum* and *Luzula campestris*.

## Conclusions

The three-cut regime reduced the height of the moderately N fertilized sward to the extent that shading of the sward was relatively low, thus allowing the survival of low species. Conversely, long-term high fertilizer doses as well as long-term nutrient removal without fertilization led to species decline.

## Acknowledgements

The long-term experiment is maintained by AREC Raumberg-Gumpenstein and writing the paper was supported by the Czech Ministry of Education, Youth and Sports (project no. 8J21AT003).

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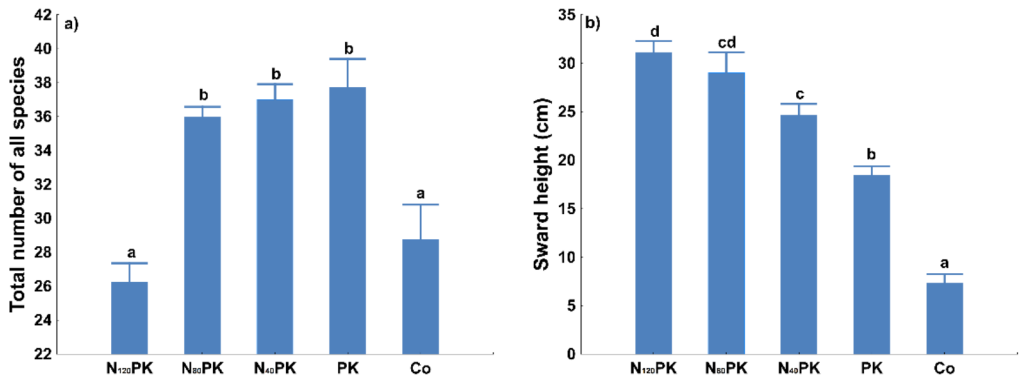


Figure 1. Effect of fertilization on (a) species richness and (b) compressed sward height before the first cut. For treatment abbreviations see Materials and methods section. The post-hoc comparison using the Tukey's HSD test was applied to identify significant differences between treatments, which are indicated by different small letters. Error bars represent standard error of the mean.

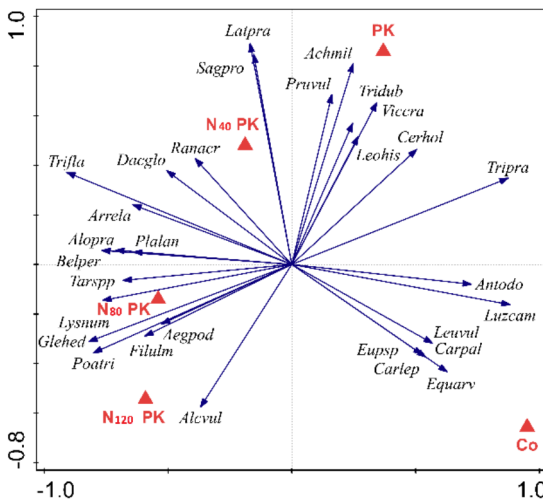


Figure 2. Ordination diagram showing the results of RDA of vegetation data. For treatment abbreviations see Materials and methods section. Species abbreviations: Achmil, *Achillea millefolium*; Aegpod, *Aegopodium podagraria*; Alcvul, *Alchemilla vulgaris*; Alopra, *Alopecurus pratensis*; Antodo, *Anthoxanthum odoratum*; Arrela, *Arrhenatherum elatius*; Belper, *Bellis perennis*; Carlep, *Carex leporina*; Carpal, *Carex palescens*; Cerhol, *Cerastium holosteoides*; Dacglo, *Dactylis glomerata*; Equarv, *Equisetum arvense*; Eupsp, *Euphrasia* sp.; Filulm, *Filipendula ulmaria*; Glehed, *Glechoma hederacea*; Latpra, *Lathyrus pratensis*; Leohis, *Leontodon hispidus*; Leuvul, *Leucanthemum vulgare*; Luzcam, *Luzula campestris*; Lysnum, *Lysimachia nummularia*; Plalan, *Plantago lanceolata*; Poatri, *Poa trivialis*; Pruvul, *Prunella vulgaris*; Ranacr, *Ranunculus acris*; Sagpro, *Sagina procumbens*; Tarspp, *Taraxacum* spp.; Tridub, *Trifolium dubium*; Trifla, *Trisetum flavescens*; Tripra, *Trifolium pratense*; Viccra, *Vicia cracca*.

# Effect of ribwort plantain (*Plantago lanceolata* L.) inclusion on the performance of high-yielding dairy cows at pasture

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## Abstract

Incorporating ribwort plantain (*Plantago lanceolata* L.) into the dairy cow diet has potential to reduce nitrogen losses, and nitrous oxide and methane emissions. However, little is known about the trade-off between these environmental benefits and milk production in European pastoral systems. This study investigated the impact of varying levels of dietary plantain on performance of grazing dairy cows. Sixty-eight spring-calving Holstein-Friesian cows were blocked on parity, days in milk, body weight, body condition score and milk yield, and assigned to one of three grazing treatments in a randomized complete block design, with two replicate groups of 11 cows per treatment. Treatments comprised: perennial ryegrass (*Lolium perenne* L.)-only (GO); low sward plantain (LP, 27% plantain); and high sward plantain (HP, 43% plantain). Results for the 2023 grazing season show that average daily milk yield did not differ significantly between treatments (GO: 29.5 kg, LP: 29.6 kg, HP: 27.8 kg). Milk protein content was similar between treatments, while milk fat content was higher in GO (45 g kg<sup>-1</sup>) than LP (43 g kg<sup>-1</sup>,  $P < 0.05$ ) and similar between GO and HP (44 g kg<sup>-1</sup>). Milk solids production did not differ between treatments. Results suggest that the inclusion of ribwort plantain, at the levels studied, reduces milk fat content but does not negatively impact milk output.

**Keywords:** plantain, grazing, dairy, yield, cow

## Introduction

In the pursuit of optimising dairy production systems there is growing interest in forages that not only enhance animal performance but also contribute to mitigating environmental impacts. Evidence suggests that including plants from contrasting functional groups (e.g., grasses, legumes, and forbs) in grazing pastures may be beneficial for herbage production and quality and the delivery of ecosystem services (Cummins *et al.*, 2021; Grace *et al.*, 2016). Ribwort plantain (*Plantago lanceolata* L.) has emerged in recent years as a promising forage that could improve the environmental footprint of pasture-based dairy farms. Studies comparing plantain with ryegrass-based swards in New Zealand have shown reductions in nitrogen (N) losses and nitrate leaching (Navarrete *et al.*, 2022) and lower nitrous oxide emissions (Vi *et al.*, 2023). It is important, however, to understand the effect that altering pasture species composition has on the animal. Studies in the southern hemisphere have noted similar or improved levels of milk production (Nguyen *et al.*, 2022) when plantain is included in the dairy cow diet; however, there is a need for further research in temperate grassland regions in Europe if farmers are to confidently incorporate plantain into their farm systems.

## Materials and methods

The experiment was carried out at the Agri-Food & Bioscience Institute in Hillsborough, Northern Ireland. A total of 68 spring-calving Holstein-Friesian cows were blocked on parity, days in milk, body weight, BCS and milk yield, and assigned to one of three sward treatments in a randomized complete block design, with two replicate groups of 11 cows per treatment. Treatments were perennial ryegrass-only (GO); low plantain (LP, 27% sward plantain content); and high plantain (HP, 43% sward plantain content). Swards were established in the previous year. Cows were rotationally grazed from April to October 2023 at a stocking rate of 3.9 cows ha<sup>-1</sup>. Average daily concentrate supplementation was 7.1 kg DM cow<sup>-1</sup>. Pre-grazing herbage mass was 3870, 3990 and 4097 kg dry matter (DM) ha<sup>-1</sup> for GO,

LP, and HP respectively, while post-grazing herbage mass was 1772, 1963, 1807 kg DM ha<sup>-1</sup> for GO, LP, and HP respectively. Herbage mass was measured using a rising platemeter (Jenquip, Fielding, New Zealand) calibrated for each sward treatment. Fertiliser applications were similar across all treatments. Weekly herbage samples were dried, milled, and analysed using the NIRS method for neutral detergent fibre (NDF), acid detergent fibre (ADF), dry organic matter digestibility (DOMD), and water-soluble carbohydrates (WSC). Crude protein (CP) was determined using the Dumas method. Individual daily milk yield was recorded at each morning and evening milking and milk fat, protein, urea and lactose concentrations were determined weekly from one successive evening and morning milking. Body weight (BW) was recorded daily and body condition score (BCS) was recorded monthly. Data were analysed using Genstat 23.1 (VSN International, Rothamsted, UK) by REML component analysis.

## Results and discussion

As outlined in Table 1, forage NDF and DM content was highest in GO and lowest in HP ( $P<0.001$ ), while CP levels were similar between treatments, which corresponds with Minneé *et al.* (2019) in their meta-analysis. Sward DOMD was highest in GO and lowest in HP ( $P=0.004$ ) which may reflect the presence of stem and seed heads in the plantain swards observed during the summer months. WSC concentration was lowest in HP and highest in GO swards, while ash was highest in HP and lowest in GO, as was noted by Minneé *et al.* (2019).

The effect of sward type on dairy cow performance is outlined in Table 2. Results show that daily milk production did not differ significantly between treatments. Milk protein and lactose were also similar; however, milk fat was lower in LP than GO ( $P=0.003$ ). This has previously been noted by Nguyen *et al.*

Table 1. Chemical composition (g (kg DM)<sup>-1</sup>) of herbage offered

	Grass-only	Low plantain	High plantain	SED	P-value
NDF	438.4 <sup>c</sup>	404.4 <sup>b</sup>	366.4 <sup>a</sup>	10.93	<0.001
ADF	256.2	254.8	248.5	6.92	NS
CP	170.5	168.3	168.1	4.34	NS
Ash	95.6 <sup>a</sup>	102.3 <sup>b</sup>	111.2 <sup>c</sup>	2.80	<0.001
DM (%)	18.1 <sup>c</sup>	15.6 <sup>b</sup>	14.4 <sup>a</sup>	0.27	<0.001
DOMD (%)	75.0 <sup>b</sup>	72.5 <sup>ab</sup>	71.5 <sup>a</sup>	1.38	0.004
WSC	163.8 <sup>b</sup>	144.0 <sup>ab</sup>	139.5 <sup>a</sup>	10.40	0.025

DM, dry matter; CP, crude protein; SED, standard error of differences; NS, not significant. Means without superscript letters in common significantly differ at  $P=0.05$ .

Table 2. Effect of sward type on dairy cow performance

	Grass-only	Low plantain	High plantain	SED	P-value
Milk yield (kg cow <sup>-1</sup> day <sup>-1</sup> )	29.5	29.6	27.8	1.12	NS
Milk fat (g kg <sup>-1</sup> )	44.8 <sup>b</sup>	42.6 <sup>a</sup>	43.8 <sup>ab</sup>	0.07	0.003
Milk protein (g kg <sup>-1</sup> )	33.5	32.6	32.6	0.06	NS
Milk solids (kg cow <sup>-1</sup> day <sup>-1</sup> )	2.37	2.31	2.22	0.07	NS
Milk lactose (g kg <sup>-1</sup> )	45.7	46.4	45.7	0.02	NS
Milk urea (mg l <sup>-1</sup> )	299.4 <sup>c</sup>	276.9 <sup>b</sup>	258.4 <sup>a</sup>	5.44	<0.001
Body condition score	248	249	244	3.88	NS
Body weight (kg)	579.8	563.1	558.9	16.09	NS

SED, standard error of differences; NS, not significant. Means without superscript letters in common significantly differ at  $P=0.05$ .



(2022) in their meta-analysis, and may relate to differences in NDF content, rumen pH and the ruminal passages of plantain and ryegrass. Milk solids output was, however, similar between treatments. Milk urea was highest in GO and lowest in HP treatments ( $P < 0.001$ ). Milk urea N has been proposed as an indicator of dietary N surplus with other studies observing a trend for reduced urinary N excretion from cows fed plantain (Minnée *et al.*, 2020). Sward type had no effect on body condition score or body weight. In terms of herbage production, GO and LP were similar (13.0 and 12.7 t DM ha<sup>-1</sup> respectively) while HP was higher than both with 14.2 t DM ha<sup>-1</sup> ( $P < 0.001$ ).

## Conclusion

Results show that dairy cow performance was similar across treatments for milk yield, protein and lactose content, and BCS and body weight. Milk fat content was lower in the high plantain treatment; however, milk solids production was similar. Herbage production was higher in high plantain swards, with lower NDF and CP; however, grass-only swards had higher WSC and DOMD content. Results indicate that the inclusion of ribwort plantain, at the levels studied, reduces milk urea and milk fat content but does not negatively impact milk output. Further examination of cow performance at higher dietary plantain inclusion levels is warranted.

## Acknowledgement

We gratefully acknowledge the Northern Ireland Department of Agriculture, Environment and Rural Affairs for their funding (E&I project 21/1/04).

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# Regulation of farm groundwater table to sustain grass production: a case study in the Netherlands

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## Abstract

Drought periods in recent Dutch summers are becoming longer and more intense. There is a need for cost-effective drought adaptation by farmers to keep and sustain grass production. A case study was performed on a dairy farm on light sandy soil by building an adaptable weir in a ditch to control the local surface water level. Monitoring tubes were placed to monitor surface and groundwater level. Satellites measured remotely the grass temperature and NDVI on four 0.5 hectare plots to determine crop evapotranspiration and need for irrigation. The surface water level in the ditch before the weir remains higher compared to the level in the surrounding area (control) shortly after rainfall. The farmer noticed that after a period of rainfall water infiltrates quickly into the soil but with no increased crop growth or decreased irrigation demand. Remote sensing with the SEBAL-model showed a lower irrigation demand on land adjacent to the ditch with the weir. Due to an insufficient rainfall in the summer season and fast water infiltration the function of the weir could be doubted.

**Keywords:** agricultural water management, grassland, groundwater level, irrigation, remote sensing

## Introduction

Drought periods in recent Dutch summers are getting longer and more intense (KNMI, 2023). Grassland on sandy soils is especially susceptible to drought depression, and irrigation is not always profitable as grass is not a cash-crop (van Oort *et al.*, 2023). Therefore, farmers need cost effective and labour flexible solutions to combat drought such as the installation of a weir in a small catchment area (<1 km<sup>2</sup>). Water boards have started projects where farmers get compensated for the installation of small weirs in ditches. The goal of these projects is higher water infiltration to groundwater and improved climate resilience with lower irrigation demand. However, the effects on grass production have not been quantified before. The goal of this case-study is to quantify the effects of increased ditchwater level on groundwater level, grass production and irrigation demand in fields adjacent to the ditch in a changing climate.

## Materials and methods

The case-study farm is located in Holten, Overijssel, the Netherlands on a sandy soil containing 10% loam and 4% organic matter (OM) in the topsoil (0–30 cm). Potential production loss on case study and catchment-area scale were carried out with the WaterWijzerLandbouw table (Werkgroep Waterwijzer Landbouw, 2018). Four monitoring tubes and the weir were installed on 10 August 2022 in pairs of 2. One pair (locations C and D) representing the business-as-usual water level (BWL) and one pair (locations A and B) representing the controlled water level (CWL). At each location one tube was installed in the ditch, and one in the field, 10 m. from the ditch border. On 24 July 2023 ten additional monitoring tubes were installed. Two monitoring tubes were added at each location (25 and 50 m from the ditch border), and two additional locations (1BWL and 1CWL) were equipped with 3 monitoring tubes each (10, 25 and 50 m from the ditch border). The bottom of the monitoring tubes reached up to 180 cm below surface level. The weir was replaced by a higher weir on 10 January 2023 to hold a higher ditchwater level behind the weir.

## Results and discussion

The ‘WaterWijzerLandbouw tabel’ calculated the effects of assumed summer ground water level rise of 20 cm on drought-related production loss (DRPL). Table 1 shows the DRPL in percentages of potential production for the BWL and CWL treatments in both normal and changed climates (Wh) for the whole catchment area (CA) and the case-study farm (CS-farm). Production loss was always lower on the CS-farm. Production loss decreased by 0.9 and 0.7 percentage point compared to between BWL and CWL within the same climate scenario for the CA and CS-farm respectively. Within a changed climate the production loss decreases by 1.3 percentage points by raising the groundwater level for both the CA and CS-farm.

The ditch and averaged groundwater level in the growing period (1 April to 1 October) is shown in Figure 1. From 1 April to 24 May the CWL ditchwater level was roughly 20 cm higher than the BWL ditchwater level, but no difference in groundwater level was recorded. From 24 May onward a period of 6 weeks without rainfall occurred, which caused the CWL ditch water level to drop since this water level is completely dependent on rainfall. Ditch and ground water levels fluctuated until 19 July due to short periods of rainfall combined with water infiltration and water evapotranspiration. From 19 July until the end of the growing season (1 October) there was sufficient rainfall to supply water for the CWL drain to be filled. In this period the CWL ditchwater was 20 cm higher compared to BWL. However, the CWL groundwater remained about 10 cm lower compared to BWL after summer.

Table 1. Drought-related production loss (% loss compared to potential yield) modelled with WaterWijzerLandbouw for the weighted average for the whole catchment area (CA) and case-study farm (CS-farm) for BWL and CWL (summer groundwater level BWL+20 cm) for a normal and Wh climate.

Treatment	BWL		CWL	
	Normal	Wh	Normal	Wh
CA	4.3	6.1	3.4	4.8
CS-farm	2.5	3.5	1.8	2.2

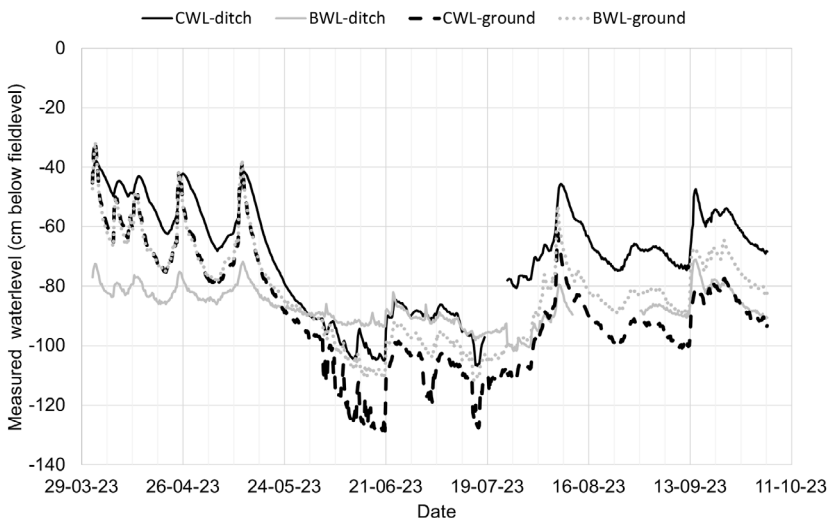


Figure 1. Ditch and groundwater level for Controlled Water Level (CWL) and Business as usual Water Level (BWL) expressed in cm below field level.

### *Advised irrigation*

The SEBAL model accessed through Irriwatch<sup>®</sup> was used to gain insight in grass performance. In line with the objective, it is relevant to see if a similar or higher crop growth is realised with lower irrigation demand. Table 2 shows per treatment per location the cumulative advised irrigation. In line with the expectations the advised irrigation for the BWL treatment was slightly higher but not significantly different. The average grass growth over the CWL compared to the BWL treatment was not different although the two extremes in grass growth were within the BWL treatment. Differences in both advised irrigation and grass growth were insignificantly small that they could also be explained slight differences in soil nutrient status and fertilisation.

Table 2. Cumulative advised irrigation (mm) and Cumulative above ground grass growth (kg DM/ ha) as calculated with the SEBAL model from Irriwatch using leaf-temperature as a proxy for two locations (A and B) under controlled water level (CWL) and two locations (C and D) under business-as-usual water level (BWL).

Treatment	CWL		BWL	
	A	B	C	D
Location				
Cumulative advised irrigation	2804	2809	2847	2845
Cumulative above ground grass growth (kg DM/ ha)	17881	17798	18325	17266

## **Conclusion**

Due to insufficient rainfall the weir could not hold the ditchwater level and groundwater level up during the growing period. Nevertheless, a slightly lower irrigation demand was observed in the controlled water level treatment. A limited production increase is possible with an increased summer groundwater level but this might disappear under the conditions of future climate change. Therefore, the instalment of a weir can be a useful climate mitigation measure for local catchment areas. But variations in local groundwater levels and crop harvest dates between farms within the same catchment areas require local alignment between farmers about instalment and management of a weir.

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# Impact of grazing herb-rich pastures on milk fatty acid profiles at Dutch conventional dairy farms

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## Abstract

The Dutch dairy sector faces significant challenges, including the reduction of greenhouse gas and nitrogen emissions, the promotion of biodiversity, and the development of more climate-resilient agricultural systems. Herbal pastures hold promise in addressing these challenges, and in the Netherlands dairy farmers are increasingly adopting herbal grasslands. However, the impact of dairy cows grazing on herbal pastures at conventional farms is not extensively studied. Therefore, a pilot study was conducted to investigate milk composition differences between conventional farms with herb-rich pastures and conventional farms with herb-poor pastures. The study involved grassland inventories conducted at various times throughout the year on dairy farms in the Netherlands. Concurrently, tank milk samples were collected and analysed for fatty acid composition, and feeding practices were documented. Our preliminary findings revealed significant differences for some fatty acids in milk from farms with partial pasture grazing on herb-rich grasslands versus herb-poor grasslands. Additionally, several significant differences were identified in the fatty acid composition of milk obtained from cows that received a winter ration compared to those with partial pasture grazing.

**Keywords:** fatty acids, herbs and legumes, dairy cows, pastures, milk

## Introduction

Many dairy farmers aim to incorporate herbs into grasslands to promote biodiversity and foster nature-inclusive agriculture. Beyond their positive impact on biodiversity, the use of herbs presents opportunities to contribute positively to animal health and emission reduction through the presence of bioactive compounds. Including herbs into pastures and animal rations may also influence the composition and quality of milk, e.g. directly by intake of fatty acids (FA) present in these herbs, or indirectly by (compounds present in these herbs) affecting rumen and fat metabolism, and/or de novo synthesis of FAs in the mammary gland. In the Netherlands the impact of dairy cows grazing on herbal pastures on conventional farms is not extensively studied. Therefore, the aim of this pilot study was to investigate differences in milk FA composition in practice between conventional farms with herb-rich pastures and conventional farms with herb-poor pastures.

## Materials and methods

Fifteen Dutch dairy farms were recruited to participate in a practical study in January 2023. Inclusion criteria to participate were application of conventional farm management, Holstein-Friesian as dominant cow breed in the herd, availability of grazing pastures with either an expected vegetation coverage with herbs and legumes >15% (herb-rich pastures) or <5% (herb-poor pastures), average milk production of about 29 l day<sup>-1</sup>. To reveal relations between herbs and legumes coverage in grazing pastures and FA composition in milk in practice, tank milk samples were obtained that reflected milk produced during pasture grazing on either herb-rich pastures or herb-poor pastures. It was required that cows have been grazing on these pastures for at least 36 hours before obtaining tank milk samples. Three research rounds at the participating farms were conducted in 2023, namely round A (13 March–4 April, winter ration, no pasture grazing), round B (1–26 May, pasture grazing) and round C (8–29 June, pasture grazing). The total feed intake of cows during winter ration was 22.5 kg DM day<sup>-1</sup> (SD 2.9) from which 13.9 kg DM day<sup>-1</sup> (SD 1.8) forages, 9.9 kg DM day<sup>-1</sup> (SD 2.4) grass silage and 3.8 kg DM day<sup>-1</sup> (SD 2.8) maize

silage. During rounds B and C, the cows were supplemented with maize (6 farms 3.5 kg DM day<sup>-1</sup>, SD 0.9) and grass silage (5.4 kg DM day<sup>-1</sup>, SD 2.8) and concentrates (all farms 5.4 kg DM day<sup>-1</sup>, SD 1.8). Average grazed herbage intake, estimated using the energy balance method, was 8 kg DM day<sup>-1</sup>.

Grassland inventories were conducted at rounds B and C. Briefly, in the surveyed grassland quadrats were randomly placed at four or more locations within the plot. Plant species were identified using a determination key describing the morphological characteristics. Grassland coverage was denoted within the quadrat by estimating the percentage of each species, starting with estimating the less prevalent species, followed by more abundant ones. Subsequently, the average coverage of the quadrat was calculated for each plot investigated.

Tank milk samples were obtained at the participating farms during rounds A, B and C and analysed on FAs by Wageningen Food Safety Research (WFSR) according to the gas chromatography-flame ionization detection method NEN-EN-ISO 16958:2020. Automatic peak integration was applied and FA content was reported in g (100 g of total identified FAs)<sup>-1</sup>. FAs for which > 33 % of the samples had a value of 0 were omitted from further analysis. Then, a Principle Component Analysis (PCA) was executed in SPSS (version 28.0.1.0) for all tank milk samples obtained during sampling rounds A, B and C. To identify FAs that are different between tank milk from farms with grazing on herb-rich pastures and herb-poor pastures, the fold difference between these groups was calculated for each individual FA and differences were statistically analysed using a Mann-Whitney U test (SPSS, version 28.0.1.0) and a Student's unpaired *t*-test when appropriate based on normality of the data (Microsoft Excel version 2311). A similar analysis was performed comparing milk from round A with milk from rounds B and C separating milk from herb-rich pastures and herb-poor pastures. FAs with a fold difference >10% and a statistical significance at *P*<0.05 were reported.

## Results and discussion

During grassland inventories it appeared that some grasslands did not meet the criteria of <5 or >15% vegetation coverage with herbs and legumes. Grassland coverage <10% was assigned to the herb-poor grassland group resulting in more farms (*n*=10) that fell within this group compared to the herb-rich group (*n*=5). Grassland coverage with herbs and legumes species at herb-rich farms varied from 15 to 94 % (rounds B and C taken together), with white clover (*Trifolium repens*) being the predominant species (average coverage of 14%, SD 12 and 36%, SD 25 in round B and C, respectively) followed by red clover (*Trifolium pratense*) (6.8%, SD 8.8 and 4.3%, SD 4.3 in B and C), and then by a variety of species which each on average were present in minor amounts (<2.2%). Perennial ryegrass (*Lolium perenne*) was on average the most dominant grass species, both in herb-poor and herb-rich grasslands.

The PCA plot (Figure 1) does not show distinctive clusters separating tank milk from farms with pasture grazing on herbs-rich or herb-poor grasslands. Our preliminary findings show that FAs C5:0, C7:0, C9:0, C22:5n3 were > 10% increased and C22:2n6 >10% decreased in milk from farms with partial pasture grazing on herb-rich compared to herb-poor pastures (*P*<0.05), while no significant difference was observed between these groups for saturated FAs, monounsaturated FAs, and polyunsaturated FAs (PUFA). Loza *et al.* (2023) reported a small difference for docosapentaenoic acid (C22:5) between grass-clover and diverse swards grazing, as well as for PUFA. The PCA did reveal two clusters of milk FA composition obtained from cows receiving a winter ration, of which one part is clearly separated from milk samples obtained from farms with partial pasture grazing in rounds B and C. Interestingly, milk FAs of sampling rounds A, B and C of farms belonging to this distinctive winter ration group form a cluster (within dashed line of Figure 1), and so do milk samples of rounds A, B and C for the other farms. The proximity of milk samples from the same farm for rounds A, B and C in the PCA plot may suggest a certain farm effect in milk FA profiles, e.g. due to farm-specific feeding regimes or other farm-specific

factors. Simultaneously, the PCA plot suggests a seasonal effect since milk samples from rounds B and C have higher PCA1 values compared with milk samples from round A from the same farm, which is in line with the seasonal effect that was observed by Frelich *et al.* (2009). Significant higher levels of individual FAs in milk from round B and C compared to round A, observed in both herb-rich and herb-poor pastures, were found for C15:0 (ante)iso, C16:0 iso, C16:1T, C16:1n9, C17:0 (ante)iso, C17:1n7, C18:1T, C18:1n9, C18:1n7, C18:3n3, C18:3n6, C20:1n9, C21:0, while C5:0 was decreased. Additionally, several FAs were increased or decreased in either milk from herb-rich or herb-poor pastures compared to milk from a winter ration.

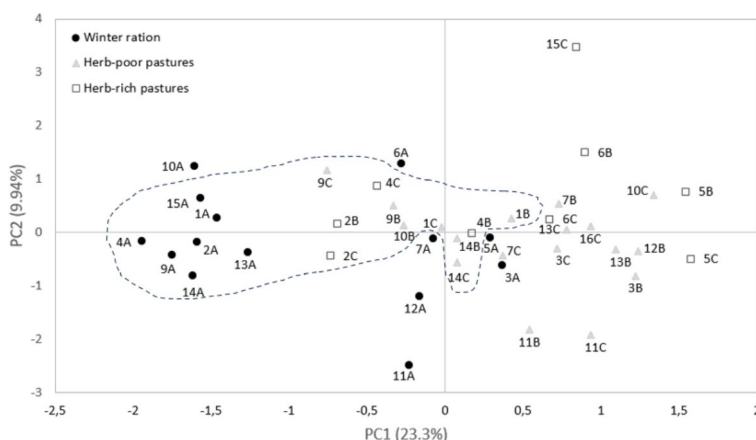


Figure 1. PCA plot of FAs of raw tank milk obtained during the different sampling rounds (A, B, C), discriminating winter ration (circle), pasture grazing on herb-rich (square) and herb-poor grasslands (triangle). The number/letter combination indicate milk samples obtained during the sampling rounds from the different farms (numbers). The dashed line includes a distinctive cluster of farms (see also text).

## Conclusion

This pilot study identified a significant difference for a few FAs between milk from conventional farms with partial pasture grazing on herb-rich grasslands versus farms with grazing on herb-poor grasslands. Moreover, the study showed that FA composition of tank milk from cows on conventional dairy farms receiving a winter ration differed from tank milk obtained during spring when partial pasture grazing was applied.

## Acknowledgements

The authors sincerely acknowledge Trijnika de Boer, Cincianne Valk and Jurgen Tekstra for performing the grassland inventories and the milk sampling. This study is funded by the project BioDiverseMelk (<https://edu.nl/pvvgg>).

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# Farm-gate nutrient balance: comparative assessment of high-productivity and low-cost dairy farm systems in Uruguay

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## Abstract

Dairy systems face the challenge to minimize environmental impact while being competitive. Dairy intensification implies a greater use of inputs like supplements (S: concentrate and conserved forages) and fertilizers (F) that can represent a potential risk of nutrient unbalance and losses to the environment. A study was carried out between July 2021 and August 2023, to evaluate the balance of Nitrogen (N), Phosphorus (P) and Potassium (K) and the main input/output sources of two contrasting dairy systems: a high productivity dairy system (HP) with a stocking rate of 3 milking cows (ha milking platform (MP))<sup>-1</sup>, bought-in forage reserves (FR) and concentrate (C), and a low-cost dairy system (LC) with 1.8 cows (ha MP)<sup>-1</sup>, self-sufficient in FR and bought-in C. Nutrients surplus (kg (ha MP)<sup>-1</sup>) in HP vs. LC were 307 vs. 194, 95 vs. 59 and 137 vs. 39 for N, P and K, respectively. Supplement was the main input in HP while for LC both S and F were important. Nitrogen use efficiency (NUE) for HP and LC were 32% and 29% respectively. HP required major nutrient surplus with major potential risk of nutrient losses to the environment, while NUE was similar between both dairy systems.

**Keywords:** environmental impact, intensification, pasture based

## Introduction

Uruguayan dairy productivity has increased steadily over the last 40 years (+3.5%), based on higher individual milk yield (+2.6%) and stocking rate (+1.6%) supported by an increase of home-grown forage reserves, concentrate (C) and dry matter intake (Fariña and Chilibroste, 2019). As intensification develops, the question arises as to what is the best combination for economic, social and biophysical factors that do not compromise the overall sustainability of dairy systems.

Source point contamination has been a common challenge in pasture-based dairy systems across Europe (Tamminga, 2003), and Uruguay is also not an exception (Barreto *et al.*, 2017). Hence, knowing how the intensification of pasture-based dairy systems impacts the environment is key to reducing negative effects.

Farm-gate nutrient balance has been determined as a predictor for potential nutrient losses to the environment (De Klein *et al.*, 2017) but its use, and particularly inefficient use, can lead to environmental losses. This paper reviews N use efficiency (NUE). In Uruguay, studies that compared different intensification strategies found a positive relation between fertilizers (F) and supplements (S), as inputs with nutrient surpluses, being a hazard for leaching loss (Stirling, 2021). Therefore, the objective of this work was to evaluate two dairy systems that represent two different intensification strategies, where the more intensive system requires greater use of external inputs as imported feed and fertilizers, resulting in higher surplus.

## Materials and methods

The experiment was conducted at Centro Regional Sur dairy farm, Agronomy Faculty, UdelaR (34°36'47" S 56°12'48" W, Progreso, Canelones, Uruguay), from August 1, 2021 to July 31, 2023. Two treatments were compared: treatment HP with an annual stocking rate of 3 milking cows ha MP<sup>-1</sup>. It included



outdoor feed pad and a resting dry lot with access to shade and water. Forage reserves (FR; silage) and C were bought-in. In contrast, treatment LC consisted of 1.8 cow (ha MP)<sup>-1</sup>, with access to shade and water in a dry lot. In LC the FR (haylage) was produced in the MP area and fed in the dry lot, whereas C was bought-in. For both treatments, C was fed in the milking parlour. Annual nutrient balance was determined for Nitrogen (N), Phosphorus (P) and Potassium (K) for both treatments using the farm-gate methodology. Nutrient balance was determined as  $\Sigma \text{Inputs} - \Sigma \text{Outputs} = \Sigma \text{Surplus}$  (kg (ha MP)<sup>-1</sup> year<sup>-1</sup>) and N use efficiency (NUE) =  $\text{Outputs}/\text{Inputs}$ . Nutrient inputs included supplements (C and FR; S), F, biological nitrogen fixation (BNF), bedding materials and animals. Accounted outputs were milk and animals. No losses of nutrient to the environment were accounted for. Weather conditions were recorded at a local meteorological station. For data analysis, descriptive statistics were used.

## Results

Rainfall was 17 and 44% less than the historical average for the years 2021–2022 and 2022–2023, respectively (average 1143 mm year<sup>-1</sup>) and summer 2023 was the driest reported in the last 60 years. Inputs for HP vs. LC were 448 vs. 274, 113 vs. 70, 183 vs. 67 kg (ha MP)<sup>-1</sup> and outputs were 141 vs. 80, 18 vs. 11, 46 vs. 28 kg (ha MP)<sup>-1</sup> for N, P and K, respectively. Their surplus was 305 vs. 179, 95 vs. 59 and 137 vs. 39 kg (ha MP)<sup>-1</sup> of N, P and K for HP and LC, respectively. For HP, S represented the highest source of inputs (mean=54.2, sd=72.7 kg ha MP<sup>-1</sup>) while for LC differences between sources was not as clear: the main source of N was F and for P was the S (mean=53.1, SD=70.8; kg (ha MP)<sup>-1</sup>). Inputs and surplus were strongly correlated ( $R^2=0.99$  and  $0.97$  for HP and LC, respectively). The correlation between surplus and output, although less evident, was also positive ( $R^2=0.68$  and  $0.89$  for HP and LC respectively). Mean NUE for HP and LC were 32 and 29% respectively, varying from 26% for both treatments in the first year to 38 and 33% in the last year for HP and LC, respectively.

## Discussion

Differences between intensification strategies determined higher nutrient inputs and surplus for HP than LC, and the main difference was due to S. The contrasting use of feeding strategies determined different grazing/confinement time, that could transform the risk of higher surplus into an opportunity for HP to manage manure. Differences in average NUE were minor between treatments although they varied between years, being pronounced in the dry year. According to De Klein *et al.* (2017) but its use, and particularly inefficient use, can lead to environmental losses. This paper reviews N use efficiency (NUE, variability in NUE is explained by agroclimatic conditions over management practices, although in this case agroclimatic conditions affected both systems, management practices impacted differently. As a result, the diminished pasture production due to the drought reduced F use and increased bought-in feed, but the quality of that feed varied among treatments and favoured HP. In national studies La Manna and Durán (2008) found NUE ranging from 22 to 66%. Farms with NUE higher than 60% underwent

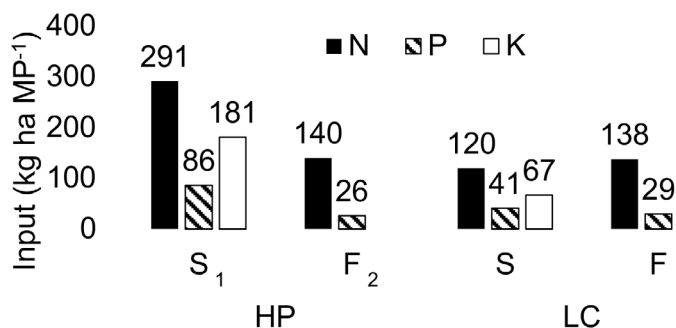


Figure 1. Main N, P and K input sources for HP and LC treatments. 1S, supplements (forage reserves and concentrate); 2F, fertilizers.

nutrient mining, explained by low productivity. Quemada *et al.* (2020) reports NUE values of 38% as ambitious, ranking HP and LC in a favourable position. It should not be overlooked that neither of the systems exports or recirculate effluents, as happens on European farms, allowing an opportunity to reduce F.

It should be noted that bought-in feeds are the main inputs in HP. If instead of considering the nutrients that enter with the bought-in feed, the nutrients required to produce that feed were also considered, as proposed by De Klein *et al.* (2017) but its use, and particularly inefficient use, can lead to environmental losses. This paper reviews N use efficiency (NUE), the inputs and surplus would be lower and therefore the NUE would decrease. In addition, if feed production efficiency were considered, NUE would decrease considerably. In this way, Quemada *et al.* (2020) proposes a 50% NUE on the net income of N from feeds. Hence, if this factor were applied in the present work, NUE of HP would decrease compared to LC, due to the greater dependence on bought-in feed.

## Conclusions

More intensified dairy systems like HP depended more in bought-in feed rather than F, while for LC this tendency was less pronounced. Understanding the implications of each intensification strategy is key to identifying potential opportunities and drawbacks. Climate conditions negatively affected both systems; LC could not express its potential, as it was designed to use forage for direct consumption and reserves. In a context where extreme climate events occur more frequently, it is crucial to investigate how systems with different levels of intensification respond and to show the path that best ensures the lowest environmental impact and system resilience. Results of NUE were within the expected ranges. However, in both systems there is an opportunity to promote manure recycling to improve NUE.

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# Effect of organic and mineral fertilization on root distribution in grassland

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## Abstract

Root biomass is one of the leading carbon inputs in grasslands, depending on environmental conditions. More information is needed on how root distribution is affected by mineral or organic fertilization in semi-natural grasslands. The total root dry matter production in depths 0–15 cm, 15–30 cm and 30–50 cm was measured in a permanent meadow experiment established in 2004. Unfertilized treatment was compared with organic (manure+dung) and mineral (NPK) fertilizers application at level 84 kg N under three-cut management in spring and autumn 2022. In all treatments, about 90% of root biomass was located in the 0–15 cm soil layer, and an average of 3% was found in the 30–50 cm layer. Fertilized treatments showed a higher root biomass below 15 cm, but no difference was observed in the top layer or between the fertilizers. Autumn sampling showed a decrease in root biomass in the layers below 15 cm. Higher root biomass at both types of fertilization implies a similar effect of mineral and organic fertilizers to support carbon storage in the deeper soil layers of grassland in well-drained soils.

**Keywords:** *Arrhenatherion* meadow, dry root biomass, nitrogen uptake

## Introduction

Grassland ecosystems can be considered particularly important for the global carbon cycle, and reliable prediction of soil organic carbon stocks under climate change or other alterations in environmental conditions requires a precise understanding of the processes and process rates (Poeplau, 2016). Belowground parts of grassland, and their spatial and time distribution in the soil, play an essential role in these processes when applied nutrients cause changes in botanical composition and forage production (e.g. Dindová *et al.*, 2019) and also affect the distribution of the root in depths or seasons (Hrevušová *et al.*, 2023). Plant species contrasting in rooting depths also may contribute to adaptation and resistance to drought stress (Hoekstra *et al.*, 2015). In addition to the nutrient doses themselves, the form of applied fertilizers can also play a role where organic fertilizers can positively impact soil carbon content in grassland under long-term management (Menšík and Nerušil, 2019). Our study aimed to compare how mineral and organic fertilization affects the root distribution of grassland communities under long-term management in association with forage production, different sampling dates, and depths in an *Arrhenatherion* meadow.

## Materials and methods

A long-term fertilization experiment is located on an *Arrhenatherion elatioris* near Jevíčko (342 m a.s.l., Czechia). The annual temperature is 7.4°C, the sum of precipitation is 545 mm. The soil is a well-drained Luvisol with a loam texture. The experiment was established in 2004 in four replicates combining harvest intensity (2–4 cuts per year) with increasing rates of nutrients in mineral (NPK) or organic form (manure+dung or slurry). In this study, three fertilization treatments were evaluated under a three-cut regime: unfertilized control, mineral NPK (annually 84 kg N, 40 kg P, 100 kg K ha<sup>-1</sup>), and manure+dung fertilization (manure+dung adjusted to 84 kg N). The measurements were taken within the EJP Soil project MIXROOT-C in 2022. The forage yield was measured from an area of 10 m<sup>2</sup> in three cuts. Before the spring and autumn cut, root samples were taken from three soil cores up to a depth of 50 cm from

three replicates, resulting in nine samples for each treatment. The soil core diameter was 8 cm, and the depth was divided into three layers: 0–15, 15–30 and 30–50 cm. Roots were separated on a 2 mm sieve, washed, and oven-dried at 60°C. The dry root biomass (DRB) was expressed in g dry root m<sup>-2</sup>. The data were analysed by two-way analysis of variance (ANOVA) within each depth. The significant differences between means were reported using the Tukey HSD test at  $\alpha=0.05$ .

## Results and discussion

The average annual forage yields of treatments were 4.5, 7.1 and 8.0 Mg ha<sup>-1</sup> for unfertilized control, manure, and NPK, respectively. Most root biomass (90%) was located in the 0-15 cm layer across all evaluated treatments and both periods (Figure 1). The layer 0-5 cm represents about 70% of roots up to 15 cm depth (Glab and Kacorzkyk, 2011). Similarly, Jackson *et al.* (1996) reported the grassland biome allocate root biomass mostly in 30 cm topsoil, where our results showed that layers 0-30 cm represent 97% of total roots.

Both fertilization treatments tended to increase DRB in deeper layers across sampling periods, but there was no clear relationship between applied nutrient doses and root biomass in the 0–15 cm layer (Table 1). A similar trend for deeper root allocation under higher nutrient supply has also been reported for *Arrhenatherion* grassland in Cambisol, when the N200 PK treatment resulted in significantly higher DRB for the 15-30 cm layer, in contrast to unfertilized treatment (Hrevušová *et al.* 2023). This effect seems more associated with species diversity than total forage yield, in line with Mueller *et al.* (2013). In the present experiment, the proportion of legumes and forbs is the highest in unfertilized control compared to fertilization treatments. Still, the number of species within botanical groups remains similar (Menšík and Nerušil, 2019). Considering forage production and root: shoot proportion, root: shoot ratio decreased under nutrient supply, in line with Poelau (2016), as plants experiencing nitrogen deficiency invest more in root formation.

Fertilized treatments consistently increased DRB in both sampling dates (Figure 1). Seasonal fluctuation of root biomass was not visible in the top layer (0-15 cm). There was still a significant trend to reduce root biomass in deeper layers at the end of the season (Table 1). A similar trend was not apparent in *Arrhenatherion* grassland in shallow Cambisol (Hrevušová *et al.*, 2023). Seasonal variation could be related to changes in the proportion of functional groups where grass, legume, and forbs proportions varied across seasons, years, and nutrient supply (Dindová *et al.*, 2019).

## Conclusions

Based on these one-year results, it can be concluded that fertilization has a noticeable effect on root biomass distribution in the deeper layer, where the impact of mineral was close to organic fertilizers. Clarifying these relationships can help optimize alluvial grassland management toward improving root carbon input.

Table 1. Effect of sampling depth, season, and fertilization treatment on root biomass accumulation (g m<sup>-2</sup>)

Layer	Treatment				Season		
	Control	Manure	NPK	P	June	September	P
0–15 cm	690	620	823	0.162	688	733	0.610
15–30 cm	33.2	50.1	64.8	0.056	62.0 a	36.7 b	0.019
30–50 cm	14.2 a	30.6 b	30.6 b	0.004	29.8 a	18.2 b	0.006

P, probability of two-way ANOVA, different letters document statistical differences between treatments or sampling period within each layer (Tukey HSD,  $\alpha=0.05$ ).

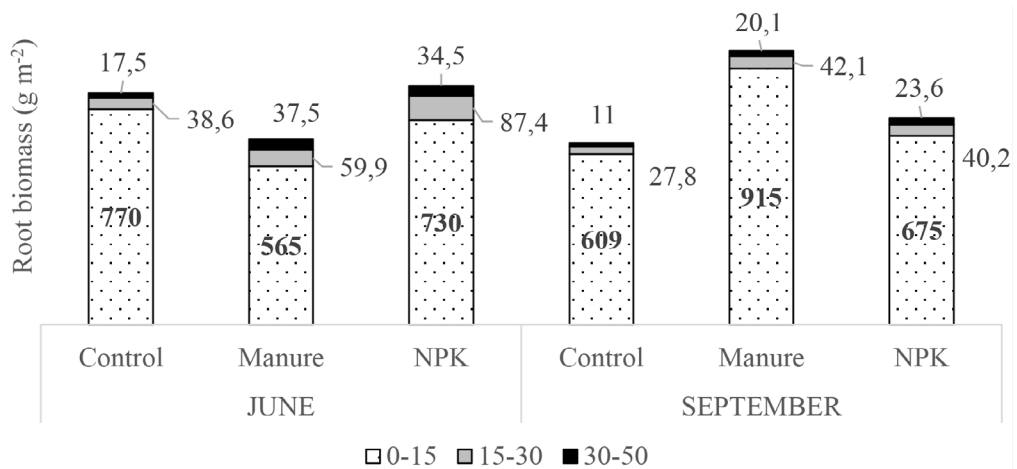


Figure 1. Root biomass in the 0–15 cm (dotted columns), 15–30 cm (grey columns) and 30–50 cm layer (black columns) at three fertilization treatments in June (before first cut) and in September (before last cut).

## Acknowledgement

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 862695 and was realized with the financial support of the Ministry of Education, Youth and Sports (MŠMT).

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# The importance of potential benefits of grasslands and livestock to stakeholders

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## Abstract

With the aim of reducing environmental impacts while addressing societal demands for safe, nutritious and affordable meat and dairy products, the Horizon 2020 project PATHWAYS seeks to identify and increase sustainable practices along the supply and production chains within the European livestock sector. In 2022, an online survey was set up to identify, rank and classify benefits to society delivered by livestock production at the territorial level. The importance of these benefits was determined on a Likert scale. Answers of 896 respondents were analysed for those benefits related to grasslands. Principal Component Analysis (PCA) was used to find underlying structures in the data considering the effect of the role of the respondent on the stated importance of benefits. The underlying variables of each of the factors could easily be linked to overarching general themes, i.e. environmental importance, personal importance, recreational importance, importance in preventing catastrophes and spiritual/religious value. There was a limited effect of the role of respondents. The results emphasize the key role of grasslands and livestock production systems in providing benefits to society.

**Keywords:** benefits of grassland, ecosystem services, livestock production, Principal Component Analysis

## Introduction

Developments in livestock production can have an effect on the different dimensions of sustainability. This creates challenges but also opportunities (Felix *et al.*, 2022). With the aim of reducing environmental impacts while addressing societal demands for safe, nutritious and affordable meat and dairy products, the Horizon 2020 project PATHWAYS seeks to identify and increase sustainable practices along the supply and production chains within the European livestock sector. PATHWAYS will improve the role of livestock in supporting a circular bioeconomy and the place of animal products in future diets for a range of stakeholders. PATHWAYS will also develop a framework that enables a holistic assessment and valuation of ecosystem services related to livestock at a territorial scale. This assessment framework will be produced with a European multi-actor platform, aligning perceptions between farmers, scientists, industry, consumers and policymakers.

The objective of this study was to identify, rank and classify benefits for society that could be delivered by livestock production with a specific focus on grasslands. Schils *et al.* (2022) previously showed that grasslands deliver a multitude of benefits that could be of value to society. This study highlights the importance of these benefits as experienced by the relevant stakeholders.

## Materials and methods

In 2022, a list of potential benefits of livestock production to society was identified by the project consortium; i.e., by an array of people from practice and science. Subsequently, an online survey was set up in which stakeholders were asked to complete the predefined benefits with additional benefits, and to rank and classify them. The survey was available in Danish, Dutch, English, French, German, Italian, Polish, Romanian, Spanish and Swedish. The survey was distributed online via the network of all participating partners in the PATHWAYS project. People could complete the survey between May 18 and October 5, 2022. The importance of benefits for individual stakeholders was determined on a Likert scale (1-7, very unimportant-very important). Respondents were not asked to assess the effect of livestock production on potential benefits, instead they were asked to assess their personal opinion on the importance of these benefits. Answers of 896 respondents were analysed for those benefits related to grasslands and livestock production: soil carbon sequestration, reduced energy use, animal health and animal welfare, nutrient cycling, reduced greenhouse gas emissions, reduced nutrient losses (e.g. nitrate, ammonia, phosphate), reduced pesticide and herbicide use, biodiversity, provision of food, generating income/profit/jobs, human health, livelihood of rural landscapes, beauty of the landscape, recreational value, cultural and historic value, prevention of flooding/ fire/ avalanche or erosion, increased resilience to extreme events and spiritual/religious value. Principal Component Analysis (PCA) was used to uncover the effects of the role of the respondent on the stated importance of benefits.

## Results and discussion

The PCA of the importance of benefits for society led to the identification of five main factors with several underlying variables (Table 1). These variables could easily be linked to overarching general themes, i.e. environmental importance, personal importance, recreational importance and importance in preventing catastrophes. The fifth factor included one variable, i.e. spiritual/religious value. The list of potential benefits to society was confirmed since most of these benefits were ranked as important. Provision of food received the highest average ranking (6.7 on a scale of 1–7). The only variable scoring ‘unimportant’ on average was spiritual/religious value (3.9).

Differences in importance between stakeholders were small. There were no significant differences for personal importance and for spiritual/religious value. For environmental importance and recreational importance, consumers scored lower than other stakeholders, while scientists and veterinarians scored the variables of environmental importance higher, and farmers, NGO, scientists, teachers and value chain actors scored the variables of recreational importance higher (Table 2). For factor 4, there was a (small) difference between farmers and consumers, but the post-hoc test did not show any differences between all other categories.

## Conclusion

This study set out to test the importance of different societal benefits for different stakeholder groups. The results emphasize the key role of grasslands and livestock production systems in providing benefits to society.

## Acknowledgement

This research has been developed within the PATHWAYS project, funded by the European Union's Horizon 2020 Research and Innovation Programme under grant agreement No 101000395.

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Table 1. Five factors (PCA-results for benefits for society) and underlying variables (average score in parentheses; ranking on a scale of 1–7).

<b>Factor 1:</b> <b>Environmental importance</b>	<b>Factor 2:</b> <b>Personal importance</b>	<b>Factor 3:</b> <b>Recreational importance</b>	<b>Factor 4:</b> <b>Importance in preventing catastrophes</b>	<b>Factor 5:</b> <b>Spiritual/religious value</b>
Biodiversity (6.4)	Provision of food (6.7)	Beauty of the landscape (5.9)	Increased resilience to extreme events (6.0)	Spiritual/religious value (3.9)
Animal health and animal welfare (6.4)	Human health (6.4)	Cultural and historic value (5.5)	Prevention of flooding, fire, avalanche or erosion (5.9)	
Nutrient cycling (6.4)	Livelihood of rural landscapes (6.3)	Recreational value (5.2)		
Soil carbon sequestration (6.2)	Generating income/profit/jobs (6.2)			
Reduced greenhouse gas emissions (6.1)				
Reduced nutrient losses (e.g. nitrate, ammonia, phosphate) (6.1)				
Reduced pesticide and herbicide use (6.0)				
Reduced energy use (5.9)				

Table 2. Importance of benefits for society for different stakeholder groups (avg = average)

	<b>Factor 1:</b> <b>Environmental importance</b>	<b>Factor 2:</b> <b>Personal importance</b>	<b>Factor 3:</b> <b>Recreational importance</b>	<b>Factor 4:</b> <b>Importance in preventing catastrophes</b>	<b>Factor 5:</b> <b>Spiritual / religious value</b>
High	Scientist Veterinarian		Farmer NGO Scientist Teacher Actor value chain		
Average	Advisor Farmer NGO Other Policy maker Teacher Actor value chain	Scientist Veterinarian Advisor Farmer NGO Other Policy maker Teacher Actor value chain Consumer	Advisor Other Policy maker Veterinarian	Scientist Veterinarian Advisor Farmer NGO Other Policy maker Teacher Actor value chain Consumer	Scientist Veterinarian Advisor Farmer NGO Other Policy maker Teacher Actor value chain Consumer
Low	Consumer		Consumer		
<i>p</i> value	0.004	0.470	<0.001	0.025	0.395



# Effect of long-term mineral fertilization on soil carbon and nitrogen in grassland

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## Abstract

Grasslands represent an important carbon sink and their natural storage potential can be enhanced further by fertilization. However, the effect of fertilization is highly dependent on site characteristics and these relationships are still not clear. We measured the soil organic carbon (SOC) and nitrogen content and the C:N ratio was calculated in depths 0–15 cm and 15–30 cm in the spring and autumn seasons. The fertilization experiment was established on an *Arrhenatherion elatioris* meadow in the Czech Republic in 1976. It is situated on a well-drained Cambisol with a sandy-loamy texture. Three levels of fertilization were evaluated in 2022: unfertilized control, N50P40K100, and N200P40K100. The carbon content was higher in the topsoil (3.21%) than in the deeper layer (1.06%) but no significant effects of season and treatment were detected. The nitrogen content showed the same pattern (0.36% and 0.18%, respectively). The C:N ratio was influenced both by the layer (8.88 and 5.69, respectively) and the season. Mean values of the ratios did not differ between fertilization treatments but decreased linearly with increasing nitrogen supply. The results suggest that the direct effects of fertilization on soil carbon content can be insignificant, but fertilization may influence soil carbon accumulation indirectly.

**Keywords:** carbon accumulation, Cambisol, *Arrhenatherion*, C:N ratio

## Introduction

Grasslands represent an important carbon sink, but their carbon storage potential is significantly affected by the management applied. Fertilization, as well as improved grazing management, irrigation, and some other treatments, tends to lead to increased soil C (Conant *et al.*, 2017). In addition, the effect of fertilization can be indirect via an increase in shoot (Dindová *et al.* 2019) and root biomass production (Hrevušová *et al.*, 2023), changes in plant diversity, and variations in soil microbial activity (Dietrich *et al.*, 2017). An application of NPK usually maintains or increases total soil C accumulation compared to unfertilized soils (Poeplau *et al.*, 2018) in temperate grasslands. However, the effect of fertilization is highly site specific and can be weaker in comparison to other site factors (Keller *et al.*, 2022). Therefore, results from more studies are needed to clarify the effect of fertilization on C accumulation in soils with different soil features in a wide range of conditions.

## Materials and methods

An experimental site is situated on a well-drained Cambisol with a sandy-loamy texture. The long-term meadow experiment is located near the village Senožaty (485 m a.s.l., Czech Republic) on an *Arrhenatherion elatioris* grassland in 1976. For more details see Dindová *et al.* (2019). The effect of three levels of long-term mineral fertilization was evaluated: unfertilized control, N50 PK, and N200 PK (50 kg N and 200 kg N, respectively, 40 kg P, 100 kg K ha<sup>-1</sup>). The soil was sampled using a soil core (8 cm diameter) in 0–15 cm and 15–30 cm depths before the spring and autumn cut in 2022. Three cores were taken from three replicates for a total of nine subsamples per treatment. All roots and the >2-mm fraction were separated. The soil subsamples from each plot (<2-mm fraction) were mixed and analysed for total carbon and nitrogen content by combustion method. The data were analysed by main effects analysis of variance (ANOVA).

## Results and discussion

The soil C content was higher in the topsoil layer (0–15 cm; 3.2 and 3.23%) than in the deeper layer (15–30 cm; 1.14 and 0.98% in spring and autumn, respectively) but no significant effect of season was detected (Table 1). Decreasing soil C content and C accumulation with increasing soil depth is a general pattern for most grasslands (Conat *et al.*, 2001). In this meadow experiment, more than 90% of roots were also found in the 0–15 cm layer (Hrevušová *et al.*, 2023), clearly indicating that the highest carbon accumulation occurs in the topsoil. Fertilization did not affect the soil C content; however, the N50 PK treatment was connected to the highest C in the top layer in both seasons. The level of fertilization was more correlated with aboveground biomass production (2.33, 4.6, and 7.73 t ha<sup>-1</sup> in control, N50 PK and N200 PK, respectively) than with root production. The greatest difference between root biomass in spring and autumn was observed in the N50 PK treatment (Hrevušová *et al.*, 2023), suggesting that root turnover and soil C content are more related to stand characteristics, e.g. botanical composition, than to the direct effect of fertilization in the topsoil layer. Poeplau *et al.* (2018) reported a positive effect of fertilization on soil organic C stock and found annual sequestration rates of 0.13 and 0.37 t ha<sup>-1</sup> year<sup>-1</sup> in NPK and increased NPK treatments, respectively, in the 0–30 cm layer. However, they recorded a negative effect of increased fertilization in the 0–10 cm layer.

The soil N content was affected by the soil profile both in the spring (0.35 and 0.18%) and the autumn (0.38 and 0.19% in the 0–15 and 15–30 cm layer, respectively). Different nutrient application rates did not result in differences in N content in the soil at the time of sampling. The N application was performed in early spring, so its effect diminished as the season progressed. Higher nitrogen application rates were compensated by higher uptake of more productive treatments (Dindová *et al.*, 2019).

The soil profile and season influenced C:N ratio, with higher ratio values in the topsoil (9.19 and 8.56) in comparison with the deeper soil layer (6.32 and 5.05 in spring and autumn, respectively). Mean values of the C:N ratios did not differ between fertilization treatments but decreased linearly with increasing N supply. Song *et al.* (2014) found that the C:N ratio in the light fraction of C (considered as labile C) showed a linear decline, the ratio in heavy fraction (recalcitrant C) did not show any clear pattern, and the ratio in microbial biomass was linearly stimulated by increasing levels of N addition in the semiarid grassland.

Table 1. Effect of season, treatment, and soil profile on soil organic carbon (SOC), nitrogen (N) content and C:N ratio in grassland soil: Results of main effects ANOVA test.

		C (%)	N (%)	C:N
Season	Spring	2.17	0.26	7.76
	Autumn	2.10	0.28	6.81
	<i>P</i>	0.539	0.060	0.001
Treatment	Control	2.12	0.26	7.50
	N50 PK	2.17	0.28	7.31
	N200 PK	2.12	0.28	7.03
	<i>P</i>	0.913	0.483	0.307
Profile	0–15 cm	3.21	0.36	8.88
	15–30 cm	1.06	0.18	5.69
	<i>P</i>	<0.001	<0.001	<0.001

## Conclusions

The results presented do not show a clear relationship between fertilization and soil organic C or N content under long-term management. However, the trend in the C:N ratio values suggests that higher N fertilization reduced this ratio and thus may influence organic matter decomposition processes in *Arrhenatherion* grasslands.

## Acknowledgements

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 862695 and was realized with the financial support of the Ministry of Education, Youth and Sports (MŠMT).

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# Delivering ecosystem services from permanent grassland: A qualitative analysis of farmer's perceptions and practices

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## Abstract

The existence and management of permanent grasslands (PG) is key to the delivery of multiple ecosystem services (ES) across Europe. However, PG maintenance and functions are under threat from sub-optimal management of inputs, cultivation in higher output farming systems, and abandonment in remote and marginal areas. The current study was conducted as part of the SUPER-G project and aimed to establish an understanding of European farmer attitudes towards the management of PG and provision of ES. A survey questionnaire was developed and completed by 352 farmers across 23 farm networks in six biogeographic regions and a qualitative analysis of perception and statistical analyses of location undertaken. Environmental features differed between regions with 53% of Continental farmers, for example, stating they have hedgerows compared with none in the Boreal networks. Similarly, 86% and 89% of Boreal and Pannonian farms respectively had species-rich grassland compared with 35% of farms in the Alpine networks. Approximately half of the farmers felt that improving PG productivity gives significant provision of biodiversity, carbon storage, flood/erosion control and good water quality. For unimproved PG, livestock feed provision was lower, but provision of other ES was viewed as being higher, particularly for biodiversity, pollination, good water quality, and flood/erosion control. These will be discussed in greater detail across farm-types and regions.

**Keywords:** ecosystem service, survey, management

## Introduction

Permanent grassland (PG) delivers a range of ecosystem services (ES) across Europe and are integral to many farming systems. The SUPER-G project aimed to explore the distribution and state of permanent grasslands (PG) across Europe. As part of this, a survey to gather information about on-farm practices, features and the locations of natural heritage and environmental features which deliver ES on farms was developed. The survey collected information from farm owners/renters, farm managers/workers, farming family members across Europe and covered nine areas, including 'Farm information', 'Grass management', 'Soil management', and 'Ecosystem services'. This paper gives a qualitative analysis of the respondents' perception of ES provided by their PG and an analysis of the location of ES features present on their farm.

## Materials and methods

The surveys were conducted by trained extension officers in the regions of interest, i.e. six biogeographic areas: Alpine ( $n=54$ ), Atlantic ( $n=112$ ), Boreal ( $n=22$ ), Continental ( $n=112$ ), Mediterranean ( $n=24$ ) and Pannonian ( $n=28$ ) for analysis. A farmer information sheet, which provided guidance for accurately filling in the survey and a glossary of terms, was provided to ensure a consistent interpretation and response to questions from all respondents. Permanent Grassland was designated as either Improved (PGI), which been improved through the installation of an underdrainage system, sowing of productive grassland plant species or the addition of lime and/or manufactured or organic fertiliser, or unimproved (PGU). Respondents were asked their opinion on the level of different ES delivered by their PG on a four-point Likert scale of "Significant provision", "Moderate Provision", "Limited Provision" or "No

Provision". Responses were grouped by grassland type and the proportion of respondents for each level of provision calculated. Respondents were also provided a list of ES features and asked to select (Yes or No) whether the feature was present on their farm and the location (PGI, PGU or both). Statistical analysis was conducted using R-3.6.3 (R Core Team, 2020). Chi-square tests were used to look for differences in the proportions of responses differences in location of various features providing ecosystem services and elements; the significance threshold was  $P < 0.05$ .

## Results and discussion

There was a notable lack of hedgerows reported in the Boreal region, with no respondents reporting them present on the PG. No respondents from the Pannonian region reported 'Grass margins', 'Historical or Archaeological features', or 'Fire breaks'. However, a high proportion of respondents in the Mediterranean region reported firebreaks on farm (79.2%). This can be attributed to the hotter, drier climate of the area leading to higher potential of a fire risk. Implementation of a 'nutrient plan' was notably higher in Atlantic region compared with other regions. A higher proportion of Pannonian farmers implemented closed periods for both grazing (75.0%) and nutrient application (82.1%) than other biogeographic regions, with the next highest proportions of respondents implementing these being Mediterranean (37.5%) and Atlantic (65.2%). It is important to note that the perception of a respondent of the features listed, such as 'hedgerows' or 'species-rich grassland' is likely to vary between different biogeographic areas. Although efforts were made to prevent this by providing guidance, the local perception may lead to some of the discrepancies.

The main ES provided by PGI was 'Grass for livestock' (Table 1). This reflects the easily accessed monetary benefit of providing animal feed, meaning that farmers were willing to invest and improve farmland to increase or maintain production of animal feed. A high proportion reported no provision for

Table 1. Farmers' perceived level of ecosystem service provision on improved and unimproved PG (% of farms stating each level of provision).

PG Type and Ecosystem service	Level of provision				n
	No Provision	Limited provision	Moderate Provision	Significant provision	
Improved PG					
Biodiversity	1%	15%	35%	48%	271
Pollination	4%	19%	36%	41%	270
Carbon storage and reducing GHG emissions	2%	10%	35%	52%	268
Flood and erosion control	12%	21%	19%	49%	268
Good water quality	2%	13%	28%	57%	270
Recreation and tourism	13%	31%	28%	29%	269
Grass for livestock	0%	0%	7%	93%	270
Grass for Biomass	82%	8%	2%	8%	260
Unimproved PG					
Biodiversity	3%	4%	15%	79%	195
Pollination	3%	4%	23%	70%	197
Carbon storage and reducing GHG emissions	13%	9%	31%	47%	188
Flood and erosion control	4%	17%	19%	60%	173
Good water quality	9%	2%	25%	64%	175
Recreation and tourism	10%	12%	25%	54%	198
Grass for livestock	2%	11%	17%	69%	202
Grass for Biomass	81%	9%	5%	5%	185

biomass from PGI; however, this can be attributed to the low number of farmers who included biomass production in their business model, with only three farms reporting to have assigned land to this. There was a higher proportion of respondents who believed there to be a significant provision of ‘Carbon storage and reducing GHG emissions’ from their PGI rather than their PGU. It would be expected that less intensive management on PGU would lead to improved C storage where the ground is undisturbed, and fertiliser is not applied. This is reflected in that their perception of significant provision of other ES was higher in unimproved than for improved PG, particularly for biodiversity pollination, good water quality and flood/erosion control.

When considering the location of the environmental features, a higher proportion of farms reported ‘Grass margins’ only in PGI (52%), compared with PGU (12%) ( $X^2$  32.7; SEM 13.4;  $P < 0.001$ ). A higher proportion of farms implemented a ‘Nutrient plan’ on PGI (79%) compared with PGU (11%) ( $X^2$  98.38; SEM 23.4;  $P < 0.001$ ). Similarly, a higher proportion of farmers had a buffer strip on PGI (63%) than PGU (17%) ( $X^2$  38.57; SEM 14.7;  $P < 0.001$ ). There was no significant difference in the occurrence of Historic and Archaeological features on PGI or PGU or both ( $X^2$  4.94; SEM 5.2;  $P = 0.08$ ), and no significant difference in the occurrence of public walkways and access to watercourses in either type of PG ( $X^2$  3.40; SEM 4.4;  $P = 0.18$ ). This might be because the occurrence of historical features or walkways are less within the farmers control whereas the other features are products of farm management decisions. ‘Agri-Tourism’ was more common on PGU than PGI ( $X^2$  12.6; SEM 8.3;  $P < 0.001$ ), potentially the manager trying to make a profit from less productive land.

The perception on ecosystem services delivered and the presence of environmental features vary depending on the biogeographic area, the type of PG (i.e. PGI or PGU) and may also be interpreted differently by each individual. Although this study does not provide sufficient evidence to direct policy, it underlines the importance of policy being tailored to specific areas, and the importance of extension to ensure farmers and custodians of PG across Europe can understand and follow updates to policy. Furthermore, an economic benefit to the manager may encourage adoption if the service has no monetary benefits; this is supported by Sollenberger *et al.* (2019) who suggest a financial incentive will encourage adoption of management to deliver ES from PG.

## Conclusions

Both PGI and PGU offer a range of ES and environmental features which deliver ES. Differences in climate, geographical location and PG type all lead to the delivery of different ES and presence of different environmental features. The results of this survey suggest that PG managers are financially motivated. As such, it is important for these factors to be considered by policy makers when developing future directives to ensure they are acceptable and achievable.

## Acknowledgements

The authors thank the extension officers who carried out the survey and the respondents who took time to fill it in. The SUPER-G project (Grant Agreement No.: 774124) has received funding from the European Union Horizon 2020 Research and Innovation Programme.

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# Farmer-led innovation in the use of multi-species swards on Northern Ireland farms

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## Abstract

There is evidence to suggest that increasing the diversity of sward species (multi-species swards (MSS)) can counteract some of the challenges faced by Northern Ireland (NI) ruminant livestock farmers. There are many suggested benefits from incorporating a mix of grass, legume and herb species into grazing platforms, such as their deep-rooting properties leading to improved soil health and reduction in the requirement for manufactured nitrogen fertiliser input. However, there is a considerable lack of information surrounding the management of MSS on farms in NI. AgriSearch and AFBI have been working with eight NI commercial farmers trialling MSS on their farms through both the SUPER-G project and a European Innovation Partnership (EIP) Operational Group. With support from AgriSearch, AFBI and Queen's University the farms involved evaluated a range of seed mixtures and establishment methods. Results have shown the MSS are significantly more drought resilient and can produce comparable dry matter yields to conventional perennial ryegrass (PRG) swards with lower fertiliser inputs. Furthermore, animals grazing MSS required less anthelmintics than those grazing PRG swards. In addition to the knowledge provided by researchers, the peer-to-peer learning and support has been invaluable, particularly in the areas of establishment and initial management of MSS.

**Keywords:** multi-species swards, herbal leys, peer learning

## Introduction

Farmers in Northern Ireland (NI) are facing financial, production and environmental challenges. Finding a suitable balance between maintaining profitable and sustainable livestock performance from grassland and improving farm ecosystem service provision is critical to sustaining farm businesses and the wider industry for the future.

Multi-species swards (also referred to as species-rich or diverse grasslands) are communities comprised of grass, legume and herb species. There is a growing body of evidence (Lowe *et al.*, 2021; Patterson *et al.*, 2022) to suggest that increasing the diversity of plant species in grassland can address many of these challenges, delivering a wide range of ecosystem services, reducing management costs and positively influencing sustainable livestock production. Since grassland is the predominant crop in Northern Ireland, incorporation of MSS presents a significant opportunity for the livestock sector. The studies reported in this paper aimed to provide information on the establishment and management of MSS on commercial beef and sheep farms in Northern Ireland.

## Materials and methods

The first project (undertaken as part of the SUPER-G project) involved the establishment of MSS on seven dairy, beef and sheep farms across NI. The swards contained perennial ryegrass (PRG), white clover (WC), chicory and plantain or a control mix containing the same PRG-WC mixture, but without the plantain and chicory. Swards were established during autumn 2019 and spring 2020.

Having been encouraged by their initial experiences, the beef and sheep farmers involved in the above project with assistance from AgriSearch applied for funding for a European Innovation Partnership (EIP) operational group to further investigate the role of MSS for beef and sheep farms. During the early stages of the project, the farmers investigated the most appropriate MSS mixtures to use on their farms. They were assisted in this by a literature review undertaken by AFBI as part of the project (Lowe *et al.*, 2021) and were supported by scientists from AFBI and Queen's University.

Dissemination was central to both projects, and included general media communication via the press and social media. Webinars were held during the pandemic and, once restrictions were lifted, two farm walks were held. The first farm walk held in September 2021 attracted approx. 100 attendees, while the second one in June 2022 was attended by over 200 people. At both the webinars and farm walks, each of the farmers communicated their experiences of establishing and managing multi-species swards. A final conference and farm visit was held in June 2023 to provide an overview of the MSS projects, and to get feedback from farmers on the practicality and feasibility of incorporating MSS on farms in NI.

## Results and discussion

The farmers involved used a range of establishment methods, which included full ploughing and cultivation, as well as minimal cultivation and surface seeding. As legume and herb species such as plantain and chicory require warmer soils for germination than a PRG monoculture, the farmers in this group were initially advised to reseed in spring. However, many of the farms involved are located in County Down, which in recent years has experienced regular late spring/early summer droughts. Due to this, the farmers expressed an interest in trying to establish a MSS in autumn (end July–early September) in the future, to avoid the potential droughts and to potentially lower the weed burden at establishment. The farmers also realised that a change of mindset was needed to manage these swards; this included reduced use of artificial N fertiliser, longer grazing rotations, along with higher entry and residual sward heights. Across both projects, the MSS produced comparable yields to grass-clover or grass-only swards. Over two years the MSS sown as part of the SUPER-G / EcoSward project on average yielded 9 t DM ha<sup>-1</sup> (5.6–12.2 t DM ha<sup>-1</sup>) using an average of 66 kg N ha<sup>-1</sup>. The control swards on average yielded 8.4 t DM ha<sup>-1</sup> (5.0–11.0 t DM ha<sup>-1</sup>) using an average of 71 kg N ha<sup>-1</sup>.

Regarding animal performance on MSS, as part of the EIP project, three farms were able to keep batches of stock exclusively grazing MSS and PRG-WC swards and monitor their performance over the 2022 grazing season. One farm compared two flocks of 74 ewes rearing double lambs that were taken through to slaughter. Slaughter weights were similar but lambs grazed on MSS were finished much earlier (–29 days), which would have significant cost saving and a lower GHG footprint relative to those grazing PRG-WC. On one beef farm, similar cattle performance was recorded. In contrast, one beef farm recorded lower performance (–0.17 kg day<sup>-1</sup>) for cattle grazing MSS relative to cattle grazing grass-only. One sheep farmer, who incorporated a single field of MSS into the grazing platform, found lamb performance dropped when grazing the MSS swards after grazing PRG swards, so more research is needed on the effect of alternating sward diet/composition on livestock productivity. In addition, the sheep farmer who closely monitored the performance of the lambs also monitored faecal egg counts (FEC) during the season. The lambs on PRG-WC received 4 anthelmintic treatments, whereas the lambs on MSS only received 2 treatments. One beef farm also monitored FEC during the 2022 grazing season and, in this case, no differences in FEC were observed between the cattle grazing PRG-WC and MSS. However, two additional beef farms reported that their animals grazing MSS received fewer anthelmintic treatments relative to those grazing PRG swards. This may potentially be linked to the year and the weather patterns within it, but it requires further research. Soil analyses across the seven farms showed no significant difference; however, there were numerically higher total carbon % values under the MSS relative to the



Table 1. Mean soil parameters on grass-clover and MSS fields from seven NI farms, after two grazing seasons

Parameter	Sward type		SED	Prob
	Grass/Clover	MSS		
Bulk density (kg l <sup>-1</sup> )	0.951	0.911	0.0293	0.197
Total carbon (%)	4.743	5.429	0.474	0.174
Organic matter (%)	8.157	9.300	0.833	0.195
Soil organic carbon (SOC %)	4.743	5.386	0.481	0.206
Organic carbon stock (t ha <sup>-1</sup> )	44.97	48.73	3.041	0.249

grass-clover control after just two grazing seasons, which also merits further investigation over a longer time period.

There has been a great deal of interest in this topic locally, which was undoubtedly due to the sharp rise in artificial fertiliser prices. Feedback was sought after each farm walk event and approximately 60% of respondents indicated that they were farmers. On both occasions, over 90% of respondents indicated they would be keen to attend a similar event in future. Specific feedback stated that the farmers' open and honest viewpoints were appreciated.

## Conclusion

While further controlled research studies on MSS are very much needed, the experiences gained by sowing a range of MSS types across a range of farms using a variety of establishment methods has been most beneficial. The research scientists involved in the study have used the farmers' experience to help draw up management protocols for follow-on research trials. Ultimately farmers learn best through peer learning and also by interacting with scientists. The farmer group has also operated as a most effective mutual support network, has highlighted areas for further research and has inspired many other farmers in NI to establish their own MSS.

## Acknowledgements

This work was completed with support from both SUPER-G (EU Horizon 2020- Grant ID 774124), EcoSward (19 4 01) Department of Agriculture, Environment and Rural Affairs) projects and AgriSearch and the Northern Ireland European Innovation Partnership scheme which is jointly funded by the European Agricultural Fund for Rural Development (EAFRD) and the Department of Agriculture, Environment and Rural Affairs (DAERA).

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# A farm level decision support tool to quantify ecosystem service delivery from permanent grassland

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## Abstract

The existence and management of permanent grassland (PG) is key to the delivery of multiple ecosystem services (ES) across Europe. A deliverable from the SUPER-G project was the development of a farm level decision support tool (DST). The aim of the DST was to provide the farmer (user) with an overview of the various ES delivered through the management of PG within their farm; and to offer guidance on steps that could be taken to enhance the delivery of each ES. A multi-actor approach, with discussions between farmers, landowners and their advisers, non-governmental organisations and researchers, was undertaken to develop the DST. The six ES considered important include food production; climate regulation; biodiversity; landscape and recreation; flood and erosion control; and water quality. The user is asked a series of questions which calculates values for each ES indicator. These ES indicators are then combined using Simple Additive Weighting to provide the user with a score for each of the six ES.

**Keywords:** ecosystem service, decision support, permanent grassland, farm level tool

## Introduction

The environmental impact of farm management is becoming more and more important at both an individual farmer level and at a governmental level. Permanent Grassland (PG) provides a range of ES; however, it can be complicated for the farmer to measure ES provided by their PG because there is not necessarily an economic benefit. Furthermore, the synergies and trade-offs in management to provide ES are complicated and difficult to understand. Previous work in the SUPER-G project found that there was appetite among farmers for a DST specific to ES (Titterington *et al.*, 2022). The objective of the study was to develop a proof-of-concept DST which could give an indication of a user's ES delivery from their PG. This paper aims to outline the development and evaluation of a scoring system for ES which the end user (farmer) can use to make informed decisions and improve ES delivery from their PG.

## Materials and methods

Six ecosystem services (ES) are included in the DST: food, wool and biomass production; climate regulation; biodiversity; landscape and recreation; flood and erosion control; and water quality. Each ES consisted of between three and five agri-environmental indicators (parameters, Figure 1). A series of co-development meetings with experts in ES were held to agree the inputs required to calculate values for each parameter within ES. Each meeting had a panel of between five and ten experts for each ES. A thematic analysis of the data requirements revealed the main inputs to be stocking rate (three ES), grassland management (six ES), nitrogen input (three ES), soil (two ES), GHG emissions (one ES,

requiring multiple questions), and on farm features (two ES). To calculate stocking rate, the livestock unit for each animal type was used (Eurostat, 2023). The parameter of GHG emissions was subsequently removed as there are existing tools which analyse this parameter. It was also important that the tool could provide a score for a point in time, so measures which would have required the user to have managed the PG for an extended period prior to using the DST were deemed unsuitable. The DST was designed to calculate a score using simple additive weighting, SAW (Nurmalini, 2017); thus experts were asked to allocate a weighting to each score, with the parameters within an ES which had a higher weighting contributing more to the final ES score.

It was important that the DST was accessible to all users; thus the language used must be clear and photo prompts were integrated where possible. Photographs were supplied by experts or, where possible, taken from published peer review articles to ensure they were appropriate. To ensure the language was clear, the DST was tested on farmers for accessibility and acceptance. This was done by observing a small sample of users (n=3) whilst using an online prototype version of the DST and through an additional paper-based trial of the DST where a group of 29 farmers were asked to answer the questions used in the DST and highlight any questions which were difficult to understand. The tool was also tested with local policy makers in Northern Ireland (NI).

## **Results and discussion**

The use of simple additive weighting was reviewed and agreed to be suitable. Although ES and their respective parameters are not necessarily additive, it was agreed the objective of the DST is to give the user the opportunity to monitor and model their ES delivery; not to provide a diagnostic score. Since it was difficult to assign a specific percentage weighting to each ES parameter the expert panels discussed and agreed the parameters ranking by importance. Subsequently, figures were applied to each parameter to reflect the rank, with a higher weighting corresponding to a higher ranking. When establishing the calculations for each ES parameter, it was necessary to agree the specific inputs which would feed into the calculations. It was agreed that the input should be minimal, with drop down lists allowing the user to choose their response. The variation between biogeographic regions made it impossible to develop a diagnostic tool for ES delivery. Thus, it was agreed that the current form of the DST is a proof of concept, which has been designed to be tailored to specific biogeographic regions and/ or smaller localities in future iterations.

The DST had a hub and spoke design for each ES, meaning that a user could view their ES score on the report page (the hub), and each parameter had a link (spoke) to the inputs required to calculate that specific ES parameter. This allowed the user to easily see how the changes impacted both the ES parameter and the ES overall. If changing the ES parameter impacted the calculations for other ES parameters these would automatically recalculate using the new inputs, allowing the user to have a full view of the impact of changing a parameter. This gave the user the opportunity to model change and also to learn how management changes would impact ES delivery. Reviewing the tool with farmers/ users led to some semantic changes which were reviewed by researchers and agreed prior to the tool launch. When NI policy makers policy tested the tool, their feedback was positive, and included some changes which would have been specific to laws and environmental conditions in NI. These changes were not introduced to the current version of the tool but demonstrate the potential to adapt it to more localised areas.

## **Conclusions**

A proof-of-concept DST was designed to give an overview of the ES provided from PG on farms across a wide geographical area. An evaluation of the DST by users identified improvements required and these were implemented by expert panel. It is planned that further iterations of the DST will be launched at

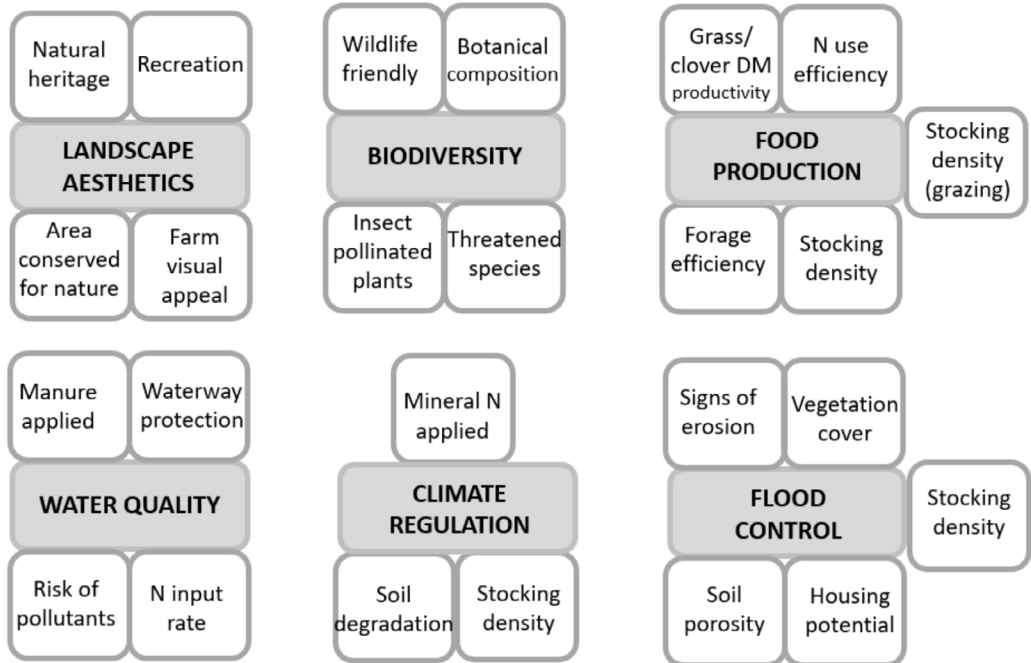


Figure 1. Ecosystem services (upper case bold text) and their respective parameters (lower case)

a more localised level, where the policy makers or advisers at a governmental level can tailor the DST to specific locations and improve ES delivery from PG.

## Acknowledgements

The authors would like to thank all those who attended the workshops. The SUPER-G project (Grant Agreement No. 774124) has received funding from the European Union Horizon 2020 Research and Innovation Programme.

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# Introducing the anecic earthworm *Lumbricus terrestris* in grasslands to improve water regulation

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## Abstract

Water regulation is an important ecosystem service of grasslands. Anecic earthworm *Lumbricus terrestris* presence in grasslands has a positive effect on water infiltration. We explored the ability of *L. terrestris* to survive and reproduce after being newly introduced into mesocosms in grasslands on sandy soils. While *L. terrestris* appeared able to survive and produce cocoons, survival rate was low (32% after 7 months, 6% after 15 months, 33% after 8 months) and the number of juveniles was low (2.6 and 2.7 ind. m<sup>-2</sup> after 7 and 15 months, resp., 2.5 ind. m<sup>-2</sup> after 8 months in re-inoculated mesocosms). Low survival rate may be related to the life history of the *L. terrestris* inoculum, soil moisture, interspecific competition for food with the native population of epigeic earthworms and the risk of predation.

**Keywords:** earthworm inoculation; mesocosm; water regulation; interspecific competition

## Introduction

Grasslands play a vital role in water regulation. Global climate changes are characterised by prolonged dry periods and intensified peak rainfall (Pachauri *et al.*, 2014). Both entail major impacts on agricultural grasslands (Beier *et al.*, 2012). As soil ecosystem engineers, earthworms cause soil bioturbation which helps to improve water infiltration (Deru *et al.*, 2018). Deep-burrowing earthworms such as *Lumbricus terrestris* create vertical, semi-permanent burrows, reaching down to 2 m. Burrows can increase the soil's infiltration rate and capacity, (Blouin *et al.*, 2013), while also increasing rooting space, which promotes drought tolerance (Edwards *et al.*, 1980). *L. terrestris* is currently present at 20–25% of Dutch dairy farms on sandy soil. It is likely that water infiltration in grasslands where *L. terrestris* is absent could be improved through *L. terrestris* burrowing activity. As natural dispersal is slow and can be hampered by obstacles (roads, waterways etc.), we explored the possibility of inoculating grassland with this species. We hypothesised that *L. terrestris* would be able to survive and reproduce, but would possibly suffer from interspecific competition with the resident earthworm population. Additionally, that loosening the soil and removing the resident earthworm population prior to *L. terrestris* inoculation increase survival and reproduction rate, as the earthworms would benefit from easier burrowing and less competition.

## Materials and methods

In April 2019, 40 mesocosms (steel pipes of 61 cm diameter, 50 cm in length) were driven 40 cm into the soil in permanent grasslands on sandy soil at two Dutch dairy farms. Twenty mesocosms per grassland, in two rows, 90 cm apart. Farm A was a conventional farm with a history of artificial fertiliser and slurry application, farm B an organic farm with a history of farmyard manure use (for details see Van de Logt *et al.*, 2023). Per farm, half of the mesocosms were inoculated with adult Canadian *L. terrestris* 52 (ind. m<sup>-2</sup>) (purchased commercially), the other mesocosms served as controls. All mesocosms were covered with a net and mowed regularly. Escape belowground was highly unlikely, as *L. terrestris* burrows vertically and disperses over the soil surface (Mather and Christensen, 1988). After 7 months, in November 2019, half of the mesocosms were harvested at both locations. The soil from each mesocosm was removed in three layers (0–20, 20–40 and 40–60 cm) and hand-sorted to collect all earthworms. Earthworms were conserved for species determination. The hand-sorted soil layers were returned to the mesocosms in their original order (hand-sorting had loosened the soil and made it devoid of earthworms, but with cocoons still present), and reseeded with grass-clover. The harvested mesocosms were re-inoculated with the same

amount of *L. terrestris* as April 2019. Fifteen months after initial installation, in July 2020, the soil of all mesocosms was harvested following the same procedure as in November. ANOVA in GenStat with location (A, B), treatment (control, inoculated) and harvesting date (November, July) as factors was used to analyse the data.

## Results and discussion

Of the *L. terrestris* that were introduced in April 2019, 32% (16.1 ind. m<sup>-2</sup>) had persisted after 7 months and 6% (2.9 ind. m<sup>-2</sup>) after 15 months. In the mesocosms that were re-inoculated in November 2019, 33% (16.9 ind. m<sup>-2</sup>) of the *L. terrestris* had persisted (Figure 1). We assumed all adult *L. terrestris* to be introduced individuals, as it can take them over one year to mature under field conditions (Daniel, 1992). Recovery 7-8 months post-introduction was comparable to earlier findings by Andriuzzi *et al.* (2015).

Loosened soil and the initial absence of a resident earthworm population (the population largely recovered over the period of the experiment) had not resulted in high survival nor reproduction rate. However, as the 7- and 8-month experiments did not run parallel in time, we cannot draw a final conclusion on the effect of introduction into hand-sorted soil.

We propose several factors that may have contributed to this low survival rate. First, as *L. terrestris* is not bred commercially, we only had access to earthworms imported from Canada. The weeks of inactivity due to the import procedure may have weakened them. It is also possible that the Dutch grassland hosted pathogens for the earthworms. Second, the spring of 2020 was exceptionally dry. In general, *L. terrestris* remains active during periods of drought (Eisenhauer *et al.*, 2008), which may make the species more susceptible to the potentially lethal effects of low soil moisture. Third, *L. terrestris* may have suffered from interspecific competition for food with epigeic earthworms, mainly *Lumbricus rubellus*, as this species also feeds on surface organic matter, but has a higher growth and reproduction rate, *L. rubellus* was the most abundant epigeic species for both locations (99% at location A and 84% at location B). The difference was non-significant but, on average, the inoculated mesocosms contained fewer epigeic worms than the control mesocosms at both locations and both harvesting dates. The discovery of juveniles showed that *L. terrestris* had successfully produced cocoons after introduction. However, it is unclear that the actual mating took place in the mesocosms, as the species can store sperm for several months. Fourth, small

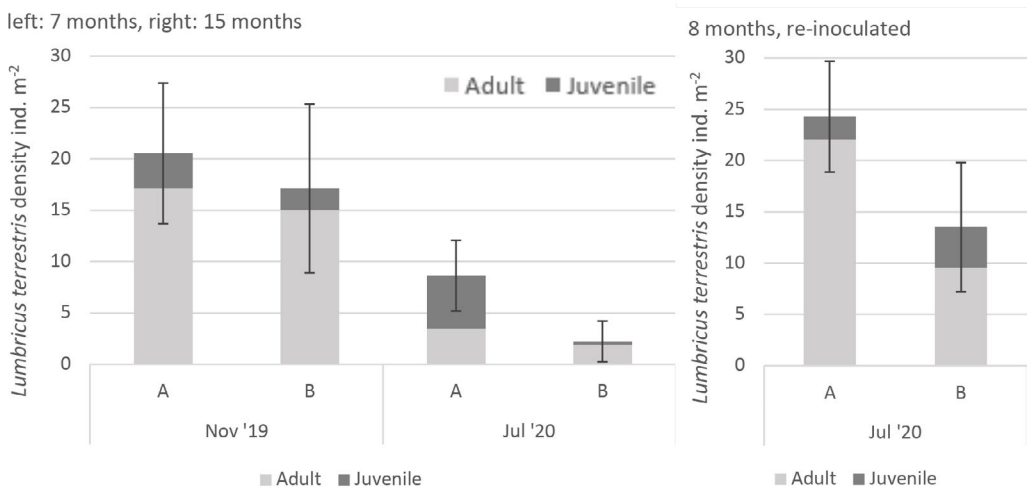


Figure 1. *L. terrestris* density at farm A and farm B after 7 months (two left bars) and 15 months (two middle bars). The two bars on the right side present *L.* density after 8 months in re-inoculated mesocosms.

holes were observed in some of the protective nets, these may be signs of attempted predation by birds. Therefore, we cannot exclude the possibility that some earthworms had been predated on.

## Conclusion

*L. terrestris* can survive for 15 months and produce cocoons after introduction into grassland on sandy soil in a mesocosm set-up. The life history of *L. terrestris*, a lack of soil moisture, interspecific competition and predation could all threaten the inoculated earthworms. Despite these challenges, a number of individuals managed to survive and produce cocoons, showing certain potential for *L. terrestris* inoculation as an ecological innovation towards climate adaptation in agricultural grasslands. However, as survival rate was low, only further experimental trials over greater time spans and in non-enclosed plots will determine whether there is realistic potential for *L. terrestris* to develop a stable population.

## Acknowledgement

The research was part of the projects Lumbricus and PPS KLIMAP. We are grateful to the farmers who granted us access to their grasslands.

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# Environmental factors determining biodiversity, productivity and fodder value of submontane grasslands

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## Abstract

In the face of global climate and socio-economic changes, it is necessary to adapt the methods of grassland management to the new realities. Extensive grazing is in line with the principles of sustainable development; however, meat production and consumption are controversial issues nowadays. The study analysed the relationship between biodiversity, productivity, fodder value and environmental conditions in submontane grasslands. Two study sites were selected in southwestern Poland, where 60 sampling plots (3×3 m) were established in transects located along the moisture and microclimate gradients. The composition and richness of vascular plant species (biodiversity), hay biomass (productivity), and chemical properties of the hay (fodder quality) were analysed and correlated with environmental data. The studied grasslands have a relatively high level of biodiversity. A positive correlation was found between the species richness, ash and calcium content in plants, and magnesium in soil. The biodiversity and species composition of grasslands were primarily shaped by soil moisture, pH, and heat index. The results could be a basis for a management plan focused on improving the ecosystem services and functions served by grasslands.

**Keywords:** biodiversity, ecosystem services, environmental conditions, fodder quality, productivity, submontane pastures

## Introduction

Climate change has already negatively impacted the grasslands in Europe, and this trend is expected to continue. As a result, the Common Policy for 2021–2027 for the EU Member States includes adaptation to climate change as a critical objective (Jacobs *et al.*, 2019). Semi-natural grasslands provide a wide range of ecosystem services and functions, ranging from forage production and carbon sequestration through recreation and tourism to maintaining a high level of biodiversity (Bengtsson *et al.*, 2019). Unfortunately, grassland areas have declined in Europe, and predictions suggest that this decline may continue in a climate-change-affected future (Schils *et al.*, 2022). To maintain the biodiversity of semi-natural grasslands, management by grazing or mowing is needed. Grazing is proven to have a positive effect on conservation values in most grasslands and is a factor linking grassland maintenance, productivity, economic use and management for biodiversity (Tälle *et al.* 2016). The mechanism of animal grazing influence on biodiversity is selective defoliation resulting from dietary choices both between species and between plant parts within species (Rook and Tallwin, 2003). However, animal keeping for meat production has become a controversial topic in public debates, as it involves multiple sustainability dimensions by the larger environmental and climate footprints than plant-based food products (Parlasca and Qaim, 2022). Worldwide, factors affecting meat consumption trends are highly complex, including ethical aspects, health considerations, economic pressures, nutritional reasons, and environmental concerns. Agricultural policies have developed worldwide, and steps toward higher levels of sustainability have already been taken (Henchion *et al.*, 2022). In the face of global climate and socio-economic changes, it is necessary to adapt the methods of grassland management to the new realities. The study aims to analyse vascular plant species composition (biodiversity), dry biomass (productivity), and chemical properties of the hay (fodder quality) and correlate these data with environmental conditions to plan the management focused on the improvement of the ecosystem services and functions served by submontane grasslands.



## Materials and methods

The study was conducted in two localities in the Lower Silesia region, southwestern Poland, in a submontane area with numerous meadows and pastures with diversified topography. We selected grassland complexes under uniform management (first pasturing, next mowing during the growing season). We located study plots along transects according to soil moisture and thermal conditions gradients, defined based on topographic wetness index (TWI, calculated based on the topography by modelling the flow of water down the slopes) and diurnal anisotropic heating index (DAH, showing thermal conditions associated with different insolation/exposition of the slope) (Sørensen *et al.*, 2006). Sixty sampling plots were established, 30 in Radomierz (147 ha grazed by 250 Charolais cattle) and 30 plots in Pasterka (72 ha, 120 sheep). The plots represented wet, medium and dry conditions, according to TWI and DAH indices. The individual plots were fenced to avoid accidental disturbance, e.g. grazing or mowing before vegetation sampling. Study plots were 3×3 m in size; soil samples were taken, and the phytosociological relevés using the Braun–Blanquet approach were done on each plot. Subsequently, the vegetation from 1 m<sup>2</sup> was mowed at a height of 3 cm, the biomass was dried and weighed, and the chemical analysis of the hay and soil samples was conducted. The soil moisture was measured in 3 points, at a depth of 6 cm, using a portable moisture meter (LB-797, LAB-EL Elektronika Laboratoryjna, Warsaw, Poland) in the second decade of June. The results were then averaged to express the mean percentage of soil moisture. The Spearman's correlations between environmental factors (TWI, DAH, slope inclination, and soil moisture, pH, N, P, K, C, Mg content in soil) and vegetation characteristics were calculated, and the redundancy analysis (RDA) was done to show the main factors shaping vegetation composition.

## Results and discussion

In both study areas, we found 170 vascular plant species in all study plots. The higher species richness was on pastures in Radomierz (120 species) than in Pasterka (90 species). The groups of plant species with the highest frequency and cover representing wet (*Alopecurus pratensis*, *Agrostis capillaris*, *Dactylis glomerata*), medium (*Festuca rubra*, *Poa pratensis*, *Trisetum flavescens*) and dry (*Deschampsia flexuosa*, *Nardus stricta*, *Hypericum maculatum*) sites were defined. The dominant species were grasses, while numerous infrequent and non-dominant species increased the species richness substantially, what seems typical to grasslands (Swacha *et al.*, 2023). A positive correlation was observed between biomass production and TWI, and a negative correlation between biomass production and DAH. A negative correlation was also found between grassland species richness and dry biomass in the Radomierz site (Figure 1a) but not in the Pasterka site. It underlines that the scheme of suitable grassland management should be adjusted to the local, and farm-specific conditions. We observed a positive correlation between grassland species richness and slope inclination and a negative with TWI (Figure 1b). We interpret it as a result of a dominance of highly competitive species in wet habitats, causing biodiversity decrease. A positive correlation was found between the number of species and the content of ash and calcium in plants, which can be considered as hay quality indices. RDA explains 15% species composition variability; the most significant variables influencing species composition are soil moisture ( $F=4.2278$ ,  $p=0.001$ ), pH ( $F=2.5432$ ,  $p=0.001$ ), and DAH ( $F=1.9432$ ,  $p=0.010$ ).

## Conclusion

The studied grasslands have a relatively high level of biodiversity, shaped mostly by soil moisture and reaction, as well as thermal conditions. Our results show that the scheme of suitable grassland management should be adjusted to the local conditions and farm specific. The results are a basis for preparing a management plan following sustainable development and nature protection to improve the ecosystem services and functions served by grasslands.

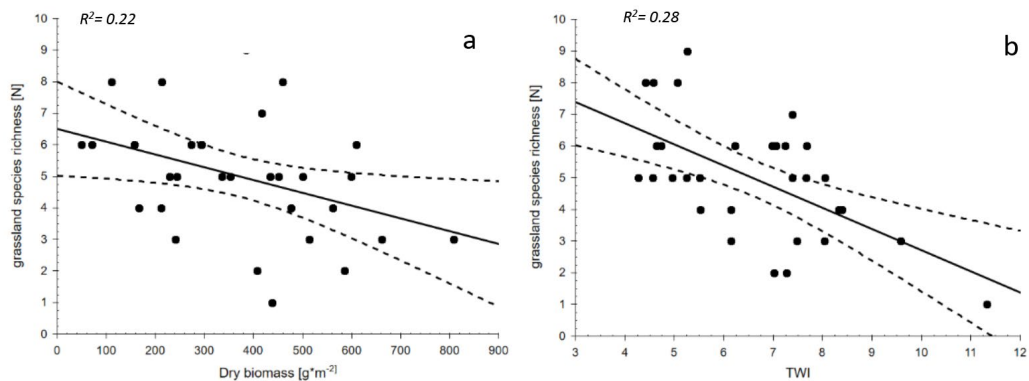


Figure 1. Correlation between grassland species richness and dry biomass (a) and TWI (b).

## Acknowledgements

This research was financed under the Leading Research Groups support project from the subsidy increased for the period 2020–2025 in the amount of 2% of the subsidy referred to Article 387 (3) of the Law of 20 July 2018 on Higher Education and Science, obtained in 2019.

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**Theme 3.**

**WHICH?**

**Which methods can be used  
to monitor, evaluate and steer  
grassland management?**



# The long path from data collection to sustainable grassland management

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## Abstract

Many different techniques are available to observe and quantify ecosystem functions and services provided by grasslands now and in the future. Measurements need to be analysed and integrated to develop and translate data into management recommendations for adoption in sustainable grassland management. Here, we provide a concise overview of different techniques available to collect data to learn about grassland functions and services. While different spatial scales of interest, i.e., field, farm or landscape, require different modes of data collection, the temporal variability of the function or service of interest also demands different techniques to capture their dynamics. Examples are provided: e.g., how high temporal resolution measurements of ecosystem greenhouse gas exchange can be used not only to quantify soil carbon sequestration but also detect trade-offs between C sequestration and N<sub>2</sub>O losses; how image analyses can help in restoration projects; how remote sensing technologies can be used to improve grassland farming; and how modelling approaches can help to predict biomass production and long-term carbon sequestration rates. The benefits of FAIR (Findability, Accessibility, Interoperability, and Reuse) data sharing as well as strengths and weaknesses of techniques will be addressed, management options outlined, but also gaps in knowledge identified.

**Keywords:** grassland functions and services, overview of techniques, greenhouse gas exchange, remote sensing, modelling, data sharing

## Introduction

Grasslands, including savannas, prairies, and steppes, are vital for ecosystems globally and particularly in Europe (White *et al.*, 2000). These landscapes are critical for agricultural productivity as well as for biodiversity and climate change mitigation. For example, grasslands significantly contribute to Europe's agricultural landscape, providing livestock forage as well as regional revenues. But European grasslands, particularly permanent grasslands, also play a key role in biodiversity conservation and carbon sequestration, aiding climate change mitigation. They are essential for soil preservation and water regulation, maintaining the ecological balance in agricultural regions. Moreover, their cultural and historical values, linked with traditional agricultural practices and recreational activities, are significant. Thus, sustainable management of grasslands is crucial for preserving their ecological functions and ecosystem services (Schils *et al.*, 2022). For Europe this means aligning food and feed production with reducing the environmental footprint of permanent grassland use, while providing income to farmers and land managers. Clearly, effective management strategies rely on accurately determining grassland functions. However, deciding which technologies and approaches to use and which strategies will be adopted by land managers is far less clear.

Many different techniques are available to observe and quantify ecosystem functions and services provided by grasslands now and in the future. Techniques vary widely, encompassing a spectrum from traditional to advanced, covering different temporal and spatial scales, and ranging from ground-based observations to airborne methods. Traditional ground surveys and manual plot sampling provide very

detailed, site-specific data, while advanced methods often include highly scientific sophisticated but also costly approaches. For example, remote sensing technologies using new satellite missions offer broad-scale monitoring capabilities, but also require complex image processing as well as ground-truthing. On the other hand, airborne methods like drone-based imaging can provide a balance between large-scale observation and detailed local data. These data are then often compiled in databases and integrated in models to predict future changes and potential responses of grasslands to various environmental and socio-economic stressors. This integration requires sophisticated data analysis techniques that can handle various data types and scales. Analyses and syntheses of data from different sources allow a more complete picture of grassland functionality; however, best practice examples are still scarce.

The ultimate goal of all these analyses is to translate complex agronomic and ecological data into practical management recommendations. This involves understanding the interplay between many ecosystem services and functions. In order to be adopted and become effective, management strategies must, on the one hand balance these services, and on the other hand be tailored to specific grassland conditions, and thus management needs as well as to agro-political and economic constraints. This paper aims to provide a concise overview of different techniques available to collect data to learn about grassland functions and services. We will address the questions “why”, “what”, “where”, “when”, and “how to measure”, but also which management options are available and what their potential to increase ecosystems functions and services might be. Finally, we briefly discuss adoption of sustainable management options and tools.

## **Methodology**

Based on expert knowledge as well as literature, we evaluated different tools and innovative techniques to inform sustainable management options for permanent grassland. We started with the management purpose, which ranged from feed production to reduction of the environmental footprint of grassland management, but also included potential solutions to different global challenges, e.g., climate change or biodiversity loss. Different tools and techniques (“what”) for those different purposes were then judged in terms of what spatial and temporal scales they are performed at (“where”, “when”), i.e., site, farm, or landscape scales, but also if they are collected or employed only sporadically (collected manually or in campaigns), more regularly (as with drones or in experiments), or even continuously. Due to the heterogeneity of purposes and spatio-temporal scales, we then addressed the many different modes and techniques of data collection (“How”), which clearly depend on the grassland functions or services of interest. We further evaluated the weaknesses of these different techniques which might limit their adoption (“Weaknesses”). Finally, we assessed management options using these techniques for their effective potential to indeed capture grassland functions and services and to be adopted by grassland farmers, and thus to improve sustainability of permanent grassland farming.

## **Results and discussion**

To develop sustainable management options, it is crucial to select data collection techniques according to the management purpose and the respective farming and production systems. While spatial and temporal scales as well as mode of data collection and weaknesses vary, some of these techniques and approaches are still used rather in academic or experimental settings. Nevertheless, some methods and techniques are already implemented in management options, both for assessing grassland vegetation as well as livestock, while others still need ‘proof of concept’ in real world, agricultural settings. In the following sections, we focus on selected examples of data capture and analysis in grassland and livestock farming systems, to illustrate the pros and cons of various techniques (Table 1).

Table 1. Overview of methods and techniques available to collect information about permanent grasslands.

What	Where	When	How	Weaknesses	Management options
<b>Feed production, grazing behaviour</b>					
Yields: quantity, quality, stability over time	site	campaigns	different manual methods	time and space resolution, time requirement, knowhow	use of different mixtures, new species, breeds or varieties, change in management intensity (cutting, grazing, fertilization, irrigation, etc.)
	site, farm	regularly	GPS collars, virtual fencing, drones, UAVs, mounted with GPS and different cameras	knowhow, privacy/permit issues, time and space resolution	
	site, farm, landscape	regularly	satellites	price, data ownership, knowhow, time and space resolution	
	animals	campaigns	feed intake observations, incl. tracers; biomass assessments	time and space resolution, rather academic/experimental approach	
<b>Resource use</b>					
Inputs via fertilization, grazing	site	regularly	farmer interviews; official statistics; legal requirements	time demand, privacy issues	different farming practices (e.g., stocking density, timing, animal breed), extensification, precision farming, change to different production systems (e.g., regenerative agriculture, organic agriculture, permaculture)
	site, farm	regularly	soil analyses	time demand, price	
	site, farm, landscape	regularly	drones, UAVs	knowhow, privacy/permit issues, time and space resolution	
	site, farm, landscape	regularly	satellites	price, data ownership, knowhow, time and space resolution	
<b>Climate change: Grasslands as drivers</b>					
Greenhouse gas emissions: magnitude of fluxes, drivers; soil carbon stocks	site	continuously	eddy covariance flux stations	price, knowhow, spatial homogeneity, rather academic approach	change in fertilization (magnitude, type, timing), precision farming (e.g., variable rate fertilization)
	site	regular campaigns	soil chambers, isotope analyses, soil analyses	time and space resolution, knowhow, rather academic/experimental approach	
	animals	campaigns	GPS collars, virtual fencing	knowhow, price, time and space resolution, rather academic/experimental approach	
<b>Climate change: Grasslands driven by climate change</b>					
Resilience to climate change, e.g. extreme events	site	campaigns, continuously	experiments, long-term data sets	academic approach, research still needed; predictability of extremes	use of new mixtures, introduction of new grassland species, irrigation/drainage, change in farming practices (e.g., timing of management)
<b>Counteracting biodiversity loss</b>					
Determining biodiversity (species and functional group diversity, plants, animals, microbiota, eDNA)	site	campaigns	manual assessments, experiments, lab assessments, long-term data sets	knowhow, time, and space resolution, academic approach, available results often site-specific	use of mixtures, new grassland species, overseeding, different animal breeds or varieties, change in farming practices (e.g., timing of management)
	farm	regularly	drones, UAVs	knowhow, time and space resolution, privacy/permit issues	
	site, farm, landscape	regularly	satellites	price, data ownership, knowhow, time and space resolution	

Different management purposes are considered, ranging from feed production to help solving global challenges. Different columns give examples of what, where, when, and how to measure, but they also address the weaknesses of these techniques. Relevant management options are given, although these are not exclusive.



## Feed production and grazing behaviour

Information about feed production in permanent grasslands and thus forage supply for animals can be collected in many different ways. While vegetation growth over time can be easily measured in meadows, such an assessment is more difficult in pastures, since not only forage supply but also animal behaviour need to be considered. GPS collars provide an opportunity to monitor the grazing behaviour of livestock over an extended period of time. The collars typically send location data every 15–20 minutes via a network. Collar device data reception can be variable, depending on field topography, vegetation type, and distance to the receiving antenna (Maroto-Molina *et al.*, 2019). However, even in uneven terrain, it is possible to generate grazing calendars and livestock use intensity maps using simple data processing; indicating grazing preferences, distances covered, habitats impacted, and how this varies with pasture availability throughout the season (Chodkiewicz *et al.*, 2023). Collars can also be used to determine where and when cows are calving, and for monitoring animal health and behaviour. However, GPS collar costs are significant, and technical support is a very important element in their adoption (Fernández-Habas *et al.*, 2020). Trade-offs between data resolution and data quality exist due to limited battery lifespans and the difficulty of replacing batteries in grazing systems. Data quality and relevance can further be enhanced in combination with additional sensors. For example, the RumiWatch holsters (Itin+Hoch, Bennwil, Switzerland) can differentiate with high accuracy between prehension bites, rumination chews, and eating chews, by using a pressure-based sensor over the cow's nose and jaw (Li *et al.*, 2023). However, while these methods enable data acquisition, further research needs to prove relevance in different grazing systems.

While GPS collars only monitor grazing behaviour, virtual fencing (VF) is a novel technology that uses a combination of audio and electrical stimuli to contain grazing livestock within a movable GPS boundary. This ability to control animal movements means that VF can provide additional productivity and conservation benefits compared with GPS collars. However, costs are greater, and in some countries, there are animal welfare concerns to address, although Hamidi *et al.* (2022) found no evidence of negative impact on animal welfare of grazing heifers. VF can facilitate more flexible and tailored conservation management in high nature value grasslands. Hiron and Wahlund (2023) demonstrated that VF can be used to create areas of short, medium, and tall vegetation (creating habitat diversity), focusing grazing pressure where needed, and temporarily reducing grazing pressure for a short period (protecting wildlife). VF can also be combined with vegetation indices (such as RGB-VI; Red, Green, Blue) data from unmanned airborne vehicles (UAV) to better allocate pasture resources to grazing livestock over large areas (Hamidi *et al.*, 2023).

## Climate change

Assessing climate change interactions with grasslands can be done with different techniques. Integrating over both, spatial and temporal variabilities, is best done using eddy-covariance (EC) measurements of ecosystem greenhouse gas exchange, accounting for soil, vegetation and animal gas exchange with the atmosphere at one EC flux station (Eugster and Merbold, 2015). Here, micrometeorological and gas concentration measurements are combined, at 10–20 Hz (i.e., 10–20 times per second), to measure the net gas exchange of carbon dioxide (CO<sub>2</sub>), water vapour (H<sub>2</sub>O), methane (CH<sub>4</sub>) or nitrous oxide (N<sub>2</sub>O) over an entire grassland. All management practices can take place as normally, so EC measurements only interfere to a very limited extent with farming practices. However, grassland areas need to be at least 1.5–2 ha, following the main wind direction, so not all grasslands can be measured with this technique. Prices for such a set-up are high, as is the knowledge demand for running such a station and analysing the EC data. Therefore, this technique is limited to academic institutions, although national research institutions are also coming on board (e.g., in Ireland). Nevertheless, the insights based on such EC stations cannot be gained in any other way. Responses of entire grasslands to changes in climatic or management practices,

sensitivities to extreme weather events like heatwaves or droughts cannot be obtained differently on site, only estimated by modelling. Moreover, combining EC measurements of greenhouse gas fluxes with management information (i.e., carbon exports with harvests; carbon inputs with organic fertilization) allows to quantify soil carbon sequestration (Feigenwinter *et al.*, 2023a) but also to detect trade-offs, e.g. between maintaining carbon sequestration while reducing N<sub>2</sub>O losses (Feigenwinter *et al.*, 2023b; Fuchs *et al.*, 2018).

In agronomy, process-based biogeochemistry models (BGMs) are often used to simulate growth and productivity of grasslands, and in environmental sciences to quantify the surface exchange of greenhouse gases. These models calculate the dynamics of carbon, nutrients, and water along the soil-plant-atmosphere continuum, often on a daily or sub-daily time step, and can be helpful in predicting and understanding ecosystem functions and services, including testing the impacts of different management decisions. However, there is a great disparity among models in terms of their ability to simulate C sequestration and status of sources and sinks of greenhouse gases in different environments, which can mostly be attributed to ill-representation of effects of pedo-climatic conditions and management in the models (Brilli *et al.*, 2017). BGMs also usually lack detailed representation of dynamics related to plant biodiversity, reducing our capacity to utilize them effectively for decision making (Van-Oijen *et al.*, 2020). Efforts to incorporate satellite and UAV-acquired data, either directly as inputs or through data assimilation, to enhance the accuracy of BGMs are highly promising. The information infrastructures for connecting models with the growing number and variety of measurements are being developed, although the widespread adoption of accessible and scalable tools remains a significant challenge (Fer *et al.*, 2020). The improvement of comprehensive data infrastructures that integrate diverse ecological measurement data, including remote sensing, with BGMs, will greatly enhance the models' functionality and effectiveness within management decision-making processes.

## Biodiversity

Evaluating biodiversity and related ecosystem services requires precise and repeated assessment of species richness or functional group richness of the organism group of interest. With regards to acquiring georeferenced vegetation data at high temporal and spatial resolutions, a number of technologies have become available. For example, satellite data, extensively available and widely utilized in various forms of vegetation indices, are integral to numerous environmental and agricultural applications. However, many practical applications for driving management decisions in grassland farming still require laborious collection of ground truth data, and often temporal and spatial resolutions are not sufficient in satellite data. The collection of aerial spectral data by unmanned aerial vehicles (UAVs) equipped with visible light, multispectral, or hyperspectral cameras presents a viable solution for applications demanding high-resolution and timely data. An example of employing UAV-derived data for making informed management decisions, such as optimizing fertilization strategies, is the precise quantification of leguminous plants within grasslands, as demonstrated in the study by Li *et al.* (2020). This task, along with many image segmentation applications, greatly benefits from the recent rapid advancements in deep learning methods for processing UAV-acquired data (Osco *et al.*, 2021; Wang *et al.*, 2022). Models that utilize ultra-high resolution imagery from UAVs or time-lapse cameras for flower mapping have also been developed (Andreatta *et al.*, 2023; Gallmann *et al.*, 2022;). They contribute significantly to efforts in developing new remote sensing tools for biodiversity monitoring, in addition to spectral variation assessments which have been hard to generalize over different environments (Thornley *et al.*, 2023). More difficult remains the quantification at the vegetative stage, as required in more intensively used grasslands. Yet at close range, analyses of RGB-images have been able to reliably differentiate between red and white clover at the vegetative stage (Skovsen *et al.*, 2021), thus providing the basis for upscaling from high resolution UAV imagery. Biomass estimation using models that combine both structural (e.g., canopy height acquired using photogrammetric 3D-models) and spectral UAV-acquired data seems to provide highest accuracy

in most cases (Bazzo *et al.*, 2023). High-resolution hyperspectral data have been utilized in assessments of forage quality with success in limited environments (e.g., Oliveira *et al.*, 2024), but generalization across different plant species and environments is still a significant challenge. Drone-in-a-box solutions, wherein an UAV autonomously executes data collection tasks based on predefined instructions and subsequently returns to a docking station for charging and data transfer, present significant potentials, particularly for research applications. By automating the process, these systems enable more consistent and frequent data gathering, thereby yielding richer datasets that are crucial for time-sensitive research and analysis.

## Open science

Many of these new approaches and technologies to develop sustainable management options provide, but also require, large amounts of high-quality reliable data and metadata. Within the context of academic and research institutions, and dependent on the methods or techniques used, making data known and available to others is thus key to push sustainable management of grasslands. Open science strategies and FAIR principles (Findability, Accessibility, Interoperability, and Reuse of digital assets) much depend on the willingness of the researchers to provide data and metadata to others, and on users who cite DOIs (Digital Object Identifiers) of such datasets, acknowledging the work of those who collected the data. These data then can feed into models of many kinds, which can be the basis of apps or online decision-support tools to help farmers in their day-to-day life.

## Assessment of management options

Developing sustainable management options, based on scientific evidence in the form of data, is best done using a participatory approach, collecting data not only from experiments, but also from real-world farms, maybe even from farm networks (Klaus *et al.*, 2020). Within an EU project called SUPER-G (Developing Sustainable PERmanent Grassland systems and policies), we used a series of co-innovation workshops with over 20 farm networks, and more than 40 experiments on commercial and research farms across Europe to identify such promising management options (Rankin *et al.*, 2023). For permanent grasslands, options were assigned to four areas:

- new grassland and animal species, diverse species swards: introduction of new grassland species, overseeding with diverse species or mixtures, new livestock breeds;
- precision grassland management: use of plate meters or other yield estimation techniques, grazing and cutting management tools, rotational grazing, satellite and/or drone technologies for yield and quality estimation and to measure ecosystem services delivery, use of precision technologies for grazing (e.g., in field weighing, virtual fencing, GPS collars, apps);
- nutrient management: quantification of nutrient balances, precision nutrient management, variable rate fertiliser application, use of slow-release fertilisers, modelling soil carbon dynamics; and
- agri-environmental options: grazing, cutting and other management strategies for productivity, biodiversity and other public goods, increasing legume cover to reduce mineral N fertiliser and decrease N<sub>2</sub>O emissions, use of locally harvested seeds for re-vegetation.

This wide range of management options, based on a broad portfolio of different methods and techniques, was subsequently ranked for their effective potential to provide increased ecosystem services, to be adopted by grassland farmers, and thus to improve sustainability of permanent grassland farming. A set of seven criteria was used for this assessment: (1) improved provision of studied ecosystem service (yes, no), (2) potential for provisioning multiple ecosystem service (yes, no), (3) technology readiness levels (TRLs, from 7 to 9), (4) ease-of-use by farmers (low, medium, high), (5) useability for pastures and/or meadows (pasture, meadow, both types), (6) potential to improve profitability (low, medium, high), and (7) relevance and/or transfer beyond local context (yes, no).

All options were ranked as being relevant for transfer to other regions, and most of them were seen as applicable to both pastures and meadows. Most options had indeed been shown to increase the one ecosystem service under study, or even to increase multiple ecosystem services. However, some of them were not seen to provide further ecosystems services as an add-on. For example, using plate meters or other yield estimation techniques such as drones and satellites were seen as rather limited to yield estimates, but not to provide further advantages. Technology readiness levels for all options were typically ranked rather high (TRL 8, i.e., system complete and qualified; TRL 9, i.e., actual system proven in an operational environment), but techniques linked exclusively to drones and satellites or modelling were ranked considerably lower (TRL 7, i.e., system prototype demonstration in an operational environment). This was reflected in the assessment that most of the options seemed to be easy to use by farmers, except drone and satellite technologies or modelling. In addition, precision nutrient management, variable rate fertiliser application as well as precision grazing management were rated considerably lower, indicating a large need for knowledge transfer from science to practice. The potential to improve profitability was rated medium to high for about 70% of all options. About 40% of the options were considered highly profitable (including use of plate meters, grazing management tools, rotational grazing, quantification of nutrient balances, precision farming for nutrients and grazing, and the use of legumes), while three options (modelling, agroforestry, and use of locally collected seeds) were considered as showing low potentials to improve profitability. This clearly showed a gap between scientific evidence, anticipated ease-of-use and readiness levels of innovative management options for permanent grasslands to their expected adoption by farmers, which is driven by many factors, including profitability.

Political boundary conditions, socio-economic factors, as well as risk attitude of farmers can limit the adoption of scientifically proven, reliable management techniques (Hofmann *et al.*, 2022). When agri-environmental policies support certain management options, or when front-runners successfully implement new technologies and are successful, their adoption is likely to be higher. When risks for farmers increase, e.g., loss of yields due to extreme climate events, using more diverse swards suddenly becomes more attractive. Knowing barriers for adoption thus becomes similarly important to collecting and providing new scientific evidence for existing knowledge gaps.

## Conclusions

Thus, although gaps in knowledge exist, data are being collected using a wide array of methods, technologies and approaches. Many data are openly shared, although FAIR principles are not yet everywhere implemented. Management options are being developed and tested for permanent grasslands, which might also be applicable to temporary grasslands within a crop rotation. Increased uptake of data processing technologies and inferred management options will require proof of concept, clear cost-benefit analyses, and both technical and financial support to farmers through the implementation of national policies, e.g. via common agricultural policy (CAP) Strategic Plans (CSP), and the careful design of eco-schemes and agri-environmental-climate commitments.

## Acknowledgements

The authors acknowledge various funding sources, among others, from the EU projects SUPER-G (project number 774124-2) and ForageDrone (project number 145346) as well as the SNF projects GrassGas (200021-105949) and M4P (40FA40\_154245).

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# Which steps are needed to go from data collection to actual management decisions?

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## Abstract

European grasslands, in the broad sense, are at the crossroads between agricultural and environmental issues at the farm and territory scales. Thus, decisions about grassland management involve not only farmers and their advisers, but land-managing entities and public policy actors as well. The amount of data about grasslands is already considerable and expected to increase in the future. Such data encompasses public satellite images, agricultural statistics, research results, monitoring data from farm networks and information pertaining to commercial farms, sometimes included in large databases owned by private companies. The way such data is analysed and put into perspective in order to support grassland management and decision-making is discussed. Indeed, research has had a key role in this respect by developing methods and tools to evaluate grassland production and services, simulating the impact of management regimes and public policies. It has contributed to the design of monitoring systems and decision support tools. Considering the multi-scale and multi-dimensional issues at stake, a priority for the future could be to build multi-stakeholder networks in order to develop a shared systemic vision. The latter could favour the development of a socio-technical framework favourable to the conciliation of a variety of services.

**Keywords:** grassland, farm networks, observatories, remote sensing, DSS (decision support systems), expert knowledge

## Introduction

According to Peeters *et al.* (2014) grasslands are “land devoted to the production of forage for harvest by grazing/browsing, cutting, or both, or used for other agricultural purposes such as renewable energy production. The vegetation can include grasses, grass-like plants, legumes and other forbs. Woody species may also be present”. Based on this broad definition, grasslands in Europe encompass temporary grasslands containing one or more selected herbaceous species, permanent grasslands containing a variety of herbaceous species, and semi-natural grasslands possibly including woody plants (e.g. mountain pastures, Mediterranean rangelands, wetland pastures, shrubland and dehesas). Permanent grasslands are an administrative category defined by the European Union (EU) as “land used permanently (for several consecutive years, normally 5 years or more) to grow herbaceous fodder, forage or energy purpose crops, through cultivation (sown) or naturally (self-seeded), and which is not included in the crop rotation on the holding” (Eurostat, 2023). The total surface area covered by permanent grasslands in the EU-27 in 2022 was estimated at 50.7 million ha (Eurostat, 2022), which corresponds to 32% of the EU’s agricultural area. Based on the same source, temporary grasslands would account for 4.5% and other plants harvested green (maize, lucerne, mixtures, etc.) for 7.6%. Grasslands are thus a major component of EU agricultural landscapes. This is especially true in France, Ireland, Germany, Romania, Spain and Poland, where permanent and semi-natural grasslands account for over 35% of the utilised agricultural area.

Since the end of the 20<sup>th</sup> century, grasslands have been increasingly valued for the diversity of services provided to agriculture and, more generally, to society: forage production, protection against soil erosion, water quality, cultural value, etc. (Lidborg *et al.*, 2022). Semi-natural grasslands in particular have drawn much attention because their higher floristic diversity supports a variety of services. To date, most of such services do not imply a direct financial compensation. Nevertheless, they have been recognised by the last Common Agriculture Policy (CAP) programmes, which incentives are higher for farms that use large surface areas of semi-natural grasslands and/or that introduce legumes in their crop rotations (Heyl, 2020). In France, a variety of semi-natural grasslands have been eligible for CAP subsidies since 2015, which has contributed to the sustainability of extensive livestock farming systems. A number of recent large-scale studies such as MAES (2020) at EU level, have identified and valued the variety of services provided by agricultural ecosystems. A major issue today is to find ways to maintain or enhance such services through appropriate management decisions at the field, farm, landscape and region/country scales.

Despite the fact that the day-to-day management of grasslands is under the sole responsibility of the farmer, a number of strategic decisions may involve other farmers (e.g. in the case of crop×livestock integration at a territorial level or for collective summer pastures), agricultural advisers and even land managers (who decide on local regulations about the multi-use of grassland, especially in nature reserves and parks) and public policy officers (who deploy and implement the CAP). Thus, strategic decisions about grasslands and their management might be taken at a variety of levels (Europe, country, local territory, group of farms, individual farm, plot) and by a variety of stakeholders that may interact directly or not. Within this framework, collecting, analysing and sharing data about grasslands and their contribution to agricultural landscapes is of utmost importance in order to ensure the consistency and efficiency of grassland management decisions at the various levels.

This paper describes the diversity of data available about grasslands and how such data may be analysed and put into perspective in order to support grassland management decisions at farm and territory scales in EU countries. Specific attention is then drawn to the case of semi-natural areas that are more difficult to document but that are of high strategic importance. Based on the French example, the potential of combining different types of data and different tools is discussed, as well as the importance of multi-stakeholder networks.

## **Heterogeneous data about grasslands: an ever-growing resource**

Data collected about grasslands (i.e. both indicators of their state and the associated metadata) are heterogeneous in nature, and of variable availability in time and space. Such data may be quantitative or qualitative and describe the amount or quality of forage production during a specific period of time, the diversity of the local flora and fauna, or assess one or more services provided by these grasslands. Data can be collected by a variety of methods: field observations or measurements carried out by humans, sensor-based collection of local conditions, smartphone or drone imagery, agricultural or ecological surveys among local stakeholders, satellite imagery, etc. Depending on the method, data collection may involve one or more stakeholders (farmers, agricultural advisors, environmental or public policy officers, scientists, technical research staff, nature management associations, private companies), as well as more or less sophisticated tools, from the eye of the local expert to the high-tech sensor. The method of data collection, management and storage might impact the availability of the data, its open or private status and the options for analysis and operational use. It also determines the precision and reliability of the data and, therefore, its and the decisions that depend on its analysis. Figure 1 summarizes the data available from the four different sources analysed in the following paragraphs.



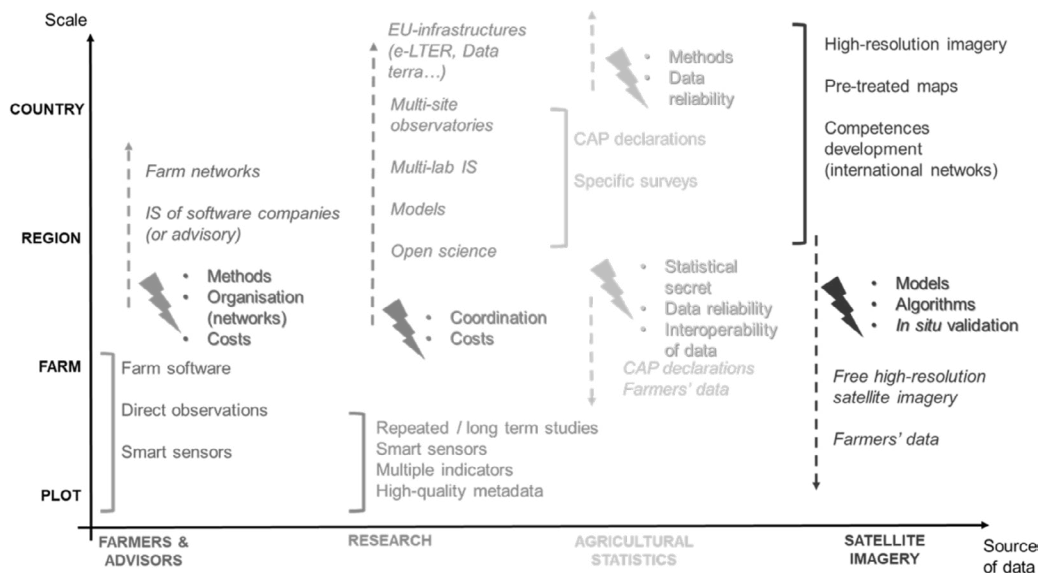


Figure 1. Data on grasslands: sources, levels of integration, characteristics and issues. The plain text refers to the characteristics of the data collected; the text in italics refers to the conditions of upscaling or downscaling the information collected, and the text in boldface near the bolts refers to the issues connected to the change of scale.

Data collected at farm scale by farmers and technical services is gathered in large amounts for the purpose of supporting technical decisions about grassland management. In this perspective, the most widespread indicator is probably grass height, generally measured using a sward stick or rising plate meter (Murphy *et al.*, 2021). An example of large-scale and long-term data collection system designed by research but implemented by farmers is PastureBase Ireland (<https://www.teagasc.ie/crops/grassland/pasturebase-ireland/>), in operation since 2013. PastureBase Ireland is a free internet-based grassland management programme associating a central national database of grassland data with an array of tools to support decisions about grazing rotations, feeding management, fertiliser/slurry application, etc. (Hanrahan *et al.*, 2017). In the last years, the massive adoption of the tool (over 6000 users) has enabled reliable grass growth forecasts to be issued and it has provided valuable data for grassland research (Ruelle *et al.*, 2021a,b). Until recently, farm data was only available to those who collected it, and was recorded on paper or sometimes in digital format. Thus, not only was the data invisible to other stakeholders, but it often lacked the necessary metadata required for an in-depth analysis. On the whole, data at the farm scale is limited by its availability, precision and interoperability (Bahlo *et al.*, 2019). The increasing availability of software able to record and display farm data, combined with the deployment of smart sensors, has paved the way to a better availability, reliability and utilisation of farm data. Data collected with commercial tools and managed by private companies is usually displayed, after analysis, on the user interface of commercial software. In a variety of EU countries, the public sector has started to design and maintain data platforms where farmers can store and access their data, and eventually share it with advisers or researchers. PastureBase Ireland is an example of such tools. Such initiatives are recent, and further work is needed in order to use more and better the large amounts of data collected.

In the last century, research produced huge amounts of data about grasslands, both in experimental situations and on commercial farms (Dumont *et al.*, 2019). Data collected within the framework of scientific programmes is site-specific when collected at the plot or farm scale in experimental stations, or refer to a network of sites and/or farms when collected in an observatory, usually in collaboration

with local stakeholders and/or technical services. In the first case, the upscaling and generalisation of the results might be difficult and may require mechanistic models; in the second case, the type and amount of data collected might vary among sites, thus limiting the extent and precision of the analysis. Another issue is that huge amounts of data are stored in non-digitalized archives. The effort required to make them widely available should be assessed against the interest of such data today, in a different context. The main advantage of scientific data is that the methods and conditions for data collection are described in detail. Another interesting point is that, for a given site and experiment, a great number of indicators are measured or estimated: forage quality and production, soil characteristics, botanical composition, GHG fluxes, biodiversity, etc. In the last decades, large efforts have been made to document the diversity of semi-natural grasslands and to acquire long-term data about grasslands and their management within the framework of observatories at regional to national scales (France: Carrère *et al.*, 2022; Michaud *et al.*, 2016; Germany: Morris *et al.*, 2014; Vogt *et al.*, 2019). The amount and availability of scientific data has benefited from the use of smart sensors (Wang *et al.*, 2022), the development of information systems shared among laboratories or institutions, such as the Sen2Grass cloud (Hardy *et al.*, 2021) and the general movement towards open science. Such evolutions are favourable to the development of references and simulation models for a variety of environments.

Agricultural statistics provide large-scale data about land use, farm types and structure, agricultural practices and the economic value of agricultural products. In the EU, the common CAP framework implies yearly declarations by farmers and, therefore, the collection of large amounts of data. However, CAP regulations and indicators may vary among member states, contributing to uncertainty in the analysis at the international level. Despite such limitations, agricultural statistics have proved useful in order to monitor major land use and management changes over time. At the European level, other approaches are possible: the Land Use/Cover Area frame Survey (LUCAS) is a harmonised *in situ* land cover and land use data collection exercise that extends over the EU's territory. The survey is conducted in two phases: (1) systematic sampling of approximately 1.1 million points spaced 2 km apart in the four cardinal directions, and photo-interpretation in order to assign them to a pre-defined land cover class; and (2) direct observation on a stratified sample of points by local field surveyors. Agricultural statistics are useful to monitor the distribution and long-term evolution of grassland types, mainly in terms of surface area of semi-natural grasslands and, for temporary grasslands, in terms of plant species sown. Thus, they are an interesting data source for measuring the impact of agricultural policies and shaping future national or international policies. Nevertheless, the precision and reliability of such data is difficult to estimate.

Satellite images contain an immense amount of information, collected *a priori* without consideration of its further analysis. The interest for grassland science and management lies in the analysis and interpretation of these data. Various steps are required to obtain indicators concerning the grassland state: (1) choose the images with the least amount of missing information (little or no clouds); (2) calculate indicators correlated with useful information about grasslands (typically, Normalized Difference Vegetation Index or infrared spectral bands); and (3) develop and apply statistical models to estimate sward characteristics, for example forage mass and quality (Bareth, 2021). In order to facilitate these steps, 'hybrid approaches' based on deep learning and data-driven learning (AI) have been developed to reduce dependence on field data (Fauvel, 2020) and to predict vegetation dynamics based on physical/environmental/agronomic constraints included in the algorithms. Another strategic utilisation of satellite images is to determine land use classes. The CORINE Land Cover database (<https://land.copernicus.eu/en/products/corine-land-cover>) was first created in 1990 and has since provided essential information on European land cover/land use. The most recent update was made in 2018, based on satellite images from Sentinel-2 and Landsat-8 for gap filling, broken down into 44 thematic classes with a minimum mapping unit of 25 ha. Although extremely valuable, the CORINE Land Cover database has a resolution grid that

is too wide to address issues at farm or landscape scales. Alternative classifications have been tried at the local and national levels in order to more precisely assess strategic land uses connected with CAP subsidies (Campos-Taberner *et al.*, 2023). The increasing availability of high-resolution free satellite data will certainly lead, in the near future, to the production of high-resolution land use maps that include information about agricultural and non-agricultural services. Two major issues will be (1) to determine and discriminate types of semi-natural grasslands that make sense in terms of management and services rendered; and (2) to validate such maps in order to ensure their reliability at local scales.

## **Tools to analyse grassland data and support management decisions at different levels**

Decision support tools may be very different in nature: references, charts, simulators, typologies, serious games (games where the players practice or acquire skills and knowledge), etc. They may come in different forms: software, spreadsheet or paper-based. Their common function is to use data in order to represent the state of a system and to put it into perspective, thus contributing to identifying solutions that can be implemented through management decisions. Recently, the H2020 SuperG project ([www.super-g.eu](http://www.super-g.eu)) published a review of existing tools used by farmers, advisers, policy-makers and other stakeholders to support permanent grassland management within the EU. Most of the 127 tools identified had not been published in the scientific literature. Fifty-nine percent of them were based on local data and thus they were specific to a particular country or region, and only a small proportion of the rest was available in more than one language. Most tools (56%) included at least one software element, and 'traditional' paper-based tools (usually available to download from the internet) were still common (44%). Software-based tools make it possible to save and store data and, consequently, to keep records as well. Such data might be pooled at a regional scale in order to feed simulation models and to add a prospective dimension. Almost all tools were intended for farmers and often their advisers as well; less than 15% included policy-makers in the target audience. Most tools (77%) were free or had a free version available, while much of the commercial farm management software involved paying a monthly or annual subscription.

Many tools supporting technical decisions about grassland management are intended to optimise the use of the forage resource at the farm level. Optimisation may rely on grass rotations, on best dates for grazing/cutting/fertilising a paddock, or on the monitoring of the available forage stock at the paddock and farm scales. Optimisation tools are usually based on data about forage biomass, extrapolated from measurements of grass height and sometimes combined with simulation models of grass growth that take meteorological conditions and farm production objectives into account. In Ireland, a variety of tools are available: on the one hand, the free national grassland database (PastureBase Ireland) associated with a web application and with a weekly report on television; on the other hand, commercial tools such as the 'Grasshopper<sup>TM</sup>' measurement system (a high precision plate meter) to be associated with the GrasslandTools (<https://www.grasslandtools.ie>) software at the farm scale, which provides an overview of the grassland state and management and of herd condition if the system is connected to an automated weighing device. Similarly to PastureBase Ireland, the 'Pâtur-Plan' French tool (Delaby and Bignon, 2015) collects grassland data at paddock and farm scale and provides the farmer with simulation results for possible management strategies. This may trigger discussions between farmer and adviser, and encourage management changes.

Another important function of grassland decision support tools is to document the variety of services rendered by grasslands, at the farm and territorial levels. This second objective might interest a variety of stakeholders and is of higher relevance in semi-natural grasslands, where the provision of forage is not the only service expected. Thus, the optimisation logic gives way to a sub-optimal agricultural utilisation of grasslands, which improves adaptation to hazards and reconciles different services. A number of tools have been developed in order to assess and discuss the impact of management decisions on the provision

of services by grasslands and grassland-based farming systems. In the early 2000s, simulation models at the farm scale enabled research to explore possible compromises between agricultural production and biodiversity conservation, carbon stocks, nutrient losses, etc. In the meantime, grassland typologies including information about non-agricultural services were published in a variety of countries including Italy (Cavallero *et al.*, 2007) and France (Galliot *et al.*, 2020). Since the 2010s, serious games at farm and landscape scales have been designed for the purpose of educating a variety of stakeholders and/or providing information to stimulate discussions and support decisions in multi-stakeholder groups. Examples of such games are AEOLE in France (Carrère *et al.*, 2022) or Vivagrass (<https://vivagrass.eu/integrated-planning-tool/>) for various regions within the EU. For the moment, a limited number of decision support tools take both the agricultural and non-agricultural services of grasslands into account. The vast majority of the decision support tools for grasslands studied in the SuperG project considered the agricultural services (provision of forage and other products); 15% or more documented non-agricultural services such as biodiversity and pollination, landscape and recreation, or water quality, and less than 8% considered flood and erosion control or carbon storage and GHG. Assessing multiple services implies considering different scale levels, and requires the development of metrics suited to be scaled up from plot to landscape (Stockes *et al.*, 2023). At the plant community level, the priority is to produce integrated information from data that is easy to acquire or access. Taugoudeau *et al.* (2024), based on academic knowledge and expertise, identified and aggregated several vegetation criteria to construct indicators of the services provided by grasslands. Such indicators were calculated based on over 2000 botanical surveys distributed over a wide biogeographical gradient. Their results show that an aggregation method based on a large dataset of botanical surveys could be appropriate for studying the temporal dynamics of services, and useful for managers to evaluate the impact of their practices.

To date, only part of the data collected about grasslands is analysed, included in decision support tools and effectively used for decision-making. The proportion of data used should increase with the development and intensification of farm networks and large-scale observatories, which make it possible to collect with similar methods, gather, normalise and analyse data at various spatiotemporal resolutions. Such networks and observatories also have the advantage of triggering collaborations between various stakeholders, with a shared tool and complementary objectives. Their major drawback remains their funding: who should pay for their development and how to secure long-term financial resources for their maintenance. Another issue will be to cross-analyse data from various sources, at various spatial resolutions. Presently, the upscaling of results obtained at the plot or farm scale might be difficult to implement due to the high impact of data collection methods and geographical location. Conversely, large-scale data are rarely applicable at local or farm scales due to the large impact of local conditions on the actual state of grasslands and the provision of agricultural and non-agricultural services. In order to secure the consistency of grassland management decisions at farm to country scales, tools able to provide indicators at multiple spatiotemporal resolutions would be most useful. In order to achieve this, they should be able to cross-analyse satellite imagery, scientific data and farm technical data. To be useful and used, decision support tools considering a variety of grassland services should combine indicators from academic research for credibility and precision and empirical results to allow the assessment of management impact; Stockes *et al.* (2023) also point to the necessity of including both ecological and socio-economic indicators and the importance of co-designing indicators with local stakeholders in order to increase their operability. Besides, there is a need for tools able to bring together public and private data for decision support (Bahlo *et al.*, 2019). The effective adoption of decision support tools by end-users is rarely documented. Tracking the number of users is possible for tools that require opening an account; even then, it can be difficult to tell if all accounts are equally active unless a special tracking system is implemented. Decision support tools are most efficiently disseminated and used when a large network of farms is involved (O'Brien *et al.*, 2015). Commercial tools that do not count enough users will probably disappear soon enough. Non-commercial tools should be managed in close collaboration

with a community of users in order to improve the existing and, if needed, propose complementary or alternative solutions.

### **Specific considerations regarding heterogeneous semi-natural grasslands**

Semi-natural grasslands are common in areas that have not undergone agricultural intensification due to unfavourable pedo-climatic conditions. They include wet areas, mountain and sub-alpine meadows and pastures, grazed steppes and dry pasture, grazed wooded areas and grazed/browsed shrubby zones (Peeters *et al.*, 2014). They are characterised by high plant diversity unevenly distributed over space, including edible and non-edible plants and plant parts. Due to the harsh conditions, annual and non-grass species are abundant and imply strong seasonal variations in the amount and quality of forage; these dynamics are enhanced by the extensive management practices applied. The consequences for data collection in view of decision-making are: (1) the sward cannot be represented by a single indicator; (2) the question of spatial resolution/spatial unit becomes crucial; and (3) metadata about the ecological, technical and seasonal context is absolutely necessary. Decision-making about semi-natural grasslands aims at two types of compromise: between a variety of services and between short-term production and long-term conservation of the vegetation.

The data about semi-natural grassland collected by farmers and advisers consists mainly of: (1) GPS tracks from animal-borne collars, which provide information about the spatial behaviour of the herd, usually displayed on a mobile application; (2) records of grassland conservation state and utilisation, collected in the framework of agri-environmental measures. Such data is often imprecise and rarely available for further analysis. In the field of research, temporary or permanent grasslands have been investigated by agricultural science, while semi-natural grasslands have been mainly studied by plant ecologists. Fortunately, research about plant functional traits has established bridges between these apparently contrasted approaches. Studying semi-natural grasslands from an agricultural perspective still raises methodological issues. Developing experimental designs is difficult, given the spatial diversity of the swards and the number of interacting factors; thus, most research has either focussed on the long-term evolution of plant biodiversity or on the direct observation of animal behaviour over seasons and years. A key issue in order to better document semi-natural grasslands is to organise plant diversity into categories which make sense for the services of interest. For forage services, such categories need to take animal behaviour into account (Mikicik *et al.*, 2023). At the landscape and country levels, the acquisition and interpretation of data about semi-natural grasslands is also difficult. In agricultural statistics, different types of semi-natural grasslands may or not be included in the CAP declarations, depending on the country, on the declaration strategy of the farmer and on the presence or absence of a contract to access the land. Satellite data is a promising source of information, although a number of methodological complications associated with the presence of rocky outcrops and woody plants still need to be solved.

Each source of data about semi-natural grasslands provides incomplete information: farmers and technical advisers collect site-specific data with little associated metadata, which is difficult to upscale and mainly serves the development of their own expert knowledge. Science documents multiple factors, but in a small range of conditions that are far from the variety of situations encountered in the field. Public data such as agricultural statistics or satellite imagery provide incomplete information due to the limitations of the underlying data acquisition methods. Nevertheless, a number of useful and powerful decision support tools are available for semi-natural grasslands. Such tools have the combination of technical data, scientific data and expert knowledge in common, as well as the support of a multi-stakeholder network. The 'Pâtur'Ajuste' network ([www.paturajuste.fr](http://www.paturajuste.fr)) includes farmers, technical advisers, environment officers, teachers and a few researchers. It was created in the 2010s to trigger discussions and innovations about the technical aspects of pasture management, within the framework of the agro-ecological transition. The network's activities include the organisation of technical exchange days and short training

courses, the creation of technical sheets combining scientific and expert knowledge, and participation in applied research programmes. The results of such activities are available on a dedicated website. Ten years after its creation, the network is well-known all over France and counts 220 members distributed in 55 'departments'. The main reason for this success is probably the fact that it has provided a bottom-up national alternative to the traditional top-down regional advisory services. 'Pastothèque' (Dodier *et al.*, 2023) is a typology of mountain and Mediterranean grasslands that summarizes the current knowledge about their diversity, their vegetation dynamics and the variety of services they render. The main difference with other typologies is that seasonal and long-term dynamics in response to management and climatic hazards are included, and the evaluation of services is related to grassland management, especially in terms of animal species, season of utilisation and grazing intensity. 'Pastothèque' is the result of years of collaboration between technical and environmental services, with the contribution of research. Although the tool would benefit from a simplified digital version, it provides a valuable and functional classification of semi-natural grasslands, which is a necessary pre-requisite for the acquisition and interpretation of data. P@stor-all (Kalenga *et al.*, 2022) is an information system devoted to the management of semi-natural grasslands. Co-designed with farmers, it gathers heterogeneous data: satellite images, GPS tracking of herds, links to technical sheets, popularised scientific results and, last but not least, feedback from farmers pertaining to their own experiences on the subject. The information system is designed mainly for farmers; advisers and education institutes interacting with them may also contribute. Farmers have access to: (1) a private area where they can upload GPS tracks and monitor various indicators of herd spatial behaviour; and (2) a public area where they can find expert knowledge, technical and scientific information about various subjects, and a forum. The information system is free and is based (similarly to PastureBase Ireland) on the exchange of services between stakeholders: farmers' data gives researchers insight into the diversity of situations, while scientists provide farmers with free analysis of farm data and access to a large pool of knowledge. The tool will be disseminated in France in 2024. Its success will very much depend on the participation of a sufficient number of farmers and the interest of other stakeholders who may use it to more efficiently interact with the farmers, or to complement training courses.

## Conclusions and perspectives

Data on grasslands is already diverse and abundant, and should further increase in the future. Rather than collecting more data, the issue is now to organise it and make it available and interoperable, with observatories and information systems that gather long-term information about grasslands. Three critical characteristics of grassland data are: (1) availability in digital format, (2) the methods of collection, and (3) the associated metadata. Research and public institutions have a key role to play in order to propose and disseminate reliable data collection methods and tools which may be used by a variety of stakeholders, but also to manage databases designed to gather large amounts of data and support a mutual benefit relation between research (who will cross-analyse data to increase grassland knowledge) and farmers (who will use the associated tools and indicators for decision support). The addition of local knowledge to the pool of data would be an asset, for example to document services for which no other data acquisition method is currently available. Research should also progress in the analysis of satellite imagery in order to extract spatial information about grassland types and production, which may help to extrapolate on-farm data collected at a local scale, especially for semi-natural grasslands which are more diverse and more difficult to characterize with simple methods.

Decision support tools already exist in most European countries. Such tools are often adapted to the regional/national agricultural context, and especially the dominant type of grassland and the main objective of grassland management. In this respect, there are two strategies that require different data and different types of decision support tools: (1) optimisation of grass utilisation, which can be based on a single indicator of grass height or biomass; (2) sub-optimal utilisation, which requires understanding and comparing different services provided by grasslands. Whatever the strategy, decision support tools



may help the farmer to develop a local knowledge about his grasslands and, if associated with simulation models, to compare the results of different management strategies. The main difference is that sub-optimal utilisation may involve other stakeholders, and thus require tools and networks shared between farmers, naturalist technicians, land managers, etc. For all types of decision support tools, the inclusion of uncertainty and hazards in the analysis and predictions is of utmost importance in the present context of climate change. Another critical aspect is to improve the methods for cross-analysis of grassland data in order to represent the multiple characteristics and services of grassland in the best possible way, at various levels of scale. This is especially true for semi-natural grasslands, which are at the crossroads of multiple agricultural and non-agricultural objectives.

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# Why and when to give concentrate to dairy cows in a grass-based system?

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## Abstract

In grass-based dairy systems, concentrate (Cc) may be allocated at various times of the lactation to support milk production and reproductive performance. To assess the benefits of Cc supplementation, an experiment combining 3 dairy breeds (Holstein, Normande and Jersey) and 4 Cc strategies was carried out at the INRAE Le Pin experimental farm. In comparison with a zero Cc group (C0), the same daily quantity of concentrate (3 kg for Je and 4 kg for Ho and No) was distributed during 100 days, at three periods of the lactation (early-C1, middle-C2 and late-C3). Each year, the 144 to 168 cows are managed under a 3-month compact spring-calving system, and grazed in a single herd with a simplified rotational grazing system (180 to 220 days). On average, the milk yield (MY) and composition response and the body condition score (BCS) show changes differ according to the breed and period of Cc allocation. The MY response of the Je cows ( $0.70 \text{ kg (kg Cc)}^{-1}$ ) is lower than the Ho and No cows ( $0.85 \text{ kg (kg Cc)}^{-1}$ ), but higher if the MY is expressed taking account the fat and protein content. Early in lactation, the Cc has no favourable effect on the BCS change, but Cc improves the BCS at the end of the 100 days, in the middle and at the end of lactation. The period of Cc allocation determines the partitioning of nutrients between MY and BCS change.

**Keywords:** dairy cow, grazing, concentrate supplementation, allocation timing

## Introduction

In well-managed grassland systems, the nutritive value of grazed grass is comparable to a 'natural' total mixed ration (Delaby *et al.*, 2022) and grass dry matter intake is the limiting factor in expressing milk yield potential (Bargo *et al.*, 2002). It may be worth allocating some concentrate (Cc) during lactation to support dairy cow performance. To maintain a low-input system and limit external dependency, the aim is to limit the amount of concentrate used. This raises the question of the best timing during lactation to allocate concentrate. This ideal moment can depend of the dairy cow breed characteristics and the farmer's objective: increase milk yield, improve milk quality (fat and protein), limit body reserve mobilization and/or improve the fertility. To assess the benefits of Cc supplementation, an experiment combining three dairy breeds (Holstein (Ho), Normande (No) and Jersey (Je)) and 4 Cc strategies was carried out for 3 years (2020–2022) at the INRAE le Pin experimental farm, Gouffern en Auge, Normandy, France.

## Materials and methods

Every year, the dairy cows are managed in one herd (between 144 to 168 cows) in a grass-based system with a 3-month compact calving period (February to April) to maximize the grazing season in lactation without forage supplementation (180 to 220 days on the 260 days outdoor). There is a global dedicated area of 88 ha of grassland with grasses, white clover and multi-species swards ( $1.85 \text{ cow ha}^{-1}$ ). Half of this area is grazed in spring; the other half (and 25% a 2<sup>nd</sup> time in summer) is harvested for pit and bale silage used in winter (nearly 105 days). Within each breed, in comparison with a no Cc group (C0), three 100-day periods of lactation with Cc are compared: early (C1), mid (C2) or late (C3) lactation. Due to the difference in BW and maintenance needs, the Je cows received 3 kg daily, whereas the Ho and No cows received 4 kg of concentrate.

Individual milk yield (MY) is measured daily during the two milkings, with automatic milk flow meters. The milk fat and protein content are evaluated by NIRS, morning and evening during two days (Mo and We). The bodyweight (BW) is measured once per week and body condition score (BCS) once per month by two well-trained technicians. The effect of concentrate timing allocation is evaluated during the 100 days of supplementation (Table 1) and also over the entire lactation performance. For all the results detailed, the statistical model applied is based on a generalized linear mixed model developed in SAS (2023) with the equation:  $X = \text{Year} + \text{Parity} + \text{Breed} + \text{Cc} + \text{Parity} \times \text{Breed} + \text{Parity} \times \text{Cc} + \text{Breed} \times \text{Cc} + \text{IdY}$ , with IdY as covariate with the genomic index for the milk performance and pre-calving BW or BCS, centered within breed and parity.

## Results and discussion

The total number of lactations included in the 3-years results is 383 (189 first lactation); respectively 125 (67) Ho, 156 (70) No and 102 (52) Je. As expected, the 44-week lactation performance of the three dairy breeds are significantly different with 5689, 4450 and 3520 kg of MY and 386, 332 and 321 kg of MS (milk solids; fat+protein) for the Ho, No and Je cows. The fat and protein content are ranked in reverse order of MY, with 38.3, 41.8 and 55.6 g kg<sup>-1</sup> of fat and 30.4, 33.3 and 36.5 g kg<sup>-1</sup> of protein, respectively, for the Ho, No and Je cows. The Je BW (376 kg) is significantly lower than the Ho and No BW (571 and 567 kg).

On average over the three 100-day periods, daily Cc consumption was 3.30, 3.30 and 2.50 kg DM, respectively, for the Ho, No and Je cows, with few difference between periods of allocation. In each lactation period and for each breed Cc significantly increases MY. In comparison to the C0 group performance, the Cc efficiency is similar in early and mid-lactation (+0.84 kg MY (kg Cc)<sup>-1</sup>, expressed in dry matter intake) and a little bit higher than observed later in lactation (+0.69). Concentrate feed reduces milk fat content significantly in mid (-0.48 (kg Cc)<sup>-1</sup>) and late (-0.81 (kg Cc)<sup>-1</sup>) lactation and increases milk protein content significantly both in early (+ 0.33 (kg Cc)<sup>-1</sup>) and mid (+0.31 (kg Cc)<sup>-1</sup>) lactation. If, for MY, the response of the Je cows seems weaker than for the No and Ho cows, this difference in response disappears when expressed on corrected MY or MS. Concentrate feed had a significant positive effect on BW at the beginning and end of lactation, but not in mid-lactation. In early lactation and for all three breeds, the allocation of Cc had no effect on the evolution of BCS and did not limit the mobilization of body reserves. In mid and late lactation, Cc improved BCS gain.

Reproductive performance, and in particular the percentage of cows in calf, varies so much from year to year and from treatment to treatment that it is too early to propose consistent and definitive results. The percentage of cows inseminated with AI after 21 and 42 days of breeding is 75 and 92%, respectively, and was unaffected by treatments. The interval between calving and 1<sup>st</sup> AI or conception is 75 and 90 days, respectively, with no significant differences between Cc allocation groups. On average, after a 90-day breeding period with 6 weeks of sexed semen use, the in-calf cows percentage is close to 70% for each breed, and slightly lower for C2 and C3 and higher for C0 and C1. The re-calving ratio is similar between breeds with 67, 69 and 68% for the Ho, No and Je cows, respectively.

## Conclusion

After 3 years of experimentation, the MY response to Cc at the different moments of lactation were slightly less than those described over the entire lactation by Delaby and Peyraud (2003).

The next 3 years of the experiment will help to consolidate these results, confirm the absence of interactions between breed and Cc allocation time responses and propose some rules of concentrate allocation in grass-based systems according to the farmers' objectives.

Table 1. Performance of the dairy cows during the three 100-day periods of concentrate allocation, in comparison with the no concentrate group.

Breed	Holstein		Normande		Jersey		RMSE	Breed	Cc
	No Cc	Cc	No Cc	Cc	No Cc	Cc			
Early lactation (Cc, kg)		3.15		3.30		2.40			
Milk yield (kg)	24.3	27.2	18.5	21.3	15.9	17.3	2.92	0.0001	0.0001
Fat (g kg <sup>-1</sup> )	37.5	36.9	40.3	38.8	50.9	52.1	2.91	0.0001	NS
Protein (g kg <sup>-1</sup> )	28.6	29.5	31.4	32.3	33.0	34.2	1.46	0.0001	0.0001
Corrected MY (kg)	23.4	25.4	18.6	21.6	18.3	20.5	2.94	0.0001	0.0001
Average BW (kg)	554	572	554	568	367	373	31.0	0.0001	0.0008
BCS change (pts)	-0.75	-0.70	-0.55	-0.45	-0.60	-0.65	0.42	0.0028	NS
Mid lactation (Cc, kg)		3.40		3.30		2.60			
Milk yield (kg)	16.3	19.9	12.9	15.6	10.4	12.3	2.11	0.0001	0.0001
Fat (g kg <sup>-1</sup> )	36.7	35.0	41.0	39.9	55.4	53.7	2.68	0.0001	0.0005
Protein (g kg <sup>-1</sup> )	29.1	30.2	32.0	33.1	36.1	36.8	1.54	0.0001	0.0001
Corrected MY (kg)	15.6	18.2	13.0	15.8	14.9	12.7	1.97	0.0001	0.0001
Average BW (kg)	557	557	556	573	370	371	29.7	0.0001	NS
BCS change (pts)	+0.05	+0.15	-0.05	+0.25	+0.05	+0.20	0.25	0.0725	0.0001
End lactation (Cc, kg)		3.30		3.40		2.50			
Milk yield (kg)	14.3	16.8	10.1	12.0	8.1	10.1	2.23	0.0001	0.0001
Fat (g kg <sup>-1</sup> )	42.3	42.1	47.7	45.8	68.8	63.3	4.37	0.0001	0.0022
Protein (g kg <sup>-1</sup> )	33.4	34.4	37.5	38.7	43.8	42.7	2.77	0.0001	NS
Corrected MY (kg)	15.1	17.7	11.4	13.7	11.7	13.8	2.30	0.0001	0.0001
Average BW (kg)	576	598	589	598	393	398	34.8	0.0001	0.0650
BCS change (pts)	+0.15	+0.25	+0.30	+0.50	+0.30	+0.25	0.33	0.0025	0.0002

Corrected MY for fat and protein content is calculated according to the INRA (2018) equation:

$cMY = MY \times (0.42 + 0.0053 \times (Fat - 40) + 0.0032 \times (Protein - 31)) / 0.42$ . Cc gross composition (%): wheat 21, maize 22, barley 20, beet pulp 20%, rapeseed meal 14, molasses 2 and salt 1.

## Acknowledgement

The authors would like to thank the INRAE Le Pin experimental dairy farm staff for managing the TripI'XL experiment and the measurement quality in this long-term experiment.

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# The influence of defoliation method on perennial ryegrass variety evaluation

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## Abstract

Perennial ryegrass variety evaluations are conducted using mechanical defoliation (cutting) which differs from how grasslands are primarily utilised on farm in Ireland (grazing). The objective of this study was to compare the herbage yield and nutritive quality of perennial ryegrass varieties assessed under both mechanical harvesting and animal grazing protocols. Fifteen perennial ryegrass varieties were sown in two studies and evaluated over 3 years. Low correlations were found between defoliation methods for herbage production traits such as herbage yield ( $r=0.29$ ), while high correlations ( $r>0.90$ ) were recorded for quality traits.

**Keywords:** perennial ryegrass, variety, animal grazed, mechanically harvested, herbage yield

## Introduction

Variety Value for Cultivation and Use (VCU) evaluation protocols should reflect the predominant farming practices of that area (Gilliland *et al.*, 2020). Perennial ryegrass VCU trials are typically conducted using mechanical harvesting machinery that rotationally defoliate plot trials. In Ireland, this is no different where herbage yield is measured from mechanical cuts used to mimic herbage offtakes from livestock in a rotational grazing system. A recent development in Ireland has been the introduction of animal grazing assessments into their VCU system (Tubritt *et al.*, 2021). Herbage yield assessments are conducted as part of these grazing evaluations but the accuracy of such evaluations is to be questioned as variety herbage yield ranking under grazing differs from their published VCU performance results. Literature is also conflicted with Creighton *et al.* (2012) finding mechanical harvesting to be reflective of grazed herbage yield while studies by Binnie and Chestnutt (1991) failed to find a relationship between both methods. The objective of this study was to unravel nuances in herbage yield and quality of perennial ryegrass varieties in grazed and mown plots.

## Materials and methods

This study was conducted at Teagasc, Animal and Grassland Research and Innovation centre, Moorepark, Fermoy, Co. Cork, Ireland (lat. 50°07' N, long. 08°16' W; 40 m a.s.l.). Two PRG plot studies containing 15 varieties each were sown in June 2020 in a complete randomised block design. Two differing defoliation methods were employed in each study, animal grazing (AG) or mechanical harvest (MH). Plot size in the AG study measured 8×4.5 m (36 m<sup>2</sup>) while plot size in the MH study measured 5×1.5 m (7.5 m<sup>2</sup>). The experiment took place over three grazing seasons, 2021 to 2023, with 27 (10+8+9) grazing events taking place. The plots AG and MH were harvested the same day, when the average pre-grazing herbage yield of the AG plots was estimated (visually) to be 1400 kg DM ha<sup>-1</sup> (above a cutting height of 3.5 cm), as is common grazing practice in Ireland. At each harvest event the following measurements were recorded in both studies: pre-grazing height was measured using a Jenquip rising plate meter. A subsection of each plot (1.2×5 m in MH and 1.2×7 m in AG), was harvested using an Etesia motor harvester to determine pre-grazing herbage yield. Harvested herbage was weighed and a 0.1 kg sample was dried at 90°C for 16 h to determine DM content. Herbage density (HD) was calculated as pre-grazing yield divided by pre-height minus post-cutting height. A second herbage sub-sample was obtained, freeze-dried at -50°C for 72 h and scanned under near infrared spectrometry to determine nutritive quality.

After these measurements were taken, a herd of dairy cows then grazed the AG plots. Post-grazing height was recorded from the grazed proportion of each AG plot. In the AG study, differing post-grazing heights were recorded from plots and therefore to avoid herbage accumulation being accounted in two rotations, the un-grazed DM above 3.5 cm (cutting height) within each plot from grazing event 'n' was subtracted from herbage yield of the same plot in grazing event 'n+1', as measured by the Etesia cut (Delaby *et al.*, 1998). Statistical analysis was conducted using the SAS statistical package (SAS Institute, Cary, NC, USA). Differences between varieties and ploidy were analysed using the PROC MIXED procedure for both studies. Pre-grazing herbage yield, pre-grazing height, post-grazing height, herbage density, Organic Matter Digestibility (OMD), Crude protein (CP) and Neutral Detergent Fibre (NDF) were the dependent variables analysed. PROC CORR was used to quantify the correlation between the AG and MH evaluations. Spearman's rank correlations were derived to compare whether variety's were ranked similarly between evaluations.

## Results and discussion

Herbage yield differed significantly between evaluations ( $P < 0.001$ ) as the average variety yield from the MH study ( $12\,713\text{ kg DM ha}^{-1}$ ) yielded  $1465\text{ kg DM ha}^{-1}$  higher than the AG (Figure 1 and 2). This is surprising as additional organic nutrients would have been supplied from the grazing animals in the AG study, but a similar result was reported by Binnie and Chestnutt (1991) who cited treading damage from the grazing animals as a causal factor. Additionally the influence of differing post-grazing height in AG study where some varieties were grazed below that of the cutting horizon (i.e. overgrazed) negatively affected regrowth rates and biases DM yield determination in AG plots (Tubritt *et al.*, 2021). Herbage density differed significantly between evaluations ( $P < 0.001$ ) as although the MH study had greater pre-grazing yields, pre-height was lower than the AG study (9.1 vs. 9.8 cm), resulting in higher HD ( $264$  and  $208\text{ kg DM cm}^{-1}$ , respectively). The adaption of perennial ryegrass to external factor such as defoliation is widely recognised (Lee *et al.*, 2008), but such HD differences question how reflective of farm practice VCU evaluations are.

Organic matter digestibility was significantly higher in the MH study than in the AG ( $P < 0.001$ ) while the opposite relationship was found for NDF, as higher levels were recorded in the AG study ( $331\text{ g (kg DM)}^{-1}$ ) compared to the MH study ( $316\text{ g (kg DM)}^{-1}$ ;  $P < 0.001$ ). This was likely due to differing and higher post-heights in the AG study which would increase stem and dead matter. Weak Spearman's rank correlations were found between the AG and MH studies for herbage yield and herbage density with correlations coefficients of 0.29 and 0.44, respectively. Strong correlations were found for nutritive quality traits with values of 0.96, 0.82 and 0.92 for OMD, CP and NDF. Such results suggest that defoliation

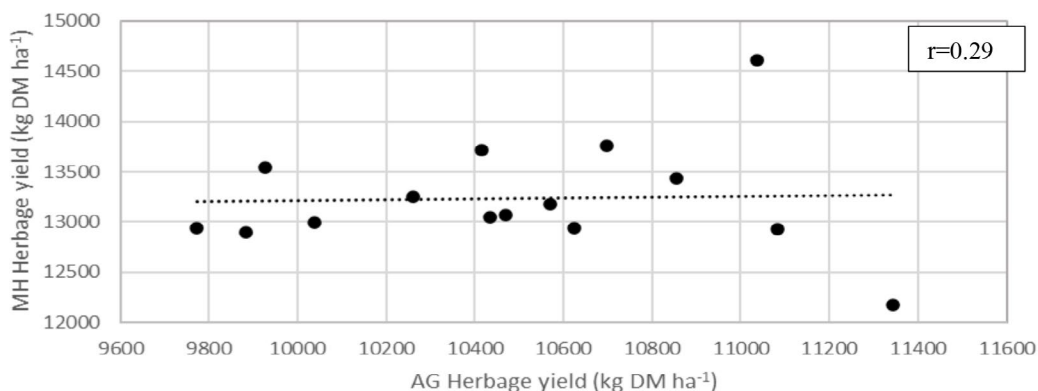


Figure 1. Relationship between Animal Grazed (AG) and Mechanically Harvested (MH) plots for herbage yield.

method influences the expression of variety herbage yield potential to a greater degree than nutritive quality traits. Cashman *et al.* (2016) reported similar correlations for nutritive quality traits between animal grazed and mechanically harvested plots but higher correlations were observed for herbage yield particularly in the 1<sup>st</sup> and 2<sup>nd</sup> evaluation years.

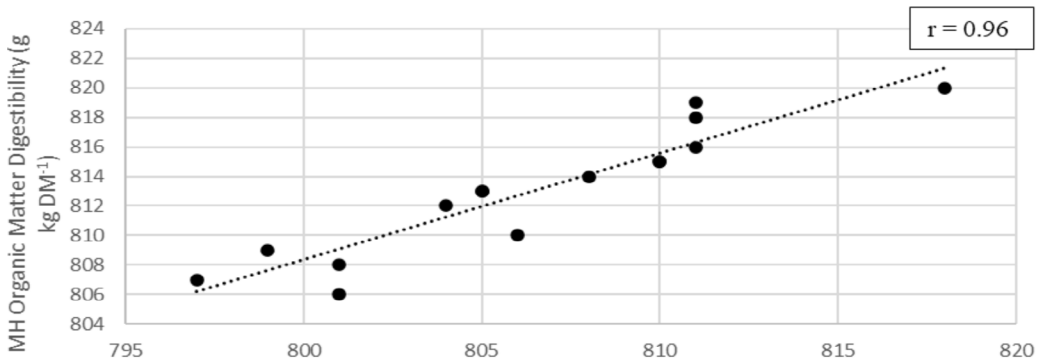


Figure 2. Relationship between Animal Grazed (AG) and Mechanically Harvested (MH) plots for digestibility traits.

## Conclusion

Large reranking of varieties was observed when assessed for herbage yield under mechanical cutting or animal grazed defoliation. This was likely due to the differing post-grazing heights recorded between varieties after animal grazing. One method of overcoming this would be to mechanically cut these plots after grazing to a common height although such practice would influence grazing efficiency measurements. Where varietal data is sought for herbage yield and grazing efficiency traits, separate trials would be required to accurately measure both.

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# Finding a needle in a haystack: case-control studies can identify measures to prevent weeds in grassland

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## Abstract

Prevention is essential to effectively manage weeds in grasslands. Yet, the identification of preventive measures can be tedious because numerous interacting management and environmental factors can influence grassland infestation by a weed. Case-control studies carried out on-farm allow a risk value to be ascribed to potential factors. We conducted a paired case-control study to assess the risk of the occurrence of *Rumex obtusifolius* in intensively managed permanent grasslands. Following a common protocol, in Switzerland, Slovenia and United Kingdom, parcels of land with high density of *R. obtusifolius* were compared with nearby parcels that had very few or no *R. obtusifolius* plants. Measured parameters included data about management practices, vegetation, and soil nutrients and texture. Analysed with multiple logistic regression, we showed that increased vegetation cover reduced the relative risk of *R. obtusifolius* occurrence while increased soil phosphorus and high soil bulk density raised the risk. Each of these effects was of comparable size across countries, as no interactions between country and any of the factors were observed. We conclude that case-control studies are a suitable tool to identify factors driving the infestation risk of grasslands regarding weeds such as *R. obtusifolius*. Results were achieved under the conditions of applied management, sometimes lasting for more than 10–20 years and allowed for direct recommendations to integrated weed management.

**Keywords:** integrated weed management, vegetation cover, soil P content, soil K content, soil bulk density, indicator species

## Introduction

Weeds are a major problem for forage production and quality in intensively managed, temperate grasslands. The management and the environment can influence weed infestation of grasslands; however, given the wide variety of farm practices, identifying preventive measures through manipulative experiments can be very laborious. On-farm case-control studies are an effective tool for identifying risk factors associated with management practices that favour a particular weed, by comparing parcels with high weed density (cases) with nearby parcels with very low weed density (controls). This type of study was used in medicine as early as the 1950s to investigate diseases (Doll and Hill, 1950) and has later been adopted to grassland systems to study weeds (Suter *et al.*, 2007; Suter and Lüscher, 2008). In the current study, a case-control design was chosen to assess the risk factors that make productive grasslands prone to infestation with broad-leaved dock (*Rumex obtusifolius* L.). Known as one of the most problematic weeds in European temperate grasslands, *R. obtusifolius* is able to tolerate frequent defoliation once it is fully established (Niggli *et al.*, 1993). *R. obtusifolius* produces large amounts of long-lived seeds that can contribute to a seed bank in the topsoil (Suter *et al.*, 2023) from which the species can potentially recruit for many years. Based on previous studies, we identified factors that could potentially influence the occurrence of *R. obtusifolius* and defined a set of variables to be measured. The objective was to identify management practices and environmental factors that affect the risk of *R. obtusifolius* occurrence with the aim of improving strategies for the integrated weed management of the species.

## Materials and methods

Following a common protocol, a case-control study was conducted in Switzerland (CH), Slovenia (SI) and the United Kingdom (UK) during the 2019-2020 growing seasons. The study was carried out on-farm by comparing parcels of land with high density of *R. obtusifolius* (case) with nearby parcels free of or with very few *R. obtusifolius* plants (control). Forty (CH), 20 (SI), and 18 (UK) pairs of parcels were sampled per country. Parameters measured included data about the environment, management practices, soil nutrients and texture, and vegetation. The influence of the recorded variables on the occurrence of *R. obtusifolius* was analysed using multiple logistic regression and forward selection, the response variable being the presence or absence of *R. obtusifolius* in high density, equivalent to case-control parcels (Agresti, 2002). Further details on design and analysis can be found in Klötzli *et al.* (2023).

## Results and discussion

The risk of the occurrence of *R. obtusifolius* in high densities was explained by three factors: percentage vegetation cover (measured by the line-point intercept method), soil P content ( $P_{\text{Olsen}}$ ), and soil bulk density (Table 1; factors were not correlated to each other). Increase in vegetation cover by 10%, for example from 80 to 90%, reduced the relative risk of *R. obtusifolius* occurrence to about half (Table 1). The two other variables raised the relative risk: an increase in  $P_{\text{Olsen}}$  of  $13 \text{ mg kg}^{-1}$  (the mean difference between case and control parcels) resulted in a relative risk of 1.24, and an increase in soil bulk density of  $0.1 \text{ g cm}^{-3}$  in a relative risk of 1.32 (Table 1). Despite differing soil conditions and a gradient of climate from Atlantic to continental, these effects were consistent across countries, indicated by non-significant interaction terms ( $P > 0.2$  each) between country and the three selected variables. Based on the selection procedure, no other recorded variable, such as soil pH, land-use intensity, or soil clay content, explained the occurrence of *R. obtusifolius*.

In this on-farm study, the factors influencing *R. obtusifolius* (vegetation cover, soil phosphorus content and soil bulk density) were all related to non-adapted management (soil bulk density most likely so), either directly or indirectly, such as unbalanced fertilisation or increased soil compaction. Importantly, these factors can be seen as the result of medium- and long-term processes of more than 10–20 years, acting much longer than a typical grassland experiment of less than five years.

Table 1. Variables with significant effects on the relative risk of the occurrence of *R. obtusifolius* in high density in grasslands of Switzerland, Slovenia, and United Kingdom.

Variable	df	$\chi^2$	P value	Relative risk
Country <sup>a</sup>	2	2.02	0.364	–
Vegetation cover	1	9.07	0.003	0.56 <sup>b</sup>
$P_{\text{Olsen}}$	1	7.63	0.006	1.24 <sup>c</sup>
Soil bulk density	1	4.20	0.040	1.32 <sup>d</sup>

Variables were tested with forward selected using a generalised linear model with a logit link function. Terms added sequentially (first to last) given country; only variables that lowered the AICc (Akaike Information Criterion) upon inclusion in the model are given.

<sup>a</sup>Inference of 'Country' based on single term deletion from the final model.

<sup>b</sup>Relative risk for an increase of vegetation cover by 10%.

<sup>c</sup>Relative risk for an increase of soil  $P_{\text{Olsen}}$  by  $13 \text{ mg kg}^{-1}$  (mean difference between case and control parcels).

<sup>d</sup>Relative risk for an increase of soil bulk density by  $0.1 \text{ g cm}^{-3}$  (mean difference between case and control parcels)



The case-control approach proved very effective in identifying risk factors among a multitude of management and environmental factors likely to influence the infestation of intensively managed, permanent grasslands by *R. obtusifolius* (under conditions practiced over several years). Moreover, this type of design has the advantage of allowing a combination of management and environmental conditions to be tested that would be difficult to establish in a manipulative experiment. However, given the potential correlations between explanatory variables under practical conditions, causal interpretations in case-control studies must be made with caution.

## Conclusion

For successful control of *R. obtusifolius* in intensively managed grasslands, preventive measures combined with direct control measures are decisive (Schaffner *et al.*, 2022). The case-control study provided evidence for the role of medium- to long-term factors driving the infestation of grasslands by *R. obtusifolius*, and all factors were indicative of poor management practices. Preventive strategies can be implemented through appropriate management, for example by adapting fertilisation to the needs of the forage plants, minimising soil compaction, and promoting dense and competitive swards.

## Acknowledgements

We thank Ellen Fletcher, Roxane Muller, Aleš Plut, and Anže Rovanešek for their support with field work and sample processing. We are grateful to Diane Bürge and her team for the analysis of soil nutrients and texture. We acknowledge financial support by the IWM PRAISE project from the European Union's Horizon 2020 Programme (grant agreement no. 727321).

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# Organic manure fertilization effect on phosphorus availability in permanent meadows

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## Abstract

Permanent meadows play a multifaceted role, not only increasing soil organic matter and reducing carbon dioxide emissions, but also mitigating nutrient percolation, particularly that of phosphorus (P). P holds essential significance for all living organisms, and understanding its availability to plants and microorganisms is pivotal for understanding the relationship between management and enhanced productivity. Our study, conducted in a mountain environment in South Tyrol (NE Italy), investigated organic fertilization effects on moderately species-poor (C1) and moderately species-rich (C2) permanent meadows at the end of the growing season. Our results show that, at this time, the application of farmyard manure (alone or combined with manure effluent) led to an increase in P availability in the soil as the P input increased, suggesting that higher organic matter content in the manures is associated with greater soil available P. We also observed a more pronounced increase in P availability in C2 compared to C1 as the P input increased. To gain a comprehensive understanding, further investigation is needed to determine if the enhanced P availability in C2 meadow class is due to a legacy effect from pre-trial management or the result of lower cut frequency associated with this treatment.

**Keywords:** permanent meadows, slurry, farmyard manure, manure effluent, soil P water extract

## Introduction

Since the global reserves and resources of phosphorus (P) are finite, organic manure fertilization helps in mitigating the depletion of P reserves in the world, as well as recirculating the nutrients that the plants need for growth (Schipanski and Bennett, 2021). Meadows serve as repositories for organic matter (Hoogerkamp, 1973), thereby reducing carbon dioxide emissions (Oyesiku-Blakemore and Dondini, 2022) and, when managed under non-intensive practices, they contribute to the mitigation of nutrient loss risk (Apostolakis *et al.*, 2022). Our study investigated the impact of organic P input from various types of manures, applied over five growing seasons, on the P availability in the soil towards the end of the growing season.

## Materials and methods

The experiment was laid out in 2017 as a split-plot design at three study sites in South Tyrol (NE Italy), at altitudes ranging between 1112 and 1714 m a.s.l.. The meadow class (MC) (C1: moderately species-poor, and C2: moderately species-rich and corresponding to a Natura 2000-status according to Tomasi *et al.* (2016) at trial start) was the main plot. The meadow class in 2022 was unchanged with respect to the initial one for 98% of the experimental plots. The combinations of manure type (MT) (S: slurry; F: farmyard manure; L: combination of farmyard manure and manure effluent with the latter providing 30% of the total nitrogen input) and total nitrogen (N) input (0, 55.5 and 111 kg ha<sup>-1</sup> year<sup>-1</sup>) were randomized within the main plots. The proportion of indicator species for intensive management was computed at the time of the first cut, based on the species cover over all vegetation layers and the indicators list of Tomasi *et al.* (2016). P input was computed based on the P content of the respective manure batch and varied depending on the respective ratio between N and P content. Four soil samples were collected in each plot in late summer 2022 just before the cut of the last growth cycle at each experimental site. Soil solution P (P availability) was extracted in sodium bicarbonate and determined by using the ammonium

molybdate tetrahydrate-malachite green reaction (Olsen *et al.*, 1954) as modified by Ohno and Zibilske (1991). Forage samples of each growth cycle were collected within four 0.25 m<sup>2</sup>-sampling areas per plot at a stubble height of 5 cm to compute a cumulative annual dry matter yield. Linear mixed models (type III sum of squares) were built stepwise backward to explain the effect of the designed factors on P availability and yield, starting from a model including the fixed terms of study site, initial meadow class, manure type, P input and all interactions of the last three factors, and including the random terms MC×study site to account for the split-plot design and the plot to account for the correlation between the soil samples taken in the same plot. This last term was omitted for the analysis of dry matter yield, as the forage samples were pooled at plot level. P input was treated as a covariate. The Akaike Information Criterion (AIC) and the *P*-value of the terms (0.1 threshold) were used to select terms to be dropped. Data were transformed (natural logarithm) to fulfil the prerequisites of the analysis. Back-transformed values are reported. A *P*-value <0.05 was regarded as significant. All statistical analyses were performed by R (RStudio 2022.07.2+576) using the *lme4* package (R Core Team, 2023).

## Results and discussion

P availability at the time of the last cut was found to be affected by MT (*P*=0.008), P input (*P*<0.001) and by the two interactions MC×P input (*P*=0.033) and MT×P input (*P*=0.007). F and L increased P availability in the soil with increasing P input, whilst P availability was not affected by P input if P was provided by slurry (Figure 1). We tentatively suggest that this may be related to the higher organic matter content of F and L as shown by their higher C input in kg ha<sup>-1</sup> (2320.5±940.9 and 1851.3±718.4 on average of all years, respectively) in comparison to S (1510.5±718.3). The quick uptake of readily available P from S lead to a low, constant P availability at the end of the growing season, whilst higher rates of organic forms of P seem to be progressively mobilised by enzymatic activity depending on P demand by plants.

The less steep relationship between P input and P availability in C1 than in C2 (Fig. 2) may be due to the higher proportion of indicator species of intensive management (55.6±13.3 vs. 17.0±11.2 on average for C1 and C2, respectively) being more competitive and consuming more rapidly available P in the soil or by the greater cut frequency applied to C1 in comparison to C2 (3 vs. 2 cuts year<sup>-1</sup>, respectively). This hypothesis is corroborated by the fact that the forage yield was found to be affected by the meadow class (*P*<0.001), with the forage yield of C1 (5.5 Mg ha<sup>-1</sup>, 95% confidence interval 5.1–5.9) being higher than that of C2 (4.0 Mg ha<sup>-1</sup>, 95% confidence interval 3.7–4.3).

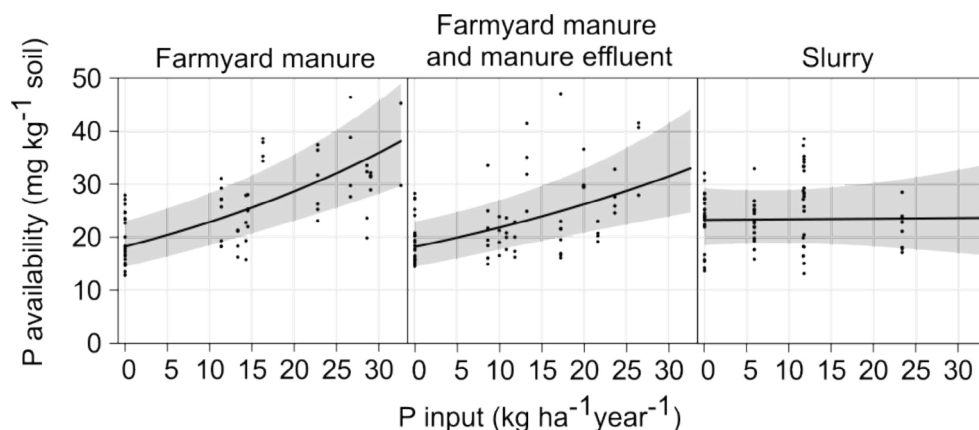


Figure 1. Effect of P input on P availability at the end of the last growth cycle depending on the manure type. Predicted back-transformed values of P availability and 95% confidence intervals are shown against partial residuals.

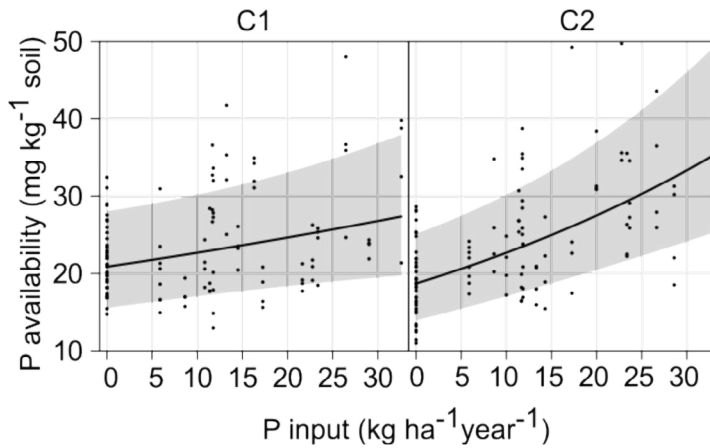


Figure 2. Effect of P input on P availability at the end of the last growth cycle depending on the meadow class at trial start. Predicted back-transformed values of P availability and 95% confidence intervals are shown against partial residuals.

## Conclusion

The manure type affects P availability towards the end of the growing season, suggesting that an increasing amount of organic matter content in the manures results in higher soil available P by a comparable P input above about 10 kg P ha<sup>-1</sup> year<sup>-1</sup>. Our study does not allow ascertaining whether the higher P availability in meadows initially moderately rich in species is a legacy effect of the management prior to trial start, or a consequence of the lower cutting frequency applied to these meadows. This requires further investigation to be clarified.

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# Drying autumn grass to improve protein quality

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## Abstract

Autumn grass generally contains high crude protein (CP), low sugar and low dry matter (DM). This often results in low protein quality silages, even with an additive. Artificially drying the grass is known to preserve protein quality, but is expensive. Alternatives for fossil energy (e.g. solar panels, residual heat) might lower costs, but can result in variable drying speed. Another option could be ensiling after artificial pre-drying. We compared intestinally digestible protein (DVE) and degradable protein balance (OEB) of fresh grass (16% DM with 160 g CP (kg DM)<sup>-1</sup>), field-wilted grass silage (27% DM), grass silage after artificial pre-drying (65°C) to 56% DM, and grass hay, oven-dried at 35, 45, 55 and 65°C (>90% DM). Based on protein solubility and rumen degradability, we estimated 81 vs. 49 g DVE and 22 vs. 32 g OEB (kg DM)<sup>-1</sup> for fresh grass vs. field wilted grass silage. Compared to fresh grass, oven-drying at 35, 45, 55 and 65°C slightly increased DVE (83, 88, 82 and 83 g/kg DM) and decreased OEB (15, 12, 6 and 16 g (kg DM)<sup>-1</sup>). The DVE and OEB (73 and 27 g (kg DM)<sup>-1</sup>) of pre-dried grass silage was in between that of fresh grass and field wilted grass silage.

**Keywords:** autumn grass; artificial drying; ensiling; protein quality

## Introduction

Autumn grass silage is generally of lower quality than spring grass silage due to its lower sugar content, lower DM content and higher amounts of rumen degradable protein, even with silage additives (Van den Bossche *et al.*, 2024). Artificial drying of grass to produce grass hay is known to preserve the nutritive value and protein quality (Edmunds *et al.*, 2014; Sefeedpari *et al.*, 2021), but is expensive and questionable when using fossil fuels (Sefeedpari *et al.*, 2021). Alternative energy sources might offer a solution, but can result in variable drying temperature and speed (Sefeedpari *et al.*, 2021). Another option could be ensiling after artificial pre-drying to an optimal DM content of 40–50%. In this study, the effect of artificial pre-drying of autumn grass at different temperatures on the grass (silage) quality was investigated.

## Materials and methods

The grass used in this experiment originated from one temporary grassland plot, sown with ryegrass (*Lolium perenne*) at ILVO (Flanders Research Institute for Agriculture, Fisheries and Food), Melle, Belgium. The grass was mown as a fourth cut on October 24<sup>th</sup> 2022 at 10:00 am (Haldrup plot harvester). Fresh grass (FG) was sampled for analysis before it was divided into the different treatments. The treatments were control silage (CS), i.e. pre-wilting in the field for 2 days before ensiling; artificial drying (AD) to 50% DM in a forced-air oven at respectively 25, 35, 45, 55 and 65°C before ensiling; and artificial drying to grass hay (GH) (90% DM) in a forced-air oven at respectively 25, 35, 45, 55 and 65°C. Drying time was dependent on temperature. Ensiling was done in PVC tubes (height 35 cm, diameter 10 cm, volume 2.75 l) closed with a cover equipped with a CO<sub>2</sub>-valve. Five of these micro-silos were filled per treatment at a silo density of about 215 kg grass DM m<sup>-3</sup>. Micro-silos were stored for 90 days at ambient temperature in a barn and, subsequently, the content was pooled per treatment. The silage fermentation characteristics (pH, ammonia, lactic acid, volatile fatty acids and alcohols) and chemical analyses of DM, crude ash, CP, NDF and sugars were done as described in Van den Bossche *et al.* (2024). The net energy content for dairy cattle (VEM) was estimated with a regression equation

(De Boever, 1999) based on the cellulase digestibility of the organic matter (OM) (De Boever *et al.*, 1986) and chemical parameters. The protein value of feeds for ruminants is described by the intestinally digestible protein (DVE) and by the degradable protein balance (OEB) (Tammenga *et al.*, 1994). The DVE value consists of rumen bypass feed protein (DVBE) and microbial protein synthesized in the rumen (DVME). The fraction of rumen bypass protein (%BCP) was calculated based on the protein solubility in water (%SCP), the CP degradation after 24 h rumen incubation (adapted from CVB, 2004) and on the undegradable protein fraction, which was estimated from OM digestibility with an own-developed regression (unpublished data). The synthesis of microbial protein depends mainly on the amount of rumen fermentable OM (FOM), which is positively related to OM digestibility and sugar content. OEB is the difference between the microbial protein synthesis based on degradable protein in the rumen and energy in the rumen (FOM).

## Results and discussion

In Table 1 the chemical composition and nutritional value is presented for FG, CS, AD at 65°C (AD65) and GH at 35, 45, 55 and 65°C (GH35, GH45, GH55 and GH65, respectively). Except for a higher DM content, artificially drying to GH did not change the chemical composition of the grass, but did preserve the sugars compared to CS. Sugars are an important energy source for the growth of rumen micro-organisms and the synthesis of microbial protein. Ensiling at a higher DM content, after artificial drying of the fresh grass, can preserve the sugars as well. Besides preservation of the sugars, heat treatment of FG slightly increases %SCP. The degradation rate of protein in FG, however, is faster ( $12\% \text{ h}^{-1}$ ) than that of GH (on average  $9\% \text{ h}^{-1}$ ), resulting in a slightly higher amount of DVBE for GH. Ensiling (CS) increases %SCP greatly, and thus it lowers %BCP as well as DVBE. Artificial pre-drying before ensiling (AD65) gives intermediate results for %SCP and %BCP and DVBE. Hartinger *et al.* (2019) underlines the importance of high intensity wilting to limit protein degradation in lucerne silage, and Edmunds *et al.* (2014) found a higher DVE value at higher DM levels. The lower %SCP and higher %BCP after artificial pre-drying also results in lower OEB-values. The energy value (VEM) is not affected by the artificial drying and/or ensiling; only CS has a lower VEM, which is due to the higher ash content ( $228 \text{ g (kg DM)}^{-1}$ ) compared to the other treatments (on average  $129 \text{ g (kg DM)}^{-1}$ ). This higher ash content could be ascribed to contamination with soil during wilting on the field.

Table 1. Chemical composition and nutritional value of the different treatments

Treatment	DM (g kg <sup>-1</sup> )	CP (g (kg DM) <sup>-1</sup> )	SUG (g (kg DM) <sup>-1</sup> )	SCP (%)	BCP (%)	DVE (g (kg DM) <sup>-1</sup> )	OEB (g (kg DM) <sup>-1</sup> )	VEM (g (kg DM) <sup>-1</sup> )
FG	159	160	135	22	28	81	22	930
GH35	920	159	133	32	33	83	15	910
GH45	929	158	143	21	33	88	12	946
GH55	948	146	155	22	32	82	6	921
GH65	945	157	151	26	29	83	16	946
AD65	534	159	108	51	26	73	27	921
CS	274	140	8	63	22	49	32	871

DM, dry matter; CP, crude protein; SUG, sugars; FOM, rumen fermentable organic matter; SCP, solubility of protein; BCP, bypass crude protein; DVE, intestinally digestible protein; OEB, rumen degradable protein balance; VEM, energy value; FG, fresh grass; GH, grass hay artificially dried at 35°C, 45°C, 55°C and 65°C; AD65, artificial drying to 50% DM at 65°C; CS, control silage pre-wilted in the field.

## Conclusion

Our results confirm that artificially drying of autumn grass is a valuable strategy to preserve the protein quality of the fresh grass, compared to ensiling after wilting on the field. The better feed value of grass hay makes it an interesting product for youngstock, dry cows and even lactating dairy cows. Nevertheless, integration in the diet of high producing dairy cows might be more challenging because of the potentially lower dry matter intake. Alternatively, grass hay can be ground (and pelleted) and valued as a concentrate feed rather than as a forage feed. All the above treatments require additional energy, which can be questionable. Alternatives to fossil fuels can lower the (environmental) costs. Drying to 50% DM followed by ensiling offers an intermediate way of preserving the protein quality in autumn grass. Although it is more difficult to reach the 50% DM and asks for more process control than does the drying to 90% DM. When the DM-content exceeds 50% it will become very difficult to ensile to reach a quality feed.

## Acknowledgement

This research was funded by the government agency Flanders Innovation and Entrepreneurship (VLAIO, Belgium; EKOPTI - HBC.2018.2222)

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# From the Surface to Space: Combining Multiscale Observations of Semi-Natural Grasslands in Ireland

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## Abstract

Field data collection and sampling are key to investigating relationships between soil carbon variability, species richness and functional diversity in Irish semi-natural grasslands. This work is exploring how multi-spectral Uncrewed Aerial Vehicle (UAV) surveys will support these field data in being scaled up to the site level, and satellite imagery in scaling up to a national level. A comparison between species richness data collected during a survey at 12 sites between 2007 and 2012, and satellite derived Normalised Difference Vegetation Index (NDVI), yielded a highly significant negative correlation ( $r=-0.45, p<0.01$ ), but with a strong seasonal trend. UAV based NDVI data collected in conjunction with species richness at 6 of the 12 sites in 2023 also produced significant negative correlations ( $r=-0.50, p<0.05$ ). The results highlight the potential of UAV data to act as a bridge between point-based ground surveys of biodiversity and decameter satellite imagery, and for these satellite data to offer valuable national scale information.

**Keywords:** semi-natural grasslands, satellite remote sensing, biodiversity, UAV, species richness

## Introduction

Natural and semi-natural grasslands provide many vital ecosystem services, from erosion control to pollination (Bengtsson *et al.*, 2019; Peciña *et al.*, 2019). In many regions, grasslands also contribute significantly to livestock farming through grazing and harvesting to produce fodder (Erb *et al.*, 2016). Natural and semi-natural grasslands are also significant sources of, and contributors to, biodiversity, as they are often characterised by high community complexity (Wilson *et al.*, 2012). Surveys of biodiversity are, however, labour intensive and normally require the extrapolation of point data to cover entire sites (Stroh *et al.*, 2020), introducing significant uncertainties. As part of the StableGrass project, we aim to scale up surveys of semi-natural grassland biodiversity in Ireland, from point-based ground data to site wide UAV and national satellite-based surveys. We present preliminary results from satellite analysis of the 2007-2012 Irish Semi-natural Grasslands Survey (ISGS) (O'Neill *et al.*, 2013), and ground-based biodiversity, UAV and satellite surveys conducted during summer 2023.

## Materials and methods

Twelve sites were chosen from the ISGS for fieldwork during 2023, four from each of the GS1 (Dry calcareous and neutral), GS3 (Dry-humid acid) and GS4 (Wet) Fossitt grassland categories. Plant species number and abundance were measured on relevés (quadrat surveys where plant species and their abundance are recorded) in all 12 sites and UAV surveys, using a DJI Mavic 3 Multispectral (<https://ag.dji.com/mavic-3-m>), were conducted on six sites (two from each grassland category) due to limited suitable weather conditions. This UAV sensor records data in the blue, green, red, red edge and near infrared wavelengths. Each site was fully surveyed from a height of between 90 and 120 m, resulting in orthomosaics with a spatial resolution of between 34 and 60 mm. Individual relevés, where the ground based surveys were performed, were surveyed at an elevation of between 5 and 15 m with the resulting orthomosaics having spatial resolutions of between 4 and 6 mm. Level 2 (L2) Landsat 5 and 7 surface reflectance data was acquired for the 12 sites on the closest available cloud-free dates to the original ISGS



from 2007 to 2012. L2A Sentinel 2 surface reflectance data were acquired for cloud-free dates between June and September 2023, to coincide with field surveys. NDVI values were generated from each satellite and UAV data set, to indicate a measure of the vegetation greenness, and compared to the species richness from the ISGS and the field survey data from 2023.

## Results and discussion

Species richness data from relevés at the 6 sites surveyed manually and by UAV in 2023 were broadly in line with the 2007-2012 ISGS, averaging 24 in both time periods. Each of the Fossitt sub-classification groups showed no significant changes between the ISGS and 2023 (Figure 1).

A highly significant correlation was found between the NDVI values from the L2 Landsat 5 and 7 surface reflectance data and species richness from the 12 sites chosen from the 2007-2012 ISGS ( $r=-0.45$ ,  $p<0.01$ ). This indicates that higher species richness occurs in areas with lower NDVI (or greenness) values, and vice versa. There was also a seasonal component (Table 1), with June and July recording highly significant negative correlations, and no significant correlations in May or August. This is likely related to the timing of flowering plants which cause the surface to appear less green when viewed from above, altering the NDVI values.

From the 2023 surveys, a similar significant negative correlation was found between the NDVI values from the UAV and the species richness (Figure 2) across relevés at the six sites ( $r=-0.50$ ,  $p<0.05$ ). However, this relationship is complicated by the fact that the UAV surveys mostly occurred towards the end of July and early August, when the correlations, according to Table 1, may be rapidly weakening. Notably, a highly significant correlation was also found between the UAV and Sentinel 2 NDVI values ( $r=0.87$ ,  $p<0.01$ ), demonstrating the close correspondence between the two datasets, and the potential for local UAV measurements to interoperate with satellite-based observations.

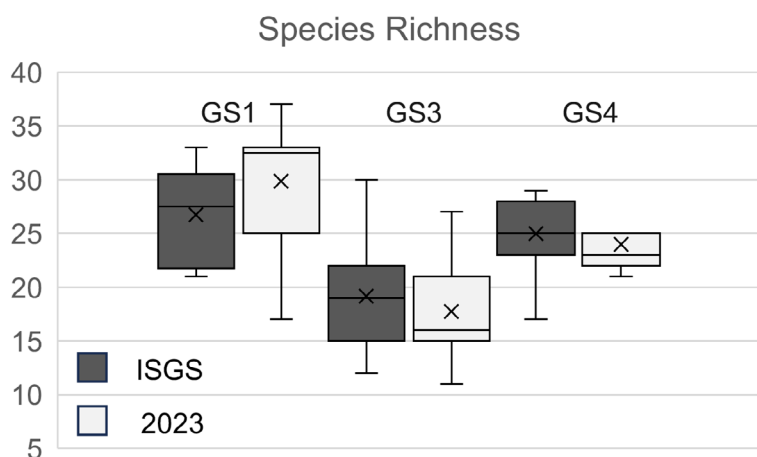


Figure 1. Species richness data across the 3 Fossitt classes from the 2007–2012 ISGS and 2023.

Table 1. Pearson correlation coefficients between species richness and NDVI

Month	May	June	July	August
Correlation	-0.11	-0.50*	-0.55*	+0.09

\* Significant at  $p<0.01$ .

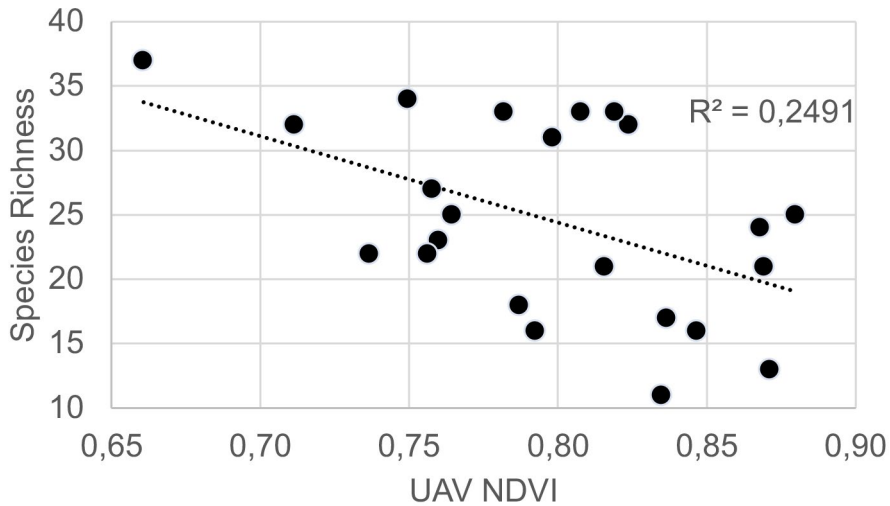


Figure 2. The relationship between species richness and 2023 UAV derived NDVI values from 23 relevés in 6 sites.

## Conclusion

A consistent negative relationship exists between summer NDVI and species richness in semi-natural grasslands in Ireland. This research shows that UAVs can produce data consistent with satellite platforms, allowing them to act as a bridge between point-based ground surveys and decameter satellite imagery. Further work on the spectral variation hypothesis and machine learning may improve the ability to map species richness at very high resolutions with UAVs.

## Acknowledgements

This research was funded by the Irish Department of Agriculture, Food and the Marine's Competitive Research Funding Programme (Project Reference 2021R529).

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# Methods and tools to routinely obtain comprehensive insight into the soil health of grassland

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## Abstract

Monitoring and steering soil health of grasslands enables dairy farmers to increase farm performance and to contribute actively to the UN Sustainable Development Goals, the European Green Deal and the EU Directive on Soil Monitoring and Resilience. Soil health encompasses physical, chemical, and biological soil characteristics. Commonly, many different tests are needed for a comprehensive soil health assessment, which is laborious, expensive, and many tests have a negative environmental impact. In many countries, a soil test report therefore includes only few soil characteristics. Broad-spectrum soil tests offer the potential to assess many soil characteristics quickly, but often face challenges with calibration and validation. Here, we describe the results of a 20-year research programme aimed at overcoming the aforementioned challenges. Two broad-spectrum techniques were selected, i.e., Near Infrared Spectroscopy (NIRS) and 0.01 M CaCl<sub>2</sub> extractions and combined with advanced analytical techniques for assessing plant-available nutrients and physical, chemical, and biological soil characteristics. The results indicate that the accuracy of NIRS determinations is high compared to the wet chemistry reference method for most soil characteristics including biological characteristics ( $R^2 \geq 0.90$ ). The CaCl<sub>2</sub> extraction is used for fifteen essential and beneficial nutrients and for nine (heavy) metals. The broad-spectrum soil test results are incorporated in several tools (Soil Carbon Check, Soil Life Monitor, and Soil Health Indicator) to provide guidance to farmers to help them attain healthier soils and productive grasslands.

**Keywords:** soil nutrients, soil health, soil test, soil carbon, soil monitoring

## Introduction

In 2015, 193 governments accepted seventeen Sustainable Development Goals (SDGs), with the European Union (EU) endorsing them through initiatives like the Green Deal in 2019 and a Proposal for a Directive on Soil Monitoring and Resilience in 2023. Soil health is integral to achieving many SDGs, including zero hunger (SDG2), good health and wellbeing (SDG3), clean water and sanitation (SDG6), sustainable consumption and production (SDG12), climate action (SDG13) and life on land (SDG15) (e.g. Bouma *et al.*, 2019). Soil health encompasses various physical, chemical, and biological soil characteristics. Usually, many different tests are needed for a full soil health assessment, which is laborious, expensive, and many tests have a negative environmental impact (e.g., aqua regia, HNO<sub>3</sub> for heavy metals). Broad-spectrum soil tests offer the potential to assess many soil characteristics quickly, but often face challenges with calibration and validation. Here, we describe the results of a 20-year research program aimed at overcoming the aforementioned challenges. Two broad-spectrum techniques were selected, i.e., Near Infrared Spectroscopy (NIRS) and 0.01 M CaCl<sub>2</sub> extraction and combined with advanced analytical techniques. Here, we summarize the analytical performance of these techniques.

## Materials and methods

Multi-nutrient 0.01 M CaCl<sub>2</sub> extraction for soil health testing was already proposed by the European Union in the 1990s as part of the research project Copernicus. Extraction with 0.01 M CaCl<sub>2</sub> (1:10

soil:solution ratio; dried soil) followed by discrete analysis and ICP-MS analyses of the extractants (Houba *et al.*, 1990) yields information about a wide range of essential nutrient elements. The extraction has a ionic strength comparable to soil solutions of most soils. Thus, the measured nutrients in the extract reflect the availability of the nutrients at the current pH and ionic strength of the soil solution (soil intensity). Plant-available macro, micro, and beneficial nutrients, as well as bio-available (heavy) metals can be measured simultaneously in a single extract, which allows the relationships between these elements to be considered.

Near Infrared Spectroscopy (NIRS) allows the quantification of key soil physical, chemical (soil quantity), and biological characteristics. NIRS was developed in the 1960s and has been used for assessing feed quality of grass and maize silage on a routine basis since the 1980s. NIRS allows for fast, quantitative, non-destructive, and cost-effective estimation of multiple physical, chemical, and biological soil characteristics. However, it requires large databases of reference analytical data for accurate calibration and validation. Between 10,000 and 100,000 samples were used, depending on the year of introduction and the type of indicator. Both the analytical calibration and the validation data sets contained samples from different soil types, widely ranging in soil texture, pH, organic carbon and CaCO<sub>3</sub> contents. Eurofins Agro started with NIRS for soil health assessments in 2003 (Reijneveld *et al.*, 2022).

## Results and discussion

All essential macro- and micro-nutrients for plants (N, S, P, K, Mg, Na, Si, Mn, Fe, Cu, Zn, Ni, B, Mo) were assessed through multi-nutrient extractions (apart from Ca, Cl). In addition, two elements that are essential for animals and humans were included (Se, Co), and pH and silicon (Si) were analysed as well. Bio-available (heavy) metals were also analyzed; extracted quantities commonly decrease in the following order Al>Ti>V>As>Cd>Cr>Pb>Sn.

The NIRS spectra were successfully calibrated to the results of the reference methods for N-total, S-total, P-total, effective CEC, soil organic matter (SOM), soil organic (SOC) and inorganic carbon (SIC), clay, and phospholipid fatty acids (e.g. Zornoza *et al.*, 2008). Next, validation studies have been performed for a wide range of samples from Belarus, Belgium, Finland, France, Germany, Lithuania, Norway, Sweden, the Netherlands and the UK (Hungary, Denmark and Spain are still in progress). Results indicate a high accuracy of determination for both the calibrations and validations ( $R^2 \geq 0.90$ ) (Reijneveld *et al.*, 2022, 2023).

Based on the successful calibration and validation studies, we developed several tools that are accessible for farmers and provide practical insights and recommendations. The first tool is the 'Soil Carbon Check' for monitoring SOC (and SIC) sequestration and guiding land users to increase SOC sequestration at relatively low cost. The tool was introduced in 2023 and translated into 11 different languages, promoted by, among others, the Finnish Ministry of Agriculture, and used by the dairy industry. Next, the 'Soil Life Monitor' was developed to routinely monitor microbial biomass of different groups of microbial taxa. In addition, the 'Soil Health Indicator' was developed, which allows farmers to optimize crop production, crop quality, and to use nutrients in a prudent way. All tools contribute to one or more SDG's. Soil Health Indicator also includes the minimum set of monitoring data required by the EU Directive on Soil Monitoring and Resilience (Electrical conductivity, soil erosion (modelling), bulk density, water holding capacity, pH-water / pH-CaCl<sub>2</sub>, biodiversity indicators, organic carbon (including SOC/clay ratio), P, and soil contamination (heavy metals). Thus, these soil-based tools are relevant for farmers, extension services, suppliers, processing industries to optimize production and forage quality, because the element composition of herbage reflects the soil health status. The contents of S, P, K, Na, Mg, and Ca in herbage are all significantly influenced by soil health characteristics (Reijneveld *et al.*, 2014). These tools are also relevant for the dairy industry to prove and claim sustainable (milk) production. The three soil-based

tools can therefore be seen as examples of translational research, linking research with relevant societal applications; their use can also be scaled to countries outside Europe relatively easily.

## Conclusions

Soil health encompasses many different soil characteristics, which can be quantified via two broad-spectrum soil tests, i.e. NIRS and 0.01 M CaCl<sub>2</sub> extraction. Based on successful calibration and validation studies, we developed Soil Health Indicator, Soil Carbon Check and Soil Life Monitor for farmers. These tools allow cost-effective soil monitoring in order to improve the economic and environmental performance of e.g., grassland-based dairy farms.

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# Validating and improving the Carnegie-Ames-Stanford Approach (CASA) for remote sensing of perennial grass biomass

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## Abstract

Estimates of net primary productivity (NPP) are useful in modelling the carbon cycle and elucidating responses to management and environment. This study improved the total radiation use efficiency (RUE)-based Carnegie-Ames-Stanford Approach (CASA) for grassland NPP. The analysis was based on multi-year field experiments with grass and grass-legume mixtures grown on sandy soil in Denmark, CO<sub>2</sub> flux chamber measurements of net ecosystem exchange to calculate NPP and long-term weather and soil moisture data for deriving environmental constraints. Canopy multispectral reflectance was collected at field- (Greenseeker, Yara N sensor) and airborne scale (drone), and several environmental constraints were utilized in the CASA model. The results showed that considering maximum air temperature and diffuse radiation considerably improved the prediction of NPP (nRMSE decrease of 7-37% compared to no constraints). Optimal total RUE was 2.1 and 1.9 g C MJ<sup>-1</sup> for ryegrass and mixture, respectively. This study provides novel insights into the environmental responses of grass biomass production and proposes an operational method for estimating grass NPP based on remote sensing and meteorology coupled with a CASA model.

**Keywords:** CASA, UAV, NPP, RUE

## Introduction

The local environmental conditions (e.g., maximum temperature) affect net primary productivity (NPP) and harvestable yields of agricultural plants even under optimal managements such as ample irrigation and fertilization. Carnegie-Ames-Stanford Approach (CASA) method for reliable estimation of biomass (NPP) based on RUE that further considers the micrometeorology and remote sensing has been demonstrated on annual crops (Peng *et al.*, 2021); however, this needs testing for perennial crops. Calculation of daily NPP is based on the concept of light-use efficiency, modified by temperature and moisture stress scalars. The aim of this study was to test the CASA method for estimating grassland NPP by explicitly accounting for spatial and temporal variability of both growth conditions and environmental factors.

## Materials and methods

Field experiments were established in 2021 in central Denmark (research station Foulum of Aarhus University) on a sandy loam soil and data were collected in 2022 and 2023. The climate is temperate and wet, characterized by mild summers and cool to cold winters, with moderate seasonal temperature variation and the agricultural systems are mostly rainfed. The perennial systems were sown on 15 August 2021 on 4.5×3 m plots in a randomized complete block design with four replicates. The field had conventional cereal cultivation in the years prior to the experiment. The perennial systems combined 19 treatments, including pure perennial ryegrass (*Lolium perenne*) treated with gradually increasing nitrogen fertilizer amounts (0, 75, 150, 300, 450 kg ha<sup>-1</sup>), and mixtures of different botanical composition, including grass with white clover and with red clover. All treatments were harvested according to the farmers' practice for grasslands, which in Denmark is three times a year, on 30<sup>th</sup> May, 2<sup>nd</sup> August and 10<sup>th</sup> October in 2022 and 2023. The biomass was harvested manually with automatic handheld shears in the centre of 1×1 m plots. We sampled total root biomass of each plot with depth 1m and separated into

large root and fine root after the last cut (10<sup>th</sup> Oct) in 2022. For the other seasons, we used the shoot-root ratio (1.05 for all the species, Bolinder *et al.*, 2002) to estimate root biomass and eventually total biomass.

Spectral measurements in 671 nm (red, ) and 771 nm (near-infrared, ) wavelengths were measured above the canopies weekly from early April until end-October in both years by a handheld Yara N sensor (Yara International, Oslo, Norway) and a multispectral camera mounted on an Unmanned Aerial Vehicle (UAV). We use the same method to calculate canopy-intercepted radiation ( $I_{par}$ ) and total RUE. Daily NPP ( $\text{g m}^{-2}\text{day}^{-1}$ ) was modelled with a modified CASA model (Peng *et al.*, 2021):

$$NPP = RUE_{opt} \times I_{par} \times f_{Tmax} \times f_{VPD} \times f_{WS} \times (1 - \mu \times f_{CI}) \quad (1)$$

where  $f_{Tmax}$ ,  $f_{VPD}$ ,  $f_{WS}$  and  $f_{CI}$  are environmental constraints for daily maximum temperature, mean vapour pressure deficit (VPD), water stress (WS) and cloudiness (expressed by cloudiness index, CI), ranging from 0 to 1 and aiming to adjust plant production for abiotic factors, and  $\mu$  is a constant set as 0.46 (Peng *et al.*, 2021).

For the field scale, five years' data from a previous study (2013, 2014 and 2015; Manevski *et al.*, 2017) were used for model building, and two years' data from the previous study (2020 and 2021) and current study (2022 and 2023) were used to validate the model.

## Results and discussion

We present only the results for the ryegrass and grass-legume mixture. The maximal total RUEs of plants present obvious spatial heterogeneity, and vary for different grass and sensors (Figure 1). At field scale

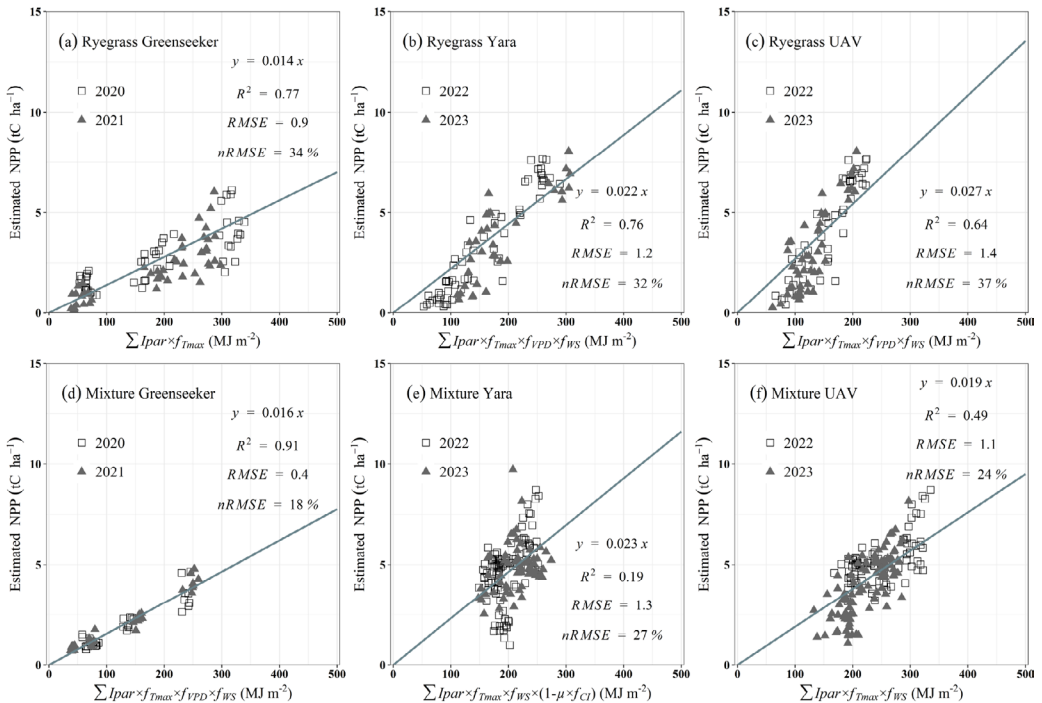


Figure 1. Relationship between measured total biomass of grass and  $Al_{par}$  derived from different remote sensing data (scales) and modified by environmental constraints. The slope means the radiation use efficiency.

and out of 16 combinations of constraint functions, the largest improvement due to inclusion of  $f_{Tmax}$ ,  $f_{WS}$  and  $f_{VPD}$  was also seen for the model built for ryegrass data (Fig. 1a–c), showing the importance of temperature variation, water stress and vapour pressure deficit on grass NPP (Macholdt *et al.*, 2023). Including the constraint of cloudiness ( $f_{CI}$ ) and vapour pressure deficit also increased the model performance, as seen for the mixture data (Figures 1 and 2). Maximum total RUE for modeling NPP was 2.1 and 1.9 g C MJ<sup>-1</sup> on average for ryegrass and mixture, respectively, which is comparable to 1.1–3.0 g C MJ<sup>-1</sup> in grassland reported by Cristiano *et al.* (2015). The accuracy in Mixture of 2022 and 2023 (nRMSE=18%) decreased compare with 2020 and 2021 (24–27%) might be due to the increase of species in later two years.

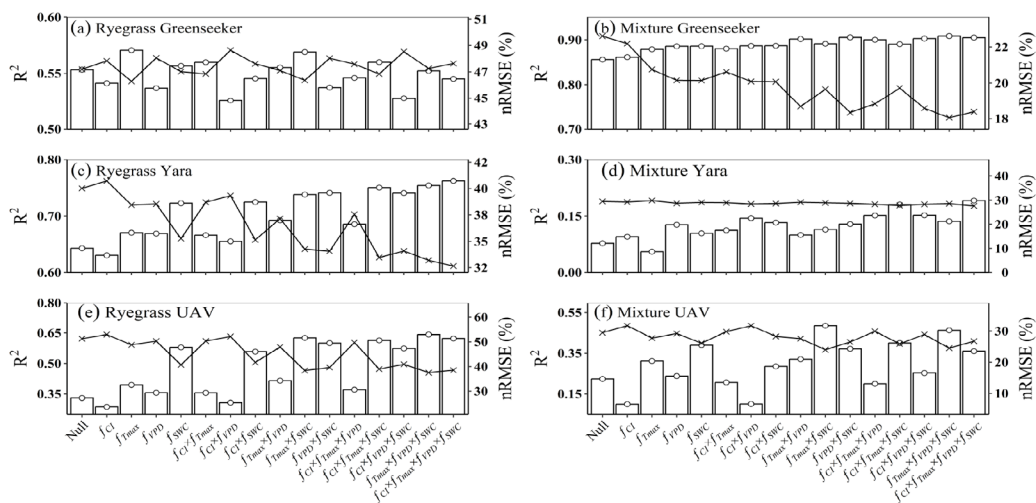


Figure 2. Impact of the studied environmental constraint functions on the performance of the model for ryegrass and grass-legume mixture from three sensors on total biomass estimation (Eq. (1)). Bar is  $R^2$  and line plot is nRMSE.

## Conclusion

Considering environmental constraints can improve the estimation of NPP for grasslands with CASA model. The study also revealed the potential for remote, fast, and accurate estimation of perennial NPP under field conditions.

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# Solar grazing – spatial distribution of sheep in free-field-photovoltaic systems on grassland

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## Abstract

In the context of climate change, the development and use of renewable energy is becoming increasingly important. However, with regard to free-field-photovoltaic (FFPV) systems the effective use of the land underneath the solar-modules (SM) remains an open question. When agricultural use and FFPV are combined, there is usually a trade-off between agricultural and energy production. Especially the required height and the smaller number of SM required for an effective arable land-management are obstacles. Grazing with small ruminants appears as a management option for an improved combination of agricultural and energy production. The aim of the present study was to analyse the spatio-temporal pattern of grazing behaviour of sheep and the grass sward response as affected by SM. We conducted a grazing experiment in a FFPV on peatland in Germany with GPS-collared sheep. The paddock was divided into two connected sectors, either with or without SM. Sheep behaviour was affected by SM: sheep had more lying time beneath SM with more trampling and more faeces deposition but preferred the section without SM for grazing. This is expected to lead to an increased heterogeneity of the grass sward in the longer-term. SM provided shade to the livestock which could improve animal welfare.

**Keywords:** photovoltaic, sheep, GPS, grazing, spatial distribution

## Introduction

For free-field-photovoltaic (FFPV) systems the efficient use of the land beneath the solar modules (SM) remains an open question. Economic, social and environmental benefits can be provided by combining energy and agricultural production on the same site (Al Mamun *et al.* 2023). Grazing with small ruminants is an option that may yield reasonable livestock products without compromising the energy efficiency of the FFPV. In addition, sheep can seek shade under the SM which then improve the welfare of the livestock under heat conditions (Kampherbeek *et al.* 2023). So far, there has been little research on the grassland status underneath SM and how sheep may respond with their behaviour at a temporal and spatial scale. The main objective of this study was thus to analyse the spatial behaviour of GPS-collared sheep grazing a FFPV-system on peatland in Germany with two sectors within paddock, i.e. either with or without SM. The target variables measured were the time and location sheep spent for lying and active behaviour, the spatial distribution of faecal spots, the compressed grass sward height (CSH), and the extent of trampling of the sward.

## Materials and methods

The present study was conducted in June and July 2023 at the ‘Solarpark Lottorf’ (54°44’55.5” N, 9°56’78.1” E), Schleswig-Holstein, Germany. Ten GPS collared sheep aged  $3.5 \pm 0.7$  years (mean  $\pm$  SD) were grazed on a 40 x 50 m trial site divided in two equally distributed sectors within paddock (with and without SM) (Figure 1). The trial site was divided in 80 5x5m grid cells (Fig. 1) resulting in 40 grid cells for each sector. Grid cells served as spatial replications for the analyses of vegetation (CSH pre- and post-grazing), trampling and distribution of faeces deposits. At pre- and post-grazing, five randomly distributed CSH measurements per grid cell were carried out using a rising plate meter. Post-grazing,

trampling (visual estimated percent of trampled grass sward per grid cell) and total number of faecal spots per grid cell were taken. According to Hamidi *et al.* (2023) GPS data for spatial analyses during active time and lying time (reciprocal of active time) were minute-wise retrieved from virtual fencing collars (® Nofence, AS, Batnfjordsøra Norway) with the virtual ncing function not being activated. Three GPS collars did not work properly. Consequently, daily aggregated active time per sector within paddock of seven animals (replicates) was used for analyses. The software environment R was used for statistical analyses. Generalised linear mixed effect models (R package ‘glmmTMB’) with the fixed effect SM (two levels) and the random term grid cell (80 levels) were used for the analyses of the target variables vegetation, trampling and number of faeces. For the analysis of active time the random term was changed to animal ID (seven levels). All data were log-transformed to improve the normality of residuals. Multiple contrast tests according to Tukey’s HSD test with Sidak’s method of confidence level adjustment (R package ‘emmeans’) were conducted to analyse the main influencing factors.

## Results and discussion

Pre-grazing CSH measurements of the grass sward revealed a higher grass stand ( $P < 0.0001$ ) in the SM sector than in the sector without SM (Table 1); the coefficient of variation (CV) was higher in the SM sector (36.6%) than in the sector without SM (24.4%). The sheep spent active time (associated with grazing) mainly in the sector without SM ( $P < 0.0024$ ). Consequently, lying time occurred mainly in the SM sector. The main grass species per grid cell in both treatments were *Holcus lanatus* and *Festuca rubra*; more *H. lanatus* in the SM sector and more *F. rubra* in the sector without SM (both sectors were mown equally during last years). *Rumex acetosa* was mainly found in the SM sector. Although grazing occurred mainly in the sector without SM, post-grazing CSH measurements revealed no significant differences; however, there was an increase of heterogeneity in the SM sector (CV of 41.5% and 30.5% for the sector

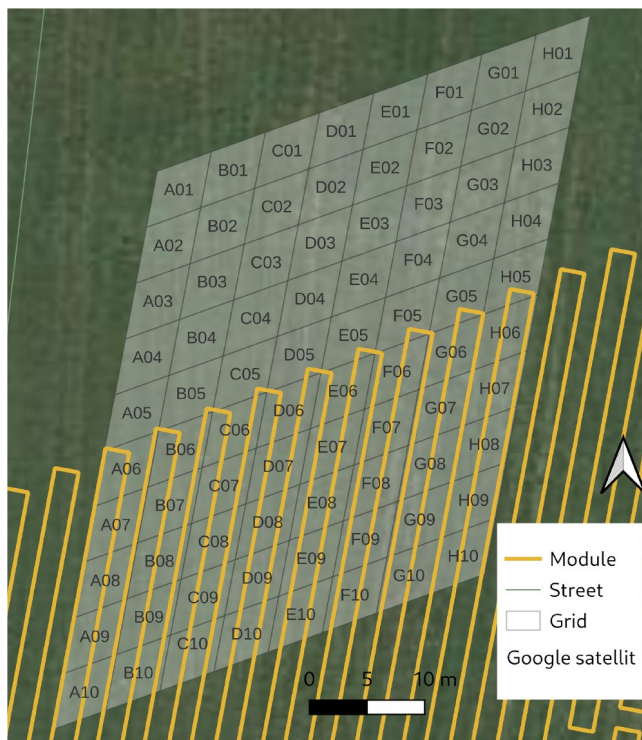


Figure 1. Representation of the study site (‘Solarpark Lottorf’).

without SM). Significantly more trampling occurred in the SM sector ( $P < 0.0001$ ; Table 1). Lying and grazing are known to be the main behaviours of sheep on pasture (Arnold, 1984). SM can be seen as anthropogenic structural landscape elements which affect the microclimate (Armstrong *et al.*, 2016) and provide shade for grazing animals (Kampherbeek *et al.*, 2023). An accompanying study by Zinken *et al.* (2024; these proceedings) marked differences of the microclimate between grasslands either covered by SM or not. Solar modules provide a preferred environment for lying of the sheep; this led to more trampling and defaecation (Table 1). This can lead to more uneven nutrient distribution in the field. As the study presented here was a short term one, no conclusions on long-term effects of grazing grasslands in FFPV on the vegetation and growth rates can be drawn. Yet, clear SM modulated short term effects on the grass sward and the grazing behaviour suggest a heterogenization of the grass sward in the longer term. The floristic and faunistic diversity may profit from this increased number of microhabitats within the grassland.

Table 1. Estimated means  $\pm$  SE (standard error) for CSH, trampling, active time and faeces.

	CSH Pre-grazing (cm per grid cell)	CSH post-grazing (cm per grid cell)	Trampling (% per grid cell)	Active time (hours per treatment)	Faeces (deposits per grid cell)
With solar modules	21.3 $\pm$ 0.5 b	7.5 $\pm$ 0.2 a	60.5 $\pm$ 2.2 b	5.8 $\pm$ 0.3 a	13.7 $\pm$ 1.0 b
Without solar modules	15.0 $\pm$ 0.4 a	7.4 $\pm$ 0.2 a	32.8 $\pm$ 2.2 a	6.7 $\pm$ 0.3 b	10.4 $\pm$ 0.78 a

Lowercase letters indicate significant differences between means within row ( $P < 0.05$ ).

## Conclusions

In our study, sheep preferred to lie under solar modules, while grazing mainly occurred where no solar modules were installed. Our study highlights the complex dynamics between solar modules and sheep grazing. It is concluded that the behaviour of the sheep can induce a greater heterogeneity of the grass sward within the field which may positively influence biodiversity in the longer term.

## Acknowledgements

We are grateful to all who support the field experiment. In particular, Monika Egerbacher for providing the GPS collars, Dag Frerichs and Holger Reimer for providing the animals and the trial site, and for the general maintenance of this trial.

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# Grazed multispecies swards: herbage production and sward botanical composition in year 4 post-sowing

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## Abstract

The use of multispecies swards (MS) for intensive grazing systems is of increasing interest for a number of reasons, including the potential to reduce chemical nitrogen (N) fertiliser application due to N from the legume components, reduce nitrate loss due to deep rooting plants, active overwinter growth, and increased drought tolerance, particularly from some herbs. A grazed plot experiment was established to investigate the herbage production of multispecies swards containing three plant functional groups: grass, legume and herb, receiving four levels of N fertilizer application (0 kg N ha<sup>-1</sup> (N0), 100 kg N ha<sup>-1</sup> (N100), 150 kg N ha<sup>-1</sup> (N150) and 200 kg N ha<sup>-1</sup> (N200)). Ten sward types were established including a perennial ryegrass (PRG, *Lolium perenne* L.) monoculture and sward mixtures of the following species: PRG, white clover (WC, *Trifolium repens* L.), red clover (*T. Pratense* L.), chicory (*Cichorium intybus* L.) and ribwort plantain (PL, *Plantago lanceolata* L.), with monoculture PRG N200 as the control. Swards were evaluated for the content of the various species in the sown mixtures and herbage production in year 4 post-sowing. Average herbage production for swards containing WC and/or PL was 531 kg DM ha<sup>-1</sup> greater ( $P < 0.001$ ) than the average of all other swards. The percentage of herbs in the sward mixtures was largely dominated by PL. The average PL content in swards with PL was 28.4±2%

**Keywords:** multispecies, persistency, grazing, DM yield, nitrogen

## Introduction

With increasing pressures to reduce nitrogen (N) inputs, N fixing legumes, usually white clover (WC; *Trifolium repens* L.) or red clover (RC; *Trifolium pratense* L.), are being more widely used in Irish grazing swards in combination with perennial ryegrass (PRG; *Lolium perenne* L.). The addition of herbs such as ribwort plantain (PL; *Plantago lanceolata* L.) and chicory (CH; *Cichorium intybus* L.) have also been thought to add resilience to grassland systems due to their increased drought tolerance and increased organic matter digestibility (Grace *et al.*, 2019). Multispecies swards have been shown to increase dry matter intake and milk production (McCarthy *et al.*, 2023; Roca-Fernandez *et al.*, 2016). However, there is still a lack of understanding of the long term (>2 years) persistency of components of multispecies swards in intensive grazing systems. The objective of this study was to investigate herbage production and sward botanical composition of binary and complex grazed multispecies swards receiving different N fertiliser application rates in year 4 post-sowing.

## Material and methods

A grazed plot trial was sown in June 2019 at Teagasc Moorepark, Fermoy, Co. Cork, Ireland. Plots (3×6 m) were sown in June 2019 a split block design with 3 replicates as described by Hearn *et al.* (2024). There were four chemical N fertiliser application rates and 10 sward types. The chemical N fertiliser application rates were 0, 100, 150 and 200 kg N ha<sup>-1</sup> year<sup>-1</sup> (0N, 100N, 150N and 200N, respectively). The ten sward mixtures were (1) PRG; (2) PRG-CH; (3) PRG-PL; (4) PRG-CH-PL; (5) PRG-CH-PL-WC; (6) PRG-CH-PL-WC-RC; (7) PRG-WC; (8) PRG-WC-RC; (9) PRG-PL-WC; (10) PRG-RC. The five species used in mixtures were: Grass - PRG (cv. AberGain), Legumes - WC (cv. Buddy) and RC (cv. Amos), and Herbs - PL (cv. Tonic) and CH (cv. Puna II). Perennial ryegrass receiving 200 kg N ha<sup>-1</sup> was the control treatment and plots were grazed when pre-grazing herbage mass was 1200–1400 kg DM

ha<sup>-1</sup> (>4 cm) on these plots. Fertilizer N was applied after grazing from February to September each year. Measurements were undertaken in year 4 of the experiment (2023).

Herbage mass was quantified 9 times in 2023 using an Etesia mower (Etesia Hydro 124D; Etesia UK). The cut herbage was weighed and a 100 g subsample dried at 90°C for 16 hours to determine DM content. Daily herbage growth rates were calculated as the pre-grazing yield divided by the number of days since the previous grazing event. Plots were grazed by lactating dairy cows for 12–24 hours to a target residual of 4 cm post herbage mass measurement. Sward botanical composition was estimated in February, May, June, August and October, prior to grazing, to establish the percentage of herbs, legumes, grass and unsown species within the sward. Random grab samples were taken diagonally across each plot using a Gardena hand shears. A 70 g subsample was separated into each of the sward components; PRG, RC, WC, PL, CH and unsown species. Once separated, the separated components were weighed and dried at 90°C for 16 hours to determine DM content.

Data were analysed using SAS 9.4. A linear MIXED procedure was used to estimate herbage mass. Chemical N fertiliser rate, sward type and associated interactions were included as fixed effects and replicate was included as the random effect.

## Results and discussion

The chemical N fertiliser application rate had a significant effect ( $P < 0.001$ ) on herbage production (Table 1). Swards receiving 200N had the greatest herbage production, while swards receiving 0N grew the least. Across all sward types, where either plantain or white clover were included, they increased yield compared to PRG at all nitrogen levels. White clover inclusion in multispecies swards has been reported to increase sward DM production (e.g. Grange *et al.*, 2021). However, when we look at the inclusion of PL as a component of a PRG-WC sward we see significant increases in yield ( $P < 0.001$ ) for all N treatments compared PRG. When we compare our control (PRG, 200N) to PRG, WC and PL at different levels of N the swards type receiving 150kg N ha<sup>-1</sup> can yield the same even when 50kg of N is removed from the system.

Plantain content was similar across all sward types containing PL (Figure 1). Sward CH was low (<7%), similar to Li and Kemp (2005) and Huwer *et al.* (2005). Sward WC content ranged from 10.9% (PRG-CH-PL-WC-RC) to 13.7% (PRG-WC). Red clover content was significantly higher ( $P < 0.001$ ) in binary mixtures compared to more complex mixes. Red clover persistence in a grazing sward is limited to approximately two years (Black *et al.*, 2009). There were higher proportions of unsown species in binary mixtures and lower proportions in swards sown with WC ( $P < 0.001$ ).

Table 1. Annual herbage production (kg DM ha<sup>-1</sup>; >4 cm) of each sward types at receiving 0, 100, 150 or 200 kg N ha<sup>-1</sup> and mean (ave.) yield across all N application rates.

kg N ha <sup>-1</sup>	PRG-RC-WC	PRG-CH-PL	PRG	PRG-CH	PRG-RC	PRG-RC-WC-CH-PL	PRG-WC-CH-PL	PRG-WC	PRG-PL	PRG-WC-PL	SE	P value
0	10 502	10 634	10 929	10 711	10 662	11 354	10 935	11 057	10 941	11 645	349.25	<0.05
100	12 101	11 659	11 220	11 742	12 601	12 002	12 291	12 158	12 522	11 995	349.25	<0.05
150	11 621	12 364	12 886	12 963	12 674	12 939	12 583	12 873	13 112	13 622	349.25	<0.05
200	12 986	14 021	13 688	13 508	13 365	13 493	14 058	13 976	13 880	14 351	349.25	<0.05
Ave.	11 798	12 169	12 180	12 231	12 325	12 447	12 535	12 516	12 613	12 903	333.83	<0.05

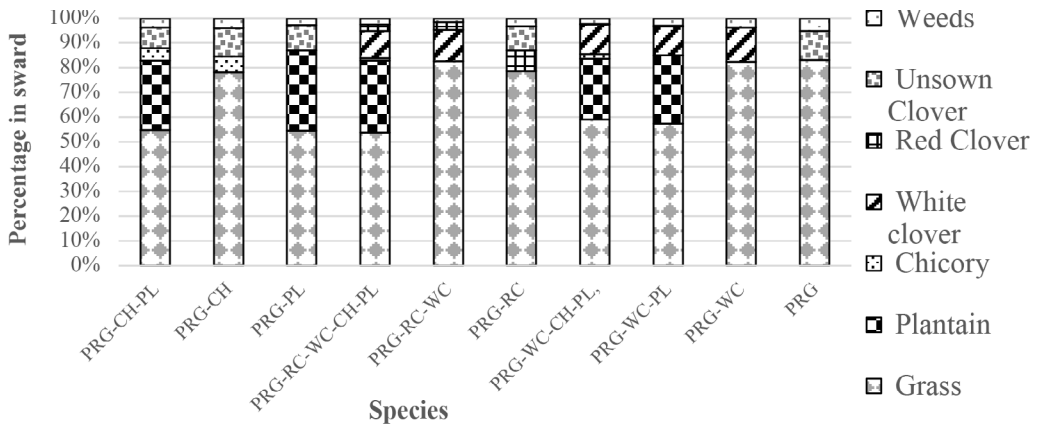


Figure 1. Annual percentage DM of botanical sward composition of each treatment.

## Conclusions

The inclusion of WC and PL had benefits in terms of overall sward DM production in year 4 of this grazing experiment.

## Acknowledgements

The financial support provided by the Teagasc Walsh Scholarship Programme, the SFIDA FM VistaMilk Research Centre (Grant no. 16/RC/3835) and the U-Protein Project funded by the Irish Department of Agriculture, Food and the Marine under the Food Institutional Research Measure (Grant no. 2019PROG702).

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# Virtual herding: Current trends and future prospects for grazing livestock

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## Abstract

Nowadays, several developments in digital technologies for herding grazing animals are arising. We conducted a systematic review on current developments in digital technologies for managing grazing animals. We highlight the most promising developments of virtual fencing used in recent research to evaluate effectiveness, animal behaviour and welfare. Moreover, we highlight current research in digital herding by drones and robots. Recent study results showed that virtual fences are highly efficient in keeping cattle within allocated pasture areas. So far, there has been no evidence for harmful impacts on animal welfare or reduction in animal performance. First findings suggest that drones can also herd and move animals. However, knowledge on the efficiency and potential effects on animal welfare when using drones is limited. First findings have shown that robots are able to gather animals to a specific location, and heart-rate and blood tests showed that the animals were less stressed by the robot than they were by a human. Digital tools provide the opportunity for precise livestock movement control.

**Keywords:** digital farming technologies, grazing livestock, herding, virtual fencing, drones, robotic

## Introduction

Digital herding such as virtual fencing is a promising, innovative technology that allows flexible and efficient grazing management by a fine-tuned control of cows' spatiotemporal grazing behaviour and precise adjustment of small-scale grazing pressure. Novel digital tools could facilitate the implementation of both productive and biodiversity-friendly grazing management approaches. Previous review papers (Anderson *et al.*, 2014; Umstatter, 2011) gave important insights into the first developments of virtual fencing. We go beyond this and address first tests of the most promising virtual fencing technologies for managing grazing animals within the landscape. Moreover, we discuss the potential of herding drones and robots for targeted control of livestock movement.

## Methods

We searched the literature for virtual fences, drones and robots. Articles in English were chosen using the 'Web of Science' section of the data base ISI Web of Knowledge (<http://pcs.isiknowledge.com>) for all available publication years. The search was based on the following search criteria: Topic= (virtual OR wireless OR fenceless OR stakeless OR automated OR digital OR drone OR robotic\* fencing OR herding) AND (dairy cows OR cattle OR sheep OR ruminants) AND (animal control OR movement control OR animal management OR animal monitoring) AND (response OR behaviour) AND (pasture OR grazing \*management) AND (animal welfare OR ethically acceptable) AND (nature OR environmental \* conservation OR landscape OR habitat \* restoration) AND (grazing OR pasture \* efficiency OR utilization) AND (agronomic outcome OR performance).

## Results and discussion

### *Virtual fencing*

Virtual fencing combines positioning techniques (e.g., GPS) with a conditioned pre-warning stimulus and an aversive stimulus to prevent animals from crossing a virtually defined border. Globally, four sophisticated virtual fencing systems have been developed and are available on the market. These systems consist of a collar that transmit positional data via GPS and devices that enable farmers to flexibly establish pasture boundary lines remotely, by shifting length and latitude coordinates using Apps on a smartphone, tablet or PC. Whereas in Australia extensive research on virtual fencing has been published since the late 2000s, studies from Europe and the USA appeared more slowly. So far, cattle, sheep, and goats have been tested using different virtual fencing systems.

Recent studies indicated that cattle and sheep successfully learn to associate the pre-warning sound announcing the electrical pulse when moving towards the virtual fence line. Furthermore, the number of received electrical pulses distinctly decreases within a few days of training, and escapes from the virtual fence border into the exclusion zone are less than 3% (e.g., Aaser *et al.*, 2022; Boyd *et al.*, 2022; Campbell *et al.*, 2019; Hamidi *et al.*, 2022). With increasing time on virtually fenced grazing areas interactions with the electrical pulse rarely occur, even if the grazing area is regularly shifted (e.g., Aaser *et al.*, 2022; Boyd *et al.*, 2022). For indicating welfare standards of virtual fencing systems, stress measures such as behavioural response and patterns (time budgets) in lying, grazing, rumination, resting, and travelled distance and herbage intake have been recently investigated. Moreover, increased fecal cortisol metabolite (FCM) concentrations can also indicate discomfort in cattle. First studies found no significant differences in fecal (e.g., Campbell *et al.*, 2019; Hamidi *et al.*, 2022) and milk cortisol metabolites (Verdon *et al.*, 2021) between virtual and conventional electrical fencing. Furthermore, differences in behavioural time budgets were low for both conventional electrical and virtual fencing or were not present at all (e.g. Campbell *et al.*, 2011; Hamidi *et al.*, 2022; Marini *et al.*, 2022). Moreover, current research uses GPS locations of individual animals to assess if the spatial distribution of animals within the grazing area is affected by the presence of the virtual fence. So far, no evidence has been reported that there is avoidance of pasture areas near the virtual fence line (e.g., Aaser *et al.*, 2022).

Virtual fencing has a potential for shifting grazing management to more flexible, controllable, precise, and less labour-intensive standards. However, the agronomic and ecological benefits of virtual fencing still need to be assessed under different socio-economic and ecological conditions across different grazing systems (Horn and Isselstein, 2022). Whereas virtual fencing is authorized for practical application in some European countries such as Norway, Sweden and the United Kingdom, in other European countries there are limitations due to restrictions in national animal welfare acts.

### *Digital herding by drones and robots*

A ground-breaking developmental step started from the Sheepdog Project in 1995. Here, wheeled robots gathered in and guided a flock of ducks to a specific location. Moreover, heart-rate and blood tests showed that the animals were less stressed by the robot than they were by either human or a stuffed fox (Vaughan *et al.*, 1998). Developments of digital herding technologies are mainly based on ground wheeled or legged robots that guide animals through coloured bars on a small vehicle (e.g., 'SCRUPAL'; Evered *et al.*, 2014) or gingerly collision by bigger sized ground robots (e.g., 'Rover' – BBC report; 'SwagBot' – IEEE Spectrum). Evered *et al.*, (2014) recorded the distance from the robot SCRUPAL' to a sheep flock when the nearest sheep first became alert to the presence of the slowly approaching robot and the distance at which the sheep began to move away from the robot in three separate trials. They found that the alert distance and flight distance dropped from the first trial to the third trial. However, among hurdles in



off-road capability for various difficult terrains, there is a current lack of sufficiently efficient algorithms to monitor, assess and adapt robot-animal- interactions and external disturbances for autonomous and remote control of herding for both ground-based robots and drones (Li *et al.*, 2022).

Several innovative livestock farmers from the Australian Outback to Irish countryside already use drones to move their sheep or cattle herds. Furthermore, in large rangelands or inaccessible terrains drones have many superior advantages regarding their speed and manoeuvrability compared to human or robotic herders. Recent studies reported similar success of drones to move wild horses into a trap for population control treatments compared to capturing by helicopters. Moreover, capturing by drones is proposed to benefit both animal and human safety and welfare (McDonnell and Torcivia, 2020). One recent study by Brunberg *et al.* (2021) investigated the response in a flock of sheep to the presence of a drone compared to a human or a dog. They found that sheep flocks exposed to drones spend more time moving around and more time with grazing compared to those exposed to more familiar humans and dogs. This demonstrates that a gently and stepwise habituation to flying drones is just as important as for human herders or sheepdogs.

## Conclusions

Digital herding technologies are likely to transcend existing paradigms of grazing management. Opportunities to provide and prevent access of grazing livestock to any subareas of the entire pasture, farm, or landscape at any spatial and temporal scale are enormous. Nevertheless, implementation of digital herding will only be as good as the knowledge and skills of farmers who operate them.

## Acknowledgement

This study was supported by the Federal Ministry of Education and Research under Grant number (031B0734A) as part of the project GreenGrass.

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# Genetics at a landscape level for better conservation of seminatural grasslands in the Karkonosze National Park, Poland

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## Abstract

The area of species-rich seminatural grasslands diminishes, along with their conservation value and wide range of ecosystem services delivered. Among the endangered values is also the genetic diversity of semi-natural grassland plant species for conservation, of which relatively little effort is undertaken. Here, we analysed the spatial pattern of genetic diversity of *Agrostis capillaris*, *Hypericum maculatum* and *Cirsium helenioides* in the Karkonosze National Park, SW Poland, and its buffer zone and vicinity, to establish genetic diversity conservation areas and strategy of genetic resources conservation. From 20 to 25 grassland patches ('populations') were sampled for each species, and the genetical structure was assessed using DArT techniques. The results allow us to find grassland patches supporting the highest genetic diversity for particular species, as well as clusters of genetically similar individuals. The least cost path method was applied to distinguish possible routes of pollen flow and seed migrations, assuming different landscape permeability for different vectors (insects, large herbivores). The results show that the protection within the National Park area is not sufficient to conserve a large part of genetic diversity. The project reveals grassland patches' priority for diversity conservation and suggests the creation of assisted gene flow migration systems.

**Keywords:** genetic conservation areas, genetic diversity, isolation by resistance

## Introduction

Semi-natural grasslands provide a wide range of ecosystem services, and many such grasslands are valued for their high biodiversity (Bengtsson *et al.*, 2019; Schils *et al.*, 2022). The area of semi-natural grasslands is declining (Isselstein *et al.*, 2005) and their biodiversity is jeopardized by land use changes (Raduła *et al.*, 2020). Within species diversity, genetic diversity is an important aspect of biodiversity (IUCN, 2024). In consequence, methods of protection of genetic diversity have been established, typically in the form of ex situ protection in gene banks, but also in the form of genetic conservation areas (further GCA) (Kramer and Havens, 2009; Maxted *et al.*, 1997). In the case of genetic conservation areas, the method has not been commonly applied, and there are no optimal procedures developed to select these areas. Moreover, the GCA is more common for woody plants and crop wild relative species, and species of less economic significance are not considered (Gradl *et al.*, 2022). Here, we show the preliminary results of our examination of the genetic structure of three common grassland plants in order to establish a conservation strategy for genetic resources in a National Park.

## Materials and methods

The Karkonosze National Park (KPN) and its buffer zone are located in Poland, Central Europe. The Park protects mostly mountain areas with their vegetation. Most of the park area is covered by forest and subalpine vegetation, with some patches of semi-natural grasslands placed at lower altitudes (Figure 1). Because of the land use system, the grassland patches are usually small and isolated, and most grasslands are placed in the buffer zone and outside of this zone. We focused on the three common semi-natural grassland plant species: *Agrostis capillaris*, *Hypericum maculatum* and *Cirsium helenioides*. The plants

are insect-pollinated (*H. maculatum* and *C. helenioides*) or wind-pollinated (*A. capillaris*), and they have a clonal growth strategy. Their seeds are wind-dispersed (*C. helenioides*), baro- and zoo-choric (*A. capillaris*, *H. maculatum*). We sampled 24 sites (populations) per species, 8 individuals from each site (Figure 1). The collected healthy leaves were transported to the laboratory where DNA was extracted. Next, the samples were analysed using Diversity Arrays Technology DArTseq sequencing, which is a form of reduced representation sequencing (RRS). The obtained results, Single Nucleotide Polymorphism (SNP) data, were checked regarding their quality and statistically analysed (Avolio *et al.*, 2012). Using the SNP data, we prioritized the populations of particular species, by indicating these with highest observed heterozygosity (1) and highest input in genetic diversity assessed by PCA ordination (2) (Gradl *et al.*, 2022).

## Results and discussion

*H. maculatum* has the highest  $t$  level of genetic diversity expressed by observed heterozygosity (0.179), following by *C. helenioides* (0.159) and *A. capillaris* (0.116). The species showed different patterns of within- and among-population diversity: *C. helenioides* had the highest value of  $F_{st}$  statistics (0.35) compared to 0.027 and 0.006 for *H. maculatum* and *A. capillaris*, respectively. According to the AMOVA results only 1.5% of genetic diversity can be attributed to different populations for *A. capillaris*, while it was 58% for *C. helenioides*. Since most of the gene flow in plants' populations is due to pollen movement (Petit *et al.*, 2005) this feature can be related mostly to different pollination strategies between these two contrasting species: wind pollination (*A. capillaris*) and insect pollination (*C. helenioides*). The depth of the tubular florets in the *C. helenioides* capitula limits nectar access to long-tongued insects, mostly *Hymenoptera* (e.g. bees or bumblebees) and *Lepidoptera*. As a result, the pollination distance typically does not exceed 10 m (Bureš *et al.* 2010; Richards 1997). In the case of wind-pollinated species, e.g. grasses, their genetic diversity is usually higher within populations than among populations (Reisch and Bernhardt-Römermann, 2014). The patches' prioritization regarding their heterozygosity shows a lack of correlations among species (detailed results not shown). It means that patches with the highest heterozygosity for one species did not support high heterozygosity for another species. Such a

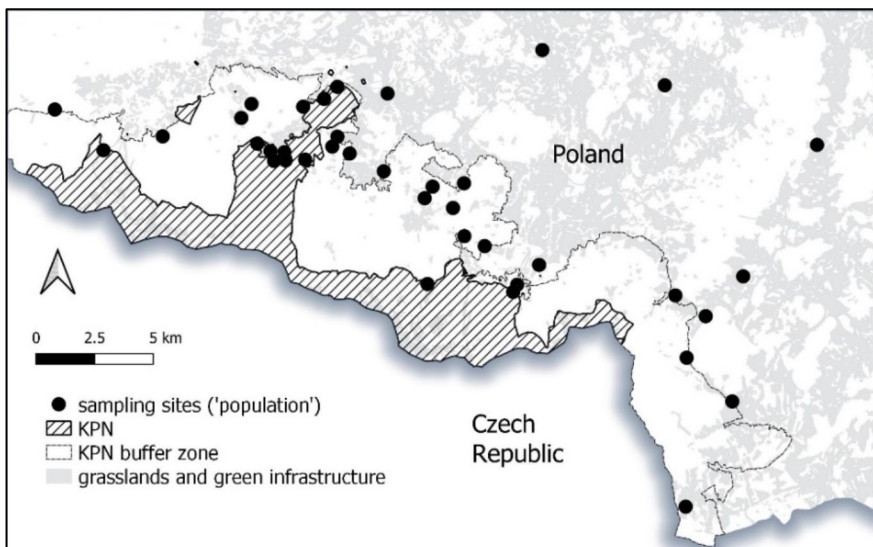


Figure 1. Location of sampling sites ('populations') on the background of grassland patches location. The low amounts of grassland within the Karkonosze National Park (KPN) is clearly visible.

phenomenon that sites which should be protected for one species are not the same as sites for another species was also observed in research of Gradl *et al.* (2022). It suggests that including multiple species in the GCA selection process will require several sites (Gradl *et al.*, 2022).

## Conclusion

The analysis of the genetic structure of populations at landscape scale will help in developing a strategy to improve ecological connectivity in the Karkonosze National Park and its surroundings, and, in a changing world, seems to be an essential element of biodiversity monitoring in protected areas. Particularly for of *C. belemnioides*, a species from the Polish Red List, the observed pattern of structured populations seems to be related to low connectivity.

## Acknowledgements

The study was financed by Karkonosze National Park as a part of the project ‘Poprawa stanu łączności ekologicznej w Karkonoskim Parku Narodowym i jego otulinie’, financed by Iceland, Liechtenstein and Norway under the European Economic Area and Polish State Budget.

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# Minimum number of spot measurements required for quantifying enteric methane production in dairy cattle

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## Abstract

The GreenFeed system (GF; C-Lock) has been developed as a static short-term measurement device for enteric methane (CH<sub>4</sub>) emission from individual animals. The objective of this study was to define the minimum number of GF spot measurements required to obtain reliable CH<sub>4</sub> production measurements in dairy cattle in three different systems: fresh grass outdoors (G), fresh grass indoors (ZG) and grass silage indoors (GS). Simple random sampling without replacement was used to analyse 9426 CH<sub>4</sub> spot measurements obtained from 138 measurement units (100 dairy cows). For each cow with more than 30 spot measurements per two-week measurement period, 100 replicates of 1 to 30 spot measurements were randomly selected to calculate the mean absolute percentage error (MAPE) for each combination of replicate and spot measurement count. A minimum of 17, 10 and 11 spot measurements is needed for G, ZG and GS, respectively, to prevent a MAPE larger than 4%. In conclusion, a larger number of spot measurements with the GF is needed for grazing studies compared to studies indoors to attain reliable enteric CH<sub>4</sub> production measurements in dairy cattle.

**Keywords:** methane emission, dairy cattle, spot measurement, method quantification, GreenFeed

## Introduction

One of the main challenges in enteric methane (CH<sub>4</sub>) emission research is to capture the diurnal emission pattern of CH<sub>4</sub>, as it can vary by up to fourfold throughout the day depending on feeding frequency (Crompton *et al.*, 2011). This circadian variation in enteric CH<sub>4</sub> production is best accounted for by using techniques that measure continuously over 24 h periods. The gold standard to measure enteric CH<sub>4</sub> emission is therefore the climate respiration chamber technique, where one or more animals are confined in airtight chambers, from which the in- and out-flowing air is monitored for gases, including CH<sub>4</sub>. However, this technique requires confinement of animals, making it impossible to measure CH<sub>4</sub> emission during grazing. Alternatively, a variety of measurement techniques have been developed, including the GreenFeed system (GF; C-lock, Rapid City, SD, USA), which can be considered a short-term measurement device (e.g. Hammond *et al.*, 2015; Manafiazar *et al.*, 2017). Because the spot measurements of the GF are not capturing the full diurnal emission pattern, it is unknown how many spot measurements of enteric CH<sub>4</sub> production are needed in order to obtain a reliable daily enteric CH<sub>4</sub> production value per cow. Therefore, the objective of this study was to investigate the minimum number of spot measurements required to reliably quantify the enteric CH<sub>4</sub> production in dairy cattle. A reliable mean was defined by a mean error of less than 4% relative to the observed mean (in agreement with Van Gastelen *et al.*, 2024). It is hypothesized that the minimum number of spot measurements needed depends on the type of diet fed to the dairy cows and the housing system (i.e. continuous grazing outdoors, zero-grazing indoors and a grass silage-based diet indoors).

## Materials and methods

The dataset used was derived from a trial conducted in 2020 at Dairy Campus (Leeuwarden, the Netherlands), described in detail by Klootwijk *et al.* (2021). In short, the trial comprised three periods, each involving two weeks of adaptation followed by two weeks of measurements: April–May, June–July and August–September. Per period, 48 Holstein-Friesian dairy cows between 80 and 200 days in lactation (mean  $\pm$  standard deviation,  $152 \pm 42.1$  days) and with a milk production of  $33.1 \pm 5.87$  kg day<sup>-1</sup> at the start of the period were enrolled. These cows were blocked into 16 blocks of 3 cows each, and within each block randomly assigned to one of three treatment groups: fresh grass outdoors (continuous grazing; G), fresh grass indoors (zero-grazing; ZG) and grass silage indoors (GS). Additionally, all cows received 2.0 kg day<sup>-1</sup> compound feed in the milking parlour and, depending on the number of voluntary visits, a maximum of 3.5 kg day<sup>-1</sup> compound feed in the GF. Each period the blocking was redone, and cows in late lactation (>200 days) were replaced by cows in earlier lactation (> 80 d). Methane production was measured using the GF. Each spot measurement was at least 2 min and on average  $4.8 \pm 1.32$  min. Six cows were excluded from the analysis because they had less than 30 spot measurements in a two-week measurement period (all from treatment G). In total 9426 CH<sub>4</sub> spot measurements from 138 measurement units (100 dairy cows) were used for the analysis. Each measuring unit corresponds to a cow per period; some cows were measured across multiple periods. Simple random sampling analysis without replacement was performed per cow  $\times$  period combination in R (version 4.2.1), using the *srvyr* package. In this process for each cow  $\times$  period combination, 100 replicates each of 1 to 30 spot measurements were randomly selected from the complete dataset. The mean absolute error (MAE; g day<sup>-1</sup>) and mean absolute percentage error (MAPE; %) for each combination of replicate and spot measurement counts was calculated as:

$$\text{Mean Absolute Error (MAE)} = \frac{\sum |Y_t - Y_p|}{n}$$

$$\text{Mean Absolute Percentage Error (MAPE)} = \frac{\sum \left| \frac{Y_t - Y_p}{Y_t} \right|}{n} \times 100\%$$

Where  $Y_t$  is the observed mean CH<sub>4</sub> production of all spot measurements per cow per period,  $Y_p$  is the mean CH<sub>4</sub> production of the 100 replicates of 1 to 30 random spot measurements per cow per period (i.e. the subsample), and  $n$  is the number of cows per period.

## Results and discussion

The mean CH<sub>4</sub> production was lowest for G (312 g CH<sub>4</sub> day<sup>-1</sup> versus 401 and 420 g CH<sub>4</sub> day<sup>-1</sup> for ZG and GS, respectively), while the within-cow standard deviation was highest (82.1 g CH<sub>4</sub> day<sup>-1</sup> versus 65.3 and 73.7 g CH<sub>4</sub> day<sup>-1</sup>). The higher within-cow variation for G resulted in a numerically larger MAE and MAPE when the number of spot measurements in the random sampling decreased (Figure 1). To prevent a MAPE larger than 4%, a minimum of 17 spot measurements is needed for G, 10 for ZG and 11 for GS in a two-week measurement period. Results should, however, be interpreted with caution, because other characteristics of the spot measurements, such as their distribution over the day or measurement length, may impact the observed variation, and these factors were not considered in this analysis. The measurements were, for example, less equally distributed over the day for G compared to ZG and GS, potentially explaining the increase of variation between spot measurements under grazing conditions compared to indoor conditions. A possible contributing factor to this discrepancy is that the GF was less frequently visited by cows with G ( $44 \pm 6.0$  measurements per cow) compared to both ZG and GS ( $81 \pm 8.3$  and  $77 \pm 15.7$  measurements per cow, respectively). This might be explained by cows being less accustomed to visiting a feeding station in the pasture compared to the barn. Additionally, it is possible that the increase in time spent eating during grazing (Dohme-Meier *et al.*, 2014) has an influence on the GF visit time as well.

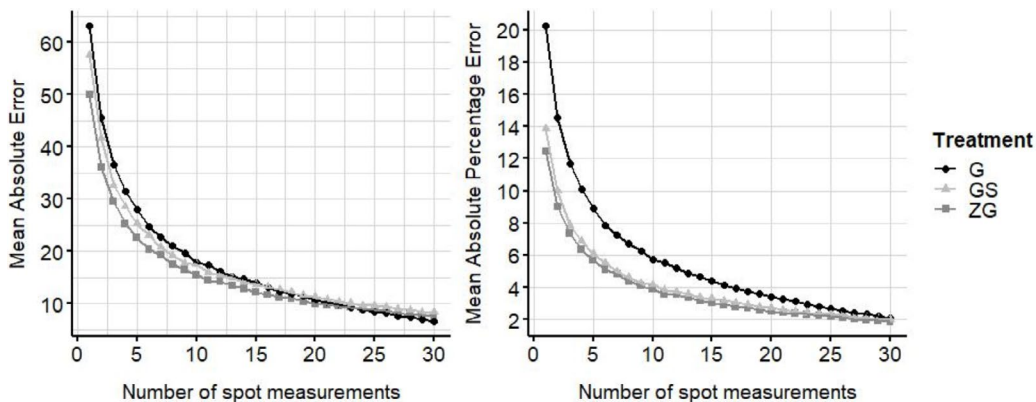


Figure 1. Mean absolute error (MAE; left panel) and mean absolute percentage error (MAPE; right panel) per treatment, where G is grazing outdoors (black circles), GS is grass silage indoor (light grey triangles) and ZG is fresh grass indoors (dark grey squares). The values are averaged over period and over the 100 iterations for each of the number of CH<sub>4</sub> spot measurements (1 to 30).

## Conclusion

To prevent a MAPE larger than 4%, a minimum of 17 spot measurements is needed for G, 10 for ZG and 11 for GS in a two-week measurement period, although the results should be interpreted with caution due to other characteristics of the spot measurements not taken into account in this study. The within-cow variation of enteric CH<sub>4</sub> production during grazing is higher, which requires a higher minimum number of spot measurements for grazing studies compared to studies indoors, regardless of diet type fed indoors.

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# From an intensively managed agricultural grassland to an extensively managed grassland: the first years of transition

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## Abstract

Intensively managed grasslands that become extensively managed require a transition period that does not neglect soil properties and gradual changes in the sward composition over time. This is necessary to prevent the grassland becoming botanically stuck in the transition phase, in which less wanted grasses such as *Elymus repens* and *Holcus lanatus* are dominant and are in heavy competition with herbs, due to nutrient rich soils and the botanical composition at the end of the intensive management. It is, however, unclear what this management exactly should be, how the farmer can use the products of these grasslands, and how and at what pace the properties of these grasslands will change over time. One paddock grassland in transition on the ILVO experimental farm is being followed up closely since fertilization and herbicide application ceased and the sward was cut only 2–3 times instead of 4–5 times per year. Soil samples, dry matter crop yield (DMY), fodder quality and botanical composition of the sward are measured. Although it is still too early to make conclusions, already some slight changes in species abundance have been observed. The new cutting management provides a fodder with a rather low to very low crude protein content with a good digestibility coefficient.

**Keywords:** transition to extensive grassland, botanical composition, DMY, fodder quality

## Introduction

Many farmers in Flanders (Belgium) will face legislation that requires them to change the management of agricultural grasslands located in or around nature conservation areas. Specific restrictions differ depending on the situation, but in all cases fertilization and herbicide use are prohibited. Reseeding and overseeding are often also restricted. Typically these grasslands are obliged to be grazed permanently or to be under a regime of maximum 2 cuts per year. The abrupt change from intensive agricultural management to extensive management neglects that the soil is typically still rich in nutrients, and with a sward that is typical of intensively managed grassland. This often results in grasslands becoming stuck for years in a transition phase, in which grasses such as *Holcus lanatus* are dominant and suppress herbs (De Schrijver, personal communication). In addition, most conventional farmers are not used to working with extensively managed grass, which is expected to be poorer in protein and richer in fibre. At the ILVO experimental farm, one paddock that had been intensively managed for decades has now been extensively managed for 2 years. Soil samples were taken, DMY, forage quality and botanical sward composition were observed and will be observed continuously in the coming years. The paddock borders another paddock with the same soil type and history that will be managed following business-as-usual, and can be used as a reference. However, no measurements are done in this reference paddock at the moment. The goals of this observational study are to understand how the grassland changes over time due to extensification of the management.

## Materials and methods

### *Site and management*

The experimental paddock consists of a 5.5 ha grassland that has been grazed (May–October) year after year for decades by >10 Belgian White Blue beef cows and heifers after the first cut. This management changed to a permanent cutting management (4–5 times a year) in 2017 and to the extensive cutting

management of 2–3 times a year since January 2022, with no input fertilizers and herbicides. Before 2022 herbicides were used every few years to control dicotyledonous weeds, and the fertilization was approximately 245 kg N ha<sup>-1</sup> and 150 kg K<sub>2</sub>O ha<sup>-1</sup> of mineral fertilizer annually. No animal slurry was used.

Measurement: Soil samples of the 0–30 cm layer were analysed chemically to determine the soil organic carbon content (SOC), soil-pH<sub>KCl</sub> and the nutrient contents in autumn 2021. Every 3 years soil samples will be taken again. The DMY of every cut was determined and sward samples were analysed for fodder quality. Fodder quality was determined immediately before baling using NIRS. Every year, three fixed quadrats (10 m×10 m) were visually observed to assess the ranking of plant species present in the sward. These measurements will be continued in the coming years.

## Results and discussion

The soil analysis revealed that SOC% and pH<sub>KCl</sub> were dependent on the specific spot in the grassland, within the range of 2.0–3.2% and 5.4–5.8, respectively. K and Ca were considered to be in the optimal zone of crop growth while Mg and Na were considered above and P was just below. This indicates that P, K, Mg, Ca and Na were still rich enough for crop growth and the soil can be considered as a productive agricultural plot. N is considered to be the limiting growth factor as no fertilization is added and very few legume species are present in the sward. The P-Olsen method is used to determine the available amount of P in the soil in the context of natural grassland. The amount of P-Olsen determines the extent to which extensification is possible. The greatest potential for the restoration of species-rich mesotrophic grassland is <10 (Gilbert *et al.*, 2009). The soil analysis revealed spatial differences ranging from 26–43 P-Olsen, far from the ideal situation. The first botanical observation (spring 2022) in the first year of extensification (Table 1) revealed an abundance of Poaceae species, typical for wet and nutrient-rich meadows. The second botanical observation (autumn 2023) showed some changes in abundance of the different species, which can also be season-dependent. However, some changes are more structural. *Elymus repens* is clearly expanding, especially in quadrat 1; *Glyceria fluitans* disappeared completely in quadrat 2 — possibly due to a very dry summer in 2022 — and *Trifolium repens* was now observed clearly in quadrat 3.

The typical cutting management for natural grasslands in Flanders is 1 cut after 15<sup>th</sup> June and 1 cut after 15<sup>th</sup> of September. However, starting from an agricultural grassland this often leads to a rapid expansion of *Holcus lanatus* and sometimes *Elymus repens*, while suppressing newly installed herbs. The grassland is then botanically stuck in the transition phase. A 3-cut system proved to prevent this situation in a long-term field trial (data not yet published) (De Schrijver, 2023). An early first cut can prevent seed formation of *Holcus lanatus* and reduces the competition with herbs. Although, the focus is on a 3-cut system, the weather conditions forced us already in the second year to go for 2 cuts. The first cut was

Table 1. Plant species in order of abundance at the start of the extensification per quadrat on 28 April 2022.

Quadrat No.	Species present
1	<i>Elymus repens</i> > <i>Lolium perenne</i> > <i>Lolium multiflorum</i> > <i>Poa annua</i> > <i>Poa trivialis</i> > <i>Holcus lanatus</i> > <i>Alopecurus</i> sp.> <i>Ranunculus repens</i> > <i>Stellaria media</i> > <i>Bromus</i> Hordeaceae
2	<i>Glyceria fluitans</i> > <i>Holcus lanatus</i> > <i>Agrostis</i> sp.> <i>Alopecurus</i> sp.> <i>Ranunculus repens</i> > <i>Lolium multiflorum</i>
3	<i>Holcus lanatus</i> > <i>Elymus repens</i> > <i>Poa trivialis</i> > <i>Lolium perenne</i> > <i>Vicia cracca</i> > <i>Cerastium</i> sp.> <i>Taraxacum officinale</i> > <i>Alopecurus</i> sp.> <i>Ranunculus repens</i> > <i>Bellis perennis</i> > <i>Cirsium</i> sp.> <i>Stellaria media</i> > <i>Alopecurus</i> sp.

later than planned due to continuous bad weather conditions. The summer cut was also postponed due to continuous rainy weather, resulting in only one autumn cut. The strategy for the coming years is again 3 cuts per year. It is still (much) too early to conclude whether this strategy will work. A possibly other advantage is that the first cut is younger material (less fibre and less protein dilution). Table 2 shows that crude fibre is not exceptional for grass forage (usually 210–280 g (kg DM)<sup>-1</sup>). However, the fibre content of both cuts in 2023 was noticeably higher when two cuts were harvested, compared to the fibre content of the 3 cuts in 2023. The crude protein content is, however, very low in all cuts, except the 3<sup>rd</sup> cut of 2022, compared to that usually harvested by farmers (210–220 g (kg DM)<sup>-1</sup>, depending on fertilization moment of the year and time since last cut). This indicates that N availability was the limiting growth factor. The sugar content remained in the optimal range for good forage quality, except for the sugar in the third cut in 2022 and second cut in 2023. However, for an autumn cut, this is expected. This resulted in moderate to good digestibility of the grass. The total DMY was 7525 and 6249 g kg DM ha<sup>-1</sup> for 2022 and 2023, respectively.

Table 2. DMY and fodder quality parameters of the silage fodder from all cuts.

Date	DM (%)	DMY (kg ha <sup>-1</sup> )	Crude protein (g kg DM) <sup>-1</sup>	Crude fibre (g kg DM) <sup>-1</sup>	Sugar (g kg DM) <sup>-1</sup>	Ash (g kg DM) <sup>-1</sup>	Digestibility (%)
10 May 2022	64.8	3150	85	223	236	62	77.6
8 Jul 2022	97.6	2667	65	239	158	104	72.9
4 Oct 2022	40.6	1708	154	230	88	148	76.2
29 May 2023	31.9	3643	71	278	192	69	71.0
8 Sep 2023	57.4	2606	86	263	88	179	72.1

Digestibility, *in vitro* cellulase digestion coefficient of the organic matter (De Boever *et al.*, 1986).

## Conclusion

Two years after ceasing fertilizer and herbicide use, the soil samples and botanical composition of the sward indicate that this grassland still has the properties of an intensive agricultural grassland and not a species-rich natural grassland. The grassland management should therefore be adapted to the transition period. It is still too early to conclude how the transition will continue. Early observed changes are a trend of expansion of *Elymus repens* and very low protein content of the fodder. It could be relevant to think about offering specific schemes and knowledge that support farmers in the transition towards extensive management. More research on the management and use of such grasslands in an agricultural context is relevant, in particular given the considerable number of farmers that will be confronted with a mandatory change from intensive to extensive management.

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# Impact of nitrogen fertilization on litter decomposition in temperate grassland—a tea bag study

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## Abstract

Nitrogen (N) inputs can alter carbon (C) cycling which may affect litter decomposition and thus soil C stocks and CO<sub>2</sub> emissions in grasslands. Nitrogen addition has been reported to result in accelerated litter decomposition in grassland. Fertilizer form and its application rate as well as the incubation time of the litter and incubation depth further affect decomposition. A widely used method to monitor standard litter decomposition is the tea bag index (TBI) method. We used the TBI method to evaluate the effects of fertilizer form (calcium ammonium nitrate vs. cattle slurry vs. mixture of both), N-application rate (170 vs. 240 kg N ha<sup>-1</sup>), and soil depth (8 vs. 17 cm) on early and late stage decomposition of the tea litter. Green tea and rooibos tea were buried for 3 and 6 months in a grassland field experiment in northwest Germany. We used tea weight loss as well as C and N content of the retrieved tea to analyse the different effects on litter decomposition rate and stabilization. We concluded that the TBI method is not efficient for discriminating the effects of depth and N management on litter decomposition after 3 months but detects a management effect after 6 months.

**Keywords:** application rate, decomposition rate, fertilizer form, soil depth, stabilization, tea bag index

## Introduction

Nutrient availability affects microbial community and its metabolism and, in turn, litter decomposition in soil (Francioli *et al.*, 2016; Poeplau *et al.*, 2018). In grassland, the application of nitrogen (N) fertiliser significantly accelerates the decomposition of litter (Ochoa-Hueso *et al.*, 2020) with organic fertilizer leading to higher decomposition rates than mineral fertilizer (Francioli *et al.*, 2016). Similarly, incubation time (Manzoni *et al.*, 2012) and soil depth (Fierer *et al.*, 2003) affect decomposition of litter and may alter C cycling and thus soil C stocks and CO<sub>2</sub> emissions in grasslands.

To investigate the above-mentioned effects on litter decomposition in a sandy grassland soil of northwest Germany we applied the tea bag index (TBI) method (Keuskamp *et al.*, 2013), which is the most standardized approach for measuring litter decomposition in soil and represents an ideal instrument for soil citizen science (Pino *et al.*, 2021). The TBI determines the decomposition rate  $k$  and stabilisation factor  $S$  from the weight loss of green tea (GT) and rooibos tea (RT) during incubation in soil. The aim of this study was to test the application of the TBI for monitoring grassland management in northwest Germany and thus to assess impacts on soil functioning.

## Materials and methods

In 2023 a grassland field experiment on tea decomposition was carried out at Markhausen, located in the northwest of Germany (Lower Saxony, 52°57'24.3" N; 7°52'22.4" E). Since 2021, treatments in a randomized blocked design varied in N fertilizer application (form and rate) and were replicated in four 12×4 m blocks. The N fertilizer varied in form (calcium-ammonium nitrate (CAN) vs. cattle slurry (CS)) and amount of input (0 (control) vs. 170 vs. 240 kg total N addition ha<sup>-1</sup>). On day 0 (21 February 2023), six weighed GT bags (Lipton Indonesian tea Sencha tradition, EAN 87 22700 05552 5) and six weighed RT bags (Lipton Infusion Rooibos, EAN 87 11327 51434 8) were buried at 8 cm soil depth in each plot of the six treatments following the protocol by Keuskamp *et al.* (2013). Additionally, in the control plots,

further six bags of each sort of tea were buried at 17 cm soil depth. After 93 days (3 months) and 189 days (6 months), respectively, half of the GT and RT bags were retrieved from each plot, cleaned and dried at 60°C before dry weight of the teas was determined. The decomposition rate  $k$  and the stabilisation factor  $S$  were calculated according to Keuskamp *et al.* (2013). Total C and N concentration of the teas was determined using an EA 3100 Elementary Analyzer (Euro Vector, Pavia, Italy). The statistical software R (v. 4.3.1; R Core Team, 2023) was used to carry out linear mixed effect models with relative tea mass loss or total C and N concentration as the dependent variable and the interaction of treatment and sampling time as independent variables with block set as random effect.

## Results and discussion

After the 3-months incubation period, GT had lost half of its original mass, while RT had lost only 22 to 26%, but no differences between treatments (control or fertiliser treatments) were detected (Table 1). Similar to our results, Poeplay *et al.* (2016) did not find any effect of mineral N-treatment which, also for our site, could suggest that microbial metabolism was not N-limited.

Table 1. Relative tea mass loss, C and N concentration of tea dry matter and C/N ratio for Green and Rooibos tea after 3 and 6 months, respectively.

Parameter	Green tea		Rooibos tea	
	3 months	6 months	3 months	6 months
relative tea mass loss	0.50–0.52 n.s.	0.51–0.59 n.s.	0.22–0.26 n.s.	0.31–0.39 n.s.
C (g kg <sup>-1</sup> )	465–480 n.s.	490–518 n.s.	483–494 n.s.	507–518 n.s.
N (g kg <sup>-1</sup> )	46–49 n.s.	52–57 n.s.	10–11 n.s.	12–15 *
C/N ratio	9.7–10.3 n.s.	9.0–9.6 n.s.	43.8–47.6 n.s.	33.2–41.6 *

Significance of treatment effect is given after minimum and maximum value of parameter (\* $P < 0.05$ ; n.s., not significant).

After 3 months, the stabilisation factor  $S$  in our study was higher (0.38 to 0.41) compared with values reported by Keuskamp *et al.* (2013) for a Dutch pasture (about 0.14) at a similarly low decomposition rate  $k$  (about 0.012 g day<sup>-1</sup>; Fig. 1). This implies that in our study chemical, physical, climatic, and/or biological effects protected the labile fraction of GT more effectively from mineralisation (Pino *et al.*, 2021).

After 6 months, relative GT and RT mass loss had increased (Table 1) resulting in significant differences between incubation times for RTs of all treatments (data not shown). Only fertilization with high amounts of easily available N (240 kg mineral N ha<sup>-1</sup>) resulted in significantly higher N concentrations of RT compared with RT of lower N-treatments (data not shown). This was in line with a significantly higher decomposition rate  $k$  (Figure 1) suggesting a very active microbial community at later stages of decomposition in this treatment.

In control plots, soil depth did not significantly affect stability or decomposition rate, suggesting that decomposition was similar within the upper 17 cm of soil. This implies that although microbial communities may have differed between depths (Fierer *et al.*, 2003) their efficiency in decomposition was similar.

## Conclusion

In our study, the standard TBI was neither affected by depth nor by N form or fertilizer rate reflecting no depth or N effect on decomposition processes after 3 months. This indicates that for NW German grassland the TBI is not efficient for discriminating between depths or N management. Only after 6 months, decomposition rate was increased by high input with synthetic fertilizer (240 kg N ha<sup>-1</sup>, CAN). Further studies are required to test whether shorter incubation times may capture early N effects.

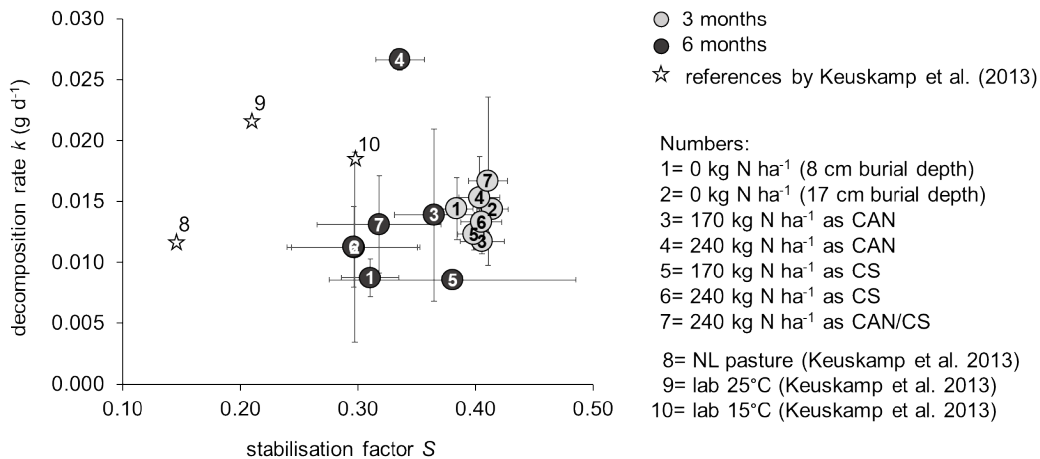


Figure 1. Stabilization factor  $S$  and decomposition rate  $k$  calculated from green tea and rooibos tea after 3 and 6 months of burial in a permanent grassland field experiment in Northwest Germany. CAN, calcium ammonium nitrate; CS, cattle slurry. Error bars represent standard deviations,  $n=28$  for 3 months,  $n=27$  for 6 months.

## Acknowledgements

The authors thank the Ministry for Science and Culture of Lower Saxony (MWK) for funding and M. Kolbeck, A. Janssen, F. Sieve and C. Moldenhauer for field and laboratory help.

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# Overseeding oat into limpoggrass: the effect of trees on animal performance

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## Abstract

The overseeding of cool-season species into dormant warm-season species and tree inclusion in pastures are two potential options for sustainable livelihoods. Our objective was to evaluate the forage and animal production of overseeding oat (*Avena sativa*) into a limpoggrass (*Hemarthria altissima*) pasture in silvopastoral vs. open pastures. A maximum gain per hectare (Gha) of 363 kg live weight (LW) ha<sup>-1</sup> was observed on limpoggrass pasture, over 139 days of grazing, after oat overseeding. The maximum Gha on oat pasture was 174 kg LW ha<sup>-1</sup>, and it was possible to obtain up to 112 grazing days in an area that would be fallow in winter. The effect of trees on forage production and Gha varied over the years, from neutral to negative impact, particularly during periods of drought.

**Keywords:** beef cattle, *Eucalyptus*, *Hemarthria altissima*, shading, silvopastoral

## Introduction

Grassland-based feedstock and tree inclusion are sustainable ways to reduce greenhouse gas emissions associated with animal production. Temperate annual forage species and tropical perennial species are key components of many grassland-based feedstock systems in subtropical regions, enabling pasture use throughout the year. However, they are mainly grown as monocultures. Overseeding of annual cool-season species into dormant warm-season species could improve forage quality and seasonal distribution of biomass production compared to monocultures, and this technique could be especially useful in a small-farm environment, because limited land area places a premium on productivity. However, few studies have investigated the use of winter overseeding species into limpoggrass pastures, particularly in silvopastoral systems. The presence of trees can affect key aspects of overseeding success, because warm-season species, with their root system competing for water and nutrients may compromise germination and growth of cool-season species. In addition to shading, the seedlings of introduced species are protected against frost events in tree-covered pastures, which minimizes temperature variations, so the competition effects may be more significant. The aim of this study was to evaluate the forage and animal production of overseeding oat into limpoggrass pasture in two systems: silvopastoral vs. open pastures.

## Materials and methods

Two side-by-side treatments were evaluated, with and without *Eucalyptus dunnii* trees, with four replicates, at the IDR-Paraná in southern Brazil. Trees were planted in 2006 at 3×14 m spacing (238 trees ha<sup>-1</sup>). During the current study, the spacing was 9×28 m after thinning. ‘Florida’ limpoggrass pastures were established in 2007. Oat was established by direct seeding, at 60 kg seed ha<sup>-1</sup> on 17 June 2019 and 27 May 2020 and of 90 kg ha<sup>-1</sup> on 13 May 2021. Liming, P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O, applied during winter, and N fertilization rates were calculated to provide non-limiting nutrition. Three topdressing N fertilizations were done using 60 kg N ha<sup>-1</sup> each (approx. 40 days after the oat sowing, at the beginning of the stocking period with limpoggrass, and approx. 50 days later). Each plot received three tester (Purunã beef heifers, average age of 10 months) and a variable number of animals (put-and-take method, Mott and Lucas, 1952) to maintain the desired sward height (SH) of 20 cm for both oat and limpoggrass under continuous stocking. The winter stocking seasons with oat corresponded to 52 days of grazing in 2019 (August to October), 84 days in 2020 (July to October) and 84 (silvopastoral) or 112 (open pasture) days in 2021 (June to

September/October). The winter stocking season ends when it is no longer possible to maintain 20 cm of SH. The short grazing period in 2019 was due a late sowing due to factors unrelated to our study. Summer stocking seasons corresponded to 139 (2019–2020) or 132 (2020–2021) days of grazing (October to March). The SH was measured at 100 points per plot every 15 days. Total herbage accumulation (THA) and stocking rate (SR) were estimated according to Kunrath *et al.* (2014). Average daily gain (ADG) was obtained by dividing weight gain by grazing days, and gain per hectare (Gha) by multiplying the number of animals per day by the ADG of the testers. Animals were weighed every 28 days after fasting from solids for 15h. All animals had unrestricted access to water and mineral supplements. The software Statgraphics Centurion XV was used for ANOVAs with systems (open pasture vs. silvopastoral) and years, as repeated factor, considered fixed effects and replicates the random effect.

## Results and discussion

During winter, the interaction between systems and years was significant for THA ( $F=4.46$ ,  $P=0.0302$ ), Gha ( $F=8.03$ ,  $P=0.0043$ ) and SR ( $F=12.22$ ,  $P=0.0007$ ). Differences between systems for THA were only significant in 2021, with a greatest THA for the open pasture, compared to the silvopastoral (Table 1). The SR and Gha were significantly greater in open pasture in winter 2019 and 2020 than in silvopastoral, with no differences in 2021 (Table 1). Significant differences were observed only among years for SH ( $F=19.56$ ,  $P<0.001$ ) and ADG ( $F=5.43$ ,  $P=0.0150$ ). The SH ranged from  $17\pm 0.51$  (2020) to  $21\pm 0.53$  cm (2019), and the ADG ranged from  $0.46\pm 0.046$  (2019) to  $0.68\pm 0.064$  kg LW animal<sup>-1</sup> day<sup>-1</sup> (2021). Regardless of the system, the maximum total Gha observed in winter ( $147$  kg LW ha<sup>-1</sup>) was far below the potential of annual cool-season pastures (i.e., between 370 and 651 kg LW ha<sup>-1</sup> as reported in many studies, e.g., Kunrath *et al.*, 2014). However, up to 112 days of grazing could still be obtained in an area that would be fallow in winter. During summer, no significant differences in ADG ( $0.53\pm 0.023$  kg animal<sup>-1</sup> day<sup>-1</sup>) were observed. Greater values were observed for THA and SR in the last summer, and in open pasture for SR (Table 2). Pontes *et al.* (2021) found a maximum THA of 11 t DM ha<sup>-1</sup> for limpopgrass, which is consistent with values observed here in the last summer (Table 2). The interaction between systems and years was only significant for Gha ( $F=6.66$ ,  $P=0.0297$ ). Differences in Gha were only found in the first summer, with a lower value for the silvopastoral ( $1.8\pm 0.20$  kg LW ha<sup>-1</sup> day<sup>-1</sup> or total Gha of  $243\pm 27.9$  kg LW ha<sup>-1</sup>) compared to open pasture ( $2.6\pm 0.11$  kg LW ha<sup>-1</sup> day<sup>-1</sup> or total Gha of  $363\pm 15.3$  kg LW ha<sup>-1</sup>). Hence, the moderate shading level provided by trees (i.e.,  $39\pm 0.87\%$ ) affected animal and forage production, but this effect of trees varied over the years. For instance, rainfall was 24% lower than historical means in the first summer, i.e., between November and March. On the other hand, in the second summer, cumulative precipitation was 8% higher than historical means (data not shown). Thus the decline in animal production throughout the first summer may denote an effect of drought more intense in silvopastoral systems, probably revealing a greater competition effect for water between tree-forage species, especially in shallow soils. Beef cattle production expressed as ADG and Gha on open limpopgrass pasture previously overseeded with oat was similar to that obtained for limpopgrass monoculture (Pontes *et al.*, 2021).

## Conclusions

The effect of trees on animal production on oat overseeding into a limpopgrass pasture varies over the years, from neutral to negative, especially in drier periods.

## Acknowledgements

We thank Agrisus-FEALQ (PA 2889/19, n° 10376-4) for financial support. Both authors are grateful to CNPq for the fellowship (304426/2022-9 and 315529/2020-2).



Table 1. Mean±standard error ( $n=4$ ) per year (2019, 2020 and 2021) and per system (open pasture vs. silvopastoral) for total herbage accumulation (THA), gain per hectare (Gha) and stocking rate (SR) on *Avena sativa* cv. Esmeralda pasture overseeded into *Hemarthria altissima* cv. Florida.

	2019	2020	2021
Open pasture			
THA (kg DM ha <sup>-1</sup> )	1047±218.1 Ab	4102±673.9 Aa	4172±247.0 Aa
Gha (kg LW ha <sup>-1</sup> day <sup>-1</sup> )	1.5±0.11 Aab	2.1±0.12 Aa	1.3±0.21 Ab
SR (kg LW ha <sup>-1</sup> day <sup>-1</sup> )	714±68.7 Aa	716±20.5 Aa	385±20.2 Ab
Silvopastoral			
THA (kg DM ha <sup>-1</sup> )	660±101.8 Ab	3515±536.4 Aa	1617±507.3 Bb
Gha (kg LW ha <sup>-1</sup> day <sup>-1</sup> )	0.73±0.106 Bb	1.4±0.10 Ba	1.5±0.09 Aa
SR (kg LW ha <sup>-1</sup> day <sup>-1</sup> )	422±84.5 Ba	507±41.7 Ba	561±37.8 Aa

The table shows the system×year interaction. Means followed by the same letter are not significantly different according to the Tukey test ( $P<0.05$ ). Uppercase letters compare means per systems, for each variable, in the same year and lowercase letters (within rows) compare years in the same system. DM, dry matter; LW, live weight.

Table 2. The effects of year ( $n=8$ ) and system ( $n=8$ ) on sward height (SH), total herbage accumulation (THA) and stocking rate (SR) during summer (on *Hemarthria altissima* cv. Florida pasture).

	2019–2020	2020–2021	F	P
Year effect				
SH (cm)	23±0.70	25±0.44	5.51	0.0408
THA (kg DM ha <sup>-1</sup> )	8146±619.7	12684±1257.1	12.18	0.0058
SR (kg LW ha <sup>-1</sup> day <sup>-1</sup> )	1247±77.5	1764±108.4	25.97	0.0005
System effect				
SR (kg LW ha <sup>-1</sup> day <sup>-1</sup> )	Open pasture 1656±108.9	Silvopastoral 1355±136.4	8.74	0.0144

F and P, F-ratio and P-value, respectively, for the year and system effect.

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# A GEO-based digital calendar for real time and site specific registration of grassland management

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## Abstract

In the Netherlands, grassland management is typically registered on a grassland calendar. The last years digital versions of the grassland calendar became available. The major challenge is to register dynamic use of paddocks during a growing season. Combining registered grassland management with geosynchronous equatorial orbit (GEO) data, e.g. data from satellites and machines is, however, difficult. To overcome these issues a digital grassland calendar was developed based upon GEO data. Polygons are used to define the paddock (or part of) for which management is registered. These polygons can be created at any moment during the growing season, which makes it possible to dynamically register management in parts of the paddock. The Grassland Calendar is a registration tool, providing an overview of the registered data with the traditional calendar view. Since it is a digital web-based calendar, registrations are digitally available and can be shared with others. Furthermore, the data can be used in other applications and advice models. The Grassland Calendar is available at the data service platform Farmmaps (<https://www.farmmaps.net/en>). The Grassland Calendar is developed in cooperation with farmers and private companies in the projects 'Precisielandbouw 4.0' and 'Netwerk Praktijkbedrijven' which are financially supported by the Dutch Ministry of Agriculture.

**Keywords:** grassland management, registration, GEO data, web-based

## Introduction

To support farmers in monitoring, evaluating, and steering their on-farm grassland management, digital registration of actual grassland use is required. In the Netherlands, grassland use is typically registered on a grassland calendar. Several versions of the grassland calendar, both paper and digital, are available. In these calendars, paddocks where grassland management is executed have to be listed at the start of the growing season. This makes it difficult to dynamically register data in parts of a paddock during a growing season. Another drawback of existing calendars, both paper and digital grassland calendars, is that the location of the paddocks is not recorded. Therefore data from the calendars cannot easily be connected with GEO referenced data, like weather data or data from satellites, machines, sensors and cows equipped with GPS collars.

However, by creating a grassland calendar based on GEO data, several restraints of current digital grassland calendars can be resolved, improving the registration of actual grassland management. The ability to create polygons with Global Position Systems coordinates throughout the year, for definition of the area concerning grassland management being carried out, makes it possible to register in parts of a paddock during the growing season. When grassland management is registered based upon GEO data, grassland management can easily be connected with GEO referenced data. This not only increases the technical options for tools to support farmers, but also reduces farmers' registration pressure. Therefore a digital GEO data and web-based grassland calendar was developed.

## Materials and methods

To obtain the requirements for a digital grassland calendar several sessions with stakeholders were organized. These included dairy farmers (the anticipated end-users of the tool) and also grassland researchers with many years of experience in analysing grassland data and developing tools to support farmers in optimizing grassland management. Based on these sessions, information was gathered on both functional requirements (e.g. regarding the type of management that needs to be registered, the level of details) and the non-functional requirements, like the user experience. From the requirements mock-ups were made to visualise input and output screens and these mock-ups discussed with the stakeholders. All this information was used to develop a first version of the application. Since the stakeholder group was limited ( $n=10$ ) statistical analyses are not a significant part of this research.

## Results and discussion

The requirements for the grassland calendar application (app) are to have the ability to manually enter data, get an overview of the registered data, be able to export the data to other advice modules, download the data for further analysis, define subplots during the growing season within existing paddocks, registration of manure and feed storages, automatic registration of grassland management with data from machines and have the ability to connect with existing external data sources (e.g. data on animals, machine data, weather data, soil data, and data from laboratories and satellite data). Furthermore, one of the most frequently mentioned requirements is the ease of use. It should be 'easy' to use; the manual input of the end user should be as little as possible. The app should be able to be used on both a smartphone as well as on a laptop/personal computer. The development of this first version is done within a research project, with the aim to develop a minimal viable product.

The opening screen of the app presents an overview of the farm's outline and provides access to the windows to register grazing, fodder conservation, fertilizing, irrigation and measurements. The paddocks identified at the start of the growing season are also shown as the sub-paddocks identified during the grazing season. Both are characterised by Global Position Systems coordinates (polygons) and referred to as 'treatment zones' in the app. Furthermore, the opening screen also gives access to the window to create new treatment zones (Figure 1). For each treatment zone an overview of the registered activities is given in the calendar (Figure 1) and within this view there is also access to the registration windows.

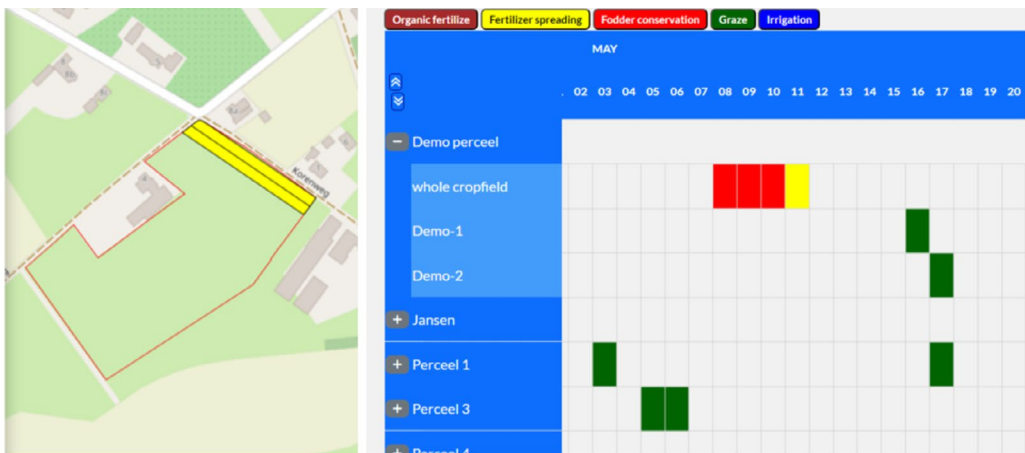


Figure 1. Treatment zones in a paddock (left) and calendar view pc (right).

Treatment zone(s), date and activity are obligatory fields when registering data. For each recording entered, additional information, relevant to the activity, can be registered. The registered data are stored in a database. The data can be exported by the end user as a csv or pdf file. In addition, via an application programming interface (API), the data can be used as input for other applications generating the necessary management information farmers need for monitoring, evaluating, and steering farmers' on-farm grassland management.

The app is available at smart farming platform Farmmaps (Been *et al.*, 2023) making use of the technical services this platform offers and the policies regarding handling data. The app is available in both Dutch and English: <https://www.farmmaps.net/en>. Since the app is GEO data and web-based, use is not restricted to the Netherlands. This version is a minimal viable product, which is still in development with respect to functionalities, technique and user friendliness. Until now the focus was to implement the basic functionalities. This version of the app was, amongst others, used by farmers in the project 'Netwerk Praktijkbedrijven'. Within this project the registered data were shared between farmers and the researchers via an API. Furthermore, user feedback from the farmers was collected to improve the app. The feedback mainly concerned issues regarding user friendliness when registering data, like handling of more complex treatment zones. Furthermore, farmers would also like to extend the grassland calendar for registration of their management of non-grass fields. The next steps in the development of the app are aimed at establishing links with other data sources, such as lab analyses, machine data and data on individual animals. Further improvements for creating treatment zones are foreseen, as well as improving the registration of strip-grazing and zero-grazing. The option of creating new treatment zones, being part of a paddock, during the growing season, results in a change of the minimal management units; which is no longer necessarily the whole paddock.

## Conclusion

The Grassland calendar is a GEO data and web-based app to register grassland management and available via <https://www.farmmaps.net/>. During the growing season, treatment zones can be created to register grassland management in parts of a paddock. The registered data are available as input for other applications, to generate management information for monitoring, evaluating, and steering farmers' on-farm grassland management. The app is, in cooperation with farmers and other stakeholders, currently still in development with respect to functionalities, links with other data sources and user friendliness.

## Acknowledgement

The Grassland Calendar is developed in cooperation with private companies and farmers in the projects 'Precisielandbouw 4.0' and 'Netwerk Praktijkbedrijven' which are financially supported by the Dutch Ministry of Agriculture.

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# Exploring the phenotypic diversity of alfalfa (*Medicago sativa* L.) in Lithuanian acidic soils: Insights from a cluster analysis

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## Abstract

Alfalfa (*Medicago sativa* L.) is a perennial forage that is widely adapted to favourable environmental conditions. It thrives on wide range of soils, including acidic soils. The experimental site was located in the western part of Lithuania. Cultivars of alfalfa (*M. sativa*, and subsp *M. varia* and *M. falcata*) were sown in 2018. Productivity and structural analysis traits were evaluated during the period of 2019–2023. In soil without mobile aluminium (Al), results showed that the cultivars of *M. varia* differed very little in stem thickness compared to *M. sativa*. In soil with mobile Al concentration (1.8–14.7 mg kg<sup>-1</sup>), the cultivars of *M. falcata* showed significant differences. These differences were observed in the height before flowering and leaf weight when compared to *M. sativa* and *M. varia*. The cultivars of *M. falcata* differed by the stem weight compared to *M. sativa*. A cluster analysis showed that *M. varia* distinguished itself by the highest stem weight, fresh and dry matter yields. The cultivar 59-109 (*M. varia*) had the highest number of stems, height before flowering and fresh matter yield.

**Keywords:** *Medicago* species, productivity trait, acidic soil, grass component, mobile aluminium

## Introduction

Alfalfa (*Medicago sativa* L.) is one of the most important forage crops in the world because of its high nutritive quality, high yield, resistance to frequent cutting, adaptation to various climatic and soil conditions (Zhang *et al.*, 2019). Grasslands with different legume species also provide more profitable production and better forage quality. Soil pH affects all phases of plant growth, disease resistance and resistance to low temperature, crop longevity, forage yield and quality (Tomić *et al.*, 2012). Different species of alfalfa or cultivars are sensitive to acidic soils and differently respond to mobile aluminium. Toxic micro-molar concentrations of soluble Al inhibit the growth and functions of roots and affect the functions of other plant parts, resulting in a large yield reduction (Yang *et al.*, 2013). The different phenotyping methods suggest that genotypes have different tolerance to mobile Al. Cluster analysis is an effective tool to distinguish diversity based on morphological traits among genotypes (Moghaddam *et al.*, 2011). The aim of this study was to investigate the response of the productivity traits and structural analysis of *M. species* to mobile Al concentrations and to select the most promising cultivars that can grow on land affected by mobile Al toxicity.

## Materials and methods

Experimental material consisted of 14 cultivars of *M. sativa*, *M. varia* and *M. falcata* of different origin, which were established in 2018 on the experimental site in Vėžaičiai Branch of Lithuanian Research Centre for Agriculture and Forestry. The experimental site was located in the western part of Lithuania (55°70' N, 21°49' E). The cultivars of alfalfa were assessed in the period of 2019–2023. The naturally acidic soil of the experimental site was a Retisol with pH<sub>KCl</sub> 4.4 and mobile Al concentration ranged from 1.8–14.7 mg kg<sup>-1</sup>. The cultivars of alfalfa were sown using a randomised block design with four replications to evaluate the productivity traits and structural analysis components: the fresh and dry matter yields (FMY, DMY, t ha<sup>-1</sup>), plant height before flowering (PH, cm), stem number (SN, m<sup>2</sup>),

weight of stems, leaf, and inflorescences (SW, LW, IW, g) and stem thickness (ST, mm) of plants. The fresh samples (1000 g) were taken from each cultivar plot, dried at 105°C and weighed. The one-factor analysis of variance (ANOVA) was used followed by Fisher's least significant difference at the  $P < 0.05$  significance levels. Using hierarchical cluster analysis we assessed how the productivity and structural analysis traits influenced the performance of the different alfalfa cultivars. Statistical programming software for all statistical analyses was SAS Enterprise (2011).

## Results and discussion

Based on the study period of 2019–2023, *Medicago* were very similar in their productivity traits and components of structural analysis in the soil with and without mobile Al (Table 1). The regional climate and soil conditions were quite variable; this resulted in different plant productivity and structural analysis components (Luo *et al.*, 2016). Leaf and stem weight is strongly related to forage quality (Julier *et al.*, 2000). In the soil without mobile Al, *M. varia* and *M. falcata* had higher stem weight compared to the acid soil with mobile Al, by 2.4 and 4.6%, respectively. In the soil with mobile Al, the leaf weight of *M. varia* and *M. falcata* was higher by 1.8 and 4.0%, respectively (Table 1). The populations of alfalfa were divided into three cluster groups, based on nine morphological traits (Touil *et al.*, 2009).

Table 1. Productivity traits and structural analysis of *Medicago* species, 2019–2023

	<i>Medicago sativa</i>	<i>Medicago varia</i>	<i>Medicago falcata</i>
SN (m <sup>2</sup> )			
0.0	477.1 a	443.8 a	460.7 a
1.8–14.7	408.8 a	432.6 a	422.0 a
PH (cm)			
0.0	116.4 a	112.8 a	118.3 a
1.8–14.7	106.7 b	107.6 b	99.8 a
FMY (t ha <sup>-1</sup> )			
0.0	88.4 a	92.6 a	84.4 a
1.8–14.7	73.8 a	79.5 a	74.0 a
DMY (t ha <sup>-1</sup> )			
0.0	24.0 a	24.8 a	23.2 a
1.8–14.7	21.8 a	21.7 a	21.2 a
SW (%)			
0.0	65.8 a	67.2 a	66.9 a
1.8–14.7	65.1 b	64.8 ab	62.3 a
LW (%)			
0.0	32.5 a	30.9 a	31.4 a
1.8–14.7	32.6 a	32.7 a	35.4 b
IW (%)			
0.0	1.7 a	1.8 a	1.7 a
1.8–14.7	2.3 a	2.5 a	2.3 a
ST (mm)			
0.0	3.3 b	3.1 a	3.2 ab
1.8–14.7	3.3 a	3.1 a	3.2 a

Averages followed by the same letter in column do not differ from each other ( $P < 0.05$ , Fisher's test).

In our study, a cluster analysis of *Medicago species* showed that *M. varia* was included in cluster 2 (Figure 1A). *M. varia* had the highest stem weight, fresh and dry matter yield. In cluster 1, *M. sativa* and *M. falcata* had the highest leaf weight, stem thickness and number of stems. Cluster analysis of the cultivars of alfalfa showed that the cultivars were included in 9 clusters (Figure 2B). Cluster 1 consisted of six cultivars Antané, Malvina, Ludelis (*M. sativa*), Biturè, Jõgeva 118 (*M. varia*) and AJC437 (*M. falcata*). Stem and leaf weight and fresh matter yield of these cultivars were similar to the cultivars in cluster 8. Inflorescence weight, stem number, height before flowering and dry matter yield of these cultivars were also similar to clusters 9, 3, 6 and 2, respectively. In cluster 2, the cultivar PGR10249 (*M. sativa*) was similar to cluster 4 and 5 due to stem thickness. In cluster 3, the cultivar PGR12489 (*M. falcata*) had the lowest stem thickness, and its dry matter yield was similar to cluster 9. In cluster 4, the cultivar Žydrūnė (*M. varia*) was similar to clusters 9 and 5 in terms of stem weight and dry matter yield, respectively. In cluster 5, the cultivar 8701 (*M. falcata*) had the highest leaf weight. In cluster 6, the cultivar AJ2024 (*M. sativa*) had the highest stem weight.

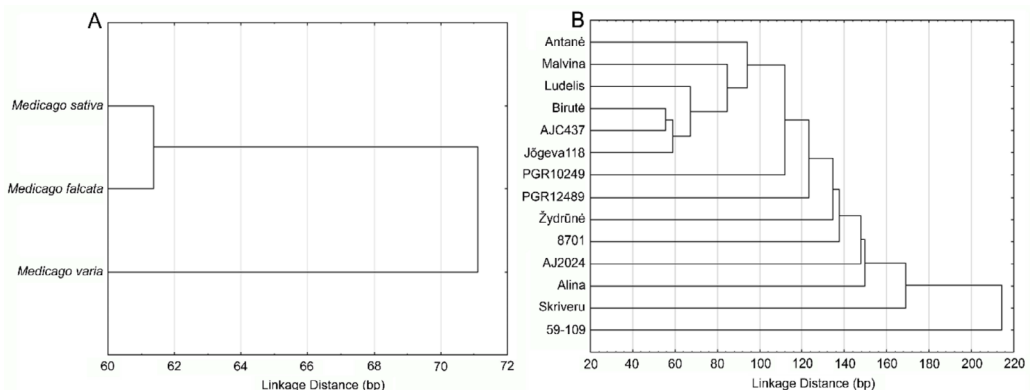


Figure 1. A cluster analysis of alfalfa (*M. sativa*, *M. varia* and *M. falcata*) in the acidic soil (A), and alfalfa cultivars (B).

In cluster 7, the cultivar Alina (*M. sativa*) had the highest inflorescences weight and stem thickness. In cluster 8, the cultivar Skriveru (*M. varia*) had the highest dry matter yield. In cluster 9, the cultivar 59-109 (*M. varia*) had the highest stem number, height before flowering and fresh matter yield.

## Conclusion

The results showed that the tested *Medicago species* varied in terms of productivity and structural analysis traits in the acidic soil, with the least toxic concentration of mobile Al (1.8–14.7 mg kg<sup>-1</sup>). *Medicago varia* was the most suitable for acidic soils and had the highest weight of stems, fresh and dry matter yields. The cultivar *M. varia* 59-109 differed mostly by the highest number of stems, height before flowering and fresh matter yield.

## Acknowledgement

The long-term programme ‘Genetics, Biotechnology and breeding for plant biodiversity and innovative technologies’ is implemented by the LAMMC Institute of Agriculture.

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# Using Digital-Surface-Models and GNSS for monitoring shade on dairy cow pasture for heat stress mitigation

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## Abstract

Providing shade in pastures can mitigate heat stress in dairy cows, which is important for animal welfare. Locating shaded areas in paddocks at specific times, coupled with global navigation satellite system (GNSS) tracking of cows, allows research into shade-seeking behaviour in different settings. A simple shade quantification method was developed using Digital Surface Models (DSM) to calculate shaded areas at a given time and place. The validation showed substantial agreement between calculated and visually observed shade with an accuracy of 82%; this confirms the reliability of our approach. Cows were tracked on two commercial farms with devices combining GNSS and internal-motion-units to obtain the cow location and the expressed behaviours. The likelihood of a cow being in a shaded area increased with rising temperature-humidity-index (THI), although heat stress was moderate with THI values not exceeding 73. The shade seeking was more expressed in sunny conditions as the effect of shading decreases with increasing cloud cover. The odds were lower when the cows were grazing compared to the spatially static behaviours of ruminating and resting. Our key results demonstrate the possibility of researching heat stress mitigating behaviours of cows in on-farm settings with a simple but reliable approach using DSM and GNSS trackers.

**Keywords:** temperature-humidity-index, digital surface model, grazing, GNSS

## Introduction

Heat stress is a concern for grazing livestock, and high yielding dairy cows especially can be severely affected by heat stress. Providing sufficient shade on pastures is a way to mitigate heat stress by reducing the impact of sun radiation (Schütz *et al.*, 2014). Shade on pastures is usually provided by hedgerows or single trees; in future, possibly also by solar systems. This work presents a simple solution for quantification and location of shaded areas on paddocks from DSMs at any given time. This shade quantification was set to use by observing shade seeking behaviour of dairy cows during different levels of heat stress while grazing, ruminating and resting. Different degrees of cloudiness were considered as these might affect the behaviour of cows during heat stress. Our hypotheses were: (i) predicting shaded areas in paddocks by DSM is reliable and accurate; (ii) it is more likely to find cows in shaded areas with increasing heat stress, (iii) cloudiness and cow behaviour affect shade-seeking.

## Material and methods

The study was conducted on two commercial dairy farms in Germany (North-Rhine-Westphalia and Lower Saxony) in 2022. Data processing and analysis was conducted in R 4.2.1. The DSMs were computed from publicly available laser scanner data (LGLN 2016 and GDI NW 2016) with a spatial resolution of one metre. Shadow casts can be calculated from a DSM for a given time and position by using the respective sun angle and sun elevation in the 'doshade' function (package 'insol'; Corripio, 2021). This approach only accounts for 2.5 dimensions because the DSM represents only the height of an object without considering covering effects and therefore not the shade underneath the object. Thus, the shaded area was expanded to include the area of the shading objects as well. The validation of this approach was conducted with the collection of  $n=798$  randomly selected time- and georeferenced points with a real-time kinematic supported GNSS device (ardusimple, Andorra, Spain) and visual observations on the

actual presence of shade (399 points observed as shaded). The non-shaded points were selected spatially close to shaded areas (mean $\pm$ SD 6.3 $\pm$ 4.5 m) to avoid the simple situation of an open field without any shade. For monitoring of cow behaviour approximately 25% of the respective herds were tracked with a self-assembled tracker including a GNSS module and Internal-Motion-Unit (IMU) operated by an Arduino microcontroller. The tracking was conducted in three separate runs per farm, resulting in a total tracking time of 16 days. The cow behaviour (rest, ruminant, graze) was classified from IMU data with machine learning and visual observations as reference (Obermeyer and Kayser, 2023). The spatial location and the behaviour were aggregated on a one-minute resolution. This approach allowed for the calculation of the shade situation at every cow position. Only the time between dawn and dusk was included in the analysis as no relevant shade is present during night time. The heat stress was quantified by the THI (NRC, 1971). The data for the calculation of the THI and for the classification of cloudiness (temporal resolution 1 h) was obtained from the German weather services (distance to farms 27 km and 15 km) and from temperature ( $^{\circ}$ C) and relative humidity (%) recordings on the farms (temporal resolution 60 s). For statistical evaluation, the binary outcome of a cow being in shade was modelled with a generalized linear mixed effects model of underlying binomial distribution with the fixed effects behaviour (resting, ruminating, grazing), cloudiness (sunny, cloudy) and the covariate THI in interaction with the two factors. The identity of the cow, the sampling occasion and farm were included as nested random effects.

## Results and discussion

The validation of the calculation of shaded areas showed a substantial agreement between observed and predicted values with a Cohen's Kappa of 0.65 (Landis and Koch, 1977). The overall accuracy was 0.82 with a 95% confidence interval (CI) of [0.80; 0.85]. The approach tended to classify false positive (non-shaded as shaded). This resulted in a comparably low specificity of 0.71. At the time of the highest THI values in each run, the farms provided an average $\pm$ SD of 66.1 $\pm$ 33.7 m<sup>2</sup> and 59.5 $\pm$ 77.8 m<sup>2</sup> of shaded area per cow in the daytime paddocks. Schütz *et al.* (2014) pointed out that the minimum shaded area of 2 m<sup>2</sup> per cow allows a location in shade for every cow in a dairy herd. On two out of eight observed paddocks the shaded area per cow was below this threshold at the time of highest heat stress, which may have influenced our findings as it was not possible for the whole herd to find a shade at any one time. Overall, we found an increase in the probability of a cow being located in a shaded area with increasing THI values; this effect was more pronounced under sunny conditions (Figure 1). The influence of the behaviours on the odds ratio change along the THI was small. Under cloudy conditions, the increases of odds ratios for a one-unit increment in THI were 1.069 CI[1.057;1.08], 1.102 CI[1.09;1.113], and 1.094 CI[1.089;1.099] for resting, ruminating and grazing, respectively. Under sunny conditions the increase of the odds ratio along THI averaged across behaviours was stronger (1.123 CI[1.112;1.133]). A THI of >72 is often considered as exceeding the comfort threshold of dairy cattle manifesting in, e.g. rising body temperatures and respiration rates (Polsky and von Keyserlingk, 2017). This indicates that true heat stress situations only occurred rarely in this study, with a mean $\pm$ SD THI of 63.5 $\pm$ 4.8 and a maximum of 73.7. Our findings agree with this, as the probability was close to 0.5 for THI values of 72 in sunny conditions for resting and ruminating. This allows the assumption of even higher probabilities of cows seeking shade in situation with heat stress beyond the THI threshold of 72. The different intercepts show an influence of the behaviour, which indicates general lower odds for finding cows in shade during grazing and explains the rise of probabilities at lower THI values for ruminating and resting (Figure 1). Therefore, when exploring heat stress mitigation strategies, a differentiation of behaviours is important. In many grazing settings few shaded areas are available. Cows cannot choose deliberately to graze in the shade, while during rumination and resting the cows can seek shade if enough shaded area is provided. This could be different in silvopastoral systems or with solar panels. Methodically, for extended understanding of heat stress situations on pasture, additional heat stress mitigating effects like wind exposure (cooling effect of wind) should be considered.

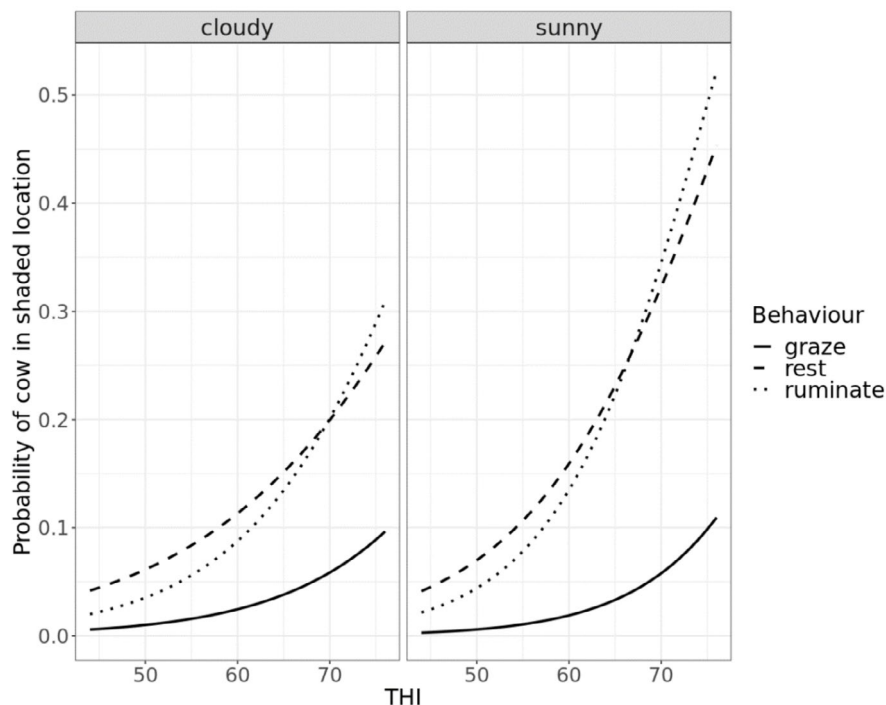


Figure 1. Probability of a cow being located in a shaded area in sunny or cloudy conditions in context of the temperature humidity index (THI) while grazing, ruminating and resting.

## Conclusion

We showed that it is possible to undertake research on shade seeking of cows by combining the definition of the shaded areas of a paddock at a given time by DSMs and the location of cows obtained from GNSS. The approach was set to use in an on-farm setting and we found increased shade-seeking of the cows with increasing heat stress, while this was less expressed in cloudy conditions and during grazing. This implies that sufficient shade should be provided in heat stress situations.

## Acknowledgement

This work is financially supported by the BMEL granted by the BLE (No. 2819MDT100).

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# Site-specific nitrogen management: N response and N uptake as basis for variable rate fertilization

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## Abstract

Precision nitrogen (N) fertilization using real-time spectral crop sensing is well established. In particular, real-time variable rate N (VRN) fertilization enables the application of adapted amounts of N to cover site-specific N demand. Fertilizer inputs are optimized, leading to improved nitrogen use efficiency and increased profitability of the crop production system. This study evaluates grass sward parameters using remotely sensed spectral data and how this information can be turned into in-field spatially VRN application. A trial with 45 sets of four plots, each with varying N rates, was conducted on a grass sward in Finland. This so-called chessboard trial design allowed the calculation of 153 N response functions. Handheld N-Sensor measurements and grass samples were taken during three cuts per year from 2019 to 2022. The results showed that spectral sensing reliably estimates N uptake in grass swards with high spatial resolution. As N uptake is closely related to optimum site-specific N rates, this enables the development of VRN fertilization algorithms for more efficient and environmentally sound fertilizer use. Observed differences between cuts and years and their impact on the applicability and accuracy of the derived algorithms are discussed.

**Keywords:** variable rate nitrogen, grass, N response, N-Sensor, chessboard trial, algorithm

## Introduction

Forage crops are of great importance for ruminant livestock, enabling the production of high-quality human food from such production systems. To maintain economic profitability and reduce emissions of greenhouse gases, efficient and precise site-specific nitrogen management in grassland is a key factor. One technology that has been developed in response to this challenge is VRN (King *et al.*, 2021). Unlike arable cropping systems, grasslands are subject to changes in composition, with the occurrence and abundance of plant species varying over time. Grass swards are highly sensitive to water stress, interacting with weather conditions across all cuts in one or more years. These circumstances make it difficult to establish the conventional precision farming applications from arable crops to grassland (Jasper, 2017). Only a few studies have reported site-specific VRN algorithms: for the United Kingdom (Berry *et al.*, 2017), Germany (Gnyp *et al.*, 2020), and New Zealand (King *et al.*, 2021). For this reason, an experiment was set up on a field with varying soil conditions, using a so-called chessboard trial design that allows the calculation of multiple N response functions across the experimental field, per cut and for several years with various weather conditions.

## Materials and methods

A nitrogen fertilizer experiment was carried out on a timothy-dominated and intensively managed grass sward situated in Kotkaniemi, Finland (60°22'35" N, 24°23'16" E) for four consecutive years from 2019 to 2022. A 'chessboard' trial set up was used, having 45 replicated sets of four plots (each 6 m by 6 m) with increasing N rates of 0 (N1), 60-40-20 (N2), 120-80-40 (N3) and 180-120-60 (N4) kg N ha<sup>-1</sup>, applied as calcium ammonium nitrate (CAN, 27% N) to cut1-cut2-cut3, respectively, in every year. The trial was fertilized using a spreader with a defined working width, applying alternating fertilizer rates of 0 and 1/4 of the dose in crosswise direction, and 0 and 3/4 of the dose lengthwise to create the chessboard design (Berry *et al.*, 2017). In total, 180 plots were established and measured with a handheld version of the Yara N-Sensor® in the spectral range of 400–1000 nm at 10 nm spectral resolution. A spectral vegetation

index (VI), using the simple ratio of near infrared and red edge, named S1, was calculated (Link and Jasper, 2003). The harvest was done with a mower by cutting a sampling area of 0.47 m×5.53 m, i.e., 2.6 m<sup>2</sup> per plot. Fresh grass yields were weighed directly in the field while dry matter yields were determined at the experimental station after drying the samples in an oven at 65 C° to constant weight. N content was analysed in the laboratory using the Kjeldahl method and N uptake calculated by multiplying N content with dry matter yield. This trial setup enables the calculation of in total 153 N response curves (2<sup>nd</sup> order polynomial, for details see Link and Jasper, 2003) and the derivation of as many economic optimum N rates ( $N_{opt}$ , calculated with a price ratio between N fertiliser and grass dry matter yield of 8.5) across the trial area for each cut. A price of €0.85 kg<sup>-1</sup> N and forage price of €100 Mg<sup>-1</sup> were assumed for Finnish conditions (Vainio, 2022). The plot-specific N recommendations ( $N_{rec}$ ) were calculated by equation 1, subtracting the applied N rate (N1–N4) from the respective economic optimum N rates ( $N_{opt}$ ).

$$N_{rec} = N_{opt} - N_{applied} \text{ (N1–N4)} \quad (1)$$

## Results and discussion

As reported in previous studies by Portz *et al.* (2017) and Gnyp *et al.* (2020), the N-Sensor value S1 was found to be a good estimator for N uptake, with one exception during the growth of cut2 in 2021 due to severe drought (Table 1). The economic  $N_{opt}$  varied widely across the field, with mean values of approx. 100 kg N ha<sup>-1</sup> for cut1 and approx. half that amount for cut3, reflecting varying soil conditions and topography. The results are comparable to the study by Preece (2022), who reported about decreased  $N_{opt}$  ranges during consecutive cuts. In contrast to cut1,  $N_{opt}$  varied more across the four years at cut2, leading to a less accurate N recommendation algorithm across all years (Figure 1, right) and with different equations per single year. On the other hand, the algorithm is more stable for cut1, even though yearly effects are visible (Figure 1, left). The algorithm indicates how much N is recommended relatively to  $N_{opt}$  when a specific N uptake was measured.

## Conclusion

This study shows that N uptake estimates derived from remote sensing data enable the development of sensor based VRN fertilization algorithms. Based on the results, the development of cut-specific VRN algorithms is recommended. While the relationship between N uptake and recommended N rate is rather stable for cut1, the most relevant for high quality forage production in the Nordics, highly variable annual growing conditions make it more challenging for cut2.

Table 1. Variability of N uptake ( $R^2$  is related to measured S1 value from the N-Sensor) and economic  $N_{opt}$  during the three cuts in 2019–2022.

	2019			2020			2021			2022		
	Cut1	Cut2	Cut3	Cut1	Cut2	Cut3	Cut1	Cut2	Cut3	Cut1	Cut2	Cut3
N uptake (kg N ha <sup>-1</sup> )												
Min	10	2	1	9	4	3	12	3	4	15	3	2
Max	143	73	80	149	111	71	125	66	68	132	57	62
Mean	58	18	14	61	41	27	59	20	29	61	18	23
$R^2$	0.75	0.81	0.85	0.88	0.91	0.91	0.83	0.06	0.81	0.92	0.81	0.62
Economic $N_{opt}$ (kg N ha <sup>-1</sup> )												
Min	38	41	27	20	40	5	25	5	14	40	15	8
Max	190	160	100	191	164	101	193	101	116	121	134	115
Mean	102	92	46	93	92	47	105	38	53	70	52	29

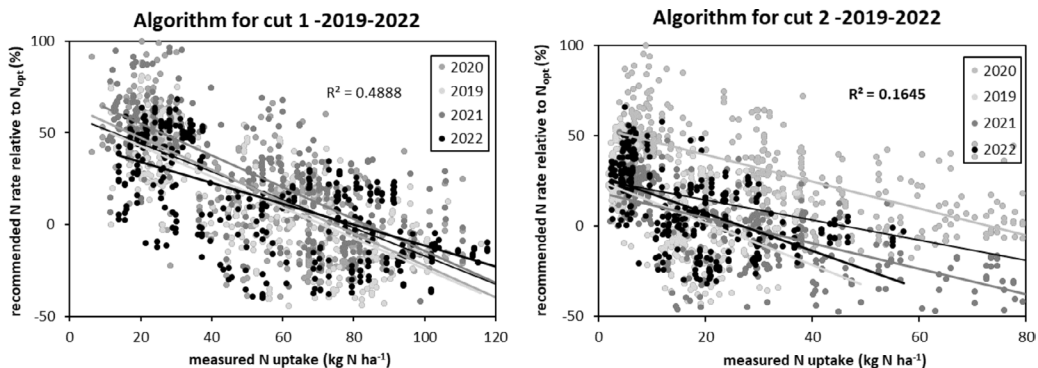


Figure 1. Relationship of N uptake and recommended N rate relative to  $N_{opt}$  for cut1 and cut2.

## Acknowledgement

We acknowledge the field team of Yara's Kotkaniemi Research Farm in Finland for collecting the data from 2019 to 2022.

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# Reducing nitrogen surplus on grassland farms by incorporating legumes

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## Abstract

The Clover150 farm project, spanning five years, was established in 2020 and has 35 farms across Ireland. The area of the farm in clover at the end of 2023 was 65%, up from <10%, in 2020, with an average sward clover content of 23%, and increase of 13% from 2020. Sward clover content between reseeded paddocks and oversown paddocks was similar, at 18%. The DM production, however, was greater on the over-sown paddocks compared with reseeded, in the establishment year (13.2 vs 9.9 t DM ha<sup>-1</sup>), respectively. Chemical N fertiliser use in 2020 was 232 kg N ha<sup>-1</sup>, with 14.4 t DM ha<sup>-1</sup> of grass grown, chemical N reduced by 81 kg N ha<sup>-1</sup> to 151 kg N ha<sup>-1</sup> in 2023 and 13.5 t DM ha<sup>-1</sup>. Farm-gate N surplus and N use efficiency (NUE) were 194 kg N ha<sup>-1</sup> and 31%, respectively in 2020 in the first year of the programme. By the end of the third year (2022), the farm gate N surplus had reduced by 55 kg N ha<sup>-1</sup> (139 kg N ha<sup>-1</sup>), while NUE had increased to 39%. This improvement in N surplus and NUE was largely driven by the reduction in chemical N fertiliser. White clover will play a key role in reducing the requirement for chemical N fertiliser and reducing farm gate N surplus on farm. A major focus in the coming years must be to increase the clover content in swards.

**Keywords:** establishment, nitrogen fertiliser, nitrogen surplus, white clover

## Introduction

White clover has an important role to play in reducing chemical fertiliser usage on grassland farms in Ireland. Recent research in Teagasc Moorepark has shown increases in milk (+30 kg MS cow<sup>-1</sup> year<sup>-1</sup>; Egan *et al.*, 2018) and herbage production (+1100 kg DM ha<sup>-1</sup> year<sup>-1</sup>; Egan *et al.*, 2018) and reductions in N fertiliser by up to 100 kg N ha<sup>-1</sup> from incorporating white clover into grass swards in high stocking rate systems (Fitzpatrick *et al.*, 2022). To date, the uptake of white clover incorporation into grass swards on commercial farms has been low. However, there has been an increase in white clover use on farm with increasing fertiliser prices and environmental concerns (Egan *et al.*, 2022). Reseeding an entire farm to introduce white clover into pastures is impractical and costly and as such there needs to be a two pronged approach (reseeding and over-sowing) to introducing white clover onto grassland farms. There are four objectives of the programme: 1. Maintain herbage production  $\geq 14$  t DM ha<sup>-1</sup>; 2. Reduce Nitrogen (N) Surplus <130 kg N ha<sup>-1</sup> and increase N use efficiency >40%; 3. Reduce N fertiliser to  $\leq 150$  kg N ha<sup>-1</sup> year<sup>-1</sup>; 4. Average sward clover content of 20–25%.

## On-farm white clover study: Clover150

In 2020, an on-farm study was launched by Teagasc Moorepark, the 'Clover150' programme, looking at establishing white clover on commercial grassland farms; the programme is currently in year 3 of a 6-year programme. A total of 36 farmers are involved in the project from across Ireland and a range of farming systems. All farms were provided with a detailed plan for clover establishment and post-sowing grazing and fertiliser management, with clover establishment commencing in spring 2021. For newly established paddocks (reseeding or oversowing) a pre-grazing herbage mass of <1100 kg DM ha<sup>-1</sup> was advised for up to 4 months and reduced levels of N fertiliser to reduce competition from the grass plant, as well as maintaining a lower pre-grazing herbage mass on these paddocks over the autumn and winter period. Sward clover content was visually assessed using the 'Teagasc Clover score card' three times per year (spring, summer and autumn), and farmers were then provided with tailored management guidelines

for their farm. For paddocks that had an established sward clover content of  $\geq 20\%$ , N fertiliser was reduced by from May onwards. All farm management (grazing events, herbage mass, N fertiliser etc.) was recorded by each individual farmers and uploaded to PastureBase Ireland. Farm gate N surplus and N use efficiency (NUE) was calculated at the end of each production year using PastureBase Ireland. All data were analysed in SAS 9.4 (SAS Institute, Cary, NC, USA) and individual farm, paddock within farm, and year were included as fixed effects, farm was included as a random effect.

### *White clover establishment blueprint*

An establishment blueprint was developed as part of the programme to establish clover on farms. A targeted multiyear approach was used in establishing a white clover system- combination of reseeding and over-sowing;

Paddocks for a full reseed were identified as early as possible in the process to avoid over-sowing clover on these. The detailed protocol for reseeding and over-sowing is outlined below:

#### Direct Reseeding

- Take a representative soil sample for analysis of P, K and pH
- Prepare a fine, firm seedbed and apply lime, P and K as per soil test results
- Sow grass ( $34 \text{ kg ha}^{-1}$ ) and white clover ( $3.5\text{--}4 \text{ kg ha}^{-1}$ ) seed mix
- Avoid sowing white clover seed too deep; sowing depth of approx. 10 mm
- Ideally cover seeds and roll well to ensure good contact between the seed and the soil

#### Over-sowing

- Over-sow directly after grazing ( $\leq 4 \text{ cm}$  post-grazing sward height; or after cutting the paddock for surplus bales (ideally only over-sow 3 to 4 paddocks at a time)
- Control weeds before 12 months before oversowing clover
- A seeding rate ( $5\text{--}6 \text{ kg ha}^{-1}$ ) for over-sowing
- Sow with a fertiliser that contains P as this will favour establishment particularly if soil fertility is poor
- Soil contact post over-sowing is one of the most crucial factors effecting germination
- Over-sow on well managed grassland – not suitable on old dense swards with a low content of perennial ryegrass – if this is the case a full reseed is best practice

## Results and discussion

In the sample of farms, the portion of the farm covered by clover has significantly expanded over four years, from  $<10\%$  of the area in 2020 to  $65\%$  in 2023, and a similar trend in yearly average sward clover content from  $10$  to  $23\%$ . The level of clover on farm is from a combination of over- sowing and reseeding, with oversowing accounting for  $50\%$  more of the established area compared to reseeding. Previous studies (MacFarlane and Bonish, 1986) have reported more success in established clover via reseeding compared to over-sowing; however, in the current data set, there was no significant variation in average sward clover content between reseeding ( $19\%$ ) and oversowing ( $18\%$ ), where the correct procedure and

Table 1. White clover establishment blueprint

	Target area reseeded	Target area over-sown	Target total area
Year 1	10%	20%	30%
Year 2	10%	20%	30%
Year 3	10%	20%	30%
Year 4	10% + any ground on which clover did not establish		100%



post grazing management was followed. Dry matter production, however, varied significantly between paddocks that were over-sown and that were reseeded: the over-sown paddocks had greater DM yield in the establishment year compared with reseeding (13.2 vs 9.9 t DM ha<sup>-1</sup>), respectively. There was a significant reduction in the level of total N applied on-farm from 2020 to 2023 (232 to 151 kg N ha<sup>-1</sup>, respectively; Table 2). Within individual years the majority of the N fertiliser reductions occurred from May onwards, to coincide with increasing sward clover content (Egan *et al.*, 2018). Although the reduction in the amount of chemical fertiliser applied is undoubtedly a significant step forward, there was a slight reduction in cumulative herbage production from 2020 to 2023 of 900 kg DM ha<sup>-1</sup>. When reducing N fertiliser, it should only be done in paddocks with adequate levels of clover content (> 20%) in order to maintain herbage production. The average N surplus for the group of farms was 194 kg N ha<sup>-1</sup> year<sup>-1</sup> in 2020, when the programme commenced; however, there has been a steady decline in 2021 and 2022 for farm gate N surplus (180 and 139 kg N ha<sup>-1</sup>, respectively) and increase in NUE (31 and 39%, respectively).

Table 2. Four-year performance data summary (2020–2023) for the Clover150

Year	Average sward clover content (%)	Average area with a clover component (%)	DM Yield (kg DM ha <sup>-1</sup> )	Nitrogen (kg N ha <sup>-1</sup> )	NUE%	N surplus (kg N ha <sup>-1</sup> )
2020	<10%	10%	14.4	232	31%	194
2021	12%	45%	15.5	206	31%	180
2022	18%	61%	13.2	159	39%	139
2023	23%	65%	13.5	156	13%	140

## Conclusion

Over the course of the four years of the programme to date, there has been significant improvements made on farms, from increasing clover content (+13%), reducing chemical N fertiliser (–81 kg N ha<sup>-1</sup>) and improving overall farm gate N surplus and NUE (–55 kg N ha<sup>-1</sup> and +8%, respectively). A clear blueprint now exists for the successful establishment of white clover on commercial farms and N reduction strategies while maintaining overall herbage productions. However, careful consideration must be taken with regard to where, and to the quantity of N reductions within individual farms, and these considerations must be based on the level of clover content within an individual farm and paddock.

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# Non-constant rate of degradation of NDF in primary growth of grass, clover, and lucerne

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## Abstract

Degradation profiles of NDF were estimated over several incubation time points for different varieties of grass, clover, and lucerne harvested at normal and late developmental stages. The fractional rate of degradation (kd) was calculated for each incubation interval to analyse the variation in kd between incubation intervals and how it differed between species. Compared to grasses, legumes display greater maximum kd and greater variation in kd. Within grasses and within legumes, kd is species dependent. Inclusion of white clover, and to a lesser degree red clover and lucerne, may account for the great kd of grass-clover mixtures compared to grass only.

**Keywords:** fractional degradation rate, grass, legume, developmental stage

## Introduction

Modern feed ration evaluation systems, such as NorFor (Åkerlind *et al.*, 2011), use a degradable fraction of NDF and a constant kd of NDF as parameters to enable practical use for feed evaluation and feed ration formulation, and to avoid overparameterization from use of, e.g., non-constant rate models. Weisbjerg *et al.* (2007) showed that the kd is far from constant, and that the variation in kd between incubation intervals is dependent on forage type. Grass-clover exhibits considerable variation in kd across incubation intervals, indicating that the heterogenous mixtures of various species of grasses and legumes have significant effects on kd of NDF. Moreover, the cross-linking of fibre components in the cell wall matrix changes as the plants mature, which may also affect the kd of NDF differently between grass and legume species. We hypothesized that the variation in kd of NDF at different in situ incubation intervals is different between various grass species and legume species, and that the difference also depends on the plants' developmental stage at harvest.

## Materials and methods

In 2021, plots (30 in total) of monocultures of seven grasses (seven species with 2-8 varieties within each species) and two varieties of white clover (*Trifolium repens* L. (WCL)), red clover (*Trifolium pratense* L. (RCL)) and lucerne (*Medicago sativa* L. (LUC)) were harvested at Research Center Foulum (Denmark) in primary growth at two developmental stages (normal and late; 16 days difference). The seven grass species were perennial ryegrass (*Lolium perenne* L. (PER)), hybrid ryegrass (*Lolium hybridum* (HYB)), tall fescue (*Festuca arundinacea* Schreb. (TALL)), festulolium (*Festulolium pabulare*, festuca and lolium types (FEST-fest and FEST-lol)), meadow fescue (*Festuca pratensis* Huds. (MED)), timothy (*Phleum pratense* L. (TIM)) and orchard grass (*Dactylis glomerata* L. (ORC)). Samples were dried immediately after harvest and then milled (1.5 mm). For each sample, NDF degradation profiles were created according to Åkerlind *et al.* (2011). Milled samples were incubated (Dacron bags with 38 µm pore size) in the rumen in triplicate (one bag in each of three cows) at 0, 2, 4, 8, 24, 48, 96 and 168 h. Sample residues were transferred quantitatively and analysed for ash-free NDF (including α-amylase). The 168 h residue was used as estimate for degradable NDF (pdNDF). The kd was calculated in each incubation interval (between the incubation times  $t_x$  and  $t_{x+1}$ ) from 0-2 h up until 48-96 h for grasses and 24-48 h for legumes due to some negative differences for the last interval:  $kd = (\ln(\text{pdNDF-degradation}(t_x)) - \ln(\text{pdNDF-degradation}(t_{x+1}))) / (t_{x+1} - t_x)$ . The dataset was divided into four subsets; grasses and legumes harvested at normal or late developmental stage. Within each subset, kd was analysed using a two-way ANOVA with species and incubation interval as factors including their interaction.

## Results and discussion

In Figure 1, the degradation profiles of grasses and legumes indicated great variation between different species in terms of pdNDF and kd. Figure 2 illustrates the relationship between kd and average time in each incubation interval, for grasses and legumes. Legumes were characterized by greater variation in kd and greater maximum kd compared to grasses. Additionally, both variation and maximum kd became smaller when the plants matured (normal vs. late developmental stage). Both at normal and late developmental stages for both grasses and legumes, the observed variation in kd between incubation intervals suggests that there are several pools of NDF with different kd values.

For grasses harvested at normal developmental stage, an interaction between species and incubation interval was found ( $P < 0.05$ ), indicating that the kd of the different species varied differently in the first three incubation intervals (0–2, 2–4 and 4–8 h) as shown in Table 1.

During incubation interval 0–2 h, kd of PER was numerically highest, and showed the greatest kd during incubation interval 4–8 h. The kd values of TIM were characterised as being similar in the first three incubation intervals, after which it decreased similar to the other grass species. The difference in development in kd in the first incubation intervals between TIM and PER suggested that the heterogenous entity of NDF, specific for each species, is prone to fast degradation at different time points. Moreover, the different kd for FEST-lol and FEST-fest in intervals 2–4 and 4–8 h aligned, as expected, with the characteristics of PER and TALL, respectively. Both types of FEST reached greatest kd already in incubation interval 2–4 h.

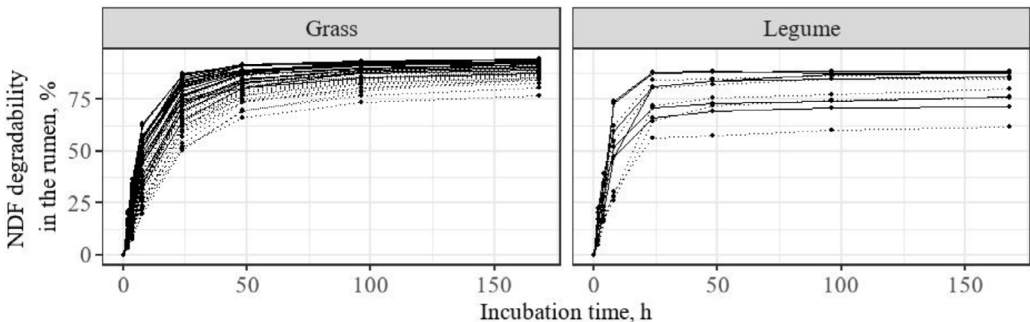


Figure 1. NDF degradation profiles for 24 and 6 varieties of grass and legumes, respectively, harvested at a normal (solid line) or late (dotted line) developmental stage in primary growth.

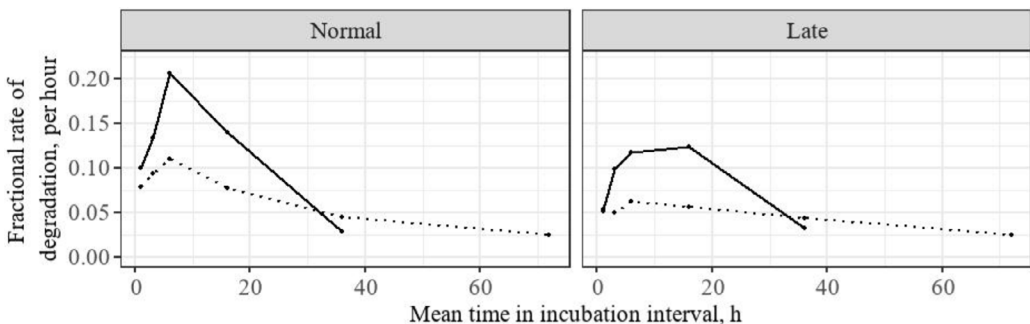


Figure 2. Fractional rate of degradation in different incubation intervals averaged across legume (solid line) and grass (dotted line) species harvested at either normal or late developmental stage in primary growth.

Table 1. Fractional rate of degradation of NDF ( $\text{h}^{-1}$ ) in different incubation intervals for grass harvested at normal developmental stage.

Species	n	Incubation interval					
		0–2 h	2–4 h	4–8 h	8–24 h	24–48 h	48–96 h
Perennial ryegrass	8	0.099 <sup>a</sup>	0.103 <sup>ab</sup>	0.137 <sup>a</sup>	0.087	0.049	0.025
Festulolium, lolium	3	0.064 <sup>bc</sup>	0.123 <sup>a</sup>	0.108 <sup>ab</sup>	0.080	0.040	0.022
Festulolium, festuca	2	0.048 <sup>c</sup>	0.070 <sup>bc</sup>	0.064 <sup>c</sup>	0.057	0.043	0.029
Tall fescue	2	0.047 <sup>c</sup>	0.051 <sup>c</sup>	0.078 <sup>bc</sup>	0.064	0.043	0.025
Hybrid ryegrass	2	0.067 <sup>abc</sup>	0.096 <sup>abc</sup>	0.120 <sup>ab</sup>	0.079	0.047	0.018
Timothy	3	0.095 <sup>ab</sup>	0.097 <sup>ab</sup>	0.098 <sup>bc</sup>	0.075	0.049	0.026
Meadow fescue	2	0.078 <sup>abc</sup>	0.100 <sup>ab</sup>	0.086 <sup>bc</sup>	0.075	0.043	0.029
Orchard grass	2	0.062 <sup>bc</sup>	0.070 <sup>bc</sup>	0.112 <sup>ab</sup>	0.071	0.032	0.023

<sup>a-c</sup>Values within the same column with different superscripts differ ( $P < 0.05$ ).

Table 2. Fractional rate of degradation of NDF ( $\text{h}^{-1}$ ) in different incubation intervals for legumes harvested at normal developmental stage.

Species	n	Incubation interval				
		0–2 h	2–4 h	4–8 h	8–24 h	24–48 h
White clover	2	0.061 <sup>b</sup>	0.157	0.334 <sup>a</sup>	0.213 <sup>a</sup>	0.020
Red clover	2	0.142 <sup>a</sup>	0.136	0.106 <sup>b</sup>	0.112 <sup>b</sup>	0.031
Lucerne	2	0.097 <sup>ab</sup>	0.109	0.177 <sup>b</sup>	0.092 <sup>b</sup>	0.030

<sup>a-b</sup>Values within the same column with different superscripts differ ( $P < 0.05$ ).

At normal developmental stage, the kd for legume species interacted with incubation interval ( $P < 0.05$ ). The kd for WCL increased from a relatively low level to reach maximum at incubation interval 4–8 h, whereas kd for RCL gradually decreased. For WCL, this indicated some lag, possibly due to that an easily degraded fraction of the NDF pool was encapsulated in a fraction of NDF, which was degraded more slowly in incubation intervals 0–2 and 2–4 h.

When forages are harvested at normal developmental stage, the current data illustrate that kd is species dependent (even within grasses and legumes). Especially varying white clover/grass ratio may account for most of the variation found in similar analyses of grass-clover mixtures (Weisbjerg *et al.*, 2007). However, species of grasses or species of legumes harvested at the late developmental stage did not differ in kd at different incubation intervals ( $P = 0.73$  and  $0.11$ , respectively, for the two-way interaction Species  $\times$  Incubation interval).

Especially when forages are harvested at normal developmental stage, the current data suggest that assuming constant fractional rate of degradation, estimated from whole degradation plots, simplifies the degradation profile.

## Acknowledgement

The research was funded by the Danish Milk Levy Fund.

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# Investigating the effects of wilting factors on the ensilage dynamics of multispecies swards

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## Abstract

Wilting plays an important role in ensuring forages with low dry matter (DM) concentrations have an opportunity to achieve effective fermentation when ensiled. This study investigated the effect of wilting duration (WD) and sward mechanical treatment (MT) on the field drying rates and silage conservation of a multispecies sward (MSS) and a perennial ryegrass + white clover sward (PRG+WC). Swards were mowed on 23 May 2023, and received either no MT or tedded with a swather at 1500 hrs on each sampling day. Within each sward, four WD sub plots (1, 21, 45 or 69 h) were established within each MT for three replicate blocks with herbage sampled from each plot ensiled in laboratory silos for 125 days, and subsequently assessed for aerobic deterioration. Increased WD and the MT significantly increased the DM concentration of the MSS silage, with an increased WD significantly increasing the PRG+WC DM concentration. The pH of silage samples increased with increasing DM. Effluent production from the silos were also affected by wilting treatments.

**Keywords:** forage, silage, wilting, dry matter, pH

## Introduction

The aim of this study was to investigate the effects of different wilting factors on the drying rate, subsequent fermentation and aerobic deterioration of a multispecies sward (MSS; *Cichorium intybus*, *Plantago lanceolata*, *Lolium perenne*, *Trifolium repens*, *Trifolium pratense*) and a perennial ryegrass+white clover sward (PRG+WC; *Lolium perenne*, *Trifolium repens*). Wilting can play an important role in aiding crops with low DM concentration achieve an effective silage fermentation by reducing excessive water activity and increasing dry matter (DM) concentration, helping lactic acid dominate the fermentation (McEniry *et al.*, 2011; Wyss, 2000). However, wilting has been found in some scenarios to increase silage pH (McEniry *et al.*, 2011) 4, 6 or 8 and excess wilting may increase the aerobic deterioration of silage (Wyss, 2000). While the incorporation of MSS into grassland systems in Western Europe has increased in recent times due to their environmental benefits (Jaramillo *et al.*, 2021) and government incentives (Department of Agriculture, Food and Marine, 2023) the low DM content of some species within MSS has been identified as a significant challenge to producing silage from these swards in a temperate climate (Moloney *et al.*, 2020). Thus, evaluation of wilting methods on the drying rate, conservation characteristics and aerobic deterioration of MSS is required to inform the design of appropriate management strategies of these swards.

## Materials and methods

Forage samples were taken from a site at Teagasc Grange Beef Research Centre, County Meath, from the 23–26 May 2023. Weather conditions were favourable for wilting: no precipitation was registered, daily global radiation values were 2462, 1602, 2381 and 1945 J cm<sup>-2</sup> across the four sampling days respectively, with corresponding mean daily wind speeds of 8.5, 9.6, 10.4 and 8.1 km h<sup>-1</sup> and maximum daily temperatures of 17.5, 16.1, 18.8 and 18.2°C. For both the MSS and PRG+WC swards, sampling plots were arranged in a split plot design within a 0.2 ha respective field sown and established in July 2021. After mowing with a Kuhn disc mower set at 4 cm cutting height, each field was randomly divided

into plots for mechanical treatment and wilting duration subplots, within three replicate blocks. The no mechanical treatment plots laid untouched while the mechanical treatments were tedded with a swather (Talex Bocian 225) each sampling day at 1500 hrs. Wilting duration subplots (7×1.9 m) were established within each mechanical treatment plot. Plots were assigned to sampling for ensiling at either 1, 21, 45 or 69 hours after mowing.

All forage within each plot was gathered by hand and representative subsamples of 5±0.1 kg from each plot were chopped and packed into laboratory silos as described by O’Kiely and Wilson (1991). Dry matter concentration of pre-ensilage subsamples was measured by drying at 98°C for 16 h in a forced air circulation oven. Botanical composition was determined through the manual separation of a 250 g subsample into individual species and dried at 60°C for 48 h. Silos were weighed and opened after 125 days and subsampled, with effluent production recorded. Silage subsamples were analysed for aerobic deterioration using the method described by Lynch *et al.* (2012). Aerobic deterioration, DM concentration and pH results were analysed as a split plot design using a mixed effect model, effluent was analysed using a nonparametric Kruskal–Wallis test, both using Minitab (2023).

## Results and discussion

The mean botanical composition of the MSS in g (kg DM)<sup>-1</sup> across all samples was: *Cichorium intybus* 26.4, *Plantago lanceolata* 186.0, *Lolium perenne* 601.5, *Trifolium repens* 71.3, *Trifolium pratense* 74.2, unsown species 12.1 and senescent material 28.4. The PRG+WC sward composition averaged 871.5 (g kg DM)<sup>-1</sup> for *Lolium perenne*, 95.7 g (kg DM)<sup>-1</sup> for *Trifolium repens*, 11.9 g (kg DM)<sup>-1</sup> for unsown species and 20.9 g (kg DM)<sup>-1</sup> for senescent material. The initial DM concentration at mowing for MSS averaged 161 g kg<sup>-1</sup>, and this concentration increased by 3.4 g kg<sup>-1</sup> h<sup>-1</sup> and 2.0 g kg<sup>-1</sup> h<sup>-1</sup> on average for tedded and untended samples, respectively. The MSS silage pH significantly increased with an increased wilting duration ( $P<0.001$ ) and also trended that way with the mechanical treatment ( $P=0.095$ ) with no significant interaction between these factors. Effluent was produced for MSS ensiled material after both the 1 h and 21 h wilt, tedded and untended, with significantly higher quantities for both 1 h samples and tedded 21h sample (Table 1). Increased effluent production with decreased DM concentration is expected (Keady and O’Kiely, 1996).

Table 1. Mean dry matter, pH, effluent and aerobic deterioration results

Mechanical treatment <sup>1</sup>	Wilting duration	MSS DM	MSS pH	MSS Effluent	MSS AD	PRG+WC DM	PRG+WC pH	PRG+WC Effluent	PRG+WC AD
Ted	1 h	163 <sup>d</sup>	3.86 <sup>d</sup>	632 <sup>a</sup>	18.2	208 <sup>d</sup>	3.77 <sup>c</sup>	114	14.6
No Ted	1 h	159 <sup>d</sup>	3.96 <sup>d</sup>	654 <sup>a</sup>	15.8	208 <sup>d</sup>	3.85 <sup>c</sup>	95	4.5
Ted	21 h	179 <sup>d</sup>	3.95 <sup>d</sup>	479 <sup>a</sup>	12.9	263 <sup>d</sup>	4.22 <sup>b</sup>	0	50.0
No Ted	21 h	177 <sup>d</sup>	4.02 <sup>cd</sup>	356 <sup>ab</sup>	3.3	248 <sup>d</sup>	4.19 <sup>b</sup>	0	5.3
Ted	45 h	307 <sup>b</sup>	4.47 <sup>a</sup>	0 <sup>b</sup>	15.6	389 <sup>bc</sup>	4.48 <sup>a</sup>	0	4.4
No Ted	45 h	234 <sup>c</sup>	4.23 <sup>bc</sup>	0 <sup>b</sup>	22.5	357 <sup>c</sup>	4.42 <sup>ab</sup>	0	22.5
Ted	69 h	395 <sup>a</sup>	4.57 <sup>a</sup>	0 <sup>b</sup>	8.8	473 <sup>a</sup>	4.61 <sup>a</sup>	0	22.9
No Ted	69 h	298 <sup>b</sup>	4.42 <sup>ab</sup>	0 <sup>b</sup>	2.0	437 <sup>ab</sup>	4.59 <sup>a</sup>	0	3.4
<i>P</i> value:									
MT		0.006	0.095	–	0.664	0.138	0.911	–	0.292
WD		<0.001	<0.001	–	0.207	<0.001	<0.001	–	0.608
MT*WD		0.001	0.004	0.005	0.674	0.614	0.539	0.058	0.227

Ted, tedding; No Ted, no tedding; DM, dry matter (g kg<sup>-1</sup>); MSS Effluent, total effluent recorded (g); AD, aerobic deterioration; –, measured in accumulated temperature difference over 192 hours (°C). Different letters denote statistically significant differences between the treatment means.

The PRG+WC DM concentration averaged 208 g kg<sup>-1</sup> initially. Its DM concentration increased at a rate of 3.9 g kg<sup>-1</sup> h<sup>-1</sup> on average for tedded samples and 3.4 g kg<sup>-1</sup> h<sup>-1</sup> for untended. The PRG+WC silage pH also significantly increased with increased wilting duration ( $P < 0.001$ ), with no significant effect from the mechanical treatment ( $P = 0.911$ ). Effluent was only produced in PRG+WC silage wilted for 1 h. No significant differences were found between any treatments for the aerobic deterioration of both swards.

These results highlight both the importance of wilting for MSS, due to its initial lower DM concentration and the high effluent production of the shortest wilted samples, and the duration of favourable wilting conditions that would be required. Achieving this DM concentration increase in the shortest possible time through a rapid wilt is important to reduce DM losses in the field (Borreani *et al.*, 2018). These results also highlight the usefulness of a simple mechanical treatment to reach desired DM concentrations in MSS in a shorter wilting duration. The evaluation of further mechanical treatment options in future studies with similar swards in similar conditions would be of value. In the current study, the MSS sward required both a 45 h wilting and tedding to reach a DM concentration of 307 g kg<sup>-1</sup> with favourable weather conditions for a mild temperate climate, whereas no mechanical treatment only reached 298 g kg<sup>-1</sup> after 69 hours of wilting. The pH of the silage samples increased with both increasing DM, and wilting duration, similar to results seen in McEniry *et al.* (2011), 4, 6 or 8

## Conclusion

Increasing the wilting duration increased the pre-ensilage DM concentration and silage pH of both sward types in favourable weather conditions for a mild temperate climate. Mechanical treatment was observed to be particularly valuable in MSS to decrease the wilting time required to achieve a satisfactory DM concentration for ensiling and reduce effluent losses.

## Acknowledgements

Funding for this project was provided by the DkIT and the Higher Education Authority Technological University Transformation Fund. We wish to thank staff at Teagasc Grange and DkIT for their support in conducting this study.

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# The effect of biochar and forage species on rumen fermentation and methane production *in vitro*

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## Abstract

Biochar is produced via pyrolysis of biomass. The pores in biochar are suggested to enhance the symbiosis of methanogenic and methanotrophic microbes. Forage species differ in starch and fibre content that may also affect methane formation in the rumen. We aimed at investigating the effects of biochar combined with several forage options on rumen fermentation and gas production *in vitro*. There were 12 treatments, formed by 3 biochar levels (0, 2.5, and 5.0 g (kg dry matter (DM))<sup>-1</sup>) and 4 forage options (grass silage alone or mixed 1:1 with red clover, faba bean or maize silage). Concentrate (350 g (kg DM)<sup>-1</sup>) contained oats and rapeseed meal. Feed grade biochar was made of spruce (Carbofex). The ratio of rumen fluid and McDougall's buffer was 1:2. The experiment (24 hours) was repeated 5 times with Gas Endeavour equipment. Biochar had no effect on DM digestibility, which was lower for faba bean than maize silage diets. Biochar or forage species had no effect on ruminal methane or carbon dioxide production per g of digested DM. Total volatile fatty acids (VFA) and the proportion of acetate in VFA were linearly decreased with biochar on pure grass silage diet but unaffected on silage mixtures.

**Keywords:** biochar, forage species, methane, *in vitro*

## Introduction

Biochar is produced via pyrolysis of biomass. The pores in biochar are suggested to enhance the symbiosis of methanogenic and methanotrophic microbes by providing them with a favourable, common habitat (Terry *et al.*, 2020). Forage species differ in starch and fibre content that may also affect methane formation in the rumen. Starchy silages such as whole crop maize (*Zea mays*) silage may shift rumen fermentation towards propionate and thus mitigate methane formation (Vanhatalo and Halmemies-Beauchet-Filleau, 2020). In addition, forage legumes typically contain less fibre than grass species with potential to decrease ruminal acetic acid formation and further methane. However, data on the effects of legume species on methane emissions is rather limited and inconsistent (Vanhatalo and Halmemies-Beauchet-Filleau, 2020). Therefore, we aimed at investigating the effects of biochar combined with several forage options on rumen fermentation and gas production *in vitro*.

## Materials and methods

The experimental silages were harvested at the University of Helsinki Viikki research farm (60°13' N, 25°1' E) in Finland. There were 12 treatments, formed by 3 biochar levels (0, 2.5 and 5.0 g (kg dry matter (DM))<sup>-1</sup>) and 4 forage options: grass silage (*Phleum pratense-Festuca pratensis*) alone or mixed 1:1 on a DM basis with red clover (*Trifolium pratense*), faba bean (*Vicia faba*) or maize silage. The diets contained forage (650 g (kg diet DM)<sup>-1</sup>), oats (300 g (kg diet DM)<sup>-1</sup>) and rapeseed meal (50 g (kg diet DM)<sup>-1</sup>). Feeds other than biochar were dried and milled through 1 mm sieve for incubations. Feed grade biochar was made of spruce (Carbofex, Nokia, Finland) and crumbled by hand for incubations.

Rumen fluid was obtained from 3 rumen-fistulated lactating Nordic Red cows consuming a standard diet based on red clover-containing grass silage. The ratio of rumen fluid and McDougall's buffer was 1:2. The experiment (24 hours) was repeated 5 times with Gas Endeavour (Bioprocess Control, Lund, Sweden) equipment. Bottles of 500 ml with alternating clockwise and counter clockwise mechanical stirring (60% of full speed, 5 minutes stirring, 10 minutes pause) were used. The amount of incubated feed was 3.8



g DM in 400 ml of rumen fluid–buffer mixture. Anaerobiosis at the beginning of the incubations was obtained by N<sub>2</sub>. At the end of incubations, a sample of rumen fluid (1 ml) was taken, pH was measured, and the dry matter of bottle contents was determined. Feed chemical composition was determined as described by Halmemies-Beauchet-Filleau *et al.* (2023). The rumen fermentation parameters were analysed as described by Lamminen *et al.* (2017).

Gas production and rumen fermentation data at 24-hour incubation endpoint was analysed by ANOVA (SAS 9.4) using experimental run and diet as fixed effects and bottle as random effect. The orthogonal contrasts tested were: (1) linear effect of biochar inclusion; (2) quadratic effect of biochar inclusion; (3) grass silage vs other silages; (4) red clover vs faba bean and maize silage; (5) faba bean vs maize silage, and (6–11) their interactions (1×3, 1×4, 1×5, 2×3, 2×4 and 2×5).

## Results and discussion

Grass silage contained 669 g digestible organic matter (DOMD) (kg DM)<sup>-1</sup>, 523 g neutral detergent fibre (NDF) (kg DM)<sup>-1</sup> and 136 g crude protein (kg DM)<sup>-1</sup>. For red clover silage the corresponding compositions were 626, 362 and 188 g (kg DM)<sup>-1</sup>, respectively. Both these silages were from 1<sup>st</sup> cut. The lower digestibility and NDF content, but higher protein content of red clover relative to grass species is in line with previous reports (Koivunen *et al.*, 2015). Of the whole crop silages, maize silage was more digestible than faba bean silage (DOMD 679 vs 593 g (kg DM)<sup>-1</sup>) as it contained more starch (278 vs 48 g (kg DM)<sup>-1</sup>) than faba bean silage. In contrast, maize silage contained less crude protein than faba bean (79 vs 180 g (kg DM)<sup>-1</sup>), which is a grain legume.

Biochar had no effect ( $P>0.10$ ) on ruminal DM digestibility, pH or the production of methane or carbon dioxide *in vitro*. When the forage was of pure grass silage, the biochar slightly reduced the rumen fluid volatile fatty acid (VFA) content and tended to reduce the molar proportion of acetic acid in VFA, but it did not affect rumen fermentation when silage mixtures were fed ( $P=0.003$  and  $P=0.075$  for interaction 1×3, respectively). Studies on the effect of biochar on methane production from ruminants are somewhat inconsistent. *In vivo* in growing cattle, biochar has slightly improved digestibility and mitigated methane production (11%) in growing, but not in finishing diets (Winders *et al.*, 2019). *In vitro*, Hansen *et al.* (2012) reported a statistically insignificant decrease of 10% in methane production for biochar in 48-hour batch culture, whereas Saleem *et al.* (2018) reported a 25% reduction in continuous culture system with 17-day periods. It is possible that longer incubation times with continuous cultures are needed to be able to detect the potential effect of biochar on ruminal methane production.

Methane total production (ml day<sup>-1</sup>) was lower ( $P=0.032$ ) with pure grass silage than with silage mixtures. However, per digested DM, this difference in ruminal methane formation no longer existed ( $P > 0.10$ ). Similarly, maize silage increased the methane and carbon dioxide total production in the rumen (ml day<sup>-1</sup>) compared to faba bean silage, but gas production per digestible DM did not differ, due to the higher digestibility of maize silage. The slightly lower rumen fluid pH in maize silage-based diets is in line with this (6.51 vs 6.57;  $P<0.001$ ). Forage species had no effect on propionic acid molar proportions in the rumen fluid ( $P>0.10$ ). Despite considerable differences in the NDF and starch contents between forage species, methane formation per digested DM remained unaffected in the present *in vitro* study. However *in vivo*, maize silage rich in starch has often decreased ruminal methane production compared to grass and legume silages, the decrease being often, but not always attributable to the shift towards propionate in the rumen (Vanhatalo and Halmemies-Beauchet-Filleau, 2020). In addition to forage species, the stage of maturity at harvest and variable climatic conditions during growing season affect forage quality (Vanhatalo and Halmemies-Beauchet-Filleau, 2020) which may mask the effect of forage species on rumen fermentation and also explain discrepancies between the experiments.

## Conclusion

The effects of the biochar and the forage species on rumen fermentation and methane production *in vitro* were small, although there were considerable differences in the fibre and starch content of the silages. It is possible that longer incubation times with continuous cultures are needed to be able to detect the potential effect of biochar.

## Acknowledgements

This study was a part of IRMA-project ‘The Climate smart feeding solutions for Finnish milk production sector’ financed in part by ‘The Catch the carbon research and innovation programme’ of the Ministry of Agriculture and Forestry of Finland.

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# Estimating fresh pasture forage quality using a mobile handheld near infrared spectroscopy sensor

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## Abstract

A handheld near infrared spectroscopy (NIRS) sensor to predict the nutritive quality of fresh herbage on heterogeneous, temperate pastures was evaluated. For this, pastures grazed by dairy cows on four commercial organic farms in South Germany were sampled every two to four weeks from May to October 2023 ( $n=207$ ). The nutritional composition of the sampled vegetation was determined via the mobile NIRS sensor pre and post cutting, and via wet chemistry as a reference. The concentrations of dry matter (DM), crude protein (CP), and acid detergent fibre (ADF) were predicted with acceptable accuracy (root mean squared error (RMSE)=16.4–20.4%) and moderate precision (coefficient of determination ( $R^2$ )=0.61–0.77), while neutral detergent fibre (NDF) was predicted with lower accuracy. There was no distinct difference between predictions based on measurements taken on the standing (i.e., pre cutting) or on cut and homogenised biomass (i.e., post cutting). Calibrations with a broad dataset covering variable structural and chemical composition of heterogeneous grasslands is needed to further improve the prediction adequacy.

**Keywords:** grassland, grazing, sensors, NIRS, dairy cattle

## Introduction

The seasonal and variable changes in the nutritional value of pasture herbage are a considerable challenge for grazing-based milk production systems. Information on the nutrient and energy concentrations of pasture herbage is needed to aid grazing management and to meet the animals' nutritional requirements throughout the grazing season. Methods to estimate the nutrient composition of the pasture feed base are, therefore, needed. The present study aimed at evaluating a hand-held near-infrared-reflectance spectroscopy (NIRS) sensor for real-time prediction of the nutritive quality of fresh herbage on heterogeneous temperate permanent pastures.

## Materials and methods

Pastures grazed by dairy cows on four commercial organic farms in South Germany were sampled every two to four weeks from May to October 2023. Six samples within a 1 m<sup>2</sup>-rectangle were harvested and weighed at each sampling date on each farm ( $n=207$ ). The dry matter (DM), crude protein (CP), neutral detergent fibre (NDF), and acid detergent fibre (ADF) concentration of each fresh herbage sample was determined via five scans within the 1 m<sup>2</sup>-rectangle using a NIRS sensor calibrated for fresh forages (NutriOpt On-Site Adviser, Trouw Nutrition, Amersfoort, the Netherlands). According to the official measurement protocol for this sensor, measurements ought to be taken on the harvested and homogenised sample. To evaluate whether a simplified measurement protocol would be a viable option, each NIRS analysis was repeated (1) before harvesting on the standing biomass (PRE), and (2) immediately after harvesting on the homogenised sample (POST). The reference concentrations of DM, CP, NDF, and ADF were determined using wet chemistry. The adequacy of NIRS predictions was evaluated separately for PRE and POST using the mean bias (MB,%),  $R^2$ , and relative root mean squared prediction error (RMSE, % of the mean reference value). The concentrations of NDF and ADF were evaluated based on

samples collected from July onwards ( $n=117$ ), because the calibrations for NDF and ADF were only available from that date. Due to the occasional lack of internet connection during the field application of the NIRS sensor,  $n=189$  PRE and  $n=182$  POST samples were available for evaluation.

## Results and discussion

A variable evaluation dataset was gathered on heterogenous, temperate pastures with DM, CP, NDF, and ADF concentrations in the ranges 134–632 g kg<sup>-1</sup> fresh matter, 54–297, 318–719, and 174–383 g (kg DM)<sup>-1</sup>, respectively. The handheld NIRS sensor predicted the concentrations of DM, CP, and ADF of the pasture herbage samples with acceptable accuracy (RMSE=16.4–20.4%) and moderate precision ( $R^2=0.61$ – $0.77$ ; Table 1). The MB indicated an overall overprediction of CP and ADF concentrations for the PRE samples by 13 and 40 g (kg DM)<sup>-1</sup>, respectively. There was no distinct difference between PRE and POST predictions. Measurements taken directly on the standing biomass (i.e., PRE samples) achieved results with similar prediction adequacy as samples taken on the cut and homogenised samples. A simplified measurement protocol can, thus, achieve results comparable with estimates based on the more time-consuming method. While concentrations of ADF i.e., the share of less digestible structural carbohydrates, were estimated with acceptable accuracy, estimates for NDF were neither adequate when measured PRE nor POST harvest. A larger dataset across the entire vegetation period is needed to validate these results.

Few studies have evaluated NIRS calibrations for fresh herbage samples, and even fewer using mobile and/or handheld NIRS devices. Murphy *et al.* (2022) developed calibrations for determining the nutritional quality of fresh herbage originating from homogenous Irish pastures using a benchtop NIRS device. Their best-performing calibrations achieved a greater prediction precision for both DM ( $R^2=0.86$ ) and CP ( $R^2=0.84$ ), than in the present study. The differences in prediction precision between the results by Murphy *et al.* (2022) and by the present study are likely owing (1) to the fact that their calibrations and its validation was based on data from the same farm (i.e., lower diversity in grassland conditions), and (2) to the greater variation in measurement conditions when using a handheld NIRS device. Parrini *et al.* (2022) developed calibrations for fresh herbage samples gathered on multi-species meadows and pastures in Italy, analysed via a benchtop NIRS device. Their calibrations achieved a high prediction precision for DM, CP, NDF and ADF ( $R^2 \geq 0.89$ ), despite using a highly variable calibration dataset collected across various sampling conditions (i.e., botanical composition, years, and geographical sites).

Table 1. Evaluation of the predictions of the nutritional composition of herbage samples collected on heterogenous, temperate pastures in South Germany determined using a mobile near-infrared reflectance spectroscopy (NIRS) sensor pre or post sample harvest ( $n=207$ ).

	Mean ( $\pm$ one standard deviation)			MB,%		R2		RSME,%	
	Reference	PRE	POST	PRE	POST	PRE	POST	PRE	POST
DM (g (kg FM) <sup>-1</sup> )	276 ( $\pm 94.8$ )	249 ( $\pm 80.6$ )	255 ( $\pm 85.6$ )	5.5	1.8	0.77	0.76	17.1	16.4
CP (g (kg DM) <sup>-1</sup> )	157 ( $\pm 55.5$ )	175 ( $\pm 41.8$ )	171 ( $\pm 46.1$ )	-8.1	-3.8	0.70	0.69	20.2	18.3
NDF (g (kg DM) <sup>-1</sup> )	484 ( $\pm 85.4$ )	581 ( $\pm 33.2$ )	583 ( $\pm 42.4$ )	-21.5	-23.0	0.28	0.19	26.2	27.6
ADF (g (kg DM) <sup>-1</sup> )	253 ( $\pm 46.3$ )	290 ( $\pm 48.7$ )	296 ( $\pm 53.3$ )	-16.1	-19.7	0.61	0.63	20.4	23.6

ADF, acid detergent fibre; CP, crude protein; DM, dry matter; FM, fresh matter; MB, mean bias in% of mean reference value; NDF, neutral detergent fibre; RMSE, root mean squared error in% of the mean reference value.

Murphy *et al.* (2022) and Parrini *et al.* (2022) demonstrate the feasibility of NIRS analyses using a benchtop device for fresh forage samples despite their high moisture content and show that precise predictions can even be achieved for multi-species grasslands via a robust dataset for model calibration. This indicates a need for further model calibrations with a similarly variable dataset as observed in the present dataset, i.e., covering heterogeneous pastures, across different farms, and the entire grazing season. It should be noted, however, that mobile, handheld NIRS devices can hardly attain predictions as precise and adequate as estimates by benchtop NIRS devices; the latter being able to analyse samples in a standardised, controlled environment. The findings of this study, nevertheless, demonstrate that adequate results reflecting the variability in the nutritional composition of heterogeneous grassland can be achieved even in a practical farming context using a handheld NIRS device.

Due to the occasional lack of internet connection during field application of the NIRS sensor, 8.7% of PRE and 12.1% of POST estimates were missing. This issue highlights a large obstacle for the use of digital tools in agriculture, where the lack of a suitable infrastructure for timely exchange of (large) data streams still hampers the application of digital tools.

## Conclusions

The tested handheld NIRS device has the potential to predict the nutritional quality of heterogeneous temperate pastures, rapidly and adequately. The prediction accuracy and precision could potentially be improved by calibrating the device using a larger diverse dataset reflecting the structural and chemical differences in pasture vegetation across the grazing season. Measurements taken on the standing biomass result in similar predictions as estimates from cut and homogenised fresh samples, simplifying the measurement protocol.

## Acknowledgements

The project DiWenkLa (Digital Value Chains for a Sustainable Small-Scale Agriculture) is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany. The Federal Office for Agriculture and Food (BLE) provides coordinating support for digitalisation in agriculture as funding organisation, grant number 28DE106A18. DiWenkLa is also supported by the Ministry for Food, Rural Areas and Consumer Protection Baden-Württemberg.

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# Are virtual fences applicable in mountain pastures?

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## Abstract

Fencing steep mountain pastures is time consuming and expensive. Consequently, these valuable grasslands are more and more abandoned. Virtual fencing (VF) is a promising technology to facilitate pasture management: animals wear GPS-collars emitting (1) audio tones (ATs) when reaching a digitally determined pasture boundary, and (2) an electric pulse (EP) when crossing it. We aimed at testing the adaptation of cattle to VF in mountain conditions. Thirty heifers were trained to VF in the lowlands and then divided into 3 independent groups during mountain grazing. Here, during 3 months, each group successively grazed 9 paddocks (6 virtual fenced, 3 wire fenced). We recorded the number of ATs and EPs per individual. The VF system worked reliably, but required careful handling when changing paddocks. During 83 days of mountain grazing, each animal received an average of  $4.9 \pm 6.9$  ATs and  $0.3 \pm 0.7$  EPs per day. These numbers were much lower when compared to the training period, indicating an effective learning by the animals. Only during days with special events occurring (e.g., wildlife presence) were ATs and EPs significantly increased. VF effectively kept the animals within the defined area. Therefore, if a 4G-GSM network is available, it can facilitate mountain pasture management.

## Introduction

Virtual fencing (VF) is a promising technology, since it can potentially optimize grazing management and reduce the workload for farmers, especially in extensive conditions (Umstätter, 2011). In VF systems, animals wear a VF-GPS collar and physical boundaries are replaced by virtual ones, which are digitally set in a smartphone app. The collars emit an audio tone (AT) when the animal approaches the virtual boundaries, followed by a weak electrical impulse (EP) when crossing it. Although livestock have been shown to successfully learn the system under flat conditions (Campbell *et al.*, 2020; Colusso *et al.*, 2021; Lee *et al.*, 2009), no research has been carried out to assess its applicability under mountain conditions. In mountain pastures, VF is especially promising because fencing is much more laborious, due to the more challenging environmental conditions. Mountain pastures are steeper, larger, rockier, with more heterogeneous vegetation of lower forage yield and quality, and weather conditions can be harsher. This may also affect animal behaviour and spatial pasture use and thereby could impair the functionality of VF. Additional challenges may arise due to lower GPS/GSM coverage, which in turn reduces animal positioning accuracy and thus may have a direct impact on animal learning. Therefore, this study aimed to investigate whether (1) VF is applicable in mountain conditions and (2) animals can deal with VF under mountain conditions in a rotational grazing system.

## Materials and methods

The study involved 30 female heifers ( $11.9 \pm 1.6$  months old) from a conventional Swiss dairy farm in the canton of Vaud and was conducted between May and August 2023. Each animal was fitted with a VF collar (Nofence, Batnfjordsør, Norway). First, the heifers were trained to VF under lowland conditions (about 700 m a.s.l.). An electrically fenced paddock was subdivided by a straight virtual boundary placed

in parallel to an outer electric fence. The training procedure was designed in several small sub-steps, over a total of 16 days to facilitate animal learning, according to the approach adopted by Hamidi *et al.* (2022). After this training period, the heifers were transported to a mountain summer pasture in the Swiss Pre-Alps (between 1300–1500 m a.s.l.), including flat and open areas as well as steeper topography with rocks, shrubs and trees. Vertical transhumance of heifers is a typical alpine management system. The outer perimeter of the farm was electrically fenced. Within this area, the summer pasture was subdivided into three electrically fenced paddocks (EF control) and six virtually fenced paddocks (VF treatment). Heifers were divided into three homogeneous groups of 10 animals each, balanced by age and breed. All groups grazed simultaneously on separate paddocks under a rotational system, where two groups always grazed a VF treatment paddock and one group an EF control paddock. Depending on the limiting factor of available forage, all groups were moved to their next paddock at the same day after, on average, 9 days (min. 7 to max. 14 days). This procedure was repeated until each of the three groups had grazed each of the nine paddocks once (i.e., six VF treatments and three EF controls per group).

We evaluated the learning success of heifers by analysing ATs and EPs recorded by the collars. Two different generalized mixed effect models (GLMs) were calculated, one with the number of ATs and the other with the number of EPs as dependent variable, respectively. For both models, the fixed factors considered were grazing period, day after paddock change, as well as their interaction, average grass height, and days with special events, i.e., a lynx prowling around the pasture, a group of deer grazing nearby or a neighbouring cattle herd breaking through the outer fence of their farm and joining the experimental group. Cow identity, nested into animal group, as well as days of the experiment, were considered as random factors.

## Results and discussion

During training, the total number of stimuli per cow per day was  $15.4 \pm 26.0$  ATs (mean  $\pm$  SD) and  $1.6 \pm 1.7$  EPs. During mountain grazing, the animals received on average  $4.9 \pm 6.9$  ATs and  $0.3 \pm 0.7$  EPs per day. Thus, both the total number of ATs and EPs decreased clearly when the animals had learned the VF system. Moreover, learning success was reflected by the animals grazing along the virtual fence without crossing it; i.e., they received several ATs, but few EPs throughout the experiment. The results of the GLMs revealed significant associations between the number of ATs and EPs and the estimated coefficients of the average grass height and days with special events (Table 1). During mountain grazing, the odds of a high number of ATs and EPs decreased by around 1% ( $(1 - 0.99) \times 100$ ) at higher grass heights ( $P < 0.05$ ), respectively. This indicates that the heifers tested the VF boundary more frequently with a decreasing amount of fodder. However, the animals respected the virtual boundary, as the VF system was still effective in keeping the heifers within their assigned grazing area. Moreover, there was a clear impact of days with special events (Table 1). On these days, the odds of a high number of ATs increased by about 103% ( $P < 0.001$ ) and those of EPs by about 391% ( $P < 0.001$ ) compared to days without special events. The number of ATs and EPs received by the animals did not significantly change over the course of the grazing periods, among days after changing the paddock or in the interaction of these two effects (Table 1).

## Conclusion

The results of this trial emphasise that the heifers learned the VF after two weeks of training in the lowlands, as well as its application in mountain pastures. The probability of special events such as wildlife contact can be increased under mountain conditions, which may ultimately affect the number of VF stimuli received by the animals. However, the VF kept the animals reliably within the defined grazing zones throughout the whole grazing period.

Table 1. Results from the fitted generalized mixed-effects models with ATs and EPs as the dependent variables and the parameter estimates for the fixed effects of the models.

Predictor	ATs			EPs		
	Estimated coefficient	Odds ratio	Significance level	Estimated coefficient	Odds ratio	Significance level
Special event	0.707	2.03	***	1.592	4.91	***
Period	-0.061	0.94	ns	-0.128	0.88	ns
Days after paddock change	0.047	1.05	ns	0.078	1.08	ns
Average grass height	-0.008	0.99	*	-0.015	0.99	*
Period×Days after paddock change	0.009	1.01	ns	0.008	1.01	ns

Significance levels: \*\*\* $P < 0.001$ ; \* $P \leq 0.05$ ; ns,  $P > 0.05$ .

## Acknowledgements

We thank the Bourgeois Bach family for their cooperation, commitment to the study and hospitality during the experiment, Olga Wellnitz for collaboration on the licence application, and Patrick Ledermann, Nicolas Cauda and Bastien Raymond for technical support.

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# Effect of mineral nitrogen application rate and strategy on productivity and quality of grass-clover swards

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## Abstract

Legumes can profit from biological nitrogen (N) fixation. Including clover into grass swards can lead to reduced mineral N requirement, and improved sward protein content and feed value. Proper mineral N management is crucial, as low N supply retards grass development and promotes clover over grass, whereas high mineral N rates decrease clover content and reduce biological N fixation in the sward. This study aimed to evaluate the impact of three mineral N application rates and three N application strategies on the performance of a grass-clover sward in Finland. Mineral N was applied in the form of calcium ammonium nitrate (CAN). Herbage samples were taken from total biomass and split into grass and clover. Each fraction was analysed for dry matter yield and N concentrations during three cuts, separately. First year results show that both the mineral N application rate and the application strategy affect dry matter (DM) yield and sward composition. Clover growth in spring was low due to low spring temperatures and was not affected by N application rate. There was a significant negative response of clover herbage yield on increasing N rates applied to cut 2. The most beneficial strategies in 2022, when drought compromised DM yield of cuts 1 and 2, were high allocation of mineral N to first harvest cut and application of the remaining N to cut 3.

**Keywords:** grass-clover, nitrogen, rate, timing

## Introduction

Leguminous plants such as clover can profit from biologically fixed nitrogen. Including clover into grass swards can result in high herbage yield with reduced mineral nitrogen (N) requirement. Previous research from Sweden show reduced grass-clover yield but increased clover content when mineral N fertilizer input is reduced or completely omitted. The main herbage yield differences were observed during cut 1 when clover content is relatively low, as compared to later cuts (Frankow-Lindberg and Geijerstam, 2014). The effects of mineral N application rate on grass-clover sward productivity have been investigated in several experiments under Nordic conditions (Kristensen *et al.*, 2022; Mela, 2003); however, information on the effects of N application strategy during the season is scarce.

Recommendations for grass-clover management from Ireland allocate a high share of total mineral N input in spring to support grass growth when clover content is low due to low soil temperature. In contrast, a lower share of mineral N is applied to later cuts when clover content is high and able to contribute to total N supply of the sward through biological N fixation (Hennessy, 2022). This field experiment was established to investigate the influence both total mineral N application rate and application strategy to grass-clover swards yield, seasonality and quality under Nordic conditions.

## Materials and methods

This experiment is conducted at Kotkaniemi Research Farm, Vihti, Finland (60°35'90" N; 24°38'11" E) to compare different N rates and application timings in a grass-clover sward managed in 3-cut system. The soil is classified as very humus loamy clay (4.5% C, pH 6.0). The trial was established in autumn 2021 using a commercial grass and clover seed mixture: *Phleum pratense* L. (60%), *Festuca pratensis* Huds. (10%), *Lolium perenne* L. (10%), *Trifolium pratense* L. (10%), *Trifolium repens* L. (10%). Nine treatments

were established in a randomized block design using four replicates per treatment. In following spring, next to an unfertilized control, three total N application rates (100, 150 or 200 kg N ha<sup>-1</sup>) were combined with different application splits, i.e. (a) standard – application of 50, 25 and 25% of total N to cuts 1, 2 and 3, respectively, (b) >60% of N applied to cut 1, rest to cut 3, (c) >60% of N applied to cut 1, rest split between cuts 2 and 3. All rate/timing combinations are described in Table 1. Yara CAN 27 (27% N) was used as N source. Base dressing with triple superphosphate, potassium sulphate and potassium chloride supplied 15 kg S ha<sup>-1</sup>, 20 kg P ha<sup>-1</sup> and 250 kg K ha<sup>-1</sup>. Dry matter (DM) biomass yield was weighed from each plot at all harvest dates, botanical analysis for grass and clover shares was done in two out of four replicates. All fractions (total biomass, grass and clover) were analysed for N concentration using Kjeldahl method. N removal was calculated as DM yield multiplied by total N concentration. Anova, followed by Tukey test was performed using Statgraphics® software.

## Results and discussion

Reducing N overall application rate reduced DM biomass yield and increased clover content (Table 1). DM yield of harvest cuts 1 and 2 was limited by drought in 2022, resulting in a high contribution of cut 3 to total DM biomass yield (Table 1). Total DM yield varied between 6.2 (unfertilized) and 8.7 t DM ha<sup>-1</sup> with an average of 7.7 t DM ha<sup>-1</sup> which is rather low compared to typical DM biomass yield which varies between 9 and 11 t DM ha<sup>-1</sup>.

Total DM yield was significantly affected by mineral N application rate. Harvest cut 1 showed significant DM yield increase of biomass with increased N rate. Biomass N concentration was lower in the control than in fertilized treatments, but no differences were found between fertilizer rates. N removal, however, was significantly increased with application rate.

Grass proportion became lower throughout the growing season, and the clover proportion increased. This was most pronounced in those treatments that received no or low fertilizer rates. A positive response to N application rate was found in all cuts for grass DM yield, but no effect of application rate on grass N concentration was observed.

Clover contributed by more than 50% to total DM biomass yield in the unfertilized treatment. Its share was lower, but still considerable with up to 43% of total DM biomass yield in the N fertilized treatments. Clover DM yield during cut 1 was low but increased in the later cuts. Due to elevated N concentrations in clover over grass, there was a trend towards higher biomass protein concentration with increasing clover share in cut 3 (not shown).

Even though N application rate was the dominating factor in this trial, there was also an effect of application strategy. Application of more than 60% of total N at cut 1 increased clover content over standard distribution. A rate of 75 kg N ha<sup>-1</sup> or more applied to cut 1 resulted in highest DM yield at first cut, together with high N concentration. At low total N rates, this strategy reduced productivity later in the season. Clover content at either cut was not affected by mineral N application to cut 1. Nitrogen application rate to cut 2 did not affect grass growth significantly, potentially due to drought conditions, which impaired growth at this stage. Clover DM yield and content responded significantly and negatively to increasing N rates applied to cut 2. This may indicate that N application to cut 2, combined with low grass growth rates due to drought conditions, increased soil N concentrations which impaired clover development. N application to cut 3 also reduced clover content, but at the same time promoted grass growth after drought. Overall, distribution of a high share of N to cut 1 and the remainder to cut 3 was the most promising strategy in 2022. Following this strategy with an N input of 150 kg N ha<sup>-1</sup> (100-0-50 kg N ha<sup>-1</sup> to cuts 1 to 3, respectively), led to a yield difference of only 125 kg DM ha<sup>-1</sup> and 10 kg N ha<sup>-1</sup> in total N uptake as compared to average N application rate of 200 kg N ha<sup>-1</sup>. If proven successful

Table 1. Effect of mineral N application rate and strategy on biomass DM yield, N concentration, clover content and total N uptake (standard N distribution pattern in italics).

	Total N rate (kg N ha <sup>-1</sup> )									p-value	HSD (5%)
	<i>0</i>	<i>100</i>	<i>100</i>	<i>100</i>	<i>150</i>	<i>150</i>	<i>150</i>	<i>200</i>	<i>200</i>		
N split to cuts 1–3 (kg N ha <sup>-1</sup> )	<i>0-0-0</i>	<i>50-25-25</i>	<i>75-0-25</i>	<i>100-0-0</i>	<i>75-38-38</i>	<i>100-0-50</i>	<i>100-25-25</i>	<i>100-50-50</i>	<i>125-25-50</i>		
DM yield (kg DM ha <sup>-1</sup> )											
Total	6219	7519	7814	7439	8159	8515	7844	8703	8577	<0.05	1197
Cut 1	2059	3111	3476	3535	3398	3595	3513	3763	3430	<0.05	646
Cut 2	997	1401	1169	1419	1595	1387	1639	1686	1641	<0.05	458
Cut 3	3160	3007	3169	2486	3166	3534	2693	3254	3506	<0.05	807
Nitrogen concentration (g (kg DM) <sup>-1</sup> )											
Total	24.2	25.0	25.5	25.6	24.9	25.1	25.0	25.5	26.3	0.45	2.7
Cut 1	19.1	25.4	26.6	26.8	26.7	26.2	26.7	26.7	29.8	<0.05	3.4
Cut 2	24.8	24.7	22.5	22.0	24.7	21.7	25.0	26.8	25.4	<0.05	5.1
Cut 3	27.4	24.8	25.5	26.1	23.0	25.2	23.3	23.4	23.2	<0.05	4.8
Clover share (% of DM yield)											
Total	56	31	39	43	25	34	39	18	31	<0.05	19
Cut 1	13	8	9	17	10	11	12	5	10	0.34	16
Cut 2	50	34	35	34	21	32	36	15	24	<0.05	29
Cut 3	85	55	70	84	44	54	72	37	55	<0.05	31
Total N uptake (kg N ha <sup>-1</sup> )											
Total	151	188	199	190	203	214	197	222	226	<0.05	39

in the following years under variable weather conditions, this might be a strategy to economically reduce N input into grass-clover without compromising yield.

## Conclusion

Reduced mineral N application rates decreased DM biomass yield, which could not be compensated by increased clover growth in 2022. N application strategies with > 60 % of total N rate applied to cut 1 and remaining N applied to cut 3 resulted in increased DM yield at comparable N concentration during the highly important first cut, and profited from clover development, providing high nitrogen concentrations later in the season. As weather conditions were suboptimal for grass production in 2022 it remains to be seen how different strategies perform in following trial years.

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# Characterization of polyphenol content and antioxidant activity of a network of French pasture grasses

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## Abstract

Pasture grasses contain many secondary metabolites such polyphenols or vitamins that have different health-promoting abilities in animals, including antioxidant effects, which may provide farmers with an opportunity to contribute to animal health improvements with minimal investment. However, knowledge on the polyphenol content and antioxidant activity of pastures is limited. The objective of this study was to assess the variability of polyphenol content and antioxidant activity (ORAC and DPPH assays) of 45 pastures located in three different areas of France and chosen to represent a diversity of environmental conditions and botanical diversity. Important differences between pastures were observed: polyphenol content varied from 8.6 to 40.8 mg of gallic acid equivalent per g dry matter (DM). Antioxidant activity varied from 9.1 to 63.5 mg trolox equivalent per g DM with DPPH and from 50.9 to 280.3 mg trolox equivalent per g DM with ORAC. The present work provided a first valuable report on the potential variability in polyphenols and antioxidant activity of pastures. Next step will be to study the correlations between botanical diversity and pasture management and these values in order to improve pastures utilization by taking account health benefits for ruminants.

**Keywords:** grassland, antioxidants, polyphenols, animal health

## Introduction

Animal health management is a key point of the agroecological transition of ruminant production. Plants contain many secondary metabolites such as vitamins or polyphenols that may have different health-promoting properties in animals. Numerous studies have demonstrated the role of these metabolites in promoting animal health via antioxidant, anti-parasitic, bloat-preventing and anti-microbial effects (Poutaraud *et al.*, 2017). In a survey on French dairy farms, Sulpice *et al.* (2019) observed that higher levels of grazing were associated with a reduction in veterinary intervention and medical drugs consumption by cows. Pastures could provide farmers the opportunity to contribute to animal health improvements with minimal investment. However, knowledge on the polyphenol content and antioxidant activity of pastures is scarce and does not provide support to demonstrate the real supplies of antioxidants and polyphenols by pastures. Moreover, potential differences on antioxidant activity and polyphenol content between pastures and the factors affecting these values remain unclear. Reynaud *et al.* (2010) suggested that botanical composition and vegetative stage could affect the polyphenol content and composition of pastures. Environmental conditions may also affect polyphenols in plants. Thus, the first objective of our project was to investigate the variability of antioxidant activity and polyphenol content within French pastures in order to objectify a potential difference in health value of pastures.

## Materials and methods

Grass samples were taken in three different areas of France: Auvergne-Rhône-Alpes, East (mainly Haute-Saône) and West (Pays de Loire and Indre). The pastures, 15 per area, were chosen to represent a diversity of environmental conditions and botanical diversity. Grass samples were collected twice during spring 2022: at temperature sum of about  $432 \pm 80^\circ\text{C.D}$  (P1) and at temperature sum of  $950 \pm 107^\circ\text{C.D}$  (P2). The sum of temperatures was calculated, as the cumulative sum of positive ( $>0^\circ\text{C}$ ) daily temperature, from 1 February to the present day considered and measured at local level. The botanical composition of each pasture was determined using 10 quadrats distributed within the parcel. Biomass contained in each quadrat was collected and mixed to constitute a representative sample of each pasture. Samples were quickly stored at  $-20^\circ\text{C}$ , then freeze-dried and ground before analysis. The antioxidant activity of pasture samples was estimated using two assays: the measurement of the free-radical scavenging activity of the DPPH\* (2,2-diphenyl-1-picrylhydrazyl) and the measurements of the oxygen radical absorbance capacity (ORAC). Total polyphenol (TP) content (Folin–Ciocalteu method) and DPPH (Galmarini *et al.*, 2013) were analysed at GRAPPE. ORAC assay was realised by the Végépolys Valley lab (Angers, France; Ou *et al.*, 2001). ORAC and DPPH results were expressed as mg trolox equivalent per g dry matter (DM) and TP were expressed as mg of gallic acid equivalent per g DM. Nutritive values of grass samples (organic matter, nitrogen and crude fibres contents) were determined by Lano Lab (Saint-Lo, France). Comparisons between sampling dates for each parameter analysed were performed (*t* test for paired data). Pearson's correlation tests were also performed to assess the relationships between the TP content, antioxidant activity and nutritive values.

## Results and discussion

Total polyphenol content varied from 8.6 to 40.8 mg of gallic acid equivalent per g DM (Table 1), which was similar to the range of values previously reported for grasses (Amrit *et al.*, 2023) or for highly diversified natural grassland (Reynaud *et al.*, 2010). Antioxidant activity assessed with DPPH method varied from 9.1 to 63.5 mg trolox equivalent per g DM and from 50.9 to 280.3 mg trolox equivalent per g DM with ORAC method. These results underlined an important difference in antioxidant activity between pastures.

Table 1. Total polyphenol content and antioxidant activity of the 45 French pastures studied

	Sampling date	<i>n</i>	Mean	Min	Max	SD
Dry matter (%)	P1	45	25.7 <sup>a</sup>	17.5	42.8	6.2
	P2	45	26.1 <sup>a</sup>	15.7	42.7	7.1
Organic matter (g (kg DM) <sup>-1</sup> )	P1	45	890 <sup>a</sup>	810	927	24.9
	P2	45	919 <sup>b</sup>	884	948	16.4
Crude protein (g (kg DM) <sup>-1</sup> )	P1	45	173 <sup>a</sup>	80	242	34.9
	P2	45	135 <sup>b</sup>	69	249	45.4
Crude fibres (g (kg DM) <sup>-1</sup> )	P1	45	195 <sup>a</sup>	134	312	34.8
	P2	45	246 <sup>b</sup>	158	323	46.2
Total polyphenols, mg gallic acid equivalent per g DM	P1	45	23.3 <sup>a</sup>	8.6	37.2	7.0
	P2	45	24.5 <sup>a</sup>	11.8	40.8	6.6
DPPH, mg trolox equiv. per g DM	P1	45	26.3 <sup>a</sup>	9.4	47.1	9.3
	P2	45	29.5 <sup>a</sup>	9.1	63.5	11.1
ORAC, mg trolox equiv. per g DM	P1	45	144.8 <sup>a</sup>	50.9	280.3	47.6
	P2	45	149.1 <sup>a</sup>	67.6	277.8	42.9

Significant differences ( $P < 0.05$ ) between sampling date within pastures are indicated by different lowercase letter superscripts. SD, standard deviation.

Total polyphenol content was significantly and positively correlated to DPPH ( $r=0.91$ ) and ORAC ( $r=0.90$ ) values, suggesting that polyphenols present in pastures have a significant antioxidant activity. These findings have been already reported by Amrit *et al.* (2023) and Rapisarda and Abu-Ghannam (2023). DPPH and ORAC values were significantly correlated ( $r=0.83$ ), but very different as both assays assess the antioxidant capacity differently. Sampling date had no effect on TP content, DPPH and ORAC values, probably due to the high variability obtained within a sampling date. Changes in polyphenols may be affected by many factors as weather, stress, botanical composition or phenological stage making it challenging to explain these observed variations. Crude protein was significantly higher in P1 ( $173 \text{ g (kg DM)}^{-1}$ ) than P2 ( $134 \text{ g (kg DM)}^{-1}$ ,  $P<0.05$ ), whereas CF was significantly lower in P1 ( $195 \text{ g (kg DM)}^{-1}$ ) than P2 ( $247 \text{ g (kg DM)}^{-1}$ ,  $P<0.05$ ). These changes are commonly found with increasing maturity of plants and values were consistent to references for these types of pasture (INRAE, 2018). No correlation was observed between CP or CF and the TP content, DPPH and ORAC values.

## Conclusion

Results of this study showed important variations in polyphenol content and antioxidant activity in a network of French pastures, suggesting that pastures could have a different animal health potential. Next step of our study will be to assess the effects of botanical diversity and pasture management (fertilization, grazing intensity, etc.) on these characteristics in order to improve pasture utilization by taking into account health benefits for ruminants.

## Acknowledgement

This study is part of the PRAIDIV project, that aims to study the health services for animals provided by grasslands and funded by a special 'agricultural and rural development account' allocation (CASADR, n°21AIP3921850) from the French Ministry of Agriculture, which cannot be held liable.

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# Breeding perennial ryegrass varieties with an improved combining ability with white clover

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## Abstract

White clover varieties, bred in Europe, are mostly evaluated in grass swards. Perennial ryegrass varieties, on the other hand, except in some rare scientific experiments, are bred in monocultures receiving high nitrogen inputs. Hence, the resulting varieties are not necessarily persistent and productive in mixtures with clover. As the transition from grass to grass-clover is essential to increase the sustainability of European agriculture, we conducted an experiment to find out whether it would be best to breed dedicated perennial ryegrass varieties for this purpose. In a three-year field trial, eighteen perennial ryegrass populations were either sown as monocultures, receiving 300 kg N ha<sup>-1</sup> year<sup>-1</sup> or in a mixture with white clover receiving 150 kg N ha<sup>-1</sup> year<sup>-1</sup> under conservation management. Although no significant yield interactions were found between population and clover presence, contrasting reactions of the populations were found. For example, the yield of population 11, when expressed relative to the average of the eighteen populations for 2021 and 2022, was 4% and 5%, respectively, higher in combination with clover, but 2% and 7% respectively, lower in monoculture. Strengthened by these results, an important part of our actual breeding effort in perennial ryegrass breeding is in the development of varieties with a good combining ability with white clover.

**Keywords:** breeding, grass-clover

## Introduction

Including white clover in grassland, instead of pure grass swards, offers a great potential to make European agriculture more sustainable. Benefits include, amongst others, reduced dependency on mineral fertiliser (and hence of fossil energy), lower emissions of N<sub>2</sub>O and NO<sub>3</sub> to the environment, lower production costs and higher protein self-sufficiency (Lüscher *et al.*, 2014). In addition, grass legumes are more resilient and can cope better with drought compared to grass monocultures (Höfer *et al.*, 2016). The benefits in terms of biomass and protein yield are maximized when the legume content is in the range of 40-50%, based on annual dry matter yield (Nyfeler *et al.*, 2011). Keeping the legume proportion within this range is challenging: often mixtures are quickly dominated by one component, and eventually one of the two species is outcompeted. Management (N fertilisation, cutting frequency) and seed mixture composition (choice of species in the mixture, seed proportions) are two important levers to influence this equilibrium, but also the choice of the varieties can have an effect on the composition and hence the performance of the mixtures (Lüscher *et al.*, 2014). In associations of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.), a mixture that is widely used for intensive grazing with dairy cows, effects of both the clover variety (Annicchiarico, 2003) and the grass variety (Komainda and Isselstein, 2020) on production were found. This offers prospects for breeding dedicated varieties (Rognli *et al.*, 2021). The state institute for plant breeding in Melle, Belgium, part of ILVO today, has been breeding perennial ryegrass and white clover since 1932. The breeding work in perennial ryegrass, has, so far concentrated on creating varieties for use in pure swards. This experiment explored the variation in ability for combining with white clover in our perennial ryegrass breeding pool.

## Material and methods

In May 2020 we established a yield trial comparing eighteen perennial ryegrass populations, either sown as pure swards (PS) or mixtures with white clover (MS). The grass was sown at a density of 1000 germinative seeds  $m^{-2}$  on all plots at a depth of *circa* 2 cm. The MS plots were oversown with the white clover (cv. Melital) at a density of 5 kg  $ha^{-1}$  at a depth of *circa* 0.5 cm. The trial was sown as a split plot design with two replicates, with clover presence as main plot factor and perennial ryegrass population as sub plot factor. Plot size was 7.8  $m^2$ . In 2020, the swards were cut three times (July, September and October) using a Haldrup forage harvester to eliminate weeds, but yield was not measured. In 2021 and 2022 yield was measured on 5 and 4 occasions (cutting every 5–6 weeks), respectively, using a Haldrup forage harvester. Annual fertilisation amounted to 300 kg N  $ha^{-1}$ , 320 kg  $K_2O$   $ha^{-1}$  and 60 kg  $P_2O_5$   $ha^{-1}$  on the PS and 150 kg N, 320 kg  $K_2O$  and 60 kg  $P_2O_5$  on the MS. Before the first cut, the fertilisation was 80 kg N  $ha^{-1}$ , 100 kg  $K_2O$   $ha^{-1}$  and 60 kg  $P_2O_5$   $ha^{-1}$  on all plots (PS and MS). The remainder of this amount was divided over the three following cuts.

The 18 populations included 6 reference varieties (Bovini ( $2n=2x$ ), Barhoney ( $2x$ ), Melonora ( $2x$ ), Soraya ( $4x$ ), Melforce ( $4x$ ), Melfrost ( $4x$ )) and were equally distributed between diploids and tetraploids. Heading date of the populations was distributed between 21<sup>st</sup> May and 5<sup>th</sup> of June. The data were analysed and graphs were plotted using R (R Core Team, 2021). A two way analysis including the factors clover presence and population and the interaction between both as fixed factors was fitted for each year separately.

## Results and discussion

In 2021, the rather wet summer resulted in very high grass yields. The average yield of the PS (18 297 kg DM  $ha^{-1}$ ) was significantly higher than that of the MS (15 419 kg DM  $ha^{-1}$ ). In 2022, marked by a very dry summer, yields were much lower, the MS (10 399 kg DM  $ha^{-1}$ ) outyielding the PS (9452 kg DM  $ha^{-1}$ ) (Table 1). There was no significant main effect of population, nor an interaction effect on the total annual dry matter yield.

Despite the absence of significant interaction effects, it was clear from the field observations and from the yield data (Figure 1) that some ryegrass populations were better adapted to grow with clover, whereas other populations were less well adapted.

The yield of population 10 ( $2n=2x$ , heading date=2 June) for example, when expressed relative to the average of all populations (population average=100), was close to that of the population average when grown in PS (100 in 2021, 98 in 2022) but lower than the population average in MS (93 in 2021; 82 in 2022). The relative yield of population 11 ( $2n=2x$ , heading date=30 May) was smaller than the

Table 1. Result of the two-way ANOVA testing the effect of perennial ryegrass population with or without white clover on the total annual dry matter yield in the years 2021 and 2022.

Factor	Year 2021		Year 2022	
	F-value	p-value	F-value	p-value
Population (df=17)	0.45	0.96	1.36	0.21
Clover (df=1)	118.7	<0.001	28.13	<0.001
Population:Clover (df=17)	0.56	0.90	1.08	0.41



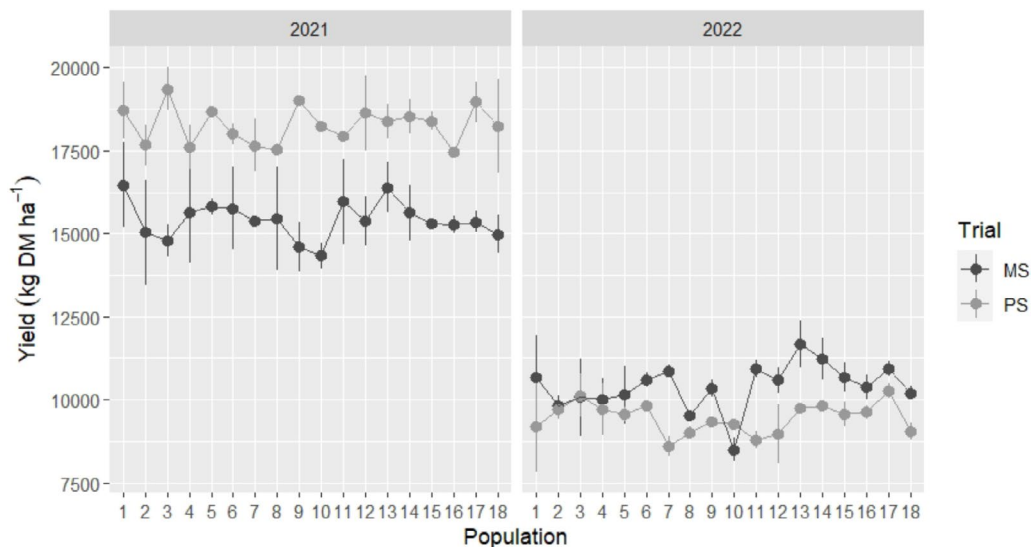


Figure 1. Dry matter yield (DMY) of eighteen perennial ryegrass populations growing either in pure swards (PS) receiving  $300 \text{ kg N ha}^{-1} \text{ year}^{-1}$  or in mixed swards (MS) with white clover receiving  $150 \text{ kg N ha}^{-1} \text{ year}^{-1}$  in two successive years (2021 and 2022). Error flags denote standard deviations.

population average in PS (98 in 2021, 93 in 2022) but greater in MS (104 in 2021, 105 in 2022). The relative yield of population 13 ( $2n=4x$ , heading date=21 May), was greater than the population average both in PS (101 in 2021, 103 in 2022) as in MS (104 in 2021, 112 in 2022).

Traits like phenology, growth form and ploidy have been found to affect clover content in mixtures (Komainda and Isselstein, 2020) but the present trial did not allow us to elucidate the effect of these traits. Further research will focus on the mechanisms explaining the ability for combining perennial ryegrass and white clover.

## Conclusions

The results of this trial indicate that our breeding pool contains variation in the combining ability of perennial ryegrass with white clover, opening perspectives to breed varieties with an improved ability for combining with white clover.

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# Identifying the optimal stocking rates depending on soil type and local weather conditions in Ireland

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## Abstract

A modelling exercise was conducted using the Pasture Base Herd Dynamic Milk model on 12 years weather data at three locations in Ireland: Johnstown Castle (south east), Ballyhaise (north) and Oak Park (centre). Simulations were run on a 40 ha farm. There were two soil types: free draining soil (FDS) or heavy soil (HS). Chemical nitrogen (N) fertiliser application was 225 kg ha<sup>-1</sup> annually. Concentrate feeding was fixed at 480 kg DM cow<sup>-1</sup> year<sup>-1</sup> and only grazed grass and grass silage were fed to the animals. Simulations were optimised in terms of stocking rate (SR) to ensure forage self-sufficiency. Grass growth, start and end of the grazing seasons, grass and silage intake and total grazing days were outputs from the model. Results show that the ideal SR was dependent on the interaction between soil type and weather. The worst combination was Ballyhaise weather combined with the HS, which could only sustain a SR of 2.4 cow ha<sup>-1</sup> at 225 kg N ha<sup>-1</sup>. This was not due to a lower grass growth but to a lower number of grazing days per cow as soils were too saturated to facilitate grazing.

**Keywords:** modelling, soil type, weather, self-sufficiency, adaptation

## Introduction

Irish dairy farm profitability is driven by increased grass productivity and improved efficiency of the conversion of grazed pasture to animal products. While Ireland is a relatively small country characterised by a long grazing season due to its temperate climate, there is disparity in grass growth and grazing days across the country. To use grass efficiently and match animal grass demand with grass growth, the stocking rate (SR) must be adapted to the farm and will depend not only on grass growth but also on its seasonality and the soil trafficability. Many factors affect grass growth such as weather, soil type and grazing management. In this paper we investigated the ideal SR based on soil type and weather at three locations in Ireland and the intra-year variability.

## Material and methods

This study aimed to predict the impact of the interaction between soil type and weather on the ideal SR of a farm to maintain forage self-sufficiency. The weather conditions at three Teagasc Research Farms at different locations (Johnstown Castle in the south-east, Ballyhaise in the north and Oak Park in the centre) and two different soil types [free draining soil (FDS) or heavy soil (HS)] were simulated. The weather data used were from the Met Eireann synoptic weather stations at the three locations from 2011 to 2022. The model used was the PBHDM (Pasture-Based Herd Dynamic Milk) model (Ruelle *et al.*, 2015) in conjunction with the Moorepark St Gilles (MoSt) grass growth model (Ruelle *et al.*, 2018). The models work with a daily time step, predicting daily grass growth (paddock level), animal intake (both grazing and indoor feeding) and milk yield, amongst other parameters. Farm size was fixed at 40 ha of grassland and included 40 paddocks. Concentrate supplementation (1.03 UFL and 120 PDI) was fixed at 480 kg DM cow<sup>-1</sup> year<sup>-1</sup>, and fertiliser applied was 225 kg N ha<sup>-1</sup>. The yearly grass silage fed, purchased and sold varied year to year.

If no paddock on the farm was available for grazing due to soil trafficability, cows were housed and fed indoors. The grazing season started after the 20<sup>th</sup> of January once the average farm cover was higher than 800 kg DM ha<sup>-1</sup>. The final grazing rotation started on 3<sup>rd</sup> October and cows were housed after all the paddocks were grazed once in the final rotation, or if none of the paddocks were available for grazing due to soil trafficability. The final possible grazing date was 1<sup>st</sup> of December. For each scenario, simulations were completed for 12 years on a continuous cycle, meaning that an event occurring in year 1 had consequences in year 2 and so on. A typical Irish system was simulated with seasonal calving and an average calving date of the 15<sup>th</sup> of February.

To determine the optimal SR of the different scenarios, an initial simulation was run with the current maximum permitted SR in Ireland of 2.75 cow per ha, corresponding to 110 cows per farm in those simulations. Then cow number was reduced until the silage balance of the simulated farm (silage harvested – silage fed) was positive (but always remaining lower than 100 kg DM ha<sup>-1</sup>) on average for the 12 years. The main outputs of the simulations were the optimal number of cows, grass growth, grass DM intake, silage fed, and start/end of the grazing season.

## Results and discussion

The scenario which sustained the lowest number of cows was Ballyhaise with a HS and the highest was Johnstown with a FDS demonstrating the interaction between weather and soil type and the necessity of adapting the SR to the local conditions (Table 1). On average, the HS grew more grass than the FDS. However, the grass DM intake and the number of grazing days were lower on the HS compared to the FDS. This was due to the number of days cows had to be housed due to paddocks being ungrazable (Figure 1). The total average number of ungrazable days was of 163, 125 and 94 days on the HS with Ballyhaise, Johnstown and Oak Park weather, respectively and was between 1 to 3 days for the FDS.

The final date to house cows was day 335. While most of the FDS simulations reached, or almost reached, this date every year, the HS had an average end of the grazing season ranging from 324 days for Oak Park to 313 for Ballyhaise due to waterlogging of soil. One surprising result is that the HS in Ballyhaise could commence earlier than the FDS at that site due to low opening farm cover on the FDS.

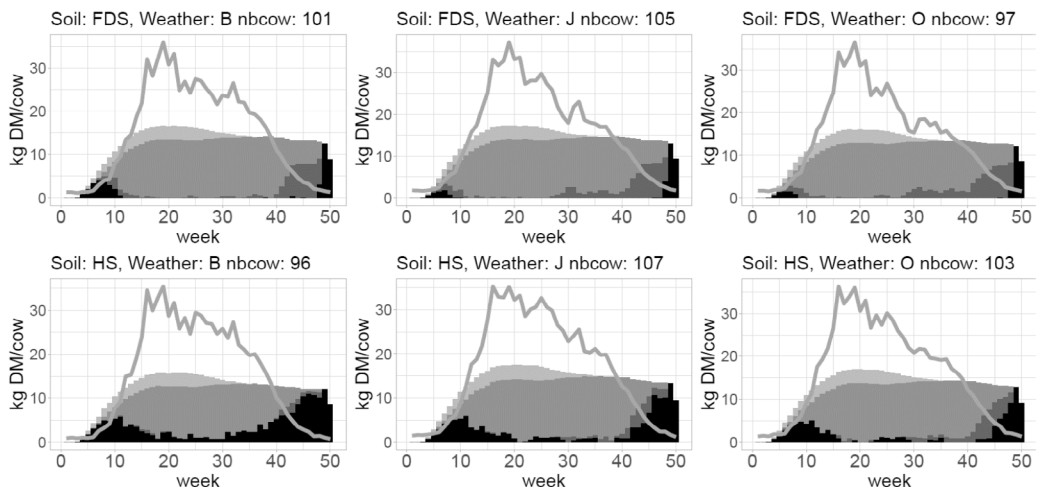


Figure 1. Weekly feed intake per cow of grass (grey), indoor silage (black), silage at grazing (darker grey), concentrate (lighter grey) as well as grass growth (grey line) in kg DM cow<sup>-1</sup> for the different soil type and weather average of the 12 years. FDS, free draining soil type; HS, heavy soil type; J, Johnstown Castle; B, Ballyhaise; O, Oak Park; nbcow, number of cows.

The scenarios were optimised to ensure silage self-sufficiency on average across the 12 years; however, a big variation existed between years (Table 1). The biggest variation in terms of surplus was in Oak Park especially for the heavy soil, and the lowest variation was in Ballyhaise. In term of grazing days, the biggest inter-year variation was with the heavy soils.

Table 1. Impact of soil type and weather on optimal number of cows, cow diet and grazing management (average of 12 years and standard deviation).

Location	Soil type	Cow number	Grass growth (kg DM <sup>-1</sup> )	Grazing day (ha <sup>-1</sup> )	Start GS	End GS	Grass intake (t cow <sup>-1</sup> )	Silage intake (t cow <sup>-1</sup> )	Silage surplus (min/max)
B	FDS	101	13.7±0.7	681±18	57±10	333±2	3.2±0.1	1.4±0.1	-0.9/1.6±0.5
	HS	96	13.8±0.8	452±62	46±19	313±20	2.5±0.3	2.0±0.3	-1.3/2.5±0.6
J	FDS	105	14.2±1.3	734±12	37±13	333±2	3.2±0.3	1.3±0.2	-2.1/1.9±1.1
	HS	107	15.1±1.2	596±79	38±17	318±15	2.8±0.3	1.7±0.3	-1.7/2.5±0.9
O	FDS	97	13.1±1.5	682±10	37±13	334±1	3.2±0.2	1.4±0.2	-2.1/1.8±1.1
	HS	103	14.4±1.3	624±54	42±19	324±12	2.9±0.3	1.6±0.3	-2.6/2.3±1.0

B, Ballyhaise; J, Johnstown Castle; O, Oak Park; FDS, free draining soil; HS, heavy soil.

Extreme events, such as the drought in summer 2018, had different effects on different parts of the country. The Ballyhaise HS simulation produced an excess of forage that year (+607 kg DM ha<sup>-1</sup>) while Oak Park HS had the greatest deficit (-2553 kg DM ha<sup>-1</sup>) followed directly by the Oak Park FDS (-2148 kg DM ha<sup>-1</sup>). The variation across the different locations highlights blanket or general advice for the country is not relevant. While having an optimal SR for their farm, farmers must adapt each year to the specific weather conditions and variations, requiring a consequent silage reserve to maintain their forage self-sufficiency.

## Conclusion

The interaction between soil type and weather was high leading to different optimal SR, start and end to the grazing seasons and grass DM intake highlighting the requirement to adapt farm management depending on location and soil type. However, even within a site the between-year variation was high. This shows that farmers need to be flexible in their grazing management. With climate change, this variation is predicted to increase further, highlighting the need for more grazing management tools to help farmer anticipate surpluses or deficits.

## Acknowledgment

The authors acknowledge the funding received through the 2022 Policy & Strategic Studies Research Call administered by the Department of Agriculture, Food and the Marine (FarmAdapt 2022PSS111)

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# PastureBase Ireland — the adoption of grassland knowledge on Irish grassland farms

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## Abstract

Grass remains the most competitive feed available on Irish livestock farms. While European systems continue their transition away from grassland systems, Ireland continues the journey to improve the standards of grassland management on farms. Grass enables low-cost systems of milk and meat production, it promotes a sustainable, green, low carbon and high quality image of grazing ruminant production. Through a combination of climate and soil type, Ireland possesses the ability to grow large quantities of high quality grass and convert it into grass-based milk and meat products. PastureBase Ireland (PBI), the national grassland database is now 11 years in operation. The objective of this development was to increase the level of pasture measurement on farms and increase/improve grazing management practice and grass utilisation. In recent years there has been a large uptake of grassland technology; the number of farm cover measurements has increased from 33 864 in 2017 to approximately 135 907 in 2023. Nitrogen (N) usage has reduced by 30% since 2021 (a reduction of 118 595 Mg) to 280 569 Mg. Grass DM production has declined on farms from the initial years of PBI development, on farm grass DM production has averaged 12 943 kg DM ha<sup>-1</sup> from 2019–2023. While there is now more grassland measurement on farms, coupled with lower N usage, improvements to grazing management need to be made, namely improving the timing of N application during the grazing season, increasing overall grass production, improving the level of clover in Irish grassland swards.

**Keywords:** PastureBase Ireland, grass production, farm cover, grassland systems

## Introduction

PastureBase Ireland (PBI) (Hanrahan *et al.*, 2017) was formally introduced to Irish grassland farmers in 2013; it is an internet-based grassland management programme for all Irish grassland farmers. It offers farmers a 'grassland decision support' and stores a vast quantity of grassland data from dairy, beef and sheep farmers in a central national database used for research, advisory and industry use. In total there are over 7 000 farms registered on PBI. Since the introduction of the PBI App there has been a steady increase in the number of commercial farms recording fertiliser data, approximately 40% of all grass covers are uploaded from the PBI app.

## Materials and methods

The number of grass covers completed by farmers using PBI has steadily increased each year: 33 864 in 2017, 35 117 in 2018, 55 217 in 2019, 66 903 in 2020, 79 020 in 2021, 91 528 in 2022 and 135 907 in 2023. The level of uptake has been high, some of which has been on the development of industry-funded grassland programs like Grass10. The incorporation of a grassland management measure within the Nitrates Action Plan Program (NAP) has led to increased usage of PBI. Farmers who are participating in the NAP are required to measure grass 20 times yearly (using plate meter/ cut and weigh, etc.). This development started fully in 2023, previously it was to measure grass three times annually and complete a grassland management courses. Generally as the number of measurements increase in the system the accuracy and dependability of the farm data increases.

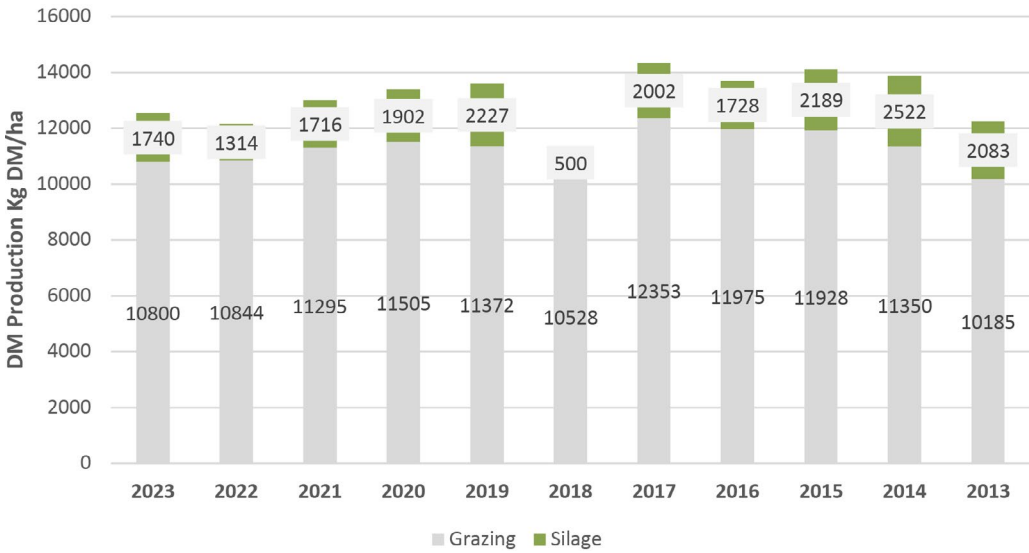


Figure 1. Total (grazing and silage) grass DM production ( $\text{kg DM}^{-1}$ ) (2013–2023).

## Results and discussion

The dataset used in Figure 1 comprises farms that completed at least 30 farm covers in PBI from 2013. The participating numbers change annually; on average >900 farmers are completing 30+ covers annually. By choosing farms with high level of farm cover measurement, the level of grass production is better quantified. The reason why farms with 30+ covers are used is because grass growth accuracy is increased the more frequently grass measurements are completed.

For farmers completing >30 measurements in PBI for the past 11 years the mean grass DM production was  $12\,977\text{ kg DM ha}^{-1}$  and ranged from  $11\,028\text{--}14\,355\text{ kg DM ha}^{-1}$  over that period. The lowest grass production year, 2018, coincided with a large moisture deficit. Grass DM production has been consistent, which shows the resilience of Ireland's grassland based production system. Figure 2 illustrates the weekly

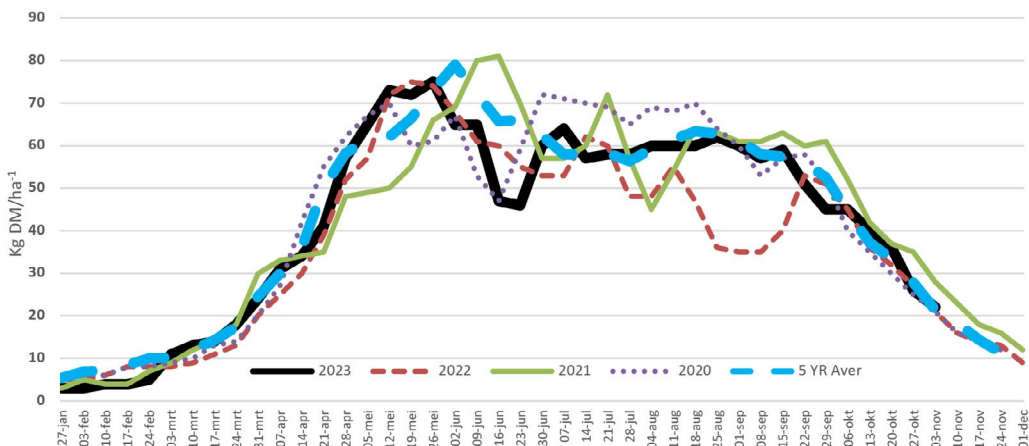


Figure 1. Total (grazing and silage) grass DM production ( $\text{kg DM}^{-1}$ ) (2013–2023).

DM production on PBI farms. It is very apparent that weekly DM production is variable and requires considerable grazing management skill for farmers to ensure adequate grass supply on their farms to feed the grazing herd. As part of the continued improvements required in grassland management, a predictive grass growth model was developed, the MoSt (Moorepark and St Gilles grass growth model). This model is now part of the feedback which farmers completing >30 covers will receive within the PBI decision support system. The number of grazing events per paddock is a grazing management efficiency measure derived from PBI. This measure shows how many actual grazing or silage harvests took place on paddocks in the growing season. Table 1 shows a matched sample of farms (534) for the last 4 years on PBI. The use of matched samples meant there was a consistent number of farms within the comparison. The number of grazing events varied from 7.6 to 8.3 on farms across the four years. There was no consistent trend in overall grass DM production, which averaged 12 775 kg DM ha<sup>-1</sup> over the 4 years. Whenever the grazing events reduced the level of grazing, DM production declined.

Table 1. Grassland parameters for a matched sample of 534 farmers in PastureBase Ireland.

Year	Number of grazings	Number of silage harvests	Grazing DM production (kg DM ha <sup>-1</sup> )	Silage DM production (kg DM ha <sup>-1</sup> )	Total DM production (kg DM ha <sup>-1</sup> )
2020	7.0	0.6	10 848	2056	12 905
2021	7.7	0.5	11 508	1727	13 236
2022	7.9	0.4	11 005	1382	12 388
2023	7.3	0.5	10 804	1745	12 569

## Conclusions

The uptake of grassland measurements at farm level can increase the level of grass utilisation on farms, and a continued focus on grazing DM production is needed. An objective on farms is to increase the grazing events on individual farm paddocks. In the past 8 years there has been organic growth in the uptake of PBI grassland technology in Ireland, this is further enhanced with the development of the MoSt grass growth model (Ruelle *et al.*, 2018). The continued adoption of best grazing management practices at farm level will be critical in meeting the challenges posed by climate change, ammonia emissions and water quality in Irish livestock production systems.

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# Evaluation of three-year farm-gate balances of dairy farms in a coastal area of northwest Germany

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## Abstract

Since 2018, many German farms have to calculate a farm gate balance according to Substance Flow Analysis Ordinance (StoffBilV). The farm gate balance considers amounts of N and P that enter or leave the farm such as mineral and organic fertilizers, feed, livestock, plant and animal products. Farm gate balances were calculated for three years, 2019–2021, on 23 dairy farms in a forage production region of Lower Saxony, Germany. Averaged over three years, gross N balances were 133 (79–190) kg N ha<sup>-1</sup> and gross P balances were 10 (5–18) kg P ha<sup>-1</sup>. The purchase of mineral N fertilizer had the greatest influence on the gross N balance while the purchase of concentrates and roughages had the greatest influence on the gross P balance. The high variability among farms and years may, at least partly, be explained by varying weather conditions and market prices for purchased mineral fertilizers or milk sold from the farm. Especially for forage farms, in literature and following our results, the three-year mean is considered the minimum requirement for the assessment of the farm gate nutrient balances.

**Keywords:** nitrogen, phosphorus, balances, farm level, forage production

## Introduction

Nitrogen (N) is an important driver of agricultural crop production. At the same time, surpluses and inefficient use of N pose a threat to the ecological balance, biodiversity, and climate. The sustainability strategy of the German government aims at reducing the N surplus calculated as a farm gate balance for a farming enterprise to 70 kg N ha<sup>-1</sup> by 2030. Farm gate balances according to the Substance Flow Analysis Ordinance (StoffBilV; BMEL, 2017) are perceived as a promising tool for mapping nutrient flows of nitrogen (N) and phosphorus (P) on farms. Lower Saxony has the second largest dairy herd of all German federal states and has seen a significant intensification of production in the grassland regions along the North Sea coast in recent years. In the project ‘Waterbuddies’ nutrient inputs from grassland areas in northern Germany via drainage ditches into the North Sea were assessed. In addition to analyses of ditch water and ditch flora and fauna, farm gate balances were calculated for 23 dairy farms for the years 2019, 2020 and 2021. The main research questions were: (i) How high are the farm gate balances of the dairy farms on a three-year average base? and (ii) What are the main factors that affect farm gate balances in dairy production systems?

## Materials and methods

The study region was located in northwest Germany, in the northern part of Lower Saxony (districts of Ammerland, Friesland and Wesermarsch). The area is characterised by three types of soil landscapes: Geest (predominantly sandy soil), Peatland, and Marshland (mainly clay soil). The study involved 23 conventional dairy farms with a wide range of farm sizes and production levels which represent the dairy production of the region quite well (Table 1). Data were gathered through personal interviews that were conducted over three consecutive years from 2019 to 2021.

The farm gate balance covers all N and P inputs and outputs during a calendar year but does not consider internal nutrient fluxes. The nutrient inputs comprise mineral fertilisers, manure imported from other farms, additional organic fertilisers, animal feed, acquisitions of livestock, and nitrogen input via legumes

Table 1. Farm characteristics (three-years mean).

Farm characteristic	Minimum	Maximum	Mean
Utilised agricultural area (ha)	49	350	160
Number of dairy cows	71	522	199
Livestock density (LU ha <sup>-1</sup> )	1.4	2.9	1.9
Total annual milk yield (kg ECM)	546 258	4 404 699	1 840 908
Annual milk yield per cow (kg ECM cow <sup>-1</sup> )	5900	11 479	9179
Concentrated feed per litre milk (kg)	0.231	0.368	0.308
Gross N balance (kg N ha <sup>-1</sup> )	79	190	133
Gross P balance (kg P ha <sup>-1</sup> )	5	18	10

which is estimated based on German law (StoffBilV). The nutrient output includes plant products, animal products, farm manure exported to other farms and livestock sold.

For statistical analyses linear mixed effect models were applied using ‘N input from mineral fertiliser’ as fixed effect and ‘farm nested in year’ as random effect. The model for P balances contained ‘P input from forage purchase’ as fixed effect and the same random effects.

### Results and discussion

We found that the purchase of mineral N fertiliser and the input of P in purchased feed (mainly concentrates but also other feedstuff) have a significant effect on the farm gate balance for N and P. The gross N balance shows an increase of 1.06 kg N ha<sup>-1</sup> with every extra kg N ha<sup>-1</sup> of mineral fertiliser ( $R^2=0.65$ ,  $P<0.001$ ; Figure 1A). The gross P balance increases by 0.439 kg P ha<sup>-1</sup> with every kg of purchased feed ( $R^2=0.36$ ,  $P<0.001$ ; Figure 1B). If no P was purchased through concentrates and forage, the gross P balance would be  $-0.434$  kg P ha<sup>-1</sup> (intercept).

The strong influence of the input of mineral N fertiliser on the gross N farm gate balance suggests a reduction in mineral N fertilisation. However, the production of high-quality grass with sufficient energy and protein content must be ensured. With less mineral N, this can usually be achieved only when the efficiency of N use within the farm is increased by applying N-reduced feeding and better utilisation of N in farm manure or by introducing legumes (Grethe *et al.*, 2021; Löw *et al.*, 2021).

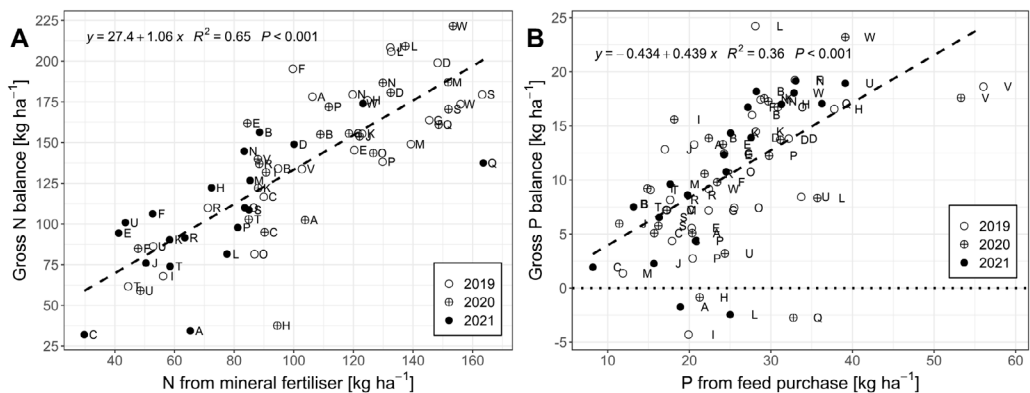


Figure 1. Linear relationship between (A) N from mineral fertiliser (kg ha<sup>-1</sup>) and gross N balance (kg ha<sup>-1</sup>) and (B) P from feed purchase (kg ha<sup>-1</sup>) and gross P balance (kg ha<sup>-1</sup>) for the three study years 2019–2021. Each point refers to one farm (A–W) and symbols indicate study years.

Phosphorus in the total purchased feed ( $\text{kg P ha}^{-1}$ ) is the primary factor influencing the gross P balance. To reduce the P intake through purchased feed it is essential to analyse the feed (especially farm forage), use concentrates with less P, adjust the P supply to the lactation course and/or generally reduce the amount of concentrates and mineral feed (Jürgens, 2021). An improved utilisation of farm forage offers the opportunity to reduce concentrates.

Generally, there is a high variability in farm gate balances resulting from a range of different farming practices and the differences across years. The yearly effects are primarily due to the strong differences in amount and distribution of precipitation and the related quantity and quality of the forage (Smit *et al.*, 2008). In addition, the general market situation, milk price, and prices of mineral fertiliser, concentrates and fuel are influencing factors (Banse *et al.*, 2019; Hartmann, 2022). In order to minimise the influence of extreme years (e.g., drought), it is recommended to use a three-year moving average to evaluate farm gate balances as it is already prescribed by German law (StoffBilV).

## Conclusion

Farm gate balances on dairy farms show a high variability and are influenced by yearly effects. The gross N farm gate balance increases with the amount of N from purchased mineral fertiliser while the gross P balance is mainly influenced by the amount of P from purchased feed.

## Acknowledgements

The study is supported by the Federal Office for Agriculture and Food (BLE) within the collaborative research project Waterbuddies, support code: 2817NA004. We thank all participating farms for the friendly cooperation and the straightforward provision of the data. For the support with the data acquisition, we thank our project partners Svenja Janssen and Mathias Paech.

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# Use of compressed sward height and growing degree days to predict herbage availability on pastures

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## Abstract

Accurate prediction of dry matter yield (DMY) in pastures is crucial for grazing management. Estimates by means of rising-plate-meter measurements are a time-effective way to determine DMY in pastures. It has been shown previously that the accuracy of DMY estimation can be improved by taking the day of the year (DOY) into account. In this study we replace DOY by Growing Degree Days (GDD) and compare both approaches. The study was performed in South Tyrol (NE Italy), where 830 paired measurements of DMY and compressed sward height (CSH) by means of rising plate meter (Grasshopper®) were taken in three different paddocks during three growing seasons (2020, 2021 and 2023) covering the whole grazing season from April to the start of November. Statistical predictive models for DMY were stepwise forward developed by linear models starting from a baseline model including paddock and CSH. Both approaches (GDD and DOY) resulted in final models predicting a curvilinear increase of DMY with increasing CSH, a stronger slope towards the end of the CSH range and a fluctuating effect of the growing season. Although GDD proved to be a valid alternative to DOY, it did not increase the accuracy of statistical models estimating DMY based on CSH.

**Keywords:** grazing, compressed sward height, seasonality, statistical predictive models

## Introduction

An accurate prediction of dry matter herbage yield (DMY) is crucial for planning and managing grazing. Traditional methods for determining yield require time-consuming manual sampling and analyses, which are both costly and labour-intensive. For this reason, using estimates of DMY based on measurements of the compressed sward height (CSH) obtained by a rising plate meter are considered a viable alternative (Murphy *et al.*, 2020). The relevance of this topic lies in the potential to enhance the efficiency of pasture farming by providing farmers with a tool to optimize the management and utilize their resources more effectively. Peratoner *et al.* (2021) found that incorporating the day of the year (DOY) to account for seasonality in statistical predictive models based on CSH enhances the accuracy of DMY estimation. In this study, we test replacing DOY by Growing Degree Days (GDD).

## Materials and methods

The study was conducted in three paddocks (PD1, PD2, PD3) being part of a compartmented short-sward grazing system in Dietsheim (South Tyrol, NE Italy). The pastures face south-west and are situated at an elevation of 890-930 m a.s.l.. During three growing seasons (2020, 2021 and 2023), paired measurements of DMY and CSH, obtained by means of a rising plate meter (Grasshopper®, True North Technologies, Shannon, Ireland) were taken from late April to the end of the grazing season at the beginning of November. The botanical composition in terms of yield proportion of all occurring species was visually assessed within each paddock in three randomly placed 25 m<sup>2</sup> quadrats at the begin of June. Growing degree days (GDD) with base temperature 0°C were computed either between the start of the growing season and the respective measurement event using temperature data collected daily on site, starting from the begin of the growing season as defined by Schaumberger (2011) (GDD<sub>sgs</sub>), or from the first of January (GDD<sub>soy</sub>). Statistical predictive models for DMY were stepwise forward developed and backwards checked by linear models (type III Sum of Squares), starting from a baseline

model including paddock and CSH, with 70% of data being used for training and 30% for testing.  $R^2$  and RMSE were used as evaluation metrics of model fit to select terms to be added, also including interactions and the adjustment of the polynomial degree of the covariates. Terms with  $P > 0.1$  were dropped from the model. The variables GDDsgs, GDDsoy or DOY were alternatively used to develop the models. A square root transformation of the dependent variable was applied to meet ANOVA assumptions, which were evaluated by means of diagnostic plots.

## Results and discussion

All approaches, using GDDsgs, GDDsoy or DOY, resulted in final models predicting a curvilinear increase of DMY with increasing CSH. Using GDDsgs or GDDsoy, the interaction of CSH and the paddock is in the final model (Table 1, Equation b), while there are no interactions in the final model using DOY (Table 1, Equation a). All three equations had similar  $R^2$  and a similar RMSE (Table 1). Thus, the use of GDD did not result in a relevant increase of accuracy.

The botanical composition varied between paddocks and years. The yield proportion of *Poa pratensis*, *Lolium perenne* and *Trifolium repens* was 86% in PD1, 60% in PD2 and 46% in PD3 in 2020. In 2021 their proportion was 96% in PD1, 68% in PD2 and 87% in PD3 and in 2023 their proportion was 79% in PD1, 70% in PD2 and 83% in PD3.

A fluctuating effect of the progress of the growing season (Figure 1) was observed, as shown previously for DOY in Peratoner *et al.* (2021): at comparable values of CSH, high values of DMY were observed in the first and in the last part of the growing season. Additionally, DMY increased faster towards the end of the CSH range. As a difference to the results of Peratoner *et al.* (2021), the model based on DOY did not detect any effect of the paddock. We tentatively suggest that this is due to the changes in the botanical composition over time, which progressively reduced the initial differences between the paddocks, as the proportion of the species tolerating frequent defoliation (*Poa pratensis*, *Lolium perenne* and *Trifolium repens*) increased in all paddocks, resulting in the species composition of the sward becoming more similar over time.

## Conclusion

Both GDDsgs and GDDsoy proved to be valid alternatives to DOY for estimating DMY based on CSH. The models with GDDsgs and GDDsoy were similar, suggesting that there is no need to compute the start of the growing season. Moreover, their use did not result in a relevant improvement of accuracy in comparison to the model based on DOY. Also, the final model using DOY is less complex because no

Table 1. Summary of parameter estimates  $\pm$  standard error (in parentheses) and coefficients of model performance for statistical predictive models using (a) GDDsgs, (b) GDDsoy and (c) DOY, to account for seasonality when estimating dry matter herbage yield (in  $\text{kg ha}^{-1}$ ) based on CSH (in cm).

Equation	$R^2$	RMSE
(a) $-13.21 (\pm 2.44) - 2.15 (\pm 1.35) \times \text{PD1} + 0.20 (\pm 1.46) \times \text{PD2} + 8.21 (\pm -0.72) \times \text{CSH} - 0.43 (\pm 0.07) \times \text{CSH}^2 + 0.01 (\pm 2\text{E}-3) \times \text{CSH}^3 + 4\text{E}-3 (\pm 1\text{E}-3) \times \text{GDDsgs} - 1\text{E}-6 (\pm 3\text{E}-7) \times \text{GDDsgs}^2 + 0.31 (\pm 0.14) \text{CSH} \times \text{PD1} + 0.02 (\pm 0.16) \times \text{CSH} \times \text{PD2}$	0.781	6.300
(b) $-13.53 (\pm 2.47) - 3.14 (\pm 1.35) \times \text{PD1} + 0.15 (\pm 1.47) \times \text{PD2} + 8.21 (\pm -0.72) \times \text{CSH} - 0.43 (\pm 0.07) \times \text{CSH}^2 + 9\text{E}-3 (\pm 2\text{E}-3) \times \text{CSH}^3 + 4\text{E}-3 (\pm 1\text{E}-3) \times \text{GDDsoy} - 1\text{E}-6 (\pm 3\text{E}-7) \times \text{GDDsoy}^2 + 0.31 (\pm 0.14) \text{CSH} \times \text{PD1} + 0.02 (\pm 0.16) \times \text{CSH} \times \text{PD2}$	0.780	6.315
(c) $-20.73 (\pm 4.51) + 0.5 (\pm 0.65) \times \text{PD1} + 0.37 (\pm 0.73) \times \text{PD2} + 8.22 (\pm 0.83) \times \text{CSH} - 0.41 (\pm 0.08) \times \text{CSH}^2 + 0.01 (\pm 2\text{E}-3) \times \text{CSH}^3 + 0.09 (\pm 0.04) \times \text{DOY} - 2\text{E}-4 (\pm 9.9\text{E}-5) \times \text{DOY}^2$	0.780	6.264

Data analysis performed with square root-transformed data.

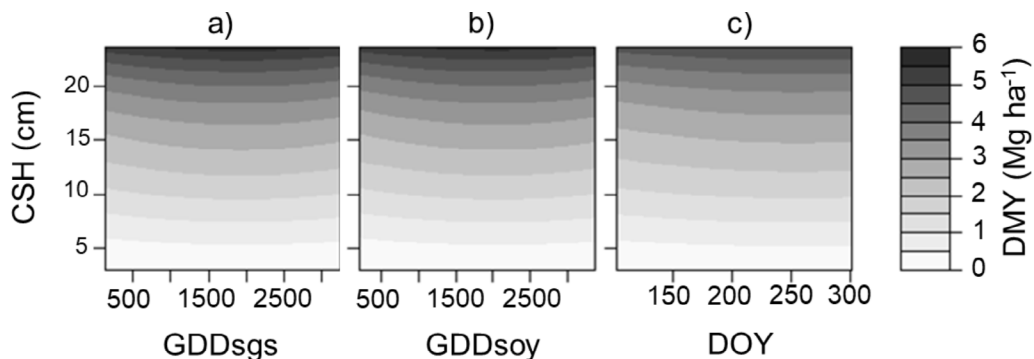


Figure 1. Dry matter herbage yield (DMY) as estimated using CSH and seasonality expressed by means of (a) GDDsgs, (b) GDDsoy and (c) DOY. Back-transformed predicted values are shown.

interaction term is needed. Thus, the use of DOY seems to be advantageous for practical aims because it is simpler, and it does not require meteorological data to be computed. Therefore, we recommend using DOY to account for seasonality in a DMY estimation. However, further research based on a larger data set and encompassing more years is advisable.

## Acknowledgements

This research was funded by the Action Plan 2016-2022 for Research and Training in the Fields of Mountain Agriculture and Food Science of the Autonomous Province of Bolzano/Bozen. We thank all current and former members of the working group Grassland Farming of the Laimburg Research Centre for assistance during data assessment.

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# Spectroscopy for assessing the nutritional value of pastures and enteric methane emissions from dairy cows in northern Sweden

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## Abstract

We aimed to estimate the nutritional value of leys using short-wave infrared (SWIR) spectroscopy. Samples were collected from 12 different field spots at seven dairy farms in Northern Sweden during summer 2021 and 2022. Sub-samples were chemically analysed for nutrient content, gas production and digestibility, and the remaining material was scanned with the Specim SWIR camera. Multivariate regression and machine learning algorithms were tested to estimate organic matter, crude protein, aNDF, ADF, non-fibre carbohydrates, energy, *in vitro* true organic matter digestibility, and enteric total gas and methane emissions predicted *in vivo*, where the SWIR spectral reflectance values were used as explanatory variables. The nutrient chemical composition of pastures varied among farms and months, and higher nutritional value was observed in June and August, irrespectively of year. Preliminary results show satisfactory performances for most quality parameters ( $R^2$  ranging from 0.83 to 0.99, normalized RMSE ranging from 0.4 to 5.1) and indicate the potential of spectroscopy to assess pasture quality, making it a viable alternative for nutritional monitoring. We plan to increase the size of the dataset to improve the robustness and generalization of the models.

**Keywords:** dairy cattle, grazing, pasture nutritional value, remote sensing, spectroscopy

## Introduction

Grazing contributes to various advantages for animal production systems and the environment, including enhanced animal welfare, improving livestock health, promoting open landscapes, and biodiversity preservation (Rivero and Lee, 2022). Allowing cows to graze can result in significant cost savings. However, meeting nutritional requirements on pasture, especially for high-yielding dairy cows, is challenging (Spörndly and Kumm, 2010). Access to timely information on pasture quality is crucial for milk producers to optimize grazing and feeding management, particularly in the face of global environmental challenges. Nonetheless, usual laboratory methods for nutritional analysis are time-consuming, expensive, and involve delays in relation to pasture growth. With remote sensing techniques, it would be possible to accelerate the estimates of the chemical composition of the pasture, providing information for diet adjustments and decision-making over the grazing season. Therefore, we aimed to estimate the nutritional value of leys by using short-wave infrared (SWIR) data.

## Materials and methods

Samples of pasture, mainly composed of grass and legumes, were collected from 12 different field spots at seven dairy farms in northern Sweden. The sampling occurred in June, July, and August in 2021 and 2022 and from the seven farms visited, five were the same for both years. At each of the 12 sampling spots, an area of 0.5×0.5 m was cut at approx. 3 cm height with grass shears. In the laboratory, after excluding the dead material, the samples were dried, milled and then merged by farm, month, and year; totalling 34 composed samples. A total of 36 samples was expected (six farms, three months and two years), however, due to raining days and wet fields, it was not possible to take samples from two farms in August 2021. Subsamples (10 g weight) were sent to DairyOne (Ithaca, NY, USA), for chemical analysis. Dry matter

(DM), ash, and crude protein (CP) were analysed according to AOAC (2005). Organic matter (OM) was determined as  $OM = 1000 - \text{ash}$  using ash content. Concentrations of aNDF and ADF were analysed following ANKOM procedures. Non-fibre carbohydrate (NFC) and metabolisable energy (ME) were calculated. The remainder of the samples underwent 48-hour gas *in vitro* production incubations using rumen fluid as an inoculum. The methods adopted are thoroughly described by Chagas *et al.* (2019) and the *in vitro* results for total gas and methane were expressed as predicted *in vivo* according to Ramin and Huhtanen (2012). The data on nutrient chemical composition and *in vivo* predicted analyses were processed using the GLIMMIX procedure in SAS version 9.4 (SAS Institute, Cary, NC, USA). Farm and month were treated as fixed effects, while year was considered as random effect (no statistical significance observed).

The dried and milled samples were scanned using a Specim SWIR sensor, which is a hyperspectral camera that measures reflected light in the short-wave infrared region of the electromagnetic spectrum (1000–2500 nm), with 288 spectral bands. Partial Least Square (PLS) and Support Vector Machine (SVM) algorithms were tested to estimate OM, CP, aNDF, NFC, ME, *in vitro* true organic matter digestibility (TOMD), methane, and total gas production *in vitro*, using the SWIR spectral reflectance as explanatory variables and a cross-validation resampling method. The best models were selected based on the coefficient of determination ( $R^2$ ) and the root mean square error (RMSE).

## Results and discussion

The observed concentrations of OM, CP, aNDF and ME differed between farms and months ( $P \leq 0.028$ ), irrespectively of the year ( $P > 0.05$ ). The average of CP ( $207 \text{ g (kg DM)}^{-1}$ ) and ME ( $10.4 \text{ MJ (kg DM)}^{-1}$ ) in June and August were higher ( $P \leq 0.017$ ) than in July, while aNDF was lower ( $P = 0.028$ ;  $454 \text{ g (kg DM)}^{-1}$ ). The NFC concentrations differed for farms while ADF only varied between months ( $P < 0.001$ ). The TOMD, total gas and methane varied between months ( $P \leq 0.022$ ) where June presented the highest values for all parameters ( $853 \text{ g kg}^{-1}$ ,  $255$  and  $40.5 \text{ ml (g DM)}^{-1}$ , respectively). The nutrient chemical composition results support the findings regarding digestibility and gas production and, based on that, it is clear that the pasture nutritional value was lower in July. This can be related to higher average temperatures observed for that month in both years.

Preliminary data show satisfactory performances for all the quality and gas parameters estimations using the SWIR spectral data ( $R^2$  ranging from 0.83 to 0.99, normalized RMSE ranging from 0.4 to 5.1). The PLS performed better than SVM, except for TOMD, methane and total gas, with good agreement between observed and predicted values for the calibration and validation data (Figure 1). Our results suggest that hyperspectral data can potentially assess forage quality and total gas and methane emissions from grazing forage. However, we need more data to ensure satisfactory robustness and generalization of the models.

## Conclusion

Our preliminary results suggest that imaging spectroscopy has good potential to estimate forage quality and enteric total gas and methane emissions predicted *in vivo*. The next step includes increasing the dataset to improve the model prediction and robustness.

## Acknowledgements

We would like to thank *Stiftelsen Lantbruksforskning* and *Regional Jordbruksforskning för Norra Sverige* for financing the project. We are also thankful for the additional support from SustAinimal.



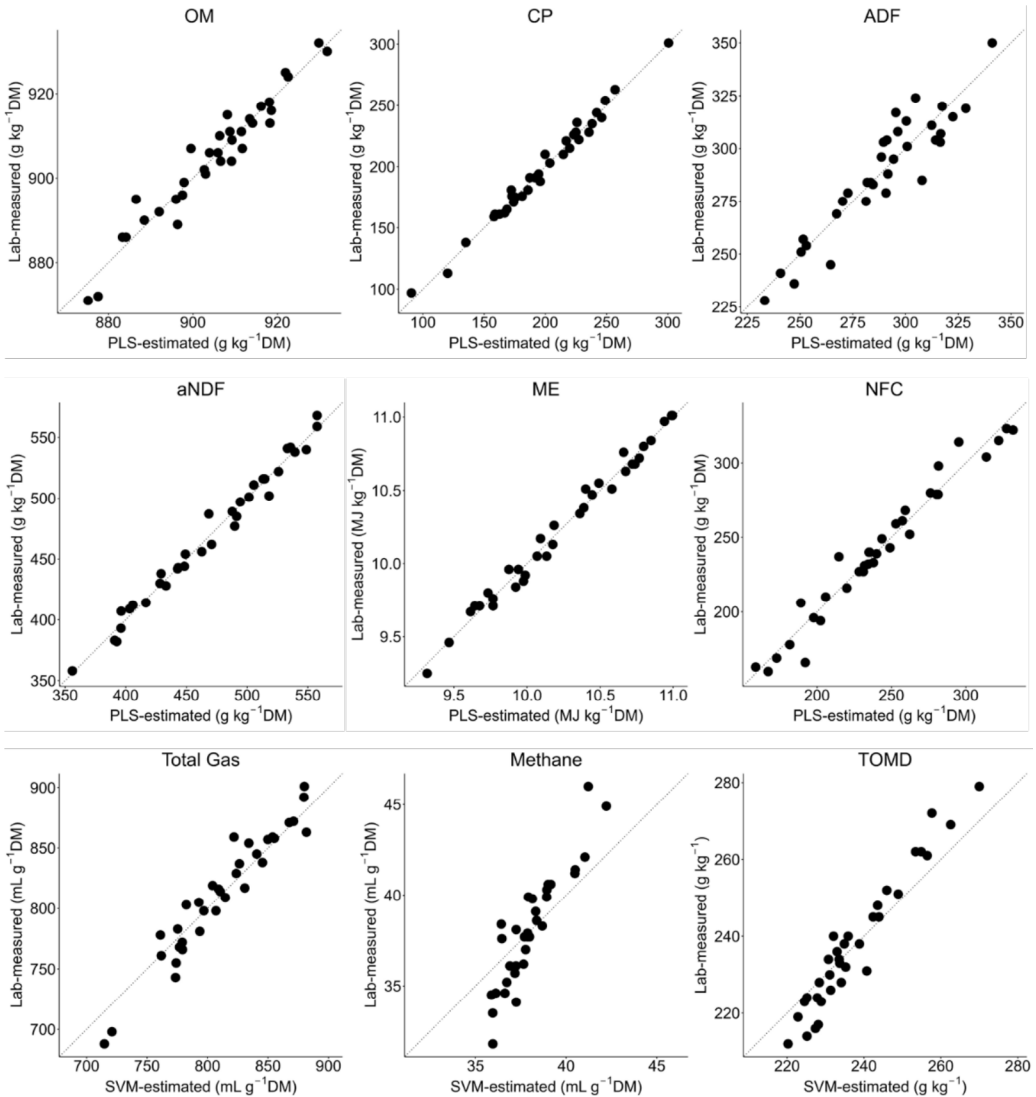


Figure 1. Scatter plots of modelling estimations versus lab-measured parameters.

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# Unlocking forage yield and field stand persistence potential of alfalfa in low pH soils through recurrent selection

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## Abstract

Alfalfa (*Medicago sativa* L.) production is limited in regions with low pH and high aluminium soils. Despite efforts to understand the mechanisms underlying low pH and aluminium stress in alfalfa, nothing has translated into acceptable cultivars. The lack of progress is most likely due to the limitations of the procedures used to identify germplasm with low pH tolerance. For this purpose, we evaluated 966 accessions from the National Plant Germplasm System (NPGS) in low-pH soil (pH 4.9). The most vigorous 135 entries were dug from the field and intermated in a greenhouse bee cage. The resulting half-sib families were established in the greenhouse and planted in rows in a low pH site (pH 5.2) and an adjusted pH site (pH 7) in randomized complete blocks with two replications. The average dry matter yield (DMY) in the adjusted pH field soil (264 g) was significantly higher ( $p < 0.01$ ) than the DMY in the low pH soil (187 g). DMY was used to calculate an Acid Soil Adaptation Index (ASAI), where values greater than one indicate acceptable tolerance. Sixty-nine families showed ASAI values greater than one, with 14 being higher than 2, suggesting a gain in low pH tolerance from the first cycle of selection.

**Keywords:** alfalfa, low pH tolerance, adaptation index, acid soils

## Introduction

Alfalfa (*Medicago sativa*) is the most widely grown forage in the world, with more than 60 million hectares harvested in the United States alone in 2022, producing 43.5 million metric tons (USDA-NASS, 2023). As a legume, alfalfa biologically fixes atmospheric nitrogen and improves soil health (Sun *et al.*, 2008).

Alfalfa production in many subtropical regions is significantly reduced in low pH soils, exacerbated by aluminium toxicity (Wolf *et al.*, 1994). Aluminium affects the cell division of root apices and increases the rigidity of the cell wall by cross-linking pectin (Zhang *et al.*, 2014) resulting in a stunted root system. Though lime and gypsum applications can improve alfalfa performance, these amendments can also be a costly and impractical long-term solution in many field settings, especially in marginal lands and developing countries (Bouton, 1996). Genetic improvement of low pH and aluminium tolerance in alfalfa has been the target of a great body of research over the last three decades (Dall'Agnol *et al.*, 1996; Khu *et al.*, 2012; Narasimhamoorthy *et al.*, 2007; Parrot and Bouton, 1990; Sledge *et al.*, 2002). However, phenotyping based on lab and greenhouse screening for AL-tolerance failed to translate to improved cultivars due to low forage yields in field production conditions.

In this study, we have undertaken a reverse approach, evaluating large collections of germplasm for forage yield in field conditions with low pH and high aluminium, select and recombine the top performers and evaluate the half-sib progeny in low and adjusted pH to identify the entries with equal or better performance in low pH condition to undergo further recurrent selection.

## Materials and methods

A subset of 966 Plant Introductions (PIs) from the USDA NPGS collection was planted in a low pH soil at Tifton, GA, in 2014. After four years, the surviving most vigorous plants were dug and planted

at the University of Georgia J. P. Campbell Research and Education Center (JPC) in Watkinsville, GA, USA (33.871513°N, 83.450632°W), in an adjusted soil pH (6.8) and evaluated for yield for three years. The top 135 PIs were dug from the field and crossed in a greenhouse bee cage. The resulting half-sib seed was established in the greenhouse and transplanted in two sites at JPC in May 2020 in two separate sites. Each trial consists of 140 entries (135 experimental and 5 commercial checks, including 2 cultivars developed for the southeast and 3 accessions used previously in lab and greenhouse studies of AL-tolerance) in a randomized complete block design (RCBD) with two replications, each with eight individual plants equally spaced in a 150 cm row with 76 cm between rows. The low pH site has a pH 5.2 and total aluminium concentration of 13 000 ppm. The adjusted pH site received 30 kg (equivalent of 1.9 Mg ha<sup>-1</sup>) of fast acting lime prior to planting to bring the pH above 6.8. Phenotypic data, including dry matter yield (DMY), plant count (as a measure of persistence), and fall dormancy ratings, were collected throughout 2021, 2022 and 2023. An identical trial was planted in the fall of 2022 at the University of Georgia Animal Science Farm in Tifton, GA, using cuttings of the same germplasm with the same arrangement as the original trial. Phenotypic data was collected throughout the growing season of 2023. Each row plot was harvested at 10–25% bloom every 25–28 days throughout the growing season (roughly April–October). Dry matter yield was determined by drying the samples 48 h in a convection dryer. Tolerance of alfalfa genotypes to low acid soil was assessed using an Acid Soil Adaptation Index (ASAI) as described in Howeler (1991).  $ASAI = \frac{Y_s \times Y_p}{\mu_s \times \mu_p}$ , where  $Y_s$  is yield in low pH condition;  $Y_p$  yield in normal pH condition;  $\mu_s$  average yield in low pH condition; and  $\mu_p$  average yield in normal condition. Analysis of variance (ANOVA) was conducted to test the effects of locations, years, genotypes, and their interactions using the “aov” and summary functions in R (version 4.2.1). Principal component analysis (PCA) biplots were plotted for the low pH and adjusted pH conditions to show the relationships among genotypes using Microsoft Excel.

## Results and discussion

The data were analysed across locations and years to estimate the effect of soil pH on yield by comparing the means for Average DMY/cut and Total DMY/year calculated for each half-sib family in both conditions.

Overall the entries in the panel, forage yield was significantly higher ( $p < 0.01$ ) in the adjusted pH (average yield cut<sup>-1</sup> entry<sup>-1</sup> = 264 g) than in the low (average yield cut<sup>-1</sup> entry<sup>-1</sup> = 187 g).

Due to significant interactions between location and pH condition, the ASAI calculations were conducted separately by location and across the two locations (Figure 1).

There were significant differences in the ASAI between the families ( $p < 0.01$ ) with more than half of the families producing lower forage yield under low pH compared to adjusted pH (ASAI < 1). However, 14 families had higher yield under low pH (ASAI > 2) suggesting they are potential candidates to be advanced for recombination for the next cycle of recurrent selection.

## Conclusion

The results of this work suggest that there is significant and usable genetic variation within the USDA NPGS germplasm collection to improve low pH and AL-tolerance in alfalfa through recurrent selection.

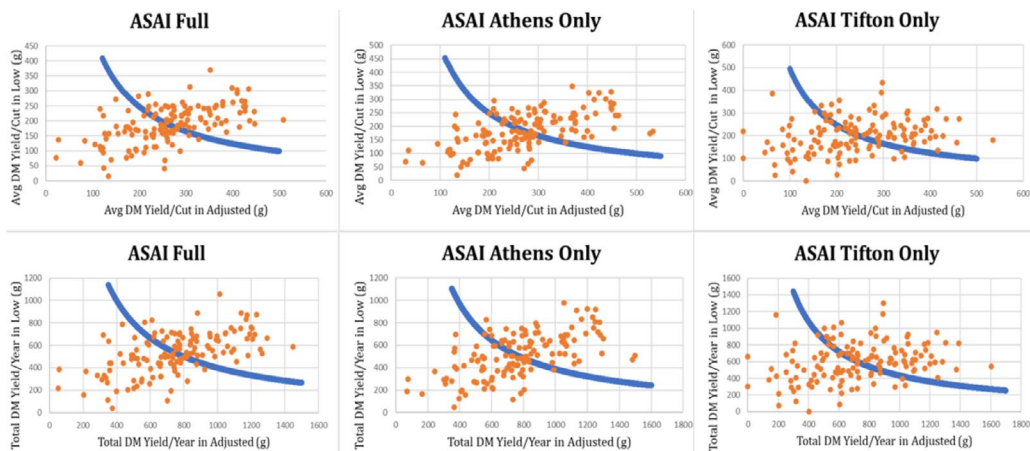


Figure 1. Acid Soil Adaptability Index scores for the population using average DM yield per cut (left panels) and total DM yield per year (right panels) for the full data set and the Athens and Tifton data separately, where the solid line indicates ASAI=1 (equal performance in low pH and adjusted pH soils) and the dots indicate each half-sib family entry. Values above the blue line indicate entries that performed higher in low pH.

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# The influence of foliar fertilizer on the productivity and quality of grass-red clover swards

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## Abstract

A field trial (2021–2022) was conducted in Latvia on an acidic Planosol (FAO) with a low phosphorus and a medium potassium content. Eight types of foliar fertilizer were studied, and dry matter yield, botanical composition, and forage quality were determined. The use of foliar fertilizers in a grass-red clover sward provided a positive effect, with increased dry matter yield. The highest dry matter yield (13.24 t ha<sup>-1</sup> year<sup>-1</sup>) was obtained in the foliar fertilizer variant with potassium, compared with 9.5 t ha<sup>-1</sup> year<sup>-1</sup> for the control. In all variants, the first cut provided most of the annual dry matter yield, 66–72% of the total sward yield. The results showed that the effects of applied foliar fertilizers on the grass dry matter quality indices were different. The highest crude protein content was produced in the variant with iron, providing a 13.4% increase compared to the control.

**Keywords:** foliar fertilizer, grass-red clover swards, productivity

## Introduction

The use of foliar fertilizers in the fertilization of grasses has not been widely studied. Unlike many other forms of fertilization, foliar fertilizers provide plants with both macronutrients and micronutrients. Only a few studies can be found on the influence of micronutrients on grasses. Examples include the effect of foliar sulphur fertilization in Poland, in which two rates of nitrogen fertilization (50 and 100 kg N ha<sup>-1</sup>) and four rates of sulphur fertilization (0, 5, 10, and 15 kg S ha<sup>-1</sup>) were compared in leys of pure sown hybrid ryegrass (*Lolium × boucheanum* Kunth.) and mixed hybrid ryegrass-white clover (*Trifolium repens* L.) swards. The use of sulphur fertilization showed a positive effect on dry matter yield increase compared to the control treatment, and the rate of 10 kg S ha<sup>-1</sup> produced the highest yield increase (Grygierzec *et al.*, 2015). In a study conducted in the Czech Republic, the response of cocksfoot (*Dactylis glomerata* L.) cv. Dana, to a foliar fertilizer containing selenium found that the use of selenite and selenate significantly increased the Se content in the green mass of cocksfoot (Caslavova *et al.*, 2017). The objective of the present research was to study the influence of different types of foliar fertilizer on the productivity of grass-red clover swards.

## Materials and methods

A field trial (2021–2022) was conducted in Latvia (56°51'22.8" N 22°21'53.6" E) on grass-red clover swards in the first year of production. The soil was a Planosol (FAO), agrochemical parameters: pH<sub>KCl</sub> 5.1; organic matter 2.6%; phosphorus (P<sub>2</sub>O<sub>5</sub>) content 55–70 mg (kg soil)<sup>-1</sup>, and potassium (K<sub>2</sub>O) content 110–130 mg (kg soil)<sup>-1</sup>. Eight fertilizer treatments were compared. Each experimental plot was 100×3 m in each replication. All research variants were set up in four repetitions. A background mineral fertilizer with 74 kg N, 35 kg P<sub>2</sub>O<sub>5</sub> and 100 kg K<sub>2</sub>O ha<sup>-1</sup> was applied in early spring to all swards in all variants (including control). In addition, different types of liquid foliar fertilizers were sprayed when the swards had reached height of 10 cm. The type and rate of foliar fertilizers are presented in Table 1. Swards were cut three times during the vegetation season, average cutting height of 7 cm. The first mowing was carried out in the first 10 days of June (beginning of budding-flowering), and subsequent mowing was done approximately after 40 days, in mid-July and at the end of August.

Table 1. Plant foliar fertilizer product treatments and plant nutrients applied with each.

Variant/Option	Foliar fertilizer	Plant nutrients (kg ha <sup>-1</sup> )
Control	No treatment	–
Iron (Fe)	Ultraferro (1 kg ha <sup>-1</sup> )	Fe, 0.102
Copper (Cu)	Tradecorp Cu (1 kg ha <sup>-1</sup> )	Cu, 0.145
Zinc (Zn)	Tradecorp Zn (1 kg ha <sup>-1</sup> )	Zn, 0.14
Boron (B)	TradeBor (1 l ha <sup>-1</sup> )	B, 0.145
NPK	Nutricomplex 13-40-13 (5 kg ha <sup>-1</sup> )	N, 0.65; P, 0.2; K, 0.65
Manganese (Mn)	Tradecorp Mn (1 kg ha <sup>-1</sup> )	Mn, 0.13
Potassium (K)	Final K (2 l ha <sup>-1</sup> )	N, 0.9; K, 0.93

The sward herbage yield, dry matter quality indices, and botanical composition of grasses, legumes (red clover) and forbs were determined after each cutting. The following qualitative indicators of forage quality were determined in “Pieno tyarimi”, the accredited laboratory of UAB in Lithuania: the content of dry matter (DM), fat and ash were determined by gravimetric analysis; crude protein (CP) content in the DM was determined by modified Kjeldahl, and mineral elements were analysed by atomic absorption spectrometry. The data were statistically analysed using the analysis of variance (ANOVA) and Microsoft Excel computer program, and the difference among means was determined by LSD test at the  $P < 0.05$  probability level.

## Results and discussion

All foliar fertilizers had a positive effect on the dry matter yield in grass-red clover swards. The highest dry matter yields were obtained using fertilizer options with K (13.24 t ha<sup>-1</sup>) and Mn (12.38 t ha<sup>-1</sup>). Generally, in all foliar fertilizer variants, the dry matter yield ranged from 10.73 t ha<sup>-1</sup> to 13.24 t ha<sup>-1</sup> (Table 2), compared with only 9.5 t ha<sup>-1</sup> for the control.

The use of foliar fertilizers contributed to the average yield increase of 2.14 t ha<sup>-1</sup>. The highest yield increase of 3.74 t ha<sup>-1</sup> was observed in the foliar fertilizer variant with potassium. The effectiveness

Table 2. Influence of the different types of foliar fertilizer on the dry matter yield of grass-red clover swards (t ha<sup>-1</sup>) (on average in two years of use in three cuts).

Variant/Option	1 <sup>st</sup> cut	2 <sup>nd</sup> cut	3 <sup>rd</sup> cut	In total
Control	6.25±0.21	1.17±0.08	2.08±0.09	9.50 <sup>a</sup>
Iron	7.19±0.19	1.39±0.02	2.15±0.02	10.73 <sup>b</sup>
Copper	7.25±0.36	1.47±0.06	2.22±0.04	10.94 <sup>bc</sup>
Zinc	7.35±0.18	1.43±0.04	2.20±0.07	10.97 <sup>bc</sup>
Boron	7.74±0.38	1.48±0.02	2.15±0.06	11.37 <sup>c</sup>
NPK	8.14±0.23	1.44±0.03	2.21±0.05	11.80 <sup>c</sup>
Manganese	8.63±0.30	1.52±0.06	2.23±0.04	12.38 <sup>d</sup>
Potassium	9.52±0.23	1.50±0.03	2.21±0.02	13.24 <sup>e</sup>
Average	7.76	1.43	2.18	11.37
LSD <sub>0.05</sub>	0.39	0.07	0.08	0.45

Different letters indicate a significant ( $P < 0.05$ ) difference among treatments.

of foliar fertilization has also been noted in previous studies; however, the effect of micronutrients on the level of productivity has not been accurately evaluated. Studies have confirmed that sulphur foliar fertilization provides yield increases by increasing the nitrogen use efficiency (Grygierzec *et al.*, 2015). Other studies have found that foliar fertilization affects the plant yield by increasing the availability of certain elements, such as stems and leaves, which, in turn, increases the biomass volume and aboveground vertical distribution (Sosnowski *et al.*, 2018).

Most of the dry matter yields were produced by the first cut. In our study, the average dry matter yield of the first cut provided 65.8–71.9% of the total annual dry matter yield. Higher dry matter yields of the first cut grass were produced in all foliar fertilizer variants. The comparison of the yield distribution between cuts demonstrated that in the first harvest, the highest share was produced by the foliar fertilizer variant with potassium, providing 71.9% of the annual harvest. In contrast, the lowest share was observed in the control variant. Comparing the productivity levels between variants and cuts, it was observed that foliar fertilization significantly affected the yield level of the first and second cut. The positive effect of foliar fertilization on the third cut yield was less expressed. Although foliar fertilization was applied once per season, the impact on the results of the second cut harvest is possible through the partial mobilization of absorbed nutrients in the root zone and their effect on root development, which accordingly improved plant growth in adverse weather conditions. There was no significant effect of foliar fertilization application on the botanical composition of swards. The proportions of the groups of herbage species were more affected by cut. In the 1<sup>st</sup> cut, the proportion of legumes ranged between 12% and 15%, in the 2<sup>nd</sup> cut between 25% and 27%, and in the 3<sup>rd</sup> cut between 20% and 21%. The proportion of forbs in all cuts was very low (1–3%).

The results suggest that the effect of applied foliar fertilizers on the grass dry matter quality indices differed. The highest protein content was produced in the variant with Fe, providing a 13.4% increase compared to the control. In the variant with K, the content of mineral elements increased compared to the control variant: calcium by 51.2%, phosphorus by 7.4% and magnesium by 35.3%. Combined use of mineral fertilizers and foliar fertilizers was economically beneficial, regardless of meteorological conditions. All types of foliar fertilizers provided an increase in income by an average of 22.5%, but the K fertilizer variant provided a 39% higher income compared to the control variant.

## Conclusion

We demonstrated that foliar fertilization is a potentially effective strategy to increase grassland yields. The application of foliar fertilizers was effective in all treatments. The highest dry matter yield of 13.24 t ha<sup>-1</sup> year<sup>-1</sup> and 0.82 t ha<sup>-1</sup> of crude protein yield in the first ley year were provided using a foliar fertilizer containing potassium. In the control variant, the dry matter yield was 9.5 t ha<sup>-1</sup> year<sup>-1</sup>. No significant effect of foliar fertilizer application on the proportion of the groups of herbage species in the swards was established.

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# Nitrate nitrogen residues measured in autumn in Flemish grassland soils

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## Abstract

To comply with the European Nitrates Directive, the Manure Action Plan (MAP) was introduced in 1991 in Flanders. Since its introduction several successive MAPs have been implemented. Flemish legislation became more differentiated and increasingly stringent due to changes in a.o. maximum nitrogen (N) fertilisation rates (or N fertiliser limits), maximum nitrate ( $\text{NO}_3^-$ )-N-residues allowed in the autumn and fertiliser application periods. The average  $\text{NO}_3^-$ -N-residue measured in grassland soils is low compared to other crops due to the long growing period and high N uptake. In the autumn period, between 2004 and 2012, the average measured soil  $\text{NO}_3^-$ -N-residues were significantly reduced, corresponding to the N fertilisation limits being lowered. However, despite further stringent legislation post-2012, the measured average  $\text{NO}_3^-$ -N-residues in soil have been stable, or even higher after dry summers. The average  $\text{NO}_3^-$  concentrations in surface waters followed the same trend in the corresponding winter year (July–June) as the average autumn  $\text{NO}_3^-$ -N-residues in soil.

**Keywords:** nitrate nitrogen residue, leaching, legislation

## Introduction

Since 1991 the European Nitrates Directive (91/676/EEC) has imposed a maximum of  $50 \text{ mg NO}_3^- \text{ l}^{-1}$  to protect ground and surface waters against agricultural pollution. To comply with the Directive, the MAP was introduced in the same year in Flanders (the northern part of Belgium). Since its introduction, several successive MAPs have been implemented. The  $\text{NO}_3^-$ -N-residues in the soil profile (0–90 cm depth) are measured before the onset of winter (1 October–15 November) as an indicator of the potential N pressure from agriculture as  $\text{NO}_3^-$  leaching occurs mainly during the winter period (Anonymous, 2008, 2023). The aim of this paper is to investigate the effect of legislation on soil  $\text{NO}_3^-$ -N-residues measured in autumn and average  $\text{NO}_3^-$ -concentrations in surface waters.

## Materials and methods

The successive maximum N fertilisation rates, fertiliser application periods and maximum  $\text{NO}_3^-$ -N-residues allowed in the autumn for grassland in the MAP legislation were assembled (Anonymous, 1995, 2002, 2006, 2011, 2016, 2019). The average  $\text{NO}_3^-$ -N-residues measured in grassland and all fields in autumn (2004–2022) were collected (Anonymous, 2008, 2023). A monitoring network for surface water measures the  $\text{NO}_3^-$  concentrations in 754 surface-water monitoring points in areas dominated by agricultural land use (VMM, 2022).

## Results and discussion

At the start of the MAP in 1991, the N fertiliser limits were  $400 \text{ kg total N ha}^{-1}$  for all crops. Afterwards the N fertiliser limits were increased for grassland (up to  $500 \text{ kg total N ha}^{-1}$  in 2004–2006) and decreased for other crops (Anonymous, 2002) based on the measured biomass yields obtained from experimental fields and a calculated soil balance of the major crops (Hofman *et al.*, 1995). Since 2011, N fertiliser limits are based on the average biomass yields of farmers' fields (Anonymous, 2011). Until 1998, all N could be applied by manure which was progressively reduced to 250 and limited to  $170 \text{ kg total N ha}^{-1}$  in



NO<sub>3</sub><sup>-</sup> vulnerable zones (except for derogation fields in 2007–2022). Gradually the NO<sub>3</sub><sup>-</sup> concentration in ground and surface water was taken into account in the N fertiliser limits. Since 2011, N fertiliser limits for grassland have been lower for sandy soils with a high NO<sub>3</sub><sup>-</sup> leaching risk than non-sandy soils (Anonymous, 2002, 2006, 2007, 2011, 2016, 2019). Currently (in 2024) the N fertiliser limits of grassland vary between 385 effective N ha<sup>-1</sup> (cut grassland on non-sandy soils in area type 0 or 1 with good water quality) and 188 effective N ha<sup>-1</sup> (grazed grassland on sandy soils in area type 2 or 3 with bad water quality) (Anonymous, 2019).

Since 2011, the maximum NO<sub>3</sub><sup>-</sup>-N-residues have become more divergent depending on crop, soil texture and water quality (Figure 1) (Anonymous, 2011; 2016; 2019). Based on research showing the link between fertilisation rate and NO<sub>3</sub><sup>-</sup>-N-residues, grassland has a lower maximum soil NO<sub>3</sub><sup>-</sup>-N-residue than other crops (Anonymous, 2019; D’Haene *et al.*, 2014).

The end of the slurry application period in grassland varied from 31 October at the start (1991–1995) to 15 August (2015–) (Anonymous, 2002, 2006, 2011, 2016, 2019).

The average autumn NO<sub>3</sub><sup>-</sup>-N-residue measured in grassland is low compared to other crops due to the long growing period and high N uptake (Figure 2). Moreover, the fractionation of the fertilisation throughout the year makes it easier to take the weather conditions into account. The average NO<sub>3</sub><sup>-</sup>-N-residues in grassland and all fields decreased significantly ( $p < 0.05$ ) between 2004 and 2012 (Figure 2). In this period, N fertilisation limits were reduced except for grassland and gradually the agricultural area became NO<sub>3</sub><sup>-</sup> vulnerable. Since about 2012, the average NO<sub>3</sub><sup>-</sup>-N-residues have been at a stable level, or even higher. The further divergence and tightening of the legislation did not result in a clear positive effect on the average soil NO<sub>3</sub><sup>-</sup>-N-residues. By contrast, dry weather conditions had a negative effect on the average soil NO<sub>3</sub><sup>-</sup>-N-residues and explain the average higher NO<sub>3</sub><sup>-</sup>-N-residues in 2018 and 2020. The rain amount in spring (April–June) has been shown to mainly affect the autumn NO<sub>3</sub><sup>-</sup>-N-residues (Odeurs *et al.*, 2020). The R<sup>2</sup> between the spring rain and average soil NO<sub>3</sub><sup>-</sup>-N-residues measured since 2012 for grassland was 0.38 ( $p < 0.05$ ). The negative correlation was slightly higher for the spring + summer rain (April–September) ( $R^2 = 0.45$  ( $p < 0.05$ )).

The average NO<sub>3</sub><sup>-</sup>-concentrations in surface water followed the same trend as the average autumn NO<sub>3</sub><sup>-</sup>-N-residues (Figure 2). The R<sup>2</sup> between the average autumn NO<sub>3</sub><sup>-</sup>-N-residues measured in grassland since 2004 and the average NO<sub>3</sub><sup>-</sup>-concentrations in the surface waters was 0.60 ( $p < 0.001$ ).

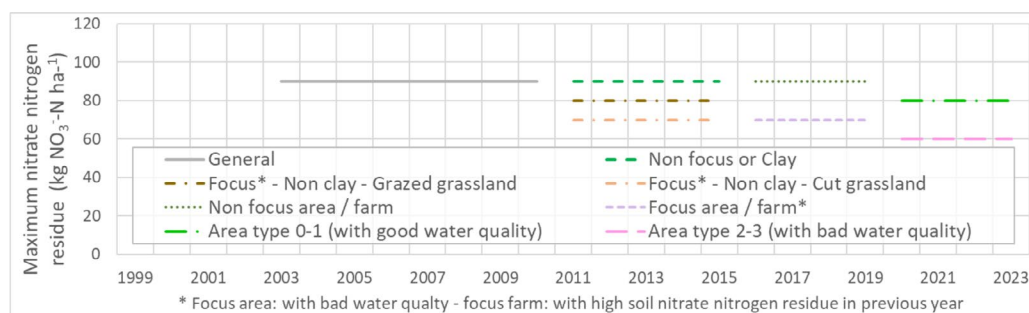


Figure 1. The maximum nitrate nitrogen residue allowed in grassland (0–90 cm, 1 October–15 November) as function of the year (Anonymous, 2002; 2006; 2011; 2016; 2019).

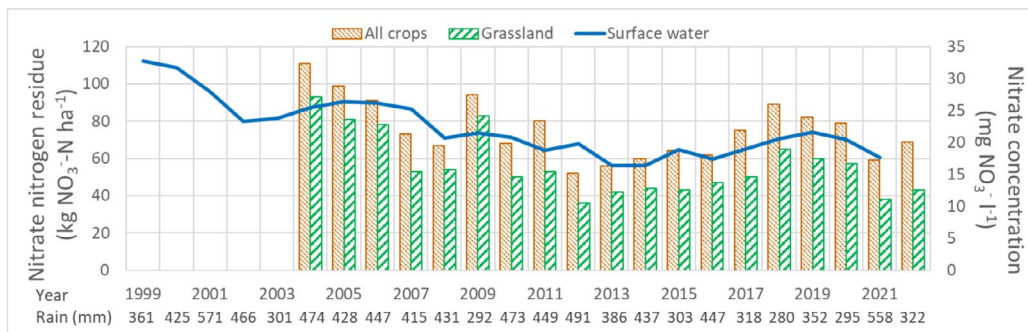


Figure 2. The average autumn soil nitrate nitrogen residue (0–90 cm), nitrate concentration in the surface water and rain in spring + summer (April–September) in function of the year (Anonymous, 2008, 2023; VMM, 2022).

## Conclusion

The maximum N fertilisation rates, fertiliser application periods and maximum soil NO<sub>3</sub><sup>-</sup>-N-residues allowed in the autumn in MAP legislation have frequently become stricter and more targeted. Until 2012 the measured average autumn soil NO<sub>3</sub><sup>-</sup>-N-residues were significantly reduced, but have been at a stable level since then or in some years even higher after dry summers. The NO<sub>3</sub><sup>-</sup>-concentrations in surface waters in the corresponding winter year (July–June) followed the same trend as the average autumn NO<sub>3</sub><sup>-</sup>-N-residue.

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# Use of two GreenFeed Emission Monitoring units in an indoor-grazing management system to estimate methane production in cows

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## Abstract

This study investigates cow behaviour when visiting two GreenFeed Emission Monitoring (GEM) units within a Part-Time Grazing (PTG) system. Two separate PTG systems were assessed in Sweden and Norway, involving Nordic Red and Norwegian Red dairy cows, respectively. In Sweden, 24 cows were allocated to treatments with restricted access to pasture, either daytime or nighttime grazing. Meanwhile, the Norwegian PTG involved 33 cows with free pasture access, categorized by varying training levels (Partially or Fully). In both PTG systems, cows were exposed to GEM units positioned indoors (Indoor) and in the grazing pastures (Pasture), with individual visitations recorded. Significant variations in visitation patterns were observed. In the restricted access PTG, Nighttime grazing access cows exhibited reduced visits to the Indoor GEM unit but increased visits to the Pasture GEM unit compared to Daytime grazing. Conversely, within the free access PTG, fully trained cows demonstrated elevated visits to the pasture GEM unit and total visits compared to their partially trained counterparts. These findings highlight the influence of temporal conditions and training levels on cow-visiting behaviour within PTG systems.

**Keywords:** part-time grazing, Norway, Sweden, training

## Introduction

The dairy production system in the Scandinavian countries is mainly based on indoor feeding (silage and concentrate) throughout the year, combined with part-time grazing (PTG) for two to four months during the summer. Grazing is beneficial for animal welfare, may lower feed costs, and reduce enteric methane (CH<sub>4</sub>) emissions. Feed intake is the main driver of milk yield and enteric CH<sub>4</sub> production from dairy cows. However, it is challenging to measure feed intake and CH<sub>4</sub> production during grazing conditions. In PTG systems, accurately monitoring CH<sub>4</sub> production over an extended period can be challenging due to the animals' movement between pasture and the barn. Consequently, the complete extent of CH<sub>4</sub> emissions in PTG systems remains only partially understood.

Advancements in monitoring systems, including the GreenFeed™ emission monitoring (GEM) system (C-Lock, Rapid City, SD, USA), offer reliable estimates of daily CH<sub>4</sub> production comparable to established methods like respiration chambers. However, ensuring accurate CH<sub>4</sub> production estimates from spot sampling measurements (during visitation) requires data spanning across time (number of visits) and incorporating a large number of animal measurements due to increase within-day and within-animal variation (Hammond *et al.*, 2016). The GEM system is a spot-sampling technique that requires a minimum of 20–30 voluntary visits per cow and treatment to significantly detect an effect, equating to 7–14 days of recordings (Renand and Maupetit, 2016). Incorporating this consideration into experimental designs is important in avoiding skewed estimates. Hence, to address potential constraints of point measurement using GEM systems, it is recommended to employ two parallel GEM units, one indoor and one in the pasture, to effectively evaluate CH<sub>4</sub> recordings under the PTG systems.

The primary objective of this paper is to examine cows' visitation patterns to two GEMs, positioned indoor or in pastures. The study aims to gain insights into two different PTG systems, distinguishing between restricted grazing with access to pastures during either Daytime or Nighttime hours, and allowing free access grazing with cows at various levels of training (Partially or Fully).

## Materials and methods

The study was conducted in two separate PTG systems using dairy cows at the Swedish University of Agricultural Sciences in Umeå (Sweden) in 2021 and at NIBIO Steinkjer (Norway) in 2023, both during the summer season. Both experiments used two GEM units simultaneously employed, with one positioned inside the barn (Indoor) and the other in the grazing pastures (Pasture), to record CH<sub>4</sub> emissions during cow visits. Each visit involved providing cows with drops of concentrate (each drop around 50 g) to encourage them to visit the GEMs unit.

In Sweden, 24 Nordic Red dairy cows were assigned to one of two treatments: Daytime (10 h daytime) or nighttime (12 h nighttime) pasture access (Lardy *et al.*, 2023). The cows in each treatment received the same *ad libitum* partial mixed ration indoor and *ad libitum* pasture allowance. Animals were acclimated to the pasture GEM unit for a 28-day training period after which GEM unit visits were recorded over a 7-day period.

In Norway, 33 Norwegian Red cows were subjected to free access pasture PTG system, with constraints on the percentage of their daily ration from grass silage and concentrate in the indoor facility, yet with *ad libitum* access to pasture forage, where a new strip was offered every second day. Two groups of cows in the free-access grazing underwent different treatments based on their training levels (Partially and Fully trained). During both training phases, researchers lured individual cows during pasture visits to utilize the pasture GEM unit. Partial training lasted for one week, while Full training lasted for two weeks, with the latter group comprising double the number of cows that successfully used the pasture GEM unit by the end of the training. This was followed by a 14-day period of data collection to observe GEM unit visits.

Under the two separate PGT systems, recordings of visits to the two GEM units, as well as their cumulative total, were documented for each individual cow within each treatment, and a descriptive analysis was conducted. Utilizing the Microsoft Excel® data analysis tool, a two-sample *t*-test, assuming unequal variances, was employed to scrutinize cow visits (Indoor, Pasture, Total) within each PTG system separately, considering restricted (Daytime or Nighttime) or free (Partially or Fully trained) access to pasture. Furthermore, within the restricted access PTG system, the percentage utilization of the two GEM units was examined for each treatment (Daytime and Nighttime). Significance for all analyses was determined at a threshold of  $p \leq 0.05$  on a two-tailed *t*-test.

## Results and discussion

During the 7-day data collection period in the Swedish trial, visits to the GEM units resulted in 380 Indoor visits (207 for Daytime and 173 for Nighttime) and 226 Pasture visits (67 for Daytime and 159 for Nighttime). T-test analysis revealed statistically significant differences in GEM unit visits between Daytime and Nighttime access. The Nighttime grazing cows had more visits to the Pasture unit compared to the Daytime cows (Table 1). Lardy *et al.* (2023) did not discuss if these differences were due to temperatures but found estimated feed intakes from pasture were equal for the two treatments (Table 1). The Daytime grazing group visited the Indoor GEM 75.5% and the Pasture GEM 24.5% ( $p < 0.001$ ). There were no differences in visits of Indoor and Pasture GEM for Nighttime grazing group (53.3% vs. 46.7%,  $p = 0.16$ , respectively). The difference observed between Daytime and Nighttime access underscores the impact of temporal conditions on GEM unit engagement. Notably, during Nighttime grazing, cows were observed to have an equitable utilization of the two GEM units.

Table 1. Average number of cows' visitation to two GreenFeed Emission Monitoring (GEM) systems, positioned indoors or in pastures in two Part-Time Grazing (PTG) systems: Restricted grazing access (Daytime vs. Nighttime; 7 days) or free access, with cows at different levels of training (Partially vs. Fully; 14 days). Average GEM visits per cow over periods (Indoor, Pasture, and Total visits) and corresponding *p*-values for within-system (PTG) differences in GEM utilization.

PTG Systems		Average visits per cow			<i>p</i> -value		
		Indoor	Pasture	Total	Indoor	Pasture	Total
Restricted grazing	Daytime (7 days)	20.7	6.7	27.4	<0.01	<0.01	0.12
	Nighttime (7 days)	12.4	11.4	23.8			
Free access	Partially (14 days)	7.9	2.3	10.2	0.99	<0.01	0.05
	Fully (14 days)	8.0	10.1	18.1			

For the free access PTG system performed in Norway, GEM unit visits recorded over a 14-day data collection period, resulted in 255 Indoor visits (135 for Partially and 120 for Fully trained) and 190 Pasture visits (36 for Partially and 151 for Fully trained). Statistically significant differences in cow visitation were observed between Partially and Fully trained cows, particularly concerning visits to the Pasture GEM and Total visits. Fully trained cows demonstrated elevated visits to the Pasture GEM unit and Total visits compared to their Partially trained counterparts (Table 1). The higher visitation to the outdoor GEM unit was not at the expense of visits to the indoor unit.

## Conclusion

The results underscore the impact of cow behaviour on CH<sub>4</sub> monitoring systems, emphasizing the effects of temporal conditions within restricted grazing access PTG systems and the correlation between animals' training levels. This is particularly relevant to the utilization of pasture GEM units in free-access grazing for obtaining realistic CH<sub>4</sub> measurements.

## Acknowledgements

Trøndelag county authority RT21.05; Steinkjer municipality 2019/198; NIBIO 19/00310-14 project 51516; SLU, Formas Council, 2020-02977.

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# What information is needed for upscaling grassland ecosystem services to landscape scale?

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## Abstract

Field measurements of ecosystem services (ES) are laborious and costly, so ES cannot be measured at larger spatial scales. Therefore, ES are upscaled from local measurements to a whole region, based on a restricted number of field-scale measurements combined with environmental and management predictors available for the whole region of interest. The data available to estimate ES are decisive for the quality of the resulting ES maps and the robustness of the conclusions that can be drawn. We present two ES measured in 92 grasslands and determine how well these can be upscaled using different data sources. We developed stepwise models using (i) field-scale agricultural census data, (ii) topographic characteristics, (iii) soil maps, (iv) soil measurement data, (v) detailed management data, and (vi) plant community information. Resulting models reveal forage protein content to be already well predicted by agricultural census data, but for soil carbon stocks considerably more information was needed for a reliable prediction. The explained variance ( $R^2$ ) of the final models ranges from 0.61 to 0.74, showing a good fit but also considerable uncertainty associated with ES maps, despite the vast data used for the final predictions.

**Keywords:** ecosystem services, management, protein content, soil carbon stocks, upscaling

## Introduction

Ecosystem services (ES) are in the focus of many agricultural policies and, potentially in the future, also part of result-based agri-environmental payments. Field measurements of multiple ES are laborious and costly (Richter *et al.*, 2021), and thus not realistic at larger spatial scales. Therefore, upscaling of ES, from local to regional, based on a restricted number of measurements used to estimate the ES of entire farms or landscapes, is used to produce ES maps, based on readily available environmental and management data (Felix *et al.*, 2022; Le Clec'h *et al.*, 2019). The upscaling process and the data used to estimate ES are decisive for the quality of the resulting maps, determining the reliability of the conclusions drawn from this information. Thus, it is important to know what data is needed to achieve a model that is sufficiently precise for upscaling ES. At present, little is known about data needs and model quality during upscaling, especially when considering the many different ES that are important to meet societal demands.

## Materials and methods

We studied 92 permanent grasslands in the Canton of Solothurn, Switzerland, which were (i) either unfertilised or fertilised and (ii) either used as meadow (mostly mown) or pasture (mostly grazed). In fertilised grasslands, the total available nitrogen in annual fertiliser applications per year was on average 85 (SE = 54) kg ha<sup>-1</sup>, going up to a maximum of 203 kg ha<sup>-1</sup>. This shows the considerable variability in management intensity in the fertilised grasslands included in the study. On these 92 plots, we measured two highly relevant ES using well-established indicators (Richter *et al.*, 2021). The first indicator was the raw protein content in aboveground plant biomass close to the first harvest date (dry matter). Protein content, a proxy for forage quality, is an important indicator for the provisioning ES obtained by grasslands. Grazing cages ensured biomass was available for sampling, even if livestock were grazing early in the season. Unfertilised meadows had a delayed date for the first cut (June 15<sup>th</sup> in the lowlands), as all

unfertilised grasslands belong to Swiss agri-environmental schemes (biodiversity promotion areas). Yet, this restriction did not apply for extensive pastures. The second indicator was a regulating ES, i.e., the soil organic carbon (SOC) stock, measured in the top 20 cm of the soil and corrected for inorganic carbon. Based on this survey data and further environmental and management information, which was partly measured in the field and partly taken from public sources, we ran stepwise linear regressions in R. The stepwise models were based on (i) agricultural census data at the field scale (i.e., management categories), (ii) topography (from digital elevation model), (iii) soil maps with four classes for agricultural suitability at the field scale (GIS maps), (iv) soil measurement data (field survey, 0–20 cm), (v) management details (based on farmers interviews), and (vi) plant community information (field survey, cover sum of plant functional groups; Tables 1 and 2). The order of the data sources stepwise entering the models was set according to their availability, from readily available data to laborious surveys. Alternative orders were not tested. All models were additionally optimised using AICc (step function in R). Predictors entering the models were allowed to correlate no more than  $r=0.45$  (Spearman correlation). See Table 1 for further details on the predictors used.

## Results and discussion

We give detailed results for the stepwise models for SOC stocks (Table 1), while for protein content we only provide model quality for each step (Table 2). We found a significant increase in explained variance ( $R^2$ ) and a decrease in AICc when including more data in the predictions. This was much less pronounced for protein content, which was already reasonably well predicted by the (interacting) management categories derived from census data. For SOC stocks, in contrast, the initial model performed poorly, highlighting the data demand to precisely predict this ES for upscaling. For the SOC stock, we found a sharp increase in model quality when particularly relevant data was included, i.e., soil measurement data. From the different data sources used, not only the management categories appeared to be important in most cases, but also topography was frequently included in final models. On the other hand, details on grassland management, i.e., fertilizer application levels (more detailed than the categorisation *unfertilized vs. fertilized* as given by the management categories based on the census data) and grazing intensities, were of surprisingly little relevance. Potentially, management categories already explained most of the variability related to management. Thus, although nitrogen applications varied widely among fertilised grasslands, the presence of fertilisation appears to be more important for the two ES than exact fertilisation rates.

Note that model 5 was the same as model 4, as additional data did not improve model performance and was thus excluded. Positive and negative estimates (and  $t$ -values) abbreviated by  $\uparrow$  and  $\downarrow$ , respectively. Significance coded as: \*\*\* $p<0.001$ , \*\* $p<0.01$ , \* $p<0.05$ , ( $\cdot$ ) $p<0.1$ , n.s.=not significant; / indicates predictors *a priori* not included in the respective model; “-” indicates predictors excluded based on the step function using AICc.

## Conclusion

We find models to require different data sources to upscale ES data depending on the specific ES considered. Especially for SOC stocks, more than one data source was needed to achieve a model  $R^2>0.6$ , which still leads to considerable errors if such models are used for upscaling. In line with previous research (Le Clec'h *et al.*, 2019), we show the vast data demand inherent to the upscaling of ES, especially if multiple ES are considered and robust results are to be obtained. Future research should seek ways to gather additional ES predictor data at low costs, such as via remote sensing (Muro *et al.*, 2022; Weber *et al.*, 2023), to further improve the quality of ES maps used for future agri-environmental decision-making.



Table 1. Results of linear regressions predicting soil organic carbon stocks, stepwise including more data from model 1 (only i) to model 6 (i to vi).

		Model quality					
		Model 1	Model 2	Model 3	Model 4	Model 5	Model 6
Adj. $R^2$ , model $p$		0.13 **	0.36 ***	0.38 ***	0.60 ***	0.60 ***	0.61 ***
AICc		-593	-620	-622	-660	-660	-662
		Effect/ $p$	Effect/ $p$	Effect/ $p$	Effect/ $p$	Effect/ $p$	Effect/ $p$
Predictor							
(i) Census data	Pasture (vs. meadow)	↑ n.s.	-	-	-	-	-
	Unfertilized (vs. fertilization allowed)	↓ ***	↓ ***	↓ ***	↓ ***	↓ ***	↓ ***
	Interaction of both previous categories	*	-	-	-	-	-
(ii) Topography	Elevation (m a.s.l.)	/	↑ **	↑ **	↑ ***	↑ ***	↑ ***
	Slope (degree)	/	↑ ***	↑ ***	↑ (.)	↑ (.)	↑ (.)
(iii) Soil maps	Soil permeability	/	/	↑ (.)	-	-	-
	Degree waterlogging	/	/	-	-	-	-
(iv) Soil measurements	Soil pH	/	/	/	↑ ***	↑ ***	↑ ***
	Clay content	/	/	/	↑ ***	↑ ***	↑ ***
(v) Management details	Fertilizer application (available N ha <sup>-1</sup> year <sup>-1</sup> )	/	/	/	/	-	-
	Livestock-unit-grazing days (ha <sup>-1</sup> year <sup>-1</sup> )	/	/	/	/	-	-
(vi) Plant community information	Non-leguminous herbs (cover)	/	/	/	/	/	↑ (.)
	Legumes (cover)	/	/	/	/	/	-

Note that model 5 was the same as model 4, as additional data did not improve model performance and was thus excluded. Positive and negative estimates (and  $t$ -values) abbreviated by ↑ and ↓, respectively. Significance coded as: \*\*\* $p$ <0.001, \*\* $p$ <0.01, \* $p$ <0.05, (.)  $p$ <0.1, n.s.=not significant; / indicates predictors *a priori* not included in the respective model; “-” indicates predictors excluded based on the step function using AICc.

Table 2. Results and quality measures of stepwise linear regression models predicting raw protein content in the first harvest using the same model predictors shown in Table 1.

		Model quality					
		Model 1	Model 2	Model 3	Model 4	Model 5	Model 6
Adj. $R^2$ , model $p$		0.68, ***	0.71, ***	0.72, ***	0.73, ***	0.74, ***	Same model as 5
AICc		-22	-29	-28	-31	-35	

## Acknowledgements

We thank the Agroscope program *Indicate* for funding the IndiGras project, and the Mercator foundation Switzerland and the Fondation Sure-la-Croix for supporting the field measurements of the ecosystem service indicators (project ServiceGrass). We further thank Sergei Schaub, Nadja El Benni and Pierrick Jan for their support within IndiGras.

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# A reference framework for evaluating the ecosystem services of grasslands and livestock farming

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## Abstract

Only a few services, mainly water quality and C sequestration, are involved in payment schemes to improve the ecosystem services (ES) provided by grasslands and livestock farming. Farmers are remunerated then solely based on their grassland area, or the length of hedgerows. An operational tool is needed to make better use of existing knowledge on ES and to develop the payment schemes for livestock farmers. We have drawn up a reference framework based on over 100 scientific and technical articles. This framework lists 16 functions, 60 indicators and 69 methods and their results classified according to their cost, expertise, equipment and time. To test its interest, we surveyed 17 stakeholders in France involved in by a payment for ecosystem services project or motivated by the approach. A first group, made up of advisers and farmers, finds the guide comprehensive and proposes an awareness-raising application. A second group, made up of payment scheme managers or coordinators, finds the guide complex, and would prefer an ES diagnostic at the farm scale. According to the survey, the two areas for improvement are to fill two gaps: a lack of information concerning the thresholds to be applied locally for many indicators, and a lack of indications of synergies or antagonisms between ecological functions.

**Keywords:** ecosystem services, grasslands, framework, stakeholder

## Introduction

Although the ecosystem services (ES) provided by grasslands are numerous, they are decreasing as a result of a reduction in the grassland areas and an intensification of the uses of the remaining areas (Couvreur *et al.*, 2019). One way of maintaining them and limiting their intensification would be to set up economic support schemes for farmers that reward virtuous practices. Such schemes include payments for environmental services (PES), i.e. services provided by farmers to the environment. To date, numerous PES initiatives have been launched in the US and Europe, both public (e.g. Conservation Reserve Programme in the US; the Green, Low-Carbon, Agri-Environment Scheme in Ireland; Payments for Environmental Services in France) and private (e.g. Friesland Campina in the Netherlands, Vittel in France; Perrot-Maitre, 2006; van Laarhoven *et al.*, 2018). In these PES, payments do not relate directly to grasslands, but to practices that help to preserve the quality and quantity of water resources, to store carbon, or to prevent leaching and erosion through the presence of soil cover. As regards to grasslands, indicators are few and not very precise, which makes it difficult to maintain them and their services. However, there is an abundance of literature on how to assess these services. Our aims were (i) to build as exhaustive a reference framework as possible for the services provided by grasslands and the indicators and methods for assessing them, and (ii) to assess how stakeholders (farmers, advisers, project managers, PES scheme developers) could use this reference framework when setting up support schemes.

## Materials and methods

The reference framework, based on an analysis of the scientific and technical literature (Sénécal *et al.*, 2024), comprises 16 functions, 60 indicators and 69 methods, divided into supporting services ( $n=4$ , 21 and 41, respectively), provisioning services ( $n=4$ , 14 and 21), regulating services ( $n=6$ , 23 and 39) and cultural services ( $n=2$ , 10 and 12). Some indicators and methods are used to assess different functions.

It has been designed to be suitable for a very wide range of stakeholders (farmers, advisers, facilitators, naturalists, public policy makers, project developers, etc.). They can collectively use it like a repertoire of functions to identify the functions provided by grasslands, and then choose to focus on the specific ones that have to be enhanced according to the issues at stake in their area (Figure 1). For each “function”, they can select indicators and methods according to different criteria (genericity, scale, time, expertise, cost). Thresholds for interpreting the results, if they exist, are indicated and bibliographical references for further reading on the subject are provided.

In order to validate the relevance of the framework designed in terms of content and potential use, a diverse panel of 17 stakeholders already involved ( $n=12$ ) or interested ( $n=5$ ) in PES schemes mentioning grasslands was surveyed: 5 PES scheme holders (1 environmental adviser, 2 task officers, 1 director of a water management syndicate, and 1 water quality engineer), 7 PES partners (2 animators and 2 grassland and livestock advisers, 1 farmer, 1 task officer, a 1 botanist) and 5 stakeholders outside the PES project (3 farmers, 1 animator and 1 grassland adviser). The interview guide consisted of 18 open questions covering the following topics: profession (mission, structure); perceptions of environmental issues, grasslands and ecosystem services; description of the PES scheme (if applicable); opinion on the content of the reference framework (interest in the function sheets, indicators, methods, layout); opinion on the use of the reference framework. The interviews, which lasted an average of one hour, were recorded and partially transcribed in order to highlight the key verbatims in the answers. The verbatims were then used to construct, using Bertin’s (1967) visual method, a classification of the stakeholders, according to their opinion of the content and the potential use they envisage for the reference framework.

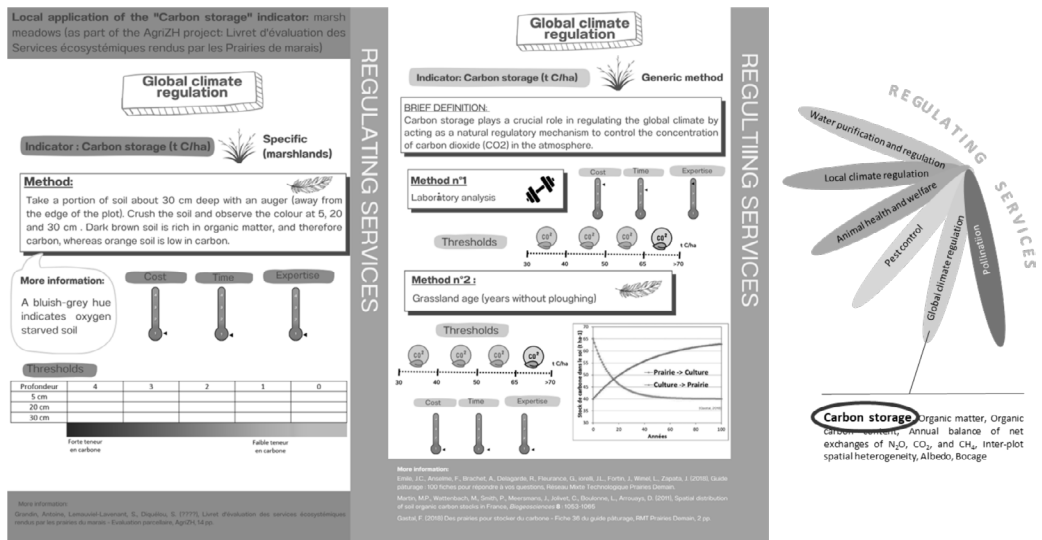


Figure 1. Example explaining how the repertoire of services provided by grasslands is organised. It details the case of an indicator used to assess the global climate regulation function (regulating service): carbon storage. For this indicator, two pages present generic evaluation methods (right) and methods specific to a pedoclimatic context (left). Each method is defined and described. The blade of grass symbol indicates that the methods are applied at plot level. The feather or altars indicate whether the method is simple or difficult to implement, with three associated criteria: cost, time and level of expertise. Where available, thresholds for interpreting the results are proposed to assess the level of service provided.

## Results and discussion

Two main user profiles were identified thanks to the verbatim analysis. The first profile consisted of technical advisers and farmers who found the reference framework comprehensive or had no opinion ( $n=6$ ). They imagined an application in the form of raising public awareness of virtuous practices provided by livestock farmers. The second profile ( $n=4$ ) consisted of PES scheme holders who found the guide complex and imagined an application in the form of a complete diagnosis of a farm before launching or at the end of a project. Only 2 stakeholders, involved in PES schemes, (an engineer and an animator, both in water management) amended the guide and envisaged using it to evaluate services providing payment and to support farmers. Some players are split between two profiles and are therefore not taken into account, which explains why all 17 players are not represented.

This lack of motivation to use the reference framework within a PES scheme seems to be linked to a lack of information in the framework concerning the thresholds to be applied locally for many indicators, and a lack of indications of synergies or antagonisms between ecological functions (e.g., soil fertility *versus* C sequestration). It can also be explained by the potential use that the users would have with the framework. Farmers and advisers prefer to see it as a tool to enhance the value of what they do, and less as a way of asking them to do things differently. PES developers, already faced with the difficulty of implementing relatively simple schemes (few functions and indicators), do not project themselves into a reference framework that is certainly more in tune with reality, but costlier and difficult to implement. A simplified and more operational framework would then suit better for them.

## Conclusion

The reference framework that has been developed stands out for its educational and precise nature but still needs to be completed with local threshold indicators and synergies between functions. It can also be used in a variety of ways according to the users' profiles involved: awareness-raising, ongoing assessment or exhaustive diagnosis. It represents a valuable tool for integrating the ES of grasslands into a variety of projects, thereby contributing to more sustainable ecosystem management. This survey has also highlighted the need for tools to assess the level of services provided to maintain virtuous farming practices while PES currently only finance changes in practices.

## Acknowledgements

The authors thank the stakeholders who participated in the surveys and the French network 'Avenir Prairies' for its financial support.

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# What is the hoof pressure exerted by different types of dairy cows at grazing?

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## Abstract

Extending the grazing season and trying to graze more early in spring and later autumn is economically worthwhile. Due to inclement weather, the risk of damage to pastures is high during these periods and not all cows are equal, due to differences in size, body weight and hoof surface area. The Tripl'XL experiment managed at the INRAE Le Pin experimental farm, including three breeds (Jersey, Normande and Holstein) of dairy cows, offers the opportunity to evaluate the hoof area contact and pressure exerted at grazing. The Jersey cows had the lowest BW (389 kg) and global hoof area (GHA: 235 cm<sup>2</sup>), whereas the Normande and Holstein cows do not differ (with 582 and 600 kg of BW and 314 and 322 cm<sup>2</sup> of GHA, respectively). Consequently, the pressure of Jersey cows is lower (1.66 kg cm<sup>-2</sup>) than that of Normande (1.84 kg cm<sup>-2</sup>) and Holstein (1.87 kg cm<sup>-2</sup>) cows, but the difference is smaller than that expected from the difference in BW. If the footprint is associated with the contact surface, the depth depends on hoof pressure. In this situation, a reduction of the adult BW of Normande and Holstein cows should be considered to reduce the risk of damage from trampling.

**Keywords:** grazing, dairy cow, hoof pressure, methodology

## Introduction

Grazing is the most economical method of feeding ruminants. At the same level of inputs, the more grazing days per hectare (GD ha<sup>-1</sup>) per year, the greater the profit for the farm (Ramsbottom *et al.*, 2015). There are two ways of increasing the number of GD ha<sup>-1</sup>: increase the stocking rate (SR) and increase the length of the grazing season. Extending the grazing season and trying to graze more in early spring and late autumn is worthwhile. Due to inclement weather, the risk of damage to pastures is increased during these periods, especially in the case of high SR. Protecting the productive potential of grasslands is essential to guarantee the long-term viability and efficiency of systems based on grazed grass. In the presence of this risk, not all cows are considered equal due to differences in size, bodyweight (BW) and hoof surface area (Greenwood and McKenzie, 2001). With the objective to anticipate and limit the risk of grazing damage, improving our understanding of the impacts of hoof surface area and the pressure exerted at grazing of diverse dairy breed is the main objective of this paper.

## Materials and methods

The experiment named Tripl'XL (Delaby *et al.*, this volume), managed since 2020 at the INRAE Le Pin experimental farm, gives us the opportunity to quantify the effect of breed and parity on the global hoof area (GHA) and global hoof pressure (GHP). The 160 dairy cows of different breeds — Holstein (Ho), Normande (No) and Jersey (Je) — are managed in a grass-based system with a compact 3-month calving season with approximately 260 days outdoor on pastures (mid of March to end of November) and 105 days indoors with pit and bale silage.

Each year, in 2021 and 2023, respectively 36 and 34 cows (in total 24 Je, 23 No and 23 Ho, 50% of first lactation) were selected within the Tripl'XL herd, weighed and placed in a restrained cage to measure the ground contact area of all 4 hooves. Two methods of area measurement were tested. One is based on photos and the tracing of the circumference of the hoof to calculate the surface area using the “ImageJ”

(Softonic – V 1.8.0) software. The second estimates the surface area using the triangulation method, assuming that the underside of each nail is made up of a whole triangle (grey in figure 1) and half of the other (transparent in the figure), which ultimately equates to a 3/4 of a rectangle (Figure 1). The formula applicable to each nail is  $(h*w)/2+(h*w)/4$ .

The GHA was defined as the sum of the surfaces of the four hooves and the GHP was estimated by dividing the BW by the GHA and expressed in  $\text{kg cm}^{-2}$ . The statistical analysis has been developed on SAS (2023) according to a general linear model including the breed, parity, their interaction and the age (in months) centered within parity as a covariate.

## Results and discussion

On average, the GHA and GPH are respectively  $289 \text{ cm}^2 (\pm 50)$  and  $1.79 \text{ kg cm}^{-2} (\pm 0.21)$  with the picture tracing method and  $290 \text{ cm}^2 (\pm 51)$  and  $1.79 \text{ kg cm}^{-2} (\pm 0.21)$  with the triangulation method. As Figure 1 shows, the two methods are very similar and the relationship does not differ significantly from the  $y=x$  line. According to this similarity, in the remainder of this text, we will focus on the tracing pictures data. Nevertheless, the results obtained with the 2 methods are presented in Table 1.

Due to a significantly lower BW ( $-130 \text{ kg}$ ) and despite a smaller GHA ( $-37 \text{ cm}^2$ ), the global hoof pressure is significantly lower for primiparous dairy cows of the 3 breeds than for multiparous dairy cows ( $1.68$  vs  $1.90 \text{ kg cm}^{-2}$ ).

As well described in the literature, the BW of the Jersey cows is significantly lower ( $-200 \text{ kg}$ ) than the Holstein or Normande cows, which do not differ ( $591 \text{ kg}$  on average). The GHA is also lower in the case of the Je cows ( $235 \text{ cm}^2$ ) than for the Ho and No cows ( $322$  and  $312 \text{ cm}^2$ , respectively). The Je cow GHA is similar to that observed by Tuohy *et al.* (2015) with Jersey $\times$ Holstein-Friesian (Je $\times$ HF) crossbreed ( $230 \text{ cm}^2$ ). In contrast, the Ho and No GHA are higher than the HF value ( $270 \text{ cm}^2$ ) reported in the Tuohy *et al.* study. The overall pressure exerted by the hooves follows the same trends, with the pressure exerted by Je cows being significantly lower ( $1.66 \text{ kg cm}^{-2}$ ) than that exerted by Ho or No cows, with respectively  $1.87$  and  $1.84 \text{ kg cm}^{-2}$ . Expressed in percentage, the ratio between the Je and other breeds is more consequential for the GHA ( $-27\%$ ) than for the GHP ( $-10\%$ ). Thus, the allometry ratio between the bodyweight and the hoof contact area is more favourable for No and Ho cows, but not enough to compensate for the higher BW, as described by Tuohy *et al.* (2015), between HF and Je $\times$ HF. At grazing, if conditions are wet, the footprint size and depth will be more important with the Ho and No than Je cows.

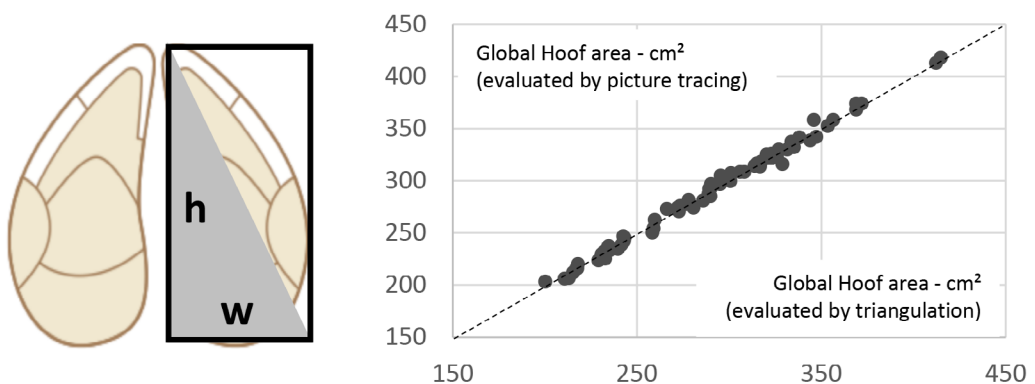


Figure 1. Schematic representation of the hoof area measurements by triangulation and relationship between the two methods.

Table 1. Effect of dairy breed and parity on the global hoof area and pressure evaluated with two calculation methodologies.

	Je	No	Ho	Primi	Multi	RMSE	Breed	Parity
Age (months)	46.1	43.2	44.9	31.6	57.9	6.5	NS	0.0001
BW (kg)	389	582	600	458	589	51.3	0.0001	0.0001
GHA (cm <sup>2</sup> )								
by picture	235	314	322	272	309	26.9	0.0001	0.0001
by triangulation	237	313	320	271	308	26.9	0.0001	0.0001
GHP (kg cm <sup>-2</sup> )								
by picture	1.66	1.84	1.87	1.68	1.90	0.15	0.0001	0.0001
by triangulation	1.65	1.85	1.88	1.68	1.90	0.16	0.0001	0.0001

Je, Jersey; No, Normande; Ho, Holstein; Primi, primiparus; Multi, multiparous; BW, body weight; GHA, global hoof area; GHP, global hoof pressure; RMSE, root mean square error.

## Conclusion

This study highlights the interest in reducing adult BW for the Ho and No cows to limit the footprint on pasture, especially if the grazing season is extended in wet weather, such as during early spring or late autumn. A better understanding of the GHP will make it possible to imagine the development of a penetrometer equipped with a hoof to assess the risk of damage due to trampling, before deciding whether or not to graze.

## Acknowledgements

The authors would like to thank the INRAE Le Pin experimental dairy farm staff for managing the TripI<sup>XL</sup> experiment, and in particular Y. Carbonnier, J. Gentil, D. Marot for their essential involvement in the hoof measurements.

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# A regression approach relating nitrogen fertilization rates with herbage yields for perennial ryegrass and multispecies swards

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## Abstract

Agricultural grassland improvement has largely focused on achieving higher and more stable yields. This has led to the simplification of swards and an increased reliance on monocultures, such as perennial ryegrass (PRG). However, PRG's high dependency on nitrogen (N) fertilization and the current need to reduce N-fertilizer use, have sparked interest in less N-dependent alternatives, such as multispecies swards (MS). We present a regression approach, which relates herbage yields of PRG and MS at varying N-fertilization rates, using Irish data as a case study. Our results show that, at the same N-fertilizer level, MS had higher dry matter yields compared to PRG. Linking this approach to other empirical datasets would result in development of site-specific models of N-fertilizer and grass growth.

**Keywords:** quadrant analysis; grasslands; nitrogen

## Introduction

The improvement of agricultural grasslands has generally focused on the homogenisation of swards to achieve more stable and higher yields (Sheridan *et al.*, 2021). This has resulted in an increased reliance on grass monocultures, such as perennial ryegrass (*Lolium perenne*; PRG). In Ireland, for instance, 95% of the seeds sold for grassland reseeded are PRG seeds (Baker *et al.*, 2023). However, PRG's productivity is heavily dependent on high nitrogen (N) fertilizer inputs and, with regulations over N-fertilizer application becoming more restrictive (Baker *et al.*, 2023), interest in less N-depending grasslands is rapidly growing. Multispecies (MS) swards are an example of such an alternative. For this study, we defined MS swards as sown swards that contain at least one species from each functional group: grasses, legumes, and forage herbs (Baker *et al.*, 2023). Despite the growing interest, knowledge on these MS swards is still rather limited and appropriate management practices, such as suitable N-fertilization rates, have yet to be established.

One approach that has been vastly used and validated to analyse, estimate and represent the relationships between nutrient application rate and nutrient uptake, nutrient uptake and dry matter yield, and, finally, nutrient application rate and dry matter yield, is the quadrant analysis approach (Van Keulen, 1977). In this study we used this approach to elucidate the relationship between N fertilization rate, N uptake and herbage yield for PRG and MS swards. Using Ireland as a case study, we performed a regression analysis based on N application rates and dry matter yields obtained in various experimental studies, to compare the responses of PRG and MS containing the three functional groups to different N fertilization rates.

## Material and methods

We used Scopus to search for studies that conducted comparative experiments between PRG and MS swards containing the three functional groups, in Ireland, and that reported N-fertilization rate and productivity, in terms of dry matter (DM) yield. We identified 10 datapoints for PRG and 8 for MS sward productivity at varying N-fertilization rates (Baker *et al.*, 2023; Grace *et al.*, 2018, 2019; Grange *et al.*, 2021; Moloney *et al.*, 2020).

Using NLREG version 6.2 non-linear curve fitting software (Sherrod, 2004), we fitted the relationship between N application rate ( $N_A$ ) and DM yield ( $Y$ ) with a parabolic equation (Equation 1), as it was the best-fitting model. The relationship between  $N_A$  and N uptake ( $N_U$ ) by the sward was derived from the fitted relation between  $N_A$  and  $Y$ , using the minimum and maximum N content of herbage (Groot *et al.*, 2003) at the lowest and highest  $N_A$  levels, respectively. We assumed that N content increased linearly between these two levels.

$$Y = Y_{\max} + a(N_A - N_Y)^2 \quad (1)$$

where:  $Y$  is dry matter (DM) yield (kg DM ha<sup>-1</sup>);  $Y_{\max}$  the maximum herbage dry matter yield (kg DM ha<sup>-1</sup>);  $a$  the shape parameter (kg DM (kg N)<sup>-1</sup>);  $N_A$  the nitrogen application rate (kg ha<sup>-1</sup>); and  $N_Y$  the nitrogen application rate at which  $Y_{\max}$  occurs (kg ha<sup>-1</sup>)

## Results and discussion

Figure 1c shows the fitted relations between  $N_A$  and the resulting  $Y$ , based on the data found on the literature search. Figure 1b shows the relationship between N yield and  $Y$ . At 0  $N_A$ , N yield was higher in the MS (306 kg N ha<sup>-1</sup>) than in PRG (114 kg N ha<sup>-1</sup>). With increasing  $N_A$ , PRG showed a fast increase in  $N_Y$  and  $Y$ , while MS only showed gradual increase. In the studies that reported MS  $Y$  by functional group (Moloney *et al.*, 2020), the proportion of legumes decreased as  $N_A$  increased. For PRG, the initial slope of the uptake curve (Figure 1a) indicated a recovery of N-fertilizer of 75.6%. For PRG and MS, maximum  $Y$  was fitted at a  $N_A$  of 370 kg N ha<sup>-1</sup> (parameter  $N_Y$ ). The intercept of the N yield curve for PRG was interpreted as the N delivery capacity of the soil (Deenen and Lantinga, 1993; Groot *et al.*, 2003), while it was hypothesized that, at the same  $N_A$ , differences in  $N_Y$  between PRG and MS could be attributed to N fixation by legumes (Høgh-Jensen *et al.*, 2004) and additional  $N_U$  due to more extensive root zone exploration expected in the MS sward (Baker *et al.*, 2023).

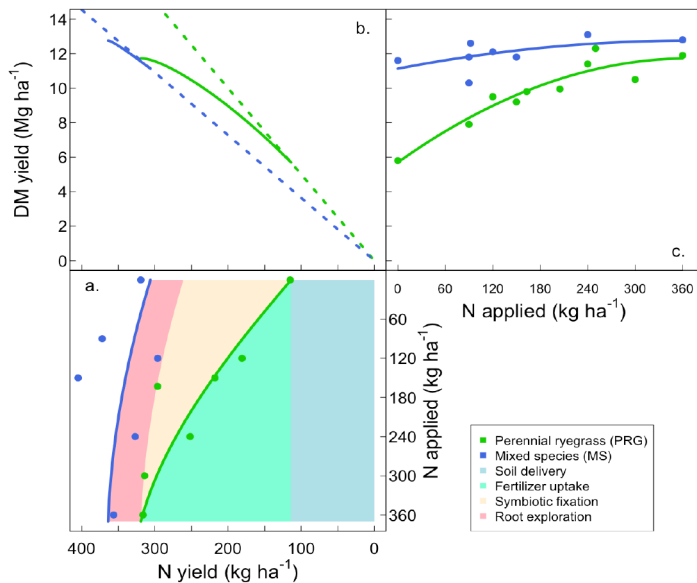


Figure 1. Relationships between nitrogen (N) fertilisation rates and herbage N-yield (a); herbage N and dry matter yield (b); and N-fertilisation rates and dry matter yield (c) for perennial ryegrass (PRG) and multispecies (MS) swards. The dotted lines in (b) represent the dry matter production efficiency at minimum nitrogen content (0.020 for PRG and 0.025 for MS). The dots indicate the observed N uptake (a) and DM yield (c) of PRG and MS in the experimental studies.

From these results, we can first see that MS swards show great potential for increasing yields, while reducing N fertilization levels, when compared to PRG. The quadrant analysis approach we used here has been vastly used and validated for PRG swards (Deenen and Lantinga, 1993). However, it had not been used for these MS swards. Interest in these types of swards is rapidly growing, as they are showing that, apart from the N-fixation benefits found in grass-legume mixes, the inclusion of the third functional group (i.e., forage herbs), may have further benefits, such as increased drought resistance and higher yields (Baker *et al.*, 2023). However, insights into appropriate fertilization rates are still lacking. This study allows N-fertilization rates to be related to PRG and MS yields and may inform decision-makers about the suitability of these sward types, particularly as  $N_A$  may be further restricted in the near future.

Although the number of experiments to fit the regression was somewhat limited, it contained all datapoints available for the Irish context. Local agroecological conditions and common practices (e.g., soil, climate, or regular  $N_A$ ) play a key role in grass productivity (Vogeler *et al.*, 2023). Hence, these response curves and ideal  $N_A$  should always be fitted locally. Furthermore the proportion of each functional group within the MS, both in the seed mixture and in their contribution to the total DM yield, should also be considered, as we observed how grasses, legumes and herbs would respond differently to varying N fertilization rates.

## Conclusion

Our study provides a methodology to assess the effects of N application rate on yields of PRG and MS and potential fertilization strategies for these different sward types. We showcase the role of legume N-fixation and root exploration on the use of less N-dependent swards.

## Acknowledgements

This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement No. 814030.

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# Plate meter assisted grass height measurement and grass yield estimation adjustments in Hungary

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## Abstract

Hungarian steppe-type natural or diverse semi-natural grasslands require modified correction formulas for the rising plate meter (RPM) yield estimation. Phenological state, dicots ratio and the cuts are the major modifying factors in dry matter (DM) calculation. Compressed sward height method by a Grasshopper G2 rising plate meter has been verified with simultaneous weekly clipping method (CLM), on a mesic *Arrhenatherum elatius* dominated meadow (Keszthely, Hungary) during the vegetation period of 2023. Though RPM constantly overestimated the sward yield compared to CLM, we proved a strong linear relationship, which differed for each cut. Applying linear regression, we obtained different models for the three cuts, but the goodness of fit was high for all three cuts (1<sup>st</sup> cut  $R^2=0.97$ ; 2<sup>nd</sup> cut  $R^2=0.91$ ; 3<sup>rd</sup> cut  $R^2=0.93$ ). The RPM method is promising even in heterogenous swards, but further validation studies are needed.

**Keywords:** rising plate meter, clipping test, mesic meadow, dry matter, yield

## Introduction

Currently, 15.5% of agricultural land in Hungary is permanent grassland, usually utilized by mowing. Most of these grasslands with a very diverse plant community are in a close-to-natural condition, are extensively cultivated, and are characterized by a low-level yield (on average 1.7 Mg ha<sup>-1</sup> DM according to the Database of the Hungarian Central Statistical Office). To improve the management of such grasslands, a non-destructive and quick yield estimation method would be an advantage. Rising plate grass yield estimation tools provide information on grass height and vegetation density at the same time. The measured data can be considered as volumetric weight (Michalk and Herbert, 1977). The use of Grasshopper/Jenquip rising plate meters (Murphy *et al.*, 2020) are widespread in Ireland, United Kingdom, Netherlands and New Zealand, where they are mainly used on intensively utilized grasslands dominated by perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*). RPM tools have been optimized for intensive pastures with high coverage and fertilizer application. Several comparative studies have been conducted on the reliability of yield estimation tools. Biomass clipping, however, gave different results. Phenophases have strong relation with biomass estimation. Swards grow sheath and inflorescence during the generative phase, which distorts the estimated yield. The Grasshopper G2's ultrasonic measuring head is more accurate than the mechanical version (McSweeney *et al.*, 2018). A difference of 14% can occur, which can mean a difference of 0.3 kg of dry matter ha<sup>-1</sup> in Irish conditions. During the tests conducted by Stumpe *et al.* (2020) in Germany, the DM yield estimated based on the standardized Grasshopper formula differed by 10% from the samples taken with biomass clipping. Steppe-type natural or diverse semi-natural grasslands probably require modified correction formulas for the rising plate meter (RPM) yield estimation therefore, the aim of the present pilot study was to compare RPM and CLM yield estimations in a Hungarian grassland.

## Materials and methods

A mesic *Arrhenatherum elatius* dominated, diverse, 2.7 ha semi natural hay meadow in Keszthely, Hungary (46.7272° N, 17.2433° E) was investigated in the vegetation period of 2023. For the yield estimation, the compressed sward height method by a Grasshopper G2 rising plate meter (RPM, 200 'spot' measurements per occasion) has been tested weekly with a simultaneous clipping method (CLM; 7 subsamples per occasion) measured or cut at 4 cm, in a pilot study. Between April 21 and October 1, we carried out 18 parallel measurements (7 in the 1<sup>st</sup> cut, 6 in the 2<sup>nd</sup> cut and 5 in the 3<sup>rd</sup> growth of grass). Average plant height and meteorological parameters (daily temperature and precipitation) were also recorded. During the study period 445 mm precipitation (37%–45%–18%) and 931°C effective sum of heat (23%–54%–23%) were recorded for each cut, respectively. The result of the RPM measurement was corrected with the actual DM content of the fresh cut sward samples, determined in the laboratory by a FOSS NIRS DS2500 using the 'Fresh grass and alfalfa silage' calibration. We used a two-sample *t*-test for sward height estimates and linear regression analysis for dry matter estimates with CLM and RPM were done with R (R Core Team, 2022).

## Results and discussion

The measured average DM was  $26.8 \pm 1\%$ ,  $29.2 \pm 1.1\%$  and  $29.6 \pm 0.9\%$  for successive cuts, respectively. The assessed total grass yield was  $5885 \text{ kg DM ha}^{-1}$  according to the clipping method (62%–29%–9% for each cut, respectively), while  $7821 \text{ kg DM ha}^{-1}$  according to the RPM method (57%–36%–7% for each cut, respectively). Two-sample *t*-test indicated similar coefficient of variation (CV) of the two methods in height measurements (CLM 21.1% and RPM 22.6%,  $p > 0.05$ ), however, the estimated yield CV was significantly smaller in RPM (22.6%) as compared to CLM (31.7%,  $p < 0.05$ ), indicating higher precision of the RPM method.

Linear regression analysis revealed a strong relationship between the two methods: the coefficient of determination was  $R^2 = 0.96$  ( $p < 0.01$ ), the equation was  $\text{Yield}_{\text{CLM}} = 3.3768 \times \text{Yield}_{\text{RPM}} - 341.2$ , indicating a systematic overestimation by RPM. Separate analysis of the individual cuts is shown in Figure 1. The

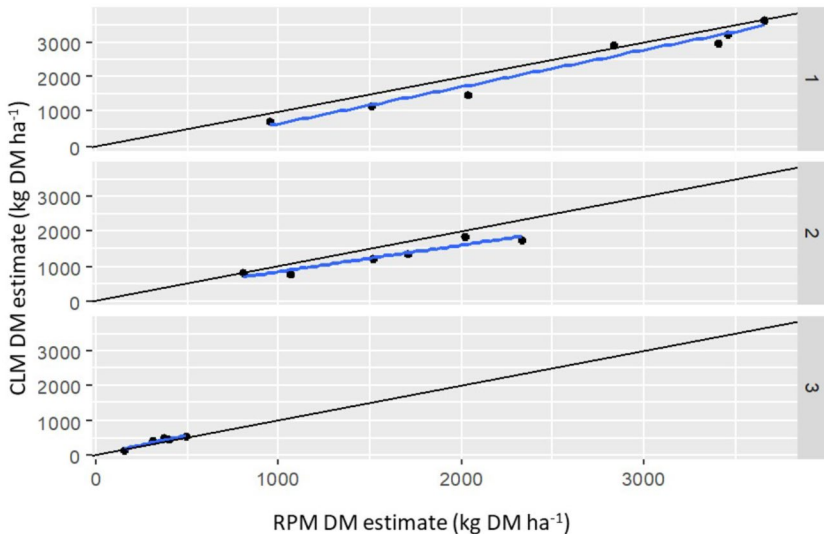


Figure 1. Linear regression analysis of DM estimates by CLM and RPM estimations in consecutive cuts. Black line indicates line of equality.

$R^2$  values were 0.97, 0.91 and 0.93 ( $p < 0.01$ ); the equations were  $\text{Yield}_{\text{CLM}} = 1.06 \times \text{Yield}_{\text{RPM}} - 19$ ,  $\text{Yield}_{\text{CLM}} = 0.765 \times \text{Yield}_{\text{RPM}} - 66.3$  and  $\text{Yield}_{\text{CLM}} = 1.1 \times \text{Yield}_{\text{RPM}} - 4.48$  for cuts 1, 2 and 3, respectively.

The determination of actual DM in the field is critical for the precise yield estimation. The use of real-time NIRS can be a solution (Bell *et al.*, 2018); however, that tool is currently not widely available.

## Conclusion

The RPM method can be a quick and more precise alternative of the classic clipping test even in heterogeneous swards; however, our first experiences in the present pilot study indicate that it tends to overestimate the yields, on average by 33%. Moreover, the extent of overestimation was different in successive cuts (up to 63% in the 2<sup>nd</sup> cut). Further validation studies are needed and planned to adjust the mathematical model behind the RPM measurements in order to successfully adapt this method to evaluate the extensive grasslands in Hungary.

## Acknowledgement

The Grasshopper G2 rising plate meter device was provided by the Hungarian Animal Breeders Association.

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# Yield and nutrient value determination in permanent grassland via sensors on the self-propelled forage harvester

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## Abstract

The aim of the study was to test sensors for volume flow detection and near infrared spectroscopy (NIRS) sensors for determination of moisture and nutrient contents of wilted grass from permanent grassland for their precision and functionality under practical conditions. The sensors, located on self-propelled forage harvesters (SFH), were tested on several farms ( $n=9$ ) in Bavaria over four years (2020–2023). Four manufacturers of forage harvesters were involved in the project and their machines were distributed among the project farms. The comparison between forage harvester data and manually recorded reference data showed that the determination of the dry matter in wilted grass had a high level of accuracy ( $R^2=0.74$ ) and was well suited for the use on commercial farms. The determination of the nutritional attributes still had potential for optimisation concerning the NIRS-calibrations but can already provide useful assistance for evaluating grassland. Data transmission to farm management information systems (FMIS) and data processing also need further optimisation to get better benefits for the farmers.

**Keywords:** near infrared spectroscopy, dry matter content, nutritional attributes, yield maps, farm management information system

## Introduction

Site-specific yields in permanent grassland are mostly an unknown parameter for the farmer (Boppel *et al.*, 2023) and can vary greatly within a year and between years and grassland sites on a single farm. Knowledge of the site-specific yield potential can help to improve roughage management with the aim of saving costs and making grassland management, especially fertilization or reseeding, more efficient. The key parameter is the annual dry matter (DM) yield, which provides the farmer with a decision-making basis for optimising the entire grassland management on the farm. Due to several grassland harvests per year, it is a challenge to record yields continuously throughout the year (Worek and Thurner, 2022). Sensor-supported yield recording on the SFH was tested as part of the DigiMilch experimental field as a possibility for year-round yield recording. The tests were performed under practical conditions to demonstrate the realistic benefits for the farmer. The aim of the study was to determine the accuracy of the sensors on the forage harvesters for yield and moisture determination as well as the nutrient content determination of further feed ingredients and to test the data transfer from the machine to a farm management information system (FMIS).

## Materials and methods

Four sensor systems on SFHs from the companies Claas, John Deere, Krone and CNH Industrial were tested on nine farms in Bavaria. On the SFH, the fresh mass yield is recorded through determination of volume flow using the degree of deflection and speed of the pre-compression rollers. To calculate the corresponding fresh mass yield, the SFH had to be calibrated regularly at the start of harvesting and when changing fields via counter weighing of the harvested material collected on a loader wagon. A total of 627 wagonloads was weighed and sampled for DM at various cutting dates of permanent grassland harvests throughout the study period in the years 2020 to 2022, to check the accuracy of the sensors. Dynamic axle load scales (Intercomp, type LS630, accuracy 2–3% at  $6 \text{ km h}^{-1}$ ) or static wagon scales (on the Bavarian State farms (BaySG), accuracy  $\pm 10 \text{ kg}$ ) were used for weighing to record the reference values



(Worek and Thurner, 2023). The DM content and the nutrient components of the crop (crude protein (CP), crude fibre (CF), crude fat (CL), crude ash (CA), starch (ST) and sugar (SU)) were recorded using near-infrared spectroscopy (NIRS) sensors on the SFH's ejection spout. As a reference for the SFH data determined by the NIRS sensor, at least twenty wagon-loads were representatively sampled per harvest day (single samples per wagon load:  $n=50$ ) (Thurner *et al.*, 2011). Two subsamples were taken from the well-mixed random samples, packed in Crispac bags, weighed on site, and dried in a drying cabinet at 105°C until complete moisture loss. To check the accuracy of nutrient content estimates, a further subsample was packed airtight and analysed in the laboratory using wet chemistry according to Weender analysis. In the laboratory, 116 samples of wilted grass were tested in the years 2021 to 2023 for ingredients.

## Results and discussion

When comparing the reference values with the SFH data, all manufacturers showed good results both in determining the fresh mass yield and in determining the DM content for wilted grass from permanent grassland. The comparison of the calculated amount of DM per wagon-load between the reference and SFH data (Figure 1) showed a high coefficient of determination ( $R^2=0.74$ ), which indicates a high accuracy of the sensors. The median of the relative deviation for the test year 2020 was 2.89%; for 2021 it was 3.40% and for 2022, 9.66%. No differences were found between the years and the cuts. The deviations of the regression line from the zero line, at both a very low amount of DM and a very high amount of DM loaded to a wagon, can be attributed to the determination of the DM content by the NIRS sensors. At very low and very high DM contents, the estimates are less accurate, e.g. due to limits in the calibration curves (Worek and Thurner, 2022). The estimation of nutrient contents by the NIRS sensors showed greater deviations from the laboratory values (Table 1). Due to the remaining high water content in the wilted grass, it is more difficult for the NIRS sensors to accurately detect the ingredients. Furthermore, the exact detection also depends on the quantity of the crop flow. The smaller database for the stored ingredients calibration curves (varying for each company, plus amount of reference values for each calibration curve is unknown) is an additional reason for the higher deviations (Worek and Thurner, 2022).

## Conclusion

The results over the years 2020 to 2022 showed a high accuracy in determining the amount of DM per wagon-load (Figure 1). Due to the fact that the material was loaded randomly on the fields, this can be used to conclude that the corresponding yields can be recorded just as accurately for each field and sub-field as for each single wagon-load. A low volume flow through the SFH due to small swaths

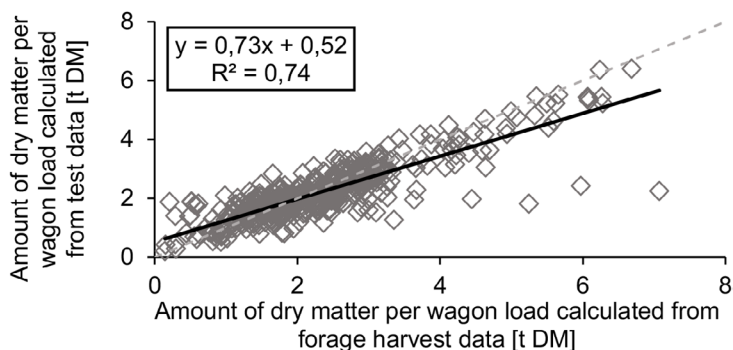


Figure 1. Comparison of calculated amount of dry matter per wagon load between reference data and forage harvester data for wilted grass in the years 2020 to 2022 (all manufacturers).

Table 1. Median, 1<sup>st</sup> and 3<sup>rd</sup> quartile of relative deviation of the forage harvester values compared to the laboratory values for the ingredient estimation for wilted grass, for crude protein (CP), crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF), crude lipid (CL), crude ash (CA) and sugar (SU), of all manufacturers (2021 to 2023).

	CP (n=116)	CF (n=52)	NDF (n=66)	ADF (n=86)	CL (n=73)	CA (n=116)	SU (n=30)
3 <sup>rd</sup> quartile	2.4%	18.1%	7.3%	9.8%	14.7%	12.2%	35.5%
Median	-10.7%	1.3%	2.2%	-1.6%	1.9%	-2.8%	30.5%
1 <sup>st</sup> quartile	-21.3%	-3.8%	-3.4%	-11.4%	-17.5%	-20.2%	24.0%

of wilted grass, which is common in harvests after long dry periods or on low-yielding grassland sites, is still a challenge for the sensor systems (Worek and Thurner, 2021). There is a need for action by the manufacturers, especially with regard to expanding the calibration curves for determining DM and nutrient contents to adapt the sensor systems to the heterogeneous permanent grassland swards that predominate in Bavaria. Despite greater deviations, the data on the nutrient contents can be used to check harvest and roughage management, for example using the CA data to get information about the quality of the harvesting process and to avoid excessive wear on the machine. To enable the farmer to work with the site-specific annual DM yields, data must be transferred from the SFH to a FMIS. This is currently the biggest challenge because data transfer is not yet fully automated. Furthermore, the FMIS does not (to date) feature multiple grassland harvests, thus not allowing a summation of yield data to determine the annual yield. Therefore, the practical benefits of sensor-based yield recording in grassland for farmers are limited.

## Acknowledgements

The project was supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany through the Federal Office for Agriculture and Food (BLE), grant number 28DE112A18.

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# Virtual fencing in rotational stocking systems: stress levels of grazing heifers

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## Abstract

Virtual fencing (VF) represents an innovative technology to simplify and reduce the labour intensity of dynamic grazing management and enables remote animal monitoring. While previous research found no significant effect of VF on stress levels of cattle on continuously stocked pastures, the effects of this novel technology on animal welfare continues to be a subject of debate. This study investigated herbage consumption, dry matter digestibility and faecal cortisol metabolite concentrations of heifers fenced with a virtual fence (Nofence, Batnfjordsøra, Norway) within a rotational grazing system, compared to heifers fenced with electric fence. 32 heifers were divided into four groups, with two groups using each fencing system. The four experimental plots were each subdivided into four paddocks. In two periods, animals grazed for 3–4 days on each sub-paddock before moving to the next. Faecal samples were collected on both the first and last day on pasture of each period and grass sward samples were taken near the ground both before and after grazing each paddock and analysed by near infrared spectroscopy. Results showed that VF did not affect faecal cortisol metabolite concentrations, live weight gain, and herbage selection differently from electric fencing.

**Keywords:** virtual fencing, rotational grazing, faecal cortisol metabolites, grassland management, precision livestock farming, sustainable intensification

## Introduction

Virtual fencing technology offers an innovative solution to reduce high labour requirements in pasture-based livestock production through remote animal monitoring and simplified dynamic grazing management (see also Hamidi *et al.*, 2024). While previous research suggests no differences in stress responses of grazing cattle with virtual fencing compared to standard electric wire fencing (e.g. Hamidi *et al.*, 2022) the VF technology (Ö Nofence, AS, Batnfjordsøra Norway, the effect of this novel technology on animal welfare is still a matter of debate. Little is known on the effects of virtual fencing on animal welfare and livestock performance in rotational stocking management. Therefore, this study investigated livestock performance, organic matter digestibility and faecal cortisol metabolite concentrations of heifers on pastures in a rotational stocking system, fenced with a virtual fence compared to heifers fenced with a traditional electric wire fence.

## Materials and methods

The study was approved by the animal welfare service of the LAVES (Lower Saxony State Office for Consumer Protection and Food Safety (Germany), ref. No. 20/3388) and conducted from July to September 2021 at the University of Göttingen's experimental farm in Relliehausen, Germany. Thirty-two heifers were randomly divided into four groups and assigned to virtual fencing (VF) or electric wire fencing (PF). Prior to the trial, all animals were released to a pasture near the experimental farm for grazing adaptation with a physical fence. VF heifers were trained in a 12-day training period preceding

the trial (Hamidi *et al.*, 2022). Each group grazed on a ca. 2-ha pasture divided into four paddocks, with an average stocking density of  $14.4 \pm 1.9$  LU ha<sup>-1</sup>. Grazing occurred in two 15-day periods (P1, P2), with a 20-day break between periods. Per period, each group grazed a paddock for 3 to 4 days before rotating to the next one.

On the first and last day of each period, animal live weight was measured for analysis of live weight gain (LWG), and faecal samples were collected to determine faecal N concentration, concentration of faecal cortisol metabolites (FCMs; Palme *et al.* 1999) and faecal organic matter digestibility (fOMD; Schmidt *et al.*, 1999). For each animal, up to three samples were collected on pasture immediately after spontaneous defecation. Samples were cooled immediately after collection and frozen for storage ( $-18^{\circ}\text{C}$ ) within eight hours after sampling. The FCMs were extracted from the defrosted faecal samples according to Palme and Möstl (1997). For this, a portion of the wet faeces (i.e. 0.5 g), suspended in 5 ml of 80% methanol, was shaken and centrifuged and FCMs were measured in an aliquot of the supernatant via an 11-oxoetiocholanolone enzyme immunoassay (EIA; Palme and Möstl, 1997). The FCM concentrations in the faeces reflect the cortisol secretion in the body approximately 12 h earlier (Palme *et al.*, 1999). Additionally, faecal samples were dried at  $60^{\circ}\text{C}$  until constant weight. Thereafter, a subsample was burned ( $550^{\circ}\text{C}$ , 3 h) in a muffle furnace to determine the ash and consequently organic matter content. Another subsample was analysed for the total N content using elemental analysis (Vario el Cube, Elementar Analysensysteme, Langensfeld, Germany) to determine the organic matter digestibility (fOMD) of the ingested herbage according to Schmidt *et al.* (1999). Grassland herbage quality on offer as *in vitro* digestible organic matter (IVDOM) was determined from hand-plucked samples taken pre- and post-grazing of each paddock. For this, three samples consisting of 5–10 manual hand pickings, mimicking cattle grazing behaviour, were obtained in each paddock (plucking the upper half of the extended sward height). Samples were frozen before analysis. The fresh matter was determined after thawing. Then samples were dried ( $60^{\circ}\text{C}$ , 48 h), milled in a two-step procedure (first 4 mm and then 1 mm) and then analysed with near-infrared reflectance spectroscopy (NIRS) on a Phoenix 5000 (BlueSun Scientific, Jessup, MD, USA) IVDOM (Schmidt *et al.*, 2004).

All data analyses were performed in R Studio (v2022.07.2; R Core Team, 2021; R Studio Team, 2020) using generalized linear mixed effects models (GLMM) from the package ‘glmmTMB’ (Brooks *et al.*, 2017). For the target variable FCM, the GLMM included the fixed effects and interaction of fencing system, period, and sampling day and the random effect of the individual animal. For the target variables fOMD (%), IVDOM (%), and LWG (kg day<sup>-1</sup>), GLMMs included the fixed effects of fencing system and period and the random effect of the individual animal (fOMD, LWG) or the experimental replicate (IVDOM).

## Results and discussion

FCM concentrations differed significantly between periods with lower FCM concentrations in P2 but not between fencing systems ( $P > 0.05$ ). In P1, FCM concentrations were  $56.0 \pm 3.46$  and  $58.1 \pm 3.69$  ng FCM (g faeces)<sup>-1</sup> and in P2  $35.3 \pm 3.30$  and  $39.5 \pm 3.25$  ng FCM (g faeces)<sup>-1</sup> for VF and PF, respectively. Similarly, Hamidi *et al.* (2022) the VF technology (Ø Nofence, AS, Batnfjordsøra Norway reported no difference in FCM concentrations between VF and PF, but lower concentrations of 14.3 and 16.4 ng/g faeces for VF and PF, respectively. This suggests that animals may have experienced some stress in rotational grazing with frequent changes between paddocks. However, this was unrelated to the virtual fence. Lower FCM concentrations in P2 compared to P1 indicate that animals adjusted to the management. Increased FCM concentrations at the end of P1 compared to the beginning of P1 suggest that the change in location and management style affected animals more than the fencing system. No significant difference was found in IVDOM or fOMD between fencing systems. Regardless of the fencing system, faecal samples showed higher organic matter digestibility (average fOMD  $74.5 \pm 0.3\%$

for VF and  $74.0 \pm 0.3\%$  for PF) compared to the herbage IVDOM (average  $68.7 \pm 0.8\%$  for VF and  $68.8 \pm 0.8\%$  for PF). The IVDOM/fOMD ratio was 0.92 and 0.93 for VF and PF, respectively, suggesting a quality difference between offered and ingested herbage due to selective grazing. The fencing system had no significant effect on LWG, which was influenced by period only. In P1, LWG was  $-0.08 \pm 0.15$  and  $0.23 \pm 0.15$  kg heifer<sup>-1</sup> day<sup>-1</sup> for VF and PF, respectively, whereas in P2 it was  $1.11 \pm 0.15$  and  $1.44 \pm 0.15$  kg heifer<sup>-1</sup> day<sup>-1</sup> for VF and PF, respectively.

## Conclusion

Rotational grazing management requires frequent paddock changes. The implementation of virtual fencing facilitates this process while maintaining livestock performance and with no effects on animal stress levels compared to standard physical fencing.

## Acknowledgements

The authors thank all students and staff for their support during fieldwork and analyses. Special thanks go to Barbara Hohlmann, Knut Salzmann, Laura Ahlers, Nele Busse, Jonas Steinkuhl and the technical team of the Department of Crop Sciences for supporting the fieldwork. This study was supported by the Federal Ministry of Education and Research under grant number [031B0734A] as part of the project 'GreenGrass'.

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# Technologies for the authentication of grass-based dairy milk samples

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## Abstract

The authentication of food has considerable importance especially when it comes to labelled products, and the need to conform the regulatory specifications. The objective of this study was to investigate the use of fatty acid (FA) profile and chemometric analysis of infrared spectra of milk samples in order to discriminate cows' milk from fresh forage feeding systems vs a non-grazing diet, as an instrument for the accreditation of milk delivered under the label "Leite de Pasto de Galicia" (Pasture Galician Milk). The databases used comprised 317 milk tank samples from commercial farms (including organic, conventional grazing and confined-silage based dairy farms) and 250 individual cows' milk samples from controlled experiments (including grazing and confined feeding regimes). The cows' diet composition associated to each milk sample was known, and the whole set of data was classified into two groups, grazing (>5% fresh grass in the diet) or no grazing (0% fresh grass in the diet). It is concluded that NIRS and FTMIR calibrations are superior to the GC-FA equations for the authentication of milk samples from grass-fed cows, the FTMIR calibration being the best option in terms of cost and speed of labour for routine use.

**Keywords:** authentication, labelled products, pasture milk

## Introduction

Consumers demand greater transparency regarding the origin of food. In the case of milk, this demand includes information on the diet consumed by the cows and the characteristics of the production system (Cossignani *et al.*, 2019). Product authentication is of particular importance, especially when consumers purchase products with high added value, in many cases covered by voluntary labelling systems (Medina *et al.*, 2019). Therefore, there is a growing need to have analytical methodologies applicable to products that enable their authentication within the framework of the protocols that specify their characteristics and requirements (Engel *et al.*, 2007). Several studies have reported the capacity of different methods to discriminate between milk obtained on different types of farms and production systems. For example, among others, based on the determination in milk of phytanic acid, a branched fatty acid (FA) derived from chlorophyll metabolism (Baars *et al.*, 2012), the analysis of isotopes of certain atoms (Ehtesham *et al.*, 2013) or the FA composition of milk (Coppa *et al.*, 2015). Of these, the determination of the FA profile is considered the most reliable method to predict the food origin (Bergamaschi *et al.*, 2020). These authors indicated that in terms of cost, speed and simplicity in the acquisition of information, infrared spectroscopy with discriminant analysis provides valuable information about the cows' feeding system. Likewise, literature provides evidence on the possibility to discriminate grass-fed products by using analytical methods that quantify specific compounds. The objective of this study was to investigate the use of FA profile and chemometric analysis of infrared spectra of milk samples in order to discriminate cows' milk from fresh forage feeding systems, as an instrument for the accreditation of the milk delivered under the label "Leite de Pasto de Galicia".

## Materials and methods

The databases used comprised 317 milk tank samples from commercial farms (including different feed systems: organic, conventional grazing and confined-silage based dairy farms) and 250 individual cows' milk samples from controlled experiments (including grazing and confined feeding regimes). Likewise, the cows' diet composition associated to each milk sample was known, allowing separation of the whole set of data into two groups, grazing (>5% fresh grass in the diet) or no grazing (0% fresh grass in the diet). The FA profile of milk samples was determined by gas-chromatography (GC) and their spectra were recorded using NIRS (Foss NIRSystem 6500) and FTMIR (MilkoScan™ 7 RM) instruments (FOSS, Hillerød, Denmark). A discriminant analysis (SAS Institute, 2009) was carried out on the FA profile of the milk to obtain discriminant equations and chemometrical analysis were performed on milk-sample spectra applying MPLS and MLR algorithms for NIRS and FTMIR, respectively, to develop the correspondent classification models.

## Results and discussion

In the cross-validation step the percentage of success observed in the assignment of a problem sample to the correct group for the FA-based discriminant equations was 79.3% for grazing and 86.5% for no grazing (Table 1). Results indicate that milk samples can be assigned to the presence or absence of fresh grass in the cows' ration with a moderately high probability of success. However, the need to determine the composition of the FA profile by reference methods (GC) reduces the practical usefulness of this method, due to the high cost and time-consuming procedure.

Table 2 shows the ability of NIRS technology to discriminate the origin of milk samples from diets with (values of 95.0%) and without (99.4% for no grazing) fresh grass. This evidenced that NIRS technology is a fast, reliable, robust and economical method, without use of reagents, thus outperforming discriminant methods that require chromatographic analysis.

Table 1. Frequencies of correct or incorrect assignment of milk samples to groups with the presence or absence of pasture in the ration (cross-validation).

Original group (n=238)	Group assigned by the discriminant equations	
	Grazing	No grazing
Grazing (n=97)	77 (79.3%)	20 (20.6%)
No grazing (n=141)	19 (13.5%)	122 (86.5%)

FA discriminant equations.

Table 2. Frequencies of correct or incorrect assignment of milk samples to groups with the presence or absence of pasture in the ration (Cross validation).

Original group (n=276)	Group assigned by the discriminant equations	
	Grazing	No grazing
Grazing (n=121)	115 (95.0%)	6 (5.0%)
No grazing (n=155)	1 (0.6%)	154 (99.4%)

NIRS calibration.

The FTMIR calibration (Table 3) permitted a discrimination success of the origin of milk samples of 99.8% for grazing and 98.2% for no grazing groups. This technology, being cheaper and faster than NIRS, can be applied to large numbers of milk samples. Accordingly, this technique allows for use in the inter-professional laboratory, for rapid identification of samples that present some inconsistencies with respect to their declared origin, and which are subject of confirmatory investigation.

Table 3. Frequencies of correct or incorrect assignment of milk samples to groups with the presence or absence of pasture in the ration (Cross validation).

Original group (n=1067)	Group assigned by the discriminant equations	
	Grazing	No grazing
Grazing (n=519)	518 (99.8%)	1 (0.2%)
No grazing (n=548)	10 (1.8%)	538 (98.2%)

FTMIR calibration. Three spectra were recorded from each of the milk samples.

## Conclusion

According to analyses NIRS and FTMIR techniques provided calibrations results, being superior to the GC-FA equations. Linked to results, FTMIR calibration was the best option in terms of cost, speed of labour for the routine and accuracy, for verification of the origin of milk samples, with and without a grass-based diet.

## Acknowledgement

The research was funded by Axencia Galega da Calidade Alimentaria (FEADER 2020-066A project)

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# An assessment of the GHG emissions in grazing and confined total mixed ration dairy systems of Atlantic NW Spain

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## Abstract

Many studies have shown that the intensification of agricultural and livestock systems improves the environmental performance per unit of product, but contrasting results are showed when carbon storage is included in the greenhouse gas (GHG) emission estimation. In addition, it is important to know the importance of the emission sources of the different production systems, helping to propose mitigation measures adapted to each system. The objective of the present work was to compare dairy cows' systems typically used in the Galician dairy region (NW Spain), in terms of the GHG emissions and with respect to grazing management. Fifty farms, representative of the most common feeding systems of Galicia, were selected and grouped accordingly into cows with grazing in organic (GO) or conventional (GC) systems, or confined systems with total mixed ration (TMR) feeding based on grass silage (GS) or maize and grass silage (MGS). Selected farms were simulated with the CAP'2ER (IDELE, France) tool to estimate GHG emissions based on technical data obtained from farm visits. Analyses show that net GHG emissions were lower for both grazing systems compared with all-silage systems, with average values of 0.83, 0.80, 0.94 and 1.01 kg eqCO<sub>2</sub> (l milk)<sup>-1</sup> for GO, GC, GS and MGS farms.

**Keywords:** carbon footprint, carbon storage, dairy production, feeding systems

## Introduction

Among the most notable demands of European consumers is the importance of adopting low-carbon production models, whose correct evaluation implies having standardized and widely accepted models to correctly assign the corresponding GHG emissions to each system. It is necessary to consider, however, that the sustainability of a farm rests on three fundamental bases: environmental protection, economic growth and social equity; therefore a measure to mitigate GHG emissions will only have a positive effect when its application leads to a situation of greater sustainability (Jacobsen *et al.*, 2016). In the livestock sector, life cycle assessment (LCA) analysis gives the opportunity to understand where the production truly stands in terms of its environmental impact, but also what approaches can be implemented to reduce the impact to make livestock farming more sustainable (Biagetti *et al.*, 2023). However, certain considerations must be taken, especially when different production systems are compared. The choices about system boundaries, functional units and allocation methods are crucial and can significantly influence the results of the LCA assessment (Bava *et al.*, 2018). Despite the obvious high scientific interest in this topic, general conclusions on the climate-friendliness of contrasting production systems can hardly be drawn and there is no clear answer to the question of whether or not grazing-based systems provide an overall more climate-friendly alternative to confinement barn-systems (Lorenz *et al.*, 2019). The objective of the present work was to compare 'feed systems' of dairy cows' typically used in the Galician dairy region (NW Spain), in terms of the GHG emissions.

## Materials and methods

Fifty farms representative of the most common feeding systems of Galicia were selected, and grouped according to cows' grazing in organic (GO) or conventional (GC) systems or confined systems with

total mixed ration (TMR), feeding based on grass silage (GS), or maize and grass silage (MGS). Selected farms were simulated with the CAP'2ER (IDELE, France) tool to estimate GHG emissions based on technical data obtained at farm visits. The statistical analysis was performed using SAS (SAS Institute, 2009) considering the group system as the class variable.

## Results and discussion

Average values per group of the main characteristics in terms of land use, herd size, milk productivity and feed composition are shown in Table 1. The variables corresponding to land use are similar in the 3 grass-dependent groups, differentiating from the MGS group in a lower utilized agricultural area but higher percentages of grasslands and permanent grasslands. The herd size is greater in the MGS group as well as production per cow, although the GS group also has a production per cow greater than 10 t.

Gross GHG emissions ( $\text{kg eqCO}_2 (\text{l milk})^{-1}$ ) were 1.26, 1.02, 1.07 and 1.08 for GO, GC, GS and MGS farms (Table 2). The proportion of total gross GHG emissions caused by enteric fermentation varied between 52.7% of GO and 39.0 of MGS, but in all groups, it was the main source of emission. Manure management had a similar proportion in all groups, near 20%, being the second source of emission in GO and GC, but not in GS and MGS where it is surpassed by the food purchase, which doubles its proportion between GO (11.6%) and MGS (23.5%). Fertilization also has a relevant weight, since it represents around 10% of emissions in all groups, reaching 13% in the GC, while energy, fertilizers purchased and animals purchased were less than 5% in all groups. Carbon storage in pastures and crop areas was different among systems, with average values of 0.43, 0.22, 0.14 and 0.07  $\text{kg eqCO}_2 (\text{l milk})^{-1}$  for GO, GC, GS and MGS farms. This resulted in net GHG emissions being lower for both grazing systems, compared with all-silage systems, with average values of 0.83, 0.80, 0.94 and 1.01  $\text{kg eqCO}_2 (\text{l milk})^{-1}$  for GO, GC, GS and MGS farms.

Table 1. Main characteristics of the farms.

	Treatment				P
	GO	GC	GS	MGS	
n	10	11	9	20	
Land use					
Utilized agricultural area (ha)	45.4	43.1	37.7	54.7	NS
% Grassland	94.7 <sup>a</sup>	96.4 <sup>a</sup>	99.3 <sup>a</sup>	74.9 <sup>b</sup>	***
% Permanent grassland	46.6 <sup>ab</sup>	51.6 <sup>a</sup>	40.5 <sup>ab</sup>	22.9 <sup>b</sup>	*
Forest area (ha)	2.9	2.0	2.2	3.7	NS
Herd size					
Number dairy cows	45.2 <sup>b</sup>	51.4 <sup>b</sup>	57.4 <sup>b</sup>	105.3 <sup>a</sup>	**
Total livestock units	61.3 <sup>b</sup>	66.4 <sup>b</sup>	81.3 <sup>b</sup>	146.8 <sup>a</sup>	**
Milk productivity (t)					
Production farm <sup>-1</sup>	219.1 <sup>b</sup>	366.6 <sup>b</sup>	556.9 <sup>b</sup>	990.7 <sup>a</sup>	***
Production dairy cow <sup>-1</sup> year <sup>-1</sup>	5.7 <sup>c</sup>	7.8 <sup>b</sup>	10.2 <sup>a</sup>	10.9 <sup>a</sup>	***
Feed composition (%Dry matter)					
Fresh grass	46.5 <sup>a</sup>	29.2 <sup>b</sup>	7.0 <sup>c</sup>	0.0 <sup>c</sup>	***
Grass silage	23.4 <sup>bc</sup>	30.7 <sup>a</sup>	28.9 <sup>ab</sup>	19.4 <sup>c</sup>	***
Maize silage	4.7 <sup>b</sup>	5.6 <sup>b</sup>	7.0 <sup>b</sup>	38.3 <sup>a</sup>	***
Dry forages	9.4 <sup>a</sup>	5.4 <sup>b</sup>	5.8 <sup>b</sup>	2.3 <sup>c</sup>	***
Concentrate	16.1 <sup>d</sup>	29.2 <sup>c</sup>	51.3 <sup>a</sup>	40.0 <sup>b</sup>	***

\*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$ ; NS,  $P > 0.05$ . Means with different superscripts differ significantly.

Table 2. Gross GHG emissions, proportion of total GHG emissions, carbon storage and net GHG emissions.

	Treatment				P
	G0	GC	GS	MGS	
<i>n</i>	10	11	9	20	
Gross GHG emissions (kg eqCO <sub>2</sub> (l milk) <sup>-1</sup> )	1.26 <sup>a</sup>	1.02 <sup>b</sup>	1.07 <sup>b</sup>	1.08 <sup>b</sup>	*
Proportion of total GHG emissions (%)					
Enteric fermentation	52.7 <sup>a</sup>	45.7 <sup>b</sup>	39.8 <sup>c</sup>	39.0 <sup>c</sup>	***
Manure management	18.6 <sup>b</sup>	19.3 <sup>ab</sup>	20.8 <sup>ab</sup>	22.2 <sup>a</sup>	*
Fertilization	8.7 <sup>b</sup>	13.1 <sup>a</sup>	11.4 <sup>ab</sup>	8.7 <sup>b</sup>	*
Energy	4.5 <sup>a</sup>	4.4 <sup>a</sup>	2.8 <sup>b</sup>	4.0 <sup>a</sup>	*
Food purchase	11.6 <sup>b</sup>	14.3 <sup>b</sup>	21.7 <sup>a</sup>	23.5 <sup>a</sup>	***
Fertilizers purchase	0.0 <sup>c</sup>	3.1 <sup>a</sup>	2.4 <sup>ab</sup>	1.6 <sup>b</sup>	***
Animal purchase	3.9	0.0	1.0	1.0	NS
Carbon storage and net emissions (kg eqCO <sub>2</sub> (l milk) <sup>-1</sup> )					
Carbon storage	0.43 <sup>a</sup>	0.22 <sup>b</sup>	0.14 <sup>bc</sup>	0.07 <sup>c</sup>	***
Net GHG emissions "net carbon footprint"	0.83 <sup>b</sup>	0.80 <sup>b</sup>	0.94 <sup>ab</sup>	1.01 <sup>a</sup>	**

\*\*\**P*<0.001; \*\**P*<0.01; \**P*<0.05; NS, *P*> 0.05. Means with different superscripts differ significantly.

## Conclusion

Grazing dairy farms showed a lower net carbon footprint than maize silage-based systems, with no differences between organic and conventional grazing systems. The results indicate the importance of considering the carbon storage in agricultural soils to adequately compare carbon footprint between systems of different production intensity.

## Acknowledgements

The research was funded by Axencia Galega da Calidade Alimentaria (FEADER 2017-022B project)

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# Split nitrogen application in spring to timothy-meadow fescue leys

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## Abstract

Climate change with fluctuations in weather patterns, environmental concerns, and increased costs of mineral fertilizers all demand adjustment of nitrogen (N) used for forage production. The aim of the study was to investigate the effects of splitting N application in spring on dry-matter (DM) yield, crude protein (CP) content and protein quality of timothy-meadow fescue leys. The trial was conducted during two years at three locations (Kvithamar and Særheim, Norway and Långhem, Sweden). Split N application with 60 kg N ha<sup>-1</sup> at onset of grass growth in April and 50 kg N ha<sup>-1</sup> in May resulted in the same DM yields and CP concentrations as a single application of 110 kg N ha<sup>-1</sup> in April in Kvithamar the first year and Særheim both years. In Långhem both years and for Kvithamar in the second year, a late application two weeks before first cut gave less DM yield than the single full application in April. Split application did not affect the contents of nonprotein N or nitrate.

**Keywords:** *Festuca pratensis*, maturity at harvest, N application strategy, *Phleum pratense* L., protein, yield

## Introduction

Climate change with fluctuations in weather patterns, environmental concerns and increased costs of mineral fertilizers increase the need for reducing nitrogen (N) losses. Split N application, instead of one full single application in early spring, is one way of adjusting the mineral N application to the need of the plants and to the N supply from soil organic matter and manure, thereby decreasing environmental concerns (Gastal and Lemaire, 2002). The aim of this study was to investigate the effects of split N application in the spring on dry matter (DM) yield, crude protein (CP) content and protein quality of timothy-meadow fescue leys.

## Materials and methods

A seed mixture of 80% timothy (*Phleum pratense* L.) and 20% meadow fescue (*Festuca pratensis*) was sown in 2017 at Kvithamar, Norway and in 2018 at Særheim, Norway and Långhem, Sweden. The soil at the Kvithamar site is a silty loam, and a sandy loam at both Særheim and Rådde. One part of the sown area was harvested as first-year ley (2018 at Kvithamar; 2019 at Særheim and Långhem) and another part of the sown area was harvested as a second-year ley (2019 at Kvithamar; 2020 at Særheim and Långhem). The experimental design was a randomized block with three blocks at each site, four fertilization treatments and three harvest times in the spring growth cycle. The treatments were A) no N fertilization, B) 110 kg N ha<sup>-1</sup> in spring, C) 60 kg N ha<sup>-1</sup> in spring and 50 kg N ha<sup>-1</sup> when the first node was palpable on the stem of timothy shoots, and D) 60 kg N ha<sup>-1</sup> in spring and 50 kg N ha<sup>-1</sup> when the second node was palpable on the stem of most of the timothy shoots. The N in the N-P-K compound fertilizer that was applied at all occasions was 54% ammonium N and 46% nitrate N. There were three plots per treatment in each of three blocks per field. One plot was harvested on the day of 1<sup>st</sup> split application, another one at the 2<sup>nd</sup> split, and the last one at early heading of timothy (i.e. when the tip of the inflorescence was visible above the flag leaf on 10% of the tillers). The stubble height was 70 mm. Crude protein was analysed as total N × 6.25, crude protein quality was analysed by chemical fractionation of the CP (Licitra *et al.*, 1996) and nitrate was analysed according to Boehringer Mannheim/R-Biopharm. Data were analysed

in Proc Glimmix (SAS). When the global P-value was significant ( $P < 0.05$ ) or tended to be significant ( $0.05 < P < 0.10$ ) LS-means were compared using Tukey's test.

## Results and discussion

Split N application with 60 kg N ha<sup>-1</sup> in spring when grass growth began, and 50 kg N ha<sup>-1</sup> in May gave the same DM yields and CP concentrations as a single application of 110 kg N ha<sup>-1</sup> in spring; the exceptions were for Långhem in both years and Kvithamar during the second year, where a late application 2 weeks before first cut (treatment D) gave less DM yield (4509 vs. 5125 kg ha<sup>-1</sup> and 4637 vs. 5298 kg ha<sup>-1</sup>, respectively,  $P < 0.001$ ) than the single full application in the spring (treatment B) (Table 1). Timing of the second N application was less important at Særheim, which might be related to a larger N uptake from the soil (425 kg CP ha<sup>-1</sup> vs. 308 kg CP ha<sup>-1</sup> and 202 kg CP ha<sup>-1</sup> at Kvithamar and Långhem, respectively, in the harvested grass and stubble of the non-fertilized plots averaged over harvests and years, data not shown). Circa 66%, 64% and 52% of the N supplied in fertilizer was recovered as N-yields above stubble height at Kvithamar, Særheim and Långhem, respectively (Table 1). As both weather and the soil N content affect the N uptake by the plant, we recommend that the second application in a split-N strategy should occur at 3 to 4 weeks before expected harvest.

The content of nonprotein N at early heading did not differ between treatments and was, on average, 235 g kg<sup>-1</sup> of CP. There was no significant difference for nitrate content at this stage either (0.43 g (kg DM)<sup>-1</sup> for split application, mean for treatment C and D, vs. 0.34 g (kg DM)<sup>-1</sup> for single application, treatment B; data not given in table). This low and non-different content could depend on a relatively moderate application rate of 110 kg N ha<sup>-1</sup>, which the plants could take up and store as true protein.

## Conclusions

Split N application did not increase DM yield and CP content of the timothy-meadow fescue ley compared to a single N application in spring at an application rate that was moderate for a grass ley with high growth potential. The low content of nitrate in the yield at all developmental stages indicated that grass plants had a large potential to take up and assimilate N.

## Acknowledgements

The work was part of the project "Improved precision in forage crop management" with financing from Matfondet, Lantmännen Research Foundation, Felleskjøpet Agri SA, YARA Norway AS, YARA GmbH & Co. KG and Strand Unikorn AS.

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Table 1. Effects of N fertilization strategy and harvest time in spring growth cycle on DM yield and CP content in timothy-meadow fescue ley at Kvithamar (K) and Særheim (S), Norway and Långhem (L), Sweden.

Site, year and N-strategy	Yield (kg DM ha <sup>-1</sup> )			Crude protein (g (kg DM) <sup>-1</sup> )		
	Maturity stage of timothy			Maturity stage of timothy		
	1 node palpable,	2 nodes palpable,	Early heading,	1 node palpable,	2 nodes palpable,	Early heading,
	6-18/5	15-23/5	3-9/6	6-18/5	15-23/5	3-9/6
K2018 – A	1106 <sup>Bb</sup>	1543 <sup>Bb</sup>	3243 <sup>Ba</sup>	n.a.	n.a.	74 <sup>B</sup>
K2018 – B	1934 <sup>Ac</sup>	2896 <sup>Ab</sup>	5617 <sup>Aa</sup>	n.a.	n.a.	129 <sup>A</sup>
K2018 – C	1547 <sup>ABc</sup>	2375 <sup>Ab</sup>	5928 <sup>Aa</sup>	n.a.	n.a.	125 <sup>A</sup>
K2018 – D	1663 <sup>ABc</sup>	2540 <sup>Ab</sup>	5444 <sup>Aa</sup>	n.a.	n.a.	136 <sup>A</sup>
<i>P</i> -value (SEM)		<0.001 (138.0)				
K2019 – A	783 <sup>Ab</sup>	1215 <sup>Bb</sup>	2592 <sup>Ca</sup>	113 <sup>Ca</sup>	108 <sup>Ca</sup>	72 <sup>Bb</sup>
K2019 – B	1335 <sup>Ac</sup>	2063 <sup>Ab</sup>	5298 <sup>Aa</sup>	219 <sup>Aa</sup>	209 <sup>Aa</sup>	111 <sup>Ab</sup>
K2019 – C	1218 <sup>Ac</sup>	1902 <sup>Ab</sup>	5306 <sup>Aa</sup>	181 <sup>Bb</sup>	224 <sup>Aa</sup>	128 <sup>Ac</sup>
K2019 – D	1424 <sup>Ab</sup>	1972 <sup>Ab</sup>	4637 <sup>Ba</sup>	185 <sup>Ba</sup>	170 <sup>Ba</sup>	120 <sup>Ab</sup>
<i>P</i> -value (SEM)		<0.001 (134.8)			<0.001 (5.1)	
S2019 – A	693 <sup>(Ab)</sup>	1670 <sup>(Ab)</sup>	4273 <sup>(Ba)</sup>	122 <sup>Ca</sup>	101 <sup>Ba</sup>	86 <sup>Ba</sup>
S2019 – B	920 <sup>(Ac)</sup>	2469 <sup>(Ab)</sup>	6486 <sup>(Aa)</sup>	227 <sup>Aa</sup>	178 <sup>Ab</sup>	110 <sup>ABc</sup>
S2019 – C	905 <sup>(Ac)</sup>	2355 <sup>(Ab)</sup>	6052 <sup>(Aa)</sup>	192 <sup>ABa</sup>	188 <sup>Aa</sup>	132 <sup>Ab</sup>
S2019 – D	1402 <sup>(Ab)</sup>	2835 <sup>(Ab)</sup>	6476 <sup>(Aa)</sup>	181 <sup>Ba</sup>	152 <sup>Aa</sup>	97 <sup>ABb</sup>
<i>P</i> -value (SEM)		0.051 (283.3)			<0.003 (8.6)	
S2020 – A	1272 <sup>Ab</sup>	1749 <sup>Bb</sup>	3079 <sup>Ba</sup>	125 <sup>Ca</sup>	117 <sup>Ba</sup>	80 <sup>Bb</sup>
S2020 – B	1752 <sup>Ac</sup>	2804 <sup>Ab</sup>	5615 <sup>Aa</sup>	206 <sup>Aa</sup>	160 <sup>Ab</sup>	115 <sup>ABc</sup>
S2020 – C	1456 <sup>Ac</sup>	2750 <sup>Ab</sup>	5458 <sup>Aa</sup>	163 <sup>BCa</sup>	172 <sup>Aa</sup>	121 <sup>Ab</sup>
S2020 – D	1397 <sup>Ac</sup>	2623 <sup>Ab</sup>	4938 <sup>Aa</sup>	174 <sup>ABa</sup>	154 <sup>Aab</sup>	124 <sup>Ab</sup>
<i>P</i> -value (SEM)		<0.001 (166.6)			<0.0182 (7.8)	
L2019 – A	232 <sup>Bb</sup>	747 <sup>Cb</sup>	1681 <sup>Ca</sup>	137 <sup>Ca</sup>	107 <sup>Cb</sup>	78 <sup>Bc</sup>
L2019 – B	958 <sup>Ac</sup>	2667 <sup>Ab</sup>	5295 <sup>Aa</sup>	258 <sup>Aa</sup>	185 <sup>Ab</sup>	124 <sup>Ac</sup>
L2019 – C	773 <sup>Ac</sup>	2224 <sup>Bb</sup>	5245 <sup>Aa</sup>	202 <sup>Ba</sup>	195 <sup>Aa</sup>	118 <sup>Ab</sup>
L2019 – D	805 <sup>Ac</sup>	2052 <sup>Bb</sup>	4786 <sup>Ba</sup>	205 <sup>Ba</sup>	138 <sup>Bb</sup>	137 <sup>Ab</sup>
<i>P</i> -value (SEM)		<0.001 (77.7)			<0.001 (4.8)	
L2020 – A	283 <sup>Bc</sup>	864 <sup>Cb</sup>	2281 <sup>Ca</sup>	148 <sup>Ca</sup>	101 <sup>Bb</sup>	74 <sup>Bb</sup>
L2020 – B	940 <sup>Ac</sup>	2645 <sup>Ab</sup>	4955 <sup>Aa</sup>	225 <sup>Aa</sup>	152 <sup>Ab</sup>	111 <sup>Ac</sup>
L2020 – C	666 <sup>ABc</sup>	2085 <sup>Bb</sup>	4769 <sup>Aa</sup>	194 <sup>Ba</sup>	157 <sup>Ab</sup>	113 <sup>Ac</sup>
L2020 – D	669 <sup>ABc</sup>	1900 <sup>Bb</sup>	4232 <sup>Ba</sup>	183 <sup>Ba</sup>	125 <sup>Bb</sup>	123 <sup>Ab</sup>
<i>P</i> -value (SEM)		<0.001 (100.2)			<0.001 (5.4)	

N strategy: (A) 0 kg N ha<sup>-1</sup>, (B) 110 kg N ha<sup>-1</sup> in spring, (C) 60 kg N ha<sup>-1</sup> in spring+50 kg N ha<sup>-1</sup> when the first node is palpable on timothy, (D) 60 kg N ha<sup>-1</sup> in spring+50 kg N ha<sup>-1</sup> when two nodes are palpable on most of the timothy shoots. *P*-value and standard error of the mean (SEM) of N-strategy-by-harvest time interaction. <sup>A,B,C</sup>LS Means with different superscripts within column and site differ (*P*<0.05) or have a tendency to differ (0.05<*P*<0.10). <sup>a,b,c</sup>LS Means with different superscripts within row and site differ (*P*<0.05) or have a tendency to differ (0.05<*P*<0.10). *N*=36. n.a., not available.

# Ryegrass and tall fescue perform better than timothy in a three-cut system in Nordic maritime climate

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## Abstract

Longer growing season has replaced two-cut with three-cut system even at higher latitudes and has an impact of persistency and use of seed mixtures. This on-going study is searching for the most appropriate seed mixtures that persist over time in a three-cut system. Six grassland seed mixtures were established at Tjøtta, 65°N coast, 11 m a.s.l in 2016, with two (2017) or three annual cuts (2018–2023) using organic fertiliser, in total 170 kg N ha<sup>-1</sup> year<sup>-1</sup>. Four seed mixtures contained timothy (*P. pratense*), 30–45% whereas two seed mixtures were based on perennial ryegrass (*Lolium perenne*). Other grass species as well as red (*Trifolium pratense*) and white (*T. repens*) clover constituted the remaining proportion of the mixtures. The species proportion in each seed mixture was determined in spring cut in 2019 and 2022. Results for seven production years show differences for dry matter yield (DMY), DMY stability and species persistence in space and time in favour of *Lolium*-containing mixtures with greater DMY in all production years and with lower interannual coefficient of variation (CV%) than timothy-based seed mixtures, indicating potential species adaptability to Nordic maritime climate. Decrease of timothy proportion in the mixtures suggest negative effects of three-cut system.

**Keywords:** grass species, legumes, long-term sward, seed mixtures, yield

## Introduction

Timothy (*Phleum pratense* L.) and perennial ryegrass (*Lolium perenne* L.) are important forage grasses at high latitudes (Höglind *et al.*, 2013). Timothy often persists better than perennial ryegrass due to its better winter survival but, in areas with a more maritime Nordic climate, perennial ryegrass can perform surprisingly well (Höglind *et al.*, 2013). Thus, the growing zone for perennial ryegrass expands to the north. Duration of growing season has increased in higher latitudes allowing the two-cut system to be replaced by a three-cut system. At the same time, the weather conditions have become more unstable and demand for more robust and resilient grassland production systems are expressed. The positive effects of species diversity on forage productivity and yield stability have been discussed earlier (Suter *et al.*, 2021). Interaction effects among species often lead to better yields, and even overyielding, than expected from the performance in monoculture (Ergon *et al.*, 2016). Sowing diverse and more persistent seed mixtures may also minimise renewal demands and costs. We have limited knowledge on how long grassland mixtures could extend the period before renewal and which species and species compositions perform best over longer periods. The main objective of this long-term study is, therefore, to find the most appropriate seed mixtures for good production and persistence over time in a three-cut system in a northern maritime climate.

## Materials and methods

A field trial was established at Tjøtta (65°5' N, 12°25' E; 11 m a.s.l., northern coastal climate) on 2 June 2016. A complete randomised trial with three blocks (plot size 10.5 m<sup>2</sup>) was used for assessment of seasonal biomass production for seven full production years. During the establishment year, a topping cut was carried out. Species, cultivars and their proportions in the different seed mixtures are shown in Table 1. Four seed mixtures (T1–T4) contained timothy, where T1 and T2 differed in one species; tall fescue (*Festuca arundinacea* Shreb.) and meadow fescue (*F. pratensis* Huds.), respectively. T3 contained

both meadow fescue and red fescue (*Festuca rubra* L.) plus common bent grass (*Agrostis capillaris* L.) and smooth grass (*Poa pratensis* L.) and T4 included red fescue, smooth grass and brome grass (*Bromus inermis* L.). Two multispecies seed mixtures (L1 and L2) without timothy were also tested (Table 1). Clovers were included in all mixtures except L2. The seed rate was 40 kg ha<sup>-1</sup> for all mixtures which were composed on a weight basis, similar to commercial seed mixtures. The field trial was organically managed, using granulated organic fertiliser. In each of the seven ley years, the trials received 170 kg N ha<sup>-1</sup> year<sup>-1</sup>. All plots were harvested simultaneously three times per year and herbage samples (approx. 700 g per plot) were collected for estimation of dry matter yield (DMY). In 2017, only two cuts were taken due to continual rainfall and wet soil conditions. Prior to the first cut in the third and sixth ley years, three plant samples were cut randomly from each plot (in total approx. 1000 g per plot) for determination of botanical composition of the herbage. Plant samples were sorted into individual species except unsown species, which were pooled together. The sorted samples were dried at 60°C for 48 h for estimation of species proportional amount in the mixture. A general linear model (GLM) was used for analysing data. The fixed effects were ley years and seed mixtures, and the blocks were random effects. For significant effects ( $p \leq 0.05$ ), Tukey's multiple comparison method was used. For each seed mixture the interannual coefficient of variation (CV) of DMY was calculated.

## Results and discussion

Mean annual herbage DM yield differed among seed mixtures (Figure 1). Averaged over all seven experimental years, the seed mixture containing *Lolium* species (L2) had a significantly greater DMY than the timothy-containing seed mixtures T1 and T3 ( $P < 0.006$ ). The seed mixture L2 also had the lowest estimated interannual CV, reflecting great DMY stability and adaptability to the three-cut system (Figure 2). In the sixth ley year, the proportion of *Lolium* species in herbage of the seed mixtures L1 and L2 corresponded to 33% and 73%, respectively (data not shown). In contrast, timothy made up between 3 and 9% of the herbage in the timothy-based seed mixtures, suggesting that timothy is sensitive to a three-cut system, particularly at high latitudes. Low regrowth capacity of timothy is surely also a reason for limited persistence over time (Tamaki *et al.*, 2010).

Table 1. Species proportion in seed mixtures (based on% weight).

Species	Cultivar	Mixture					
		T1	T2	T3	T4	L1	L2
<i>Phleum pratense</i>	'Grindstad'/'Liljeros'	45	45	40	30		
<i>Festuca arundinacea</i>	'Swaj'		25				20
<i>Festuca pratensis</i>	'Vestar'	25		20		20	
<i>Festuca rubra</i>	'Leik'			10	10	10	
<i>Lolium perenne</i> 4x	'Figgjo'					20	40
<i>Lolium hybridum</i>	'Fenre'						20
<i>Festulolium</i> **	'Frosta'						20
<i>Poa pratensis</i>	'Knut'	20	20	10	20	20	
<i>Agrostis capillaris</i>	'Leikvin'			10			
<i>Bromus inermis</i>	'Leif'				30		
<i>Dactylis glomerata</i>	'Laban'					20	
<i>Trifolium pratense</i> 2x	'Gandalf'	5	5	5	5	5	
<i>Trifolium repens</i>	'Litago'	5	5	5	5	5	

\*Half-and-half of each cultivar.

\*\* *Festulolium* crossings (4x) between *F. pratensis* and *L. perenne*.



The seed mixtures T1 and T2 contained either meadow fescue or tall fescue, respectively. In all seven experimental years, mean annual herbage yield tended to be higher for the tall fescue-containing seed mixture T2 than for seed mixture T1 (Fig.1). In the seventh ley year, the T2 showed the greatest total DM yield among the tested seed mixtures. On average, herbage DMY decreased in the order of T2>L2>T4>L1>T1>T3 in the 7<sup>th</sup> year. High CV for the seed mixture T2 suggests that tall fescue needs time to be established and companion species have lower yield capacity (Todnem and Lunnan, 2017). Evaluation of botanical composition in 2019 and 2022 showed that tall fescue dominated in seed mixture T2, making up 81 and 74%, respectively. In the seed mixture T1, 33% comprised unsown species, suggesting low persistence of sown grasses and legumes. The content of red and white clover ranged between 0.5 and 5% in the mixtures containing clovers.

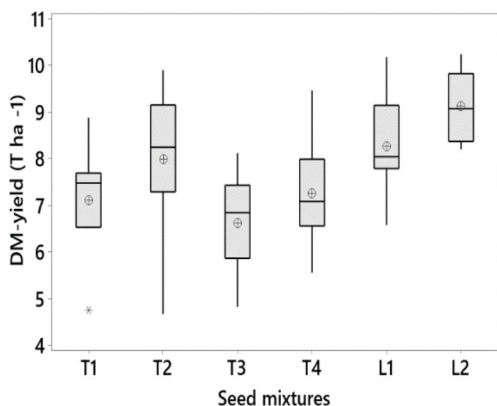


Figure 1. Box plots of the mean annual yield (2017–2023) for seed mixtures (see Table 1).

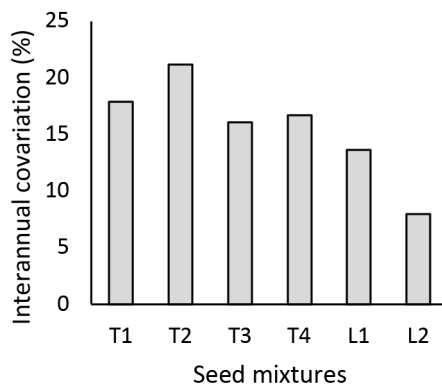


Figure 2. Yield stability shown by interannual coefficient of variation of seed mixtures.

## Conclusions

This study suggests that seed mixtures containing ryegrass cultivars and tall fescue persist well in the Nordic maritime climate, partly replacing timothy and meadow fescue. These species should be part of future seed mixtures designed for leys with long lifespan.

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# How does the heterogeneity of sward height evolve under moderate grazing intensity?

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## Abstract

Heterogeneity is an inherent property of grasslands, while grazing management practices try to eliminate it. Uniformity only occurs naturally during overgrazing, impairing animal and herbage production and related ecosystem services. Most studies that investigated heterogeneity together with grazing intensities described neither its evolution, nor how animals exploit it. We evaluated the evolution of heterogeneity in sward structure, focusing on height as a descriptor over the stocking season and managed under two initial contrasting levels of heterogeneity at moderate grazing intensity. Lambs were set to graze continuously five paddocks kept the same average sward height (10 cm), but the homogeneous treatment started with two paddocks evenly cut at 10 cm and the heterogeneity treatment had three paddocks divided into 80 25-m<sup>2</sup> quadrants randomly allocated to an initial 5-cm or 15-cm cut. Semivariograms and interpolated maps of spatiotemporal patterns showed that the grazing process in a matter of 2 weeks creates heterogeneity in an initially homogeneous vegetation, converging between the heterogeneous and homogeneous paddocks. Intra-paddock variations vary over the course of a grazing season. These results provide a baseline for understanding the dynamics of heterogeneity, detailing the relationships between plants and herbivores, which is essential to propose sustainable grazing management at moderate intensity.

**Keywords:** vegetation dynamics, spatio-temporal heterogeneity, sward structure, perennial ryegrass, patch grazing, temporal pattern

## Introduction

Despite evidence regarding the importance of heterogeneity in grassland ecosystems, many management practices try to eliminate it using mechanical interventions or high animal densities that aim to generate a more uniform distribution of livestock grazing by suppressing the selective behaviour of animals (Fuhlendorf and Engle, 2001). In grazing situations, vegetation uniformity only occurs in overgrazing situations, which is well known to impair both the growth performance of animals, herbage production, and related ecosystem services. Thus, understanding the role and dynamics of heterogeneity at moderate grazing intensity is essential to propose nature-based solutions for pastoral ecosystems that integrate knowledge about the processes of natural ecosystems into production systems. Starting from swards with contrasting heterogeneity, we tested how the vegetation heterogeneity evolves in the short term under moderate grazing intensity.

## Materials and methods

A grazing experiment was conducted in Gembloux (Belgium) on a *Lolium perenne* sward with sheep (lambs) managed under continuous stocking method as animal model. The two treatments were applied on experimental paddocks as follows: Treatment 1: a randomized heterogeneity treatment at the patch level (25 m<sup>2</sup>); Treatment 2: a homogeneous treatment, the entire paddock with a homogeneous initial

sward height. We generated two initial levels of heterogeneity, considering the sward height as a metric, through taking a cut before the animals accessed the area, to obtain a 10-cm average sward height on all paddocks. In the homogeneous treatment the whole area was mowed to a 10 cm sward height. In the heterogeneous treatment, each paddock was subdivided in 80 randomly distributed patches of 25 m<sup>2</sup> each. Half of the patches were allowed to reach a sward height of 15 cm, the other half were mowed to reach 5 cm when the animals were set to graze. The 10±5 cm sward height was used as target for a moderate grazing intensity. The experimental design was a randomized complete block with three replicates of the heterogeneous treatment and two replicates of the homogeneous treatment. The stocking season lasted 92 days, starting in 8 May 2021. The sward surface height was measured using a sward stick, and the plant species were also recorded at 200 geolocated points once a week, totaling 12 measurements. Data were collected on May 5 2021 (pre-grazing), May 12, May 19, May 26, June 2, June 9, June 16, June 23, July 7, July 21, July 28 and August 4.

Geostatistical analyses were conducted for spatial heterogeneity of the investigated grassland. Isotropic experimental semivariograms of each paddock were calculated using GS+ software version 10 (Robertson, 2008), considering the 2×2 m<sup>2</sup> grid separation distance between points and a maximum separation distance of 80 m to obtain a minimum of 30 pairs of points, as recommended by Journel and Huijbregts (1978). We obtained a minimum of 95 pairs. The data were interpolated using ordinary kriging and used to generate interpolated maps of spatiotemporal patterns of sward height using Smart-Map plugin in QGIS software version 3.22 Białowieża (QGIS Development Team).

## Results and discussion

Before grazing, the paddocks showed different semivariances with a pattern according to treatment (May 6, 2021). The heterogeneous treatment presented higher semivariance (Figure 1) and met the initial conditions proposed for the two treatments (heterogeneity vs. homogeneity). Grazing rapidly altered the vegetation structure: 4 days after the grazing started the spatial heterogeneity increased in the homogeneous treatment but remained stable in the heterogeneous treatment (Figure 1), as shown by the semivariograms (May 12, 2021) and maps (Figure 2). The semivariance threshold gradually changed. In most measurements, significant semivariance fluctuations were observed beyond the first peak (semivariogram), thereby indicating that over the stocking season the grazing process shaped the vegetation so that both small-scale patchiness and regular arrangement of those patches (patches arranged regularly across the paddock) occur, as demonstrated in previous studies (e.g. Palmer and McGlenn, 2002). Our results clearly showed that the grazing process quickly creates heterogeneity in the vegetation under moderate grazing intensity. It is important to highlight that heterogeneity is an inherent and unavoidable property in grassland ecosystems. However, further research should look to the extent (time) to which patterns imposed via anthropic intervention, such as those generated in this study, are stable.

## Conclusion

Our findings especially highlight that the grazing process rapidly creates heterogeneity in grassland vegetation. Fluctuations in sward structure heterogeneity levels at moderate grazing intensity occur throughout the stocking season and are associated with the average target sward height, being impacted by stocking rate adjustments and morphological and phenological changes in plants.

## Acknowledgement

We thank CAPES/CNPq and Erasmus+ for funding.

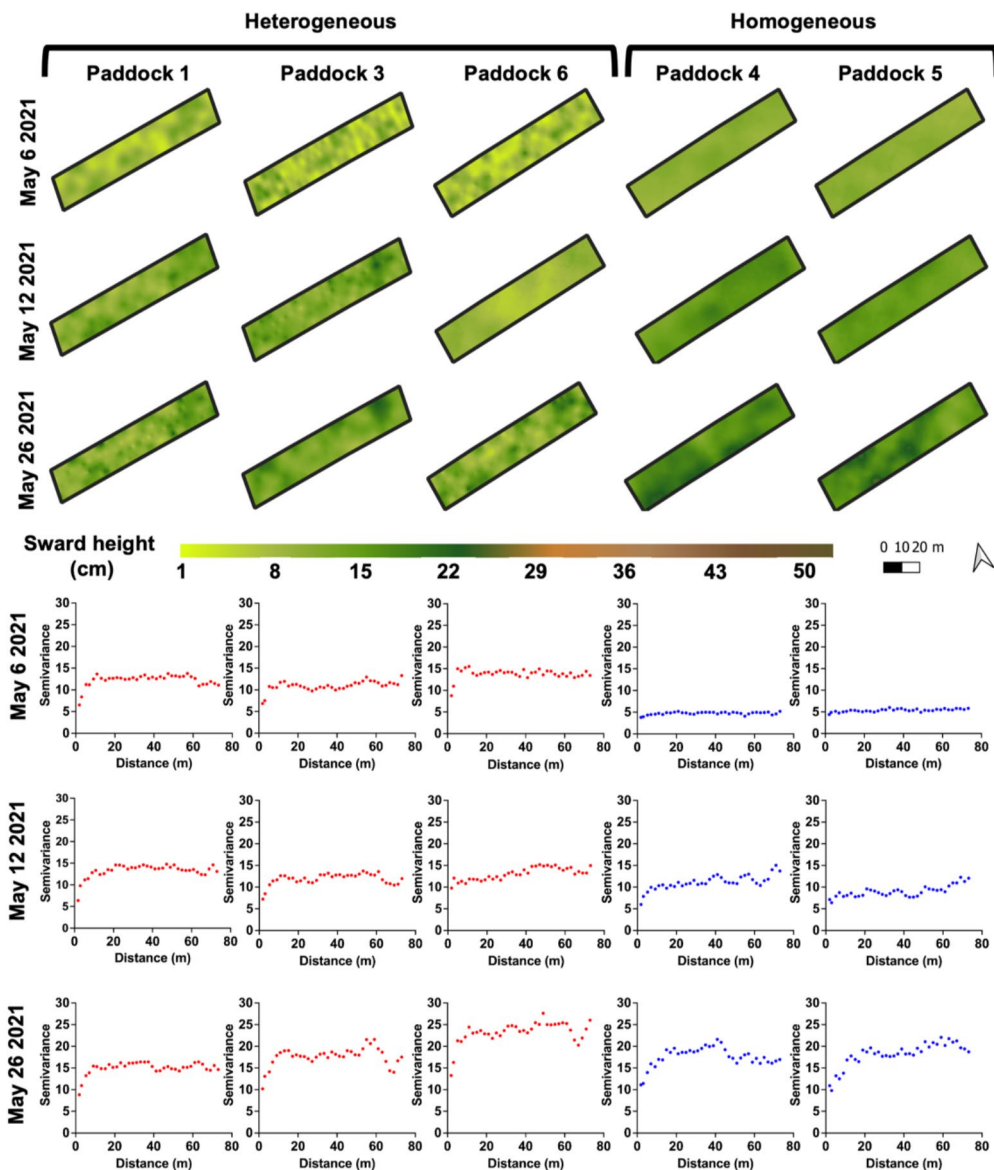


Figure 1. (Top) Sward structure heterogeneity maps derived from ordinary kriging of sward heights (cm). (Bottom) Semivariograms of sward height spatial patterns in paddocks with two initial levels of heterogeneity.

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# Assessing root biomass in timothy and tall fescue via minirhizotron imaging and core sampling

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## Abstract

Grass root systems play a pivotal role in soil processes and are important in carbon cycling. Despite their significance there remains a lack of understanding around the roots of species in intensively managed northern grasslands. This study aimed to assess and compare the root biomass (RM), depth distribution, and growth patterns of root systems in timothy (T) and tall fescue (TF) using root imaging and root sampling techniques. Results from the analysis of images were compared to the RM results. The study was conducted on sandy loam mineral soil in central Finland during 2020 to 2022. Root samples were taken from the 0–40 cm soil depth profile at each cut. Root images were captured using a minirhizotron system during the growing seasons. AI-based image segmentation was used to estimate the root area per unit area in the observed profiles. The results from root biomass measurements indicated substantial differences in the quantity, depth distribution and development patterns of roots among species. According to the image analysis, the percentage of the imaged surface area occupied by roots in TF is significantly larger than that of T at depths below 40 cm.

## Introduction

Roots play a crucial role in agroecosystems, not only in terms of yield production but also from an environmental perspective. In grasses commonly used in northern Europe, the formation of root biomass (RM), depth distribution, and growth patterns of root systems are poorly understood (Bolinder, 2002; Palosuo *et al.*, 2016). The main aim of this study was to enhance our understanding of root biomass, root depth distribution, and root growth patterns over the course of three grass production years.

## Material and methods

A field experiment was conducted between 2019 and 2022 at the Natural Resources Institute Finland (Luke) in Maaninka (63°09' N, 27°20' E). The experiment was sown on sandy loam soil with a 1.7 % organic matter content in the soil layer of 0–20 cm. The experiment was carried out as a randomized complete block design with four replicates. The treatment was species: timothy (*Phleum pratense* L. cv 'Nuutti'; T) and tall fescue (*Lolium arundinaceum* (Schreb.) Darbysh; TF). The ley was intensively managed (2–3 cuts per year, N fertilization 200–240 kg N ha<sup>-1</sup> year<sup>-1</sup>); the cultivation practices can be found in more detail in Kykkänen *et al.* (2022). Root biomass was assessed by soil coring to a depth of 40 cm at each cut in 2020 and 2022. Roots were separated from the soil in four different profiles (0–2, 2–10, 10–20 and 20–40 cm) using a hydropneumatic elutriation system (Smucker *et al.*, 1982) and by hand picking. Separated roots were dried at 50°C until constant weight. Subsequently, the dried roots were analysed to determine total dry matter (DM). Minirhizotron imaging was performed using the Bartz VSI MS-190 system in proximity to the harvest dates. In 2019, during the establishment phase, acrylic plastic tubes were installed in each plot at a 30-degree angle. The tubes reached a depth of 70 cm (43 imaging layers), and they were imaged in three directions (both side sections and upper section). The area covered by roots in each image was analysed using RootPainter (Smith *et al.*, 2022). The segmentation model, based on convolutional neural networks (U-net), was trained using a subset of 200 images that were randomly selected in a balanced manner from each image capture session. Images without visible roots were removed from the training data, resulting in a total of 182 images for training. The model was trained to recognise all visible roots, excluding root hairs. Performance was deemed satisfactory when rolling average ( $n=10$ ) of the dice score reached 0.8. Statistical analyses were calculated using ANOVA (SAS 9.4, Mixed-procedure). The RM for each soil profile and the total profile was initially analysed

for each cut. The species factor was treated as a fixed effect and replicate as a random effect. As year had significant interactions with species, the years were analysed separately. Statistical calculation of data from root image analyses was implemented like analyses for RM data.

## Results and discussion

In this study, the species and the year were the most significant factors affecting the RM (0–40 cm). TF exhibited significantly higher total RM values compared to T in almost all cuts and years ( $P < 0.05$ , Table 1). Specifically, the total RM of TF was 13–68% higher in the 0–40 profile. The observed effect was evident in all profiles, especially in the second cut and over the years. In the first year, the total RM of both species significantly higher compared to the third year ( $P < 0.05$ ). This finding contradicts previous reports (Bolinder *et al.*, 2002), but aligns with the observation made by Chen *et al.* (2016) regarding grass-legume ley. The decline in RM can be attributed to a reduction in the yield potential of grasses, which is typical for T and TF over the years (Virkkajarvi *et al.*, 2012). On the other hand, the growing conditions could also be a contributing factor. In 2020 the growing conditions were exceptionally dry until the first harvest. The observation necessitates additional analysis of the data. The phenomenon of TF's development of RM into deeper soil profiles throughout the 2020 growing season, as observed by Kykkänen *et al.* (2022), was also observed as a tendency in 2022 in the 2–10 cm profile ( $P < 0.10$ ). T exhibited contrasting behavior as RM was observed to decrease in the profile 20–40 cm ( $P < 0.05$ ). Chen *et al.* (2016) also reported the presence of a seasonal pattern and interannual variability in root traits.

The root profile up to a depth of 70 cm was captured by a minirhizotron camera. The total root area (totRA) of TF was consistently and significantly higher than that of T across all years ( $P < 0.05$ ; Figure 1). Specifically, in 2020 and 2021, the totRA of T was 50%, and in 2022, 40% lower than that of TF. Below the 40 cm depth, the difference in root area between the two species appeared to increase. TF consistently exhibited a higher root area below 40 cm compared to T across all cuts, except for the first cut in 2021. Interestingly, when focusing on the 0–40 cm profile, no differences were observed between the species, contradicting the conclusions drawn from the RM measurements. In the regression analysis  $RM = a + B$  (root area) the model showed a weak explanatory power in relation to the variation in RM

Table 1. Effect of species (tr; T: timothy, TF: tall fescue) on root biomass (RM kg DM ha<sup>-1</sup> year<sup>-1</sup>), in soil profiles 0–2 cm (1), 2–10 cm (2), 10–20 cm (3), 20–40 cm (4) and 0–40 cm (tot) in 2020 and 2022.

	1 <sup>st</sup> cut				2 <sup>nd</sup> cut				3 <sup>rd</sup> cut				P		
	T	TF	p	SEM	T	TF	p	SEM	T	TF	p	SEM	tr	Cut	tr*cut
2020															
1	1290	1800	*	120	1500	1950	*	156	2080	2130	ns	123	**	*	ns
2	1320	1640	o	121	1560	2090	**	87	1360	2140	*	114	***	o	ns
3	880	1150	ns	101	810	1120	*	57	700	1260	***	27	***	ns	ns
4	490	570	ns	53	500	810	*	62	490	830	**	33	**	o	o
Total	3990	5160	o	289	4360	5960	**	194	4620	6360	**	182	ns	*	**
2022															
1	1230	1050	ns	239	860	1180	*	55					ns	ns	*
2	1230	1560	ns	130	1090	2160	***	85					***	ns	*
3	530	690	*	32	370	540	o	48					**	*	ns
4	380	510	*	25	240	450	**	22					***	*	ns
Total	3360	3800	o	306	2570	4320	**	143					***	ns	***

\*\*\* $P < 0.001$ , \*\* $P < 0.01$ , \* $P < 0.05$ , o $P < 0.10$ , ns  $P \geq 0.10$ ; SEM, standard error of mean.

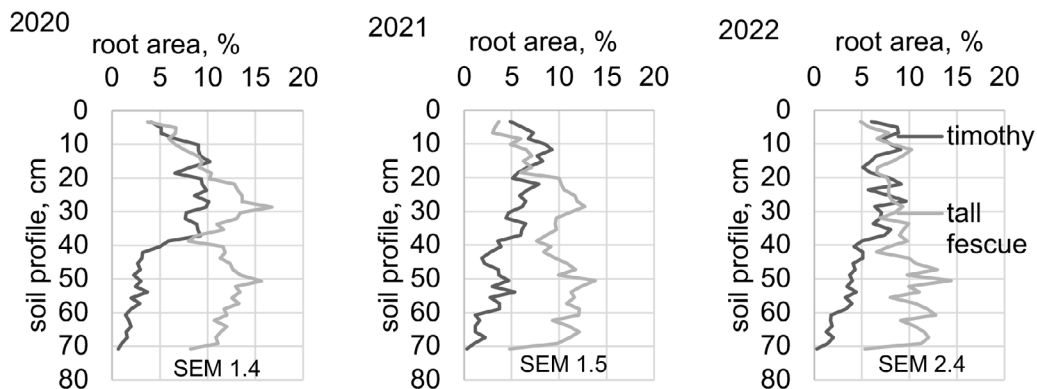


Figure 1. The percentage of the imaged surface area occupied by roots (%) assessed for timothy and tall fescue within a depth profile 2–70 cm during the years 2020, 2021 and 2022, over the cuts. The Standard Error of the Mean (SEM) is calculated for the total root area within the total depth profile (2–70 cm) over the cuts.

( $r^2=0.17$ ,  $p=0.009$ ) within the 2–40 cm layer. The images obtained from the upper layers demonstrated a higher tendency to show roots at a greater distance from the surface of the imaging tube, and soil being less densely packed against the tube surface. This observation may contribute to the interpretation of certain conflicting results.

## Conclusion

Tall fescue forms a larger root system than timothy under the Nordic humid climate conditions. Root imaging showed the disparity among species was more pronounced in soil profiles below 40 cm. The observed species-specific differences, seasonal patterns, and year-to-year variations underscore the multifaceted nature of belowground processes. The methodology employed for root observation had a substantial influence on the interpretation of the results, highlighting the need for further advancements in root image acquisition and analysis.

## Acknowledgements

We thank the Ministry of Agriculture and Forestry of Finland ('JuuriHiili' and 'OrmiNurmi'-projects) and The European agricultural fund for rural development ('Sustainability from grass'-project) for financially supporting this research.

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# Methodological study for optimising a digital grazing schedule plan from GPS data

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## Abstract

Recording the parcels where cattle have grazed during the grazing season (grazing schedule) is useful for optimising grazing management, reducing certain health problems and promoting farms where grazing practices are implemented via dedicated labels. Most farmers do not record their grazing schedule accurately. The use of a GPS collar could track the location of the animals to trace the paddocks and to get the number of grazing days both per paddock and over the grazing season. As GPS collars are expensive, the number of animals to equip must be as low as possible while keeping up the accuracy of the tracking using a suitable sampling frequency. Our objective was to identify the best pairings of 'number of equipped animals\*sampling rate' to get a grazing schedule. Fourteen cows out of 75 were fitted with GPS collars in a rotational grazing with front wire, with a measurement every 10 seconds. Pairings of 10, 5 and 2 cows (C) and measurements every 10, 20, 30, 60 and 120 seconds (s) were used. To compare the pairings, the area of the paddock, the position of the front and back wires and the distance between the two wires got with the GPS data were calculated relative to the actual ones (measured in the field and via data retrievable from the land register). The best pairings were 2C-10s, 2C-20s, 5C-10s, 5C-20s, 10C-10s, 10C-20s and 10C-30s.

**Keywords:** grazing schedule, bovine, grassland, GPS

## Introduction

Grasslands provide many ecosystem services, especially when they are semi-natural and grazed, in line with public (carbon storage, biodiversity, animal welfare, tourism) and consumer expectations (product quality). They can also represent certain animal health, nutritional and welfare risks. Tools that trace the grazing schedule exist to help farmers manage these risks and improve the services they provide by managing their grasslands. However, few farmers make use of them, often because they are too demanding to provide information. GPS collar systems, which are more reliable than RFID and UWB systems (Ganskopp and Johnson, 2007), can now be used to track animals in time and space and thus record a grazing schedule. These recording systems usually require a large number of equipped cows, and the cost can be a major obstacle for farmers (Knight *et al.*, 2018). In addition, high measurement frequencies affect the charge of the batteries, reducing the use time of these systems between two recharges. These two parameters have a significant impact on the worktime farmers would allocate to using this tool. One study showed that a low number of measurements (every 5 minutes) made it possible to increase up to 54 days of monitoring cattle on pasture with a lithium-ion battery without the need for recharging (McGranahan *et al.*, 2018). However, an increase in this interval can affect the quality of information retrieved. Collecting data once a day rather than every 5 minutes increased the misinterpretation of the tracking order of the plots visited by the cattle (Johnson and Ganskopp, 2008). However, few studies have been carried out on the simultaneous optimisation of increasing the measurement interval and reducing the number of collars.

The aim of our work was to assess with GPS whether optimising the number of cattle equipped, combined with optimising the data acquisition interval, made it possible to accurately characterise the paddocks visited (areas covered, location of front and rear wires, etc.) to reconstruct a reliable grazing schedule.



## Materials and methods

The data were collected on a dairy farm (Lion d'Angers, France, 47°37'43" N, 0°42'42" E) with 71 Prim'Holstein dairy cows. The cows grazed on a rotational system on temporary grassland (sown with *Lolium perenne* and *Trifolium repens*). Every day, the front wire was moved to allocate a paddock surface suited to the cows' requirements. Access to the grassland was by day and night. Data were collected over 3 days of grazing (from 4 to 6 May 2017) on two paddocks, ranging from 0.3 to 2 ha.

Cows were fitted with GPS collars. Data (longitude and latitude) was acquired every second from 14 cows with a standard error of  $\pm 1.5$  m. A total of 15 pairings were made by reducing the number of cows equipped (10C, 5C and 2C) and increasing the time intervals between two acquisitions (10 s, 20 s, 30 s, 60 s and 120 s). From the GPS data, four indicators were created to compare the pairings with the reference data collected on the grassland, hereafter referred to as 'actual': the ratio of the detected surface/actual surface, the ratio of the detected position of the front wire/actual position, the ratio of the detected position of the rear wire/actual position and the distance between the two wires detected/actual distance. Finally, an average (Final score) was calculated to compare the pairings. A threshold of less than 5% was chosen to identify the pairings closest to the field measurements. The data were processed using R software (version 4.3.0).

First, 1001 combinations were created for each pairing with 10 cows, 2002 combinations for each pairing with 5 cows and 91 combinations for each pairing with 2 cows. The values of the 4 indicators and their averages are presented in Table 1. Then, to study whether 2 cows selected for their exploratory nature could be a solution to characterise the plot visited, the best pairing generated from the 91 batches was selected for each increase in time interval (10 s, 20 s, 30 s, 60 s and 120 s) and this is presented in Table 2.

Table 1. Results of indicators and final score compared to grassland reality for each pairing. The lower the indicators are the more accurate is the measurement compared to actual one.

Pairing	Observations	Back wire (%)	Front wire (%)	Area of paddock (%)	Distance wires (%)	Final score (%)	SE Final score (%)
10C-10s	1001	2.2	0.9	12.9	1.8	4.4	0.04
10C-20s	1001	2.2	0.9	9.6	1.9	3.6	0.04
10C-30s	1001	3.4	2.3	6.8	4.5	4.3	0.03
10C-60s	1001	3.5	6.6	27.2	8.3	11.4	0.07
10C-120s	1001	3.5	11.7	65.4	12.8	23.4	0.13
5C-10s	2002	4.5	1.8	5.2	3.9	3.8	0.04
5C-20s	2002	4.8	2.3	5.7	4.5	4.3	0.04
5C-30s	2002	6.0	3.1	7.7	6.2	5.8	0.04
5C-60s	2002	7.1	11.0	48.6	14	20.2	0.11
5C-120s	2002	7.7	17.5	76.1	19.8	30.3	0.11
2C-10s	91	12.2	3.9	9.8	7.6	8.4	0.41
2C-20s	91	13.8	5.4	16.8	9.6	11.4	0.47
2C-30s	91	15.1	7.6	25.2	12.1	15.0	0.54
2C-60s	91	17.2	24.5	74.4	28.7	36.2	0.76
2C-120s	91	19.0	29.9	89.1	34.3	43.1	0.73

The final score is the average of the 4 indicators. C, the number of cows used among the 14 fitted (10, 5 or 2 cows); s, the different frequencies used, 1 measurement every 10, 20, 30, 60 or 120 s.

The final score is the average of the 4 indicators. C, the number of cows used among the 14 fitted (10, 5 or 2 cows); s, the different frequencies used, 1 measurement every 10, 20, 30, 60 or 120 s.

Table 2. Effect of acquisition frequencies on the final score with cows selected for their exploratory nature

Optimization	Final score (%)
2C-10s	1.1
2C-20s	2.2
2C-30s	5.2
2C-60s	18.5
2C-120s	24.7

C, the number of cows used among the 14 fitted (10, 5 or 2 cows); s, the different frequencies used, 1 measurement every 10, 20, 30, 60 or 120 s.

## Results and discussion

As the number of cows equipped decreases and the measurement interval increases, the final score (average of the 4 indicators) deteriorates in relation to the data measured in the plots (Table 1). Indeed, when the number of cows equipped is large (10C) and the measurement interval is small (10s), the deviation from reality is small (Final <5%). In the opposite case (2C-120s), the deviation is large (Final >40%). According to the final score, the pairings following 10C-10s, 10C-20s, 10C-30s, 5C-10s and 5C-20s show a variation of less than 5% compared with the field measurements. Table 2 shows the best final scores from all pairings made with 2 cows selected from the 14 cows available. It is shown that when equipped cows are chosen, the final score error compared with the field measurements is less than 5% for measurements taken every 10 or 20 s.

## Conclusion

This study shows that measuring grazing schedules by GPS can be optimized in two ways, by reducing the number of cows equipped (up to 5C if randomly selected) and the time between two measurements (up to 20 s). Some cows seem to show a more exploratory nature when grazing. If these cows are equipped with GPS, this behaviour could be an asset for monitoring the herd on grassland without having to equip a large number of cows. However, these results still need to be validated on different grazing methods and different types of grassland, particularly on permanent grassland where the type of terrain can affect the movement of the animals.

## Acknowledgements

We thank the commercial farm GAEC Beloin (Le Lion-d'Angers, France) where the experiment took place.

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# The effect of chemical nitrogen fertiliser on red clover production in silage swards

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## Abstract

Interest is growing in red clover (*Trifolium pratense* L. (RC)) in silage swards due to its potential to displace chemical nitrogen (N). The objective of this study was to investigate the impact of N fertiliser rate and RC variety on dry matter yield (DMY) across a three-cut silage protocol. There were three N rates; Low (0 kg N ha<sup>-1</sup>), Medium (75 kg N ha<sup>-1</sup>) and High (150 kg N ha<sup>-1</sup>), applied in a 44, 33 and 22% split for Cut 1, 2 and 3, respectively and 8 RC varieties. Plots were defoliated in May, July and September. There was an interaction present between N rate and cut number on yield: the Low had the lowest yield on cut 1 (6156±170.9 kg DM ha<sup>-1</sup>), compared to the Medium and High (7420±170.9 kg DM ha<sup>-1</sup>), with no difference in the second or third cuts. An interaction ( $P<0.05$ ) between variety and N rate on red clover content was also detected. Under the High N treatment variety Pastour had a significantly lower clover content (470±45 g (kg DM)<sup>-1</sup>) than Low and Medium (both 740±45 g (kg DM)<sup>-1</sup>). In conclusion, N fertiliser can be applied for the first cut to increase yield and omitted thereafter with no negative impact on DMY. Although nitrogen application reduces overall red clover content, the current study shows that some varieties are less affected than others.

**Keywords:** red clover, nitrogen fertiliser, silage yield

## Introduction

There is a renewed interest in red clover (*Trifolium pratense* L. (RC)) in silage swards due to its potential to maintain herbage production and quality at reduced rates of nitrogen (N) fertilisation (Clavin *et al.*, 2016). Red clover has the potential to fix up to 200 kg N ha<sup>-1</sup> through biological N fixation thereby displacing chemical N requirements (Black *et al.*, 2009). This can aid Ireland in meeting its target to reduce chemical N to under 325,000 tonnes by 2030 (DAFM, 2020). There have been conflicting reports on RC response to chemical N fertiliser to increase DMY. Clavin *et al.* (2016) reported similar annual dry matter yield (DMY) from grass-only swards receiving 412 kg N ha<sup>-1</sup> and perennial ryegrass (*Lolium perenne* L. (PRG))-RC swards receiving zero N, whereas Søegaard and Nielsen (2012) reported increased DMY as N fertilisation increased from 0 - 220 kg N ha<sup>-1</sup> year<sup>-1</sup>; however, in that study a reduction in sward clover content was reported. In contrast, Clavin *et al.* (2016) reported a reduction in annual DMY when spring N increased from 0 to 50 kg N ha<sup>-1</sup>. The application of N fertiliser (Søegaard and Nielsen, 2012) on a range of RC varieties (Marshall *et al.*, 2017) can have varying impacts on herbage production and sward RC content. The impact of RC variety and how N fertiliser can impact herbage production and longer term persistence under an intensive silage protocol needs to be investigated further. The objective of this experiment was to determine the effect of N fertiliser application rate and RC variety on herbage DM production under a three cut silage protocol.

## Materials and methods

The current experiment was conducted at Teagasc, Moorepark, Fermoy, Co. Cork, Ireland (52°9' N; 8°16' W) in 2023. The soil type was a free-draining, acid brown earth of sandy loam to loam texture. Soil phosphorus and potassium were high (both Index 4). A total of 81 plots (7×1.5 m) were established in May 2022, with 8 red clover varieties Aberclaret (Diploid - D), Fearga (D), Sinope (D), Spurt (D), Bonus (D), Garant (D), Pastour (D) and Amos (Tetraploid, T) sown (7 kg ha<sup>-1</sup>) with two perennial ryegrasses

Gracehill (T; 14 kg N ha<sup>-1</sup>) and Astonconqueror (D; 14 kg N ha<sup>-1</sup>). Each variety received one of three N rates: (0, 75, 150 kg N ha<sup>-1</sup>; Low, Medium, High, respectively), applied in a 44, 33 and 22% split for Cut 1, 2 and 3, respectively. All plots were defoliated for silage on 3 occasions, in May, July and September, to a post-cutting height of 6 cm. Herbage mass was determined by harvesting a strip of each plot using an Agria 3600 BM mower, the harvested material was weighed and DM content was determined by drying a 100 g sub-sample at 60°C for 48 h. Red clover content was determined by separating a 200 g subsample into grass and red clover portions and drying at 90°C for 16 h to determine the DM proportion of each in the sward. Data were analysed using PROC MIXED in SAS 9.4 (SAS Institute, Cary, NC, USA, 2002) with N rate, variety, cut number and associated interactions included as fixed effects. Repetition was included as a random effect and plot as subject.

## Results and discussion

There was a significant effect ( $P < 0.05$ ) of N rate on annual DM production. The Medium N treatment had the greatest annual DMY (17 136 ± 301.2 kg DM ha<sup>-1</sup>) compared to the Low N (16 044 ± 301.2 kg DM ha<sup>-1</sup>) with the High N (16 793 ± 301.2 kg DM ha<sup>-1</sup>) intermediate. The results are similar to Søegaard & Nielsen (2012) who reported an increase in DMY of > 2 t DM ha<sup>-1</sup> as N fertilisation increased from 0 to 220 kg N ha<sup>-1</sup> year<sup>-1</sup>. There was a significant effect ( $P < 0.0001$ ) of cut number on yield: Cut 1 had the highest (6999 kg DM ha<sup>-1</sup>), followed by Cut 2 (5307) and then Cut 3 (4353 kg DM ha<sup>-1</sup>), similar to Clavin *et al.* (2016). There was a significant interaction ( $P < 0.0001$ ) between N rate and cut number. In Cut 1 the Low N had the lowest yield (6156 ± 170.9 kg DM ha<sup>-1</sup>) with no difference between the Medium and High N treatments (7420 ± 170.9 kg DM ha<sup>-1</sup>) and no difference between the N treatments in Cut 2 or Cut 3. This could have been as a result of increased N fixation at higher clover contents after the first defoliation (May), (260 ± 18 g kg<sup>-1</sup>) compared to second and third cut (830 ± 20 g kg<sup>-1</sup> and 850 ± 19 g kg<sup>-1</sup>, respectively), similar to Hennessy *et al.* (2022). This could have also accounted for the impact on DM production reported on the Low N treatment in Cut 1 in the current study, due to lower levels of N fixation expected from legumes in spring (Hennessy *et al.*, 2022). Therefore, the results indicate that, by applying 33 kg N ha<sup>-1</sup> for 1<sup>st</sup> cut and zero N for 2<sup>nd</sup> and 3<sup>rd</sup> cut, annual DM yield will be greater than the Low N treatment and similar to the Medium N treatment. Nitrogen rate had a significant impact ( $P < 0.05$ ) on annual mean sward clover content. The Low N treatment had the greatest proportions of RC (670 ± 20 g kg<sup>-1</sup> DM) compared to the Medium and High N treatments (620 ± 20 g (kg DM)<sup>-1</sup>) similar to Søegaard and Nielsen (2012). Previous studies also reported that higher rates of N fertiliser have a greater negative impact on RC content over multiple applications across years. The current study is only reporting data from the first full production year, and further investigation is warranted to examine the longer term impact of high N fertiliser on sward clover content.

Red clover variety had a significant effect ( $P < 0.05$ ) on annual DM yield (Figure 1). Aberclaret had a greater yield compared to Garant and Pastour (18 093 ± 491.8, 15 479 ± 491.8 and 15 692 ± 491.8 kg DM ha<sup>-1</sup>, respectively) with all other varieties intermediate (16 615 kg DM ha<sup>-1</sup>). Similarly, Marshall *et al.*, (2017) reported a significant effect ( $P < 0.01$ ) of RC variety on DM yield, with the highest RC yields reported from Aberclaret and Milvus. There was no impact of RC variety on clover content, similar to Clavin *et al.* (2016); however, there was a significant interaction ( $P < 0.05$ ) between variety and N rate. Pastour had the lowest clover content on the high N treatment (470 ± 45 g (kg DM)<sup>-1</sup>) compared to the Low and Medium N treatments (740 ± 45 g (kg DM)<sup>-1</sup>). Pastour under High N was also significantly lower in clover content than the Low Amos, Spurt, Sinope and Aberclaret as well as the High Sinope and Aberclaret varieties. There was no interaction between variety and cut number on clover content.

Figure 1. Effect of variety on cumulative dry matter yield (black bar graph) and average sward clover content (grey line graph).

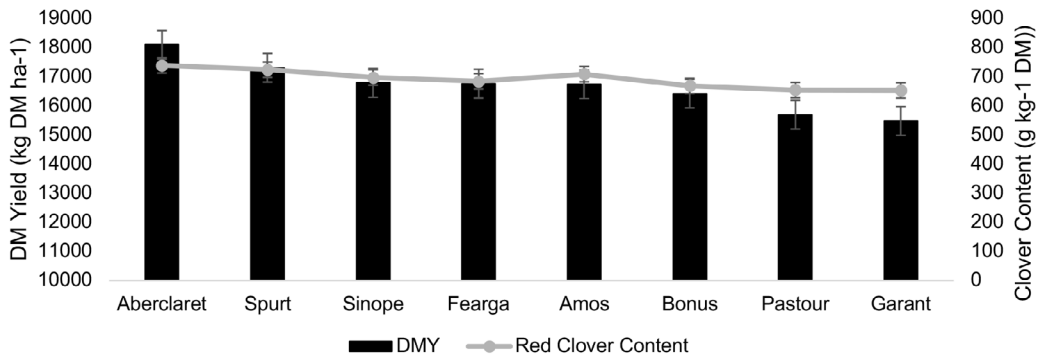


Figure 1. Effect of variety on cumulative dry matter yield (black bar graph) and average sward clover content (grey line graph).

## Conclusion

Applying N fertiliser increased annual DMY, predominantly through increased production in first cut. The results indicate that annual yield can be optimised by applying approximately 33 kg N ha<sup>-1</sup> for the first cut only with minimal impact on clover content. Red clover variety has a major impact on annual DMY and careful consideration should be made when selecting a suitable variety to increase dry matter production. Although nitrogen application can reduce clover content this study shows that certain varieties are less affected than others. Further study is required to determine the extent of the impact of N on clover content over more productive years.

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# N, P, K balances for six grazed or cut plots in an agroecological dairy cattle system

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## Abstract

The low-input dairy cattle ‘OasYs’ system aims to maximise grazing through the use of diversified multi-species grasslands and annual crops. The aim of this study was to assess whether the management of organic fertilisation or grazing led to deficits or surpluses of N, P and K at plot and rotation levels. We assessed N, P and K inputs and outputs on 6 plots in a grassland-crop rotation, 3 of which were in a 7-year grazed rotation and 3 in an 8-year cut rotation. The amounts of herbage grazed were evaluated at paddock level by the Herbvalo method, and used to quantify inputs by faeces. Urinary-N excretions at grazing were assessed by the Urea model. Direct excretion by grazing animals were major inputs of N, P and K at plot level. The 3 grazed plots had a fairly balanced N ( $-19$  to  $28 \text{ kg ha}^{-1} \text{ year}^{-1}$ ), P ( $-4$  to  $3 \text{ kg ha}^{-1} \text{ year}^{-1}$ ) and K ( $-27$  to  $23 \text{ kg ha}^{-1} \text{ year}^{-1}$ ) balance. One cut plot was in balance and the other two in deficit, especially for K (maximum of  $-100 \text{ kg ha}^{-1} \text{ year}^{-1}$ ).

**Keywords:** mixed crop-dairy system; ley pastures; OasYs

## Introduction

Several authors agree that grasslands and ruminant livestock are a key to improving nutrient circularity in agroecosystems, particularly when grasslands are grazed and alternate with crops (Lemaire *et al.*, 2015). However, for these systems to be virtuous and sustainable, organic effluent and grazing management practices need to be consistent with the production and NPK absorption capacities of the grasslands (Vertès *et al.*, 2019). Exports outside the system or transfers of fertility between plots must not deplete the soil’s P and K reserves (Möller *et al.*, 2018). The low-input ‘OasYs’ system experiment (72 dairy cows, 91 ha), which aims to maximise grazing through the use of diversified multi-species grasslands and annual crops, showed good N efficiency and N balance (Novak *et al.*, 2022b). However, a balance sheet at farm level can mask disparities at plot level. The aim of this study was to assess N, P and K balances for 6 plots in the OasYs system, grazed or not, over their entire rotation, in order to provide guidelines for improving organic fertilisation practices in low-input systems with a view to ensuring long-term soil fertility.

## Materials and methods

We quantified the inputs and outputs of N, P and K over the entire rotation of 6 plots of approximately 3 ha monitored in the OasYs system experiment located on an INRAE facility (Vienne, France), 3 plots of which were grazed and 3 of which were not. The all-year grazed rotation alternates 5 years of temporary grassland and 2 years of annual crops, while the cut rotation is made up of 4 years of temporary grassland and 4 years of annual crops (Novak *et al.*, 2022a). The 6 plots started their rotation with different crops. The stocking rate was  $1.05 \text{ LU ha}^{-1}$ . We assessed N, P and K balances from 2015 to 2021 for the 7-year grazed rotation, and from 2015 to 2022 for the 8-year cut rotation. Urinary N excretion by dairy cows was calculated from milk urea using the Urea model (Edouard *et al.*, 2018) and excretion in faeces was calculated as  $7.2 \text{ g N (kg ingested dry matter)}^{-1}$  (Delaby *et al.*, 1997), which was itself assessed using the Herbvalo method (Delagarde *et al.*, 2018). P and K excretions were calculated using the  $\text{N/P}=6.6$  and  $\text{N/K}=0.783$  ratios given by Richner *et al.* (2017). Symbiotic fixation was assessed as representing 90% of the N contained in the leguminous part of the cover (A.S. Voisin, personal communication). The proportion of legumes in the covers was assessed visually in spring. Non-symbiotic N fixation by free-living soil microorganisms was set at a constant value of  $5 \text{ kg N ha}^{-1} \text{ year}^{-1}$  (Smil, 1999) and atmospheric deposition

at 6 kg N ha<sup>-1</sup> year<sup>-1</sup> (M.L. Decau, personal communication) and at 2 kg K ha<sup>-1</sup> year<sup>-1</sup> (Le Gall *et al.*, 2004) and were considered in the inputs. Fertilisation and harvested crops were weighed and analysed. The amounts of herbage grazed were evaluated at paddock level by the Herbvalo method (Delagarde *et al.*, 2018) and their N, P and K contents came from analysis results.

## Results and discussion

As very little mineral fertiliser was used, N inputs were mainly linked to cattle excreta on grazed plots, as well as symbiotic fixation and organic fertilisation, in liquid form for grazed plots, and in liquid or solid form for cut plots (Table 1). Cattle excreta on pasture were also the main source of P and K (Tables 2 and 3) for plots M1 and M2, but slurry inputs were greater for plot M5. For cut plots, P and K were almost exclusively supplied by organic fertilisation in liquid and solid forms. Input-output balances varied according to the plots and elements.

Table 1. Mean (min;max) N-balance (kg N ha<sup>-1</sup> year<sup>-1</sup>) of 6 plots, grazed (M1, M2, M5) or cut (G22, G23, V12), over 2015-2021 and 2015-2022, respectively.

	M1	M2	M5	G22	G23	V12
Cattle excreta	59 (16;80)	85 (33;131)	83 (23;153)	–	–	–
Slurry	19 (0;136)	9 (0;62)	52 (0;110)	45 (0;158)	8 (0;43)	12 (0;56)
Solid manure	–	–	–	40 (0;155)	42 (0;228)	29 (0;135)
Mineral N fertiliser	4 (0;30)	–	5 (0;34)	26 (0;118)	27 (0;60)	11 (0;50)
Symbiotic fixation	21 (0;70)	45 (7;122)	27 (1;47)	57 (0;194)	25 (0;85)	22 (0;65)
Total N inputs	114 (27;256)	150 (70;239)	177 (49;242)	176 (46;297)	96 (17;239)	87 (15;160)
Grazing	124 (20;190)	143 (56;223)	148 (3;273)	–	–	–
Harvested crops	10 (0;52)	7 (0;48)	1 (0;8)	182 (123;269)	125 (55;200)	140 (58;207)
Total N outputs	133 (72;190)	151 (88;223)	149 (3;273)	182 (123;269)	125 (55;200)	140 (58;207)
N balance	–19 (–116;146)	0 (–35;29)	28 (–94;170)	–4 (–82;149)	–11 (–99;184)	–55 (–131;34)

Table 2. Mean (min;max) P-balance (kg P ha<sup>-1</sup> year<sup>-1</sup>) of 6 plots, grazed (M1, M2, M5) or cut (G22, G23, V12), over 2015-2021 and 2015-2022, respectively.

	M1	M2	M5	G22	G23	V12
Cattle excreta	9 (2;12)	13 (5;20)	13 (3;23)	–	–	–
Slurry	6 (0;45)	4 (0;26)	14 (0;35)	16 (0;46)	4 (0;20)	6 (0;34)
Solid manure	–	–	–	10 (0;37)	9 (0;46)	7 (0;31)
Mineral P fertiliser	–	–	–	4 (0;29)	–	–
Total P inputs	15 (2;56)	16 (8;31)	26 (3;43)	23 (0;70)	7 (0;46)	15 (0;34)
Grazing	13 (3;21)	19 (9;28)	22 (2;49)	–	–	–
Harvested crops	2 (0;7)	1 (0;6)	2 (0;17)	25 (17;33)	21 (15;30)	20 (9;25)
Total P outputs	15 (10;21)	20 (12;28)	24 (2;49)	25 (17;33)	21 (15;30)	20 (9;25)
P balance	1 (–10;35)	–4 (–12;16)	3 (–46;41)	4 (–30;53)	–9 (–26;31)	–7 (–23;15)

The 3 grazed plots were fairly well balanced: N ( $-19$  to  $28 \text{ kg ha}^{-1} \text{ year}^{-1}$ ), P ( $-4$  to  $3 \text{ kg ha}^{-1} \text{ year}^{-1}$ ) and K ( $-27$  to  $23 \text{ kg ha}^{-1} \text{ year}^{-1}$ ). In the cut zone, the N, P, K balances were close to equilibrium for plot G22 ( $-4$ ,  $4$  and  $0 \text{ kg ha}^{-1} \text{ year}^{-1}$  respectively), but in deficit for the other 2 plots, especially G23 for K ( $-100 \text{ kg ha}^{-1} \text{ year}^{-1}$ ) and V12 for N ( $-55 \text{ kg ha}^{-1} \text{ year}^{-1}$ ). The better balance of G22 was due to greater inputs of slurry (rich in N, P and especially K) than G23 and V12, and to higher symbiotic N fixation.

Table 3. Mean (min;max) K-balance ( $\text{kg K ha}^{-1} \text{ year}^{-1}$ ) of 6 plots, grazed (M1, M2, M5) or cut (G22, G23, V12), over 2015–2021 and 2015–2022, respectively.

	M1	M2	M5	G22	G23	V12
Cattle excreta	75 (20;102)	109 (42;168)	106 (29;196)	–	–	–
Slurry	46 (0;320)	28 (0;199)	156 (0;330)	120 (0;384)	25 (0;135)	40 (0;183)
Solid manure	–	–	–	58 (0;216)	46 (0;282)	34 (0;143)
Mineral K fertiliser	–	–	–	2 (0;17)	–	–
Total K inputs	127 (22;414)	146 (68;243)	270 (31;399)	164 (2;511)	43 (2;284)	86 (2;185)
Grazing	135 (24;224)	154 (77;232)	217 (18;549)	–	–	–
Harvested crops	15 (0;63)	9 (0;62)	24 (0;166)	182 (59;269)	173 (36;321)	141 (43;195)
Total K outputs	150 (87;268)	163 (83;232)	241 (18;549)	182 (59;269)	173 (36;321)	141 (43;195)
K balance	$-27$ ( $-219$ ;220)	$-24$ ( $-114$ ;104)	23 ( $-518$ ;381)	0 ( $-267$ ;371)	$-100$ ( $-258$ ;208)	$-66$ ( $-177$ ;14)

## Conclusions

On the grazed plots, most of the inputs were provided by cattle excreta, and their management resulted in N, P, K balances close to equilibrium. Managing fertilisation with grazing thus seemed to be an interesting practice in low-input systems. The contrasting results obtained on the cut plots showed that slurry spreading is a strong lever for balancing the N, P, K balances.

## Acknowledgements

We thank N. Edouard and R. Delagarde for their invaluable advice and INRAE for funding.

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# Forage seed mixtures adapted to the number of cuts per season

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## Abstract

Dry matter (DM) yields, concentrations of metabolisable energy (ME), and crude protein (CP) of forages are all important on a dairy farm. This study examined DM yield and concentrations of ME and CP of forage mixtures of early or late maturing cultivars. Three mixtures were used in a randomised block design at Rådde, Långhem, Sweden, for three years. The field trial results were used to calculate silage production costs and diet costs at 38 kg ECM day<sup>-1</sup>. Mixtures of late maturing cultivars of timothy, red clover and white clover harvested three times resulted in similar yields of DM, ME and CP and concentrations of ME and CP as a forage mixture of earlier maturing cultivars of timothy, meadow fescue, perennial ryegrass, red clover and white clover harvested four times. When comparing number of cuts, four cuts resulted in higher concentrations of ME (+0.35 MJ (kg DM)<sup>-1</sup>) and CP (+20 g (kg DM)<sup>-1</sup>) and larger CP yields (+150 kg ha<sup>-1</sup>) compared to three cuts ( $P < 0.05$ ). Lower machinery costs resulted in lower production costs, but the lower nutritional content of the forage resulted in higher diet costs for three cuts compared to four cuts.

**Keywords:** cultivars, forage mixtures, number of cuts, nutrient composition, yield, diet cost

## Introduction

Highly digestible forage is important for high-yielding dairy cows. Therefore, dairy producers harvest the ley more frequently to increase forage digestibility and to improve forage intake by the dairy cows. Also, longer growing season due to climate change makes it possible to take more cuts of the ley per season. Forage digestibility varies between species and cultivars depending on differences in maturity at harvest (Mitchell *et al.*, 2020). The aim of this study was to investigate dry matter (DM) yield and concentrations of metabolisable energy (ME) and crude protein (CP) of forage mixtures of early or late maturing cultivars, and its effect of diet costs.

## Materials and methods

A field trial with a randomized block design and four blocks was established in 2017 at the Research farm Rådde of the Rural Economy and Agricultural Society Sjuhärad, Långhem, Sweden. The seed mixtures used were: A) 80% timothy (*Phleum pratense* L.) cv. Tryggve, 15% red clover (*Trifolium pratense*) cv. SW Ares and 5% white clover (*Trifolium repens*) cv. SW Hebe; B) 45% timothy cv. Switch, 20% meadow fescue (*Festuca pratensis*) cv. Tored, 15% perennial ryegrass (*Lolium perenne*) cv. SW Birger, 15% red clover cv. Vicky and 5% white clover cv. SW Hebe; and C) 45% timothy cv. Rakel, 35% tall fescue (*Festuca arundinacea*) cv. Swaj, 15% red clover cv. Vicky and 5% white clover cv. SW Hebe. The forage mixtures were harvested during 2018, 2019 and 2020. Forage mixture A, which contained late maturing cultivars, was harvested two or three times per year, while forage mixtures B and C were harvested three or four times per year (Table 1).

Nitrogen (N) fertilization during the harvest years was 90 kg ha<sup>-1</sup> before the first cut and 60 kg N ha<sup>-1</sup> before the second cut in all the systems. In the 3-cut system, 40 kg N ha<sup>-1</sup> was used for the third cut, while 50 kg ha<sup>-1</sup> and 30 kg ha<sup>-1</sup> were used before the third and fourth cut, respectively, in the 4-cut system. Data were analysed using Proc Glimmix (SAS ver. 9.4), where forage system was treated as a fixed

Table 1. Harvest dates for treatments (trt) averaged over years.

Seed mixture	No. cuts	Trt	Cut 1	Cut 2	Days after previous cut	Cut 3	Days after previous cut	Cut 4	Days after previous cut
			Date	Date		Date		Date	
A	2	A2	June 10	Aug 1	50				
A	3	A3	June 1	July 10	40	September 1	50		
B	3	B3	June 1	July 10	40	September 1	50		
B	4	B4	May 26	June 27	32	Aug 1	33	September 10	40
C	3	C3	June 1	July 10	40	September 1	50		
C	4	C4	May 26	June 27	32	Aug 1	33	September 10	40

effect and block as random effect. When the global  $P$ -value was significant ( $P < 0.05$ ), LS-means were compared using Tukey's test. Economic calculations were performed on forage production costs and diet formulation costs. Diets were formulated for 38 kg ECM day<sup>-1</sup>, with limits to 17% CP and 20% starch in DM. Furthermore, using feedstuff with different prices and nutrition values affects the optimizing, and diet compositions differ between silages and periods, e.g., the content of silage varied between 46% and 59% silage of DM intake.

## Results and discussion

Mixtures of late maturing cultivars of timothy, red clover and white clover harvested three times (A3) resulted in similar yields of DM, ME and CP, and similar concentrations of ME and CP, as forage mixtures of earlier maturing cultivars of timothy, meadow fescue, perennial ryegrass, red clover and white clover harvested four times (B4) and of earlier maturing cultivars of timothy, tall fescue, red clover and white clover (C4); Table 2). There were only minor differences between A3, B4 and C4 in the economic calculations for feed costs. Using forage mixtures of late maturing cultivars and forage mixtures of early maturing cultivars on a farm increases the harvest window by 5 days in the first cut; this gives different harvest dates in the regrowths, which is important for farmers with low harvest capacity or that use contractors (Table 1). When comparing number of cuts for forage mixtures B and C, four cuts resulted in higher concentrations of OMD, ME and CP and larger CP yields compared to three cuts ( $P < 0.05$ ). There was, however, a lower DM yield ha<sup>-1</sup> for four cuts compared to three cuts for forage mixture C (Table 2). The improved OMD and CP contents with four cuts compared to three cuts decreased the need for supplementary protein concentrate, which decreased the feed costs, especially at higher feed prices (Figure 1). Production costs for the forage was 0.20–0.29 SEK (kg DM)<sup>-1</sup> higher for four cuts compared to three cuts, which gives lower total costs for four cuts compared to three cuts mostly due to decreased need of protein concentrate with 1.5 and 1.9 kg<sup>-1</sup> for B4 and C4 respectively. Two cuts resulted in higher feed costs compared to three cuts for forage mixture A as the lower nutritional value of the forage required more supplementary concentrates (Table 2, Figure 1). The forage mixture C harvested three times per year (C3) had the largest yields of DM and ME (Table 2).

Table 2. Annual yields, digestibility and nutrient composition for forage mixtures A, B and C harvested 2, 3 or 4 times per year averaged over three years.

	A2	A3	B3	C3	B4	C4	SEM	P value
DM yield (kg ha <sup>-1</sup> )	10 860 <sup>d</sup>	11 890 <sup>c</sup>	12 370 <sup>b</sup>	12 990 <sup>a</sup>	11 950 <sup>bc</sup>	12 010 <sup>bc</sup>	133.8	<0.001
<i>In vitro</i> OMD (g (kg OM) <sup>-1</sup> )	817 <sup>d</sup>	868 <sup>ab</sup>	852 <sup>c</sup>	857 <sup>bc</sup>	883 <sup>a</sup>	877 <sup>a</sup>	4.0	<0.001
<i>In vivo</i> OMD (g (kg OM) <sup>-1</sup> )	715 <sup>d</sup>	761 <sup>ab</sup>	746 <sup>c</sup>	752 <sup>bc</sup>	775 <sup>a</sup>	769 <sup>a</sup>	4.0	<0.001
ME (MJ (kg DM) <sup>-1</sup> )	10.5 <sup>d</sup>	11.1 <sup>ab</sup>	10.8 <sup>c</sup>	11.0 <sup>bc</sup>	11.3 <sup>a</sup>	11.2 <sup>ab</sup>	0.1	<0.001
CP (g (kg DM) <sup>-1</sup> )	120 <sup>c</sup>	146 <sup>a</sup>	130 <sup>b</sup>	130 <sup>b</sup>	148 <sup>a</sup>	152 <sup>a</sup>	1.9	<0.001
NDF (g (kg DM) <sup>-1</sup> )	575 <sup>a</sup>	507 <sup>d</sup>	511 <sup>cd</sup>	527 <sup>b</sup>	502 <sup>d</sup>	522 <sup>bc</sup>	2.5	<0.001
CP yield (kg ha <sup>-1</sup> )	1309 <sup>d</sup>	1737 <sup>abc</sup>	1613 <sup>c</sup>	1688 <sup>bc</sup>	1777 <sup>ab</sup>	1823 <sup>a</sup>	27.3	<0.001
ME yield (MJ ha <sup>-1</sup> )	114 147 <sup>c</sup>	131 982 <sup>b</sup>	134 743 <sup>b</sup>	142 770 <sup>a</sup>	135 754 <sup>b</sup>	134 585 <sup>b</sup>	1581	<0.001

(A) 80% timothy cv. Tryggve, 15% red clover cv. SW Ares and 5% white clover cv. SW Hebe, (B) 45% timothy cv. Switch, 20% meadow fescue cv. Tored, 15% perennial ryegrass cv. SW Birger, 15% red clover cv. Vicky and 5% white clover cv. SW Hebe and (C) 45% timothy cv. Raket, 35% tall fescue cv. Swaj, 15% red clover cv. Vicky and 5% white clover cv. SW Hebe; *in vitro* organic matter digestibility (OMD) of organic matter (OM); *in vivo* OMD =  $-2.0 + 0.90 \times \text{in vitro OMD}$ ; ME, metabolisable energy; CP, crude protein; NDF, neutral detergent fibre; a–dLS-means with different superscripts within a row differ ( $P < 0.05$ ).

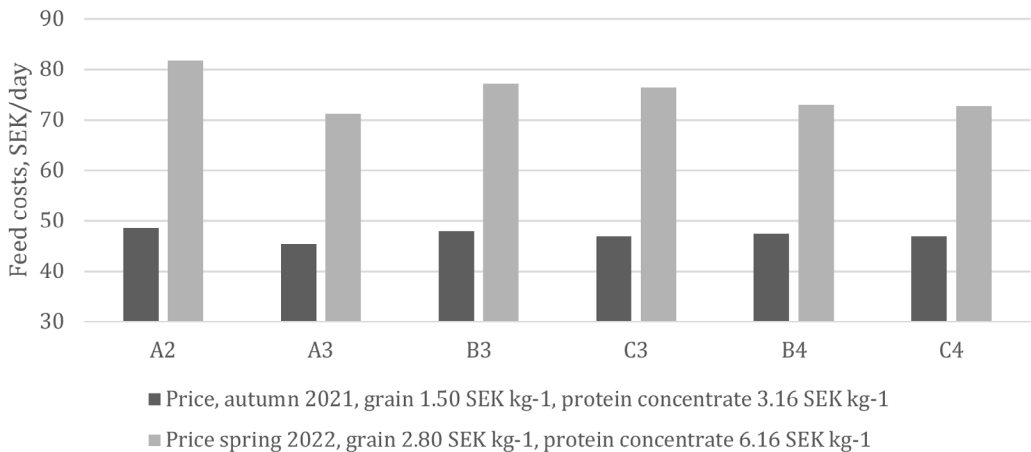


Figure 1. Feed costs at 38 kg ECM at two different price levels on grain and protein concentrate, optimized at different levels of feed stuff.

## Conclusions

Choice of forage mixtures containing cultivars of grass and legume species, which vary in growth rates, will determine the number of cuts per season on a dairy farm. Forage nutrient composition affects the need for supplementary concentrates and total feed costs, which needs to be balanced with the forage production costs, which are affected by the number of cuts.

## Acknowledgement

The trial was funded by Agroväst Dairy program.

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# Validation of behavioural-based models to estimate pasture herbage dry matter intake of dairy cows

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## Abstract

The individual pasture herbage dry matter intake (PHDMI) of dairy cows can be important for efficient and sustainable milk production. Depending on the PHDMI, supplementation can be implemented indoors to cover the animal's requirements, and pasture management can be improved. In a previous study, different behavioural-based models were developed to estimate the PHDMI of Holstein dairy cows. In our study, these models were validated with an independent dataset ( $n=72$ ) from different experiments. One observation is the 7-day average of the PHDMI of a dairy cow and the corresponding 7-day average of the predictors. Behavioural data were collected with a noseband sensor (RumiWatch). The PHDMI was measured using the n-alkane double marker technique. Based on these measurements, the weekly values of the measured PHDMI and the estimated PHDMI of the models were compared. The mean bias was generally low, ranging from  $-0.1$  to  $0.81$  kg dry matter (DM) with a standard deviation around  $1.9$  kg. However, there was little agreement for individual cows' PHDMI, with a concordance correlation coefficient of  $0.33$  at best. Therefore, it is concluded that the behavioural models predict a good herd average of daily PHDMI, but not for an individual dairy cow.

**Keywords:** behaviour, grazing, decision support, grazing management, herbage intake

## Introduction

Decision support for farmers to optimise their management is the key aim of digital technologies applied on farms. In dairy production systems, especially in confinement systems, many sensors are already applied, whereas the application of sensors for grazing animals is still limited. In particular, the main aim of estimating the individual PHDMI of dairy cows to optimise grazing management and supplementation schemes remains challenging (Tedeschi *et al.*, 2019). There are various approaches to improving estimations by farmers, which are either based on visual estimations or expertise. In the literature, there are studies using mathematical models, mainly regression models, to estimate PHDMI based on behavioural parameters or production values, such as milk yield or energy requirements (Perdana-Decker *et al.*, 2023; Rombach *et al.*, 2019; Schori *et al.*, 2020).

Estimating PHDMI values close to the real intake of dairy cows is one reason for developing a decision-support tool. Finding solutions to visualise or integrate this information into useful applications is another important element in implementing more digital solutions at the farm level. Therefore, an attempt was made to visualise the individual PHDMI of dairy cows using the Intake Analyser software, either based on a model including exclusively behavioural parameters or a model including additional production variables, such as milk lactose content and body weight. Behavioural parameters were measured using the noseband sensor RumiWatch.

The aim of this study was to validate the developed behavioural-based and production-based models, which were integrated into the Intake Analyser software. An independent reference dataset gathered under temperate pasture-based dairy production conditions was used for this purpose.

## Materials and methods

Six grazing trials on multispecies temperate pastures were conducted in western Switzerland at the organic farm Ferme Ecole de Sorens during the vegetation periods of 2018 and 2019. The results of these trials were used as a reference dataset to validate the PHDMI models. Each trial consisted of 13–14 dairy cows. A total of 38 individual dairy cows were used, including 22 Holstein and 16 Swiss Fleckvieh cows. These cows were either primi- or multiparous, with a mean body weight of  $603 \pm 45$  kg, a mean age of  $45 \pm 7$  months, and mean days in milk of  $154 \pm 32$  at the beginning of the trials. In addition to grazed herbage, the cows were fed a bait feed (pelleted dried whole maize plant), an energy-rich concentrate and a mineral mixture. On average, the cows consumed  $0.7$  kg DM of supplements  $\text{day}^{-1}$  (ranging from 0 to 3.25 kg DM). Each cow was equipped with a RumiWatch halter (Itin + Hoch GmbH, Liestal, Switzerland), a noseband sensor to record daily behavioural parameters, which was previously validated under grazing conditions (Rombach *et al.*, 2018). Finally, 72 seven-day measurements of the PHDMI (mean PHDMI =  $13.9 \pm 1.6$  kg DM  $\text{day}^{-1}$ ) were taken using the n-alkane double indicator technique (Rombach *et al.*, 2019) and used as a reference intake. Behavioural sensor data were processed using the RumiWatch Converter V.7.3.36 (Itin + Hoch, Bennwil, Switzerland) and were averaged first per day and second by the daily pasture access time to provide an average weekly dataset. As pasture access times differed between the four trials in June 2018 and 2019 and the two trials in September 2018, the one-hour summaries from 5 am to 7 am and 12 pm to 6 pm (June) or 4 pm to 6 pm (September) were excluded to calculate behavioural parameters limited to cows being on pasture. Two models estimating PHDMI were evaluated against the reference dataset. The behavioural-based model with eight predictors (Schori *et al.*, 2020), referred to as S5, and one production-focused model by Rombach *et al.* (2019), referred to as WSB3, were evaluated. Both models were integrated into the Intake Analyser software V.1.1.7.0. (Itin + Hoch) to visualise individual PHDMI based on the RumiWatch sensor data (S5) and body weight and milk lactose content with behavioural data (WSB3).

## Results and discussion

The mean bias of PHDMI and its standard deviation estimated with the S5 are  $-0.13 \pm 1.95$  kg DM  $\text{day}^{-1}$ , compared to  $0.81 \pm 1.85$  kg DM  $\text{day}^{-1}$  with the WSB3. This result demonstrated a slight underestimation of the behavioural-based model compared to the reference intake, whereas the WSB3 model, including production parameters, overestimates the PHDMI of individual cows. However, the root mean square error (RMSE) of S5 with  $1.93$  kg DM  $\text{day}^{-1}$  is comparable to  $1.94$  kg DM  $\text{day}^{-1}$  for WSB3. Also, the relative prediction error (RPE) is similar, with 14.0% for S5 and 14.4% for WSB3. These results are comparable to the development datasets of Schori *et al.* (2019), who found an RPE of around 15% for S5. Rombach *et al.* (2019) obtained an RPE of 11–13%, even though the development dataset of WSB3 included a higher level of supplementation with roughage and concentrates. However, the correlation of the estimated PHDMI at the individual cow level with the reference intake based on S5 and WSB3 was very low, with an  $R^2$  of 0.11 for S5 and 0.06 for WSB3, and a concordance correlation coefficient (CCC) of 0.33 for S5 and 0.24 for WSB3. This demonstrates a low level of precision in estimating the individual PHDMI, but considering the mean bias of both models, the estimations at the herd level were acceptable. The  $R^2$  and CCC may be improved using a more versatile dataset covering the whole range of PHDMI values, from low to high herbage intakes. In our dataset, the measured PHDMI ranged from 11.4 to 18.2 kg DM  $\text{day}^{-1}$ . Including both models in the Intake Analyser software will help farmers understand herd intake dynamics and quantify the differences of individual cows, even though the numerical values per individual cows are not accurately estimated. Furthermore, behavioural-based models perform similar to, or even better than, production-based models. This gives scope to use these models in the future, not only with lactating cows but perhaps also with non-lactating cows. The inclusion of production parameters or body weight may hamper farmers from using these intake estimation models, as those values are not

frequently recorded and sometimes not precisely measured, whereas the behavioural parameters might be easier to measure with advanced sensor technologies.

## Conclusion

The estimation models, either behavioural- or production-based, were evaluated and appear to be valid for estimating mean herd PHDMI, but seem only moderately suitable for estimating individual PHDMI. Furthermore, behavioural-based models perform similarly to or even better than production-based models. A larger validation dataset with more values in the range of 2–12 kg PHDMI per day may increase the correlation coefficient of the models.

## Acknowledgements

This study was supported by the Federal Office for Agriculture, the Foundation Sur-la-Croix, and Itin + Hoch GmbH.

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# Calibration of grass growth model Lingra-N-Plus for Flemish conditions

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## Abstract

Alongside maize, grass is the most important fodder crop in Europe. Increasingly, intensive grassland cultivation faces challenges such as fertilisation limits, scarcity of land, and climate change. In order to help tackle these, a decision support tool to optimise grassland management will be developed. Therefore, an existing open-source grass growth model, Lingra-N-Plus, is calibrated for productive grassland in Flanders. Lingra-N-Plus is a mechanistical model which simulates the interaction of climate, soil and management on grassland yield and N mineralisation parameters. The model will later be integrated into an online platform (WatchITgrow), which allows the model output to be combined with satellite imagery. The WatchITgrow platform integrates publicly available data about weather (KMI), soil texture (DOV) and elevation (DMHV II), based on field coordinates. These data are used to run field-specific simulations with the model.

**Keywords:** GrasSat, Lingra-N-Plus, satellites, WatchITgrow, yield estimation, grass growth

## Introduction

Apart from the climatic and environmental benefits of grasslands, such as carbon sequestration and low nitrate leaching to ground water, grass is an important feed for cattle. However, changing climatic conditions, strict limitations of N fertilisation and scarcity of land make it challenging to get optimal yields from grassland. A management optimisation tool for farmers can be an additional help to increase grassland yield under stringent conditions.

Grass growth in western Europe is mainly limited by drought, N-limitation or low temperature (Harpole *et al.*, 2007; Wingler *et al.*, 2016). A mechanistic model fed with climatic and soil data can estimate the severity of these limiting factors and could be useful in tailoring management practices to alleviate them. Lingra-N-Plus is such a model, which can be used for predicting the effects and interactions of different pedoclimatic conditions with management decisions, such as harvest intervals and N application rates, on both annual green leaf and total dry matter grass yields in England and Wales (Giannitsopoulos *et al.*, 2021). Weather conditions and soil properties in England and Wales are obviously different from those in Flanders, which has its impact on grass growth and yield. Hence, the GrasSat-project aims to recalibrate the grass growth model to Flemish conditions based on historical and recent data.

## Materials and methods

Data needed for calibrating the existing Lingra-N-Plus model were obtained from current and historical data originating from four regions across Flanders, each with different soil types. The amount and application date of N-fertilisation, and mowing regimes varied since they were aligned with varying weather conditions. Grass height measurements were done weekly with a rising plate meter to estimate aboveground biomass, together with sampling of fresh grass to determine crude protein content. In addition, shortly before mowing, the dry matter yield of the grassland was determined via the cut and



weigh method. Weather data were obtained from a weather station on the field, which recorded air temperature, precipitation, relative air humidity, soil moisture, soil temperature and suction tension. In addition, historic data were obtained from other projects or trials such as variety trials and fertiliser trials.

A sensitivity analysis was conducted to identify the most influential model input parameters. The morris function from the sensitivity package in R was employed for this purpose (Pujol *et al.*, 2015). The sensitivity analysis involved testing 57 potentially influential input parameters, on yield and crop N uptake as output variables. Parameters were systematically retained until achieving a cumulative sensitivity measure ( $\mu^*$ ) of 95% of the total sensitivity. This step ensured that the most influential parameters were identified for subsequent analyses. Following the sensitivity analysis, 21 parameters were retained for optimisation to enhance the overall performance and predictive accuracy of the Lingra-N-Plus model. The parameter optimisation was done using the modFit function of the FME package (Soetaert and Petzoldt, 2010), using the yield estimates and the crude protein content obtained from experiments as described above. All analyses were conducted using the R programming language.

## Results and discussion

In the sensitivity analysis 21 parameters were retained for the model optimisation. Some of these selected parameters include the LAI after cut, base temperature for grass development, minimum optimum temperature for grass development and initial total crop weight. The Lingra-N-Plus model adapted to Flemish conditions will allow farmers to make estimates at the level of their own fields.

The modified Lingra-N-Plus model is currently presented visually through Shinyapps. It displays the harvestable biomass, distinguishing between leaf and stem mass. Variation is possible based on the year, soil type, mowing regime and fertilization level. Under the traditional mowing regime, mowing is done every 6 weeks, while under the shortened interval, mowing occurs every 4 weeks. Fertilization options include 200, 250, or 300 kg N ha<sup>-1</sup>. Generally, the shortened mowing regime shows a slightly lower yield compared to the traditional mowing regime. However, the shortened mowing regime removes more nitrogen during harvest, indicating that the harvested grass has a higher crude protein content.

Figure 1 shows the harvestable biomass for 2021 under traditional mowing and 300 kg N ha<sup>-1</sup> for clay ground. As is commonly known, the yield of the grass is highest in the first cut and gradually decreases towards later cuts. The ratio of leaves to stem is higher in the first cuts, indicating that the potential quality of the grass silage will also be better because of the higher protein concentrations in leaves than in pseudo-stem and flowering stem, which contain more cell walls. The model for these management parameters gave a harvested biomass of 14.2 ton dry matter per ha. Field measurements under the same management showed a yield of 14.6 ton DM ha<sup>-1</sup>. Thus, the model currently underestimates the harvested biomass by 0.4 ton DM ha<sup>-1</sup>.

## Conclusion

The recalibrated grass growth model Lingra-N-Plus provides good estimation of grassland yields. However, further fine-tuning of the model can potentially provide even better results.

## Acknowledgements

These results originated from the VLAIO-LA project 'Optimisation of grassland exploitation by means of a decision support tool'. We would also like to thank our co-financers: Boerenbond, Vereinigte Hagel, Alzchem Trostberg, DLF, For Farmers, Jorion Philip-Seeds, LIBA, Quartes and Yara.

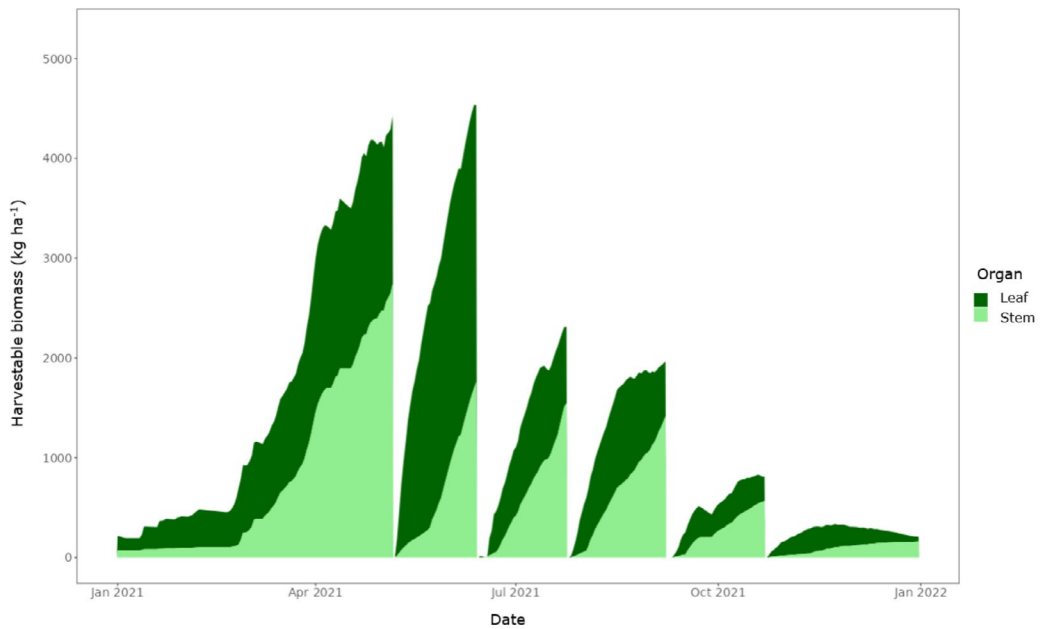


Figure 1. Visualisation of the model for harvestable biomass differentiating between leaf and stem mass per cut for 2021.

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# Tillerbox: an easy-to-use tool to efficiently assess tiller density in grassland

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## Abstract

Perennial ryegrass (*Lolium perenne* L.) is an important grass species in temperate regions due to its high forage quality and its high productivity. To maintain this productivity over time, high persistence is key. Tiller density is a trait strongly associated with persistence and is traditionally measured by manually counting tillers in swards. Even though this method is still considered state-of-the-art, it is intensive in labour and time. Therefore, a reliable high-throughput method would be highly valuable. Here we suggest such a method to assess tiller density using images taken with a self-designed ‘Tillerbox’. The ‘Tillerbox’ is a portable, waist-high box, containing a camera, enabling both standardised conditions and measurements independent of weather conditions. An image covers approx. 1800 cm<sup>2</sup>, which is at least ten times more than what is usually covered by manual counting. This makes our method especially suitable for tiller density analysis of heterogeneous swards. The images of swards of perennial ryegrass revealed significant differences in tiller density between diploid and tetraploid cultivars, which is in line with other studies. We anticipate our method to be a valuable tool for breeders to facilitate the monitoring of persistence of ryegrass in grassland.

**Keywords:** Perennial ryegrass (*Lolium perenne* L.), tiller density, ‘Tillerbox’

## Introduction

Perennial ryegrass (*Lolium perenne* L.) is an important grass species in temperate regions (Wilkins and Humphreys, 2003). Beside its high forage quality and productivity, perennial ryegrass is valued for its high persistence (Bruinenberg *et al.*, 2002). Persistence of swards is key for consistent yield and forage quality as persistence is inversely proportional to the occurrence of weed species (Wilkins and Humphreys, 2003). Tiller density is strongly associated with persistence, and it has a significant effect on the yield of a sward (Creighton *et al.*, 2012; Sills *et al.*, 1982). Therefore, tiller density provides valuable information about the potential productivity of a sward. Tiller density is traditionally measured by manually counting tillers from swards. This method is still the benchmark but intensive in time and labour (Jewiss, 1993). The number and size of plots that can be measured with the traditional method might be too low to reliably capture the variability in tiller density of an entire sward. Therefore, high-throughput methods to reliably monitor tiller density of heterogeneous swards would be highly valuable. The main goal of this study was to develop an easy-to-use tool for assessing tiller density in swards. Perennial ryegrass cultivars grown in swards under different management types were used as a proof-of-concept for the developed tool.

## Materials and methods

A field experiment was launched in 2019 at two farms in Switzerland. At both locations, three diploid and three tetraploid perennial ryegrass cultivars were managed under three management types. The cultivars were sown as single-cultivar swards and as mixtures of two cultivars. The swards were managed under regular cutting, grazing by cows and grazing simulation and defoliated five, eight, and seven to nine (according to the practice of the farmers) times per year to heights of 4, 8 and 4 cm, respectively. The plots were arranged in a randomised complete block design with three replicates, and the mixtures were grown

under regular cutting or grazing by cows. After the third defoliation event of each management type in 2023, images of the plots were taken using a self-designed box. This 60 cm high ‘Tillerbox’ consisted of an aluminium frame fixing five wood-plastic composite boards protecting the inside of the box from sunlight. A digital single-lens reflex camera (Canon EOS 250D, Canon, Tokyo, Japan) was attached to the inner top of the ‘Tillerbox’ pointing to the bottom. The camera was equipped with a ring flash (Godox ML-150II, Godox, Shenzhen, P.R. China) enabling standard light conditions. For this study, one third of the plots (one replicate per location) were selected and one image per plot was taken with the ‘Tillerbox’, which covered a sward area of approx. 1800 cm<sup>2</sup>. These full-size images were cropped to nine sub-images in a 3×3-arrangement using ‘ImageMagik’ v7.0.10 and three sub-images along the diagonal were selected for further analysis. Therefore, each ploidy-management-combination is represented by two biological replicates (one per location) and three technical replicates (three images per plot). Tillers were counted by manually annotating the images using ‘VGG Image Annotator’. The statistical analysis of the tiller densities was conducted with R v4.2.2 using RStudio v2023.6.0.421. The packages ‘dplyr’, ‘tidyr’, and ‘ggplot2’ were used for data processing and visualisation.

## Results and discussion

The ‘Tillerbox’ was successfully used to efficiently assess tiller densities of perennial ryegrass cultivars. The sampled area covered was up to twelve times larger than that of traditional methods (Vipond *et al.*, 1997). Additionally, an experienced person was able to annotate one sub-image within 3 minutes, which is similar to the time needed to just cut a sample using a scalpel (Jewiss, 1993). The results we obtained using the ‘Tillerbox’ in the field experiment are mostly in accordance with previous studies. On average, tiller densities ranged from ~ 3600 to 4600 tillers m<sup>-2</sup> (Figure 1). Diploid cultivars (approx. 4600 tillers m<sup>-2</sup>) had more tillers than tetraploid cultivars (approx. 3600 tillers m<sup>-2</sup>) across all management types, which has also been described by Griffiths *et al.* (2016) or Tubritt *et al.* (2020). Tiller densities of the mixtures (approx. 4000 tillers m<sup>-2</sup>) were closer to those of the tetraploid cultivars, which might indicate a higher persistence of tetraploid cultivars. On average, the stands under regular cutting, grazing by cows, and grazing simulation showed tiller densities ranging from ~ 4,400, 4,000 to 3,800 tillers m<sup>-2</sup>, respectively. This contradicts other studies, where tiller densities under grazing and simulated grazing were generally higher than those under regular cutting (Cashman *et al.*, 2016; Evans *et al.*, 1998). This contrasting result could be explained by the age of the experimental swards. The measurements of tiller densities were conducted in the fourth year after sowing, which is one year longer than the period during which the cultivars were tested in the process of breeding and variety testing.

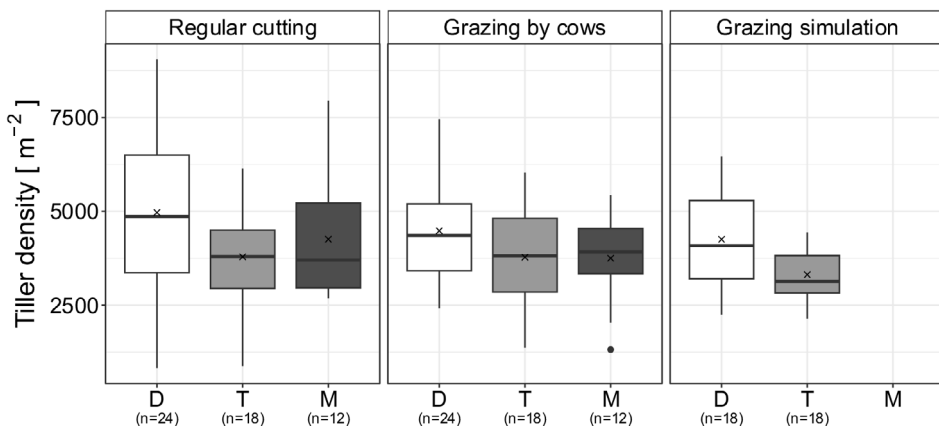


Figure 1. Tiller densities of *Lolium perenne* cultivars (D, diploid; T, tetraploid; M, diploid and tetraploid mixed) defoliated under three management types. Arithmetic means are represented by crosses.

## Conclusion

The ‘Tillerbox’ is an easy-to-use tool to reliably measure tiller densities of managed grassland. The method can cope with the limitations traditional methods have by being effective in time and labour. The number of tillers counted with our method is similar to, and the results are mostly in accordance with, the results of other studies. Additionally, image segmentation could be applied to our method to further facilitate the monitoring of persistence of perennial ryegrass in grassland.

## Acknowledgement

We thank Patrick Flütsch, Steven Yates, Simon Jäggi, and Micha Wyss for constructing the ‘Tillerbox’, giving scientific advice, and/or annotating the tillers.

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# Monitoring C<sub>3</sub> and C<sub>4</sub> species change over time in Kentucky cattle pastures

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## Abstract

Botanical composition measurements indicate ecological diversity, forage quality and palatability, and encroachment of undesirable species. The objective of this study was to determine change over time in mixed C<sub>3</sub>/C<sub>4</sub> pastures based on climate and management. Six farms with two pastures each were sampled three times per year in Kentucky USA from fall 2020 to fall 2022 using the point quadrat method. An expected shift from C<sub>3</sub> to C<sub>4</sub> species was observed during the warmer months of the growing season. The annual warm-season species crabgrass (*Digitaria sanguinalis* L. (Scop.)), increased on farms 1, 2 and 3 and decreased or remained the same on farms 4, 5 and 6. These increases were significant and coincided with drought conditions, but were most dramatic on overgrazed farms. In contrast, the perennial unpalatable warm season nimblewill (*Muhlenbergia schreberi* J.F. Gmel.) showed an increase based more on drought conditions than management. Drought conditions and less-than-ideal management strategies caused yield to decline over the study period and a shift in species abundance. In summary, change over time of C<sub>3</sub> and C<sub>4</sub> species was most affected by climate and pasture management.

**Keywords:** grasslands, botanical composition, biodiversity, management, climate, measurement methods

## Introduction

The distribution of grass species is an important part of any grassland or rangeland ecosystem. Species inventories and various botanical composition methods are used to measure species distribution. Two distinct groups of grasses exist in grassland or pasture-based systems, cool season or C<sub>3</sub> species and warm season or C<sub>4</sub> species. Determining the fluctuation in C<sub>3</sub> or C<sub>4</sub> species during the growing season can be used to assess pasture condition and level of management being implemented. In Kentucky and temperate regions of the USA, C<sub>3</sub> species dominate pasture systems and are considered the most desirable forages, whereas C<sub>4</sub> species are often considered undesirable. Previous studies on potential effect of climate change in pasture systems have shown that as temperatures increase, C<sub>4</sub> grass species will begin to encroach during the summer in many C<sub>3</sub> dominated systems (McCulley *et al.*, 2014). Catorci *et al.* (2021) showed that pasture management influences the shift from C<sub>3</sub> to C<sub>4</sub> species and can be used to mitigate the effects of climate change in pasture systems. Crabgrass, a common annual C<sub>4</sub> grass in Kentucky, has been shown to be a high-quality summer forage and despite its reputation as weedy species cattle will readily graze it (Blount *et al.*, 2013). The main limitation of crabgrass is that it dies off after frost leaving bare soil for 7 months. Properly planning and managing a forage system is key to providing a flourishing pasture ecosystem while ensuring there is always enough forage to provide animals with proper nutrition.

## Materials and methods

Six farms with two pastures each across Kentucky were monitored using a point quadrat grid method at 20 randomly selected locations in each pasture (25 points per location). The point quadrat method was chosen to take the species inventory due to its reputation as being accurate, efficient, and thoroughly tested through years of grassland and rangeland research (Bråkenhielm and Liu, 1995; Heselhurst, 1971; Nagel, 1967; Tinney *et al.*, 1937; VanKeuren, 1957). The farms were monitored from the fall of 2019 to the fall of 2022. The measurement dates included spring (April–May) to establish a baseline of cool season grass growth; summer (July–August) to determine composition change due to warm season grass (WSG)/ forbs; and fall (September–October) to contrast the senescing WSG and actively growing cool

season grasses. For the purposes of this study, drought index was the factor being compared to the changes seen in pastures over the study period. The drought index used was created by the NOAA National Integrated Drought Information System. Amounts of cool season ( $C_3$ ) and warm season grasses ( $C_4$ ) were quantified by combining the primary  $C_3$  species (tall fescue, Kentucky bluegrass, orchardgrass) and comparing to the combined  $C_4$  species (nimblewill, crabgrass, yellow foxtail, goosegrass) to simulate a growth chart. Seasons and their interactions were determined in JMP using ANOVA ( $p > 0.05$ ) and Tukey-Kramer HSD test for pairwise comparison of  $C_3$  and  $C_4$  species with time of year.

## Results and discussion

Species distribution varied from farm to farm because each of the six participating study farms were located in a different county of Kentucky, had different field histories, and were managed differently. The participating farms were all active livestock operations. Each of the farms managed their pastures differently. Management factors that were considered were grazing system, stocking rate, mowing, and weed control (Table 1). The farms with high stocking rates had less cool season species, more bare soil, and were more likely to have higher broadleaf weed presence. Farms 1, 2 and 3 were set stocked and/or had very high stocking rates, which damaged the pastures' ability to retain a high percentage of desirable grasses. Crabgrass remained at a relatively low percentage (0–15%) on farms 1, 2 and 3 until summer of 2022 when these farms experienced an increase. Most notable was the 30.9% crabgrass observed on farm 3. In contrast, farms 4, 5 and 6 had decreased amounts of crabgrass or remained the same over the study period. During the final data collection in the summer and fall of 2022, periods of moderate to severe drought affected most of Kentucky. In June 2022 farm 3 began to experience a long period of moderate to severe drought coinciding with the final sampling season. Normal fluctuations of  $C_3$  and  $C_4$  grasses had been observed at this farm until this time period. When species composition was overlaid on this county's drought monitor, the severely dry conditions offered a plausible explanation for the rapid increase in warm season grass species (Figure 1). Over all the farms in this study, the effect of weather was exacerbated by overgrazing and high stocking rates so the  $C_3$  species in these pastures had a competitive disadvantage to  $C_4$  species.

## Conclusion

In conclusion, proper management of pasture systems is needed to maintain desirable grasses ( $C_3$ ) and reduce warm season grass ( $C_4$ ) encroachment due to summer droughts. Warm season grass species thrive in warm conditions and are more photosynthetically efficient, and while they may not be eliminated, steps can be taken to ensure they do not completely take over a pasture. This research suggests that proper management may reduce the effects of our warming climate on  $C_4$  grass encroachment.

Table 1. Management practices on pastures at each participating farm in Kentucky

Farm	Rotational grazing	Stocking rate	Mowing	Weed control
1	No	High	No	No
2	No	Moderate	Yes	Yes
3	Yes	High	No	Yes
4	Yes	Low	Yes	Yes
5	Yes	Moderate	Yes	No
6	Yes	Low	Yes	Yes

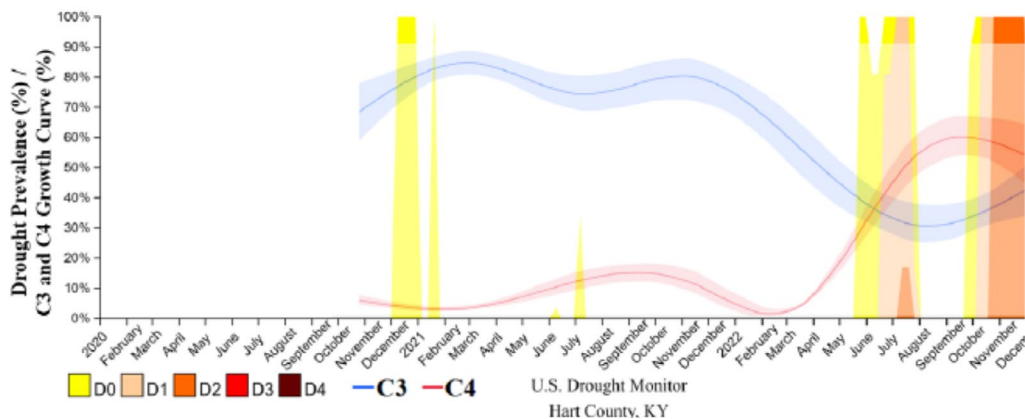


Figure 1. The US Drought Monitor for Farm 3 in Kentucky (coloured bars) overlaid with the seasonal composition change for C3 and C4 species during the growing seasons lines. Coloured band width represents 95% confidence interval (CI) of the mean botanical composition.

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# Does sowing rate affect establishment of ribwort plantain when over-sown in grass-clover swards?

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## Abstract

The inclusion of ribwort plantain (*Plantago lanceolata* L. (PL)) in grazing swards is of considerable interest to researchers as it has been shown to impact on N cycling in both dairy cows and grassland soils, where it may be responsible for a reduction in N losses from grazing systems. There is substantial interest in the establishment of PL in existing mixed swards of perennial ryegrass (*Lolium perenne* L. (PRG)) and white clover (*Trifolium repens* L. (WC)) in Ireland. This study examined the effect of PL sowing rate on the establishment of PL in existing mixed swards of PRG and WC. In April 2023, 12 plots (36 m<sup>2</sup>) were grazed to 3.5 cm by dairy cows and subsequently over-sown with PL at 2.5, 5.0 or 7.5 kg seed ha<sup>-1</sup>. The PL germinated and established in all plots; the interaction of sowing rate and grazing rotation had an effect on establishment ( $P < 0.05$ ) where PL accounted for 30, 29 and 13% of September sward dry matter in the 7.5, 5 and 2.5 kg ha<sup>-1</sup> sowing treatments, respectively, but no differences were observed between sowing rates in May.

**Keywords:** white clover, spring, compressed sward height, complementarity

## Introduction

Ribwort plantain (*Plantago lanceolata* L. (PL)) has been shown to impact N cycling at animal and soil interfaces (Bracken *et al.*, 2020; Mangwe *et al.*, 2019; Welten *et al.*, 2019). Thus, PL can play a role in reducing N losses from grazing systems where ruminants graze pastures in situ. In order for PL to impact the N cycling in grazing systems it must be present in pastures in sufficient quantity – now believed to be at least 30% of sward dry matter (DM) (Minnée *et al.*, 2020). Recent studies have reported various contents of PL in grazing swards (Baker *et al.*, 2023; Hearn *et al.*, 2024); sward PL content can also vary significantly across seasons within years (Hearn *et al.*, 2023). Where PL content is reduced to less than 30% of sward DM, remedial action is required to maintain sward ecosystem service functionality. Researchers in New Zealand and Australia have had some success with over-sowing PL into existing pastures (Bryant *et al.*, 2019; Raedts and Langworthy, 2020) while there is a history of effective establishment of WC in PRG-dominant grazing swards in Ireland (Egan *et al.*, 2022). The objective of this trial was to determine whether the sowing rate of PL could impact on the level of PL establishment in existing grazing swards of PRG and WC in Ireland within 6 months post over-sowing.

## Materials and methods

The experiment took place in 2023 at Teagasc Moorepark (52°16' N; 8°26' W) where the soil type is a free draining acid brown earth soil of sandy loam texture; soil nutrient status of the plot area in January 2023 was 11.9 and 187 mg l<sup>-1</sup> of P and K, respectively, and soil pH was 6.4. Plots from a PRG-WC grazing experiment completed in 2022 were used for the current study — the existing plots consisted of mixed swards of PRG (cv. AberGain and AberChoice) and WC (cv. Buddy) and had not been treated for weed control post establishment — the pre-experimental botanical composition of these plots were 73, 19, 0 and 8% PRG, WC, PL and weeds, respectively. The protocol for this trial was developed to incorporate best practices from previous over-sowing studies (Egan *et al.*, 2022; Raedts and Langworthy, 2020). As per Raedts and Langworthy (2020) the PL sowing rates used in the current trial were 2.5, 5 and 7.5 kg ha<sup>-1</sup>. In April 2023 all plots (36 m<sup>2</sup>) were grazed to 3.5 cm compressed sward height (CSH)

by 40 lactating dairy cows; CSH was measured using a Jenquip rising platemeter. Plots were arranged in a block design with 4 replicates of each sowing treatment within each block. Plantain (cv. Tonic) seed was applied on to the soil surface immediately post grazing using an Einböck Pneumaticstar PRO Seeder, for each of the sowing rate treatments. Compound chemical fertiliser was applied following the sowing, at a rate of 12 kg N ha<sup>-1</sup>, 12 kg P ha<sup>-1</sup> and 24 kg K ha<sup>-1</sup>.

Plots were grazed on 7 occasions post-sowing in 2023 (May – September). Plots were grazed when the average plot area pre-grazing CSH was 8 cm; all plots were grazed together over a period of 24-36 h to a target residual CSH of 4 cm. Botanical composition of each plot was measured immediately prior to each grazing event as per Hearn *et al.* (2024). Briefly, herbage samples were collected from each plot and a subsample was physically separated into all sown and unsown species; all components were dried at 90°C for 16 h to determine botanical composition on a DM basis. Compound chemical fertiliser was applied at a rate of 12 kg N ha<sup>-1</sup>, 12 kg P ha<sup>-1</sup> and 24 kg K ha<sup>-1</sup> following each grazing to ensure appropriate N was provided to a grazing sward including WC (Murray *et al.*, 2023) while not limiting the availability of soil P or K. Data analysis was conducted using SAS 9.4; a mixed model was used to estimate species sward DM content differences where sowing rate, grazing rotation and associated interactions were included as fixed effects, and replicate was included as a random effect.

## Results and discussion

The level of PL was associated with the interaction of over-sowing rate and rotation ( $P < 0.05$ ). The level of PL was similar for all sowing rates until rotation 4, but in rotations 5, 6 and 7 significant differences were clear between sowing rates (Figure 1). The magnitude of sward PL differences changed between rotation: there was 8% more PL in the 7.5 kg ha<sup>-1</sup> over-sown swards compared to the 5 kg ha<sup>-1</sup> over-sown swards in rotation 6 ( $P < 0.05$ ) but there was only a 1% difference in sward PL content between the same swards in rotation 7. The results of this trial suggest that an over-sowing rate of 5 kg ha<sup>-1</sup> of PL seed, in combination with a similar grazing protocol to that commonly used for WC establishment, is optimal as it maximises sward PL content while minimising seed costs. Further, the level of establishment achieved in these swards by the end of the experimental period was similar to the target of 30% of grazed sward DM as suggested by Minnée *et al.* (2020). The time lag between sowing and detection of significant levels of PL in swards is of some interest and provides an avenue for future investigation. Previous work

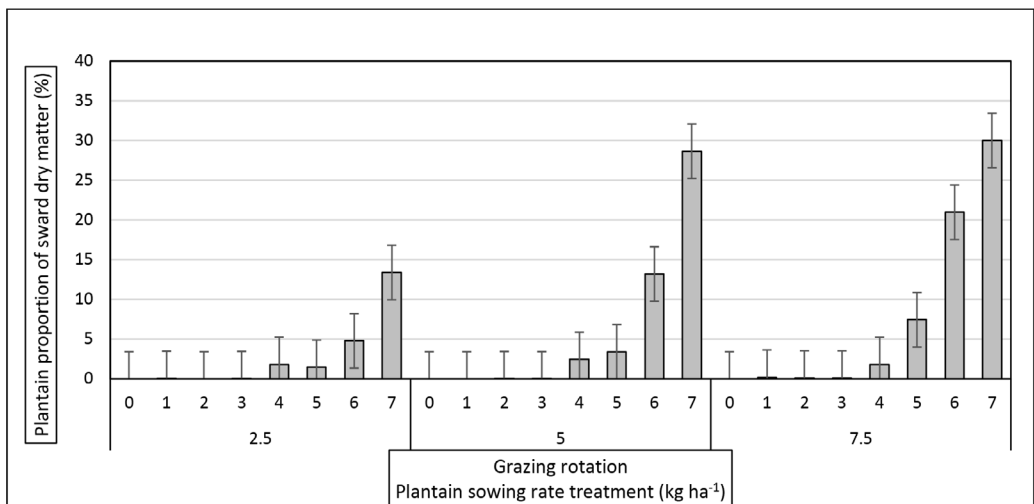


Figure 1. Botanical composition of swards at 8 time points throughout 2022; error bars represent standard error. T0, pre over-sowing.

did not report such a lag (Raedts and Langworthy, 2020) although other work reported little success in establishment of PL using over-sowing (Bryant *et al.*, 2019). Ongoing monitoring of these plots can provide data on the persistency of PL in the years post over-sowing.

## Conclusion

Over-sowing PL into existing mixed swards of PRG and WC using the protocol outlined here is an effective method of establishing PL in grazing swards. Using a sowing rate of at least 5 kg ha<sup>-1</sup> is required to establish PL at a level where it can provide sward ecosystem services in the year of spring over-sowing.

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# Assessment of perennial ryegrass variety performance on commercial farms

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## Abstract

Perennial ryegrass (*Lolium perenne* L. (PRG)) varieties are most commonly assessed for dry matter (DM) production and quality parameters in mechanically defoliated plots. More recently, efforts have been made to establish a system of assessing PRG variety performance on commercial farms in Ireland in order to gain insight as to how these varieties perform in real-world conditions. Data published from similar analyses to date has shown that PRG persists on commercial farms with little difference between varieties in DM production trends as they age. That data was limited by a small number (8) of PRG varieties used whereas the current study analysed a much larger set of varieties (20). Results of the current study show that higher performing varieties can be identified within 3 years post-sowing, where this group of varieties produced an average of 1248 kg DM kg ha<sup>-1</sup> more than the lowest performing group of varieties ( $P < 0.05$ ). This analysis provides evidence for the use of on-farm data in PRG variety evaluations.

**Keywords:** dry matter production, grazing, variety evaluation

## Introduction

In the context of grazing-focussed farms, the dry matter (DM) production potential of individual varieties of perennial ryegrass (*Lolium perenne* L. (PRG)) is of great importance as higher sward DM production allows farmers to increase stocking rates and increase overall farm profit. Previously, the evaluation of varieties on commercial farms was seen as prohibitively expensive (Grogan and Gilliland, 2011) but PastureBase Ireland, a dual functioning grassland decision support tool and national grassland database, has allowed for the development of a cost efficient protocol to assess pasture species on commercial farms (Byrne *et al.*, 2017; Hearn *et al.*, 2023). In previous on-farm analyses of long-term PRG variety performance a limited number of varieties (8) were evaluated (Hearn *et al.*, 2023), whereas more conventional plot-based assessments can evaluate >50 varieties at a time. The aim of the current study was to evaluate 20 PRG varieties on commercial Irish farms using similar methodology to that used by Hearn *et al.* (2023).

## Materials and methods

This study of PRG variety performance was based on data collected from 98 commercial Irish grassland farms between the years 2012 and 2021 inclusive; all farms were grazing-based ruminant production systems in the Republic of Ireland. All participating farmers used PastureBase Ireland (Hanrahan *et al.*, 2017) to assist with grassland management decisions. Data capture and storage were as per Hearn *et al.* (2023). Briefly, farmers built a profile for each paddock, including details such as size and sown varieties (Byrne *et al.*, 2017) in PastureBase Ireland. Subsequently, farmers entered grass cover estimates in to PastureBase Ireland for each paddock on a weekly or bi-weekly basis - a minimum of 30 cover estimates per year was required for a farm to be included in this analysis; farmers were trained on DM estimation on an ongoing basis throughout the trial period and used their preferred measurement method of either rising plate meter, cut and weigh, or visual estimation (O'Donovan *et al.*, 2002).

Swards used in the current analysis were all sown, using best practice methods (Teagasc, 2014), between 2012 and 2021 but swards were only considered for analysis up to 3 years post sowing; a minimum of 5 replicates per variety per year post sowing were required for a variety to be included. The varieties

analysed in the current study (along with their associated ploidy and heading date) were: AberChoice (D; 9 June), AberGain (T; 4 June), Astonenergy (T; 2 June), Drumbo (D; 7 June), Kintyre (T; 6 June), Majestic (D; 1 June), Twymax (T; 7 June), Tyrella (D; 4 June), Clanrye (D; 6 June), AberGreen (D; 31 May), Meiduno (T; 3 June), AberClyde (T; 25 May), AberPlentiful (T; 8 June), Aspect (T; 3 June), Kerry (D; 1 June), Solas (T; 10 June), Xenon (T; 7 June), Glenveagh (D; 1 June), Dunluce (T; 29 May) and Oakpark (D; 2 June).

The association between variety and annual DM production was estimated using linear mixed models in SAS using PROC MIXED (SAS Inst., Cary, NC, USA). A two-way interaction between farm and year was included as a random effect to account for any possible changes in farm management practices or conditions (e.g., weather) over the trial period. The fixed effects in the model were variety, year, sward age, and a two-way interaction between variety and sward age (sward age was defined as measurement year minus sowing year).

## Results and discussion

Results of this analysis showed that varietal differences in DM production can be identified within 3 years post sowing (Figure 1). The higher performing group of varieties produced, on average, 1248 kg DM ha<sup>-1</sup> more than the lowest performing group of varieties ( $P < 0.05$ ). In grazing systems this can equate to an extra grazing event per year (Wims *et al.*, 2013) in paddocks where higher performing varieties are sown compared to paddocks where lower producing varieties are sown.

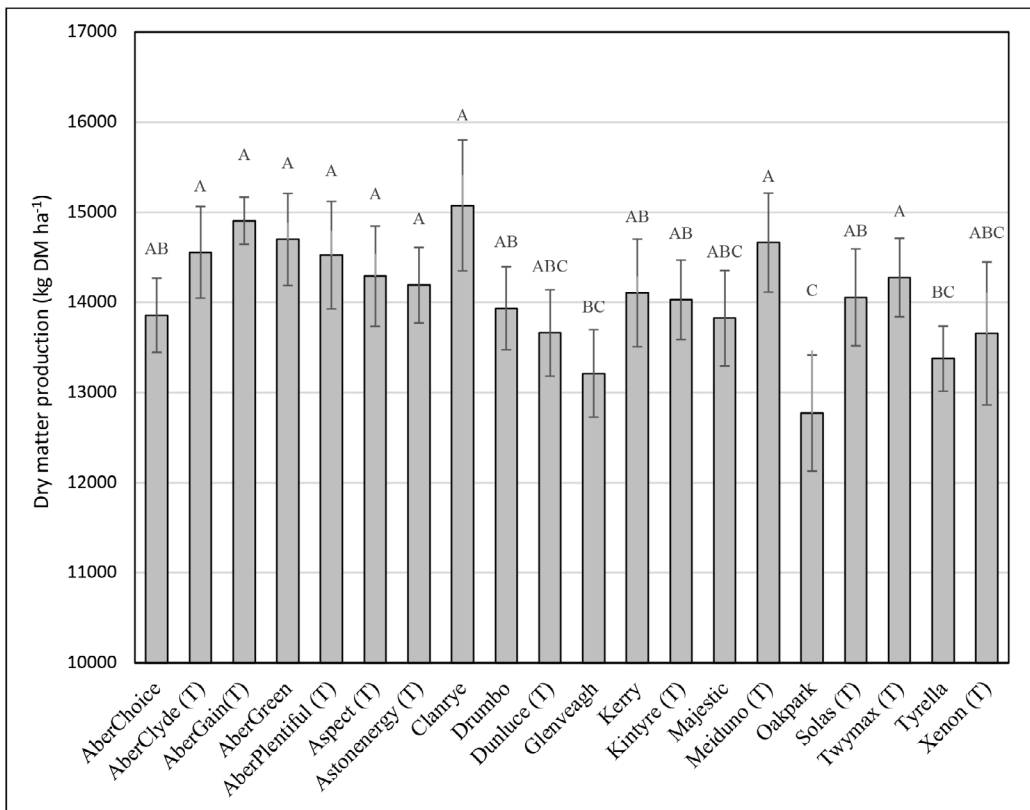


Figure 1. Annual dry matter production (kg DM ha<sup>-1</sup>) of 20 perennial ryegrass varieties over 3 years on commercial grassland farms. Varieties with different letter labels differ significantly ( $P < 0.05$ ); error bars represent standard error.

Comparing this analysis to previous, longer term work from commercial farms, shows that similar trends for individual varieties are observed (Hearn *et al.*, 2023). Those longer term trials highlight the persistence of DM production across varieties up to 7 years post-sowing, so it can be speculated that differences observed in the current data will manifest into significant differences over time – up to 12 t DM ha<sup>-1</sup> over a 10-year period in this case. As such, variety selection at reseeded becomes increasingly important and the inclusion of actual farm data in selection indexes must now be strongly considered by researchers (Lee *et al.*, 2012). These results emphasise the robustness of this methodology where up to 20 varieties can be effectively evaluated, and differences detected between individuals and groups of varieties, in real world situations on commercial grassland farms. Moving forward, this methodology can be modified for the evaluation of other pasture species using PastureBase Ireland data from commercial farms.

## Conclusion

This analysis provides further evidence for the use of on-farm data in PRG variety evaluations as it highlights the possibility of effectively assessing a large number of varieties over a relatively short time period.

## Acknowledgement

The authors would like to thank VistaMilk (16/RC/3835) for providing the financial support which facilitated the current study.

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# Robotics on leys: An Unmanned Ground Vehicle to monitor forage grasslands

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## Abstract

Remote sensing information supports farmers to adjust practices and increase the forage grassland production and efficiency of dairy and meat production. Unlike satellites, which can be affected by weather conditions, or drones, which face regulatory restrictions and payload limitations, Unmanned Ground Vehicles (UGVs) could become an efficient scouting tool to help farmers in their decision-making processes. In the scope of the “Robotics on leys” pilot project, we developed a compact agricultural robot equipped with GPS, cameras and spectrometers. An initial trial occurred in a ley field (a mixture of grass and red clover) in Northern Sweden in 2023. We successfully captured georeferenced images, videos and spectral data. The robot showed a potential to be used in field conditions to acquire spectral data and pictures, which could be used in the future to estimate botanical composition, biomass production, and winter survival. The next steps will be communicating with stakeholders and implementing more processing and improvements.

**Keywords:** forage, monitoring, UGV, remote sensing, farming practices

## Introduction

Over the last decades, remote sensing has offered relevant solutions for monitoring forage grasslands (e.g., Morel *et al.*, 2022; Näsi *et al.*, 2018). It collects spectral information from sensors coupled on different platforms, such as satellites, drones, and ground vehicles. Spectral images acquired from satellites can cover a large geographical extent at the cost of pixel size. Moreover, sensors covering the optical domain can be affected by clouds, which limits data availability. Drone-mounted sensors are another method for acquiring spectral images, providing high spatial resolution information. However, their use is constrained by weather conditions, restrictive regulations, and a limited weight-carrying capacity. Ground vehicles are not affected by the abovementioned issues; however, they are geographically limited to smaller areas and prone to physical constraints in the fields. Unmanned ground vehicles (UGVs) could become alternative scouting tools to help farmers in decision-making since they can work accurately and efficiently without or with limited human intervention. UGVs are becoming more popular in agricultural operations, such as for weed management (Utstumo *et al.*, 2018) and plant phenotyping (Underwood *et al.*, 2017). Here, we present the preliminary outcomes of a pilot project, wherein we developed a compact agricultural UGV robot for monitoring grassland fields (e.g., botanical composition, winter kill and biomass) in Northern Sweden. We explored the operational potential of the robot to collect reliable images and spectral data in field conditions. We expect this pilot project to provide the initial operational framework towards supporting technological solutions in forage management.

## Materials and methods

In the first phase of the project, we defined the requirements for the robot, considering the most suitable aspects in terms of design, platform, and navigation capacity to collect pictures and spectral data over the fields, which would be relevant for visual surveys and estimation of characteristics, such as botanical composition and biomass. More specifically, the robot was projected for use in grassland fields with pasture and leys. The robot was also designed to be low-cost, durable, reconfigurable, and lightweight,

facilitating its mobility in the field while preserving the plants. The robot was equipped with: two RGB-D cameras (Intel RealSense Depth camera D435); two spectrometers (Blue-wave miniature, Stellarnet; 350 to 1150 nm), one pointing downward to measure the ground radiance and the other upward registering the incoming irradiation (sun) and compute a reflectance factor of the field (field of view = 3°); a high-precision GPS (Here3); an Intel NUC PC to register the RGB-D data and a compact tablet to register the spectral data; an Inertial measurement unit (IMU; Cube); and batteries. The RGB-D cameras come with a classic RGB sensor, complemented with a depth sensor, which provides true-colour and distance (from the sensor to target, such as the leaves in the field) images and videos (1920×1080 frame resolution, up to 1280×720 depth resolution), which can be used as a proxy to estimate botanical composition and biomass. Spectrometers are non-imaging sensors that collect spectral information over different regions of the electromagnetic spectrum. This spectral information can be used to assess several biophysical characteristics of the vegetation. The GPS is a navigation system that supports autonomously driving the robot and collects geo-located information. The IMU controls the robot, and batteries provide the life span for moving and acquiring data. After assembling the robot and sensors, field trials were performed in a ley field at the Öjebyn Agro Park in Piteå, Sweden, in May and September 2023. Some of the characteristics evaluated were the stability, movement according to the sowing lines, payload configuration, and data collection. Based on these field trials, we could address the main challenges, needs, and future recommendations.

## Results and discussion

The robot was adaptable to mount equipment with different setups, using both the rover structure and the two size-height-angle flexible rods (Figure 1a and 1b). The prototype was teleoperated and the cameras were mounted with both downward (attached in the rod) and forward views (attached in the rover). The robot successfully operated in the field for several runs and approaches, such as: parallel and transversal to the sowing direction, and through a zig-zag moving over the field using a viewing angle downward to the ground (0°); parallel to the sowing direction using a viewing angle of 45°; and in a static position rotating the rod. The sensors registered the data with different starting points and distances inside a field of circa 0.5 ha and a speed of circa 3 m s<sup>-1</sup>. The battery life span efficiently performed without needing to be recharged. The robot did not damage the field, but depending on the canopy height, it could cause some lodging. A future improvement could be to change the rover height according to the canopy height.

Figure 1c shows an example of the RGB images collected (viewing angle of 45°), which illustrates the potential for using these images to estimate the botanical composition. The next step will be to estimate it using the already trained algorithm described in Sun *et al.* (2021). Figure 2 illustrates an example of a post-processed reflectance spectra acquired over the different locations when moving the robot transversally to the plant lines. It is general spectra, representing the field characteristics (including vegetation and soil contributions), which could be used to explore the spectral variation over the fields. However, more data collection and analysis are still necessary to estimate forage characteristics, such as quality and biomass. A future approach will be to perform field trials with the robot together with sample collection to build models and statistical analysis for estimating these characteristics using both the spectral and RGB-D data. We also plan to adapt the sensors to register data using the same computer system to improve data harmonization and geo-location. Also, there is still a need to test the impact of the robot's speed on data acquisition and estimations.

## Conclusion

The robot showed potential to be used in field conditions for continuously acquiring pictures and spectra data that can be useful to monitor forage fields. The next steps will be communicating with stakeholders and implementing the necessary processing and improvements.





Figure 1. Frontal (a) and superior (b) photos of the robot in the field trial, and an example of an image acquired by the camera with a viewing angle of 45° (c). Photos: Julianne Oliveira.

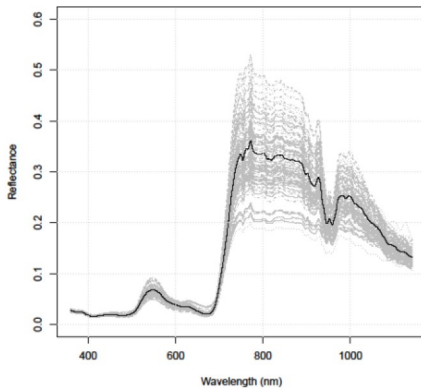


Figure 2. Reflectance spectra (grey lines) and average spectra (black line) acquired by the spectrometers over the different locations when moving the robot transversally to the plant lines.

## Acknowledgements

We thank the Regional Jordbruksforskning för Norra Sverige (RJN) and Norrlandsnavet for financially supporting this research. We also thank the Öjebyn Agro Park for the field trial.

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# Efficiency of methods of managing meadows on sloping land in Ukraine

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## Abstract

About 1.3 million hectares of arable land in the Ukraine are located on slopes prone to water and wind erosion. Grass vegetation covering slopes can reduce erosion by 2–5 times compared to tilled land by stabilising the soil. Surface improvement or radical renovation techniques exist for forage land enhancement, the latter being more potent for severely degraded land. Accelerated improvement utilises adapted species to rapidly create productive swards, minimising costs and reducing soil erosion. This study aimed to develop a rapid method to establish highly productive fodder on slopes. Trials conducted in 2018–2022 in Ukraine evaluated various perennial mixtures and establishment techniques on sloping terrain for hay use. Results showed meadow agrophytocenosis formed within the first year, most rapidly with legume mixtures. Sainfoin was most active initially, before lucerne took over in later years. Regenerative legume swards with taproots had the highest yield and crude protein. The method developed establishes productive perennial communities for effective hay management, enabling efficient forage production for the reclamation of sloping arable land in Ukraine while preventing erosion and degradation.

**Keywords:** accelerated improvement, slope land reclamation, legume mixture, forage productivity

## Introduction

Water and wind erosion affect approximately 1.3 million hectares of arable land in Ukraine (Petrychenko *et al.*, 2018). To stabilise the soil, certain areas need a reversion to meadow. Restoration of degraded slopes to grassland reduces soil loss by 2–5 times compared to arable land (Kurhak *et al.*, 2023). There are methods for superficial and radical improvement of forage areas (Holoborod'ko *et al.*, 2022). In cases of severe land degradation, radical measures are required, including complete removal of the old turf, followed by a comprehensive programme of reclamation and agro-technical measures (Veklenko *et al.*, 2020). Accelerated seeding of adaptive grasses that can quickly form a productive stand can also be used (Olifirovych *et al.*, 2021). The primary objective was the development of a method for the rapid establishment of a highly productive fodder agrophytocenosis on eroded slope land.

## Materials and methods

A study was conducted from 2018 to 2022 at the experimental plots of the Institute of Feed Research and Agriculture of Podillya of the National Academy of Agrarian Sciences of Ukraine to evaluate the impact of slope land reclamation (SLR) and perennial mixtures ( $P_{\text{mix}}$ ). The experimental plots are located in Vinnytsia district of Vinnytsia region on model slopes with a steepness of 8–10° (49°10'32" N, 28°22'54" E). The soil is grey forest podzolised with a medium degree of erosion. The study focused on three types of perennial mixtures ( $P_{\text{mix}}$ ): a three-component grass mixture ( $G_{\text{mix}}$ ), a three-component legume mixture ( $L_{\text{mix}}$ ), and a complex six-component legume-grass mixture ( $LG_{\text{mix}}$ ). The  $G_{\text{mix}}$  was composed of bromegrass (*Bromopsis inermis* (Leyss) Holub) cv. 'Vseslav', tall fescue (*Festuca arundinacea* (Hack.) V. Krecz. Et Bodr.) cv. 'Lyudmyla' and perennial ryegrass (*Lolium perenne* L.) cv. Ruslana. The sowing rate for each component was  $3.3 \times 10^6$  seeds  $\text{ha}^{-1}$ , resulting in a total sowing rate of  $1 \times 10^7$  seeds  $\text{ha}^{-1}$ .  $L_{\text{mix}}$  was composed of sainfoin (*Onobrychis arenaria* (Kit) DC.) cv. 'Smarahd' ( $3.3 \times 10^6$  seeds  $\text{ha}^{-1}$ ), lucerne (*Medicago sativa* L.) cv. 'Snyukha' ( $3.3 \times 10^6$  seeds  $\text{ha}^{-1}$ ) and bird's-foot trefoil (*Lotus corniculatus* L. p.p)

cv. 'Ajax' ( $3.4 \times 10^6$  seeds  $\text{ha}^{-1}$ ). The sowing rate for  $L_{\text{mix}}$  was  $1 \times 10^7$  seeds  $\text{ha}^{-1}$ .  $LG_{\text{mix}}$  was composed of the above species at a sowing rate of  $1.7 \times 10^6$  mln. seeds  $\text{ha}^{-1}$  for each component. All components of the studied mixtures were sown at the same ratio of their quantitative sowing rate in each  $P_{\text{mix}}$ . These mixtures were sown under surface (SI), radical (RI) and accelerated improvement (AI) slopes. The experiment consisted of nine variants, each repeated in triplicate, resulting in 27 plots. Protective strips of at least 0.6 m separated the sowing plots. The field plots were arranged in randomized blocks. The perennial mixtures were mowed for hay. The efficiency of SLR methods was evaluated in terms of  $P_{\text{mix}}$  performance, which was expressed in green mass (GM), dry matter (DM), feed units (FU), crude protein (CP), digestible protein (DP), gross energy (GE), and DP content in FU. We used generally accepted methods for determining quality indicators in feed in Ukraine (Bogdanov *et al.*, 2012). The statistical programme Statistica 10 was used for a two-way ANOVA with SLR and  $P_{\text{mix}}$  levels as factors.

## Results and discussion

Slope land reclamation by using different systems of SI and RI with sowing perennial mixtures makes it possible to create fodder agrophytocenoses already in the year of sowing. The species composition in the perennial mixtures has the biggest impact on the grassed cover in the first year following seeding. The fastest rates of sod formation were observed in the  $L_{\text{mix}}$  and  $LG_{\text{mix}}$ . Grasses have a higher tillering coefficient than legumes in the early stages of development. However, they grow more slowly, close rows and form a crop. It was found that the  $L_{\text{mix}}$  was twice as productive as the  $G_{\text{mix}}$  and  $LG_{\text{mix}}$  in terms of green mass and dry matter in the year of sowing - 23.6 and 4.55  $\text{Mg ha}^{-1}$  versus 11.4–13.4 and 2.15–2.33  $\text{Mg ha}^{-1}$ , respectively. Among the legume species, sainfoin was dominant in the grass stands in the first years; its share in the yield of the respective variants was the highest, but in the following years lucerne showed the greatest phytocenotic activity. Table 1 shows the weighted average GM and DM yields of  $P_{\text{mix}}$  according to slope land reclamation.  $L_{\text{mix}}$  based on taproot species was best in terms of productive potential. Average GM yield was 34.28–45.70  $\text{Mg ha}^{-1}$  and DM yield was 8.51–11.16  $\text{Mg ha}^{-1}$  during the season. These indicators were the highest in the experiment. Legumes grown under unfavourable water regime and mineral nutrient deficiency on sloping land showed the best ecological resistance to growth conditions. They formed cenotically closed grass stands with a minimum proportion of herbs. On average, over the four years of research, the  $P_{\text{mix}}$  variants produced by the different reclamation methods yielded 3.36–6.89  $\text{Mg ha}^{-1}$  of feed units (FU) and 0.39–1.29  $\text{Mg ha}^{-1}$  of crude protein (CP). At the same time, studies have shown the superiority of  $L_{\text{mix}}$  in these indicators, which, depending on the SLR methods, yielded 4.67–6.89  $\text{Mg FU ha}^{-1}$ , 0.91–1.29  $\text{Mg CP ha}^{-1}$  and 0.61–0.93  $\text{Mg}$  of digestible protein (DP)  $\text{ha}^{-1}$  from the forage area during 4 years. These indicators were the highest in the experiment. The highest content of DP was also in the  $L_{\text{mix}}$  variant, which included sainfoin, lucerne and bird's-foot trefoil - 132.31–134.16  $\text{g FU}^{-1}$ . The highest output of gross energy (GE) with the harvest of biomass was observed in the variants of  $L_{\text{mix}}$ , where it was 110.81–158.47  $\text{GJ ha}^{-1}$ . It was lowest on the  $G_{\text{mix}}$  with grass species, ranging from 77.39 to 84.85  $\text{GJ ha}^{-1}$ , depending on the SLR. Although the slope land reclamation methods did not show a significant statistical difference, the use of AI for the reclamation of eroded slopes is justified.

## Conclusions

The study aimed to assess the effectiveness of slope land reclamation and perennial mixtures in establishing highly productive fodder agrophytocenosis on eroded slope land. Three types of perennial mixtures were examined: a grass mixture ( $G_{\text{mix}}$ ), a legume mixture ( $L_{\text{mix}}$ ), and a complex legume-grass mixture ( $LG_{\text{mix}}$ ). Results demonstrated that the  $L_{\text{mix}}$  exhibited twice the productivity of the  $G_{\text{mix}}$  and  $LG_{\text{mix}}$  in terms of green mass (GM) and dry matter (DM) in the sowing year. Sainfoin dominated the grass stands initially, while lucerne showed greater phytocenotic activity later on. Throughout the four-year study period, the  $L_{\text{mix}}$  variants, produced using various SLR methods, yielded the highest feed units (FU), crude protein (CP), and digestible protein (DP), indicating their suitability for erosion-prone areas. Although the SLR

Table 1. Fodder productivity of perennial mixtures ( $P_{mix}$ ) depending on slope land reclamation (SLR) (average 2018–2022).

SLR (A)	$P_{mix}$ (B)	GM ( $Mg\ ha^{-1}$ )	DM ( $Mg\ ha^{-1}$ )	FU ( $Mg\ ha^{-1}$ )	CP ( $Mg\ ha^{-1}$ )	DP ( $Mg\ ha^{-1}$ )	GE ( $GJ\ ha^{-1}$ )	Content DP in the FU ( $g\ kg^{-1}$ )
SI	$G_{mix}$	22.21	5.72	3.73	0.42	0.27	84.85	71.50
	$LG_{mix}$	32.83	8.17	4.76	0.66	0.44	106.92	92.67
	$L_{mix}$	34.28	8.51	4.67	0.91	0.61	110.81	132.31
AI	$G_{mix}$	19.16	4.82	3.85	0.42	0.27	84.31	70.41
	$LG_{mix}$	44.11	10.73	6.55	0.90	0.61	146.15	94.05
	$L_{mix}$	45.70	11.16	6.89	1.29	0.93	158.47	133.83
RI	$G_{mix}$	19.85	4.97	3.36	0.39	0.24	77.39	71.57
	$LG_{mix}$	40.27	9.82	5.40	0.73	0.51	120.18	94.02
	$L_{mix}$	41.16	10.03	5.49	1.05	0.74	128.05	134.16
A		$P=0.273$	$P=0.334$	$P=0.084$	$P=0.125$	$P=0.148$	$P=0.110$	$P=0.310$
	B	$P=0.008$	$P=0.009$	$P=0.019$	$P=0.003$	$P=0.004$	$P=0.017$	$P<0.001$
A	B	$P=0.073$	$P=0.711$	$P=0.601$	$P=0.392$	$P=0.127$	$P=0.721$	$P=0.999$

SLR, slope land reclamation. SI, surface improvement. RI, radical improvement. AI, accelerated improvement;  $P_{mix}$ , perennial mixtures;  $G_{mix}$ , grass mixture;  $L_{mix}$ , legume mixture;  $LG_{mix}$ , legume-grass mixture; FU, feed units; CP, crude protein; DP, digestible protein; GE, gross energy.

methods did not exhibit significant statistical differences, the use of accelerated improvement (AI) for eroded slope reclamation was deemed justified.

## Acknowledgement

We thank the National Academy of Agrarian Sciences of Ukraine for financial support.

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# Introducing goats to virtual fencing

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## Abstract

Virtual fencing (VF) is a GPS-enabled fencing technology that replaces the visual cue of a physical fence with an acoustic signal and a potential subsequent electric impulse and therefore facilitates the grazing of livestock in areas that are environmentally sensitive, protected, or difficult to access. So far, research and commercial trade of VF have concentrated mainly on cattle, but the Norwegian system Nofence® is also available for sheep and goats. In this study, two groups of ten adult 'Blobe' goats wearing 'Nofence' collars with offspring were successively introduced to VF. The twelve-day schedule was divided into three phases, in which the VF-line was marked by a physical fence for the first two days, followed by a phase with a VF only and a VF shift on day eight to enlarge the accessible grazing area. An evaluation of the ratio of acoustic signals without a following electric impulse to all acoustic signals showed a significant difference between the phases. The ratio was significantly higher in the second and third phase than in the first one, which suggests that goats learned to respond to the acoustic signal alone, thus avoiding the electric pulse. However, further research is needed to support these findings.

**Keywords:** associative learning, grazing, goats, Nofence, virtual fencing

## Introduction

Virtual fencing (VF) is a modern fencing technology, which replaces the visual cue of a physical fence (PF) with an acoustic signal and if the signal is disregarded a subsequent electric impulse. The animals are required to wear GPS-enabled VF collars, which emit the acoustic signal when an animal approaches the VF line. When the animal keeps moving forward, an electric impulse (1.5 kV, 0.1 J, 0.5 s duration) gets sent out by the collar. The successful application of VF requires the animals to associate the acoustic and electric signals so that the electric impulse can be avoided by responding appropriately to the acoustic signal (Lee *et al.*, 2009). Otherwise, when the animals keep getting electric impulses, chronic stress can occur with a negative impact on the animals' welfare (Lee *et al.*, 2018). In this study, we investigated whether 'Blobe' goats were capable of this associative learning process, while undergoing a VF training protocol after Hamidi *et al.* (2021). We used the ratio of acoustic signals without a following electric impulse to all acoustic signals and its development over the experimental time as a learning indicator.

## Material and methods

The study was conducted from May until June 2023 on a farm in Längenfeld, Tyrol, Austria. In two subsequent periods (two time replicates: 11 May–22 May and 25 May–5 June) two groups of ten adult 'Blobe' goats (with offspring) each were observed for four hours per day while grazing in different paddocks. The VF animals wore activated Nofence® collars (Nofence, Batnfjordsøra, Norway) and had a VF line at one side of the paddock, which divided the pasture into an inclusion and exclusion zone. The PF goats had a standard electric fence instead of a virtual one. After the first period, the groups were moved to new paddocks and swapped the fencing system, so that every goat had experienced the virtual fence at the end of the study. The twelve-day-schedule (Figure 1) of each VF group was split into three phases, which included a visual support of the VF line (posts and wire on day one; posts only on

day two), the virtual fence on its own (days three until seven), and an enlarged grazing area after a fence shift on day eight. The PF group also had their fence shifted on day eight. The VF collars recorded the daily number of acoustic and electric signals for each day. Because of technical problems, the data for one collar in period one was missing. Therefore, only nineteen animals were included in the statistical analysis. The ratio of acoustic signals without a following electric impulse to all acoustic signals (including electric impulses) was calculated for each animal and day. When an animal did not receive an acoustic signal, the ratio was zero ( $n=12$ ) and had to be removed from the calculation (this could cause a bias as those animals could react properly but undetected to other animals' signals without receiving a signal themselves). According to the VF schedule, the three phases were classified in: Training (days 1 and 2), pre-shifting (days 3–7), and post-shifting (days 8–12). Linear mixed effect models were used with phase and group as fixed effects and animal ID as a random effect to evaluate the effect of phase and group on the ratio. The trial was approved by the Department of Agricultural Education and Agricultural Law of the Office of the Provincial Government Tyrol (LW\_LR-4022/210-2021).

## Results and discussion

Phase had a significant effect on the ratio ( $P=0.0006$ ). When investigating for significant differences between the phases, 'training' proved to be significantly different from 'pre-' and 'post-shifting' ( $P<0.001$ ). In the phase of training, the mean ratio was lower ( $0.596 \pm 0.0433$  mean  $\pm$  SE) than later in the experiment, which shows that at the beginning of the experiment the goats were not able to respond properly to the acoustic signals and still received electric impulses. In the pre- and post-shifting phases, the ratio was higher ( $0.844 \pm 0.0315$  and  $0.894 \pm 0.0286$  mean  $\pm$  SE, respectively), which suggests that the animals seem to have learned the association between the signals and avoided the electric impulses. However, individual variation in learning behaviour and factors like location learning need to be considered and investigated

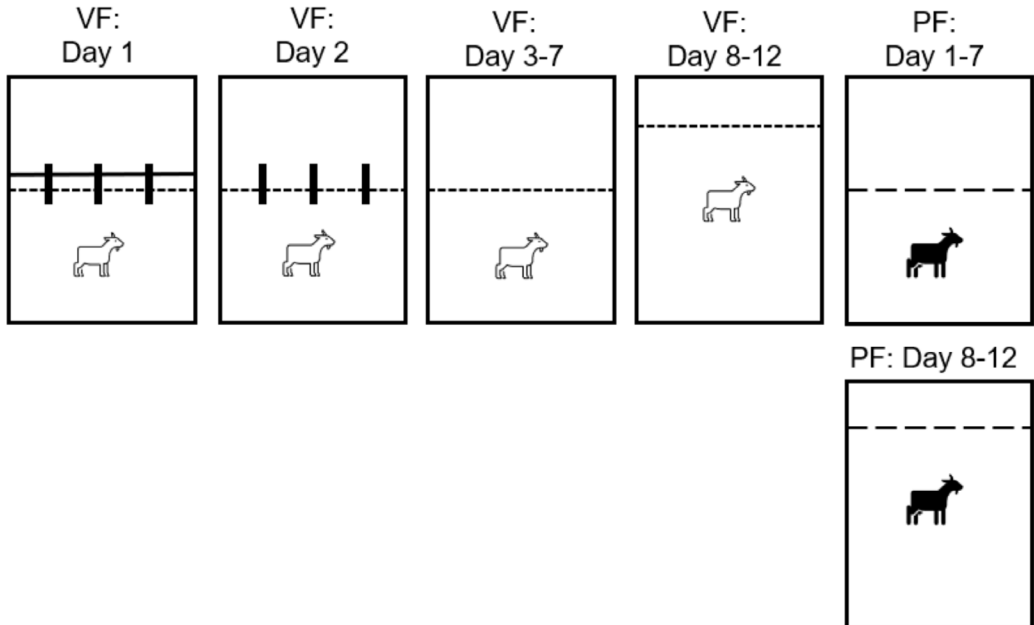


Figure 1. (Left) The 12-day-schedule of the virtual fence group: On day 1 posts and wire of a physical fence plus virtual fence (dotted line), day 2 with posts and virtual fence (both days together as 'training' phase), days 3–7 virtual fence only ('pre-shifting' phase) and fence shift to enlarge the grazing area after day 8 ('post-shifting' phase). (Right) The 12-day-schedule of the physical fence group: Physical fence (dashed line) with fence shift on day 8.

in further studies. So far, there are only two other studies that have analysed the effect of VF on goats. Fay *et al.* (1989) introduced goats to a shock-collar system in a training pen, where it took them about thirty minutes to associate the acoustic and electric signals as afterwards no more electric impulses could be observed. Eftang *et al.* (2022) used a success ratio similar to ours and showed that it increased with time. Especially, goats that were naïve to VF presented a sharp rise in their success ratio after day two, which is comparable to our results. The authors concluded that goats were able to associate the signals and therefore avoid receiving electric impulses (Eftang *et al.*, 2022).

## Conclusions

In this study, we investigated the ratio of acoustic signals without following an electric impulse to all acoustic signals of goats wearing VF collars. A significant increase in the ratio after the first two days of training suggests that the goats were able to associate the VF signals and, therefore, actively avoid the electric impulse. This learning process is necessary to ensure the successful application of VF without compromising animal welfare. Further analyses are necessary to confirm VF as a learnable fencing technology for goats and to investigate interaction effects with landscape and goat breeds.

## Acknowledgements

Thanks to Ferdinand Haid, who provided his farm and animals for the experiment. This study was funded by the Federal Ministry of Agriculture, Forestry, Regions, and Water Management of Austria. Lisa Wilms was supported by the Federal Ministry of Education and Research under grant number 031B0734A as part of the project 'GreenGrass'.

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# LIFE *Nardus* & *Limosa*: The challenge of harmonizing *Nardus* grassland restoration with meadow bird populations

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## Abstract

Species-rich *Nardus* grasslands in Western Europe have suffered extensive loss due to intensified land-use. Elevated soil phosphorus (P) concentrations particularly impede the re-establishment of typical *Nardus* grassland plant species. Restoration strategies primarily focused on P-reduction are often not compatible with the protection of local meadow bird populations. For instance, removal of P through biomass harvesting, either by traditional mowing (without fertilization) or the more intensive P-mining technique (with fertilization of nitrogen, N, and potassium, K) can inadvertently damage nests. Here, we present the first results of a field experiment (3 years) featuring four sites with each four plots ( $n=16$ ) where we evaluated P-removal by (i) postponed mowing without fertilization, (ii) intensive P-mining with an early fertilization-and cutting-date and (iii) two meadow bird-friendly P-mining variants with a postponed cutting-and fertilization-date and with less NK-fertilizer input. Phosphorus removal with intensive P-mining was significantly higher than with the other treatments. The two bird-friendly P-mining variants did not hasten up P-removal compared to the unfertilized mowing, at least after three years of measurement. It remains unclear whether both *Nardus* grassland restoration and meadow bird-conservation can be combined at the same time in the same field.

**Keywords:** ecological restoration, biodiversity, P-mining, high nature value grasslands

## Introduction

The United Nations declared 2021–2030 as the decade of Ecosystem Restoration, acknowledging the importance of biodiverse ecosystems with high resilience against future environmental changes. In Flanders (Belgium), the conservation and restoration of *Nardus* grasslands is a priority under the European Habitat Directive. Restoration of these nutrient-poor *Nardus* grasslands on land with a fertilization history implies large abiotic and biotic changes (Wasof *et al.*, 2019). Land-use intensification has caused the plant community to shift from the slow-growth strategy species in *Nardus* grasslands to fast-growth strategy species in eutrophic grasslands where nutrient cycling is fast and plant species richness is low. Increased soil-P-concentrations pose a key challenge for the restoration of *Nardus* grasslands (Goossens *et al.*, 2021). Phosphorus can be extracted from the soil with plant biomass, i.e. through phytomining (by mowing or P-mining). Mowing implies the unfertilized cutting and removing of hay two or three times a year, and P-mining implies yield maximization by adding growth-limiting nutrients other than P (i.e. N and K). With P-mining, the restoration time is considerably shorter (Goossens *et al.*, 2021). Phytomining may, however, negatively impact meadow birds, particularly in areas hosting remnant populations. In this context, the European LIFE *Nardus* & *Limosa* project aims to address the dual objectives of *Nardus* grassland restoration and meadow bird conservation. Recognizing the challenges posed by conventional restoration methods, the project explores two new meadow bird-friendly variants of the P-mining technique.

## Materials and methods

From an extensive soil database ( $n=434$ ), four sites were selected in the nature reserve ‘Turnhoutse Vennengebied’ (Belgium) with the selection criteria: (i) permanent grassland, (ii) low to medium



bioavailable P-concentrations (25–40 mg Olsen-P kg<sup>-1</sup>), (iii) moist to wet soil conditions (drainage class 'd' or 'e' in the Belgian soil map), and, (iv) similar vegetation (i.e. grass-dominated phase 2 according to Schippers *et al.*, 2023). The four sites are regarded as real-world replications. The grasslands were unfertilized for at least 15 years but received relatively high amounts of atmospheric N (20–30 kg N ha<sup>-1</sup> year<sup>-1</sup>). In June 2021, at each site, four 3 m by 3 m plots separated by a 1 m buffer were installed. Each plot received a different treatment. Three treatments had a delayed first cutting date (July), compatible with breeding meadow birds, and a second cutting date in October: mowing 0N0K bird-friendly; P-mining 80N165K bird-friendly; and P-mining 120N125K bird-friendly. A fourth treatment was the P-mining 170N180K intensive, which had four cuttings in May, June, August and October. Fertilization of the P-mining treatments was performed with ammonium nitrate (27% N) and patentkali (30% K<sub>2</sub>O). For 3 consecutive years (2021–2023), biomass was sampled in 0.43 cm by 0.43 cm quadrats with grass clippers, dried at 60°C for at least 48 h, weighed and prepared for analyses of P, N and K content. To investigate the effects of the treatments on P-removal, we fitted linear mixed models with the function lmer (package lme4, site as a random effect) in R. We tested the variance between the groups with the anova function (stats package). Post-hoc pairwise comparisons were performed with the function glht (multcomp package, with Tukey adjustment).

## Results and discussion

In 2021 we observed an unexpectedly large site effect ( $P < 0.01$ ; data not shown) but no treatment effect on the yearly P-removal. This may have been because we left out the fertilization in March 2021 and harvest in May 2021, as the experiment started in June 2021. Overall, the first cut, in July 2021 (before the first fertilization), showed moderate to severe limitation by nitrogen (35–77% Nitrogen Nutrition Index, NNI, *sensu* Duru and Thélier-Huché, 1997) and severe to very severe limitation by potassium (15–60% Potassium Nutrition Index, KNI, *sensu* Duru and Thélier-Huché, 1997). From the cut in September 2021, significant differences in P-removal were found between the treatments ( $P < 0.001$ ; data not shown), with P-mining 170N180K intensive removing most P. In the following cuts of P-mining 170N180K intensive, growth limitation by N and K was less severe due to fertilization (NNI 52–101% and KNI 54–95% per cut). In 2022 and 2023, the site effect was absent, and the treatments became more distinct (Fig. 1). On average, yearly P-removal was higher than had been expected, given the relatively low bioavailable soil P-concentrations (e.g. 20–30 kg P-removal ha<sup>-1</sup> year<sup>-1</sup> in Liebisch *et al.* (2013) in P-rich soils), even in the unfertilized plots. This may be due to the wet growing conditions where soil-P is quickly replenished due to redox reactions (Van Rotterdam *et al.*, 2021). After three years, the field experiment revealed significant differences in cumulative P-removal among the treatments, with P-mining 170N180K intensive being 26% more effective (80±7 kg P ha<sup>-1</sup>) than the other treatments (64±6 kg P ha<sup>-1</sup>;  $P < 0.001$ ; Figure 1). With this, P-mining 170N180K intensive removed between 17% and 39% of the theoretical residual P-stock from the top 20 cm soil layer, depending on the site. The other treatments removed between 13% and 30% of the theoretical residual P-stock. We expect that P-removal will slow down due to decreasing bioavailable P-concentrations and that P-removal with the treatments may converge (Schelfhout *et al.*, 2019). Both P-removal and soil-P-concentrations will be further monitored in the future, as these initial patterns may change over the long term. The effective impact of bird-friendly P-mining on breeding meadow birds as well as the economical feasibility should be investigated further.

## Conclusion

During the first three years, the two bird-friendly P-mining variants did not remove more P than unfertilized mowing, while intensive P-mining was significantly better at removing P, leading to a 26% increase in cumulative P-removal. However, intensive P-mining is incompatible with breeding meadow birds. Therefore, it remains unclear whether both *Nardus* grassland restoration and meadow bird-conservation can be combined at the same time in the same field. Harmonization may imply spatially distinguished management choices.

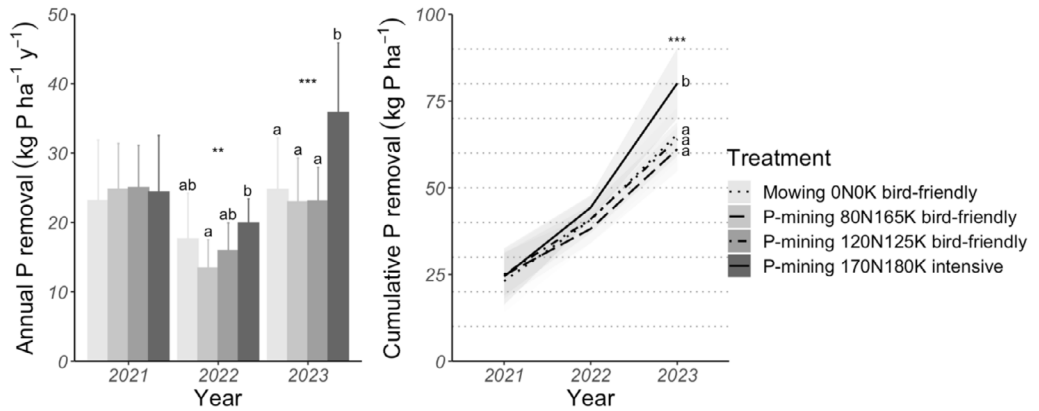


Figure 1. Mean±SD annual (left) and cumulative (right) phosphorus removal over the four sites from July 2021 until October 2023. Significant differences between treatment levels are indicated by asterisks, \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , and by letters (mixed model with site as a random effect).

## Acknowledgements

This research was supported by LIFE *Nardus & Limosa* LIFE18 NAT/BE/000576. We further thank Robbe De Beelde, Ellen De Vrieze, Luc Willems, Greet De Bruyn and students helping with fieldwork.

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# Asking grazing cattle: using virtual fencing collars to make forage availability dynamics visible

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## Abstract

Virtual fencing (VF) promises a future with more grazing and more sophisticated grazing management due to simplified fencing. However, identifying the need to shift the virtual boundaries can be challenging. It was assumed that active time of grazing livestock is spatially linked to decreasing forage availability. Therefore, relevant information to support sward monitoring and grazing management should be provided by using GPS-data of VF-collars. In this study, four groups of eight heifers (fitted with VF-collars) were assigned to four pastures each divided into four paddocks for rotational grazing. Unmanned-aerial-vehicle (UAV) imagery was applied pre- and post-grazing to determine changes in forage availability. Active time of heifers was deduced from detecting the time for lying using the VF-collars that use a battery-saving feature that transmits the same GPS-position. The minute-wise validation of lying time with observational data of lying proved promising (92% precision (confusion matrix)). We combined the active time obtained from the VF-collars and the UAV-based changes in forage availability on a polygon-grid (2.5m × 2.5m) and calculated a random forest model ( $r^2:0.43$ ) to verify the relationship. The spatial patterns of both data sources are almost identical, highlighting the potential of animal spent active time to estimate changes in forage availability.

**Keywords:** smart farming technology, Fleckvieh heifers, grazing, UAV, remote sensing

## Introduction

Optimal pasture utilisation with standard fencing technologies is difficult (Stevens *et al.*, 2021), as temporal and spatial control of grazing animals is challenging. Virtual fencing (VF) provides new options to improve the efficiency and flexibility of grazing systems (Umstatter 2011) and this should enable an optimal utilisation of the available grazing area. A remaining question is how to determine when to move the virtual boundaries. Unmanned-aerial-vehicle (UAV)-based remote sensing already helps to measure pasture depletion during grazing (Alvarez-Hess *et al.*, 2021). Using animal monitoring data provided by the VF-collars to detect changes in forage availability via pasture utilisation analyses (summed active time of grazing livestock per polygon-grid-cell) should be useful especially for large and remote areas and could possibly reduce the frequency of UAV-flights. The main objective of this work is to investigate (i) the suitability of GPS data from VF-collars for animal monitoring and (ii) the combination of active time (excluding lying) and UAV-based changes in forage availability on a polygon-grid.

## Materials and methods

The present study was conducted at the experimental farm of the University of Göttingen in Rellichhausen, Solling Uplands, Lower Saxony, Germany during August and September 2021 and has been part of a larger rotational grazing trial. In total, 32 Fleckvieh heifers were fitted with VF-collars (Nofence®, Batnfjordsøra, Norway) and divided into four groups of eight animals each. Groups were similar in age and liveweight (for details see Grinnell *et al.*, 2024, these proceedings). In two groups, the fencing function was activated, while in two (physically fenced) groups only the GPS function of the VF-collars was used, which provides

minute-wise data. Each group was allocated an area of 2 ha, which was divided into four paddocks of equal size. The grazing period per paddock was 3-4 days. The time on the first paddock and the days of paddock change were considered to be the animals' acclimatisation period to the new environment and were not included in the data analysis. In total, data for three days on three paddocks were analysed. UAV overflights with a Phantom 4 Obsidian drone were carried out before and after each grazing period to determine changes in forage availability (calculation of the difference pre-grazing and post-grazing). In addition, animal observations (continuously observed main cattle behaviour) were carried out for 2 hours on 6 days during the trial. For each minute, the value lying or not lying was extracted from the observation data. Based on this data, the lying time recorded by the automatic battery saving function of the VF-collars was validated using a confusion matrix (to define the performance of the algorithm) and then removed as time not associated with grazing. The remaining active time of all animals per paddock was spatially assigned to a polygon-grid (2.5 m×2.5 m). The same polygon-grid was used to calculate the UAV-based changes in forage availability. Consequently, one polygon-grid cell refers to one forage availability value and one value of active time spent by all cattle within this polygon-grid cell. A random forest regression was used to analyse the relationship between UAV-based changes in forage availability and cattle spent active time on a grid base. The trial was approved under Ref. No. 33.19-42502-04-20/3388).

## Results and discussion

The results of the confusion Matrix revealed a precision of 92%, a recall of 78% and an accuracy of 91% (5270 data points in total) for the validation of VF-collar-detected lying time using observed lying time. These results are higher than comparable studies using conventional GPS collars, which are known to have difficulties in distinguishing between standing and lying (Ungar *et al.*, 2011). The evaluation of the random forest model with the test data yielded a rmse of 5.59 min and  $r^2$  of 0.43. The standard deviations of rmse and  $r^2$  were 0.19 min and 0.30, respectively. Additionally, visual comparison of the active time per grid cell with the UAV-based changes in forage availability highlighted similar patterns (Figure 1), which reveals the close relationship between grazing animal and sward (Opitz von Boberfeld, 1994). The UAV data are necessary to record the actual condition of the pasture as a starting point. The development of changes in forage availability can then be deduced, *ceteris paribus*, from the active time data provided by the VF-collars. This demonstrates the potential of using VF-collars for more than fencing purposes. The individual polygon-grid cell, which is used for pasture utilisation analyses and continuous animal monitoring (no data presented here) can form the basis for fine-scaled, sustainable pasture and animal management, independent of ground-based fencing systems. The fencing function of the VF-collars could be used to respond to the pasture utilisation analyses, for example by fencing out overgrazed areas or generally, giving access to new grazing areas.

One observer per group continuously recorded the behaviour of each of the four heifers per group. The two groups (n=24 cattle in total) were grazed in adjacent paddocks on permanent grassland during each successive period. One GPS-coordinated VF-line separated the pasture of the VF-group into an exclusion and an inclusion zone. The control group had a physical fencing (PF)-line dividing it into the exclusion and inclusion zone (see Figure 1). The data needed for the research question was retrieved from the observational data (electrical impulse from the physical fence) and the Nofence collar report (electrical impulse from the VF collar). In total, n=156 electrical impulses from the VF collars and n=93 electrical impulses from the physical fence were recorded across periods and fencing system treatments. These data points were included in one generalized least squares model that estimated the effect of impulse type on the time until grazing was continued after having received one of the electrical impulse classes during the observation periods. Data was log-transformed before analysis in order to improve the normality of residuals.

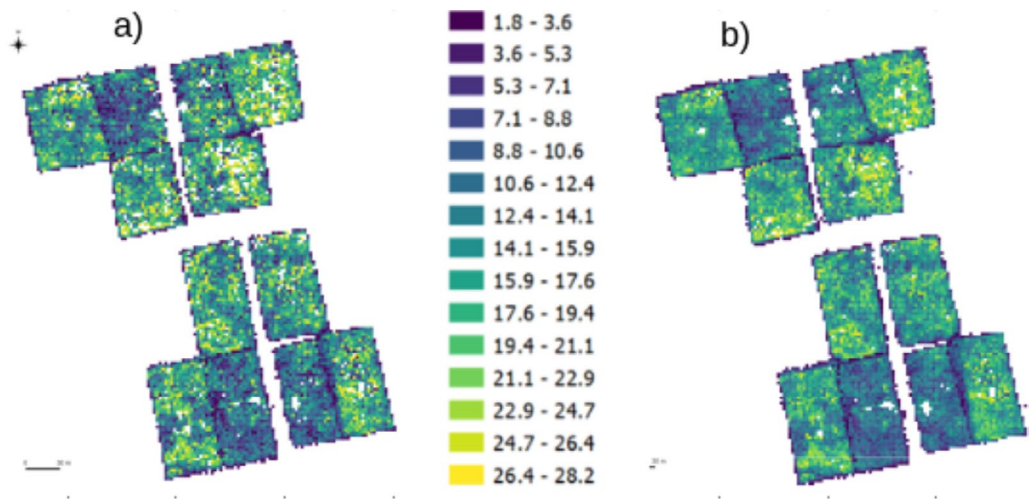


Figure 1. (a) Active time (min) of all animals per grid cell (2.5 m×2.5 m) from the GPS data of the virtual fence collars. (b) UAV based prediction of the active time per grid cell.

## Conclusions

UAV data of forage availability is needed to create a baseline of herbage availability. From this baseline, pasture utilisation data from VF-collars on a polygon-grid can make forage availability dynamics visible. Using VF-collar data for animal monitoring, pasture utilisation analyses and fencing, i.e. shifting virtual boundaries, illustrates the potential of VF to increase the efficiency and sustainability of future grazing systems.

## Acknowledgements

We are grateful to all students and staff for supporting the field experiment. This study was supported by the Federal Ministry of Education and Research under grant number (031B0734A) as part of the project “GreenGrass”.

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# Does sward type affect lamb performance and the eating quality of the meat produced?

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## Abstract

Improvements in animal performance when grazing diverse swards has resulted in increased farmer interest. Potential impacts for the eating quality of the meat produced must be evaluated, ensuring they align with consumer preferences. This study investigated the impact of perennial ryegrass (*Lolium perenne* L.) (PRG) and four binary sward types, namely; PRG+white clover (*Trifolium repens* L.) (PRG+WC), PRG+red clover (*Trifolium pratense* L.; PRG+RC), PRG+chicory (*Cichorium intybus* L.) (PRG+Chic) and PRG+plantain (*Plantago lanceolata* L.) (PRG+Plan), on lamb performance and meat-eating quality. Post-weaning, lamb live-weight (LW) was assessed fortnightly. Lambs were slaughtered targeting a carcass weight of 20 kg. Meat samples were obtained from the *longissimus thoracis et lumborum* muscle (LTL). Trained and consumer panels conducted sensory analysis. Results show that companion forage inclusion increased average daily gain ( $P<0.01$ ) by up to 74 g day<sup>-1</sup> (PRG+RC), reducing days to slaughter by 41 days ( $P<0.01$ ), on average. Meat from PRG+Plan lambs had reduced tenderness and flavour according to both panels ( $P<0.01$ ). Consumers generally scored meat from PRG+Plan lambs lower for overall liking ( $P<0.01$ ).

**Keywords:** trained sensory panel, consumer sensory panel, herbs, legumes, average daily gain

## Introduction

As the demand for low carbon products increases, extensive lamb production systems prioritising animal welfare and sustainability are gaining interest. Production of red meat is consistently under scrutiny as producers and consumers become more aware of the environmental and ethical implications of their food choices. Pasture-based feeding systems are economically advantageous to producers, yield a product perceived as healthier by consumers and present an opportunity for market capitalization. The nutritional profile (López-Andrés *et al.*, 2014) and taste attributes of ruminant meat can be influenced by the ingested feed. Mid-season lambing flocks account for approximately 80% of the Irish national sheep flock and aim to operate as low-input grass based systems. However, post-weaning lamb live-weight (LW) gains are frequently below target on perennial ryegrass as its nutritional value decreases once the plant reaches maturity, reducing sward digestibility and hindering animal performance. This results in lambs requiring concentrate supplementation to reach their desired sale weight. Increasing the botanical diversity of pastures can boost sward quality, support superior animal performance, in turn, reducing the reliance on concentrate supplementation (McGrane *et al.*, 2022). This study aimed to determine the effect of sward type on lamb performance and the sensory perception of lamb meat.

## Materials and methods

The study was performed at Teagasc, Athenry, Co. Galway, Ireland, during the 2022 production year. A complete randomised block design was used to assess the effect of sward type on lamb performance and meat-eating quality. Sensory analysis of meat samples was carried out by trained and consumer panels. Five sward types were investigated: perennial ryegrass (*Lolium perenne* L.) (PRG), PRG+white clover (*Trifolium repens* L.) (PRG+WC), PRG+red clover (*Trifolium pratense* L.) (PRG+RC), PRG+chicory (*Cichorium intybus* L.) (PRG+Chic) and PRG+plantain (*Plantago lanceolata* L.) (PRG+Plan). Five

farmlets were established, stocked at 11.8 ewes ha<sup>-1</sup> and managed in a rotational grazing system. Seeding rates implemented were; 25 kg ha<sup>-1</sup> of perennial ryegrass for the PRG treatment, plus 6 kg ha<sup>-1</sup> of white clover for PRG+WC, 8 kg ha<sup>-1</sup> of red clover for PRG+RC, or 4 kg ha<sup>-1</sup> of herb for PRG+Chic and PRG+Plan treatments. Actual sward companion forage content (%) and dry matter digestibility (DMD; g (kg DM)<sup>-1</sup>) achieved post-weaning were: 25% and 788 g (kg DM)<sup>-1</sup> (PRG+WC), 39% and 735 g (kg DM)<sup>-1</sup> (PRG+RC), 21% and 770 g (kg DM)<sup>-1</sup> (PRG+Chic), 31% and 719 g (kg DM)<sup>-1</sup> (PRG+Plan). The PRG treatment had a DMD of 763 g (kg DM)<sup>-1</sup>. One hundred and twenty Texel×Belclare ewes ( $n=24$  per sward type) were mated to Texel rams. Male lambs were castrated within 24 hours of birth. Ewes and lambs were turned out to pasture 24–36 hours post-partum. Lambs were weaned at an average of 107 days of age and a leader follower grazing system was implemented. At weaning, sixty lambs ( $n=12$  per sward type) were selected and further balanced for LW, sex and reared litter size. Lambs were drafted to a minimum carcass weight of 20 kg. The *longissimus thoracis et lumborum* (LTL) muscles were obtained, vacuum-packed, aged for 7 days at 4°C and then frozen at -20°C. The LTL muscles were cut into steaks of 2.54 cm thickness, thawed at 4°C for 24 hours prior to sensory analysis and cooked according to Meat Standard Australia protocols. Cooked steaks were cut into equal portions and offered to panellists and consumers accordingly. Both panels rated samples on a 10 cm continuous line scale, where trained panellists scored samples ranging from 0=not at all to 10=high and the consumer panel scored samples ranging from 0=dislike extremely to 10=like extremely. Data were analysed using a mixed model in SAS 9.4. Dam parity, sex and sward type were included as fixed effects for lamb performance data with dam included as a random effect. Slaughter date was included as a random effect for days to slaughter (DTS), dressing proportion, and carcass weight while drafting weight was also included as a random effect for DTS. Sward type and sex were included as fixed effects, animal as a repeated effect and slaughter date, session and panellist/consumer were included as random effects for sensory analysis.

## Results and discussion

Lambs grazing PRG+WC, PRG+RC and PRG+chic had a higher average daily gain (ADG) ( $P<0.01$ ) and reduced DTS ( $P<0.01$ ) compared to PRG lambs, who performed similarly to the PRG+Plan lambs (Table 1). Sheep are selective grazers, with a preference for nutritionally rich, digestible forages (Mohammed *et al.*, 2020), a likely driver of the enhanced performance with WC, RC or chicory inclusion. Plantain has been associated with poor palatability in summer and reduced intakes (Pain *et al.*, 2014), potentially contributing to the lack of performance enhancements with Plan inclusion in the current study.

Meat from lambs grazing PRG+Plan scored lower for initial tenderness and overall tenderness ( $P<0.001$ ). Consumers generally scored meat from PRG+Plan lambs lower, resulting in reduced overall liking ( $P<0.01$ ) (Table 2). A reduction in one sensory attribute can result in reductions across other

Table 1. Lamb performance

	Dietary treatment					SEM	P-value
	PRG	PRG+WC	PRG+RC	PRG+Chic	PRG+Plan		
Average daily gain (g day <sup>-1</sup> )	110 <sup>a</sup>	174 <sup>b</sup>	184 <sup>b</sup>	142 <sup>bc</sup>	125 <sup>ac</sup>	7.7	<0.01
Days to slaughter	249 <sup>a</sup>	197 <sup>c</sup>	199 <sup>bc</sup>	213 <sup>bc</sup>	225 <sup>ab</sup>	6.2	<0.01
Dressing proportion	0.49	0.45	0.47	0.48	0.47	0.008	NS
Carcass weight (kg)	22.8	21.3	22.2	22.6	22.0	0.51	NS

PRG, perennial ryegrass; PRG+WC, perennial ryegrass and white clover; PRG+RC, perennial ryegrass and red clover; PRG+Chic, perennial ryegrass and chicory; PRG+Plan, perennial ryegrass and plantain; SEM, standard error of the mean; a, b, c within row means with different superscripts are significantly different.

Table 2. Sensory attributes of lamb meat

	Dietary treatment					SEM	P-value
	PRG	PRG+WC	PRG+RC	PRG+Chic	PRG+Plan		
Trained panel							
Initial tenderness	7.49 <sup>a</sup>	7.57 <sup>a</sup>	7.42 <sup>a</sup>	7.26 <sup>a</sup>	6.59 <sup>b</sup>	0.248	<0.001
Overall Tenderness	7.01 <sup>a</sup>	7.08 <sup>a</sup>	6.89 <sup>a</sup>	6.72 <sup>a</sup>	6.05 <sup>b</sup>	0.261	<0.001
Juiciness	6.80	6.60	6.88	6.77	6.71	0.324	NS
Flavour	5.71 <sup>a</sup>	5.27 <sup>ab</sup>	5.70 <sup>a</sup>	4.84 <sup>b</sup>	4.99 <sup>b</sup>	0.261	<0.001
Consumer panel							
Tenderness	7.50 <sup>a</sup>	7.29 <sup>a</sup>	7.08 <sup>ab</sup>	7.01 <sup>ab</sup>	6.46 <sup>b</sup>	0.278	<0.05
Juiciness	7.22	7.32	7.17	7.03	6.47	0.265	NS
Flavour	7.24 <sup>a</sup>	7.18 <sup>a</sup>	6.96 <sup>ab</sup>	6.93 <sup>ab</sup>	6.25 <sup>b</sup>	0.279	<0.01
Overall liking	7.42 <sup>a</sup>	7.36 <sup>a</sup>	7.07 <sup>ab</sup>	7.21 <sup>a</sup>	6.30 <sup>b</sup>	0.285	<0.01

PRG, perennial ryegrass; PRG+WC, perennial ryegrass and white clover; PRG+RC, perennial ryegrass and red clover; PRG+Chic, perennial ryegrass and chicory; PRG+Plan, perennial ryegrass and plantain; SEM, standard error of the mean; a, b, c within row means with different superscripts are significantly different.

parameters due to halo effects (Lawless and Heymann, 2010), which was seen in the current study. The lower acceptance of meat from PRG+Plan lambs may be linked to the reduced sward DMD. However, further investigations into meat fatty acid composition and a comprehensive analysis of forage nutritive value are necessary to confirm this theory.

## Conclusion

Binary swards can enhance lamb performance, increasing the production efficiency and sustainability of pasture-based lamb finishing systems. All meat produced in the current study had palatability scores that were above average; however, a distinct pattern emerged where meat derived from PRG+Plan lambs was generally scored lower by both panels. This study supports the existing literature, emphasising the positive potential of pasture diversification to promote superior animal performance. However, this study also highlights the importance of considering lamb finishing diets in tandem with the eating quality of the meat produced.

## Acknowledgement

Postgraduate funding provided by the Teagasc Walsh Scholarship Programme.

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# Evaluation of host plant suitability of *Festuca arundinacea* and *Festuca rubra* for plant parasitic nematodes

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## Abstract

Crop rotation is an important measure for the control of soil-borne diseases and pests. In the Netherlands, when growing maize on sandy soils, a nitrogen-catch (N-catch) crop is required. To establish a “smart” crop rotation, knowledge about the host plant status of crops is necessary. A pot and a field trial were conducted to investigate the host plant status of different grasses for, respectively the root lesion nematode *Pratylenchus penetrans* (Pp) and the root knot nematode *Meloidogyne chitwoodi* (Mc). The results of the pot experiment indicate that *Festuca arundinacea* (Fa) and *Festuca rubra* (Fr) are poor hosts for Pp. The distinct responses between Fa turf and forage suggest genetic variation. Results of the Mc field experiment showed that Fa varieties co-sown with maize have no significantly effect on the Mc population compared to monoculture of maize. Fa-1 monoculture decreased the Mc population significantly which indicates poor host status of Fa for Mc. Resistant Fa or Fr varieties will contribute to the control of Pp and Mc as part of a smart-crop rotation to control these harmful nematode species.

**Keywords:** *Festuca arundinacea*, *Festuca rubra*, *Pratylenchus penetrans*, *Meloidogyne chitwoodi*, host suitability, N catch crops

## Introduction

In the management of soil-borne diseases, such as soil fungi and plant-parasitic nematodes (PPN), crop rotation plays a pivotal role. A strategic crop rotation can mitigate or prevent damage caused by nematodes. The root knot nematode *Meloidogyne chitwoodi* (Mc) and the root lesion nematode *Pratylenchus penetrans* (Pp) are common nematode species worldwide and can be harmful to various crops. Damage is often manifested in yield and quality loss Sikora, *et al.* (2021). Understanding the interaction between crops and these nematode species is crucial for developing sustainable crop protection strategies.

## Methods

We carried out a pot experiment and a field experiment. The pot experiment, with artificial infestation, was conducted to define the host status of different grasses for Pp. Six crops were selected: turf and forage types of *Festuca arundinacea* (Fa), two varieties of *Festuca rubra* (Fr) and the references *Avena strigosa* (black oats, non-host) and *Lolium multiflorum* (Italian rye-grass; good host). The pot experiment was conducted in 4-litre pots filled with artificial soil consisting of silver sand and a nutrient solution, with five replicates per crop. The soil was artificially infested with a standard population of Pp reared by Wageningen University Research (WUR) Field Crops. The inoculum density was set at five Pp nematodes per ml soil. Crops were grown outdoors for a period of approximately 12 weeks from end of May until the end of August. After this cultivation period, soil samples were taken from each pot to determine the final population density of Pp. Soil was mixed and a representative sample of 100 ml was taken for analysing nematode population density. The nematodes were extracted by using the Oostenbrink elutriator. After elutriation of the suspension with particles <180 µm (mineral fraction), the material that was caught on the 180 µm sieve (organic fraction) was incubated for four weeks to facilitate hatching of juveniles and eggs.

The field experiment was conducted to determine the effect of N-catch cover crops in maize on the infestation of Mc. Crops were grown according to standard agricultural practice during the 2021–2022 growing season (May 2021–February 2022) at the Mc trial field of WUR-OT in Vredepeel Netherlands. This sandy soil is naturally infested with Mc.

The effect on the Mc population of the N-catch crops, three Fa varieties and a mixture of Fa varieties sown simultaneously with maize was compared with the effect of maize monoculture and monocultures of black oats and Fa-1. Plots of 3×12 m were sown in four repetitions. Soil samples from the top 25 cm of the soil surface were taken prior to maize sowing (May) and after incorporating the cover crops (Feb.). Approximately 1 litre of soil was collected per plot; a sub-sample of 100 ml was taken for nematode assessment, as described in the Pp-experiment.

### Statistical analysis

The nematode data were statistical analysed using Genstat Windows 22<sup>nd</sup> edition. Data of nematode counts were <sup>10</sup>log transformed to stabilize the variance (meet normal distribution) and analysed with ANOVA. The means obtained after 10log transformation are back transformed. Object means were compared with each other using a Student (or *t*) test by the Genstat procedure ATTEST

## Results and discussion

The initial population density in the pot experiment was 500 Pp (100 ml soil)<sup>-1</sup>. The population of Pp increased up to 825 Pp (100 ml soil)<sup>-1</sup> soil when Italian ryegrass was grown. All other grass varieties showed a decrease of the Pp population (Figure 1). Both Fa and Fr showed a reduction in nematode population and, based on the results of this pot experiment, can be categorized as poor hosts. However, a notable observation was the significantly higher population density of Pp on the turf-type Fa compared to the forage-type of Fa, indicating potential differential host plant suitability within this species.

The field was moderately infested, with an average Mc density of 170 Mc (100 ml soil)<sup>-1</sup> across all plots. The average initial infestation in plots with N-catch crops did not differ significantly from the average infestation in maize monoculture. The lowest average initial infestation was observed in the Fa monoculture plot, which was significantly lower than the other N-catch crops. The cultivation of black oats increased the Mc infestation to nearly 500 Mc (100 ml soil)<sup>-1</sup> (Figure 2). The infestation after maize monoculture averaged 250 Mc (100 ml soil)<sup>-1</sup>, a density expected after cultivating a moderate host for Mc. However, due to the variability in results, the final population density of maize-mono did not differ significantly from the infestation found after growing black oats. N-catch crops did not have a statistically

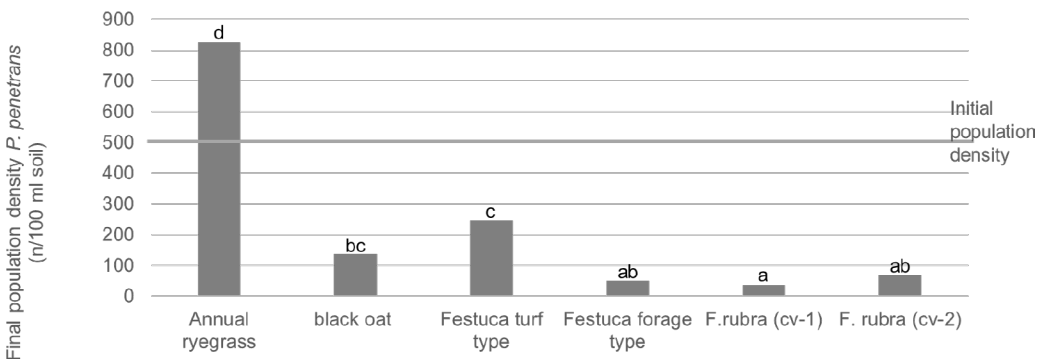


Figure 1. Effect of cover crops on the population density of *P. penetrans* (average initial population density = 500 Pp (100 ml soil)<sup>-1</sup>) in the pot experiment 2021.

significant effect on Mc infestation compared to maize monoculture without N-catch crops. The lowest infestation was observed in the N-catch crops Fa-1 (mono). Here, the infestation level was significantly lower than in the monoculture of maize, black oats and the Fa varieties co-sown with maize. Despite a substantial difference in final infestation between the Fa mixture co-sown and maize monoculture without N-catch crops, this difference did not reach statistical significance. Fa-1 (mono) demonstrated to be a very poor host, exhibiting a high level of resistance to Mc, with infestation of only 4 Mc (100 ml soil)<sup>-1</sup>.

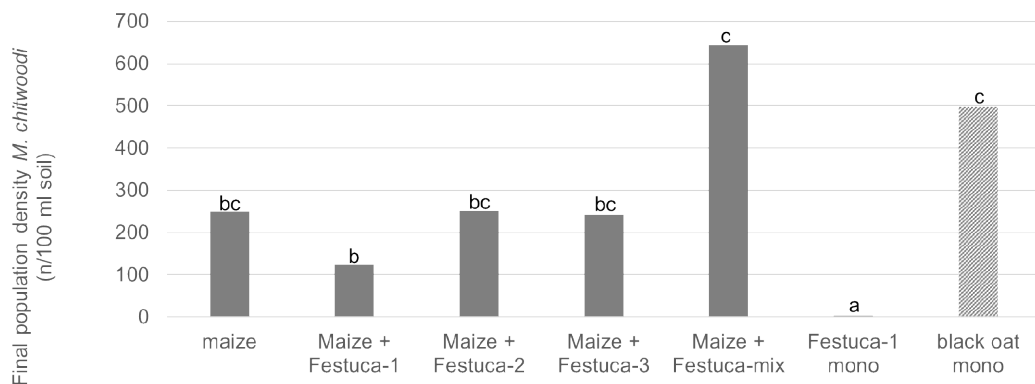


Figure 2. Effect of *Festuca arundinacea* as a N-catch crop in maize and as a monoculture-grown cover crop on the population density of *M. chitwoodi* (average initial population density = 190 Mc (100 ml soil)<sup>-1</sup>) in the field experiment 2022.

## Conclusion

The results of the pot trial with artificial infestation give a good indication that Fa and Fr are poor hosts for the root lesion nematode Pp. The results of this pot trial are confirmed by research of Thies *et al.* (1995) and Knoetze *et al.* (2023). Field research is needed to confirm the results for the varieties in the pot trial. The distinct responses between Fa turf and forage suggest genetic variation, but further study is needed. The N-catch crops can have a significant impact on Mc infestation. Fa-1 (mono) showed a significant reduction of the Mc population compared to maize and black oats. The Fa varieties that were co-sown with maize showed no significant difference compared to monoculture maize. This indicates that the Mc population was not significantly affected by the co-sown Fa. Although no statistically reliable differences between Fa-varieties were found, the results do seem to indicate that differences in host status may exist. Resistant Fa or Fr varieties will contribute to the control of Pp and Mc as part of a smart-crop rotation to control these harmful nematode species.

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# Does precision N-fertilizer application in grassland make sense?

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## Abstract

Productive grassland is under pressure worldwide due to environmental, ecological and economic conditions. This study aims to increase N-use efficiency of grassland with variable rate application (VRA) of fertilizer. We compared a VRA strategy with equal spatial distribution of N fertilizer. A two-year trial was conducted on a sandy soil in the Netherlands. In the first year (2019), the spatial variability of the N yield without N fertilization ( $YN_0$ ) and the maximum N yield at high N fertilization ( $294 \text{ kg ha}^{-1}$ ) ( $YN_{\text{max}}$ ) was assessed. N recovery ranged from 32 to 64%. In the second year (2020), a VRA strategy was tested, based on the results from 2019. Neither average yields (dry matter and N) nor average N-use efficiency were higher with VRA compared to equal N distribution. However, with VRA, a more equal distribution of N use efficiency was found within a field, suggesting the potential to prevent local N surplus or N depletion.

**Keywords:** VRA, N recovery, spatial variation, N-use efficiency

## Introduction

Variable rate application (VRA) machinery (e.g. Kubota, John Deere) and GPS driven techniques (e.g. prescription maps) are being developed for VRA of nutrients and pesticides in order to (1) reduce nutrient and pesticide losses and (2) to reduce costs of fertilizer and pesticide use. For pesticides, different methods and strategies have already been successfully tested and implemented, predominantly in arable farming (Cammarano *et al.*, 2023). VRA in grassland is developing, but a strategy with proven better N-use efficiency (NUE) is still lacking.

Classical N response curves under different conditions show that the agriculturally optimal N rate is determined by the N yield without N fertilization ( $YN_0$ ) and the maximum N yield at high N fertilization ( $YN_{\text{max}}$ ). Low  $YN_0$  indicates low soil N supply, so more applied nitrogen is needed; high  $YN_{\text{max}}$  indicates good growing conditions and high N demand so high nitrogen supply (from all sources) is needed. On this basis, a N fertilization strategy can be defined, provided that the locations with high and low  $YN_0$  and with high and low  $YN_{\text{max}}$  in a field can be determined.

The aim of the experiment was to determine whether a strategy for VRA can increase the yield (dry matter (DM), N) and/or NUE of production grassland compared to undifferentiated (i.e. equal) fertilization. If successful, this strategy could form the basis for VRA of N in production grassland.

## Materials and methods

A field experiment on grassland was conducted in 2019 and 2020 on a sandy soil (4% SOM) at the De Marke experimental dairy farm in the Netherlands. The field experiment had 32 plots of  $10 \text{ m} \times 6 \text{ m}$ , laid out in two strips of 16 plots. In 2019, each plot was divided into two half plots with an area of  $10 \times 3 \text{ m}$ . For each plot, the half plots were numbered separately from 1 to 64.

In the first year (2019), we determined  $YN_0$  and  $YN_{\text{max}}$  at each of the 32 half plots leaving the uneven numbered half plots unfertilized and applying a 120% recommended N rate (CBGV, 2023) to the even numbered plots to reach near maximum achievable N yield. Even half plots received N rates of  $119 \text{ kg}$

ha<sup>-1</sup>, 89 kg ha<sup>-1</sup>, 59 kg ha<sup>-1</sup> and 27 kg ha<sup>-1</sup> from the 1<sup>st</sup> cut to the 4<sup>th</sup> cut, reaching a total of 294 kg ha<sup>-1</sup>. In addition, sufficient phosphate and potash was given before the 1<sup>st</sup> cut to avoid P or K limitation (90 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and 180 kg K<sub>2</sub>O ha<sup>-1</sup>).

In the second year (2020), a ‘King John strategy’ (Kindred *et al.*, 2016) was tested based on the results in 2019: more N fertilizer to plots with high N recovery. This VRA strategy was compared with the current practice (control; equal distribution). The 32 plots were split into two treatments (control and VRA) by ‘controlled random selection’. First, the 32 plots were ranked by the measured N recovery in 2019. Second, for the first two highest ranked plots, one of both treatments was randomly selected. This procedure was continued for the other (ranked) plots. The ratio between N fertilizer rates of VRA plots was equal to the ratio of measured N-recovery in 2019. The average of all (16) VRA rates was equal to the rate of the (16) control treatment plots. The average N rates in 2020 were 120 kg ha<sup>-1</sup>, 88 kg ha<sup>-1</sup>, 60 kg ha<sup>-1</sup>, 28 kg ha<sup>-1</sup>, and 9 kg ha<sup>-1</sup> from the 1<sup>st</sup> cut to the 5<sup>th</sup> cut, resulting in a total of 306 kg ha<sup>-1</sup>.

All half plots ( $n=64$ ) were harvested on the same day using a forage harvester from Haldrup (<https://www.haldrup.net/>). The harvesting area of each half plot was 10×1.5 m in the centre of each half plot. The weight of harvested fresh grass on each plot was measured. The DM content was determined for a randomly collected subsample (300–500 g). The subsamples were dried for 48 hours at 70°C and homogenized by grinding in a 0.5 mm mill. Samples were taken from the resulting material to assess the remaining moisture content by drying at 105°C. Samples were taken from the remaining materials to assess the total N content (NC) by destruction with H<sub>2</sub>SO<sub>4</sub>-H<sub>2</sub>O<sub>2</sub>-Se and subsequent SFA-Nt/Pt (Novozamsky *et al.*, 1983).

The purpose of the design of the experiment in 2019 was to determine the spatial variation in N recovery by quantifying N yield without N fertilization (YN<sub>0</sub>) and by the maximum N yield at high N fertilization (YN<sub>max</sub>). For each plot with two half plots (1 and 2, 3 and 4, etc.), the N recovery can be determined according to:

$$\text{N recovery (plot 1and2)}=100*(\text{YN}_{\text{max}}(\text{half plot 2})-\text{YN}_0(\text{half plot 1}))/\text{N fertilization (half plot 2)}$$

For robustness of the spatial variation in N recovery, results are presented as a moving average. For example:

$$\text{N recovery plot 5 and 6}=(\text{N recovery 3 and 4}+\text{5 and 6}+\text{7 and 8})/3.$$

N surplus is defined as N fertilization rate minus N yield and NUE is defined as 100\*(N yield/N fertilization rate).

## Results and discussion

The spatial variability of YN<sub>0</sub>, YN<sub>max</sub> and N recovery (results first year) was high. N recovery ranged from 32 to 64% (data not shown). The correlation between YN<sub>0</sub> and YN<sub>max</sub> was 0.88.

In the second year, the spatial variability in yield (DM and N) was higher with VRA (King John) compared to the control (equal distribution), whereas the spatial variability in N surplus and N use efficiency was lower with VRA compared to the control (Figure 1).

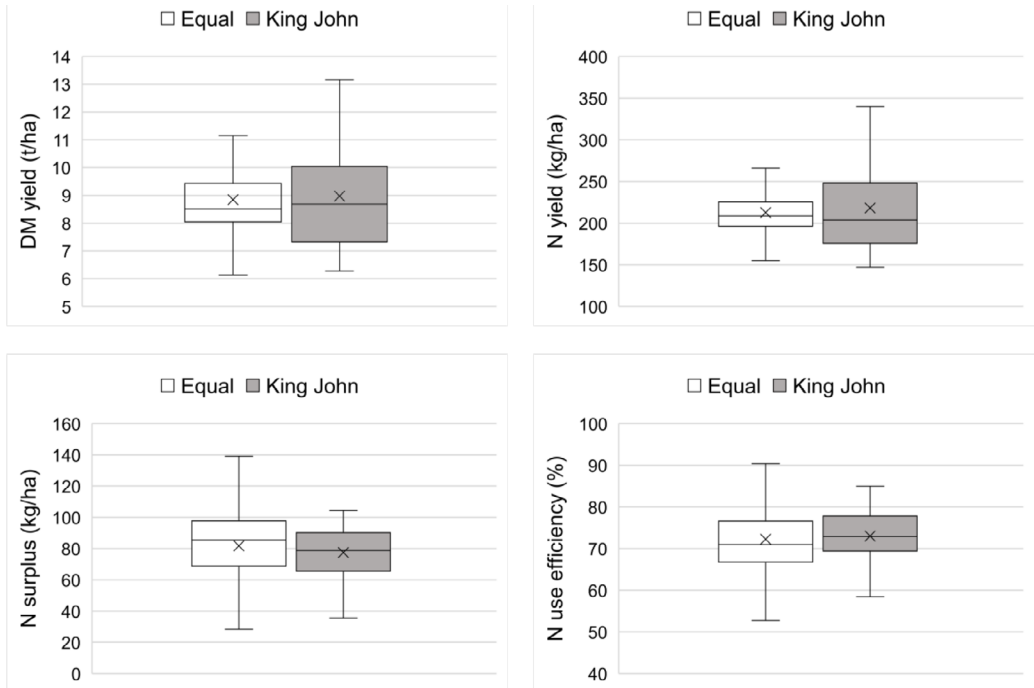


Figure 1. Box plots of DM yield ( $\text{Mg ha}^{-1}$ ), N yield ( $\text{kg ha}^{-1}$ ), N surplus ( $\text{kg ha}^{-1}$ ) and N use efficiency (%) for the control (equal distribution) and VRA strategy (King John) at De Marke in 2020. Boxes indicate the 25 (bottom) and 75 (top) percentile values of the 16 plots, the line in the box represents the median, and the whiskers indicate the 5 and 95 percentile values.

## Conclusion

So far, our experiment has not indicated any added value of a spatial VRA strategy for N fertilization to improve NUE on production grassland. However, with VRA, a more equal distribution of NUE was found within a field, suggesting the potential to prevent local N surplus or N depletion.

## Acknowledgements

We acknowledge the Topsector AgriandFood which made the budget available for the work presented. The fieldwork was organized in two public private projects: DISAC and Precision Agriculture 4.0. The budget is related to two donors: Ministry of Agriculture, Nature and Food, and Kubota / Kverneland Group. Writing of the paper was funded by the European Union Horizon 2020 research and innovation programme, under grant agreement 774124, project SUPER-G (Developing Sustainable Permanent Grassland Systems and Policies).

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# The effects of varying Nitrogen, Potassium, and Sulphur treatments on grass clover sward production and composition

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## Abstract

Benefits of high forage quality in tandem with nitrogen (N) fixation benefits have placed a renewed emphasis on optimising nutrient advice for grass/white clover (WC) swards. The objective of this study was to optimise nutrient advice for grass/WC swards to enhance DM production, while optimising WC proportion in the first production year. The experiment consisted of 4 N treatments (0, 100, 150, 200 kg ha<sup>-1</sup>), 4 Potassium (K) treatments (0, 50, 100, 150 kg ha<sup>-1</sup>), and 4 Sulphur (S) treatments (0, 15, 30, 45 kg ha<sup>-1</sup>) with three replicates. Herbage mass was estimated at each grazing ( $n=8$ ) and sward clover proportion were measured on 3 occasions; April, July, and September. Nitrogen rate significantly ( $P<0.001$ ) effected cumulative DM production; increasing as N rate increased. There was no significant effect of K or S on cumulative DM yield. Neither K nor S effected clover proportions within the sward. There was a significant effect ( $P<0.001$ ) of N rate on clover content, the 0 N treatment had the greatest clover content ( $358.0\pm 12.00$  g (kg DM)<sup>-1</sup>), compared to the 100, 150 and 200 N treatments. This study demonstrates that WC proportions and sward DM production is not effected by additional K, when soil K status is above minimum requirements, nor by additional S. Moreover, this study highlights the need for N fertiliser strategies on grass clover swards to avoid reductions in DM production.

**Keywords:** white clover, nitrogen, potassium, sulphur, dry matter production

## Introduction

White clover (*Trifolium repens* L.; WC) is the most important legume species in Europe and New Zealand (Whitehead, 2000). In an Irish context, WC has been shown to fix up to 128 kg N<sup>-1</sup> ha<sup>-1</sup> year (Burchill *et al.*, 2014), which can lead to a reduction in chemical nitrogen (N) use on farms. In mixed swards, there are three primary factors effecting biological N fixation (BNF); clover production and persistency, soil N status, and competition with companion grasses (Ledgard and Steele, 1992). The negative effect of increasing N rates on WC production is well-documented (Enríquez Hidalgo, 2014). Contrary to N, the application of potassium (K) has been shown to increase the proportion of WC within swards (Simpson *et al.*, 1988), whilst K can also effect BNF through regulation of nitrogenase activity (Hogh-Jensen, 2003). White clover competes poorly with grasses for nutrients other than N (Whitehead, 2000), and with minimum soil K requirements according to Chestnutt and Lowe (1970) of 170–200 mg l<sup>-1</sup> for WC, additional K may be needed to sustain this requirement and alleviate against seasonal K fluctuations (Metson and Saunders, 1978). Due to reductions in atmospheric Sulphur (S) deposition, application of S is important for WC persistency (Webb *et al.*, 2016), and has been shown to increase WC content (Sinclair *et al.*, 1996). The objective of the current study was to investigate the effect of differing N, K, and S treatments on WC production and persistency under grazing conditions in a grass WC sward.

## Materials and methods

A grazing plot experiment was carried out at the Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Ireland (Latitude 52°16' N Longitude 8°26' W) in 2023. The soil type was a free-draining acidic brown earth (Cambisol) of sandy loam-to-loam texture. Soil had a pH of 7.2, phosphorus index of 4 (25.1 mg l<sup>-1</sup>; Morgan, 1941), and K index of 4 (208 mg l<sup>-1</sup>; Morgan, 1941), which places the experimental site above the optimum P and K soil fertility targets for grass production in Ireland.

The site used was sown in August 2022 with 10 kg of perennial ryegrass (PRG; cv. Astonenergy) and 2 kg of medium leaved WC (cv. Chieftain). The experiment was conducted on 6×3 m plots, arranged in a randomised block design consisting of 4 N treatments (0, 100, 150, 200 kg ha<sup>-1</sup>), 4 Potassium (K) treatments (0, 50, 100, 150 kg ha<sup>-1</sup>), and 4 Sulphur (S) treatments (0, 15, 30, 45 kg ha<sup>-1</sup>) with three replicates. Nitrogen was applied in the form of 46% protected urea, K applied as 50% Muriate of Potash, and S applied as Kieserite (20% S, 15% Mg). All plots were grazed on 8 separate occasions (rotations) between March and November by lactating dairy cows. Grazing commenced once the experimental area reached a pre-grazing herbage mass of 1600 kg DM ha<sup>-1</sup> and was grazed to a target residual height of 4 cm, both of which were visually assessed on the whole experimental area. Pre-grazing herbage mass (>4 cm) was measured using an Etesia motor harvester by cutting a sub section of each plot. Harvested material was weighed and a 0.1 kg subsample was taken and dried at 60°C for 64 h to determine DM content. Clover separations were taken on 3 occasions throughout the grazing season (April, July, and September) using a Gardena hand shears by cutting to 4 cm at six random points along the diagonal line of each plot. Samples were then mixed and a 100 g subsample was weighed and ultimately separated into sown species and dried at 90°C for 16 h to determine DM content of the sown species. Metrological data was recorded at the onsite metrological station. Statistical analysis was carried out using a mixed model in SAS 9.4. Fixed effects included N treatment, K treatment, S treatment, rotation and associated interactions.

## Results and discussion

Nitrogen treatment had a significant effect on WC content (g kg<sup>-1</sup>) within the sward ( $P < 0.001$ , Table 1), with the 0 N treatment recording the highest average WC (358.0±12.00 g (kg DM)<sup>-1</sup> year<sup>-1</sup>). Seasonal WC variation was also evident ( $P < 0.001$ ); with an increase in WC content at each measurement for April, July and September, similar to Enríquez Hidalgo (2014) who reported that increasing N fertiliser rate had a negative effect on WC content within the sward as referred to previously in this paper. Previous studies (Simpson *et al.*, 1988) have reported increases in WC content after the application of K, however in the current study; K did not have an effect on WC proportions within the sward. White clover requires a minimum K availability of 170-200 mg l<sup>-1</sup> (Chestnutt and Lowe, 1970); the experimental site used by Simpson *et al.* (1988) were K deficient soils (28 mg l<sup>-1</sup>), whereas, the current study had a K index of 4 (208 mg l<sup>-1</sup>), above the optimum index 3 for grassland in Ireland. Application of S had no effect on WC content, which contrasts with Sinclair *et al.* (1996) found an increase in sward WC content from 30 to 46% when increasing S application from 0 to 30 kg ha<sup>-1</sup>. During spring and early summer, DM yield was greater ( $P < 0.001$ ) as N fertiliser input increased (0 N to 200 N). This is due to lower average soil temperatures experienced during the start and end of the growing season (8°C and 12.5°C, respectively) which reduces BNF by WC (Frame and Newbould, 1986), thereby reducing N supply to the swards with lower levels of chemical N. From mid-summer until early autumn, there was no additional response to increasing N rates, which coincides with increased WC sward content and higher average soil temperatures (17.7°C). There was no interaction of treatments on cumulative DM production. Biological N fixation was not measured as part of the current study, however it could account for the lack of difference in DM yield over the summer period between the 4 different N treatments. Burchill *et al.* (2014) reported that the level of clover in swards is directly related to the level of BNF, which was seen in the current study with a 206 g (kg DM)<sup>-1</sup> difference in sward WC content between the 0 N and 200 N treatments. Results of our study showed no significant response to additional K, which suggests that the soil used in the current study is sufficient to mitigate against seasonal herbage K fluctuations reported by Metson and Saunders (1978) and any implication this may have on DM yield, and as such there is no additional benefit of applying K fertiliser to high K index soils. The addition of S yielded no increase in either cumulative or rotational DM production nor did it effect WC content (Table 1). While this contrasts with other literature (Sinclair *et al.*, 1996; Webb *et al.*, 2016), a study on temperate grasslands in Ireland found no response to S when N rates of 200 kg ha<sup>-1</sup> and less were applied (Murphy & O'Donnell, 1989), aligning with the results of the present study, where 200 N was the maximum application rate.



Table 1. White clover content, cumulative DM production across fertiliser treatments.

Nitrogen treatment	0 N	100 N	150 N	200 N	SE	P value
Total DM (kg ha <sup>-1</sup> year <sup>-1</sup> )	12 935	13 247	13 991	14 036	185.09	<0.0001
WC content (g (kg DM) <sup>-1</sup> year <sup>-1</sup> )	358.0	229.5	189.9	152.1	12.00	<0.0001
Potassium treatment	0 K	50 K	100 K	150 K		
Total DM (kg ha <sup>-1</sup> year <sup>-1</sup> )	13 356	13 629	13 718	13 505	185.09	NS
WC content (g (kg DM) <sup>-1</sup> year <sup>-1</sup> )	250.54	212.52	236.42	230.06	12.00	NS
Sulphur treatment	0 S	15 S	30 S	45 S		
Total DM (kg ha <sup>-1</sup> year <sup>-1</sup> )	13 396	13 350	13 892	13 569	185.09	NS
WC content (g (kg DM) <sup>-1</sup> year <sup>-1</sup> )	216.04	232.05	248.41	233.04	12.00	NS

## Conclusions

This study highlights the needed for effective N strategies to optimise early and late season herbage production in grass WC swards. This study has also confirmed that there is no response to addition levels of K on soil with optimal K indexes, while yielding no benefit to additional S application on grass WC swards. It must be emphasised that this study only consists of one year of measurement; further research is needed to quantify the effect of varying levels of N, K, and S on grass WC swards under a grazing regime.

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# The effect of sward species on nitrate leaching: a lysimeter study

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## Abstract

A lysimeter study was carried out to assess the efficacy of Ribwort plantain (PL) monocultures to reduce Nitrate-Nitrogen (NO<sub>3</sub>-N) leaching from bovine urine patches, compared to perennial ryegrass (PRG) monocultures, across two soil types in autumn in Ireland. The study was conducted as a 2×2×2 factorial design with two soil types (free draining Cambisol (FD) and poorly draining Gleysol (PD)), two sward types (PL and PRG) and two urine application dates (October and November) with 3 replicates per treatment. Urine (equivalent to 575 kg N ha<sup>-1</sup>) was applied to lysimeters twice in autumn (October and November) 2022. Leachate was collected at two-week intervals from the date of urine application until March 2023 and analysed for total chlorine and nitrogen species. There was an effect of application date on NO<sub>3</sub>-N concentrations ( $P < 0.001$ ) with higher concentrations in the November application (61.8±5.11 mg l<sup>-1</sup>) compared to October (35.4±4.12 mg l<sup>-1</sup>). Both sward types leached similar total NO<sub>3</sub>-N (kg ha<sup>-1</sup>) following the October application. The PRG sward had a greater total NO<sub>3</sub>-N leached (272.9±18.23 kg ha<sup>-1</sup>) compared to the PL sward (199.1±19.48 kg ha<sup>-1</sup>) following the November application ( $P < 0.05$ ). Significantly higher rainfall, and thus drainage, following the October (473.3±18.87 mm) compared to the November (275.4±36.68 mm) urine application is likely to have affected the ability of PL to reduce NO<sub>3</sub>-N leached.

**Keywords:** nitrate-nitrogen leaching; urine; sward type; soil type

## Introduction

Under the EU Water Framework Directive (WFD) all EU member states are required to ensure that all waters are protected and restored to at least good status by 2027 (EPA, 2022). Contrary to this target, water quality in Ireland declined in the period of 2016–2021 (EPA, 2022). To achieve the WFD targets, agriculture, as well as other sectors, must minimise nutrient losses to waterways. In pasture-based systems, urine excreted from grazing animals is a major source of nitrogen (N) loss (Selbie, 2014), with increased risk at times of high drainage, such as the autumn-winter period when evapotranspiration and grass growth is reduced (Di and Cameron, 2002). Urine patches represent 'hot spots' for N loss due to high N loading rates of up to 1000 kg N<sup>-1</sup> ha<sup>-1</sup> (Haynes and Williams, 1993), which exceed the nutrient requirement of the plant, leading to potential N losses (Jarvis *et al.*, 1995). Recent research has highlighted the potential of different forage species such as Ribwort plantain (PL, *Plantago lanceolata* L.) to reduce inorganic N leaching (Carlton *et al.*, 2019; Welten *et al.*, 2019). Increased over winter growth rates, increased evapotranspiration, and the impact of biological nitrification inhibitor compounds exhibited by PL (Welten *et al.*, 2019), indicate the potential of PL to reduce N leaching. There is a need to evaluate the effect of PL under differing soil types and climatic conditions to establish its efficacy at reducing N leaching in pasture systems. The objective of the current study was to assess the efficacy of PL monocultures to reduce Nitrate-N (NO<sub>3</sub>-N) leaching over the autumn-winter period relative to perennial ryegrass (PRG, *Lolium perenne* L.) across free and poorly draining soils.

## Materials and methods

The experiment commenced in October 2022 using the existing lysimeter facility at the Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Ireland (52°16' N, 8°26' W). The facility contains 24 lysimeters of high-density polyethylene casing (300 mm internal diameter and 700

mm depth). The study was conducted as a 2×2×2 factorial design with two soil types: an acidic brown earth Cambisol (free draining (FD,  $n=12$ )) and a silt loam Gleysol (poorly draining (PD,  $n=12$ )); two sward types, PL (cv. Ceres tonic ( $n=12$ )) and PRG (cv. Aberchoice ( $n=12$ )) sown as monocultures and two application dates (October and November). On October 10<sup>th</sup> 2022, fresh urine was collected from late-lactation dairy cows grazing PRG-white clover swards, and frozen. Prior to application, frozen urine was thawed at room temperature and a sub-sample analysed for N components. Prior to each urine application event, herbage was cut to 5 cm to simulate a grazing event. Urine was applied on the 17<sup>th</sup> October 2022 to half of the lysimeters ( $n=12$ ), and to the remainder on 14<sup>th</sup> November 2022 at a rate of 575 kg N ha<sup>-1</sup>. Leachate was collected at two-week intervals from the date of urine application (October and November) until March 2023 and analysed for total chlorine, total N, total oxidizable N, nitrite-N, ammonium N and total organic carbon. Nitrate-N was calculated by subtracting nitrite-N from total oxidizable N. Analysis of inorganic N components was conducted using an Aquakem 600A automated analyser (Thermo Electron, Joensuu, Finland). Herbage on all lysimeters was harvested in March 2023 to determine dry matter yield. A sub-sample of the fresh herbage was oven dried at 60°C, milled and analysed for total N (Leco FP-528 N Analyzer, Leco, St. Joseph, MI, USA) to calculate total N uptake in the herbage. Metrological data was collected from an onsite metrological station. Statistical analysis was carried out using a mixed model in SAS 9.4.

## Results and discussion

Cumulative rainfall from October 2022 to March 2023 was 607 mm, with 152 mm and 76 mm falling within 2 weeks of urine application for October and November treatments, respectively. There was an effect of application date on NO<sub>3</sub>-N concentrations ( $P<0.001$ ), with higher concentrations in the November application ( $61.8\pm 5.11$  mg l<sup>-1</sup>) compared to the October application ( $35.4\pm 4.12$  mg l<sup>-1</sup>). No difference in leachate NO<sub>3</sub>-N concentrations was observed between PL and PRG or between the PD and FD soil types post-October urine application. There was a tendency ( $P=0.08$ ) for PL ( $59.4\pm 8.73$  mg l<sup>-1</sup>) to have lower leachate NO<sub>3</sub>-N concentrations than PRG ( $82.6\pm 8.16$  mg l<sup>-1</sup>) post-November urine application, but no difference between the PD and FD soil types was observed.

In the November treatment, sward type had a significant effect ( $P<0.05$ ) on total NO<sub>3</sub>-N leached (in kg ha<sup>-1</sup>; Table 1). Results from the November treatment align with results from Welten *et al.* (2019) and Carlton *et al.* (2019) who reported a reduction in NO<sub>3</sub>-N leached under PL compared with PRG swards. There was no difference in NO<sub>3</sub>-N leached between the sward types following the October application (Table 1). There was significantly higher drainage ( $P<0.001$ ) for the October ( $473.3\pm 18.87$  mm) compared to the November ( $275.4\pm 36.68$  mm) application dates, driven by above average rainfall for the month of October (230 mm compared to a ten-year average of 103 mm). The findings of the current study suggest that heavy rainfall and subsequent drainage following urine application may have impacted the ability of PL to mitigate NO<sub>3</sub>-N leaching. Di and Cameron (2002) suggested that leaching risk increases during periods of high drainage and this may have led to the lack of difference

Table 1. Total NO<sub>3</sub>-N (kg ha<sup>-1</sup>) leaching for sward type (perennial ryegrass and plantain) and soil type (free draining and poorly drained) after October and November urine applications.

Leachate	NO <sub>3</sub> -N leaching (kg ha <sup>-1</sup> )				P-value	
	Free drained	Poorly drained	Perennial ryegrass	Plantain	Species	Soil type
October application	278.0±28.25	214.6±24.47	273.2±26.43	219.6±26.43	NS	NS
November application	278.7±18.23	193.3±19.48	272.9±18.23	199.1±19.48	<0.05	<0.05

between the two sward species following the October application. This finding is important in the face of future climate scenarios that predict more extremes in weather events, including higher rainfall events in autumn and winter (Nolan and Flanagan, 2020) and which could lead to increased N loss through elevated drainage levels. Soil type also had a significant effect ( $P < 0.05$ ) on total  $\text{NO}_3\text{-N}$  leached ( $\text{kg ha}^{-1}$ ) following the November application (Table 1); this aligns with reports from Di and Cameron (2002) of lower leaching from fine, as opposed to coarse, textured soils, due to slow drainage and an increased potential for denitrification.

Cameron *et al.* (2013) reported that more N can be utilised by plants if N leaching is reduced; however, in the present study when total  $\text{NO}_3\text{-N}$  leaching was reduced in the November treatment, there was no significant effect in total N plant uptake for PL ( $22.2 \pm 2.69 \text{ kg}^{-1} \text{ ha}^{-1}$ ) or PRG ( $17.6 \pm 2.50 \text{ kg}^{-1} \text{ ha}^{-1}$ ). This is similar to a finding by Welten *et al.* (2019) who reported no difference in total plant N uptake, even while reporting a reduction in  $\text{NO}_3\text{-N}$  leaching. The reduction in total  $\text{NO}_3\text{-N}$  leaching between sward types in the current study, in the absence of a difference in drainage or in total herbage N uptake, signals that other mechanisms are responsible for the reduction in  $\text{NO}_3\text{-N}$  leaching. Carlton *et al.* (2019), attributed lower N leaching losses to a combination of lower drainage and the effect of biological nitrification inhibitor compounds released by PL. These BNIs, such as aucubin, have been shown to play an active role in the suppression of soil N nitrification (Dietz *et al.* 2013) and could be responsible for reducing  $\text{NO}_3\text{-N}$  available to be leached from soils, as reported by Carlton *et al.* (2019).

## Conclusions

Findings from this study further demonstrate the potential for PL to be utilised as a plant species to reduce  $\text{NO}_3\text{-N}$  leaching from urine patches. However, the results also demonstrate that this potential may not be consistent and suggest that periods with high drainage can reduce the effectiveness of PL in reducing  $\text{NO}_3\text{-N}$  leaching from urine patches.

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# Grassland species identification and mapping with UAS imaging

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## Abstract

Identifying grassland species and estimating their coverage is crucial for effective forage management and biodiversity monitoring. There is significant promise in leveraging multispectral drone imagery and machine learning algorithms, while the potential for multi-species identification from drone imagery is still unclear. Here, we aim to investigate which grassland species can be accurately identified and to assess the impact of time and location to classification accuracy. During the experiment drone images were captured and several plant species of interest identified, particularly those relevant to forage quality and biodiversity issues. Images were processed further and utilised to generate a synthetic dataset for training neural networks on a semantic segmentation task to recognise these species in drone imagery throughout the vegetative period. Results have demonstrated significant potential for identifying common species and even distinguishing between various grass species with the help of additional infrared bands in multispectral imagery. The incorporation of multi-temporal analyses has enhanced classification accuracy, especially in areas with mixed species. Multi-species detection in grassland seems possible and will be enhanced by further model training and continuous learning processes. Moreover, robust classification models have the potential to improve grassland management strategies and contribute to more effective biodiversity monitoring methods and conservation efforts.

**Keywords:** remote sensing, biodiversity, phytodiversity, machine learning, semantic segmentation

## Introduction

Grasslands covers roughly 40 percent of earth's land surface area, including parts that are managed intensively through fertilisation, regular defoliation (grazing or mowing), as well as water drainage to increase economic yield rates (Plantureux *et al.*, 2005). However, this kind of management is accompanied by a loss of habitats for invertebrates, leading to fewer pollinators and a lack of food sources for higher order species (Morris, 2000). To avoid further loss of biodiversity, it is necessary to establish methods for grassland species monitoring. Here, remote, or proximal sensing methods with unmanned aerial systems (UAS) equipped with cameras in the visible (RGB) and additionally in the infrared spectrum (multispectral) can help to monitor phytodiversity in grasslands (Ghajar and Tracy, 2021; Muro *et al.*, 2022).

Numerous studies have already proven the possibility for detecting single grassland species with RGB and multispectral drone images (Lyu *et al.*, 2022). However, the potential for multi-species identification in grasslands from remote sensing is still unclear and therefore, we aim to (1) investigate which grassland species in can be accurately identified and to (2) assess the impact of time and location to classification accuracy.

## Materials and methods

This study was conducted at three different grassland sites in Mecklenburg-Western Pomerania in North-Eastern Germany. The management systems ranged from extensively to intensively used grasslands and included seed breeding areas. The selection of investigation sites was based on the abundance of both common fodder and poisonous non-fodder species, as well as diverse species indicating species-rich

grasslands which are relevant for governmental support. Individual plants for the species of interest were identified and labelled. UAS images were captured with RGB (visible red, green, and blue bands) and multispectral cameras at each location at 20 m height and a frequency of 2 weeks from May to October 2023.

The collected RGB and multispectral images are processed to orthophotos and stacked to a single data cube with RGB and multispectral information. According to the optical or GPS-tracked location, the single individuals are annotated and extracted along the resulting polygons. These data and background images were used for synthetic data generation as artificial creation of further images, serving as training and initial validation dataset.

Different machine learning models are applied to solve different regression, object detection, and segmentation problems depending on the issue at hand. Ranging from bounding box regression for larger individual plants to image-wide semantic segmentation for extensive grass stands, the necessary amount of annotated training data varies. As such, the experiment started with low-cost maximum likelihood classification and object detection to get preliminary results for a feasibility assessment.

For further development training on semantic segmentation models like DeepLabV3 on realistic synthetic composites of all our annotated plant species is being undertaken.

To validate the results of this image-wide segmentation, the model was applied on multiple staked out plots on the experimental sites and manually compared to the predicted positions of individual species and the shape of larger grass patches to the actual ground truth in the field. To facilitate this process, each 2×2 metre plot is further divided into a square grid of 25 cm with all vegetation in each cell charted and then compared to the model's prediction.

## Results and discussion

The preliminary results of a maximum likelihood classification show the potential to detect common grassland species (Figure 1) was successfully identified and grass species, e.g., *Lolium perenne* L. and *Poa pratensis* L. were distinguishable. Furthermore, it was possible to identify the four species in one algorithm. It was also found that infrared bands using multispectral imagery increased the accuracy of species identification, especially in distinguishing between *Lolium perenne* L. and *Poa pratensis* L. The training of a neural network (Mask-RCNN) on an object detection task of *Rumex obtusifolius* L. resulted in an accuracy of approximately 85%.

Higher accuracies are expected with the enhanced method using synthetic data generation and model training with neural networks. Furthermore, images from species in different growth states and from various locations and years may increase prediction accuracies, due to broader variation in the plant phenotype capture of training images. The resulting maps of species distribution and individual detection also allow the localisation of species with an accuracy of less than 10 cm.

## Conclusion and outlook

Multi-species detection in grassland seems possible and will be enhanced by continuous model training and learning processes. Further image data collection of the various species is expected to lead to improved species detection accuracies. These are crucial prerequisites for farmers to use UAS imagery with detection software as a decision tool for plant protection measures, to measure grassland quality, monitoring biodiversity, as well as a proof for governmental support of species-rich grassland. This may enhance grasslands productivity by more targeted management measures by forage species monitoring and contribute to more effective conservation efforts by phyto diversity monitoring.

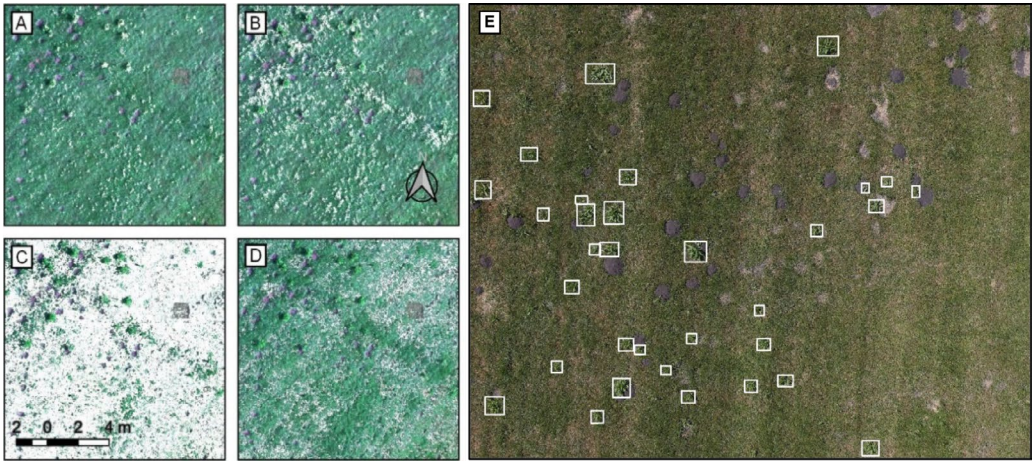


Figure 1. Result map with white pixels of *Rumex obtusifolius* (A), *Achillea millefolium* (B), *Lolium perenne* (C) and *Poa pratensis* (D) detection and distribution by maximum likelihood classification using RGB and multispectral images. Neural network detection using bounding boxes for *Rumex obtusifolius* (E) from an RGB drone image.

## Acknowledgements

This study was funded by the German Ministry of Education and Research (BMBF) through the collaborative research initiative “Biogenic value creation and smart farming” for the Fraunhofer Gesellschaft. We further thank Sebastian Krauleidis for his support in drone data collection and the land managers for providing access to their fields.

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# Exploring the genetic basis of cattle grazing behaviour for the sustainable use of the Swiss Alps

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## Abstract

Sustainable use of pastures requires suitable grazing livestock. Novel research has revealed the glutamate metabotropic receptor 5 gene (*GRM5*) to be associated with the movement of Hereford cows grazing steep and rugged grasslands in New Zealand. The study also reported a predominance of variants *C* and *B*, and much lower frequency of *A* in this population. In the current exploratory study, we asked if similar *GRM5* variant frequencies and behavioural relationships are observed in different cattle breeds elsewhere. Grazing behaviours were derived from GPS-tracked mature cows ( $n=17$ ; 12 Highland and 5 Original Brown) when grazing in the Swiss Alps. Gene variation was determined using PCR-single-strand conformation polymorphism analysis. The Swiss cattle had only three of a potential six *GRM5* genotypes: *AC*, *BC* and *CC* (3:3:11). Variant *C* was the most common (frequency 82%) and present in all the cows, while the *A* and *B* variants were equally represented (9%). The results suggest differences in grazing behaviour among *GRM5* genotypes in Swiss cattle, although such differences did not reflect those observed in the New Zealand cattle. The unbalanced frequency of *GRM5* genotypes found in the Swiss cattle herds may bring opportunity for genetic selection to adapt their grazing behaviour to alpine landscapes. A larger sampling is needed to establish the effects of *GRM5* variation on grazing behaviours.

**Keywords:** genetic associations, grazing personalities, home range, GPS-tracking collars

## Introduction

Differences in space use and movement between and within cattle breeds suggest a genetic basis for grazing behaviour (Bailey *et al.*, 2004; Pauler *et al.*, 2020). Recent research has revealed that variation in the glutamate metabotropic receptor 5 gene (*GRM5*) is associated with the behaviour of Hereford cows grazing in steep and rugged grasslands in New Zealand (Moreno Garcia *et al.*, 2022): Cows' home range, movement tortuosity, elevation range and travelled distance were related to *GRM5* genotypes. The study also reported a predominance of *GRM5* variants *B* and *C*, and a much lower frequency of *A*. A subsampling analysis on the New Zealand data revealed linkages between cattle *GRM5* variation and fifteen key grazing behaviours, and highlighted the consequences on rangelands functioning (Moreno Garcia *et al.*, 2024). We therefore investigated if *GRM5* variant frequencies and relationships with grazing behaviours are similar in cattle farmed elsewhere.

## Materials and methods

The Swiss GPS data originated from two grazing experiments on subalpine pastureland in Switzerland. In the first experiment, Pauler *et al.* (2020) compared the grazing behaviour of cattle breeds in a latin-square design in independent repetitions of relatively small grazing areas (0.3–1.2 ha). In the second experiment, Svensk *et al.* (2021) investigated the grazing behaviour of Highland cattle in shrub-encroached pastures. These paddocks were larger (approx. 5 ha) and included patches of dense shrub as well as open grassland. Nine cows (four Highland and five Original Brown) from Pauler's study and eight cows (Highland) from Svensk's study were still available for blood sampling ( $n=17$ ), and grazing behaviours were calculated by combining 5-min GPS data and digital elevation models as described in Moreno Garcia *et al.* (2022).



Blood samples were collected from the tail vein of the cows (animal testing authorization GR/16/2021). These samples were air dried onto FTA papers for subsequent genotyping of *GRM5* exon 5 using PCR-single-strand conformation polymorphism analysis (Moreno Garcia *et al.*, 2022). After genotyping, linear mixed models were fitted to four grazing behaviours that had been previously associated (home range and movement tortuosity) or trended towards association (horizontal distance travelled and elevation range) with variation in the gene. The models were initially constructed with the New Zealand data of Moreno Garcia *et al.* (2022) and *GRM5* genotype and cow age class were fitted as explanatory variables (fixed factors) when significant. The random structure was nested by cow identity (15-day repeated measurements) and adjusted by herd. Models with lowest Akaike information criterion were then fitted to the Swiss data. The models' predicted mean grazing behaviour for the New Zealand and Swiss cattle were determined for subsequent comparisons.

## Results and discussion

The *GRM5* genotypes found in the Swiss cattle were *AC*, *BC* and *CC* in the proportions of 2:3:4 in Pauler's experiment (Pauler *et al.*, 2020) and 1:0:7 in Svensk's study (Svensk *et al.*, 2021). The overall frequency of variant *C* was 82% and a 9% frequency was revealed for both variants *A* and *B*. Although the *CC* genotype was the most common (65% of tested individuals), cows in the Pauler's study had three genotypes with two to four individuals in contrast with the two genotypes found in the Svensk's herd being seven *CC* cows and one *AC* cow. A possible explanation might be that in the Svensk's herd, all the cows belonged to one farmer, while Pauler's herd was put together with animals from different farmers who may have used diverse selection criteria. The lack of *AB* and *BB* genotypes in the Swiss cattle tested might be attributable to the small sample size, the breeding decisions made by the farmers, or because these genotypes are generally uncommon: within the 306 New Zealand cows all six possible genotypes were present; however, the *AA* genotype was found in only three cows, and *AB* and *BB* (not found in the Swiss cattle) represented only 6% and 12% of the New Zealand herd, respectively (Moreno Garcia *et al.*, 2022). In contrast to the Swiss cattle, the New Zealand cattle seem to be more commonly *BC* or *CC*, with 35% and 36% of individuals respectively, and 10% of cows had the *AC* genotype. These frequencies are similar to the composition found in Pauler's herd with lower proportion of *AC* and higher proportion of *CC*. Despite the geographic distance between the Swiss and New Zealand cattle populations, and the differences in the breeds and livestock systems, we still found an unbalanced frequency of *GRM5* genotypes in favour of variant *C* and, a much lower presence of *A*.

The *GRM5* genotypes in the Swiss cattle seemed to have behavioural relationships that were different to the associations observed in the New Zealand study, where *BC* cows travelled longer distances (significantly different; Figure 1A) but had smaller elevation ranges (Figure 1B), than was displayed by the *AC* and *CC* cows. The three genotypes had similar searching patterns with no differences observed (Figure 1C). The *CC* genotype tended to have a larger home range than the *AC* or *BC* cows (Figure 1D). In contrast, with the New Zealand cattle, Moreno Garcia *et al.* (2022) reported larger home ranges for *AC* (7.82 ha day<sup>-1</sup>) than *CC* cattle (7.29 ha day<sup>-1</sup>) or the *BC* cattle (7.25 ha day<sup>-1</sup>), while the opposite was observed with the Swiss cattle. The *AC* New Zealand cows displayed smaller searching patterns than *BC* and *CC* genotypes, whereas no differences were observed for the Swiss cows.

## Conclusion

This preliminary study exploring relationships between *GRM5* genotypes and grazing behaviours in Swiss cattle found different results to those found with a previous study from New Zealand (Moreno Garcia *et al.*, 2022). It also investigated *GRM5* variant frequencies and revealed the *GRM5* variant *C* to be common, suggesting a potential opportunity for genetic selection to adapt grazing behaviours of cattle to the heterogeneity of the Swiss Alps.

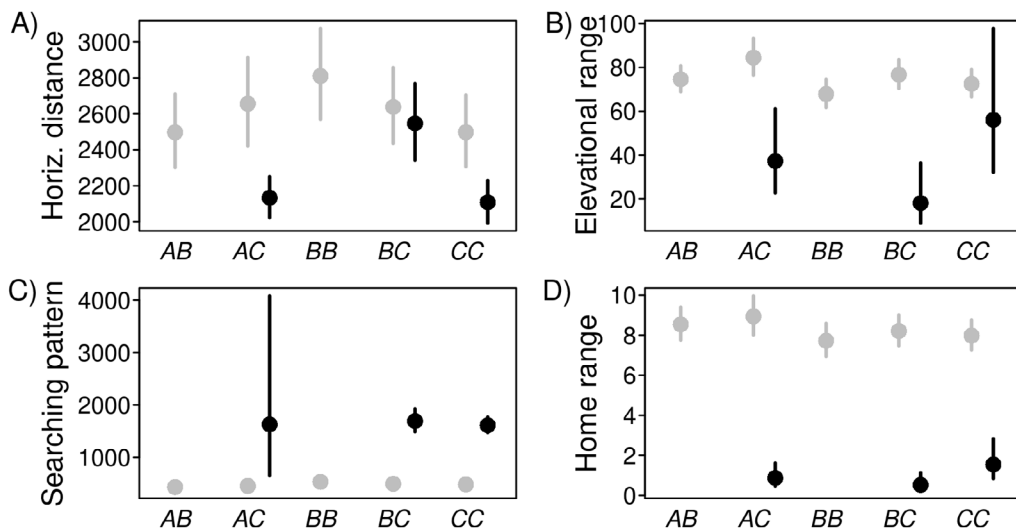


Figure 1. Daily grazing behaviours for Swiss (black) and New Zealand (grey) cows. Dots indicate the marginal means predicted by the linear mixed models for five *GRM5* genotypes. The bars indicate the back-transformed standard errors.

## Acknowledgements

We acknowledge field support by Beat Baier, David Frund and Andreas Wyss and financial support by Swiss National Science Foundation, Deutsche Studienstiftung and Fondation Sur-la-Croix.

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# Comparing grassland management on boreal mineral and peat soils

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## Abstract

Milk production in Finland relies heavily on grassland management. The growing season is short, varying from 105 days in the north to 185 days in the south. The region is characterized by having two soil types: mineral soils and organic soils. Mineral soils are typically well-drained, and have a low organic matter content, whereas organic soils are characterized by high organic matter content and high-water retention capacity. Grasslands have the potential to store substantial amounts of carbon in their roots and soil, making them important for soil carbon sequestration. With this in view, we have initiated a long-term GHG monitoring framework for a sustainable grassland management and agriculture at the Natural Resources Institute Finland (Luke) across several agricultural research sites in Finland. The results presented in this study highlight that boreal legume grasslands managed on mineral soils are environmentally sustainable, whereas those on drained organic soils emit large amounts of GHGs to the atmosphere.

**Keywords:** grasslands, GHG exchange, C sequestration, sustainability, milk and beef production, Finland

## Introduction

Finland relies heavily on forage grasslands cultivated both on mineral and drained organic soils (Kivinen, 2005). In the northern part of the country, grassland cultivation occurs mostly on organic soils. Mineral soils vary from being a small sink to a small source of GHGs emitted to the atmosphere. However, drained organic soils, depending upon their hydrology in terms of whether they are well or poorly drained, can be significant sources of CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O. The aim of this paper is to compare the performance of grasslands in terms of the characterization of their annual GHG balance and grass yield as affected by the seasonal weather, vegetation (legume grassland vs. non-legume forage grasses), soils (mineral vs. organic) and management practices.

## Materials and methods

The following three grasslands under investigation are located near the Luke Maaninka research station in Eastern Finland. (A) Anttila is a 6.3 ha site on a mineral soil with a land use history of bioenergy crops and grasslands, and currently is a legume grassland with a mixture of timothy, meadow fescue and red clover. (B) Särkisuo is a timothy-meadow fescue grassland (6.8 ha) on a drained organic soil with normal and elevated water table levels. (C) Pappilansuo is also grassland (6.9 ha) on a drained organic soil with seminatural vegetation under different tillage options. At each of these sites, CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O flux measurements are being carried out using the eddy covariance (EC) technique (Shurpali *et al.*, 2010). The EC system is standardized at all these sites and consists of a Metek 3D sonic anemometer for turbulent wind components, an IRGA (LiCor 7200 RS) for water vapour and CO<sub>2</sub>, and a laser spectrometer (Aerodyne, Billerica, MA, USA) for water vapour, N<sub>2</sub>O and CH<sub>4</sub> mixing ratios. The 10Hz EC data are stored locally on 16 GB USB disks and on Luke cloud servers. The data are processed, 30 min fluxes are calculated, and missing data gaps are filled as per the standard EC data handling and statistical procedures (Pastarello *et al.*, 2020). After filling the data gaps, the daily, monthly and annual sums of GHG fluxes are computed. Meteorological variables such as air temperature, humidity, shortwave radiation, net radiation balance, PAR, precipitation, atmospheric pressure, soil moisture and temperature, snow depth, soil oxygen content and water table level are monitored as 30 min averages corresponding to 30 min GHG flux values.

Table 1. Various grassland management activities carried out during 2022 at the three research sites in eastern Finland.

Management activity	Anttila	Särkisuo	Pappilansuo
Ditch widening*	–	14 March	–
Date of first fertilization event	23 May	30 May	24 May
Amount of synthetic N applied (N kg ha <sup>-1</sup> )	52	90	84
Date of herbicide treatment	–	8 June	17 June
First grass cut	21 June	30 June	27 June
Summer ploughing	–	–	7 July
Second fertilization event	22 June	4 July	8 July
Amount applied (N kg ha <sup>-1</sup> )	44	69	69
Second grass cut	8 September	15 August	–
Ditch widening	–	9 August	–

Särkisuo, one of the grassland sites on a drained peat soil needed work on widening the ditch for better drainage of moisture. The ditch preparation at this site was started in the middle of March in 2022 and another drainage work carried out in the middle of August.

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With the onset of spring and after snowmelt, all three sites were treated with the first application of fertilizers in late May, and the first grass cuts were made in late June. Within a week of the first cut, Anttila and Särkisuo sites received their second dose of fertilizers. The Pappilansuo site, however, was ploughed in the first week of July, fertilized a day after that for summer establishment of a new grassland cycle. The second grass cuts were taken in early September at the Anttila site, and in mid-August at the Särkisuo site. The planned second cut could not be taken at the Pappilansuo site as it was too wet for the harvesting machines to be worked in the field.

## Results and discussion

From January until early May, all sites emitted low daily amounts of CO<sub>2</sub> to the atmosphere, and no winter associated CH<sub>4</sub> and N<sub>2</sub>O emissions were detected. The onset of spring occurred at the three sites on different dates (Table 1). Despite the delayed onset of spring at the mineral site, it started sequestering atmospheric CO<sub>2</sub> much earlier than the organic soils. The daily GHG flux patterns corresponded well with the management operations carried out, the prevailing weather conditions, and soil characteristics at the respective grassland sites. Considering the net ecosystem CO<sub>2</sub> exchange, the Anttila grassland being a mineral site accumulated the highest amount of CO<sub>2</sub>, the Särkisuo site was a smaller sink, while the Pappilansuo site was a source of CO<sub>2</sub> in 2022. Owing to poor drainage conditions, the organic soils had standing water in the field, thus providing anaerobic conditions conducive for active methanogenesis. The organic grasslands were large sources of methane. The mineral site cultivated with timothy and red clover was efficient in utilizing the applied N fertilizer and was a small source of N<sub>2</sub>O. Owing to poor vegetative growth under wet conditions, the Särkisuo site was a bigger N<sub>2</sub>O source than Anttila. The Pappilansuo site, however, was the largest N<sub>2</sub>O source in 2022 (Table 2). This was because the site did not recover well after the summer ploughing and, as a result, the vegetation growth was too poor to utilize the applied N. During the eight weeks following the summer ploughing the site had high CO<sub>2</sub> losses, implying an enhanced rate of decomposition and N mineralization.

Table 2. Annual individual (CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O) and total GHG balance of grasslands in eastern Finland for the year 2022.

Site	Soil type	CO <sub>2</sub>	CH <sub>4</sub>	N <sub>2</sub> O	Total GHG balance
Anttila	Mineral	-9.2	0.1	1.2	-7.9
Särkisuo	Drained peat	-3.5	3.1	5.1	4.7
Pappilansuo	Drained peat	2.6	1.3	9.0	12.9

All GHGs are expressed as megagram of CO<sub>2</sub>-equivalents per ha per year. Negative number in the table implies that CO<sub>2</sub> is taken up by the ecosystem, while a positive number indicates that CO<sub>2</sub> is lost to the atmosphere.

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## Conclusion

Our results stemming from continuous monitoring of year-round GHG fluxes are very useful in characterizing the grassland biogeochemical processes as affected management practices, soil and vegetation types and the prevailing climatic conditions. Such data are useful in calibrating and validating grassland models for predicting grassland responses to future climate. Our results also show that grassland or any other crop cultivation is not a sustainable option on some organic soils such as at Pappilansuo with poor drainage. While it may be easy to suggest that such soils should be discontinued from traditional agriculture, asking the farmers to adopt alternative organic soil management options such as wetland restoration and paludiculture is complicated under the present circumstances. There are rural employment and socio-cultural issues that need to be considered. Such soils will continue to be cultivated until the national governments intervene with subsidies and incentive plans for adopting alternative, environmentally friendly options.

## Acknowledgement

This work was supported by funding from the Ministry of Agriculture and Forestry Finland (Helsinki, FI) (Project: NC-GRASS: VN/28562/2020-MMM-2).

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# Remote sensing services and drone technology for optimizing grassland management on cattle farms

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## Abstract

The consumer-friendly drone technology has significantly expanded its utilization in agriculture, although widespread adoption to bring economic added value on farms has faced multiple obstacles. Challenges include the absence of efficient operational practices, evolving regulations and technology, specialized camera requirements, complex software needs, data processing complexities, data reliability and the verification of benefits. However, promising applications also exist, including in grassland farming. The 'ForageDrone' project explored the role of service providers in drone operations and the potential drone applications on cattle farms in grass sward management. Beyond conventional data generation and analysis, the project envisioned a comprehensive remote sensing service utilizing the latest technology interfaces in commercial applications. Issues of responsibility remain a challenge. The project examined and piloted applications such as grass sward quality and quantity assessment, weed mapping, flower density estimation, botanical composition analysis, silage volume measurement, quantity change tracking, field anomaly detection and data integration into crop models. Also, various drone-related work tasks were evaluated. Assessment of the technological readiness and commercial potential of these applications revealed that competing with satellite imagery was generally impractical, with the most promising solutions relying on drone-in-a-box-solutions, close-up imaging, or specific wavelengths. Overall, achieving versatility is essential for profitable drone operations.

**Keywords:** grass, forage production, UAV, aerial imaging, drone services

## Introduction

The integration of unmanned aerial vehicle (UAV), or drone, technology in agricultural operations on cattle farms holds considerable promise for achieving sustainable intensification and optimising resource utilisation on farms. However, widespread adoption of this technology faces hurdles not only due to technological limitations but also due to economic challenges and the complexity of applications that are not user-friendly. The objective of this paper is to present the outcomes of the 'ForageDrone' project, which addressed these challenges and explored the potential and level of readiness of various drone applications in optimising grassland management on cattle farms in Finland.

## Materials and methods

The 'ForageDrone' project aimed to evaluate the potential use of UAV technologies in grassland farming operations in Finland. Demonstrations were conducted to showcase and develop various methods; for instance, for mapping grass biomass and quality across different scales, ranging from small experimental plots to larger field parcels. The study, conducted as a part of the project by Oliveira *et al.* (2024) aimed to compare the performance of various imaging technologies, such as typical visible light (spectral bands: red, green, and blue), multispectral (spectral bands: red, green, blue, red edge, and near infrared), and hyperspectral (hundreds of narrow spectral bands) cameras, with an objective of gaining a comprehensive understanding of their potential in estimating productivity and quality development in a typical intensively managed grass ley for silage. Our studies also encompassed an assessment of the performance of different methodologies for processing and interpreting spectral data. This assessment included the utilization of deep neural networks, as discussed by Karila *et al.* (2022). Other piloted

applications included various tasks such as weed mapping, flower density estimation, bunker silo volume measurement, botanical composition analysis, biomass change tracking, field anomaly detection and data integration into crop models. Based on a comprehensive analysis of various studies and pilots, and earlier research conducted by the project team, including those by Näsi *et al.* (2018), Viljanen *et al.* (2018), Oliveira *et al.* (2020) and Kaivosoja *et al.* (2020), we reconciled the current state-of-the-art drone technology and identified the most promising services for enhancing farming practices.

## Results and discussion

Drone technologies are experiencing rapid global advancements, which are continuously expanding their potential applications in the field of agriculture, also in the domain of grassland farming. Additionally, the scope of potential services provided is continuously expanding and evolving, as demonstrated by the diverse range of exemplary services listed in Table 1.

Typically, drone services generate a variety of informative maps that serve to facilitate decision-making processes or that can be utilized as precision farming maps. This kind of service consist of three main components: (1) data capture, (2) pre-processing of the data, and (3) the analysis of the pre-processed data. Offered services may encompass all or a subset of these components. The issue with numerous existing services lies in the absence of the final step, wherein the end-user does not receive the obtained information in a meaningful or practical format. Additionally, farmers may find certain tasks, such as flying the drone, to be excessively laborious or complicated to execute. Drone and imaging technologies, as well as data interpretation, for certain tasks, are still in early stages of development. Consequently, their economic applicability on farms is limited, thereby impeding their widespread implementation. For example, this project found the forage quality mapping to be currently constrained by the high

Table 1. Drone services assessed for their applicability and highest estimated technology readiness levels (TRL; EU) for cattle farms in Finland.

Service type	Service product	Required instrumentation	TRL (1–9)
Aerial canopy imaging	Plant biomass map	MS, RGB, LiDAR	7
	Forage quality map	(RGB), MS, (HS)	5
	Plant density map (overwintering)	RGB	6
	Canopy water stress map	MS, (HS)	6
	Continuous plant development monitoring	RGB, MS (autonomous drone-system)	3
	Nitrogen uptake map	MS, HS	6
3d-mapping	Canopy height map	LiDAR, RGB	8
	Feed volume estimation (e.g. silos)	RGB, LiDAR	7
Canopy close-up imaging	Legume proportion estimation	RGB (high-resolution)	6
	Information for weed protection	RGB (high-resolution)	6
Large area mapping	Field parcel rating	RGB, MS, BVLOS drone	7
Aerial soil imaging	Soil moisture map, drainage monitoring	Thermal camera, RGB, MS	6
Pasture monitoring	Nutrient dispersion map (manure, urine)	RGB, MS	6
	Animal location and movement monitoring	RGB, thermal camera	6
	Grazing pressure optimization map	MS, RGB, LiDAR	3
	Plant biodiversity map	RGB, MS	3
Spreading and spraying	Oversowing, reseeding	UAV-mounted spreader	6
	Fertilizer application	UAV-mounted spreader	8
	Plant protection	Spraying system	6

MS, multispectral camera; RGB, red-green-blue camera (visible light); LiDAR, light detection and ranging; HS, hyperspectral camera; BVLOS, beyond visual line of sight.

complexity of hyperspectral cameras and data processing (Oliveira *et al.*, 2023). Drone-in-a-box solutions, which involve drones flying autonomously and collecting data according to pre-programmed instructions, and sending the data to a cloud processing services, are rapidly advancing and they appear to hold great promise for comprehensive service production. The crucial stage for wider adoption of drone technologies on farms involves integrating the generated data into digital agricultural management software, facilitating easy retrieval and implementation of the information.

## Conclusion

In conclusion, the project has shed light on the promising applications of drone technology by assessing, testing and demonstrating them in grassland farming, and presented the challenges associated with adoption of these technologies on farms. While challenges exist, the potential benefits of drone and remote sensing technologies in optimizing grass sward management on cattle farms make the continued exploration of drone technology essential to improve yields, mitigate environmental impacts, mitigate food security risks, and realise potential economic benefits on farms.

## Acknowledgement

We thank The European Agricultural Fund for Rural Development for financially supporting the 'ForageDrone' project.

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# Effect of vegetation-related parameters on the relationship between Leaf Area Index and yield in meadows

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## Abstract

Leaf Area Index has been proven to be a valid proxy for grassland biomass estimation, but little focus has been put so far on different vegetation-related variables possibly influencing this relationship. In this study we evaluated the effect of vegetation parameters on the LAI–yield relationship. During field campaigns in 2021 and 2022, 1112 samples of both yield and LAI were taken at eight different permanent meadows. Moreover, the phenological stage (PS) of grasses, the yield proportion of grasses (G), forbs (F) and legumes (L) and the resulting sward type, lodging (LO) and herbage moisture (HM) were estimated by visual/sensory assessment and the growth cycle (GC) recorded. A baseline linear mixed effects model including just a quadratic polynomial accounting for LAI was compared to a more complex model including further vegetation-related explanatory variables, accounting for which allowed improving the model fit ( $R^2$  improved from 0.72 to 0.91 and RMSE from 0.94 to 0.53). Dry matter yield was strongly increased by the advanced phenology of grasses and high yield proportion of them. Moreover, the slope of the increase in yield with increasing LAI decreased from the first to the following regrowths.

**Keywords:** botanical composition, growth cycle, remote sensing, phenology, lodging

## Introduction

As global warming threatens forage production due to recurring droughts (Auer *et al.*, 2007; Spinoni *et al.*, 2018), it is crucial to have tools to estimate and monitor grassland productivity, in order to develop or implement adequate management strategies (Vroege *et al.*, 2019). One possible way of estimating grassland yield is the use of the Leaf Area Index (LAI). Due to its relationship with biomass and the possibility to retrieve LAI data at high spatial and temporal resolution from satellite imagery, LAI has a potential to be used as a proxy measure for monitoring grassland productivity (Castelli *et al.*, 2023). In this study we evaluated the impact of several parameters related to vegetation on the relationship between LAI and dry matter yield (DMY).

## Materials and methods

Field campaigns took place on eight permanent meadow parcels per year (altitude: 970–1340 m a.s.l., 2–4 cuts per year) in the Region Trentino–Alto Adige (NE Italy) during the growing seasons 2021 and 2022. At each measurement event, four different spots per parcel were sampled, and coupled measurements taken of LAI with a LAI-2200C plant canopy analyzer (LI-COR, Lincoln, NE, USA) and yield (4 cm cutting height) within a metal frame of 0.25 m<sup>2</sup>. Moreover, the phenological stage (PS) of the ubiquitous grass species *Dactylis glomerata* (from 0=vegetative stage to 8=senescence), the yield proportion of grasses (G), forbs (F) and legumes (L) and the resulting sward type (following the methodology reported in Peratoner *et al.*, 2018; Peratoner and Pötsch, 2019), the extent of lodging (LO) (0=no lodging, 1=light lodging, 2= moderate lodging, 3=strong lodging), and herbage moisture (dry, humid, wet) were estimated by visual/sensory assessment and the growth cycle (GC) recorded. Statistical analysis was performed by means of linear mixed models, fit by maximum likelihood estimation and using a type III sum of squares ANOVA. Denominator degrees of freedom were computed with the Satterthwaite method. A baseline model consisting of a quadratic polynomial accounting for LAI and a random term accounting for the environment (parcel × year) was compared to a model including

further vegetation-related explanatory variables having been found to improve model fit (using the Akaike Information Criterion as an indicator) and having significant effect ( $P < 0.05$ ). As the sum of the yield proportions of grasses, legumes and forbs sum up to 100, only the two functional groups that mostly improve accuracy were used. The independent variables were preliminarily tested for lack of collinearity using the Variance Inflation Factor. The model selection approach was stepwise forward. Due to non-normality of residuals and heteroscedasticity, a square-root transformation was performed on the dependent variable. Both models were validated by splitting the dataset into a training set (70%), and a test set (30%). Model performance metrics ( $R^2$  and the RMSE) were computed based on observed and predicted back-transformed values. All statistical analyses were performed in R (4.2.2).

## Results and discussion

The addition of several vegetation-related variables to the baseline model moderately improved model performance with an increase of  $R^2$  from 0.72 to 0.91 and a decrease of RMSE from 0.94 to 0.53 (Figure 1). In the final model, in accordance with Peratoner *et al.* (2021), besides LAI and LAI<sup>2</sup> (both  $P < 0.001$ ), four other vegetation-related parameters turned out to have a significant effect: PS ( $P = 0.048$ ), its interactions with LAI ( $P = 0.001$ ) and G ( $P < 0.001$ ), GC ( $P < 0.001$ ), LO ( $P < 0.001$ ), and the interactions of LAI with G ( $P = 0.03$ ), L ( $P < 0.001$ ) and GC ( $P < 0.001$ ). PS was the variable that in the first step of model development improved the model the most. As PS refers to *Dactylis glomerata*, a grass species, only the interaction with G was significant. DMY was found to be strongly increased by high values of PS and G (Figure 2a). The second variable that improved the model fit was GC and its interaction with LAI. The slope of the increase in yield with increasing LAI decreased from the first to the fourth regrowth, with DMY differences between growth cycles increasing by increasing LAI (Figure 2b).

The reason for this might be the high proportion of stems during the first growth cycle compared to the following ones, which have a high impact on yield but contribute less to LAI. Also, lodging had a significant effect, as strong lodging seemed to increase the yield prediction. This might be explained by a combination of factors: firstly, lodging occurred only at the later stages of vegetation development. This leads to data gaps at low LAI and therefore to possible uncertainties in intercept estimation. Secondly, the change in canopy structure, where lying grass and stems possibly alter the conditions of light transmittance we would have under no lodging conditions and therefore influence the LAI measurements.

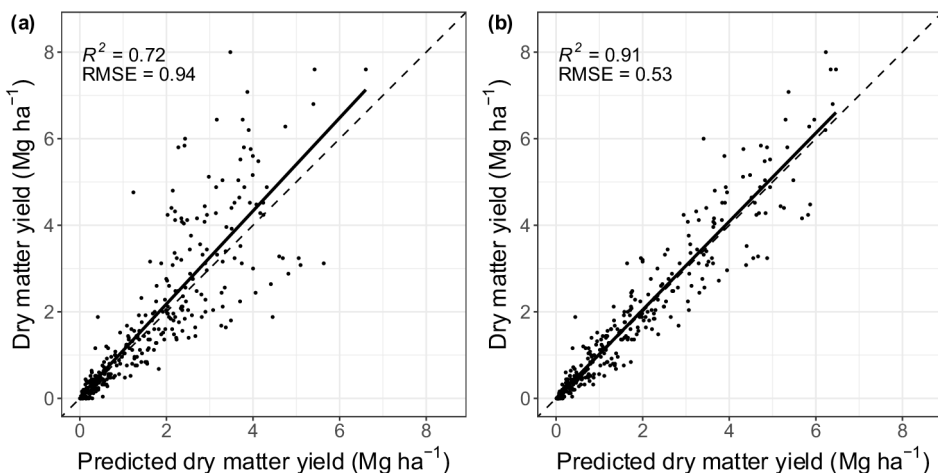


Figure 1. Observed vs. predicted DM yield for (a) the baseline model and (b) the final model including vegetation-related parameters. The dashed line is the 1:1 identity line.

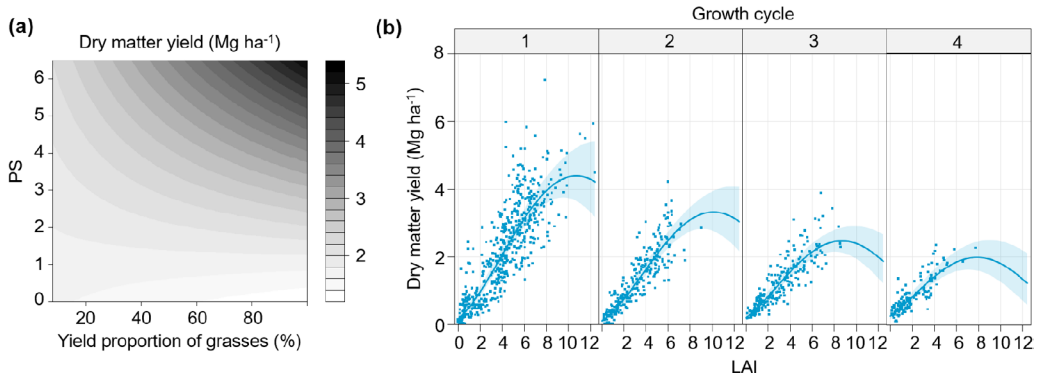


Figure 2. Effect of (a) the yield proportion of grasses and of their phenological stage and (b) the growth cycle on the dry matter yield predicted using LAI. Back-transformed values are shown. In (b), the predicted values and the 95% confidence intervals are shown against partial residuals.

## Conclusion

The results of our investigation highlighted the appropriateness of LAI as a proxy for yield estimation in permanent meadows. However, we found that several vegetation parameters affect this relationship. For the estimation of yield through remote sensing, knowing the phenological stage, growth cycle, yield percentages of grasses and legumes and the extent of lodging would help increasing the estimation accuracy.

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# The plasticity of *Festulolium* varieties in response to contrasted climatic conditions

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## Abstract

*Festulolium* is a productive, good-quality species, which, in comparison with *Lolium perenne*, is more adaptable to unfavourable growing conditions. Its inclusion in grass mixtures therefore, not only improves productivity but it can also promote overall farm efficiency. Research was carried out under different agro-climatic conditions, in Dotnuva, Lithuania and Skriveri, Latvia during 2020–2022. The study aimed to determine the productivity of *Festulolium* varieties and to select the most stable variety for the Baltic region. Three Lithuanian and two Latvian varieties were included in the trials. Dry matter yield, winter survival, and spring and post-harvest growing intensity were evaluated. Overwintering conditions were favourable and no significant differences between varieties were found. Dry and hot weather prevailed in 2021. In contrast, in 2022 (especially during the formation of the first harvest in Lithuania) it was warm and rainy, and the yield of *Festulolium* was 40% higher than in 2021. The location of the study area had a major influence on the yield of the varieties. The most productive varieties were Lina DS (12 859 kg ha<sup>-1</sup>) and Punia DS (12 712 kg ha<sup>-1</sup>), but Vētra with parental origin of *Lolium multiflorum* and *Festuca arundinacea* demonstrated higher plasticity in response to changing climatic conditions.

**Keywords:** *Festulolium*, stability, productivity, winter survival, growing conditions

## Introduction

New strategies, including use of novel varieties with an improved resilience to extreme weather events, are required to ensure sustainability of European-based grassland management systems suitable for livestock production in the face of changing climatic conditions (Berzins *et al.*, 2019; Kopecky *et al.*, 2016). In recent decades, great efforts have been made to combine the persistence and stress tolerance of *Festuca* with the rapid establishment, high productivity and excellent feeding quality of *Lolium* species into one single plant species (Ghesquiere *et al.*, 2010). Sharply fluctuating temperatures in winter and spring months, as well as increasingly frequent periods of drought and heat in summer in regions where they have not been pronounced before, impose new challenges on forage producers and grass breeders (Berzins *et al.*, 2018; Kemesyte *et al.*, 2020). Plasticity and high yield are the main aspects to consider when assessing the performance of *Festulolium*. This study aimed to determine the productivity of *Festulolium* varieties and to select the most stable variety for the Baltic region.

## Materials and methods

The investigations were carried out in two agroclimatically different locations in terms of their rainfall and temperature: in Dotnuva, Lithuania (55°23' N, 23°57' E) and Skriveri, Latvia (56°37' N, 25°07' E), during 2021–2022. Three Lithuanian (Lina DS, Punia DS, Vētra) and two Latvian (Saikava, Vizule) varieties of *Festulolium* were included in the trials. All studied varieties were of the *Lolium* type, but the parental species differed (Nekrošas and Kemešytė, 2007; Berzins *et al.*, 2018). The experiment was set up in 11.1 m<sup>2</sup> test plots using a randomized complete block design with three replications in two cycles sown in 2020 and 2021. The soil conditions were: *Endocalcari–Epihypogleyic Cambisols* (CMg-p-w-can), characterized by sandy loam texture: pH<sub>KCl</sub> 7.7, humus content 22.8 g kg<sup>-1</sup>, available P 215 mg kg<sup>-1</sup> and K 203 mg kg<sup>-1</sup> (by the Egner-Rim-Doming (A–L) in Dotnuva; and *Eutric Retisol* (Aric, Cutanic,

Drainic, Loamic, Ochric) with humus content of 25.1 g kg<sup>-1</sup>, pH<sub>KCl</sub> 6.4, 82 mg kg<sup>-1</sup> P and 92 mg kg<sup>-1</sup> K in Skriveri.

Dry matter yield (kg ha<sup>-1</sup>), winter survival, spring growth and regrowth after cuts were evaluated. The productivity was determined 3 times per season, except at Dotnuva in 2022 (4 times). The winter survival and spring growth were evaluated visually and scored using a 1–9 score scale, in which 1 represents the lowest and 9 the highest value of the performance.

In both years of the study, Skriveri recorded higher rainfall and lower temperatures than Dotnuva. In 2021, a difference of 320.7 mm of precipitation and 0.59°C temperature was observed. In 2022, the difference was 122.1 mm and 0.68°C, respectively.

Shukla's stability variance was calculated using the function 'Shukla', and the genotypic confidence index was calculated using the function 'Schmidt' from R package "metan" v1.18.0 (Olivoto and Lúcio, 2020). Analysis of ANOVA and Tukey HSD tests were conducted using R package 'agricolae' (de Mendiburu and Yaseen, 2020).

## Results and discussion

2021 in Lithuania was characterised by a lack of precipitation during the vegetative season accompanied by high temperatures. Hot and humid weather prevailed as the first cut herbage was formed in spring 2022. This was also reflected in the dry matter yield (DMY) results (Table 2). In Latvia, the most favourable condition was during the formation of the second cut. Annual yield in this location was similar in both years. In contrast, *Festulolium* varieties were twice as productive in the second year than in the first year in Dotnuva. Location had a significant ( $P < 0.05$ ) influence on overwintering, spring growth, and regrowth after cuts. In both study years, the most favourable conditions for *Festulolium* were in Lithuania.

Table 1. The application time and rates of fertilisation for *Festulolium* trials.

Location	Before sowing NPK	In spring	After 1 <sup>st</sup> cut	After 2 <sup>nd</sup> cut	After 3 <sup>rd</sup> /4 <sup>th</sup> cut	Total in the harvest year
Dotnuva	10-120-180	N60	N45	N45	N45*/0	150/195-0-0
Skriveri	24-26-75	105-20-37	N45	N45	15-13-62	210-33-100

NPK, nitrogen, phosphorus, potassium.

\*When 4 cuts.

Table 2. Average performance values at locations and classification of growing conditions according to the genotypic confidence index.

Location, year	DMY (t ha <sup>-1</sup> )					Winter survival	Spring growth	Regrowth after cuts
	1 <sup>st</sup> cut	2 <sup>nd</sup> cut	3 <sup>rd</sup> cut	4 <sup>th</sup> cut	Total			
LT-2021	4 946	2 020	2 070		9 036 <sup>c</sup>	8.89 <sup>a</sup>	8.53 <sup>a</sup>	7.35 <sup>a</sup>
LT-2022	15 232	2 254	432	438	18 356 <sup>a</sup>	9.00 <sup>a</sup>	8.65 <sup>a</sup>	7.48 <sup>a</sup>
LV-2021	6 256	3 593	999		10 849 <sup>b</sup>	8.07 <sup>b</sup>	5.13 <sup>b</sup>	5.87 <sup>b</sup>
LV-2022	7 765	1 854	971		10 590 <sup>bc</sup>	7.80 <sup>b</sup>	6.87 <sup>c</sup>	5.67 <sup>b</sup>

DMY, dry matter yield; italicised values, favourable growing conditions; roman values, unfavourable growing conditions; LT, Dotnuva, Lithuania; LV, Skriveri, Latvia. Growing conditions were determined from DMY, winter survival and regrowth. Letters indicate significant differences ( $P < 0.05$ ).

The highest total dry matter yields were obtained by varieties Lina DS and Punia DS (12 859 and 12 712 kg ha<sup>-1</sup> respectively) (Table 3). Winter survival and spring growth were similar for all varieties. The Lithuanian varieties (Lina DS, Punia DS and Vėtra) showed better regrowth after cuts in Lithuania. The *Festulolium* parent species had a significant influence on the plasticity of the varieties: *Lolium multiflorum* and *Festuca pratensis* hybrids (Lina DS, Punia DS, Vėtra) demonstrated more stable productivity (the rank of Shukla variance from 1 to 3), whereas hybrids between *Lolium perenne* and *Festuca pratensis* (Saikava, Vizule) or between *Lolium multiflorum* and *Festuca arundinacea* (Vėtra) were characterised by better stability in terms of overwintering and tolerance to unfavourable conditions during the growing season.

Table 3. Average values and ranking of varieties according to the studied characteristics.

Variety	Mean				The rank of Shukla variance			
	TDMY	WS	SG	RG	TDMY	WS	SG	RG
Lina DS	12 859	8.64	7.40	7.80	1	5	3	3
Punia DS	12 712	8.42	7.67	7.19	2	4	1	4
Saikava	11 628	8.37	7.12	5.75	4	1	2	5
Vėtra	12 389	8.31	7.38	7.28	3	3	4	1
Vizule	11 449	8.46	6.92	5.46	5	2	5	2

TDMY, total dry matter yield (t ha<sup>-1</sup>); WS, winter survival (score); SG, spring growth (score); RG, regrowth after cuts (score).

## Conclusion

The productive varieties were less tolerant of unfavourable growing conditions. The presence of *Lolium multiflorum* and *Festuca arundinacea* genetic material ensured good performance of the Lithuanian variety Vėtra, which was characterised by stable productivity, overwintering and regrowth after cuts.

## Acknowledgement

The work was funded by the long-term research programme ‘Genetics, biotechnology and breeding for plant biodiversity and innovative technologies’ implemented by LAMMC.

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# Optimizing manure application rate to grass sward ground coverage before and after the winter season

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## Abstract

Manure spreading often leads to nutrient losses with negative environmental impacts, especially in cold climates where harsh winters can affect grass sward density. Nutrient efficiency in cattle slurry depends on the plant coverage at the start of the growing season. To simulate winter damage variation, random mechanical disturbance was applied to a grass field. Aerial images were obtained and analysed using the Grasision® tool to estimate plant cover. Three fixed treatments with uniform cattle slurry and N fertilizer application across all plots, and two treatments adjusting slurry and N fertilizer based on autumn or spring plant coverage were tested. Above-ground yield was measured post-first and second cut. Adjusting N rates based on spring plant coverage or using a low N rate resulted in similar agronomic N use efficiency as high N application rates, albeit with lower dry matter yield.

**Keywords:** fertilizer, Grasision, GIS, image analysis, nitrogen, remote sensing

## Introduction

Spreading of cattle slurry is often associated with losses of nitrogen with negative environmental consequences, especially in animal dense regions. In grasslands, the use-efficiency of nutrients depends on plant density, soil nutrient status and turnover, amount of applied nutrients and cutting regime. In cold temperate regions, harsh winter conditions can also affect the plant coverage at the start of the growing season. Remote sensing tools, including unmanned aerial vehicles (UAVs) cameras, are increasingly used to determine grassland plant coverage and other aspects of grassland status (Wachendorf *et al.*, 2018). Grasision® was developed to determine the coverage of living and dead plants, and bare soil in grasslands from images that can be taken by, e.g. UAV- or tractor-mounted cameras (Rueda-Ayala and Höglind, 2019). The objective of this study was to compare the yield and nitrogen use-efficiency of non-perennial grasslands that were either fertilized uniformly over the whole field or at variable within-field rates based on the plant coverage estimated from images acquired immediately before or after the winter season.

## Materials and methods

The experiment was conducted on forage grass field at the NIBIO Særheim Research Station (58.76° N; 5.65° E). To simulate a wide range of variability in living plant cover, the grass plots were superficially tilled using mechanical tools in autumn 2022 to create three suppression levels (low, high and no suppression) randomly distributed within three repetition blocks. These suppression treatments allowed the provision of areas with a range of living plant cover between <10% and ca. 90%. Red-Green-Blue (RGB) were acquired over the experimental field, using a UAV mounted camera (21 megapixels images resolution) on October 31<sup>st</sup>, 2022 and February 28<sup>th</sup>, 2023. The Grasision® image analysis tool was used to estimate plant cover and create the site-specific fertilizer application map. The experiment included 5 cattle slurry application treatments, including three fixed doses on the whole treatment-plot level (45 m length and 7.5 m width): (i) 0 kg N ha<sup>-1</sup>, “zero”, (ii) 60 kg N ha<sup>-1</sup> applied at start of the growing season, on April 5 and 45.5 N ha<sup>-1</sup> on June 8 (after the first cut), “low”, (iii) 120 kg N ha<sup>-1</sup> applied on April 5 and 91 N ha<sup>-1</sup> on June 8, “high”. There were also two variable-rate slurry doses with site-specific adjustment on each of the 5 subplots (9 m×7.5 m), along the 45 m long treatment-plot. Slurry recommendation was based on the subplot plant coverage, using images taken either on October 31, 2022 (site-specific (autumn))

or on February 28, 2023 (site-specific (spring)) (Table 1). In the spring and after cut 1, 43% and 28% respectively of the total N was provided from manure and the rest from mineral fertilizer. The slurry was applied by a precision manure spreader and the doses were based on the N content in slurry calculated from chemical analyses and the NIBIO Nitrogen animal manure calculator (Husdyr N-Kalkulator) <https://lmt.nibio.no/husdyrn/>. P applications varied between 1.45 and 3 kg ha<sup>-1</sup> and K applications between 15–25 kg ha<sup>-1</sup>; these rates are all above recommended levels for intense forage grass production based on their soil P and K status.

The field was harvested on May 31, 2023 (cut 1) and on August 2, 2023 (cut 2). Dry matter yield was determined after drying samples at 60°C for 48 hours. The Agronomic Efficiency index, i.e. the difference between yield from fertilized plots and yield from the zero fertilization divided by the amount of N applied (Congreves *et al.*, 2021), was used as an indicator of how efficiently the N were used by the variable plant coverage levels to effectively produce harvestable forage matter. A linear mixed-effects model, fitted by the restricted maximum likelihood approach (REML) was applied using R, version 4.3.1 (R Core Team, 2023) and the package nlme (Pinheiro *et al.*, 2023). Dry matter forage yield of the two cuts and their corresponding agronomic efficiency were analysed as the response variables to the suppression and fertilization treatments. The amount of nitrogen applied and the estimated living plant coverage were covariables. Marginal means adjusted with the Tukey HSD ( $\alpha=0.05$ ) method were used to explore differences in model predictions, for the fertilization treatments.

## Results and discussion

The first-cut dry matter yield was significantly higher in the even-fertilizer treatments than in the site-specific, based on images in late October or late February. For the second cut, only the yield in the high fertilizer treatment was higher than the yield in the other treatments, while there was no yield difference between low even-fertilizer and site-specific fertilizer treatments (Figure 1). For the first cut, the N Agronomic Efficiency index was significantly higher in the low-even fertilizer treatment than in the site-specific fertilizer treatments. Also, high-even fertilization resulted in a higher N Agronomic Efficiency index than the site-specific treatment based on images in October. However, for the second-cut yield, there were no significant differences in agronomic N efficiency between the fertilized treatments besides higher efficiency in the low-even treatment than in the site-specific October image-based treatment (Figure 1). These results may not necessarily underline the benefits of applying site-specific image-based fertilizer strategies to increase N-use efficiency and reduce N waste in grassland and ruminant based farming systems. However, yield was affected by rather unusual weather conditions, including a long drought early in the growing season and large amounts of rainfall later. Further analyses from more fields and more than one year are required for better understanding the field plant cover heterogeneity

Table 1. Estimated plant cover (with min and max) and applied N fertilization based on images taken in autumn (October 31, 2022) and in spring (February 28, 2023) on five 9m x 7.5m subplots (45 m long plot).

Suppression level	Coverage autumn (%)	Coverage spring (%)	N applied April 5 (kg N ha <sup>-1</sup> )	N applied June 8 (kg N ha <sup>-1</sup> )
High	0.87 (0.85–0.90)	7.73 (4–11)	0	0.0
High	3.00 (1.56–4.36)	18.14 (18–19)	60	45.5
Low	0.53 (0.12–1.49)	–	0	0.0
Low	5.24 (5.00–5.48)	23.67 (13–36)	60	45.5
Low	–	40.96 (38–44)	120	91.0
None	–	34.74 (30–34)	60	45.5
None	35.34 (0.00–79.66)	50.69 (38–65)	120	91.0



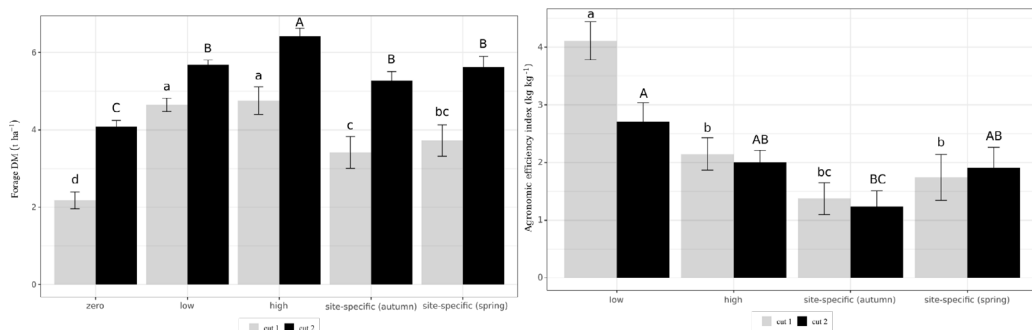


Figure 1. Average DM forage yield (kg 1000 m<sup>-2</sup>) (left) and Average calculated Agronomic Efficiency index (right) per fertilization treatment. Letters indicate differences between the Tukey HSD adjusted marginal means. Lowercase letters represent cut 1, uppercase cut 2.

and N fertilization effects on grassland yield and N-use efficiency. Future assessments will also allow analysing the effect on forage nutritive value. Given the problem with manure surplus in animal dense regions, manure budget estimates for the different fertilization strategies could be extrapolated to farms and regions.

## Conclusion

These initial results enlighten identifying a positive effect of site-specific N fertilisation based on plant cover identification in spring, just before the growing season starts. However, it is still too early to visualise the true value of site-specific fertilisation. More experiments over a longer period of time will clarify and further our understanding of the benefits of site-specific fertilizer application. Additional aspects, such as forage quality and the total amount of manure used per field, should also be taken in consideration.

## Acknowledgement

This project was funded by the Norwegian Agriculture Agency through Climate and Environment Programme (project number 2021/39760, Agros 163320).

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# Seasonal development of *Ostertagia ostertagi* antibodies in milk of grazing dairy cows

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## Abstract

The aim of this study was to evaluate the seasonal development of antibodies in milk associated with *Ostertagia ostertagi*, the most pathogenic gastrointestinal nematode (GIN) species in dairy cows and relate it to different grazing management parameters. Bulk milk samples from Swiss dairy farms were analysed monthly with an ELISA antibody test to determine the optical density ratio (ODR) in 2021 ( $n=15$ ) and 2022 ( $n=25$ ). Milk ODR was used as an indicator of the degree of exposure of dairy cows to *O. ostertagi*. On average, an ODR of 0.82 (SD  $\pm 0.17$ ) and 0.95 (SD  $\pm 0.18$ ) was measured in 2021 and 2022 across all farms. The results showed no significant differences between rotational and continuous grazing systems; however, the ODR differed in relation to the proportion of grass intake on pasture. High variations in the presence of antibodies of *O. ostertagi* between farms were observed. These results may indicate that exposure to GIN could be reduced by optimizing grazing management.

**Keywords:** gastro-intestinal nematodes, grazing management, ruminants

## Introduction

Grazing animals are inevitably exposed to gastrointestinal nematodes (GIN). As GIN become increasingly resistant to anthelmintics (dewormers) (Rose Vineer *et al.*, 2020) a decrease in the pressures associated with infection on pasture would be highly appreciated and help to maintain animal health and performance. Based on the literature, grazing and herd management factors, such as pasture exposure and the date of turnout to pasture, are related to antibody levels in bulk milk (Charlier *et al.*, 2005; Forbes *et al.*, 2008). To the best of our knowledge, the exposure to GIN in ruminants during the grazing season is not well studied. There is a lack of knowledge regarding risk factors related to grazing management such as grazing systems or post-grazing pasture height. Therefore, the aim of this study was to analyse the seasonal development of antibodies in milk associated with *O. ostertagi*, and to relate it to different grazing management parameters.

## Materials and methods

In 2021 and 2022, monthly bulk milk samples from 15 and 25 dairy farms, respectively, were analysed with an ELISA antibody test from Svanovir®. The optical density ratio (ODR) was determined from June to September in 2021 and was extended in 2022 from February to December, to obtain values that cover the entire grazing period. The ODR in bulk milk was used as an indicator of the degree of exposure of dairy cows to *O. ostertagi*. Additionally, in 2022, information on the type of grazing system, stocking density, post-grazing pasture height and proportion of grazed pasture to total feed intake was collected. Pre- and post-grazing height on pasture was measured by the farmers using a ruler, a commercial rising plate meter or a self-made rising plate meter that makes use of a plastic lid as a substitute for the rising plate. If the grass height was measured using a rising plate meter or plastic lid, uncompressed grass height was calculated using the formula described by Steinwigger and Starz (2015). The daily grass intake was calculated based on the feeding ratio reported by the farmers or for full-grazing farms without reported quantities by the farmers. A daily dry matter intake per dairy cow of 18 kg was assumed based on Thomet (2007) and Münger *et al.* (2021). Due to incomplete grazing management records, only data from 13 farms were included in the analysis (Table 1). The cows grazed leys and permanent pastures.

Table 1. Summary of grazing management factors on 13 dairy farms from February to November 2022.

Grazing system	No. of farms	Post-grazing height (cm)	Proportion of grazing DM intake (kg DM (kg daily DM intake) <sup>-1</sup> )	Grazing hours	Stocking density (LU ha <sup>-1</sup> )
RG					
Full-time	4	6.4 (n=3)	0.72	12.0	12.6
Part-time	5	7.1 (n=3)	0.39	8.2	25.8 (n=4)
CG					
Full-time	3	8.3	0.69 (n=2)	13.0 (n=2)	5.5
Part-time	1	2.3	0.23	18.1	37.3

DM, dry matter; RG, rotational grazing; CG, continuous grazing.

Statistical analysis was performed with the mixed effects model package, lmer, in R. Month was included as fixed factor and farm and year as random factors; the monthly ODR in bulk milk was given as the response variable. Additionally, a reduced data set of 13 farms from 2022 was analysed to correlate grazing management parameters (i.e. proportion of grazed grass of total feed intake, pre- and post-grazing height and pasture stocking density) with ODR. A mixed effects model with the respective grazing parameter as fixed effect and the farm as random effect, to account for repeated measurements, was applied.

## Results and discussion

A mean ODR of 0.82 (SD ± 0.17) and 0.95 (SD ± 0.18) was measured in 2021 and 2022 across all farms. The mean ODR values found in this study are comparable to ODR value (0.83) reported by Frey *et al.* (2018) on Swiss dairy farms. The results obtained with the antibody test consider an ODR greater than 0.8 as a threshold for the beginning of production losses due to ostertagiosis (Charlier *et al.*, 2005). Based on this threshold, production losses could be assumed for the majority of the analysed farms as the mean ODR value in 2021 and 2022 was greater than 0.8.

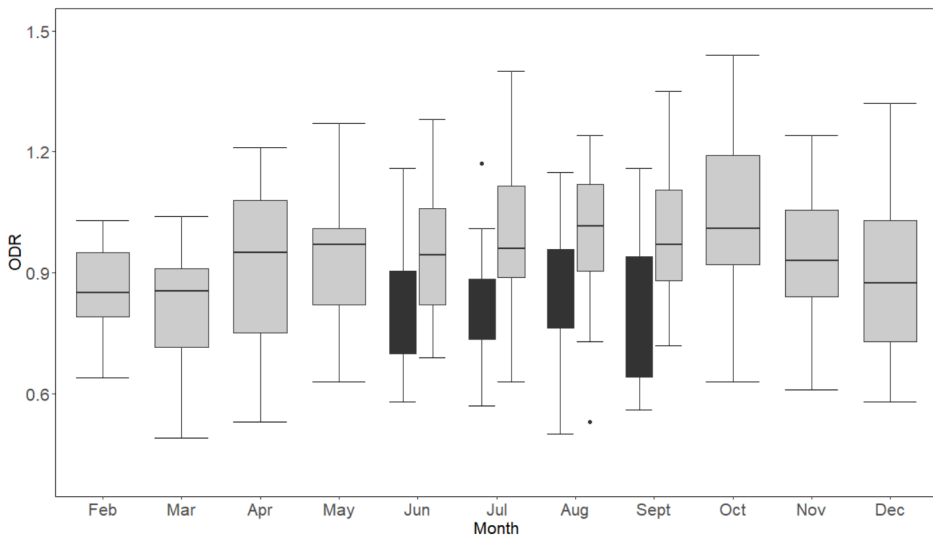


Figure 1. Boxplots depicting monthly exposure of lactating dairy cows to *O. ostertagi* measured as optical density rate (ODR) in bulk milk from Swiss dairy farms from June to September in 2021 (black, n=15) and from February to December in 2022 (grey, n=25).

The highest median ODR in 2021 was reached during August, whereas in 2022 the highest median ODR was reached in October (Figure 1). No differences in the ODR were found between rotational and continuous grazing systems. Nevertheless, a large variation in ODR values between individual farms was observed. Due to incomplete data records, grazing management factors could only be analysed for 13 farms in 2022 (Table 1). In previous studies that analysed the ODR, access to pasture was a key risk factor for infections with *O. ostertagi* (Frey *et al.*, 2018). In our study, farms with proportions of grazed grass lower than 0.25 in the feed ration had a higher ODR than farms with proportions of grazed grass lower than 0.50 ( $P=0.021$ ) and 0.75 ( $P=0.074$ ) in the ration. No difference in ODR was found between farms with a proportion of grazed grass in the ration lower than 0.25 and lower or equal 1.0. The correlation between proportion of grazed grass and ODR in individual months showed a linear correlation between the proportion of grazed grass and ODR with  $R^2=0.43$  and  $R^2=0.37$  in May and June 2022, respectively (data not shown). Our findings should be interpreted with caution as they may only apply to certain months of the year and may be influenced by other factors such as date of turnout to pasture or the type of pasture plant species. Additionally, genetic factors of the grazing animals cannot be neglected.

## Conclusion

Based on the results from this study, the average exposure to GIN increased over the grazing season and dropped when cows were stabled. The grazing system did not affect exposure to GIN. No clear positive correlation between increasing proportion of grazed grass in the ration and exposure to GIN was observed. The best correlation between grazed grass intake and ODR was found in May and June. This may indicate that an evasive grazing management strategy as described by Barger (1997) in spring and early summer could help to postpone high ODR values and thus possible milk yield loss until later in the year. Due to the high variation of ODR values across farms and the relatively small numbers of farms involved in this study, precise conclusions are difficult to define and further studies on the impact of specific grazing factors are required.

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# Spatio-temporal transferability of drone models to predict forage supply in a dryland savannah

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## Abstract

Drone technology offers a cost-effective and reproducible approach to monitor ecosystems over larger areas, but the transferability of developed models is rarely tested. Because rangelands are dynamic, this may limit the prediction of forage resources in space and time. Here, we evaluated the accuracy of drone-based models to predict proxies of forage provision in semi-arid rangelands. We tested models developed for specific scenarios and a comprehensive model, with the assumption that the comprehensive model that captures the broadest range of variability expected in the rangeland system would transfer better than the context-specific models. In line with our expectation, the landscape model consistently outperformed or matched models developed for specific contexts. This study not only addressed the critical concern of transferability but also revealed potential limitations associated with applying context-specific models. Overall, our research advances the integration of drone technology for monitoring resources in spatially and temporally heterogeneous ecosystems.

**Keywords:** model generality, phenological differences, rangeland monitoring, spatial variation

## Introduction

Despite the increasing use of drone technology for monitoring natural resources in expansive areas like rangelands (Gillan *et al.*, 2020), the transferability (Wenger and Olden, 2012) of developed models, often context-specific, is rarely tested. For example, when predicting available forage in semi-arid savannas, multiple factors that affect model generality need to be considered, including the timing and distribution of rainfall, the proportion of bush to herbaceous plants, livestock density and duration of grazing in an area (De Klerk, 2004). Therefore, a crucial step toward reliable long-term monitoring with efficient tools like drones requires developing models that effectively predict resources. Here, we tested three context-specific models: (1) spatial; based on land tenure system, (2) temporal; based on time of the growing season, and (3) spatio-temporal; based on land use type at a specific time of the growing season) and a landscape model to predict herbaceous biomass and land cover. We expected the highest transferability to be achieved by the landscape model, as it encompasses the largest variability of the rangeland system.

## Materials and methods

Our study was conducted in central Namibia's semi-arid savanna, a region that is predominantly used for cattle grazing under two land tenure systems (freehold farms vs. communal areas). The region experiences a typical dryland climate with summer rains (350 mm mean rainfall) exhibiting high variability (Mendelsohn *et al.*, 2002). This results in forage production that is patchy both in space and time. To capture the spatial and temporal dynamics of forage supply in this rangeland system, we collected drone data using a Micasense RedEdge-MX sensor mounted on a DJI Matrice 200v2 quadcopter and ground-truth data to determine herbaceous biomass and land cover. The data acquisition and processing methods

are detailed in Amputu *et al.* (2023). To evaluate the prediction accuracy of the forage supply proxies (using common quality metrics like  $R^2$ , nRMSE and classification accuracy), we conducted four model transfers (Figure 1).

## Results and discussion

We show that all the drone-based models demonstrated high transferability in predicting herbaceous biomass ( $R^2 > 0.798$ ,  $nRMSE \leq 0.195$ ) and land cover (overall classification accuracy  $> 80.2\%$ ). As we expected, the landscape model achieved the lowest prediction error for herbaceous biomass ( $nRMSE = 0.102$ ) and outperformed or matched the case-specific models for land cover (overall classification accuracy =  $91.7\%$ ) (Table 1).

This suggests that this model indeed effectively captured the spatial and temporal variations of forage supply within this rangeland system. It supports the strong recommendation by Rousseau and Betts (2022) about the value of incorporating the entire range of potential ecosystem variation when developing predictive models. In our study system, this meant integrating data that is representative of various rangeland conditions (due to management practices) throughout the growing season (due to phenological differences).

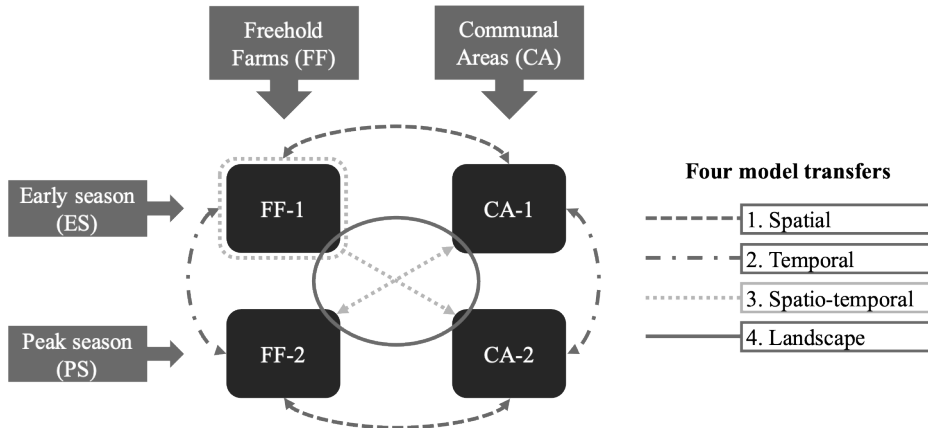


Figure 1. Schematic diagram of the four model transfers conducted across the rangeland system.

Table 1. Model transfer accuracy when predicting herbaceous biomass and land cover.

Model type	Model transfer	Herbaceous biomass ( $R^2$ ; nRMSE)	Land cover (overall accuracy $\pm$ SE (%))
1. Spatial	FF to CA	0.84; 0.13	88.9 $\pm$ 9.5)
	CA to FF	0.86; 0.11	82.5 $\pm$ 9.0)
2. Temporal	ES to PS	0.90; 0.14	80.2 $\pm$ 9.5)
	PS to ES	0.79; 0.17	88.0 $\pm$ 9.1)
3. Spatio-temporal	FF-ES to rest	0.88; 0.11	90.4 $\pm$ 6.1)
	CA-ES to rest	0.85; 0.12	93.9 $\pm$ 2.3)
	FF-PS to rest	0.88; 0.11	91.1 $\pm$ 6.3)
	CA-PS to rest	0.88; 0.20	91.7 $\pm$ 6.4)
4. Landscape	To all scenes	0.87; 0.10	91.7 $\pm$ 4.7)

Lower accuracies for predicting the two forage supply proxies resulted mainly from the temporal transfers. This is likely attributed to the rapid phenological changes in of the herbaceous vegetation that results in varying biomass and spectral features (Wan *et al.*, 2020). Particularly because the study area is dominated by annual herbaceous plants (Ward and Ngairorue, 2000), that undergo fast changes (i.e., greener biomass during the early season vs. more senescent plants later in the season), thereby increasing the predictive errors. Other studies (Théau *et al.*, 2021; Wan *et al.*, 2020) also observed a reduced predictive ability of plant biomass during the growing season due to phenological differences. It is thus worth considering temporal aspects in model development in systems that undergo such rapid and dynamic changes.

## Conclusion

We examined the transferability of drone-based models beyond their training conditions, addressing this often-overlooked aspect. The comprehensive model that effectively captured the spatial and temporal variation of forage supply in this rangeland system achieved the highest prediction accuracy. In contrast, the context-specific models, particularly, the temporal comparisons showed lower transferability, likely due to the rapid phenological changes in the growing season. This underscores a significant limitation in the generalization of such models within dynamic rangeland systems and the constraints that they may impose for broader applicability. Our insights contribute to the advancement of integrating drone technology for monitoring dynamic ecosystems.

## Acknowledgement

The research received funding from the German Federal Ministry of Education and Research (BMBF) under the NamTip project (FKZ 01LC1821B).

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# Ley field management using satellite based digital tools

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## Abstract

Forage grasslands are the most important crop in Nordic countries and the main feeding source for ruminant industries. Improving the economic and ecological performances of farms requires useful and timely field-specific information. Sentinel-1 and Sentinel-2 satellites provide high-resolution open-access imagery that can be used for near-real-time monitoring of vegetation. In this study, forage yield and quality across Sweden were estimated using satellite data from the Sentinel-1 and -2 missions from the European Space Agency (ESA) and weather data from the European Centre for Medium-Range Weather Forecasts (ECMWF) processed using machine learning-based random forest algorithms. The combination of Sentinel-1 and -2 satellites and weather data showed good potential for estimating forage crop parameters ( $R^2$  ranging from 0.42 to 0.73 for the model validation). In the future, the forage biomass and quality estimation models will be integrated into an online open-access decision support system ([www.cropsat.se](http://www.cropsat.se)), which can help farmers to make decisions, such as fertilization and harvest timing.

**Keywords:** forage, biomass, quality, Sentinel-1, Sentinel-2, Sweden

## Introduction

Leys consist of mixed forage crops, and occupy a dominant part of the agricultural landscape in Sweden. Forages from leys form the basis of ruminant diets, and forage intake and quality directly affect milk and meat production. Farmers aim to maximize harvest yield while maintaining a high level of forage quality, a balance that can be challenging to achieve. Satellite time series can be used to assess crop status with promising accuracy (e.g. Söderström *et al.*, 2017). A recent study reported using Sentinel-2 (S2) satellite data to estimate forage biomass (Peng *et al.*, 2023). However, the practicality of using satellite data to create estimation models for various forage quality properties and measures is still limited. Creating robust models to predict crop properties is a way to create a detailed decision tool both in time and space that effectively describes the status of a growing crop. In this study, we used satellite and weather data to estimate forage crop parameters, which could be used to create a helpful decision support system (DSS) for field management.

## Materials and methods

Four field stations across Sweden were selected to take field samples across three harvest periods during summer (from May to September) in 2023. At each field station, two typical ley fields, with timothy grass (*Phleum pratense L.*) as dominant species, were chosen and sampled in two locations, three times before each harvest. The area of each field ranged from 3–26 ha. Lying within a S2 satellite 10-m pixel, at each location, two duplicate plots were marked out and sampled by cutting 5-m long strips using a lawnmower, resulting in a total of 134 pixels sampled. Sample fresh weights were measured, and the dry matter concentration and yield were calculated after oven drying at 60°C for 48 hours. All dried samples were sent to a laboratory to measure forage quality characteristics, including organic matter digestibility (OMD), crude protein (CP), and neutral detergent fibre (NDF).

The spectral data from Sentinel-1 (S1) and S2 satellites and the climate data from ERA5 datasets covering the field sampling periods in each location were downloaded using Google Earth Engine. The downloaded S1 data are synthetic aperture radar data with the VV (vertical transmit/vertical receive) and VH (vertical transmit/horizontal receive) bands from ground range detected scenes under interferometric wide swath



mode, with a 10-m spatial resolution and a revisit frequency of 2–5 days. The downloaded S2 data are multispectral images with blue, green, red, red-edge, near infrared and short wave infrared spectral bands with spatial resolution of all bands resampled to 10 m and a revisit frequency of 2–5 days. The S2 data were pre-processed for cloud masking. The S1 and S2 data were spline interpolated to a daily basis. Several vegetation indices (e.g., NDVI) were calculated based on S1 and S2 data (Blickensdörfer *et al.*, 2022; Peng *et al.*, 2023). The weather data consisted of daily data of common parameters, such as precipitation, air temperature, global radiation and evapotranspiration.

The forage sample parameters (e.g., dry matter yield (DMY) and quality characteristics) were linked to S1, S2 and weather data using the random forest algorithm. With each forage parameter as the dependent variable and the S1, S2, and vegetation indices calculated from S1 and S2 data as well as climate data as independent variables, 75% of the datasets were randomly selected for model calibration, and the remaining 25% were utilised for model validation. The coefficient of determination ( $R^2$ ) was used to evaluate model accuracies.

## Results and discussion

Among different field stations, fields, harvests and samplings, the DMY, OMD, CP and NDF ranged from 43–454 g m<sup>-2</sup>, 640–850 g kg<sup>-1</sup>, 138–296 g kg<sup>-1</sup> and 368–612 g kg<sup>-1</sup>, respectively, which shows variations across the locations (Table 1). The DMY had a greater range than other parameters. The daily temperature and accumulated precipitation during growth season varied from 12–16° and 360–529 mm, which shows clear differences among different sites.

Figure 1 shows the accuracies and agreements between the observed and predicted parameters. Accuracies for model calibration (with  $R^2$  values from 0.94 to 0.97) were higher than the validation (with  $R^2$  values from 0.42 to 0.73), showing an overfitting problem from the random forest algorithm. Peng *et al.* (2023) reported similar overfitting, which can probably be alleviated by merging more data, since machine learning algorithms need large training datasets (Belgiu and Drăguț, 2016). The validation accuracies for the forage quality parameters (OMD, CP, and NDF) were lower than DMY, indicating that predicting these parameters was more difficult. The probable reason is that DMY had a wider range compared to the forage quality data, which was probably derived from the different climate conditions (Table 1). By merging new datasets in the future, the estimation models will be updated and utilised for DSS development.

## Conclusion

In this study, DMY showed greater variability compared to the forage quality parameters. The combination of Sentinel-1, Sentinel-2 and weather data could predict forage crop parameters with promising accuracies. The estimation models will be updated with merging new datasets and utilised to build a DSS for farmer decision making, e.g., timing of harvesting.

Table 1. Coordinates, average daily temperature and accumulated precipitation during May to September as well as distributions (mean ± standard deviation) of DMY (dry matter yield, g m<sup>-2</sup>), OMD (organic matter digestibility, g kg<sup>-1</sup>), CP (crude protein, g kg<sup>-1</sup>) and NDF (neutral detergent fibre, g kg<sup>-1</sup>) for each field station.

Field station	Longitude	Latitude	Temperature (°C)	Precipitation (mm)	DM (g m <sup>-2</sup> )	OMD (g kg <sup>-1</sup> )	CP (g kg <sup>-1</sup> )	NDF (g kg <sup>-1</sup> )
Långhem	13°15'53"	57°35'25"	15±3	529	247±78	740±50	205±27	499±71
Umeå	20°13'45"	63°47'49"	14±4	368	159±53	740±40	206±14	458±36
Uppsala	17°45'34"	59°50'04"	16±3	360	189±88	750±40	230±30	476±45
Östusund	14°20'42"	63°01'15"	12±4	497	215±119	730±50	183±23	502±74

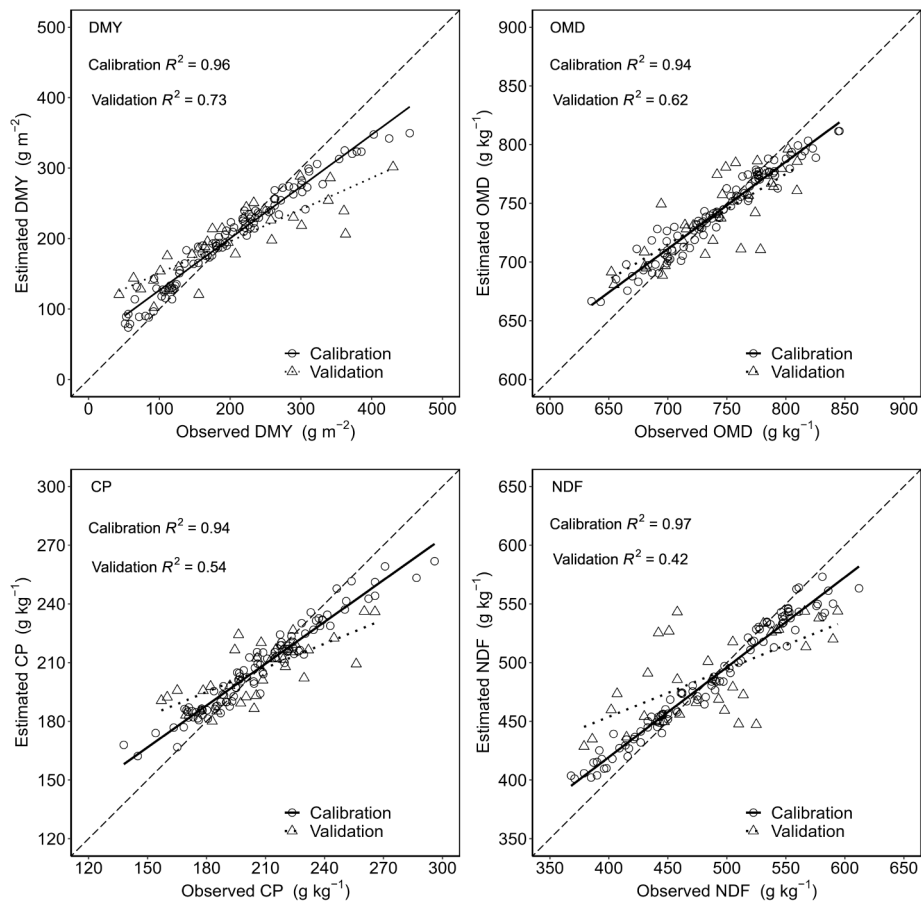


Figure 1. Comparisons of observed and estimated dry matter yield (DMY, g m<sup>-2</sup>), organic matter digestibility (OMD, g kg<sup>-1</sup>), crude protein (CP, g kg<sup>-1</sup>) and neutral detergent fibre (NDF, g kg<sup>-1</sup>).

## Acknowledgements

This study was supported by SLF (Stiftelsen Lantbruksforskning). Appreciation is given to technicians at all field stations for their intensive sampling work.

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# Grass biomass assessment in Wallonia (Belgium) based on satellite imagery and a grass growth model

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## Abstract

In Wallonia (Belgium), grasslands cover 367 200 ha and represent the dominant land cover class (47% of utilized agricultural area). Grassland yields are fluctuating according to different factors such as the pedoclimatic region, the floristic composition, the intensification level as well as management, especially in the context of climatic change. A multi-approach concept based on a large reference field data set, satellite imagery and a grass growth model (GGM) is being developed in the frame of the SUNSHINE project. The objective is to co-construct a Decision Support Tool (DST) adapted to breeders' needs. Intensive field campaigns in grassland parcels have been conducted in 2022 to calibrate/validate the GGM and information extracted from satellite images. Satellite remote sensing (RS) offers opportunities for large-scale monitoring and quantification of grass production across and within fields. This study aims to assess the potential of Sentinel 2 (S2) images to monitor grassland growth. Grass height *in situ* observations were related to Leaf Area Index (LAI) derived from ten S2 images acquired between May and October. Coupling the GGM with RS data allows estimation and prediction of grass growth with an RMSE between 367 and 482 kg dry matter ha<sup>-1</sup> on a daily step, fitting this to breeders needs.

**Keywords:** Sentinel-2, grass growth model, grasslands yield

## Introduction

The management of grasslands is complex and a great challenge in a changing climate. In the case of grazed parcels, farmers need to adjust their practices regularly depending on the spatial and temporal distribution of available grass biomass. This information is usually estimated from time-consuming *in situ* observations or measurements (e.g., with a rising plate meter). Remote sensing (RS) data could be used to help farmers to adapt their practices (Dusseux *et al.*, 2022). Satellite optical sensors provide region-scale spatial information on the status of the vegetation and could be used to map within-field heterogeneity. However, cloud cover can hamper the efficiency of the system, and RS data cannot be used to predict the status of vegetation. On the other hand, process-based dynamic crop models, relying on sets of ecophysiology-related mathematical equations, can predict the productivity of crops using weather forecasts, but due to their formalism they do not account for every factor that can affect plant growth. Moreover, crop models provide field-wise information, whereas in the case of grazing, more detailed (paddock-wise) information is required. Coupling crop models with RS data can provide a tool to estimate and predict the growth of grasslands at field and paddock-scale. In this study, the coupled use of the ModVege grass growth model (Jouven *et al.*, 2006) and leaf area index (LAI) extracted from Sentinel 2 (S2) satellite data (Weiss *et al.*, 2020) has been evaluated to estimate and predict the dry matter yield of grassland fields.

## Materials and methods

Field data were collected over 16 farms located in six different Walloon pedoclimatic regions in 2022. From 56 monitored grassland fields, the only five monitored mowing fields (three temporary grasslands: CO1, CR2, DF4 and two permanent grasslands: RA3, VA4) were considered for this study (Fig. 1). Areas of fields are between 1.2 ha (RA3) and 3.0 ha (CR2) and dominant plant species is perennial ryegrass (*Lolium perenne* L.) and tall fescue (*Lolium arundinaceum* (Schreb.) Darbysh). The field measurements consisted of geolocalized compressed sward height (CSH) measured weekly (50–65 measurements per ha) using an electronic rising plate meter (RPM).

The ModVege model is used in this study to simulate in daily time steps the growth of a homogeneous field of grass based on soil and plant traits, farming practices and weather data.

Bottom of atmosphere (Level 2A) optical RS data were obtained from the S2 constellation, which provides open-access 10m resolution multispectral images with a high revisit frequency (approximately 2.5 days for Belgium). The number of cloud-free images available in 2022 over monitored fields is DF4-12, CR2-15, RA3-32, CO1-34 and VA4-36. The LAI values per field were calculated for all cloud-free available S2 images.

ModVege and LAI-S2 models were calibrated and validated using independent samples of field data. The calibration ( $n=21$ ) of the ModVege was based on data collected on seven micro-plots (9 m<sup>2</sup>) monitored over three cutting events in the period spring to mid-summer. The calibration parameter employed is a plant coefficient (Kc) used to adjust the evapotranspiration of the grass. The coefficient is adapted monthly using data from the Grassim model (Kokah *et al.*, 2023).

## Results and discussion

The ModVege validation was conducted on the five mowing parcels considered for this study in 2022:  $n=122$ , RMSE=481.5 kg,  $R^2=0.76$ .

The LAI values extracted from S2 images acquired simultaneously with CSH field measurements for the 56 grassland parcels in 2022 ( $n=117$ ) were used to calibrate a linear model ( $n=79$ , without the five mowing fields). The validation result using an independent sample, including the five considered parcels, for  $n=38$  is RMSE=366.9 kg,  $R^2=0.78$ .

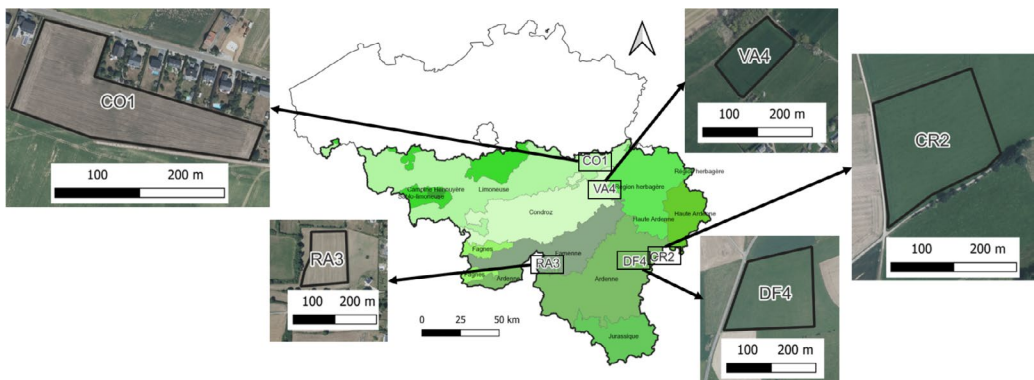


Figure 1. Map of the locations of the monitored fields in 2022. The different pedoclimatic regions from Wallonia are presented.

The simulated values of dry matter (DM) are presented in Figure 2. The CSH values were converted to DM using the formula:  $\text{kg DM ha}^{-1} = 215 \text{ kg DM cm}^{-1} \text{ ha}^{-1}$  (Lefèvre *et al.*, 2022).

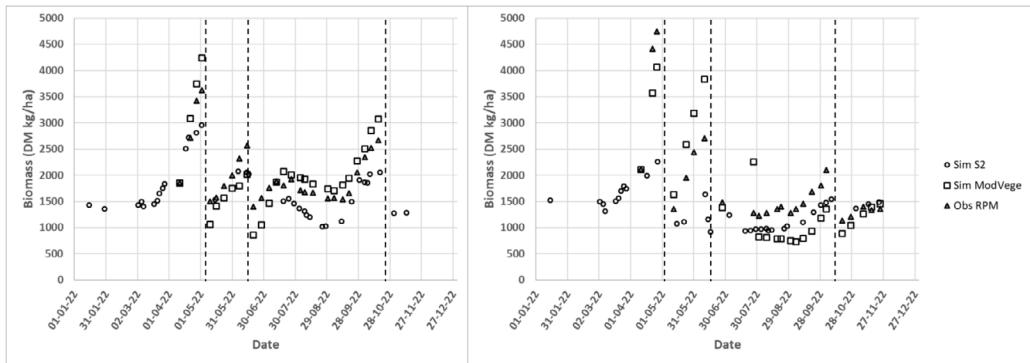


Figure 2. Example of dry matter (DM) temporal development for two grass parcels in 2022. The reference values (from CSH) and results from S2 and ModVege are presented for VI4 (left) and C01 (right) fields. The mowing dates are presented with dot lines.

A better DM estimation from ModVege than LAI-S2 can be observed during the spring-summer period when S2 values are underestimated for higher values of biomass. A possible explanation could be the saturation of LAI estimated from S2 for high values of biomass.

## Conclusion and perspectives

This study demonstrated that Sentinel-2 images provide quantitative information of the biomass status in Wallonia grasslands. The coupling of RS estimates with a grass growth model allows the obtaining of grass biomass information daily. Better calibration of models (RMSE < 400kg) and an adapted coupling method between RS and ModVege will be tested using an enlarged data set.

## Acknowledgement

We thank the Walloon Government for financially supporting the Sunshine research project in the framework of the Walloon Recovery Plan.

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# A preliminary examination of white clover variety performance influenced by leaf size and varying nitrogen levels in grass swards

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## Abstract

The current white clover (*Trifolium repens* L.) varieties within the Irish Recommended List system are only evaluated for total yield and autumn clover proportion. This offers insufficient information to identify varieties capable of maintaining herbage production while supporting Irish targets for reduced N fertiliser use and lower GHG emissions. Seventeen white clover varieties were sown with AberChoice, perennial ryegrass (*Lolium perenne* L.) and one AberChoice monoculture, and evaluated over one year under three nitrogen rates; 0, 75 and 150 kg ha<sup>-1</sup> year<sup>-1</sup>. While there were significant differences in total herbage yield and sward clover content between the varieties and the expected trends associated with leaf size were evident, some varieties did not perform true to type.

**Keywords:** white clover varieties, leaf size, dry matter yield, nitrogen rates

## Introduction

Recent increases in the prominence of white clover use in Ireland can be attributed to the amplified restrictions on artificial nitrogen use and their above-inflation price rises. Additionally, nitrogen fertilisation has environmental impacts through GHG emissions which legislation is seeking to reduce by 25%, by 2030. In response to these challenges, a better understanding of how different clover varieties react to applied nitrogen in PRG swards, is required to guide farmers' reseeding decisions. Enriquez-Hidalgo *et al.* (2016) recorded grass-only swards producing an average of 13 200 kg ha<sup>-1</sup> year<sup>-1</sup> at 240 kg N ha<sup>-1</sup> year<sup>-1</sup>, with PRG-WC swards producing an average 14 080 kg ha<sup>-1</sup> year<sup>-1</sup> at 0N, highlighting the potential to improve economic and environmental sustainability. Additionally increased milk production of 1.6 kg cow<sup>-1</sup> day<sup>-1</sup> has been reported from dairy cows grazing *Lolium perenne* L. (PRG)- *Trifolium repens* L. (WC) swards (Egan *et al.*, 2017). The Irish white clover variety Recommended List system only examines herbage yield and clover content at a single nitrogen level. Variety comparisons of nitrogen fixation capacity, nutritive quality, N tolerance consistency and persistency would identify varieties best able to lower GHG emissions. A first step is to better understand the influence between clover leaf size and N fertilisation rate on variety performance, which was the objective of this study.

## Materials and methods

Seventeen white clover varieties, spanning a range of leaf sizes (Table 1) were sown with AberChoice PRG in 7×1.5 m plots in April 2022, at the Teagasc, Animal and Grassland Research Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland (lat. 50°07' N, long. 08°16' W, 40 m asl). A three-replicate randomised block design, including a PRG monoculture control, were fertilised at 0, 75 and 150 kg N ha<sup>-1</sup> year<sup>-1</sup>, (0N; 75N and 150N, respectively). The plots were mechanically harvested when the average cover reached 1400 kg DM ha<sup>-1</sup>. Pre-grazing heights were measured using a rising plate meter (Jenquip) dropped ten times in each plot. Spring, summer and autumn clover proportions were measured in the ≥4 cm horizon. A 70 g sample, cut by Gardena shears (Gardena, Ulm, Germany) was separated into clover, grass and weed fractions, dried at 90°C for 24 hours and weighed to define DM proportions. Pre-grazing herbage mass ≥4 cm was harvested using an Etesia mower (Etesia UK, Warwick, UK), with a 0.1 kg subsample dried at 60°C for 72 hours for DM determination. Urea (46% N) was hand-applied after each rotation. All data were analysed in the SAS statistical program (SAS Institute, Cary, NC, USA), using

the PROC MIXED function. Dependent variables of herbage yield, clover percentage and pre-heights were analysed with block used as a random variable and class variables of nitrogen application rate, leaf size and leaf category implemented.

## Results and discussion

As expected, herbage yield significantly increased as nitrogen application rate increased ( $P < 0.001$ ). Average herbage yields ranged from 6422 kg ha<sup>-1</sup> (AberChoice/0N) to 12 303 kg ha<sup>-1</sup> (Clodagh/150N), giving a strong positive correlation ( $r=0.71$ ) between nitrogen rate and herbage yield across all varieties and N rates. On average, small-leaved varieties yielded 9888 kg DM ha<sup>-1</sup> and had a clover content of 15.7%, while medium leaf varieties produced 9896 kg DM ha<sup>-1</sup> with an average clover proportion of 15.5%. Large-leaf varieties had 19.2% clover and yielded 9993 kg DM ha<sup>-1</sup>. There was also a significant yield increase associated with greater leaf size ( $P<0.001$ ). A similar trend was also evident when clover content was examined. For example, the large leaved Clodagh had the highest clover percentage (25.1%) which was significantly higher than Iona, at 7.4% ( $P<0.001$ ). These overall responses were as expected, given that Evans *et al.* (1996) have shown that larger leaved varieties tend to achieve greater yields. The varieties differed significantly ( $P<0.001$ ) in herbage yield, with Clodagh giving the highest average yield (10 545 kg DM ha<sup>-1</sup>) and Iona the lowest (9131 kg DM ha<sup>-1</sup>), across all N rates.

Importantly, not every variety conformed to these overall trends. For example, Tasman produced less herbage at 75N than at 0N (9196 and 9322 kg DM ha<sup>-1</sup>, respectively; Table 1). There was also a significant variety×nitrogen rate interaction ( $P<0.001$ ) as variety rankings changed between nitrogen treatments. For example, AberHerald ranked highest at 0N and 75N, but equal lowest at 150N. Likewise AberPearl was lowest at 0N but intermediate at 75N and in the higher group at 150N. Some varieties

Table 1. Relationship between varieties, leaf size, herbage yield and clover proportion

Variety	Leaf size	Leaf category	Herbage yield (kg DM ha <sup>-1</sup> )				Annual clover (%)
			0N	75N	150N	Mean	
AberChoice	–	–	6422	9227	9669	8439	0.7
AberChoice+AberAce	0.26	Small	8728	9998	10834	9853	16.3
AberChoice+Galway	0.36	Small	8376	9656	10776	9603	11.4
AberChoice+AberPearl	0.51	Small	8319	9848	11032	9733	15.1
AberChoice+Coofin	0.51	Small	9521	10251	11316	10363	19.8
AberChoice+AberHerald	0.56	Medium	9951	10443	10618	10337	18.1
AberChoice+Crusader	0.56	Medium	8735	10022	10811	9856	16.5
AberChoice+Iona	0.56	Medium	7551	9225	10617	9131	7.4
AberChoice+Tasman	0.57	Medium	9322	9196	10664	9728	15.9
AberChoice+Buddy	0.58	Medium	8848	9886	10840	9858	13.8
AberChoice+AberSwan	0.68	Medium	9627	9710	10748	10028	19.4
AberChoice+Chieftain	0.68	Medium	8490	9749	11487	9909	15.1
AberChoice+W140134	0.69	Medium	9485	9732	11738	10318	17.9
AberChoice+Alice	0.70	Large	8653	9543	11093	9763	17.5
AberChoice+Violin	0.75	Large	8746	9733	10952	9811	21.9
AberChoice+Clodagh	0.76	Large	9438	9893	12303	10545	25.1
AberChoice+Barblanca	0.76	Large	8658	9863	10734	9751	15.2
AberChoice+Brianna	1.07	Large	9245	9953	11093	10097	16.5
SE	–	–	227.5	227.5	227.5	314.9	4.4

Leaf size and category as determined by the DAFM Recommended List of clover varieties.

also over or under performed the general trend in clover content. For example, Barblanca and Brianna underperformed and Coolfin and AberHerald over performed, based on their leaf sizes. Given the work of Ledgard *et al.* (1996), it would be expected that large leaf varieties that grow more erect and taller, increase in sward proportion because they get access to increased sunlight. It is clear that some clover genotypes are able to dissociate from this trend. Figure 1 compares the extra herbage yield at 0N that each clover attained, compared to the AberChoice monoculture. For example, AberHerald yielded an extra 3529 kg DM ha<sup>-1</sup> ( $P < 0.001$ ). There was also a moderate correlation between autumn clover % and ‘yield-over-AberChoice’, at 0N ( $r = 0.62$ ) which indicates that some varieties may be differing in the amount of herbage they support per plant. It is tempting to attribute this to extra N supplied from biological N fixation, but until this is measured directly, it cannot be assumed. Nonetheless, the differences between the varieties in Figure 1, confirm that varietal variation in this critical parameter may exist.

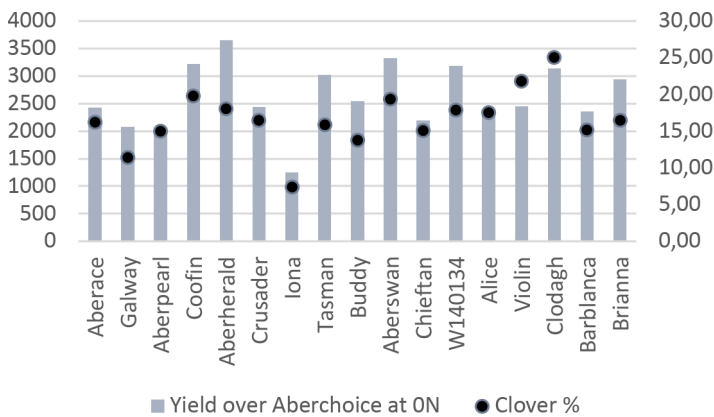


Figure 1. Annual clover percentage and yield advantage of cultivars ranked in order of leaf size over AberChoice at 0N.

## Conclusion

In this preliminary study of clover variety performance, the expected trends of increasing yield and declining clover content with increasing levels of applied nitrogen were observed. Importantly there were varieties that did not perform as would be generally predicted, but supported higher yields and persisted better than others in the same category. If elite clovers are to be identified or bred to assist lower N use and reduce GHG emissions from Irish agriculture, then understanding the causal factors in these outliers could prove critical. Further work will be necessary to understand why some varieties persist better at different N rates, by examining stolon proliferation. The ultimate goal will be to identify easily measured traits that can identify elite clovers for an improved Irish recommended list and to inform breeders' selection criteria.

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# Comparative grazing behaviour and dry matter intake of dairy-beef steers

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## Abstract

Limited knowledge exists on the grazing behaviour of dairy-beef animals, and its subsequent impact on animal performance. The objective of this study was to determine the grazing behaviour and dry matter intake (DMI) of three dairy-beef genotypes: high beef genetic merit Angus×Holstein-Friesian (High AA), low beef genetic merit Angus×Holstein-Friesian (Low AA) and Holstein-Friesian (HF), managed under two contrasting pasture supplementation strategies: Grass-only (GO) and pasture-concentrate (PC). In May and July 2021, DMI (kg day<sup>-1</sup>) was measured using the n-alkane marker technique, and grazing behaviour was measured using RumiWatch recorders (grazing minutes day<sup>-1</sup>, bout duration (min bout<sup>-1</sup>), DMI bout<sup>-1</sup>, bite mass (g), bite rate minute<sup>-1</sup>). In May, High AA had a greater ( $P<0.05$ ) DMI bout<sup>-1</sup> and a longer bout duration ( $P<0.05$ ) than Low AA, with HF being similar ( $P>0.05$ ) to both AA genotypes for both metrics. In May, HF steers consumed an additional 1.0 kg DM of pasture ( $P<0.05$ ) than both High AA and Low AA; however, by July no difference in DMI existed between genotypes ( $P>0.05$ ). In July, no significant differences ( $P>0.05$ ) were detected in grazing behaviour between genotypes. Supplementation at pasture increased total DMI in July for PC ( $P<0.05$ ) for all steer genotypes, whilst also increasing average daily gain. Improving lifetime performance, reduces time to slaughter and inputs required, contributing to increased efficiency of production.

**Keywords:** grazing behaviour, dairy-beef, intake

## Introduction

Grazed herbage is widely acknowledged as the cheapest feed source in ruminant systems (Finneran *et al.*, 2012), and with feed accounting for 75% of variable costs in dairy-beef systems, maximising the quantity in the diet is an integral component of maximising profit (Ashfield *et al.*, 2014). Despite this importance, limited literature exists on the feeding behaviour characteristics of dairy-beef steers, with previous studies examining progeny of the suckler-beef herd (Doyle *et al.*, 2021). Dairy breeds have been shown, when expressed per kg of live-weight, to have a higher intake than early-maturing beef breeds (Keane and Drennan, 2008). Previous studies have shown the weakness of predicting pasture DMI from indoor measurement, due to the low correlation in feed intake measured during the grazing season and indoor period (Clarke *et al.*, 2009). Therefore the objective of this study was to determine the grazing behaviour and DMI of dairy-beef steers of divergent genetic merit managed under two contrasting pasture supplementation strategies.

## Materials and methods

A grazing behaviour and DMI measurement study was completed as part of a wider farm systems study investigating dairy beef systems in 2021 at Teagasc, Animal and Grassland Research and Innovation Centre, Grange, Co. Meath, Ireland. This was set up as a three-by-two factorial design, in which three dairy-beef genotypes: high beef genetic merit Angus×Holstein-Friesian (High AA) ( $n=40$ ), low beef genetic merit Angus×Holstein-Friesian (Low AA) ( $n=40$ ), and Holstein-Friesian (HF) ( $n=40$ ) were managed under two contrasting pasture supplementation strategies: Grass-only (GO) and Pasture-concentrate (PC), with steers assigned to each treatment as calves post-weaning, balanced for genotype, sire, live-weight and age. Pasture-concentrate steers received 2.7 kg DM concentrate per head from July 1<sup>st</sup>. Dry

matter intake was measured using the n-alkane technique (Dillon, 1993), on two occasions (mid-May and mid-July) in 2021, at approximately 15 and 17 months of age, respectively. Steers received a paper bolus (Carl Roth, Karlsruhe, Germany) containing C32-alkane (n-dotriacontane) for 11 days. Representative herbage samples from each pasture allocation were taken from day 6 to 11. Faecal samples from each steer were collected from day 7 to 11 in the morning and afternoon, faecal samples were mainly collected during field observations, with rectal grab sample taken from steers not seen to defecate at pasture.

Grazing behaviour was measured concurrently with DMI on all genotypes in the PC group using the RumiWatch noseband sensor (Iten and Hoch, Liestal, Switzerland). Data collected was converted into 1-hour summaries using the RumiWatch convertor V 0.7.3.36, validated by Norbu *et al.* (2021) for use in grazing studies. Statistical analysis was completed using a linear mixed model in SAS (version 9.4; SAS Institute, Cary, NC, USA), with genotype and treatment as fixed effects.

## Results and discussion

During the early grazing season (May), HF steers had a greater DMI than both High and Low AA ( $P < 0.001$ ) (Table 1). Accordingly, total DMI (TDMI)  $100 \text{ kg}^{-1}$  was greater for HF steers than both AA genotypes. This difference did not persist throughout the grazing season, with all genotypes having a similar ( $P > 0.05$ ) DMI by July (Table 1). Mean substitution rates of concentrate for pasture DM for each genotype were 0.68, 0.79 and 0.84 for HF, High AA and Low AA, respectively. When management was similar (May), no statistical difference ( $P > 0.05$ ) was observed for DMI between management groups; however, once concentrate supplementation was introduced, DMI and TDMI  $100 \text{ kg}^{-1}$  increased ( $P < 0.001$ ) for PC compared to GO (Table 1) as per French *et al.* (2001).

The greater TDMI  $100 \text{ kg}^{-1}$  for HF steers than for either AA genotypes in May is in agreement with previous studies (Keane and Drennan, 2008); however, these differences in TDMI are negligible by July. In May, High AA grazing bout duration was longer ( $P < 0.05$ ) than Low AA, with HF similar to both AA genotypes. Low AA displayed a shorter bout duration, and lower DMI  $\text{bout}^{-1}$  than both High AA and HF; no other statistical differences were noted in grazing behaviour in May ( $P > 0.05$ ). In July, no significant differences were observed in key grazing behaviour characteristics. Notably, during the May grazing behaviour measurement period (GO diet), steers on the current study displayed an eating time ( $\text{min day}^{-1}$ ), bout duration ( $\text{min bout}^{-1}$ ) and bite rate ( $\text{bite min}^{-1}$ ) similar to that reported in Doyle *et al.* (2021) in suckler-beef steers grazing a grass-only diet. Steers in the current study displayed a greater mean bite mass (0.363 g vs. 0.235 g) allowing achievement of a greater total DMI.

Table 1. Effect of genotype<sup>1</sup> and treatment<sup>2</sup> on steer DMI and growth.

	Genotype			SEM	Treatment		SEM	P		
	HF	High AA	Low AA		GO	PC		Geno.	FT	Geno x FT
May DMI ( $\text{kg day}^{-1}$ )	9.03 <sup>a</sup>	7.99 <sup>b</sup>	8.03 <sup>b</sup>	0.211	8.40	8.30	0.172	***	NS	NS
May TDMI ( $100 \text{ kg}^{-1}$ )	2.29 <sup>a</sup>	2.02 <sup>b</sup>	2.05 <sup>b</sup>	0.053	2.13	2.11	0.043	***	NS	NS
May bodyweight	393	398	392	8.1	400	388	6.5	NS	NS	NS
July DMI ( $\text{kg day}^{-1}$ )	9.10	8.96	9.30	0.317	8.80 <sup>a</sup>	9.44 <sup>b</sup>	0.231	NS	**	NS
July TDMI ( $100 \text{ kg}^{-1}$ )	1.97	1.93	2.05	0.064	1.91 <sup>a</sup>	2.06 <sup>b</sup>	0.046	NS	***	NS
July bodyweight (kg)	461	465	456	9.2	461	460	6.4	NS	NS	NS
ADG (May–September) ( $\text{kg day}^{-1}$ )	1.17	1.18	1.15	0.033	0.96 <sup>b</sup>	1.36 <sup>a</sup>	0.027	NS	***	NS

a–b Least square means within rows with different superscripts differ ( $P < 0.05$ ) from each other. High AA, high sire DBI beef sub-index; Low AA, low sire DBI beef sub-index; HF, Holstein-Friesian; GO, grass only; PC, pasture concentrate; DMI, dry matter intake; TDMI, total dry matter intake.

## Conclusion

The inclusion of concentrate late in the grazing season increased DMI and ADG across all three genotypes. Maintaining DMI and growth potential late in the grazing season is difficult due to decreasing pasture quality, therefore concentrate supplementation is an important tool to maintain growth rates closer to the steer's potential, which is vital to improve lifetime performance.

## Acknowledgement

Funding from the Irish Department of Agriculture, Food and Marine Research STIMULUS research grant DAIRY4BEEF is gratefully acknowledged.

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# Evaluating the relationship between sheep grazing offtake and the nutritive value of perennial ryegrass varieties

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## Abstract

In Northern Ireland grazing livestock are supported by grassland, of which perennial ryegrass (*Lolium perenne*) is the major component when reseeded. One key factor is the utilisation of grazed grass, and scientific studies have sought to quantify the animal intake during grazing and the effect of species, variety, ploidy and heading date of forage. For this study, four plot-based ( $n=24$ ) trials were sown in autumn 2020 and 2021 in a randomised block design with intermediate diploid, intermediate tetraploid, late diploid and late tetraploid ryegrass varieties. Each trial was rotationally grazed by 15 ewes across a two-year period. Sward heights were measured with a rising plate meter and the difference between pre- and post-grazing sward heights used as a measure of grazing offtake. Fresh grass samples were collected, dried and milled for NIRS qualitative analysis. In 2022, five of the eight trials analysed showed significant differences in grazing offtake between individual varieties of the same maturity and ploidy. However, there was no discernible relationship between nutritive values and grazing offtake. These results indicate that other factors may need to be investigated such as nitrogen fertilisation, grass leaf lamina and tiller mass.

**Keywords:** grazing offtake, nutritive value, perennial ryegrass, sheep grazing, sward height

## Introduction

Animal productivity has been shown to be linked to forage intake, with grazing efficiency defined as the proportion of herbage ingested relative to that presented (Tubritt *et al.*, 2020). Studies have sought to identify methods for evaluating forage intake in grazing environments and to quantify the effects of species, variety, ploidy and maturity of the forage crop (Byrne *et al.*, 2018; Marley *et al.*, 2007; Tubritt *et al.*, 2020). Few studies have precisely determined the relative importance of each of these factors, although significant differences in variety intake have been shown, albeit on an inconsistent basis (Young *et al.*, 2022). Furthermore, sward management practices, grazing management and environmental conditions have all been shown to affect forage quality (Johansen *et al.*, 2022; Marley *et al.*, 2007; Tozer *et al.*, 2017), which could further impact on grazing efficiency. The aim of this study was to use a grazing offtake evaluation platform combined with near-infrared reflectance spectroscopy (NIRS) herbage assessment to ascertain if differences in grazing offtake between varieties of perennial ryegrass correlate with nutritional value.

## Materials and methods

This study was carried out at AFBI Loughgall (54°27' N, 6°04' W) between 2020 and 2023 (offtake data for 1 year of the 2020-sown trial published in Young *et al.*, 2022). Four separate trials were sown in September 2020 and 2021 in a randomised block design containing eight varieties of perennial ryegrass (intermediate diploid, intermediate tetraploid, late diploid and late tetraploid) from the AFBI forage grass breeding programme. Each block contained 3 replicates sown in 24 plots measuring 2 m × 4.5 m (9 m<sup>2</sup>) each. Diploid varieties were sown at 25 kg seed ha<sup>-1</sup> while tetraploids were sown at 37 kg seed ha<sup>-1</sup> to account for differences in seed size. Fertiliser was applied four times to a total of 288 kg N ha<sup>-1</sup> and 156 kg K<sub>2</sub>O ha<sup>-1</sup> throughout each growing season.

Over a 2-year period, each trial was rotationally grazed by 15 ewes. Sward height was measured pre- and post-grazing using a rising plate meter (Jenquip, EC09). Grazing commenced once the compressed sward height was between 7 and 9 cm and ceased at 4 cm with the difference between pre- and post-grazing sward height calculated as the offtake. To avoid any carry-over of ungrazed herbage to the subsequent grazing event, plots were mown back after each grazing. Fresh herbage samples were collected and dried at 80°C for 48 hours before being ground through a 0.8 mm sieve and analysed using NIRS using calibrations as published in (Archer, 2015) to determine percentage digestible organic matter content (DOMD), water soluble carbohydrate (WSC) and neutral detergent fibre (NDF). Differences in offtake and quality were analysed using analysis of variance to assess the effects of variety testing using Trial Wizard statistical analysis software. Means were separated by Fisher's least significant difference (LSD) test at  $P < 0.05$ .

## Results and discussion

As found by Young *et al.* (2022), in this study five of the eight trials showed significant differences between varieties in grazing offtake (intermediate diploid, late diploid and late tetraploid sown 2021 and intermediate and late tetraploid sown 2020; data not shown). Table 1 presents the grazing offtake and nutritional values from the late tetraploid trial (2020 sowing). There was a significant correlation between grazing offtake and percentage DOMD in the late tetraploid trial ( $r = -0.79$ ); however, this pattern was not reflected in the other trials of this study. Furthermore, there was no consistent pattern throughout all trials between nutritive values (WSC, NDF, DOMD) and grazing offtake.

There are other possible explanations for differences in grazing efficiency that are more closely linked to morphological characteristics, such as the free leaf lamina or tiller mass. Environmental conditions could also play a role such as the addition of nitrogen fertiliser, soil moisture content and the effect of drought or other prevalent weather on growing conditions affecting grazing offtake. Further studies are needed over a number of seasons, or under controlled conditions, to assess the effect of each variable.

Table 1. Grazing offtake and nutritional values for late tetraploid perennial ryegrass varieties\* rotationally grazed by sheep in 2021

Variety	Grazing offtake	Water Soluble Carbohydrate (WSC, %)	Neutral detergent fibre (NDF, %)	Digestible organic matter content (DOMD, %)
Tetraploid Variety 1	8.2 a	7.5 c	46.8 a	84.5
Tetraploid Variety 2	6.9 b	8 bc	46.1 ab	84.5
Tetraploid Variety 3	6.7 b	9.6 a	45.3 bc	86.9
Tetraploid Variety 4	6.3 b	9.7 a	45.5 bc	87.6
Tetraploid Variety 5	6.2 b	8.9 abc	45 c	86.7
Commercial Tetraploid A	6.2 b	7.8 bc	46.1 ab	88.4
Commercial Tetraploid B	6 b	9.1 ab	45.5 bc	87.5
Tetraploid Variety 6	6 b	9 abc	45.9 abc	86.7
CV (plots)	10.8	9.6	1.2	2
LSD (0.05)	1.2	1.5	0.9	3.1
F varieties	3.2	2.9	3.3	-1.9
P-value	<0.05	<0.05	<0.05	NS

Tetraploid varieties 1–6 were bred in 2017 from the AFBI perennial ryegrass breeding programme pipeline at Loughgall, NI. Letters denote significant differences between varieties (Fisher's least significant difference test;  $P < 0.05$ ).

## Conclusion

During the 2022 harvest, five of the eight trials analysed showed significant differences in grazing offtake between individual varieties (of the same maturity and ploidy), suggesting that animal studies for this trait can reveal differences in animal preference regarding grazing offtake even in similar varieties. However, the impact of trial management factors remains to be fully elucidated. This study aimed to assess the impact of nutritive characteristics, such as percentage WSC or DOMD on grazing offtake; however, there was no discernible pattern noted across the trials. These results indicate that other factors may yet prove to be significant characteristics in studying grazing uptake in perennial ryegrass and that further research is necessary to quantify the effect of these such as nitrogen addition, free leaf lamina and tiller mass.

## Acknowledgements

The authors thank the contribution made by AFBI staff at Loughgall and Hillsborough. This work was funded by DAERA in Northern Ireland. Late-stage funding for varieties included in these trials was provided by the Royal Barenbrug Group.

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# Satellite imagery to categorize botanical composition in an alpine pasture

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## Abstract

Pastures frequently exhibit a high heterogeneity and monitoring vegetation canopy can improve sustainable management strategies. Satellite imagery provides a unique opportunity to analyse pasture cover at spatial and temporal scales. The study seeks to demonstrate the use of satellite imagery to categorize botanical composition in an Italian alpine pasture. Pasture vegetation was grouped into three categories based on species abundance from Braun-Blanquet surveys: (1) vegetation dominated by typical local pasture species; (2) vegetation dominated by broad-leaf weeds; and (3) a mix of 1 and 2 vegetation types. High-resolution satellite Sentinel 2 imageries were used to calculate vegetation indices (i.e., NDVI, EVI, NDRE) for categorizing botanical composition of types 1, 2, and 3. Some of the vegetation indices assessed showed the potential to discriminate pasture vegetation types and satellite imagery can be utilized to characterize and monitor vegetation diversity and aid farmers and institutions in determining specific pasture management.

**Keywords:** remote sensing, vegetation categories, vegetation indices, degraded pasture

## Introduction

Grasslands provide multiple ecosystem services. Proper management of pastures is crucial for the maintenance of these ecosystems and their benefits. Remote sensing imagery can be used in managing decisions collecting data from different sensors (Li *et al.*, 2017) or satellites (Wang *et al.*, 2022). An important parameter estimated through remote sensing is aboveground biomass (Xu *et al.*, 2020). Vegetation biophysical attributes have also been estimated (Masenyama *et al.*, 2022) as well as biodiversity indicators (Imran *et al.*, 2021). Furthermore, multispectral images of optical sensors have been successfully used to model vegetation type (Rapinel *et al.*, 2019), vitality (Reinermann *et al.*, 2019), and phenology (Miao *et al.*, 2017). The purpose of this study is to evaluate the use of satellite-acquired data to characterize three different types of vegetation on a degraded alpine pasture to help farmers in monitoring vegetation changes. and manage pastures efficiently.

## Materials and methods

This study was conducted in a pasture in Caltrano (Vicenza province, north-eastern Italy; 45.8093° N, 11.4656° E, 1200 m a.s.l.) characterized by high vegetation heterogeneity. The mean annual temperature of the study area is 10.5°C, and the annual rainfall is 1502.2 mm. The pasture surface was split into 25 areas with homogeneous vegetation type and ground morphology. In each area a botanical survey (Braun-Blanquet method) was carried out on a 100 m<sup>2</sup> plot recording all species and their cover percentage. Botanical surveys were used to map the grazing surface into three vegetation types: (1) slightly sloping area with vegetation dominated by typical local species such as *Phleum pratense* L., *Achillea millefolium* L., and *Trifolium pratense* L.; (2) slightly sloping area with vegetation dominated by broad-leaf weeds i.e. *Urtica dioica* L.; and (3) slightly sloping area with a mix of vegetation types 1 and 2. These areas with the vegetation types 1, 2, and 3 were overlapped with 30 random plots of 1 m<sup>2</sup> wide (19 for type 1, 4 for type 2, 7 for type 3) where the above-ground biomass was collected and oven-dried at 105°C for 48 hours to determine dry matter (DM) yield. For each plot, high-resolution satellite Sentinel 2 imageries were used to calculate vegetation indices (Xue and Su, 2017): Enhanced Vegetation Index (EVI), Normalized Difference Vegetation Index (NDVI), Green Normalized Difference Vegetation Index

(GNDVI), Normalized Difference Water Index (NDWI), LAI determining index (LAI), Normalized difference red edge index (NDRE), Canopy Chlorophyll Content Index (CCCI), Chlorophyll Green (Chlgreen), Chlorophyll Red-Edge (Chlred-edge), Three Band Dall’Olmo Index (DO), Normalized Area over reflectance curve (NAOC), Normalized Difference Tillage Index (NDTI). Generalized linear models were built to explain variation in each index depending on vegetation type, and its significance was determined by likelihood-ratio tests (LRT) of reduced versus full model. All statistical analysis was performed in R (R Core Team) using the ‘nlme’ package.

## Results and discussion

The vegetation indices affected by vegetation type were Chlgreen, NAOC, and NDTI, while for EVI, LAI, NDRE, CCI, and Chlred-edge, the significance was lower than 0.1 (Table 1).

The considered indices target different regions of the electromagnetic spectrum, thus detecting various physical and chemical features of the earth’s surface and vegetation cover. As an example, the Chlgreen index, developed by Gitelson *et al.* (2006), uses the reflectance in the green and near-infrared regions to evaluate the chlorophyll content of plants’ leaves. Carmona *et al.* (2015) created the NAOC index to measure chlorophyll content using 643 and 795 nm red-edge wavelengths. However, since the exact two spectral bandwidths are unavailable, the closest available bands from Sentinel-2A satellite sensors, which

Table 1. Significances based on likelihood-ratio tests and standard error of differences of the vegetation type in generalized linear models built using vegetation indices.

Index	P $\chi^2$	Standard Error
EVI	0.098	–
NDVI	0.233	–
GNDVI	0.171	–
NDWI	0.172	–
LAI	0.098	–
NDRE	0.061	–
CCCI	0.061	–
Chlgreen	0.012	0.006
Chlred-edge	0.061	–
DO	0.256	–
NAOC	0.049	0.007
NDTI	0.033	0.012

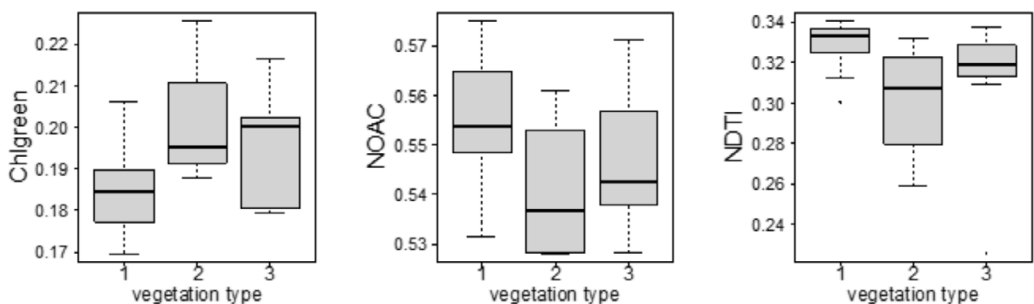


Figure 1. Boxplot for the vegetation Chlgreen, NAOC, and NDTI as affected by vegetation type: 1, vegetation dominated by typical local species; 2, vegetation dominated by broad-leaf weeds; 3, mix of vegetation types 1 and 2.



are 665 and 783 nm, were used instead. Normalized Difference Tillage Index has been created and used to evaluate the response of soils to tillage operations (Stern *et al.*, 2023). It uses the bands belonging to the short-wave infrared spectral region, so it focuses its action on the evaluation of humidity and water content, which also may be useful to discriminate different populations of plants.

The vegetation type influenced in a different way the vegetation indices (Figure 1). Chlgreen mean value was lower in pasture areas dominated by typical local species (type 1) than in pasture slightly invaded by broad-leaf weeds (type 3). NOAC and NDTI values were higher in pasture areas dominated by typical local species (type 1) than in areas dominated by broad-leaf weeds (type 2). Although vegetation type 3 combines type 1 and 2, it showed values closer to type 2 than to type 1, not only for Chlgreen, NOAC, and NDTI, but also for indices with a significance lower than 0.1 (data not shown).

## Conclusion

Most vegetation indices showed the potential to discriminate vegetation type when analysed individually, even with different levels of significance. Thus, the development of statistical models combining different indices exploiting the power of machine learning algorithms may further improve our ability to characterise and monitor pasture vegetation using remote sensing technologies.

## Acknowledgements

This study was carried out within the Agritech National Research Center and received funding from the European Union NextGenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022). This manuscript reflects only the authors' views and opinions, neither the EU nor European Commission can be considered responsible for them.

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# Application of the repellent TRICO as a method to reduce mortality of fawns at grass harvest

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## Abstract

Grasslands provide many ecosystem services, including a suitable habitat for wildlife such as deer and birds. However, previous studies have reported an expected mortality of 8000–20 000 fawns per year during the grass harvest season in Denmark. The purpose of this study was to evaluate the effectiveness of spray application of the repellent TRICO in grasslands before mowing, so the doe would remove her fawns before mowing. The study was conducted in May/June 2023 on 300 ha grasslands. Detection of fawns was conducted during the night with UAV with both thermal and RGB cameras. Twelve fawns were identified before the application of TRICO. The repellent was applied in a dose of 15 l ha<sup>-1</sup> in a 50% dilution with water the following day, in 3 m bands with a UAV (DJI Agras T10). The repellent was only applied in 3 m bands as a perimeter 20 m from the field boundary, so the treated area was only 6.7%. In the night after application, the areas were monitored again with the same cameras used before the application. The result of the study was that the doe removed 11 out of 12 fawns from fields applied with the repellent, while no fawns were removed from a field where a UAV had been flown, but without spraying with TRICO. Our study suggests that spray application of TRICO can be an effective measure to reduce mortality of fawns in grasslands used for cutting but this needs further tests regarding the required dose, repellent resistance to rain, and longevity.

**Keywords:** fawn, mortality, grass, harvest, repellent

## Introduction

Grasslands provide many ecosystem services, including a suitable habitat for wildlife such as deer and birds. However, previous studies have reported an expected mortality of 8000–20 000 fawns per year at mowing grass in Denmark (Olesen *et al.*, 2017). During the harvesting of grasslands, agricultural machinery negatively affects a vast number of animal species. European hare (*Lepus europaeus*) and roe deer (*Capreolus capreolus*) are most threatened, as juveniles in danger press against the ground trying to protect themselves from predators (Steen *et al.*, 2012). Roe deer fawns are “hidiers” and during the first 4–6 weeks mostly stay hidden alone in the vegetation (Jarnemo and Liberg, 2005) and their mother visits them 3–7 times/day (Espmark, 1969). For this reason, there is a high risk of young roe deer being killed by the cutter bars. The harvesting season of fodder crops in Central Europe also overlaps with the roe deer birth season, whose peak falls between 20<sup>th</sup> May and 10<sup>th</sup> June (Linnell *et al.*, 1998).

Placement of flags and other unfamiliar objects in the field has proven to be effective (Jarnemo, 2002), but time-consuming when large areas require a preventive action. Monitoring using UAVs with infrared cameras is superior for detecting the fawns but requires human replacement of the fawn, which might implicate a higher risk of mortality by mammalian predators and birds of prey. New harvest equipment like Pöttinger Sensosafe, where the cutter bar is lifted when fawns are detected, also reduces the instantaneous mortality at harvest but this may also implicate a higher risk of mortality by mammalian predators as the fawns are exposed in uncut patches in the fields. The objective of this study was to evaluate the effectiveness of spray application of the repellent TRICO in permanent grasslands before mowing, so the deer would replace the fawn before mowing. TRICO contains sheep fat as the active

ingredient in a liquid suspension originally designed as a deer repellent in forestry to protect emerging trees from the browsing and rubbing of deer.

## Materials and methods

The study was conducted in May–June 2023 at 300 ha of extensive permanent grasslands, managed for haymaking. Detection of fawns was conducted during 3 nights with 2 DJI Matrice 300 with both thermal (640×512px @30Hz) and RGB (20Mp 23x Zoom) cameras with 60 m height at 3.8 m s<sup>-1</sup>. Thermal images were stitched using the software Pix4D. Subsequently, all thermal signals >20 pixels (15×30 cm) were GPS-tagged for verification the following morning also with the DJI Matrice 300 UAV with the RGB zoom camera at 60 m height to verify whether the thermal signal was caused by a fawn or an artefact.

In fields with verified fawns the repellent TRICO was applied in the afternoon at a dose of 15 l ha<sup>-1</sup> diluted 50% in water and sprayed in 3 m bands as a perimeter at 20 m from the field boundary with a UAV (DJI Agras T10) at 2 m height mounted with 4 Lecher 040 injection nozzles. The area treated was only 6.7% of the entire field area, as only 3 m bands were applied.

Two random fields with detected fawns were overflown by the UAV, but without application of repellent to test whether the UAV itself could cause a replacement of the fawns. The following night after application, the areas were monitored again with the same thermal camera during the night and the RGB camera for subsequent verification.

To test if the application of the repellent harmed the palatability of the feed, a small experiment was made comparing grass (tall fescue cv. Swaj) with or without TRICO at 15 l ha<sup>-1</sup> in a 25% dilution applied with a field sprayer the day before cutting. The grass was subsequently wilted to 52–67% dry matter and baled (500 kg) and ensiled for 142 days. The treated and untreated grass silages were fed simultaneously to a herd of 15 Hereford suckler cows in a barn. The obtained data was analysed in R using a chi<sup>2</sup>-test.

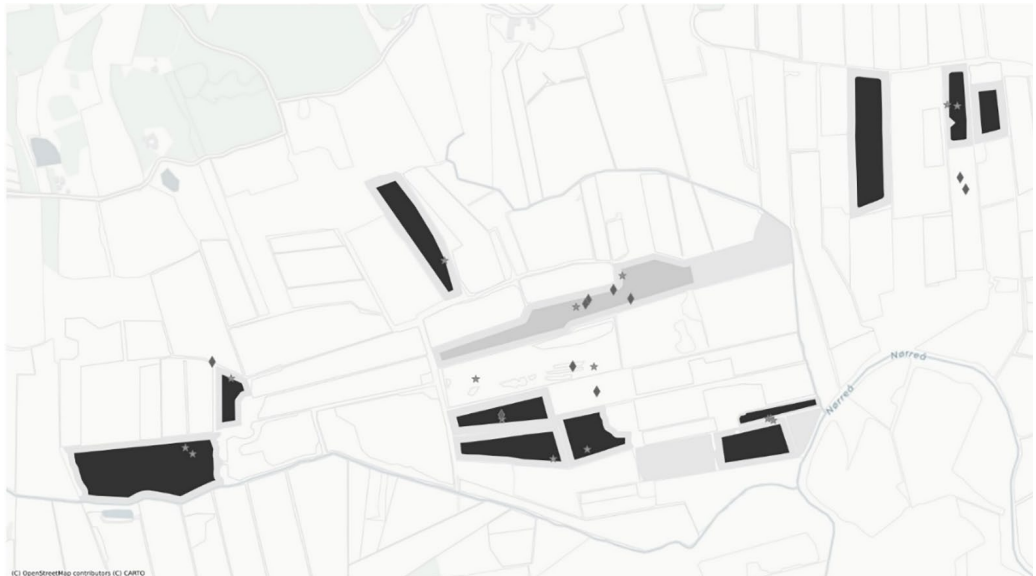


Figure 1. Localisation of fawns before (stars) and after (diamonds) application of the repellent TRICO. Black fields indicate fields applied and grey fields are not applied with the repellent as control.

## Results and discussion

Thermal monitoring for three nights and subsequent verification with an RGB zoom camera resulted in the identification of 12 fawns located at 9 different fields as shown in Figure 1.

Eleven out of 12 fawns were removed by the doe from the fields after application of the repellent, while the number of fawns in fields without application of the repellent increased despite the disturbance of the UAV as control treatment. Using a chi<sup>2</sup>-test, the decrease in fawn intensity at the level of individuals or fields is significant at 0.03. The fawns were not tagged, so it was not possible to record the movements of the individual fawns and hence not possible to ensure whether the fawns identified before the application were the same fawns located in adjacent fields after the application. The small feeding test with Hereford suckler cows did not show any effect of the repellent application on the palatability when grass was ensiled; whether the repellent would induce a negative effect when grass is conserved as hay is unknown. At current prices, the treatment with TRICO costs appr. €20 ha<sup>-1</sup> including repellent and application. The price could be reduced to 11 euros ha<sup>-1</sup> if an application rate of 7.5 l TRICO ha<sup>-1</sup> is sufficient. In this study, the application of the repellent was made exactly the day before monitoring or mowing in good conditions without any precipitation between application and monitoring. In real life, the weather influences the timing of mowing and hence the time from application of the repellent and mowing changes according to the weather conditions. This study cannot quantify the longevity of the repellent, nor the resistance to rain. Olesen *et al.* (2017) and Osada *et al.* (2014) reported an aversive effect of pyrazine cocktails like predator urine. Pyrazine compounds might also be an effective measure to reduce the mortality of fawns and European hares in grasslands.

## Conclusion

Based on this study, we conclude that spray application of the repellent TRICO can be an effective measure to reduce mortality of fawns in grasslands used for cutting, but needs further testing regarding the required dose, repellent resistance to rain and longevity.

## Acknowledgement

We would like to thank “Jægernes Naturfond” who founded this study.

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# Examination of grazing sustainability of grasslands in southwest Hungary

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## Abstract

There is little information on plant composition, quality, productivity potential, and the technical management in Hungarian grasslands. The aim of the project was therefore to carry out a phenological survey to determine grazing sustainability. The 10 examined grasslands have been used for cattle grazing. A phytocoenological table was set up, including the T (temperature), W (water), R (pH), N (nitrogen) and nature conservation indicator values of species. We assessed the coverage rates and determined the dominant species. We also determined the quality value of grazing of the grasslands. Based on the results we examined what agricultural sward maintenance, repair and renovation options could be considered in the light of sustainability. Depending on the use and intensity, some grasslands were poor (<20 species), while others were diverse (>40 species). In five of the examined grasslands *Cynosurus cristatus* was dominant, which would indicate the natural state of the grasslands. However, in these grasslands the number of disturbance-tolerant and weed species was high. The quality of *Cynosurus* grasslands as forage was better than that of the other investigated habitats. Degradation problems from overgrazing have also become visible, where it is absolutely recommended to cover the stock primarily with drought-tolerant and pH-neutral or lime-loving species.

**Keywords:** sustainability, quantity, quality, phytocoenology, indicator species

## Introduction

There is little information about plant composition, quality, productivity potential, and the technical management of Hungarian grasslands. The aim of the project was therefore to carry out a phenological survey in order to determine grazing sustainability. Maintenance and renovation plans will eventually be necessary to improve the species composition of grasslands. Collecting emergence or absence data of bioindicator species is a practical tool to make a quick assessment about grassland condition. In the EU, grassland preservation also needs corner stones to evaluate certain species and help in maintaining swards in Europe.

## Materials and methods

Ten sward areas were examined in the South-Transdanubian region of Hungary, (Kistotvaros settlement, owned by Zselicfarm; 46.249668° N, 17.913792° E). The areas are grazed by cattle, grazing period April to October every year, during which time the cattle are constantly in the fields. There was no previous topping or replanting in these grasslands. The swards were measured using the modified Braun-Blanquet method. We determined the occurrence of plant species and their percentage cover, and recorded the GPS coordinates. Sampling took place in five quadrats (2×2 m) per homogeneous habitat, in which we measured percentage cover value of each plant species, separately for the *Gramineae* and for other plant species. The plant associations were identified based on the works of Borhidi (1995). During the recordings, a phytocoenological table was set up, which contains Ellenberg's (1974) scale-system values of the association-forming plant species: T (temperature, i.e. heat value) to determine the climate of the given area; W (groundwater, soil moisture), to determine the water balance characteristics of the area; R (pH) to determine the soil reaction, N (nitrogen demand) to determine the nitrogen supply of the area. The conservation value categories (TVK) of individual plant species were also defined. Finally,

applying the Braun-Blanquet's six-degree abundance-dominance (A–D) scale (Poore, 1995) with regard to the previously determined plant species, the obtained cover rates were summarized. We determined the possibilities of use for livestock, and also formulated the necessary grassland maintenance and amelioration tasks in order to achieve sustainable grazing.

## Results and discussion

Based on heat value (T), all the sample areas belong to the temperate deciduous forest climate (*Cynosurus cristatus*, *Elymus repens*, *Festuca ovina*, *Alopecurus pratensis*), but in all areas the sub-Mediterranean character appears. Two areas (1, 2) also have a minimal Mediterranean effect, indicated by *Festuca myuros*, *Cynodon dactylon*, and *Bromus commutatus*. According to the water balance (W), the habitats are basically between the moderately dry and moderately wet categories. The highest cover values (32–45%) represent *Cynosurus cristatus*, *Festuca ovina* and *Trifolium repens*, which is ideal for the utilization of grasslands. Species with particular dry habitat requirements, such as *Festuca myuros*, *Potentilla incana*, *Cerastium arvense* L. are present only in low percentages (0–20%), as well as those with wet-water requirements such as *Dactylis glomerata* L. and *Alopecurus pratensis* L. (5.3–20%). In the case of the individual habitats, it can be seen that the first habitat is more towards the moderately dry, while the sixth grassland is more towards the cool (Table 1).

Based on the soil reaction values (R), the habitats can be considered slightly calcareous in general. In addition, the proportion of species with neutral requirements is also significant. Acidification of the area can be observed, at least in patches. The annual weather can significantly affect the cover of the particular species, e.g. in the case of a lot of precipitation, it shifts these values towards acidic, and in the case of drought, towards calcareous.

Table 1. Number of species according to the W, N, TVK criteria, in each grassland habitat. Grey rows 1-10: the number of the investigated grassland.

	1	2	3	4	5	6	7	8	9	10
W value (water supply)										
1 extremely dry	2	0	2	3	1	2	3	1	1	1
2 dry	2	0	1	2	2	2	1	0	1	1
3 moderate dry	6	4	2	3	11	6	4	2	6	7
4 moderate cool	4	4	6	4	11	7	6	4	5	6
5 cool	4	5	5	4	8	5	5	3	5	6
6 moderate wet	4	4	4	7	6	5	3	5	5	6
7 wet	1	2	0	0	2	1	1	2	1	2
8 moderate aqueous	2	1	2	2	1	2	2	2	2	2
9 aqueous	0	0	0	0	0	0	0	0	0	1
TVK value (naturalness)										
E (association-forming species)	0	0	0	1	1	2	2	0	2	3
K (accompanying species)	5	2	7	7	15	10	5	3	3	7
TP (pioneer species)	1	0	0	1	1	1	1	0	0	0
TZ (disturbance-tolerant species)	13	11	11	12	12	12	11	12	16	18
GY (weeds)	6	7	4	4	11	5	7	4	6	6
G (economic plants)	0	0	0	0	1	1	0	0	0	0

Based on nitrogen supply (N), the grasslands are between nutrient-poor and medium nutrient supply areas, which shows that animal manure deposited during grazing is the only nutrient supply. In terms of their forage value, the total coverage of the harmful species *Rosa canina* L. and *Carduus acanthoides* L. is below 10%, but the unfavourable pioneer *Ambrosia artemisiifolia* L., poisonous *Ranunculus acris* L. and unpleasant-tasting *Rumex acetosa* L. also appear. It is favourable that the number and cover of species suitable for grazing, e.g. *Cynosurus cristatus* L., *Dactylis glomerata* L., *Alopecurus pratensis* L. is significant (Table 1). In terms of the naturalness of the investigated grasslands, there is a predominance of species indicating natural conditions. Within this, the 'disturbance-tolerant' species with the greatest proportions are *Dactylis glomerata* L., *Bromus hordeaceus* L. subsp. *hordeaceus*, *Bromus racemosus* L. and *Cynodon dactylon* (L.) Pers. It is very positive that the proportion of weed species and economic plants *Elymus repens* (L.) Gould, and *Convolvulus arvensis* L. is low, a maximum of 35%, but on average around 20%. Thus, these grasslands are as close as possible to natural grasslands in terms of utilization (Figure 1).

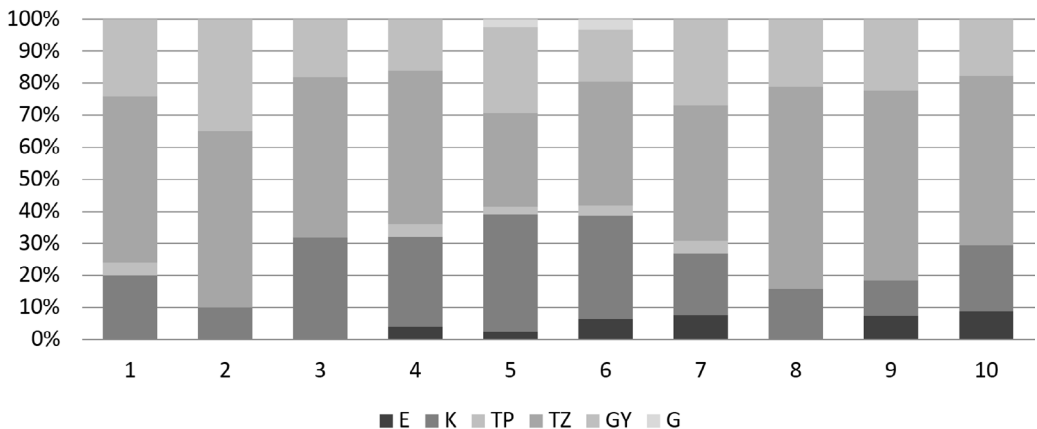


Figure 1. Naturalness of the examined grasslands (TKV). E, association-forming species; K, accompanying species; TP, pioneer species; TZ, disturbance-tolerant species; GY, weeds; G, economic plants.

## Conclusions

The assessment of grassland areas and their characteristics as well as the knowledge of the species living there are key to sustainable grassland utilization. The close-to-natural habitats are threatened by overgrazing and the drying climate. The utilization of these areas in the future can only be imagined as pasture. In the case of four habitats where trampling-tolerant species indicate habitat deterioration, reducing the grazing load should be considered. All grasslands require a finisher-mowing at the end of the grazing season, to prevent weed growth and improve feed value in the next year. As overgrazing is frequent in Southern Hungary, our results should be taken into account in planning the management of these grasslands.

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# Investigating the effects of herbage mass and harvest date on perennial ryegrass nutritive value

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## Abstract

Pasture nutritive value, which can have an effect on animal performance, is typically influenced by both management and environmental factors. The objective of this experiment was to investigate the effect of herbage mass (HM) and harvest date (HD) on the nutritive value of perennial ryegrass. A 4×6 factorial randomised complete block design was assigned to 80 plots. Four target HM (Low=1000 kg DM ha<sup>-1</sup>, Medium=1500 kg DM ha<sup>-1</sup>, High=2000 kg DM ha<sup>-1</sup> and Very High=2500 kg DM ha<sup>-1</sup>) were sampled at 2-week increments totalling 6 HD between April to July. Grass samples from each HD were snap frozen in liquid nitrogen and stored at -20°C prior to nutritive value analysis. No differences in NDF or ADF were observed among the medium, high and very high treatments; however, the low herbage mass was significantly higher than all other HM. Organic matter digestibility was reduced for the very high HM when compared with the medium treatment. Increases in HM led to a reduction in CP concentrations. Harvest date also had an effect on the nutritive value parameters. This experiment highlights the impact of HD and HM on grass nutritive value.

**Keywords:** perennial ryegrass, herbage mass, nutritive value

## Introduction

Pasture grown feeds, such as perennial ryegrass (*Lolium perenne* L. (PRG)), offer a sustainable source of nutrients in ruminant diets. However, the nutritive value of this pasture has a direct effect on nutrient supply and resulting animal performance (Schroeder *et al.*, 2004). Perennial ryegrass has two distinct growth phases, vegetative and reproductive, with PRG entering the reproductive growth phase in late spring and early summer (i.e., May to July; Hurtado-Uria *et al.*, 2014) where the dominant objective changes from tiller/leaf to seed production. The nutritive value of the grass is altered as a reduction in the leaf blade proportion is observed (Witkowska *et al.*, 2008). During the transition from vegetative to reproductive, the herbage mass or stage of maturity of the plant can also fluctuate drastically leading to further variability in the nutritive value of the pasture. Milk yield, and milk fat concentration in particular, have been demonstrated to be highly dependent on pasture nutritive value (Elgersma, 2015) leading to anecdotal suggestions that pasture herbage could be deficient in plant cell wall concentration at times during the grazing season. To maximise the production efficiency of grass-fed ruminants, a greater understanding of the temporal trends and the factors affecting grass nutrient value are needed. Thus, the objectives of this experiment were to investigate the effect of (1) herbage mass (HM) and (2) harvest date (HD) on the nutritive value of PRG during the late spring and early summer period.

## Materials and methods

This experiment was conducted at the Teagasc, Animal and Grassland Research and Innovation Centre (Moorepark, Fermoy, Co. Cork, Ireland; 50°07' N; 8°16' W). The experiment took place from the 27<sup>th</sup> of April to the 6<sup>th</sup> of July 2022. The soil type was free-draining, acid brown earth and had a sandy loam to loam texture. A 4×6 factorial randomised complete block design with treatment replication within each block was allocated across 80 perennial ryegrass plots (1.5×5 m). Treatment in the form of target HM was applied at the plot level with each treatment being represented within each block at all harvest time points. The 4 target HM (Low=1000 kg DM ha<sup>-1</sup>, Medium=1500 kg DM ha<sup>-1</sup>, High=2000 kg

DM ha<sup>-1</sup> and Very High=2500 kg DM ha<sup>-1</sup>) were harvested at each of the six HD (HD1=27 April, HD2=11 May, HD3=25 May, HD4=8 June, HD5=22 June and HD6=6 July). Plots were defoliated to a residual sward height of 4 cm using an Etesia mower. Dry matter yield was measured by weighing the total defoliation sample of the plot area. A 100 g sub-sample was collected and dried at 90°C for 16 h to determine DM content. Pre- and post-defoliation compressed sward heights were measured using a rising plate meter. At harvesting, a sub sample was collected from each plot prior to defoliation for chemical analysis. Samples were immediately snap frozen in liquid nitrogen prior to being stored at -20°C. Samples were freeze dried (LS40+chamber, MechaTech System LTD) at -55°C for at least 72 h before being milled through a 1 mm screen (Foss, Hillerød, Denmark). Samples were analysed following AOAC (1995) methods for neutral detergent fibre (NDF; method 973.18), acid detergent fibre (ADF; method 973.18) and ash (method 942.05). Crude protein (CP; Leco FP-428) and organic matter digestibility (OMD) were performed using the method described by Morgan *et al.* (1989). All data were analysed using SAS linear models, which included the fixed effects of treatment, harvest date, their interaction and block. Multiple comparisons between means were made using the Tukey–Kramer method.

## Results and discussion

A moderate drought was experienced during the experimental period, resulting in lower than predicted grass growth rates. As a result, the recorded DM yields for the low, medium and high treatments were lower than the original target HM. Nonetheless, the HM investigated had a significant effect on all nutritive value parameters (Table 1). The low HM had significantly higher NDF and ADF concentrations compared to the medium, high and very high treatments. Typically, as HM increases the plant matures and deposits greater amounts of plant cell wall, which can be quantified as greater concentrations of NDF (Van Soest, 1994).

In this experiment, we did not observe this increase in NDF, which is in agreement with other investigations studying similar HM (Pérez-Prieto *et al.*, 2012). Although cell wall concentration did not increase with increasing HM, OMD was reduced for the very high treatment when compared with the medium treatment (814 vs 828 g (kg DM)<sup>-1</sup>, respectively). Reduced OMD in heavier herbage masses can impact ruminal digestion leading to the lower production of volatile fatty acids which are the precursors of milk fat and milk lactose. Raffrenato *et al.* (2018) demonstrated that mature grasses contain significantly higher quantities of etherified ferulic acid. Such etherified cross-linkages could explain, at least in part, the reduction in digestibility observed as HM increased independently of increasing NDF concentrations. As HM increased CP concentrations reduced (Table 1). This reduction in CP concentration can have important implications on animal performance as low rumen ammonia concentrations can reduce fibre

Table 1. Effect of target herbage mass on perennial ryegrass nutritive value during late spring and early summer period (April–July).

Item	Target Herbage Mass				SEM	P-value
	L	M	H	VH		
DM (g kg <sup>-1</sup> )	20.5 <sup>ab</sup>	20.2 <sup>a</sup>	20.9 <sup>b</sup>	21.6 <sup>c</sup>	0.1	<0.01
DM yield (kg DM ha <sup>-1</sup> )	574 <sup>a</sup>	1104 <sup>b</sup>	1660 <sup>c</sup>	2733 <sup>d</sup>	45.1	<0.01
OMD (g (kg DM) <sup>-1</sup> )	823 <sup>ab</sup>	828 <sup>a</sup>	818 <sup>ab</sup>	814 <sup>b</sup>	2.4	<0.01
CP (g (kg DM) <sup>-1</sup> )	184 <sup>a</sup>	151 <sup>b</sup>	134 <sup>c</sup>	119 <sup>d</sup>	2.7	<0.01
NDF (g (kg DM) <sup>-1</sup> )	428 <sup>a</sup>	405 <sup>b</sup>	409 <sup>b</sup>	406 <sup>b</sup>	3.9	<0.01
ADF (g (kg DM) <sup>-1</sup> )	228 <sup>a</sup>	214 <sup>b</sup>	215 <sup>b</sup>	216 <sup>b</sup>	1.8	<0.01
Ash (g (kg DM) <sup>-1</sup> )	74 <sup>a</sup>	68 <sup>b</sup>	65 <sup>c</sup>	61 <sup>d</sup>	0.7	<0.01

L, Low (1000 kg DM ha<sup>-1</sup>); M, Medium (1500 kg DM ha<sup>-1</sup>); H, High (2000 kg DM ha<sup>-1</sup>); VH, Very high (2500 kg DM ha<sup>-1</sup>); 3DM, dry matter; OMD, organic matter digestibility; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; SEM, standard error of the mean. Within each row the superscripts (abcd) refer to the significant (P<0.05) difference.

digestion and microbial protein supply (Van Soest, 1994). Finally, HD had a significant effect ( $P < 0.01$ ; data not shown) on all nutritive value parameters with large variation observed across the experimental period. This could be due to the swards transitioning between the vegetative and reproductive stages. However, the climatic conditions seemed to differentially affect HD1, 5 and 6 making it difficult to interpret the effect of HD.

## Conclusion

In this experiment, during the late spring and early summer period, herbage mass and harvest date had a significant effect on grass nutritive values. The very high herbage mass reduced OMD and CP when compared with the medium herbage mass; however, NDF concentrations were similar. Although difficult to interpret, harvest date had an effect on all nutritive value parameters. These outcomes highlight the effect of herbage mass and harvest date on sward nutritive value, which ultimately can affect animal performance.

## Acknowledgement

This experiment was funded by Teagasc Core Funding and Dairy Research Ireland.

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# Simulating grassland winter survival in high latitude regions using the BASGRA model

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## Abstract

In high latitude regions, variability in weather and climate conditions during the winter season cause a considerable variation in forage grass productivity and animal feed supply between years and locations. Tools to estimate or predict winter survival and yield, such as ground registrations, satellite image analysis and process-based simulation models, can be combined in decision support for grassland management. In this study, we simulated grassland winter survival using the Basic GRAssland (BASGRA) model. The model was initialized after the last cut in the autumn. Its performance to simulate ground coverage in the early spring, either assessed by on-site ground registrations or from Sentinel-2 satellite images, was evaluated. Grass fields at Malangen and Målselv in Northern Norway were simulated for the winter seasons 2020–2021 and 2021–2022. Model input including daily air temperature, precipitation, relative humidity and wind speed data were obtained from weather stations nearby the grass fields. The initial values of biomass, leaf area and tiller density in the autumn were based on ground registration in October. Preliminary results show considerable variation in both simulated winter survival and prediction accuracy of observed spring ground coverage between the locations and two winter seasons.

**Keywords:** forage grass, process-based models, remote sensing, winter kill, yield security

## Introduction

Forage grass is the main component of ruminant feed in many world regions and production systems. In high latitude regions, variability in weather and climate conditions during the winter season cause a considerable variation in forage grass productivity and animal feed supply between years and locations. Early and accurate predictions or estimates of grassland winter survival can help farmers make decisions about reseeding, fertilisation regimes and procurement of extra feed, thereby reducing weather- and climate-related risks and increasing the production stability. However, predicting and estimating winter kill in grass fields is a difficult task. The hardening and dehardening processes in plants are regulated by weather and plant genetics and determine the lowest temperature a plant can survive. Besides snow cover, ice encasement and soil frost conditions modify the conditions the plant is exposed to (Rapacz *et al.*, 2014). Moreover, assessments of winter kill early in the spring can result in the misrepresentation of non-green but still living plants as dead plants, thereby overestimating the winter kill. More accurate information from later assessments might, on the other hand, come too late to be useful in decision making about reseeding and fertilizer regimes. Hence, in practice, early information about winter survival is often imprecise or inaccurate.

Recent development of remote sensing tools and process-based simulation models potentially opens new avenues to obtain earlier and more precise knowledge for winter survival. The goal of this study was to simulate grassland plant survival during autumn, winter and early spring using a process-based model and evaluate its prediction performance against information of winter survival and biomass production early in the growing season, obtained either from remote sensing images or from ground observations.

## Materials and methods

Forage grass performance on 5 fields at Malangen (69.4° N, 18.9° E) and 4 fields at Målselv (69.2° N, 18.5° E) in Norway was simulated during the autumn, winter, and early spring 2020-2021 and 2021-2022 as a function of daily weather and soil data using the BASGRA model (Höglind *et al.*, 2016). Initial values of biomass, leaf area and tiller density in the autumn, which are needed to run the model, were set according to ground registrations. Plant parameter values were calibrated against a combination of ground registrations and satellite-sensed data from Northern Norway. Ground cover from satellite images was derived using a machine learning model that used unmanned aerial vehicle (UAV) images as a reference measurement to generate high-resolution training data for the model. Daily weather data used as input to the BASGRA model were obtained from the Norwegian Meteorological Institute or the AgClimate network of the Norwegian Institute of Bioeconomy Research. The model performance was first evaluated against observations of spring ground cover, which was either determined visually by the human eye or by a satellite-based classification of the percentage of living plant material. The ground cover is not directly simulated by the BASGRA model. Therefore, we assumed that the ratio between the simulated number of tillers per area and the number of tillers per area in a dense stand with no gaps (which was previously assessed in controlled field trials in Norway) corresponds to the percentage of living plants. The model performance was evaluated against the first registration of above-ground plant biomass by destructive samplings in late May or early June.

## Results and discussion

There was no correlation between the simulated tiller density and the estimated share of ground area covered by living plants when assessed either by the human eye or from satellite images (Figure 1a,b). However, it is difficult to know if this lack of correlation was due to a misprediction of ground cover by the BASGRA model, by incorrect estimations of winterkill in the field, or by a combination of these two factors. However, the fact that covered ground area differed between the field observation methods indicates that these results are uncertain, and we do not know which of the methods gave the most accurate assessments. The observed above-ground biomass at the first registration as assessed by destructive samplings was simulated with a higher accuracy than the area of plant coverage (Figure 1c).

In total, these results suggest that there is a need for more reliable estimations of winter kill early in the season. A temporal mismatch between the start of the growing season, and the observation days is one possible source of error. Remote sensing tools enable more frequent area imaging than measurements that

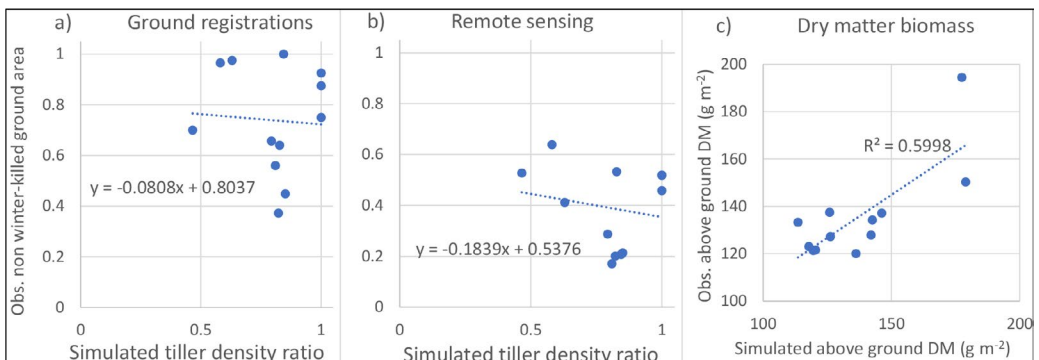


Figure 1. (a, b) Observed share of ground area (*y*-axis) covered by living plants based on ground registrations (a), and by satellite sensing (b), versus the share of maximum number of living tillers simulated by the BASGRA model. (c) Observed versus simulated above-ground plant biomass dry matter in late May–early June.

can be done practically by traditional ground-based tools. One way forward could be to first use satellite or drone mounted cameras together with detailed weather data to identify the visual character of fully survived fields at the start of the growing season, and assess winter damage related to images of the fully survived field. Such an approach would probably benefit from including images from regions and fields that include a diversity in winter harshness.

## Conclusion

The prediction of the estimated share of ground area covered by living plants in the spring by the BASGRA model was poor, while the prediction of the first cut dry matter yield was better. Future research should include the development of methods to determine more accurately the coverage of living plant material at the start of the growing season.

## Acknowledgements

This study was financed by the Polish-Norwegian Research Programme (Project ID NOR/POLNOR/GrasSAT/0031/2019) under the 3<sup>rd</sup> edition of the EEA and Norway Grants under the “Applied Research” Programme. Additional funding was provided by the Fram Centre (grant number 369924) and Troms and Finnmark County municipality (grant number TFFK2020-309). We also thank advisors at the Agricultural Extension Service in Northern Norway (NLR Nord-Norge) for field registrations and assistance when selecting study locations and fields that were analysed in this study.

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# Does the Soil Index relate to differences in grassland and forage crop yields between farms?

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## Abstract

The Soil Index (SI) is an application offered to farmers that assigns a single score for soil quality on a scale of 1 (poor) to 10 (excellent) at the field and farm level, providing a benchmark for soil fertility. The total SI score is based on the scores of four items: (1) water holding capacity; (2) capacity to supply nitrogen; (3) plant available nutrients; and (4) soil physical structure. Each score is based on soil characteristics obtained from standard soil analyses. We postulated that SI, as an indicator for soil quality, may possibly explain differences in yields of grassland and forage crops and hence differences in soil N and P surpluses between farms. To test this assumption, a study was performed wherein SI scores, soil characteristics, grassland and forage maize dry matter yields and feeding values were collected from 35 farms in the north of the Netherlands during 3 years (2019–2021). The data were analysed with linear mixed models for repeated measures, with farm as the random factor, and year and weighted SI (weighted by field size) or soil characteristics (both weighted mean of the SI of different fields on one farm) as fixed factors. The data analysis revealed that neither SI scores nor the underlying indicators were suitable for indicating differences in the annual total whole farm grassland and forage production. Further research is needed to investigate whether a field-specific relationship between SI scores of individual fields and forage production exists.

**Keywords:** Soil Index, soil fertility, forage yield

## Introduction

In the Netherlands and the EU, there is increasing interest in soil fertility and soil health. Healthy soils contribute to reducing climate change impacts, preventing soil land degradation, reversing biodiversity loss, and ensuring food security and food safety. In the Netherlands, the Council for the Environment and Infrastructure concluded that the quality of agricultural soils is under threat due to intensive land use and that measures are desired to improve soil quality (Rli, 2020). The EU has developed a soil strategy for 2023 as a key deliverable of the Green Deal (EU Soil strategy). Assessing soil quality is complex due to numerous physical and chemical factors. Accordingly, indicators that provide a quick insight into soil quality can be a helpful tool in farmers' daily lives. An example of such a tool is the Soil Index (SI), which provides an assessment of water holding capacity, capacity to supply nitrogen, plant-available nutrients, and soil physical structure, and it integrates these assessments into a single score for soil quality on a scale of 1 (poor) to 10 (excellent) at the field and farm level (<https://vibconsulting.nl/bodem/>). The inputs for the SI are typical soil characteristics obtained from routine analysis. Clay content, soil organic matter (SOM), and pH are used as proxies for water holding capacity; soil nitrogen supply (SNS) and C/N for available nitrogen; plant-available phosphorus (PAE;  $\text{CaCl}_2\text{-P}$ ) and plant-available potassium (KAE;  $\text{CaCl}_2\text{-K}$ ) for plant available nutrients, and Ca fraction of the cation exchange capacity Ca-CEC for soil physical structure. A composite sample of 40 sub samples per 5 ha max is used for the SI. Grassland is sampled at 0–10 cm depth. Arable land is sampled at 0–25 cm depth.

Farmers within study groups can compare their SI scores with each other (benchmarking) and take measures for improvement. It is therefore interesting to examine whether the SI score is also related to grassland and forage crop yields, which may be a potential incentive for further improvement of the SI score.

A preliminary comparison analysis (unpublished), using the Annual Nutrient Cycle Assessment (ANCA; van Dijk *et al.*, 2022), has indicated that a high SI score correlates with lower soil surpluses of nitrogen (N) and phosphorus (P). Results further suggested that a high SI score may link to a higher removal of N and P by vegetation and thus relate to an increased yield of grassland and forage crops. To test this assumption, a study was conducted to explore the correlation between the SI score and grassland and forage crop yields, as well as to determine if the SI score serves as a suitable indicator of grassland and forage productivity.

## Materials and methods

Thirty-five commercial dairy farms along a gradient of soil types in the central and north of the Netherlands (Table 1) participated in this study. For each farm, during 2019–2021, comprehensive data were collected. At field level, the data consisted of the results of routine soil analysis for SI (sampling depth 0–10 cm for grassland and 0–25 cm for arable land; clay content, SOM, pH, SNS, C/N, CaCl<sub>2</sub>-P, CaCl<sub>2</sub>-K, CEC, Ca-CEC), the weighted average SI score (weighted by field size), GIS-field information, and groundwater table. At the farm level the data consisted of simulated grassland and forage crop yields and feeding values (net energy, VEM (kg DM<sup>-1</sup>); N, P (g (kg DM<sup>-1</sup>))), organic fertilization with manure obtained from the ANCA model. Forage crop yields in ANCA are based on an analysis of the feed composition, sampling and measurements of the feed stocks, purchased feeds and nutrient requirement of the herd (van Dijk *et al.*, 2022). The years were analysed separately, and therefore the year effect of DM production was taken into account. The data were analysed with linear mixed models for repeated measures, with farm as the random factor, and year and weighted soil characteristics (weighted mean of the SI of different fields size on one farm) as fixed factors, using SPSS version 29.

## Results and discussion

The pilot study showed no significant correlation between the ANCA calculated yield (kg DM ha<sup>-1</sup> year<sup>-1</sup>) and SI, for both grass and maize. Table 2 shows the slope direction and fit ( $R^2$ ) of the calculated correlation between ANCA calculated crop yield (kg DM ha<sup>-1</sup> year<sup>-1</sup>) and parameter weighted SI, fraction of Ca of the Cation Exchange Capacity, and pH-CaCl<sub>2</sub>. Table 3 shows the calculated weighted average of different soil parameters for different yield classes of grass and maize.

Table 1. Distribution of commercial farms in the plot study over main soil types.

Main soil type	Number of farms	Grassland area (ha)	Maize area (ha)
Sand	20	1057	178
Clay	7	260	42
Sand-peat	4	297	41
Sand-clay	2	102	13
Peat	2	71	–
Total	35	1787	274

Field size ranged between 0.5 and 10 ha.



Table 2. Slope direction and calculated fit (R-squared) of calculated correlation between calculated crop yield from Annual Nutrient Cycling Assessment (ANCA) and Soil Index (SI), specific parameters Ca-CEC (%) and pH-CaCl<sub>2</sub> for 2019, 2020 and 2021.

Crop	Year	SI		Ca-CEC (%)		pH-CaCl <sub>2</sub> (-)	
		Slope	R <sup>2</sup>	Slope	R <sup>2</sup>	Slope	R <sup>2</sup>
Grass	2019	+	0.013	+	0.076	+	0.081
	2020	+	0.024	+	0.158	+	0.161
	2021	-	0.029	+	0.066	+	0.013
Maize	2019	-	0.018	-	0.034	-	0.006
	2020	+	0.048	-	0.001	+	0.305
	2021	-	0.004	+	0.019	-	0.016

Table 3. Weighted average of soil parameters per yield class per crop for 2019, 2020 and 2021.

Crop	Yield class (kg DM ha <sup>-1</sup> year <sup>-1</sup> )	C/N ratio	soil nitrogen supply (kg DM ha <sup>-1</sup> year <sup>-1</sup> )	P-CaCl <sub>2</sub> (mg P kg <sup>-1</sup> )	K- CaCl <sub>2</sub> (mg K kg <sup>-1</sup> )	pH	Soil organic matter (g kg <sup>-1</sup> )	Clay content (g kg <sup>-1</sup> )	Ca- CEC (%)
Grass	<7500	15	180	2.6	16	5.2	7.9	10	67
	7500–8499	14	167	2.1	117	5.3	6.9	7	71
	8500–9499	15	155	2.6	110	5.2	8.0	7	71
	9500–10 499	15	155	2.1	96	5.3	8.4	4	71
	10 500–11 499	15	156	2.6	141	5.4	10.0	7	70
	>11 500	13	159	2.7	111	5.2	8.7	13	75
Maize	<13 000	14	165	1.5	95	5.3	8.9	9	69
	13 000–14 999	14	153	2.3	113	5.3	8.0	8	72
	15 000–16 999	15	154	2.7	117	5.3	7.7	8	70
	17 000–18 999	14	164	2.0	102	5.2	8.5	9	71
	19 000–20 999	16	140	3.4	120	5.4	7.6	8	73
	>21 000	16	167	2.4	113	5.3	8.6	9	70

## Conclusion

No correlation could be found between the average weighted Soil Index and the calculated yields of grass and of maize on farm level for the 2019-2021 period. The outcomes of the research suggest that there are indications of a correlation between yield and Ca-CEC and yield and pH-CaCl<sub>2</sub>. Further research is needed on field specific relations between SI scores of individual fields and forage production.

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# Effect of pasture allocation frequency on the milk production of Holstein grazing dairy cows

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## Abstract

The objective of this study was to test the effect of two different pasture allocation frequencies (1 vs 7 days) on milk production of grazing dairy cows. Ten lactating Holstein cows were randomly assigned to the treatments in a 2×2 Latin square design with two 7-day periods repeated three times (autumn-winter, early and late spring). During autumn-winter all the cows had one grazing session and were supplemented with 10 kg DM of a mixed ration. In spring, the cows were not supplemented and were removed from the pasture only for milking. The pasture used was a mix of *Festuca arundinacea*, *Lotus corniculatus* and *Trifolium repens*. Neither milk production ( $27.0 \pm 2.38$ ,  $20.8 \pm 0.69$  and  $14.7 \pm 0.66$  kg cow<sup>-1</sup> day<sup>-1</sup>), post-grazing sward heights ( $10.6 \pm 0.23$ ,  $11.9 \pm 0.83$  and  $16.3 \pm 1.37$  cm), nor daily grazing time ( $196 \pm 10$ ,  $535 \pm 22$  and  $592 \pm 14$  min cow<sup>-1</sup> day<sup>-1</sup>) were different between treatments in autumn-winter, early spring, and late spring, respectively. This finding opens a viable option for farmers to simplify daily management and reduce labour demands.

**Keywords:** grazing systems, pasture allocation frequency, milk production, labour demand

## Introduction

In the context of an evolving dairy sector, it is essential to comprehend the relationship between pasture allocation frequency and milk production to address challenges presented by competition for resources and labour demand. This understanding is crucial for ensuring the cost-effectiveness and long-term sustainability of grazing systems. The objective of this study was to test the effect of two different pasture allocation frequencies (1 vs 7 days) on milk production and grazing behaviour of grazing dairy cows.

## Materials and methods

The experiments were carried out at the Experimental Research Station 'Dr. Mario A. Cassinoni' of the Faculty of Agronomy, University of Uruguay, Paysandú, Uruguay. Two treatments were evaluated: weekly strip (1 plot for 7 days; WS) and daily strip (7 plots with 1 day of occupation; DS). Ten lactating Holstein cows were randomly assigned to the treatments in a 2 × 2 Latin square design with two 7-day periods repeated in autumn-winter, early and late spring. The groups were homogenised by milk production, parity, days in milk (DIM), and live weight (LW) at the beginning of each experimental period. In autumn the cows were producing  $26.8 \pm 6.43$  kg cow<sup>-1</sup> day<sup>-1</sup>, with  $2.8 \pm 1.83$  lactations,  $53 \pm 24.5$  DIM and  $578 \pm 85.9$  kg LW. In early spring the cows were all primiparous cows producing  $22.9 \pm 2.63$  kg cow<sup>-1</sup> day<sup>-1</sup>,  $198 \pm 7.4$  DIM, and  $530 \pm 33.9$  kg LW. In late spring the cows were all primiparous cows producing  $16.1 \pm 1.36$  kg cow<sup>-1</sup> per day,  $249 \pm 7.1$  DIM and  $539 \pm 37.9$  kg LW. An initial period of 3 days was used for adaptation, followed by 7 days of data collection throughout the 3 experimental periods. The pasture used during autumn-winter was *Festuca arundinacea* (tall fescue cv. INIA Fortuna) and *Lotus corniculatus* cv. San Gabriel, and during early and late spring cows grazed a mixture of tall fescue, *Lotus corniculatus*, and *Trifolium repens* cv. Estanzuela Zapicán. During autumn-winter the cows had one grazing session with 20 kg DM cow<sup>-1</sup> day<sup>-1</sup> of forage allowance (at ground level) between 8:00 and 14:00 and were supplemented with 10 kg DM cow<sup>-1</sup> per day of a mixed ration consisting of ryegrass and corn silage and concentrate. In spring, the cows were not supplemented and grazed with a forage allowance of 50 kg DM cow<sup>-1</sup> day<sup>-1</sup> and were removed from the pasture only for milking at 5:00 and 15:00. Sward height was

measured with a sward stick (Barthram, 1986) pre- and post-grazing, and every day during the paddock occupation period. The readings of sward height were taken from ground level every one step along zigzag transects. The sward mass was estimated using an adaptation of the double sampling technique of Haydock and Shaw (1975) with a rising plate meter (RPM; Ashgrove, Palmerston North, New Zealand), as described by Mattiauda *et al.* (2013). The grazing time was assessed by visual observations every 5 min during autumn-winter experiment on days two, five and seven, and continuously by behavioural recorders during spring experiments. Individual milk yield was recorded automatically. On the second and seventh day of the occupation period in WS, and an equivalent day in DS treatment, individual milk samples were collected in the morning and afternoon milking to determine concentrations of fat, protein, and lactose in milk. In the autumn-winter experiment, 300 tillers of tall fescue were marked in the WS treatment in a 3×3 m gridline on a 0.3 ha plot. This was done by wrapping a flexible wire covered with red tape around the base of the tiller to facilitate their location and identification. The identified tillers were measured daily during the seven days identifying whether they were grazed or not. Data were analysed using the SAS University Edition. The variables milk production and composition, grazing time and pasture sward heights were analysed considering the treatment, day (1 to 7), and interactions between them (except for pasture sward height) as fixed effects. Analyses were performed for each experimental period separately.

## Results and discussion

There were no significant differences ( $P>0.05$ ) for any tested effect for milk production and composition, post-grazing sward heights or grazing time in any experimental period (Table 1).

Overall, our findings indicate that the milk production was not affected by the pasture allocation frequency. This was likely because the herbage allowance was not constraining the animals, regardless of how they were distributed in space and time. As argued by Carvalho *et al.* (2019), the most crucial factor in defining animal production is the amount of forage offered to the animal in its structure of greatest ingestion efficiency, and less important is how fragmented the offer is. Sward management is critical for animal performance in pastoral environments. The post-grazing sward height in the autumn-winter and spring experiments was similar to the post-grazing sward heights that optimized dry matter intake and milk production in the trials reported by Fast (2020) for autumn, and Menegazzi *et al.* (2021) which consequently determines animal performance. Despite that, few studies have explored the potential to increase milk production by managing post-grazing sward height. An experiment was carried out to evaluate the effect of three defoliation intensities on a *Lolium arundinaceum*-based pasture on frequency and length of grazing meals and ruminating bouts, daily grazing and ruminating time, feeding stations and patches exploration, and dry matter intake and milk production of dairy cows. The treatments imposed were three different post-grazing sward heights: control (TC, for spring conditions. Nutrient supply is determined by ingestive behaviour, thereby exerting a substantial impact on milk production.

Table 1. Effects of pasture allocation frequency on the milk production, grazing time and post-grazing sward height in autumn-winter, early spring and late spring periods.

	Experimental period			P-value		
	Autumn-winter	Early spring	Late spring	T	D	T×D
Milk production (kg cow <sup>-1</sup> )	27.0±2.38	20.8±0.69	14.7±0.66			
Milk fat (g (100 g) <sup>-1</sup> )	3.55±0.104	3.89±0.069	4.00±0.082			
Milk protein (g (100 g) <sup>-1</sup> )	3.43±0.082	3.49±0.055	3.49±0.040			Non-significant
Daily grazing time (min cow <sup>-1</sup> day <sup>-1</sup> )	196±10	535±22	592±14			
Post-grazing sward heights (cm)	10.6±0.23	11.9±0.83	16.3±1.37			

T, treatment; D, day of occupation.

Although it was expected, we did not observe an impact of pasture allocation frequency on the grazing behaviour of the cows. In contrast, Pollock *et al.* (2022) grazing and ruminating activities are essential in nutrient capture and ultimately animal performance however these activities can demand significant time and energy. This study evaluated the effect of three different pasture allocation frequencies (PAF's; 12, 24 and 36 h found that offering fresh pasture every 12, 24, or 36 hours resulted in increased competition for resources in the 12-hour treatment. This negatively affected primiparous animals, leading to greater grazing and ruminating times and lower milk production when managed in the 12- or 24-hour treatments compared with the 36-hour treatment (Pollock *et al.*, 2020) which subsequently may impact on animal performance. Limited research to-date has investigated grazing management methods to improve the performance of high production dairy cows whilst also achieving high grass utilisation rates. This study evaluated the effect of three different PAF's (12, 24 and 36 h. This emphasizes the influence of resource competition in intensive pasture grazing systems, especially in mixed-parity herds of high yield cows.

In autumn-winter, the WS cows explored daily (took bites from) 12% of the marked tillers. The second defoliation of the same tiller never exceeded 3% per day, except on the last day in the plot, where it reached 7%. The number of tillers grazed for the third time did not reach a total of 1% and only occurred on the fifth day of grazing onwards. A common concern on long occupation-period practices is the potential for the same tiller to be grazed several times in a short period, thereby compromising its regrowth. However, our findings suggest that when moderate post-grazing sward heights are used, re-grazing of the same tiller was not a significant issue. The labour requirement in the milk production system and the lifestyle of farmers should be considered when assessing the sustainability of grazing dairy systems, given that it has been a challenge in the sector in recent years (Fariña and Chilibroste, 2019).

## Conclusion

The milk production was not affected by the pasture allocation frequency (1 vs. 7 days). This finding opens a viable option for farmers to simplify daily management and reduce labour demands.

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# Can a liquid nitrogen fertiliser produce similar herbage dry matter yields as granular fertilisers?

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## Abstract

A plot study was undertaken to determine whether a liquid nitrogen (N) fertiliser could be as effective in terms of herbage dry matter (DM) production as granular N fertiliser options in an Irish grass-based system. The plot arrangement was a 3×2 factorial to compare calcium ammonium nitrate+sulphur (CAN), urea+N-(n-butyl) thiophosphoric triamide+sulphur (PU), and an acidified chemical liquid N (LN) on ryegrass-only swards at 120 and 200 kg ha<sup>-1</sup> year<sup>-1</sup>. The study was conducted at three experimental sites during 2023. Before each grazing event, plots were sampled for pre-grazing herbage yield and the fertilisers were applied after each grazing event. Results from the three sites show significant differences ( $P<0.05$ ) between fertiliser types and rate on pre-grazing herbage yield with liquid N yielding less than the granular fertiliser treatments. There was also a significant reduction ( $P<0.05$ ) in herbage DM production for the liquid fertiliser compared to the granular fertilisers. Liquid N fertiliser has potential in grass-based systems; however, the data from one year this study show it was unable to meet the same production levels provided by granulated fertilisers.

**Keywords:** calcium ammonium nitrate, urea+NBPT, liquid nitrogen fertiliser, perennial ryegrass, herbage production

## Introduction

Fertiliser nitrogen (N) application is a common practice of intensive pasture-based systems globally, especially those in temperate grassland regions (Forrestal *et al.*, 2017). In Ireland, granulated fertilisers, like calcium ammonium nitrate (CAN) and urea, have been the predominant forms of fertiliser used due to their availability and relative low cost (Harty *et al.*, 2016). However, in 2022, due to the conflict in Ukraine, fertiliser prices increased by 145% from 2021 (CSO, 2022) and with a requirement to reduce the environmental impact of agriculture, a 20% reduction in fertiliser use must be achieved by 2030 (Climate Action Plan, 2023). One mitigation strategy available to reduce the environmental impact of fertiliser is protected urea, which is urea that has a protective coating that reduces ammonia volatilization (Harty *et al.*, 2016; Rahman *et al.*, 2021). Liquid fertiliser is slowly gaining integration into Irish farms as an alternative fertiliser type for its varying forms, modes of action, uptake and nutrient composition. However, there has been very little research completed to date on the effectiveness of liquid fertiliser use on grassland swards. Therefore, the objective of this experiment was to investigate the effectiveness of a liquid fertiliser in terms of herbage dry matter (DM) production compared to traditional granulated fertilisers. The hypothesis of the experiment was that the LN would result in similar pre-grazing herbage yields and total herbage DM production to traditional granulated fertilisers under a grassland grazing system.

## Materials and methods

The experimental design was a 3×2 factorial complete random block design with four replications per treatment across three experimental sites (Teagasc Moorepark, Cork (52.16° N, 8.24° W), Clonakilty Agricultural College, Cork (51°63' N, 8°85' W) and Mellows Campus, Athenry, Galway (54°80' N; 7°25' W)). The treatments compared were CAN+sulphur (CAN), urea+NBPT+sulphur (PU) and

an acidified chemical liquid N containing sulphur (LN) at two annual N rates (120 and 200 kg N ha<sup>-1</sup> year<sup>-1</sup>). Each plot site had the same arrangement and size (8×6 m), except for Athenry where the size of the plots were smaller (9×4 m). Each treatment had four replicates, which gave a total of 24 plots. Granulated fertiliser was applied by hand-broadcasting after each grazing. Liquid N was applied with a 3 m long boom powered by an electrical pump system and attached to a Gator (John Deere, Moline, IL, USA) that was driven over the plots. Forward speed and application volume were calculated for the LN beforehand. The total fertiliser spread for all sites was 120 kg N ha<sup>-1</sup> and 200 kg N ha<sup>-1</sup> for the CAN and PU. The LN totals varied slightly for each site: Teagasc Moorepark (130 kg N ha<sup>-1</sup> and 216 kg N ha<sup>-1</sup>), Clonakilty (130 kg N ha<sup>-1</sup> and 195 kg N ha<sup>-1</sup>), and Athenry (132 kg N ha<sup>-1</sup> and 206 kg N ha<sup>-1</sup>). Pre-grazing yield was measured before grazing by cutting one strip (5×1.2 m) from each plot at a height of 4 cm using an Etesia mower (Etesia UK., Warwick, UK) at Moorepark, Clonakilty, and Athenry. A subsample of 100 g was dried at 60°C for 48 hours to determine DM. A rising plate meter (Jenquip, Fielding, New Zealand) was used to measure pre- and post-cutting heights on each strip and also for pre- and post-grazing sward heights on each plot. Two sites were grazed with lactating dairy cows, whereas sheep were used in Athenry. The first grazing took place in March, six weeks after the first N application and thereafter when the CAN-200 treatment had a pre-grazing herbage yield of approximately 1500 kg of DM ha<sup>-1</sup> (assessed visually). The CAN-200 treatment was used as a control as the treatment represents standard practice in Ireland and created uniformity across sites. All plots were grazed simultaneously with the aim of reaching a post-grazing sward height of 4 cm. Statistical analysis was undertaken using PROC MIXED in SAS (SAS 9.4). Fixed effects used in the model were site, fertiliser type, and fertiliser rate, with rotation included as a repeated effect and their corresponding interactions were also included in the model. Tukey's test was used to determine differences between treatment means.

## Results and discussion

There was no significant interaction ( $P>0.05$ ) between fertiliser type and rate, therefore the interaction term is not presented in Table 1. Table 1 shows that within fertiliser rate, fertiliser type had a significant effect ( $P<0.001$ ) on pre-grazing herbage yield, pre-grazing height, and overall herbage DM production. There was no significant difference between CAN and PU for pre-grazing herbage yield and overall herbage DM production, following the trends observed by Murray *et al.* (2023) that PU is a suitable alternative to CAN. However, LN had significantly lower pre-grazing herbage yield (–227 kg DM ha<sup>-1</sup>) compared to CAN and PU. Overall herbage DM production was significantly lower for LN (–1852 kg DM ha<sup>-1</sup>) compared to the other fertiliser types. A previous study in Ireland, with a similar LN product used on winter wheat, found varying results in total yield compared to granulated fertilisers (Burke *et al.*, 1999). Burke *et al.* (1999) concluded that soil and weather conditions can have a large impact on the utilisation and leaching capabilities of LN. Few, if any, studies have been undertaken with LN on grassland in Ireland. Fertiliser rate also had a significant effect on pre-grazing herbage yield ( $P=0.009$ ), pre-grazing height ( $P=0.004$ ), and overall herbage DM production ( $P=0.002$ ), which was expected and

Table 1. Effect of fertiliser type and rate on herbage production<sup>1</sup>

	200 kg N ha <sup>-1</sup>			120 kg N ha <sup>-1</sup>			SEM	FT	FR
	CAN <sup>3</sup>	PU	LN	CAN	PU	LN			
Pre-grazing yield (kg DM ha <sup>-1</sup> )	1,728	1,717	1,476	1,627	1,581	1,369	51.0	<0.0001	0.009
Density	322	336	315	323	330	316	7.0	0.053	0.809
Pre-grazing- Ht (cm)	9.36	9.24	8.70	9.06	8.84	8.36	0.150	<0.0001	0.003
Post-grazing- Ht (cm)	4.20	4.00	3.91	4.11	3.94	3.93	0.060	<0.0001	0.328
Herbage production (kg DM ha <sup>-1</sup> )	14 474	14 675	12 728	13 708	13 593	11 837	351.7	<0.0001	0.002

All data are means of the three sites for 2023. Ht, height; SEM=Standard error of means; CAN, calcium ammonium nitrate+sulphur; PU, urea+N-(n-butyl) thiophosphoric triamide+sulphur; LN, acidified chemical liquid N containing sulphur.

follows similar trends to previous research (Murray *et al.*, 2023). There was no significant differences for sward density within fertiliser rate or type.

## Conclusions

The results from this one year study show that LN was unable to produce a similar herbage production to that achieved by the two granulated fertilisers used. Liquid nitrogen shows potential as an alternative source of N; however, given the results from multiple sites over one year, further research needs to be conducted on LN fertilisers and their use in grassland systems.

## Acknowledgements

The authors wish to acknowledge the Teagasc Walsh Scholarship for financial support. The authors would also like to thank the staff and students that took measurements and samples.

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# Exploring the productivity puzzle: an examination of fast- and slow-growing forage grasses

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## Abstract

Fast-growing plants typically exhibit higher specific leaf area (SLA), leaf area index (LAI), and leaf elongation rate (LER) than slow-growing plants, which are often associated with increased forage production. Conversely, slow-growing grasses tend to have a higher leaf weight ratio (LWR), leaf lifespan (LLS), and phyllochron (Ph), which are beneficial in supporting stressful conditions. However, we hypothesized that slow-growing grasses possess specific canopy structure attributes that counterbalance the advantages of fast-growing grasses, ultimately allowing both to achieve similar productivity when cultivated in non-nutrient-limited environments. To test this, we conducted a field experiment using *Arrhenatherum elatius* and *Festuca arundinacea* as fast- and slow-growing species, respectively. Both were cultivated in 45 m<sup>2</sup> field plots under identical cutting and fertilization regimes. Plant functional traits and canopy attributes were monitored for one year. *A. elatius* exhibited higher LER, leaf appearance rate (LAR), and SLA, whereas *F. arundinacea* exhibited higher LLS, tiller population density (TPD), and LWR. Forage productivity was similar between the two species. Slow-growing *F. arundinacea* compensated for its lower functional traits (SLA and LER) by exhibiting advantageous canopy structure traits (higher TPD and LWR). This compensation mechanism enabled *F. arundinacea* to be as productive as fast-growing *A. elatius*, particularly in fertile soils.

**Keywords:** forage yield, plant functional traits, canopy attributes

## Introduction

Leaf extension pattern, as represented by the leaf elongation rate (LER), is a pivotal plant functional trait that delineates distinct growth strategies among grass species (Bucher and Römermann, 2021). Notably, the lower LER observed in slow-growing species has been associated with reduced nutrient loss (Lambers and Poorter, 1992), contributing to their success in nutrient-limited habitats (Reich *et al.*, 1992). Such habitats inherently exhibit lower relative growth rates (RGR) in comparison to more productive environments, typically inhabited by fast-growing species. Our research was driven by an interest in exploring how grass species with contrasting growth strategies optimize forage productivity, particularly in fertile environments. We hypothesized that slow-growing grasses may possess specific canopy structure attributes capable of offsetting the inherent advantages of fast-growing counterparts, potentially resulting in comparable yield when cultivated in non-nutrient-limited conditions.

## Materials and methods

This study was conducted at Santa Catarina State University, Brazil, between 2013 and 2015. A fast-growing (*Arrhenatherum elatius* L.) and a slow-growing grass (*Festuca arundinacea* Schreb.) were established in 45 m<sup>2</sup> plots serving as experimental units (EUs), arranged in a completely randomized design with three replicates. The plots were allowed unrestricted growth until full establishment over one year, with data collection carried out from June 2014 to April 2015 through ten harvests. The management criteria were defined as: cutting grasses when they reached a height of 20 cm (95% of light interception in vegetative stage), and the post-cutting height was set at 10 cm. During the experimental period, no water deficit was observed, and nitrogen fertilization events occurred every 40 days at 30 kg



N ha<sup>-1</sup> (Nitrogen Nutrition Index >0.8 (Americo *et al.*, 2021)). Immediately after each cut, 20 tillers per plot were selected to assess development of leaves using the tissue flow technique (Davies, 1993). Tiller leaves were measured at regular intervals (3–5 days) using a ruler. From these measurements, the following variables were estimated: leaf elongation rate (LER, cm °C<sup>-1</sup> day<sup>-1</sup>), leaf senescence rate (LSR, cm °C<sup>-1</sup> day<sup>-1</sup>), phyllochron (Ph, °C day<sup>-1</sup>), number of green leaves per tiller (Ln; Haun, 1973), and leaf lifespan (LLS, °C day). The tiller population density (TPD), specific leaf area (SLA, cm<sup>2</sup> g<sup>-1</sup>), leaf area per tiller (LA), leaf weight ratio (LWR), canopy density (CD, g cm<sup>-1</sup>) and weight per tiller (WT, g tiller<sup>-1</sup>) were also determined. The forage yield per cycle (kg ha<sup>-1</sup>) was calculated as described by Duchini *et al.* (2019). Observations from each species (treatment) throughout the year were analysed by cycle. Data collected for each species were analysed by cycle using analysis of variance (ANOVA) and principal component analysis (PCA) conducted in RStudioTeam.

## Results and discussion

Under our experimental conditions, the analysis of variance indicated significant differences ( $P < 0.05$ ) among grasses for all functional traits at both the plant scale and canopy attributes. Specifically, *A. elatius* exhibited nearly twice the leaf extension rate and leaf senescence rate as *F. arundinacea*. However, the leaf growth balance (LER-LSR) remained similar throughout the cycles (data not shown). Contrary to the suggestions of Skinner and Nelson (1995) who linked forage production to LER, our results did not substantiate such a relationship. Additionally, *A. elatius* displayed a higher leaf number, lighter leaves (lower leaf weight ratio), greater specific leaf area, and greater leaf area than *F. arundinacea*. Conversely, *F. arundinacea* had a higher phyllochron and leaf lifespan than *A. elatius*. Moreover, the higher leaf weight ratio and weight per tiller in *F. arundinacea* partially compensated for the lower LER, leaf area, and leaf number, which are typical indicators of potential productivity (Brougham, 1960; Ludlow *et al.*, 1974). Regarding canopy structure, *F. arundinacea* exhibited higher tiller population density, weight per tiller, and canopy density than did *A. elatius*. Nevertheless, we observed a similar forage yield accumulation among grasses throughout the cycles ( $P > 0.05$ ) (Figure 1A), except for cycles three and seven, when *F. arundinacea* was more productive than *A. elatius*. Garay *et al.* (1999) proposed that herbage production could be attributed to a combination of tiller density and tiller weight, with increases in either or both factors contributing to enhanced primary forage growth. The PCA revealed that the first two principal components (PCs) explained 63% of the total variability. The autovector values in the first PC showed specific relationship between grasses and attributes. Observations for *A. elatius* showed strong correlations with SLA, LER, LA and Ln, whereas for *F. arundinacea* they showed strong correlations with Ph, WT, CD, LLS, LWR, LSR and TPD (Figure 1B), suggesting different pathways for forage accumulation in the two grass species.

## Conclusion

*Festuca arundinacea* demonstrated a higher number of tillers and canopy density, which may compensate for its slower growth rate, thereby explaining its forage production at the canopy level. This suggests that in fertile environments that experience moderate and frequent defoliation, *F. arundinacea* exhibits characteristics that buffer against the expected higher net forage yield of *Arrhenatherum elatius* at both individual and canopy scales. This highlights the complex interplay between growth strategies and environmental conditions that influences forage production in grasses.

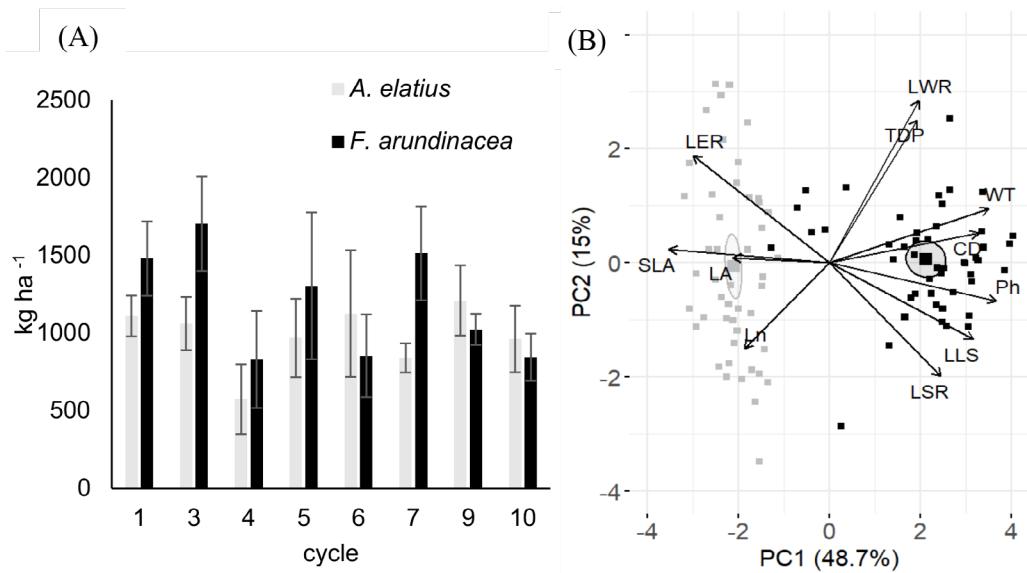


Figure 1. (A) Forage yield (kg ha<sup>-1</sup>) throughout the study period (mean±standard error; n=6). (B) Biplot representing the main effects and interactions of plant functional traits and canopy structure attributes (vectors) in *A. elatius* (grey squares) and *F. arundinacea* (black squares) with confidence ellipses for each pasture.

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# Establishing grass-clover leys in winter cereals

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## Abstract

In Northern Europe, leys often consist of grass-legume mixtures established in spring cereals. Changing climatic conditions, with recurring droughts in spring and early summer, increase the risk of poor ley establishment. This field experiment evaluated establishment of leys undersown in winter cereals, comparing grass-clover mixtures (red or white clover, timothy, meadow fescue, with or without perennial ryegrass), winter cereal (wheat, triticale, rye) and harvest time of winter cereal (as whole crop or threshed), at three locations in Sweden. Ley biomass (first cut in first harvest year) ranged from 3500 to 6100 kg DM ha<sup>-1</sup> and percentage of clover from 2% to 17%, averaged over sites and treatments. Type of grass-clover mixture affected yield and clover proportion, with no or varying interactions with winter cereal and/or harvest time depending on site. Mixtures with red clover generally gave higher yields and more clover than mixtures with white clover, but winter cereal and its harvest time had site-specific impacts. At the northernmost site, clover proportion depended on three-way interactions between grass-clover mixture, winter cereal and harvest time of winter cereal ( $P < 0.0281$ ). Clover percentage was highest (20–28%) in red clover mixtures after rye and triticale harvested as whole crop.

**Keywords:** climate change, harvest systems, ley establishment, ley mixtures, undersown

## Introduction

Ley-based farming systems are important in sustainable food production systems. In Europe, leys occupy 40% of total arable land (Huyghe *et al.*, 2014), with a large proportion used exclusively for ruminant feed. The climate in the Northern hemisphere demands winter feeding with large amounts of high-quality fodder, which often consists of mixtures of grasses and legumes. The high production cost of ley-based fodder due to ley establishment is often difficult to recoup with short-term leys (Larsson *et al.*, 2007). Changing climatic conditions, with more frequent droughts in spring and early summer, increase the risk of unsuccessful establishment. However, prolongation of autumn has led to a longer growing season, making it possible to establish leys in autumn-sown crops, e.g. winter cereals, which could decrease the risk of poor ley establishment and the risk of soil nutrient losses. Spring sowing is generally known to favour legume survival (e.g. Younie, 1998), but their ability to cope with late sowing has only been studied in pure stands (Hallin, 2023). Under climate change, more research on the establishment of mixed grass-legume leys when sown late is needed. In this field study, four grass-clover mixtures were undersown in autumn in different winter cereals with varying time of harvest. The aim was to assess the effect on ley establishment of: (i) grass-clover species mixtures, (ii) winter cereal crop, and (iii) harvest system.

## Materials and methods

In autumn 2021, replicated field experiments were established at three sites in Sweden (Färjestaden, 56° N, 16° E; Långhem, 57° N, 13° E; Uppsala, 59° N, 17° E). The management regime at the Uppsala and Färjestaden sites is conventional, while at Långhem it is organic. Four grass-clover seed mixtures (Table 1) were undersown in the winter cereals rye (*Secale cereale* L.), triticale (*Triticale rimpai* L.) and wheat (*Triticum aestivum* L.), which were harvested either early as whole-crop cereals or threshed at maturity. The four grass-clover seed mixtures contained red clover (*Trifolium pratense* L.) or white clover (*Trifolium*

Table 1. Species, variety and seed rate (kg ha<sup>-1</sup>) for grass-clover mixtures 1–4.

Mixture 1	Mixture 2	Mixture 3	Mixture 4
Red clover, Vicky, 7	White clover, SW Hebe, 3	Red clover, Vicky, 7	White clover, SW Hebe, 3
Timothy, Switch, 9	Timothy, Switch, 9	Timothy, Switch, 6.6	Timothy, Switch, 6.6
Meadow fescue, Tored, 6	Meadow fescue, Tored, 6	Meadow fescue, Tored, 4.4	Meadow fescue, Tored, 4.4
		Perennial ryegrass, SW Birger, 4	Perennial ryegrass, SW Birger, 4

*repens* L.) with timothy (*Phleum pratense* L.) and meadow fescue (*Festuca pratensis* Huds.), with and without perennial ryegrass (*Lolium perenne* L.).

The experiment had a split-split-plot design (sub-sub-plot size 2 m × 12 m), with three replicates per site. The three factors were grass-clover mixture, winter cereal and harvest system. During the ley year 2023, three cuts were made, with dry matter (DM) yield determination. This paper presents data from the first cut in the first harvest year, at which botanical analysis through DM determination was performed. The statistical program JMP Pro 16 was used for three-way ANOVA to determine effects of the three factors on ley yield and clover proportion. When ANOVA identified a significant difference ( $P < 0.05$ ), the Tukey HSD test was used to identify treatments that differed significantly.

## Results and discussion

Recorded average ley-herbage yield in the first cut per site varied from south to north and was 3500, 3900 and 6000 kg DM ha<sup>-1</sup> at Färjestaden, Långhem and Uppsala, respectively. Corresponding clover proportion, based on botanical analysis of first-cut biomass at the three sites, was on average 13, 17 and 2%. In general, across sites the grass-clover mixtures with red clover (with and without perennial ryegrass) gave higher DM yield and had higher clover proportions than white clover mixtures, averaged over winter cereal type and harvest system (Figure 1). This was possibly because white clover is more sensitive to shading than red clover, due to its growth pattern with creeping light-requiring aboveground stolons (Frame and Newbould, 1986). Species of winter cereal did not have a strong effect on ley yield. However, clover proportion after rye (when threshed) was lower than after the other two winter cereals. This was probably due to the dense stand of high-yielding rye reducing light penetration to the undersown clover.

Harvesting system had a stronger impact on clover proportion than ley yield, with higher clover proportion when winter cereals were harvested early as whole crop rather than grown to maturity (threshing). However, the impact of winter cereal and harvest system varied between sites, and was less important at the southernmost site (Färjestaden) and most important at the northernmost site (Uppsala). At Uppsala, only 0–1% clover remained when winter cereals were grown to maturity. The longer growing season at the two southerly sites may have favoured clover establishment before winter, resulting in higher clover proportions.

## Conclusions

Yield of grass-clover leys was higher for red clover mixtures than mixtures with white clover. Red clover mixtures also provided a larger proportion of clover than those with white clover. The effect of different winter cereal species was small at early harvest (whole crop), but greater when cereals were grown to maturity (threshed), in particular for rye. Early harvesting of winter cereal as whole crop was favourable for obtaining a sufficient amount of clover in the ley. When winter cereals were grown to maturity (threshed), less clover was generally recorded. This pattern was more pronounced at the northernmost site (central Sweden) and decreased towards the south.

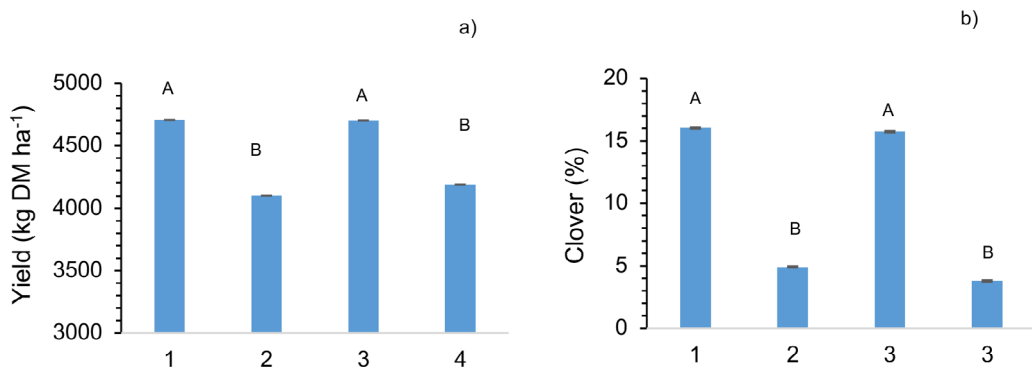


Figure 1. (a) Mean first-cut yield (kg DM ha<sup>-1</sup>) and (b) clover proportion (%) of grass-clover mixtures 1–4, averaged over sites, winter cereal and harvest system. Mixture 1, red clover, timothy, meadow fescue; mixture 2, white clover, timothy, meadow fescue; mixture 3, red clover, timothy, meadow fescue, perennial ryegrass; mixture 4, white clover, timothy, meadow fescue, perennial ryegrass. Bars with different letters differ significantly ( $P < 0.05$ ).

## Acknowledgements

The study was carried out within the collaborative research center SustAinimal, funded by the Swedish Research Council Formas (No. 2020-02977), Behms fund, the Rural Economy and Agricultural Society and SLU.

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# Identification and assessment of the distribution of fungal diseases within the main grassland species in Norway

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## Abstract

With rising temperatures and shifting rainfall patterns driven by climate change, conditions for pathogen-plant interactions will be affected based on the specific pathogen and plant species involved. In general, increased pathogen activity is expected in Norwegian grasslands. Recent breeding efforts in Norway have concentrated primarily on developing varieties resistant to fungal diseases that cause winter damage. However, their resistance against other diseases may fall short, as they have not been targeted in the Norwegian breeding programme. As a result, a comprehensive evaluation of the current situation is essential. This ongoing project aims to identify foliar fungal species and disease distribution in breeding lines and varieties of four prominent meadow species: timothy, perennial ryegrass, meadow fescue and red clover. The study encompasses four locations in Norway, spanning from 60 to 69° N. Observations from the first season indicated relatively good resistance to both winter and growing season-related fungi in the investigated breeding material of timothy. The observations indicated that perennial ryegrass is more susceptible to winter diseases, whereas its resistance to growing-season diseases is relatively good. Conversely, meadow fescue and red clover displayed moderate susceptibility to fungal diseases during the growing season but demonstrated commendable resistance to overwintering fungi.

**Keywords:** Norwegian grassland, fungi diseases, breeding programme

## Introduction

Forage production on grasslands forms the primary foundation for milk and meat production in Norway, constituting two-thirds of the country's agricultural area. As a result, the nation's food security relies significantly on stable and abundant forage production. Elevated temperatures, increased precipitation, and consequently reduced radiation during autumn can disrupt the natural acclimatization of plants to winter conditions. This acclimatization is crucial for developing maximum resistance against the most widespread overwintering fungi under Nordic conditions, snow mould (Tronsmo *et al.*, 2001), as well as other overwintering fungi in grasslands (Rapacz *et al.*, 2014). Significant yield losses occurred in 2022 due to snow mould across large regions in Western Norway. Some winter fungi have also become more prevalent in Northern Norway, affecting areas that previously experienced fewer problems. Altered rainfall patterns, coupled with higher summer temperatures, will also affect the occurrence of diseases during the growing season. Recently, there has been a growing prevalence of such diseases in Norway (Havstad, 2017). This increased risk applies to diseases already established and also those that have, historically, occurred to only a limited extent or not at all (Østrem *et al.*, 2018). A crucial strategy to mitigate vulnerability to a changing climate is the development of forage crops with high tolerance or resistance to diseases. Norwegian variety development has historically given little attention to diseases during the growing season, leading to Norwegian varieties having comparatively low resistance, for instance, the low resistance to crown rust (*Puccinia coronata*) observed in Norwegian varieties of meadow fescue (Østrem *et al.*, 2018). There is large uncertainty regarding both how the climate will evolve in the future (Hansen-Bauer *et al.*, 2015) and the potential consequences of the expected climate changes on the development of plant diseases. This necessitates a diverse set of strategies, ranging from plant breeding to grassland management. This calls for adopting more participatory approaches and engagement in interdisciplinary science. An essential initial step involves mapping the incidence of diseases under various cultivation conditions and understanding the variability in susceptibility among different varieties in

Norway's most crucial forage grass species. Subsequent efforts can then be directed towards both plant breeding and guidelines on which species, cultivars and seed mixtures to grow in different regions. The aim of this ongoing study is to identify foliar fungal species and disease distribution in breeding lines and varieties of four prominent meadow species: timothy, perennial ryegrass, meadow fescue and red clover. Here, we present preliminary result from one season.

### Materials and methods

The occurrence of leaf fungal diseases and overwintering fungi was surveyed in four forage grasses (timothy, ryegrass, meadow fescue and red clover). Data were collected from fourteen ongoing experimental fields (Figure 1), where different species and varieties are being tested under various climatic conditions between latitude 60 and 69° N (Fureneset, Tromsø, Steinkjer and Hamar). Three of these are localities of the Norwegian Institute for Bioeconomy Research (NIBIO) and Hamar field belong to the Norwegian breeding company (Graminor). These localities represent the agroclimatic variation that characterizes feed production in Norway in relation to the varying risk of disease attack. Symptoms of plant diseases vary based on the species, variety, attack percentage, and the attacking organism. While visible symptoms could provide clues about the disease affecting a plant, a reliable diagnosis requires laboratory tests conducted by experts with the necessary equipment. Fungal disease severity was scored on leaves as the percentage of leaf area infected, using a modified Cobb scale (0 to 100% infected leaf area) (Peterson *et al.*, 1948). These assessments included the scoring of overwintering fungi in early spring, before each cut (2-3 cuts depending on length of growing season on-site) in 2023. Samples of

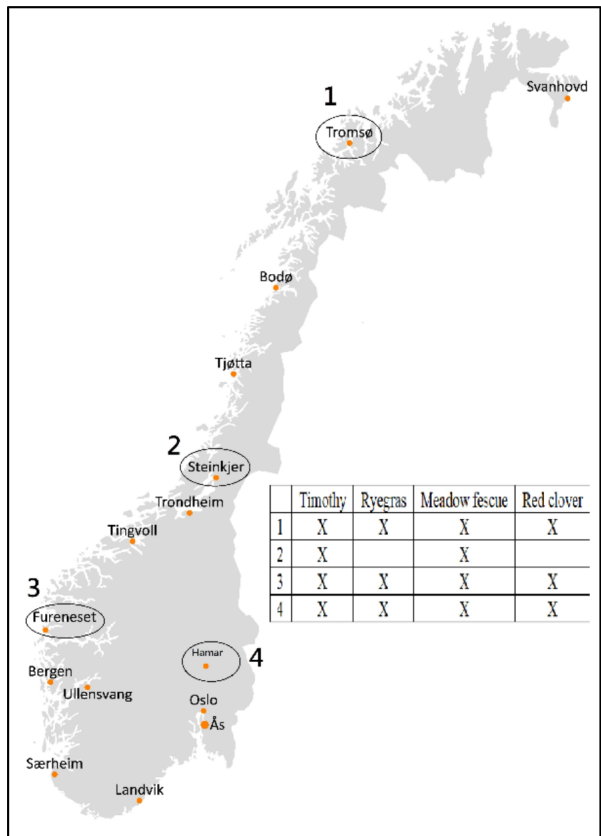


Figure 1. Distribution of NIBIO localities. Fields location for this project are indicated with circles.

disease-affected plant material were also sampled for identification at the NIBIO laboratory (inoculation method) and documented with pictures on the site.

## Results and discussion

Winter disease severity was slight or unnoticeable in early spring 2023, which indicates that the winter conditions were not favourable for fungal diseases distribution. Laboratory analysis of field samples revealed the presence of ascomycete of *Fusarium avenaceum*, *Microdochium nivale*, but without major disease severity. Conversely, in the growing-season diseases the severity was more pronounced, with large variation between species. Meadow fescue and red clover showed the largest disease severity in the 2023 growing season, with up to 40% maximum disease severity in both species based on environment. Perennial ryegrass and timothy showed lower disease severity, ranging from 0 to 15% based on environment. These findings indicate that the investigated breeding material of timothy and perennial ryegrass have a potentially good resistance to growing-season-related fungi. An alternative explanation is that the 2023 growing season did not favour the distribution of these fungal diseases.

## Conclusions

Preliminary observations from the initial year (Table 1) indicate a lack of discernible differences in disease resistance among Norwegian varieties and breeding lines within the investigated species. The study will continue in 2024 for comprehensive analysis and conclusive findings.

## Acknowledgement

This project is funded by Research Funding for Agriculture and the Food Industry (FFL/JA).

Table 1. Observed diseases in fields assessments, with (\*) confirmed by laboratory.

Diseases	Fureneset	Tromsø	Hamar	Steinkjer
<i>Microdochium nivale</i>	Mf* Rg*			
<i>Fusarium avenaceum</i>	Rg* T*	Mf* Rc* Rg* T*		
<i>Fusarium culmorum</i>		Rc*		
<i>Fusarium spp.</i>	Rg*	Mf*		Mf
<i>Pyrenophora spp. (Drechslera spp.)</i>	Mf* Rg* T*	Mf*	Rg	
<i>Rhizoctonia spp.</i>	Rg*			
<i>Colletotrichum sp.</i>	T*			
<i>Pseudopeziza trifolii</i>	Rc*	Rc*	Rc	
<i>Botrytis sp.</i>		Rc*		
<i>Ascochyta sp.</i>		Mf Rc* Rg* T*		
<i>Kabatiella caulivora</i>	Rc*			
<i>Stemphylium sarcinaeforme</i>	Rc*		Rc	
<i>Cladosporium phlei</i>				T
<i>Blumeria graminis</i>	Rg			
Unspecified ascomycete		T		

T, timothy; Rg, ryegrass; Mf, meadow fescue; Rc, red clover.



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# The impact of warm-season pasture management on the following cool-season annual ryegrass growth

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## Abstract

Diverse pastures have been proposed as alternatives to monocultures, as the combined use of species allows for better efficiency in utilizing available resources in space and time. Under several climates, it is possible to cultivate forage plants in sequence ( $C_4$  during the warm season and  $C_3$  plants during the cool season). However, little is known about how the management of the predecessor species affects the performance of successor species. We hypothesized that management of canopy heights and nitrogen doses applied in the summer may alter the growth dynamics and tillering patterns of annual ryegrass (*Lolium multiflorum* L.) cultivated in the subsequent cold season (winter and spring). The experimental design was a randomized block with a  $2 \times 3$  factorial arrangement, with three replicates per treatment. The experimental treatments consisted of two pre-grazing canopy management heights (17 and 23 cm) and three nitrogen rates (50, 150 and 250 kg ha<sup>-1</sup>) applied during the warm season. Canopy height and nitrogen dose applied in the previous summer did not affect the tiller population density or forage accumulation rates of annual ryegrass cultivated in the following winter and spring.

**Keywords:** biodiverse pastures, nitrogen, perennial pasture, canopy height

## Introduction

Southern Brazil is characterized by a predominantly subtropical climate (Cfb), with consistent year-round precipitation and mild summers (average temperature <22°C), without a well-defined dry season. This climate allows for cultivation of warm- and cool-season forage species ( $C_4$  and  $C_3$ ) sequentially in the same area throughout the year (Sbrissia *et al.*, 2017). The widespread practice of combining complementary forage species in space and time is a common approach in these regions (Barreta *et al.*, 2023a), and utilization of these systems contributes to greater temporal productivity stability in pastoral environments (Griffin *et al.*, 2009; Hector *et al.*, 2010). Additionally, it enhances multi-functionality by providing increased plasticity to adverse events (Suter *et al.*, 2021), optimizing resource efficiency, and augmenting the overall ecosystem services provided by grasslands (Duchini *et al.*, 2016; Jing *et al.*, 2017). Despite these benefits, there is still limited knowledge about the management practices employed in these pastures and how the management of predecessor species might affect the performance of successor species. We hypothesized that canopy height and nitrogen (N) application rates during the summer season change the growth dynamics and tillering patterns of annual ryegrass (*Lolium multiflorum* L.) cultivated in the subsequent cool season.

## Materials and methods

The experiment was conducted at the Center for Agroveterinary Sciences of Santa Catarina State University (CAV-UDESC) in Lages, Santa Catarina, Brazil (27°48'58" S; 50°19'34" W). The climate of the region, classified as Cfb (Köppen classification) is humid subtropical with mild summers (Alvares *et al.*, 2013). The soil at the experimental site was identified as a Haplic Aluic Leptic Cambisol (Embrapa, 2013). The study spanned two years, from December 2019 to October 2021. Experimental treatments were implemented during the warm season, when pastures were predominantly (75–80%) composed

of kikuyu grass (*Cenchrus clandestinus*) and Tifton 85 Bermuda grass (*Cynodon* spp.). Evaluations were performed in the subsequent cold season (winter and spring) on annual ryegrass (*Lolium multiflorum* L.) pastures. The experimental design was a randomized complete block with a 2×3 factorial arrangement and three replications per treatment. The experimental treatments included two grazing canopy heights (17 and 23 cm) and three N rates (50, 150 and 250 kg N ha<sup>-1</sup>), which were applied exclusively during the warm period (Southern Hemisphere). During the cool season, a uniform management approach was adopted, maintaining a grazing canopy height of 20 cm, coupled with N fertilization of 50 kg N ha<sup>-1</sup> applied to all treatments at the beginning of the annual ryegrass tillering. Tiller population density (TPD, tillers m<sup>-2</sup>) was determined by counting all annual ryegrass tillers inside three frames (0.5 m<sup>2</sup>) per plot. Morphogenic and structural characteristics were assessed using the marked tiller technique (Davies, 1993), with tillers evaluated from post-grazing to subsequent pre-grazing. Net forage accumulation rate was determined as the difference between the growth and senescence rates. Statistical analysis was performed using the MIXED procedure of SAS® (Statistical Analysis System) version 9.2, with means compared using the Tukey test at *P*<0.05.

## Results and discussion

The tillering patterns and morphogenic and structural characteristics of the plant (Table 1) were not affected (*P*>0.05) by warm-season management (height and/or N rate). Similarly, the gross, senescence, and net accumulation rates of annual ryegrass were not significantly influenced, with average values over two years of 51, 16 and 35 kg DM ha<sup>-1</sup> day<sup>-1</sup>, respectively.

This amount of accumulated forage in the annual ryegrass pastures aligns with findings of Grange *et al.* (2022), who compared 150 and 300 kg N ha<sup>-1</sup> applied to monocultures of *Lolium perenne* and observed no increase in annual ryegrass forage production in the subsequent season. The authors suggested that additional N was not retained in the soil but was instead lost from the system. Our study suggests a similar occurrence, as mineral N values in two different soil depth layers (0–10 and 10–20 cm) were similar across treatments, indicating no residual N from the summer crops for annual ryegrass in winter. In a complementary study in the same area, Barreta *et al.* (2023b) found higher accumulation of summer forage species subjected to moderate and high N doses (150 and 250 kg N ha<sup>-1</sup>) than at a lower dose (50 kg N ha<sup>-1</sup>). Nitrogen Nutrition Index values of 0.97 and 0.92 for kikuyu grass and 0.77 and 0.70 for Tifton 85, at higher and lower N doses, respectively, suggest that these grasses cultivated in the summer may have absorbed most of the N applied during this season, and thus could be limiting its availability in the subsequent season.

Table 1. Morphological and structural attributes of annual ryegrass tillers overseeded in perennial summer pastures managed at different heights and N application rates.

Item	Value	SEM	P-value		
			Height	N rate	H*N
LER (cm tiller <sup>-1</sup> day <sup>-1</sup> )	1.42	0.11	0.644	0.678	0.624
LSR (cm tiller <sup>-1</sup> day <sup>-1</sup> )	0.47	0.10	0.738	0.653	0.532
Phyllochron (degrees day <sup>-1</sup> leaf <sup>-1</sup> )	112	13	0.223	0.855	0.825
Leaf longevity (degrees day <sup>-1</sup> leaf <sup>-1</sup> )	399	51	0.181	0.744	0.985
No. living leaves (leaves tiller <sup>-1</sup> )	3.5	0.1	0.721	0.736	0.118
TPD (tiller m <sup>-2</sup> )	2289	465	0.672	0.327	0.296

Values are average of two years of evaluation. Means followed by different letters in the line differ from each other using the t-test (*P*<0.05). SEM, standard error of the mean; LER, leaf elongation rate; LSR, leaf senescence rate; TPD, tiller population density.

## Conclusion

Grazing heights ranging from 17 to 23, and N rates ranging from 50 to 250 kg N ha<sup>-1</sup>, applied in perennial pastures during the warm season did not affect the forage accumulation of annual ryegrass in the subsequent cool season in a Cfb climate in Southern Brazil.

## Acknowledgement

Thanks are due to Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina – FAPESC (Grant number 2023 TR 242).

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# Fresh grass diets supplemented with essential oils for dairy cows: effects on milk and urea

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## Abstract

This study evaluated the effect of essential oils (EO) on fat-and-protein corrected milk (FPCM) and milk urea of 42 cows in 3 systems: (1) fresh grass outdoors (day and night grazing,  $n=14$ ); (2) fresh grass outdoors during the day, grass silage indoors at night (daytime grazing,  $n=14$ ); and (3) fresh grass indoors during the day, grass silage indoors at night (daytime zero grazing,  $n=14$ ). In every system, half of the number of cows were fed a concentrate with EO, the other half was fed a concentrate without EO. The study consisted of 2 periods in the growing season, both with 2 weeks acclimatisation and 2 weeks measurements. The concentrate with or without EO was fed during both complete periods and the 2 weeks measurements were analysed. For cows receiving EO, the FPCM was  $30.9 \text{ kg day}^{-1}$  and milk urea was  $19.5 \text{ mg dL}$ , compared with  $30.4 \text{ kg day}^{-1}$  ( $P=0.42$ ) and  $19.7 \text{ mg dl}^{-1}$  ( $P=0.75$ ) for cows not receiving EO. Feeding EO did not affect FPCM and milk urea in different grazing systems between April and July, although numerically FPCM was a bit higher and milk urea was a bit lower.

**Keywords:** grazing, fresh grass, essential oils, protein, nitrogen

## Introduction

In the Dutch dairy sector, one of the aims is to reduce the emission of ammonia ( $\text{NH}_3$ ) and nitrogen (N). Cows excrete urea, an N compound, via urine, and the amount excreted is related with protein intake and N-efficiency of the cows. Essential oils (EO) have antimicrobial properties and could improve nutrient utilization of dairy cows (Benchaar *et al.*, 2008), which may finally improve their N-efficiency. With this intention, a supplement consisting of a blend of EO compounds was developed. The aim of the study was to analyse the effects of feeding EO to dairy cows in different fresh grass systems on milk yield, body condition, and milk content of fat, protein, urea and lactose.

## Materials and methods

A grazing trial with a randomized block design was performed at Dairy Campus (Leeuwarden, the Netherlands) in 2023. The trial consisted of two different periods in the growing season with each two weeks of adaptation and two weeks of measurements: April–May (P1) and June–July (P2). Per period, 42 Holstein Friesian dairy cows between 80 and 200 days (on average  $142 \pm 4.7$  days) in lactation and with a milk yield between 20 and 50 kg (on average  $30.1 \pm 0.59 \text{ kg day}^{-1}$ ) at the start of the period were blocked in 7 blocks of 6 cows and within block randomly divided over 6 groups: 3 fresh grass systems, each with 2 supplement treatments (with or without EO). The fresh grass systems were: (S1) fresh grass outdoors (day and night grazing,  $n=14$ ), (S2) fresh grass outdoors during the day, grass silage indoors during the night (daytime grazing,  $n=14$ ), and (S3) fresh grass indoors during the day, grass silage indoors during the night (daytime zero grazing,  $n=14$ ). Each period, the blocking was redone to ensure that in both periods the lactation stage and milk yield of the cows was comparable. As selection and blocking of the cows was redone for each period, observations of the same cows in both periods were considered to be independent. Cows in all 3 systems were milked twice daily, with an interval of 11–13 hours. Daily, cows in all 3 systems received 1 kg of bait pellet in the milking parlour, 1 kg of concentrate pellet (with EO ( $12 \text{ g kg}^{-1}$ ) or without EO), and up to 3.5 kg of bait pellet in the Greenfeed systems that were used for measurements of methane emission. Cows in S1 and S2 were offered a new plot with fresh grass daily.

Cows that were not day-and-night grazing received ad libitum grass silage at night (S2 and S3) and fresh grass during the day (S3). Daily, individual milk yield was recorded. Milk samples were taken weekly, and analysed for their content of fat, protein, urea, and lactose by using Fourier-transform mid-infrared. With these contents, fat- and protein-corrected milk (FPCM) yield was calculated as  $FPCM = \text{milk yield (kg day}^{-1}) \times (0.337 + 0.116 \times \text{fat \%} + 0.06 \times \text{protein \%})$  (CVB, 2016). Body weight (BW) was measured twice daily after milking, and body condition score (BCS) was evaluated by camera (DeLaval BCS camera, DeLaval, Steenwijk, the Netherlands). All variables were averaged for the 2 weeks measurement period and analysed in SAS 9.4 (SAS Institute, Cary, NC, USA), by using the PROC MIXED procedure with fixed effects of supplementation (EO+ or EO-), fresh grass system (S1, S2, or S3), period (P1 or P2) and all interactions, and a random effect of block. Normality of the data was assessed visually. Results are presented as least-squares means (LSM), significance was declared at  $P < 0.05$  and tendencies at  $0.05 \leq P \leq 0.10$ .

## Results and discussion

Supplementation with EO did not statistically affect milk yield or milk urea of dairy cows (Table 1). The effect of EO on lactose content depended on the period. In P1, lactose content was the same for cows with or without EO (4.59 vs 4.61% respectively,  $P = 0.59$ ). In P2, cows receiving EO had a higher lactose content (4.56%) compared with cows not receiving EO (4.49%,  $P = 0.048$ ). Body condition was not affected by EO.

Both fresh grass system and period affected some of the milk yield characteristics. Overall, fat yield was higher in S3 compared with S1 ( $P = 0.01$ ) and tended to be higher in S3 compared with S2 ( $P = 0.07$ ). Thus, compared to the zero-grazing system, day-and-night grazing or daytime grazing resulted in a reduced fat yield. This is in line with results from an earlier similar study (Klootwijk *et al.*, 2021) and is probably caused by an increased feed intake during zero grazing compared with day and night grazing. Moreover, protein yield was lower in S2 (0.96 kg day<sup>-1</sup>) compared with S1 (1.02 kg day<sup>-1</sup>,  $P < 0.01$ ) and S3 (1.06 kg day<sup>-1</sup>,  $P < 0.01$ ).

Table 1. Milk yield characteristics (kg day<sup>-1</sup>, unless stated otherwise) and body condition of dairy cows in 3 different fresh grass systems (S1: day and night grazing, S2: daytime grazing, S3: daytime zero grazing) receiving as treatment (Trt) a supplement with essential oils (EO+) or not (EO-) in 2 periods (P) ( $n = 84$ ).

	Treatment		Fresh grass system				P-value					
	EO+	EO-	S1	S2	S3	Trt	S	P	Trt*S	Trt*P	S*P	Trt*S*P
Milk yield	29.2	28.7	29.2	28.4	29.1	0.41	0.50	<0.01	0.65	0.27	0.97	0.97
FPCM yield	30.9	30.4	30.1	30.2	31.5	0.42	0.16	0.02	0.93	0.64	0.93	0.60
Fat yield	1.32	1.30	1.26	1.29	1.37	0.44	0.03	0.38	0.92	0.98	0.94	0.49
Protein yield	1.02	1.01	1.02	0.96	1.06	0.88	<0.01	<0.01	0.69	0.52	0.40	0.96
Lactose yield	1.36	1.33	1.36	1.31	1.37	0.28	0.22	<0.01	0.50	0.52	0.73	0.87
Urea yield (g day <sup>-1</sup> )	5.97	5.98	6.22	5.17	6.53	0.99	<0.01	0.10	0.72	0.54	0.02	0.93
Fat (%)	4.48	4.44	4.22	4.56	4.59	0.73	<0.01	0.03	0.37	0.24	0.67	0.14
Protein (%)	3.42	3.47	3.43	3.37	3.53	0.30	0.04	0.06	0.52	0.44	0.32	0.86
Lactose (%)	4.57	4.55	4.56	4.56	4.56	0.30	0.96	<0.01	0.78	0.08	0.83	0.75
Urea (mg dl <sup>-1</sup> )	19.5	19.7	19.6	17.8	21.4	0.75	<0.01	0.10	0.86	0.91	0.02	0.96
BW (kg)	597	601	593	583	621	0.73	0.02	0.69	0.74	0.90	0.22	0.27
BCS	3.0	3.0	3.0	3.0	3.0	0.80	0.75	0.18	0.20	0.44	0.64	0.91

FPCM, fat-and-protein corrected milk; BW, body weight; BCS, body condition score (1–5).

The difference in urea yield and content among systems depended on the period. In P1, cows that were day-and-night grazing tended to have a higher milk urea content ( $20.1 \text{ mg dl}^{-1}$ ) compared with cows that were daytime grazing ( $18.0 \text{ mg dl}^{-1}$ ,  $P=0.06$ ), and the milk urea content of the daytime zero grazing cows was in between ( $19.3 \text{ mg dl}^{-1}$ ). In P2, cows with daytime zero grazing had a higher milk urea content ( $23.6 \text{ mg dl}^{-1}$ ) compared with cows that were day and night grazing ( $19.1 \text{ mg dl}^{-1}$ ) or cows that were daytime grazing ( $18.0 \text{ mg dl}^{-1}$ ,  $P<0.01$  for both comparisons). The results for urea yield were similar. This may be explained by the effect of a difference in season and fresh grass quality and nutritional value, between June and July (P2), compared to April and May (P1).

## Conclusion

In conclusion, although not significant, cows that received EO had a numerically higher milk and FPCM yield and a numerically lower urea concentration in the milk. This could point towards an improved N-efficiency and may have implications for the impact of  $\text{NH}_3$  emission, including the local impact from grazing dairy cows.

## Acknowledgements

The authors thank the staff of Dairy Campus (Leeuwarden, the Netherlands). This study was financed by IDENA (Sautron, France).

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# Monitoring the effect of grass production strategies within the ‘Koe and Eiwit’ project

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## Abstract

In the ‘Koe en Eiwit’ (Cow and Protein) project, 150 Dutch dairy farmers aim to reduce dietary CP level to 155 CP g (kg DM)<sup>-1</sup> to lower ammonia losses. Ration CP level is influenced by CP content in grass silage. The aim of this research is to study: (1) what are the goals in CP level that farmers set for grass silages in the beginning of the year? (2) how are these goals translated into fertilization plans, and (3) what was the success rate of obtaining the goal set for CP level of grass silages. Farmers were visited in 2023 and questioned on their goal for CP level in grass silage, intended fertilization, and the realized CP level in grass silage. On average, farmers did not set lower goals for the CP level in grass silages than realized in 2022. Intended fertilization levels seemed consistent with goals for grass silage CP level. Success rate (as a fraction) of reaching the intended CP level category varied from 0.50 to 1 for the 9 groups, consisting of the first 3 cuts of grass silage on 3 soil types (peat, clay and sand). Success rate was not statistically different between groups.

**Keywords:** crude protein, dietary, reducing, dairy, grassland

## Introduction

The Netherlands has set a goal to reduce ammonia emissions from dairy farming by 50% by the year 2035. Crude protein (CP) level of the ration of the dairy cow has a large influence on N and ammonia losses. The ‘Koe en Eiwit’ (Cow and Protein) project aims to help farmers reach a lower CP level in their total farm diet towards a goal of 155 CP g (kg DM)<sup>-1</sup> for all (150) participating dairy farmers. The portion of fresh grass and grass silage, together with their CP level, are important determinants of the ration CP. Studies have shown that fertilization strategies influence the CP content of grass and grass silages. Higher N fertilization increase the CP content of grass silage (Valk *et al.*, 2000). There is already much advice and information available on the effect of fertilization on crude protein content in grassland (Verstraten *et al.*, 2023). How and whether this advice is applied in farming practice is unclear. The aim of this research as part of the ‘Koe en Eiwit’ project is to study: (1). What are the goals in CP level that farmers set for their grass silages, (2). How are these goals translated into fertilization plans, and (3). What was the success rate of obtaining the goal set earlier in the year.

## Materials and methods

The 150 participating dairy farms are stratified to represent the numbers of farmers on the three main soil types in the Netherlands (clay, peat, sand). Farm advisers visited participating farmers up to 5 times per year. The first visit focused on identifying the goal for the average CP level of all grass silages in 2023. The second visit focused on the goal for the CP level of the first 3 cuts as well as the fertilizer strategy the farmers intended to apply to reach the goals. For the goals for CP level farmers could choose from the categories: <150; 151–160; 161–170; 171–180; 181–190; 191–200; and >200 CP in g (kg DM)<sup>-1</sup>. The fourth visit the realized CP levels of grass silages were registered. During the visits the farmer information was registered in an online tool, which automatically created a data file. Grass silage composition for the year 2022 was available from visits in 2022. The collected data were analysed to: (1) gain insight into the way farmers want to decrease the dietary CP level and whether the goal is consistent with the desire to decrease the CP level in silage, (2). Determine whether the intention of applied fertilizer is consistent



with the goal set, and finally (3). Determine whether the result of the first three cuts is in agreement with the goal set at the beginning of the year (success is defined as a realized CP level within the predetermined goal category). For points 1 and 2, data are treated as descriptive. For point 3 the success rate was analysed with proc reliability with a binomial distribution within SAS 9.4 for windows. This analysis was conducted on 9 groups which comprised the 3 soil types each with 3 separate cuts.

## Results and discussion

Table 1 shows the CP levels for grass silages obtained in 2022, as well as the goals farmers set for 2023 for grass silage CP levels. On average, clay- and peat-based farmers wanted to maintain the amount of CP in their grass and grass silage (goal was within 1 g CP (kg DM)<sup>-1</sup> of 2022). Sand-based farmers wanted to increase the level of CP in the grass silage (goal of +5 g (kg DM)<sup>-1</sup> relative to 2022). Sand-based farmers mentioned they would like to lower the CP level in the other roughage. All farmers aimed to decrease the CP level in concentrates by 14 g (kg DM)<sup>-1</sup> on average (data not shown). On average farmers did not intend to change CP level of grass silage through grassland management in 2023. However, an average 21% of all farmers indicated to aim for a lower CP category; with percentages of 27% for clay, 23% for peat and 15% for sand-based farmers, and 30% of all farmers indicated a desire to maintain the same level. Especially sand-based farmers (46%) indicated the aim to increase the CP level.

Figures 1 and 2 show the planned amount of animal manure and artificial fertilizer that the farmers intended to apply, divided by soil type and by intended goal for the CP level of the first 3 silage cuts. Up to the category of 161-170 g CP (kg DM)<sup>-1</sup> the average fertilization levels increase numerically, which is consistent with the increasing goal for CP in grass silage.

Table 1. Number of total farms in the project, number of farms with a set goal for average CP level in grass silage, CP level of average grass silage in 2022, the goal for 2023, and the difference in CP between these for the farms on the three main soil types.

Soil	Total farms (n)	Farms with goal (n)	CP in 2022 (kg DM) <sup>-1</sup>	Goal CP 2023 (kg DM) <sup>-1</sup>	Δ CP (kg DM) <sup>-1</sup>
Clay	60	46	166	165	-1
Peat	31	27	165	166	+1
Sand	59	51	163	168	+5

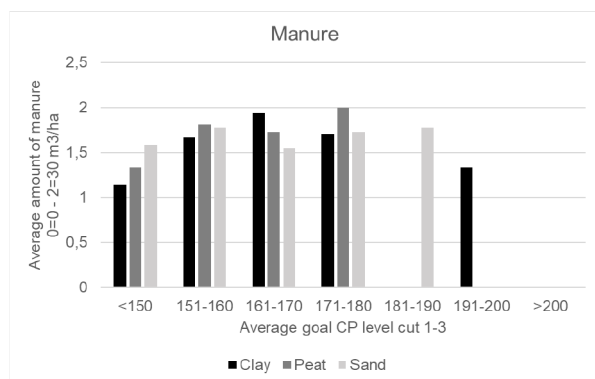


Figure 1. Intended fertilization by manure.

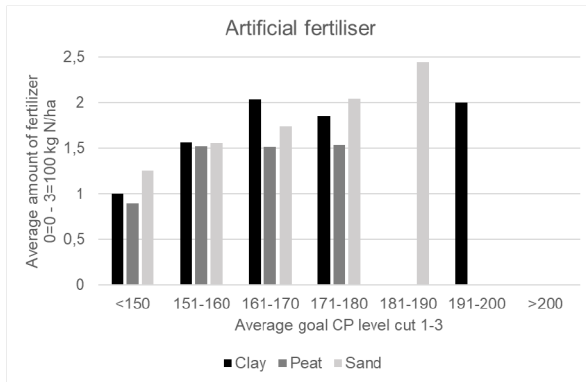


Figure 2. Intended fertilization by fertilizer.

Table 2. Number of analysis (n) and fraction where the CP category goal was achieved (success rate) for the first 3 cuts of 2023 of the farms on the different soil types.

Soil	1 <sup>st</sup> cut		2 <sup>nd</sup> cut		3 <sup>rd</sup> cut	
	n	success	n	success	n	success
Clay	25	0.72	25	0.64	23	0.52
Peat	12	1.00	10	0.50	8	0.63
Sand	32	0.66	30	0.70	27	0.78
Total	69	0.74	65	0.65	58	0.66

Table 2 shows the success rate of reaching the CP goal category set in the beginning of the year. The success rate varies from 0.50 to 1 depending on cut number and soil type. The nine groups (3 soil types with 3 cuts) did not significantly differ in success rate ( $P=0.17$ ). Numerically success rate of the first cut seems higher than for second and third cuts. There is no consistent effect between soil types.

Awareness of the effects that can be achieved within grassland management to reduce CP level could be further stimulated. This could improve the aim of reducing dietary CP level to  $155 \text{ CP g (kg DM)}^{-1}$  on Dutch grassland-based dairy farms.

## Conclusion

In 2023 farmers participating in the ‘Koe en Eiwit-project’ generally did not set lower goals for the CP level in grass silages than their 2022 realized CP levels in order to reach a lower CP level in the dairy ration. A relatively low percentage of farmers (21%) did set lower goals for the grass silage CP levels. On average, the intended fertilization levels seemed consistent with the different intended goals for grass silage CP levels. The success rate of reaching the intended CP level was not significantly different for soil types or cuts.

## Acknowledgements

We thank the Ministry of Agriculture, Nature and Food Quality (The Netherlands) for financially supporting this project. And special thanks to the involved farmers and advisors for their effort and contribution to this project.

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# Mattenklee in mixed swards with timothy for sustainable organic forage production

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## Abstract

Mixed swards with a high proportion of forage legumes will provide high-quality forage and ensure self-sufficiency of nitrogen in organic farming. Red clover (RC; *Trifolium pratense* L.) is the most common forage legume species in Swedish mixed grass-legume leys (MGL), but has poor persistence, often due to root rot caused by soil-borne pathogens. In the presented field trial, three Swiss cultivars of the RC type Mattenklee (MK), cvs. Milonia, Columba and Semperina, were compared with the Swedish-bred RC cultivar SW Vicky grown in mixed swards with timothy cv. Switch (TI; *Phleum pratense* L.). The total yield for the first production year, 2022, ranged from 17 235 kg ha<sup>-1</sup> for RC Vicky to 19 231 kg ha<sup>-1</sup> for MK Semperina, but there were no significant differences between treatments. Regarding each cut, the yield for cv. Semperina+TI was significantly higher than for cv. Vicky+TI the second and third cut. Root health was assessed in late autumn 2022, and all examined roots were affected by root rot. The disease incidence RC Vicky was significantly higher than for MK Semperina.

**Keywords:** red clover, root rot, disease severity index

## Introduction

The purpose of the field trial was to compare three modern cultivars of the Swiss RC type Mattenklee, henceforth called mattenklee (MK), with a common Swedish RC cultivar grown in mixed swards with timothy to assess yield and disease prevalence. Mattenklee is highly persistent and showed a high level of survival after three growing seasons in swards mixed with grass (Hoekstra *et al.*, 2018). The aim of the project was to identify and develop strategies for high-quality forage production and to provide nitrogen in organic farming. Leys with RC may, however, have weak persistence since RC plants disappear after winter prior to the second or third production years mainly due to root rot. Cultivation of more sustainable RC cultivars will create conditions for an increased local supply of high-quality protein forage over time, which in the long run improves soil fertility and a more sustainable and resource-efficient milk and beef production.

## Materials and methods

The field trial was established on 30 June 2021 at a single location at Åkerby, Örebro (59°17' N; 15°3' E) with three MK cultivars: Milonia (9.2 kg ha<sup>-1</sup>), Columba (7.5 kg ha<sup>-1</sup>) and Semperina (7.7 kg ha<sup>-1</sup>), all from Dely Seed, Reckenholtz, Switzerland, and a traditional Swedish bred RC cv. SW Vicky (9.4 kg ha<sup>-1</sup>) from Lantmännen Lantbruk, in mixed swards with TI cv. Switch (8 kg ha<sup>-1</sup>). The nurse crop was a mixture of pea and oats (180 kg ha<sup>-1</sup>), harvested as wholecrop silage in July 2021. The field trial with three replicates has a randomized block design with a plot size of 2.70 m x 12 m. Potassium sulphate corresponding to 80 kg ha<sup>-1</sup> K and 35 kg ha<sup>-1</sup> S was applied in May 2022. The field trial was harvested three times: 20 June, 2 August, and 26 September 2022, respectively, with a forage harvester (Haldrup 1500) to plot-wise determine dry matter (DM) yield and DM content. On subsamples, species separation for botanical composition (sown legumes, sown grass, and weeds) was measured as percentage of DM. Analyses of forage quality was made on the pool of the three replicates for each cut.

In late autumn, ten randomly selected clover plants from each plot were carefully uprooted and brought to the laboratory, washed, and split with a scalpel. The external and internal damage (discoloration) was visually assessed according to Rufelt (1986), where 0 is healthy roots and 4 represents all dark roots. External and internal disease severity indices ( $DSI_E$  and  $DSI_I$ ) and the proportion of roots with external and internal infection (disease incidence,  $DI_E$  and  $DI_I$ ) were calculated for each treatment.

## Results and discussion

The yield of MK Semperina was significantly higher in comparison with RC Vicky at the second and third cut (Table 1). The total forage yield for timothy in mixed swards with MK Milonia was 14 617 kg ha<sup>-1</sup>, Columba 15 019 ha<sup>-1</sup>, Semperina 16 008 kg ha<sup>-1</sup> and RC Vicky 14 842 kg ha<sup>-1</sup>, but there were no significant differences between the treatments (Table 1). The average DM content for MK was significantly higher than for RC (Table 1). The proportion of forage legume increased significantly in the second (71% on average) and third (82% in average) cuts for all treatments compared to the first cut (51% on average) (Table 1). The proportion of sown grass decreased in the third cut (data not shown). Accumulated over the production year, there was no difference in botanical composition between treatments.

The amount of metabolizable energy (ME) ranged from 8.4 to 10.0 MJ (kg DM)<sup>-1</sup>, which is lower than required. Mattenkleee cultivars had a fast regrowth and were harvested too late in crop development to reach optimal ME. The crude protein levels were within the range of guideline values for forage quality, 131–212 g (kg DM)<sup>-1</sup> (Figure 1).

All examined clover plants were infected by root rot ( $DI_E=100\%$ ) after the first forage production year assessed in December 2022 (Table 2). The external disease index ( $DSI_E$ ) ranged from 42 to 50 and Semperina showed significantly lower  $DSI_E$  compared to the other three cultivars. The  $DSI_I$  ranged from 24 to 36 and there were no significant differences between the cultivars, however, the internal disease incidence ( $DI_I$ ) for Semperina was significantly lower than for Vicky.

Disease severity index (DSI) and disease incidence (DI) was assessed externally (E) and internally (I) for mattenkleee (MK) cultivars Milonia, Columba and Semperina and red clover (RC) cultivar Vicky. ANOVA-procedure were used for the statistical analyses. Different characters indicate significant differences according to Tukey's HSD test ( $p<0.05$ ).

Table 1. Field trial of mattenkleee (MK) Milonia, Columba and Semperina and red clover (RC) Vicky grown in mixed swards with timothy (TI). Yields and legume content for each of the three cuts, total yield and average dry matter (DM) content the first production year 2022 at Åkerby, Örebro, Sweden.

Treatment	Cut 1 (kg DM ha <sup>-1</sup> )	Legume content Cut 1 (%)	Cut 2 (kg DM ha <sup>-1</sup> )	Legume content Cut 2 (%)	Cut 3 (kg DM ha <sup>-1</sup> )	Legume content Cut 3 (%)	Total yield	Average DM (%)
RC Vicky+TI	7612 a	56	3589 b	61	3641 b	77 b	14 842	15.9 b
MK Milonia+TI	5837 a	49	4511 ab	74	4270 ab	83 a	14 617	18.7 a
MK Columba+TI	5676 a	46	4670 ab	77	4673 a	85 a	15 019	18.2 a
MK Semperina+TI	6531 a	52	4835 a	72	4642 a	84 a	16 008	18.8 a
<i>P</i> -value	0.0476	ns	0.0313	ns	0.0254	0.008	ns	0.0075
Coefficient of variation	11.7	11.4	9.9	8.8	8.3	2.7	5.0	4.6

Dates of cuts in 2022; 20<sup>th</sup> June, 2<sup>nd</sup> August and 26<sup>th</sup> September. ANOVA-procedure were used for the statistical analyses. Different characters indicate significant differences according to Tukey's HSD test ( $p<0.05$ ).

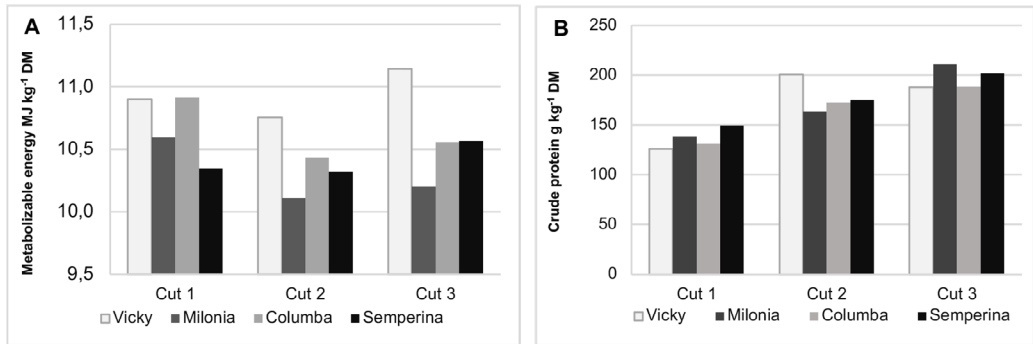


Figure 1. Forage quality presented as (A) metabolizable energy and (B) crude protein, for each treatment: mattenklee cultivars Milonia, Columba and Semperina and red clover cv. Vicky grown in mixed swards with timothy cv. Switch for 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> cut in the field trial at Åkerby, Örebro.

Table 2. Red clover plants assessed for root rot after the first production year 2022 in the field trial at Åkerby, Örebro.

Treatment	DSI <sub>E</sub>	DSI <sub>I</sub>	DI <sub>E</sub>	DI <sub>I</sub>
RC Vicky	48 a*	36 a	100	100 a
MK Milonia	47 a	24 a	100	90 ab
MK Columba	50 a	33 a	100	93 ab
MK Semperina	42 b	24 a	100	83 b
P-value	0.0022	0.0244	–	0.0068
Coefficient of variation	3.7	15.2	–	4.5

Disease severity index (DSI) and disease incidence (DI) was assessed externally (E) and internally (I) for mattenklee (MK) cultivars Milonia, Columba and Semperina and red clover (RC) cultivar Vicky. ANOVA-procedure were used for the statistical analyses. Different characters indicate significant differences according to Tukey's HSD test ( $p < 0.05$ ).

## Conclusion

Cultivars of MK grown in mixed swards with timothy showed competitive DM yield in south-central Sweden (59° N). The MK cultivars were infected by root rot, and two of the three MK cultivars showed similar disease severity indices as RC Vicky. The results from the first production year show that MK cultivars are an important resource in mixed grass-legume leys in south-central Sweden. The early maturation of MK cultivars could suit production systems with 4–5 cuts. In this trial all cultivars were harvested at same time point, and thus the forage quality of the MK cultivars was sub-optimal. The field trial was harvested in 2023 and will continue for the third production year in 2024, and thereafter a final evaluation of the resilience of MK cultivars compared to RC Vicky will be undertaken.

## Acknowledgement

The project was financed by Behm's fund, Swedish University of Agricultural Sciences.

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# Development of rising plate meter calibration equations for mixed perennial ryegrass and ribwort plantain (*Plantago lanceolata* L.) swards

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## Abstract

Accurate estimates of herbage mass (HM) are necessary for effective pasture management. The rising plate meter (RPM) can rapidly measure compressed sward height and estimate HM using simple regression equations. However, the accuracy of these equations varies depending on factors such as species composition, seasonal growth, and sward management. The use of plantain in European grasslands is gaining interest and there is a need to identify accurate plate meter calibration equations for such swards. Using quadrat calibration clips collected over a seven-month period between May and November 2023 ( $n=189$ ), equations were developed for three types of perennial ryegrass swards with differing levels of plantain inclusion. The sward treatments were ryegrass-only (GO), low plantain content (LP; average 27% of sward DM), and high plantain content (HP; average 43% of sward DM). Compressed sward height was measured using a RPM and compared with clipped quadrats using a regression analysis. Best-fit equations were identified, and analysis shows the accuracy of these equations did not differ significantly ( $P=0.065$ ). This suggests that herbage mass can be accurately estimated in swards containing up to 43% plantain using a standard perennial ryegrass equation.

**Keywords:** plantain, rising plate meter, calibration, best-fit equations

## Introduction

Pasture-based livestock systems require accurate and timely estimates of sward herbage mass (HM). Cutting and weighing grass is an accurate method to estimate HM; however, it is time intensive. The rising plate meter (RPM) is an easy-to-use tool which provides rapid estimates of herbage mass (HM) and enables farmers to make grassland management decisions that optimise animal and sward performance (Sanderson *et al.*, 2001). The RPM measures the resistance of the sward toward the plate to determine compressed sward height (CHS). Sward height is translated into HM in kilograms of dry matter per hectare ( $\text{kg DM ha}^{-1}$ ) using a calibration equation that includes a factor to represent the linear relationship between sward height and biomass based on cutting and weighing (Klootwijk *et al.*, 2019). The accuracy of these equations, however, varies depending on species composition, seasonal growth and sward management (Rayburn, 2020). Calibrations have typically been developed for grass monocultures or swards with evenly distributed plant composition (Sanderson *et al.*, 2001); however, increased use of multi-species swards necessitates the development of new calibration equations. The incorporation of ribwort plantain (*Plantago lanceolata* L.) into ryegrass swards has gained recent interest, largely due to its potential environmental benefits in dairy grazing systems (Navarrete *et al.*, 2022; Vi *et al.*, 2023). Despite this, few studies to date have explored the use of the plate meter to predict HM in mixed ryegrass and plantain swards in temperate grassland regions in Europe.

## Materials and methods

The experiment was carried out at the Agri-Food and Bioscience Institute in Hillsborough, Northern Ireland. Three farmlets were established as part of a larger dairy grazing study, each sown with either perennial ryegrass-only (GO), low plantain (LP; average 27% plantain), and high plantain (HP; average 43% plantain). Perennial ryegrass cultivars used were Aberbann and Aberchoice and the plantain cultivar

was Hercules. Once per month, three paddocks (0.14 ha each) from each farmlot were selected based on visual estimates of herbage mass to give a range of low, medium and high sward cover. Three quadrats (0.25m<sup>2</sup>) were placed randomly within representative areas of each paddock, avoiding dung patches. Biomass therein was cut with battery powered hand shears to 3.5cm above ground level on average, and weighed. Compressed sward height was measured before and after cutting using a rising plate meter (model EC10, Jenquip, Feilding, New Zealand), taking five measurements per quadrat. The harvested biomass from each quadrat was separated into grass and plantain proportions before being oven-dried at 60°C for 48 hours. The dry matter (DM) concentration was calculated and used to estimate the above-ground plant biomass per hectare (kg DM ha<sup>-1</sup>) for each quadrat. A total of 63 quadrat measurements were taken from each treatment between May and November 2023. Simple linear regression analyses were conducted to derive an equation for each of the three sward types. The prediction accuracy of the regression equations was expressed in terms of the root mean square error of prediction (RMSEP) and  $R^2$  was used as a measure of correlation.

## Results and discussion

The regression lines for the three sward types are illustrated in Figure 1. The best-fit equations identified in the study (Equations 1–3 in Figure 1) had an  $R^2$  value of 0.82, 0.74 and 0.80 for GO, LP and HP respectively, which indicates a strong prediction accuracy. The average error margin in the calibration equations, expressed as RMSEP, was 20%, 19% and 17% for GO, LP and HP respectively. Analysis of variance between equations however showed that they do not differ significantly ( $P=0.065$ ).

## Conclusion

Results confirm that herbage mass can be estimated in mixed ryegrass-plantain swards using a rising plate meter. The equations for estimating herbage mass in ryegrass-only swards did not differ significantly from equations calibrated for the swards containing plantain. This suggests that herbage mass can be accurately estimated in swards containing up to 43% plantain using a standard perennial ryegrass platemeter conversion equation.

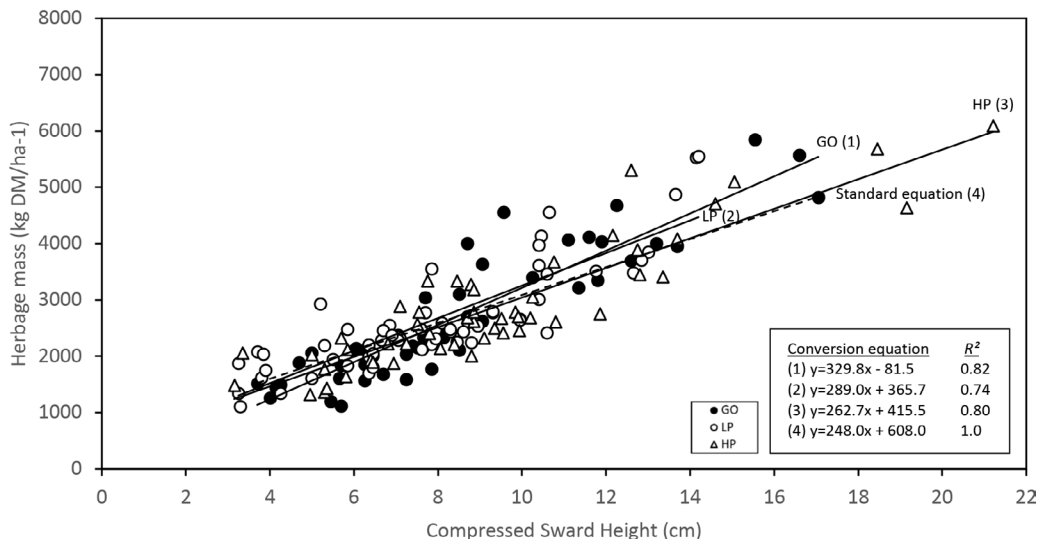


Figure 1. Linear regressions between compressed sward height (x-axis) and herbage mass (y-axis) for three sward types. Symbols differentiate between ryegrass only (GO), low plantain (LP) and high plantain (HP) swards. Conversion equations 1, 2, 3 relate to GO, LP and HP respectively, while equation 4 is a standard grass for grass.



## Acknowledgements

We wish to thank the AFBI Hillsborough staff for their assistance and the Northern Ireland Department of Agriculture, Environment and Rural Affairs for funding the study (EandI project 21/1/04).

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# Investigating factors that affect cow throughput on farm roadways

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## Abstract

In pasture-based dairy systems, farm roadway networks provide a pivotal link between the grazing paddocks and the milking parlour. The objective of this study was to examine the characteristics of farm roadways that impact the movement of a grazing dairy herd on a pasture-based production system. Roadways in the study were classified into three surface condition (SC) categories: Poor, Moderate and Optimal. Surface Condition 'Poor' was the lowest quality surface, while SC 'Optimal' was the most favourable surface for animal movement. Cow throughput on roadways was defined as the number of cows per minute (CPM) passing a specified point on the farm roadways. The impact of roadway width (RW) on CPM was also examined, with RW ranging from 1-4 m. The current study used three herds of 60 cows before milking. Roadway SC ( $R^2=0.78$ ) and RW ( $R^2=0.96$ ) were positively correlated with CPM. There was also an interaction between RW and SC, whereby CPM was limited by SC at greater RW, as the herd showed preferences towards specific parts of the roadway where the SC was Poor, thereby reducing its effective width. Conversely, at narrower RW, increasing SC had a reduced effect on CPM due to the inability of animals to overtake one another. Nonetheless improvements in SC resulted in an increased CPM relative to increases in RW.

**Keywords:** roadway width, surface condition, cow throughput

## Introduction

Roadways on Irish farms have developed over time as the farm has developed or herd sizes have grown and as grazing platforms have increased in size (Kelly *et al.*, 2020). Since the abolition of milk quotas in 2015, dairy farmers in Ireland and across Europe have experienced substantial growth of their herd sizes (Läpple *et al.*, 2022). This was particularly evident in Ireland with the average herd size increasing from 64 cows to 93 cows between 2012 and 2022 (Dillon *et al.*, 2023). This has placed additional strains on existing farm roadway infrastructure on many Irish dairy farms (Maher *et al.*, 2023). To date there has been little investigation of the impact that roadway surface condition and roadway widths have on the movement of the dairy herd. While previous studies have investigated floor surface for animal movements, many of these studies have investigated surfaces with the use of a single cow or two cows walking on the surface at a time (Telezhenko and Bergsten, 2005). This, however, does not represent the practice of moving large numbers of animals to the milking parlour on commercial pasture based dairy farms. The objective of this study is to examine if roadway width (RW) or surface condition (SC) may influence cow throughput of a dairy herd on farm roadways.

## Materials and methods

The study was carried out at the Dairygold Research Farm, Animal and Grassland Research Centre, Moorepark, Fermoy, Co. Cork, between May and July 2021. Three herds of 60 mid-lactation (DIM=180), balanced for breed (Holstein-Friesian, Jersey), milk yield (24.1 $\pm$ 1.4l), parity and calving date, were used to assess the cow throughput on a range of roadway types. Roadways examined in the study were selected based on their SC prior to the examination procedure. Three roadway SC were investigated: Poor, Moderate and Optimal quality surfaces. The Poor quality surface was determined as the roadway on the farm with a large proportion of loose stones present, while the Optimal surface was described as

a smooth walking surface with no loose stones present. Four RW were investigated ranging from 1 m to 4 m wide on one section of farm roadway. The three SC were assessed at a constant RW of 4 m. An additional analysis then investigated if there was any interaction between SC and RW where Poor and Optimal surfaces were examined with a RW of 2 m and 4 m wide. These surfaces were selected as they were the most optimal and least optimal surface for animal movement present on the farm.

An acclimatisation period was firstly under taken before the study began to allow the herd to walk alongside the tape and cones which were used to vary the width of the roadway. Cows were walked along a 60 m section of farm roadway, with the time recorded as the total time taken for the herd to move past the start, mid and end point of the examination section. A herds person walked 5 m behind the last cow. Instances where the herd began to trot or stopped to graze along grass verges were removed from the data set as anomalies, as this may have been caused by factors not influenced by the roadway such as a lack of feed available in their previous allocation. A tape was placed across the roadway 20 metres before the start of the examination period; this allowed the herd to gather, which reduced the spread of cows on the roadway that may have been caused by poor surfaces on the roadway leading to the examination section. The study was undertaken prior to a.m. and p.m. milkings, as a labour unit is required to move the herd from pasture to the milking parlour, while cows often make the return journey independent of any labour requirement. Results of the study were reported as the number of cows per minute (CPM) passing a specified point on the roadway.

Data were statistically analysed using Rstudio through Rx64 4.0.2, using a One way ANOVA to determine significant difference between SC or RW, the correlation of determination was used to determine the proportion of variance in CPM that could be explained by SC and RW.

## Results and discussion

There was a strong positive linear relationship between RW and CPM ( $R^2=0.95$ ) where the SC remained constant, resulting in CPM increasing from 16.28 CPM to 47.42 CPM on roadways 1 m to 4 m. This was due to cows being more dispersed across the roadway. Surface condition also strongly impacted CPM ( $R^2=0.78$ ) across the three surfaces examined. The CPM observed with an Optimal SC was 65.8 CPM, and this decreased to 28.6 CPM for Poor SC. This agrees with work carried out by Telezhenko and Bergsten (2005) where smoother surfaces resulted in faster walking speed. Although the current study did not define walking speed, higher CPM may be associated with increased walking speed. This study also indicated a negative impact of narrow RW on CPM and increased labour input to move the herd along given sections of farm roadways where there was a narrower roadways. Additionally, Browne *et al.* (2022) previously reported that narrower roadways were also a potential cause of increased lameness due to increased pushing and overcrowding on roadways.

The second part of our analysis observed an interaction between RW and SC ( $P<0.001$ ). This revealed where RW was limited to 2 m, increasing SC had a limited impact on CPM. This was due to the inability of the animals to pass one another when the RW was low, while at greater RW (4 m), SC became a limiting factor, as animals tended to follow specific paths on Poor SC roadways, due to the presence of an unfavourable walking surface, resulting in a lower CPM. This is in agreement with a study by Buijs *et al.* (2019) who reported cows will select a pathway with the smoother surfaces when given a choice.

This research provides informative results in relation to roadway upgrades. Although improving roadway SC will improve CPM, its effect is limited by the RW. On large farms where herd sizes have increased, investments in RW may be required to fully benefit from SC improvements. Due to the strong correlation between RW, SC and CPM ( $R^2=0.87$ ,  $P<0.05$ ), a guide was created which indicated the projected CPM passing a given point on farm roadways with specific RW and SC (Table 1). This guide will allow farmers

Table 1. A projection of the cows per minute (CPM) passing a given point on farm roadways based on the roadway's width and surface condition.

Roadway width (m)	Surface condition		
	Poor	Moderate	Optimum
1	13.8	15.2	16.6
2	17.4	27.8	33.6
3	25.0	40.3	50.7
4	32.6	52.8	67.8

to benchmark the roadway network quality of their own farms and use this information to identify which specific improvements are required to improve cow throughput.

## Conclusion

This study reported that RW and SC had strong linear correlations with CPM. Increasing the width of farm roadways will lead to increased cow throughput. Cows per minute increased as RW increased; however, this was strongly influenced by the SC of roadway, whereby roadways which were SC Poor hindered CPM on roadways of greater RW. Future investments should also focus on improving the SC of farm roadways to improve cow throughput.

## Acknowledgements

This research was funded by the Irish Dairy levy administered by Dairy Research Ireland. P.J. Maher was in receipt of the Teagasc Walsh Scholarship.

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# Evaluation of satellite data for estimation of legume proportion in clover-grass swards

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## Abstract

Temporary grasslands in Denmark are mostly used for cutting and typically consist of mixtures of grasses (perennial ryegrass, festulolium) and legumes (white and red clover) as the yield and forage quality is more stable compared to monocultures of species. However, the legume proportion differs within and between fields, which results in different optimal nitrogen fertilization, as the nitrogen requirement is negatively correlated with the legume proportion. Determination of the legume proportion can be challenging and time-consuming. The purpose of this study was to evaluate the use of Sentinel 2 data to estimate the legume proportion in grass-clover fields. The ground truth (validation data) was primarily NIR data on forage choppers from 397 fields and data based on image analysis from 13 fields: in total a dataset of almost 300 000 observations. The legume proportion in the fields measured by NIR sensor on choppers ranged from 0 to 95% with an average of 22%. The final model with satellite images as features showed a mean absolute error of 7.8 percentage points at field level. This study indicates that Sentinel 2 data can be useful for differentiated nitrogen fertilisation according to the legume proportion but requires further refinement.

**Keywords:** legume proportion, estimation, satellite data

## Introduction

Inclusion of legumes in grasslands generally increases yield stability and forage quality compared to fertilized grass-only leys (Egan *et al.*, 2018; Johansen *et al.*, 2017; Lüscher *et al.*, 2014; Søgaard, 2009). The legume proportion in grasslands has a major impact on the nitrogen requirement of the crop as the legume perform symbiotic fixation of atmospheric N. Eriksen *et al.* (2019) illustrated the beneficial effects of differentiated nitrogen fertilization according to the legume proportion. Estimation of the legume proportion can either be done by visual estimation with a high uncertainty, or by botanically fractionation which is time-consuming. Skovsen *et al.* (2017) and Hennesy *et al.* (2021) demonstrated a method for estimation of the legume proportion using analysis of RGB images of mixed swards – however, good quality images typically require flash or intensive light to reduce the effects of shadows. For commercial use such systems require further development before a wide implementation. More self-propelled forage choppers are equipped with NIRS sensors to measure different quality traits in the forage, i.e., content of dry matter, fibre crude protein, but also the legume proportion. However, these NIRS sensors are few and require calibration to deliver useful information. The purpose of this study was to evaluate whether a model based on satellite data could estimate legume proportions by the measurements of NIRS sensors on forage choppers.

## Materials and methods

A dataset based on measurements of the legume proportion in 1185 grass-clover fields in Denmark in 2018, 2022 and 2023 was used as input. The NIR sensor on the chopper measures the legume proportion every three seconds. The result is a cloud data of measurements of each field harvested and the average is calculated for each field. The estimation by NIR on choppers shows a mean absolute error of 12.5 percentage points.

A rather large proportion of the fields (775) had either negative values for the legume, a proportion above 100%, or had only a sparse coverage, and these were discharged from the analysis leaving a training set of 410 fields with reliable data. Of these, 373 originate from 2023. For these 410 fields, the number of registrations per field ranged from around 100 to well above 4000, with an average of 730.

As the data source is primarily the NIRS sensors on forage choppers, the data are recorded as the farmers are cutting the fields – typically 3-5 times a year. Consequently, the number of fields measured by the NIRS sensors differs in each month, resulting in a data set of 96, 86, 182 and 46 fields for May, June, September and October, respectively. For each field, the clover measures were aggregated to 10×10 metre grids (by averaging all the measures inside a grid). The satellite images were collected on a 10m resolution as well, aligned with the field grids. The model was trained on observations corresponding to the 10×10 metres on the field, and afterwards the predictions were averaged over the entire field.

The cells of the images have not been averaged but the predictions of the model were averaged. A collection of 13 bands from the Sentinel 2 satellite's cloud-free image was made nearest to the harvest day. Vegetation indices such as NDVI, gNDVI and GCI were calculated to capture information not contained in the raw bands. Additionally, the altitude of each field was incorporated into the model. The final model included these indices, all atmospheric-corrected (L2A) Sentinel 2 bands, field altitude, and a day-of-the-year indicator for the harvest date.

To evaluate the effectiveness of the model, different models like RandomForest and Shrinkage were tested, but XgBoost performed better. A cross-validation technique was used. In this process, each farm was excluded one by one from the training data. The model was then trained using data from the remaining farms and applied to predict the outcomes for the excluded farms' fields. The model's overall performance was determined by calculating the average of the mean absolute error (MAE) from the predictions of each farm that was left out. This method provided a clear measure of how accurately the model could predict across different farms.

## Results and discussion

The legume proportion was estimated to vary from 0 to 69% at field level with an average of 21%. On a monthly basis, the average legume proportions were 20.8, 10.8, 25.3 and 24.1% for May, June, September and October respectively. Data from mid-June until September were excluded from the analysis as the estimation accuracy in these months was lower — perhaps due to the flowering of the legumes resulting in different satellite values.

The best model predicts the legume proportion with a mean absolute error of 7.8%.

The scatter plot reveals a clustering of data points near the 45-degree line, which symbolizes the line of perfect prediction. This clustering indicates that the model's predictions are, on average, closely aligned with the actual values. Despite this, there is a notable dispersion of points around the line, reflecting considerable variability in prediction accuracy.

This variability manifests as a broad spread of values, suggesting that while the model is generally reliable, there are instances where its predictive capacity deviates from the measured values. We did not find any pattern in the deviations, indicating the uncertainty of the NIR measurements also influences the predictive capacity of this model.

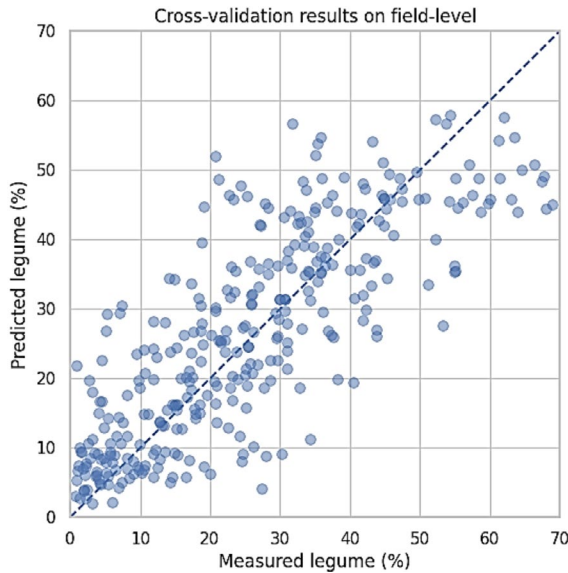


Figure 1. Cross-validation of estimation of legume proportion by Sentinel 2 data as function of legume proportion estimated by NIR sensors on choppers measured at 410 fields.

## Conclusion

The model needs further development and refinement but indicates that satellite data can be useful for a vast estimation of the legume proportion as a tool for farmers and advisers to differentiate nitrogen fertilisation according to the legume proportion. The outcome might be a higher net margin for the farmer, lower GHG emissions and lower potential nitrogen leaching from grasslands and the following crop.

## Acknowledgement

We would like to thank “Promilleafgifsfonden for Landbrug”, who supported this study.

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# How to resurvey historic grassland records from the turn of the 19<sup>th</sup> century?

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## Abstract

Grasslands host a significant share of Europe's species diversity but are among the most threatened vegetation types of the continent. Resurvey studies can help to understand patterns and drivers of changes in grassland diversity and species composition. The Historic Square Foot Dataset, comprises several hundred vegetation plots carefully sampled at the turn of the 19<sup>th</sup> century, covering a wide range of temperate grassland types. In order to use the vegetation data for a resurvey study, the historic species abundance measure of the plots, biomass fraction, had to be translated to percentage cover estimation. Mean vascular plant species richness was 19.7 species for a plot size of 30 cm×30 cm, with a maximum of 47. Historically, species richness did not vary with elevation, whereas present-day pattern shows a hump shaped curve along an elevational gradient in temperate climate conditions. Fractional biomass could be related to fractional cover with an allometric function, while graminoids and forbs showed clear differences in function parameters when analysed separately. The dataset provides a unique insight into what grasslands in Switzerland looked like more than 100 years ago, and thus offering manifold options for studies on the development of grassland biodiversity and productivity.

**Keywords:** biomass estimation, long-term vegetation dynamics, resurvey study

## Introduction

Semi-natural grasslands are among the most threatened habitat types in Europe (Janssen *et al.*, 2016). Their plant diversity is generally assumed to be declining (Dengler *et al.*, 2020; Janssen *et al.*, 2016). However, it is hard to quantify the amount of diversity loss and the direction of compositional change over longer periods, as there are few quantitative datasets that can serve as baseline for comparisons with today's diversity.

The Historic Square Foot dataset (Riedel *et al.*, 2023) comprises 580 vegetation records conducted on small squares of 30 cm×30 cm between 1884 and 1931 in grassland habitats throughout Switzerland. To our knowledge, it is the largest standardised vegetation dataset in grasslands from the late 19<sup>th</sup> to the early 20<sup>th</sup> century. In contrast to recent methods, the researchers of the historic study dug out the survey area, including a few centimetres of topsoil. Aboveground biomass was only harvested and sorted by species in the laboratory before air-drying and weighing the species. These data are unique in providing precise descriptions of how grasslands in Switzerland were composed more than a century ago.

## Materials and methods

We located each historic plot geographically based on the information on the record sheets, which were mainly name of the village, field name and the elevation. We translated this information into a potential area, in which the original plot could have been located. The 580 plots with a size of 0.09 m<sup>2</sup> are distributed throughout Switzerland, covering an elevational range, from 212 to 2547 m above sea level.



For further characterisation of the historic dataset, we calculated unweighted mean ecological indicator values for moisture and nutrients of each plot. To analyse species richness in relation to elevation, moisture and nutrients, we ran general linear models.

One major challenge in resurveying the historical plots had been to find a method for translating the historically measured biomass fraction into percentage cover estimates, which we used for the resurvey study. To address this, carrying out the resurvey study, we estimated the cover of each species in 40 permanent grassland plots and subsequently cut the biomass, sorted it by species, air dried and weighed them separately. We ran linear mixed effect models in order to obtain an allometric function to translate biomass to cover.

## Results and discussion

The species richness in the plots of the historic dataset ranged from 2 to 47 species, with an average of 19.7 species. The maximum of 47 vascular plant species in a 0.09 m<sup>2</sup> plot originates from a “semi-dry perennial calcareous grassland (meadow steppe)” at 1480 m above sea level in the village of Sais in the canton of Grisons. Apart from this plot, five more plots show very high species richness. This indicates a recurring pattern of high small-scale species richness in this dataset, especially when compared to current mean species richness of 11.8 for this plot size in palearctic grasslands.

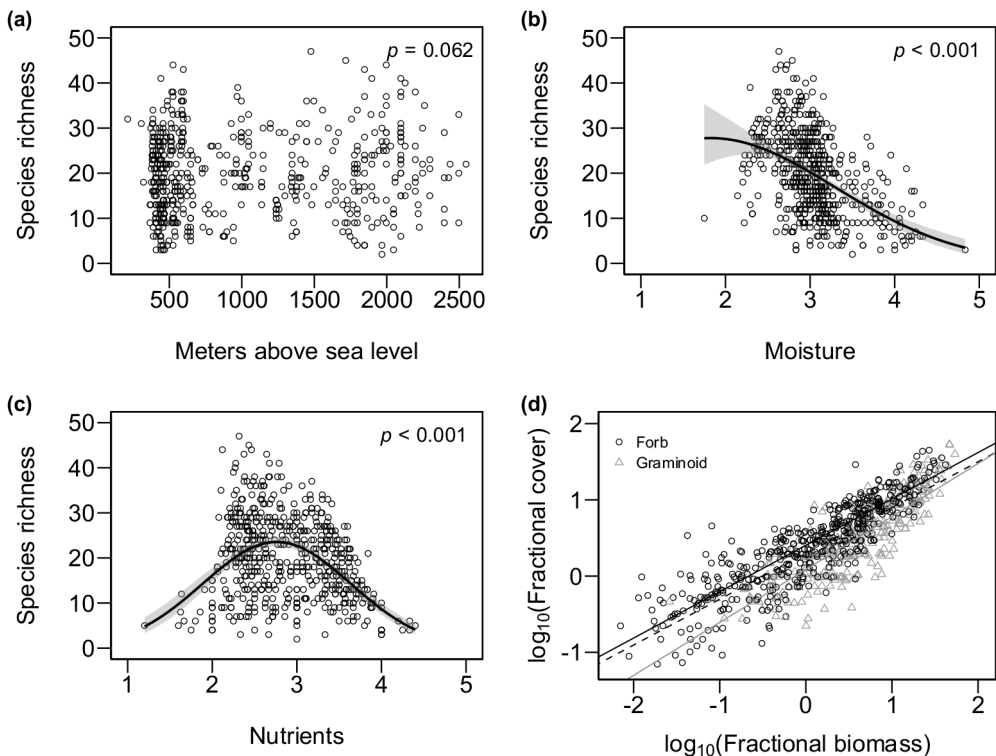


Figure 1. Vascular plant species richness in 0.09-m<sup>2</sup> plots of the Historic Square Foot Dataset ( $n=580$ ) along three main environmental gradients: (a) elevation, (b) mean indicator values for moisture (1=very dry to 5=flooded) and (c) mean indicator values for nutrients (1=nutrient poor to 5=nutrient-rich and over-rich). The lines in (b) and (c) indicate significant relationships in quasi-Poisson GLMs of species richness vs. the respective variable. (d) Relations of the  $\log_{10}$ -transformed weighted biomass and the  $\log_{10}$  transformed estimated cover. The dashed line indicates regression for all species combined, the black line for forbs and the grey line for graminoids.

Species richness showed a small and insignificant increase with elevation ( $p=0.062$ ) (Figure 1a). By contrast, species richness showed a unimodal relationship with the two niche axes determined by the mean indicator values for moisture ( $p<0.001$ , pseudo- $R^2=0.242$ ) and nutrients ( $p<0.001$ , pseudo- $R^2=0.213$ ). With increasing moisture, predicted richness decreased, whereas for nutrients, predicted richness was highest for intermediate nutrient availability (Figs. 1b–c).

The linear mixed model using biomass as a predictor for estimated cover showed a high conditional  $R^2$  (conditional  $R^2=0.78$ ,  $p<0.001$ ). If this model was run separately for forbs with (conditional  $R^2=0.79$ ,  $p<0.001$ ) and without rosettes (conditional  $R^2=0.86$ ,  $p<0.001$ ) a higher proportion of the variance could be explained (Fig. 1d). By contrast, the predictive power was worse for graminoids alone (conditional  $R^2=0.70$ ,  $p<0.001$ ). The regression analyses supported the view that this is an allometric relationship that follows closely a power law. This indicates that a relatively reliable transformation from biomass to cover or vice versa is possible. The parameters of our regression functions add to the hitherto small body of knowledge of such values.

## Conclusions

We demonstrated that biomass and cover can be converted into each other with a good reliability and cover estimation can serve as a good predictor for biomass fractions of grassland species. These findings can also be used for answering questions on grassland productivity, using classical vegetation surveys. While we provide regression functions for temperate permanent grasslands, similar empirical regressions would still have to be established for other ecosystems.

In the ongoing Square Foot Project, the historic plots are resampled with modern approaches of resurvey studies. Ongoing analyses will give major insights into the changes in species diversity and composition which occurred over approximately 120 years.

## Acknowledgement

This research is part of the Square Foot Project (Patterns and drivers of epochal changes in grassland biodiversity in Switzerland) funded by the Swiss National Science Foundation (SNSF) (project no. 197641).

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# A survey analysis of permanent grassland management practices across six European biogeographical regions

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## Abstract

Permanent grasslands (PG) play a key role in providing numerous economic and environmental benefits on European farms. There are however many complexities in the management of these swards, and therefore understanding how farmers meet the challenges of managing PG is vital to promoting best practice. The current study aimed to establish an understanding of PG management practices across European farms. A survey questionnaire was developed and completed by 352 farmers across 23 farm networks in six biogeographic regions. In terms of grass production, farmers in the Atlantic networks targeted 10–15 t DM ha<sup>-1</sup> year<sup>-1</sup>, while the majority of farmers in the Boreal, Mediterranean and Pannonian networks targeted up to 5 t DM ha<sup>-1</sup> year<sup>-1</sup>. Most respondents deemed ‘cutting at the optimal growth stage’ and ‘grazing efficiency’ as highly important, however attitudes towards certain management practices differed between regions. Grass measuring and soil fertility for example were more important to farmers in the Atlantic region, while inclusion of clover was the most important factor for sward performance in Mediterranean and Pannonian networks. Several challenges were identified, with weather being the most significant of these in all regions apart from the Mediterranean.

## Introduction

The SUPER-G project aimed to explore the distribution and state of permanent grasslands (PG) across Europe and to give a better understanding of PG, its impact on the environment and how farmers are managing it. This has been achieved through various activities, one of which was a detailed farm survey designed to gather information on farm characteristics and PG management practices, intentions and outlook from farmers participating in SUPER-G farm networks. The survey’s objective was to collect information from farm owners/renters, farm managers/workers and farming family members across Europe about their PG management practices and expectations. The survey included questions on farm structure; how the farmer used their permanent grassland; grass yields; and soil, grassland and sward management.

## Materials and methods

The surveys were conducted by trained extension officers in the region of interest. Due to the large geographic area, the continent was split into six biogeographic regions: Alpine ( $n=54$ ), Atlantic ( $n=112$ ), Boreal ( $n=22$ ), Continental ( $n=112$ ), Mediterranean ( $n=24$ ) and Pannonian ( $n=28$ ). Respondents were provided with a farmer information sheet that ensured interpretation and responses between farm networks and biogeographic regions were consistent. Both the survey and the farm information sheet are available from the authors on request.

Responses were collated and standardised in an anonymous datafile for analyses. Statistical analysis was conducted using R-3.6.3 (R Core Team, 2020). Differences in livestock stocking densities (LU ha<sup>-1</sup>), land type area (ha), percent of PG receiving different types of fertiliser, fertiliser product use on improved and unimproved PG, between farm networks in contrasting biogeographic regions were analysed by ANOVA. Chi-square tests were used to look for differences in the proportions of responses to questions concerning farm management practices, factors viewed as important to improve PG performance,

farmer target yields from cutting and grazing swards, and challenges when trying to improve grassland performance. The significance threshold was  $P < 0.05$ .

## Results and discussion

There were four types of exploitation system, these were: 'Grazing and cutting', where more than 25% of PG is both grazed and cut for hay or silage; 'Predominantly cutting', where more than 75% of PG surfaces are cut for hay or silage; 'Predominantly grazing' where more than 75% of PG surfaces are directly grazed by animals; and 'Non feeding' where the grass biomass produced goes to an anaerobic digester or is rarely or inconsistently used. Exploitation system differed significantly between farm networks grouped by biogeographic region ( $X^2=104.55$ ,  $df=15$ ,  $P < 0.001$ ). The Boreal and Pannonian respondents reported that their PG was predominantly used for cutting (62% and 57% respectively). The majority of farms in the Alpine region allocated their PG to grazing and cutting (65%), and the majority of Mediterranean farms (87%) were predominantly grazing. Atlantic and Continental farms were mixed, with most Continental farms reporting to be predominantly cutting or grazing and cutting (49 and 36% respectively) and Atlantic farms being either predominantly cutting or predominantly grazing (45%) or cutting and grazing (43%).

Target yields for PG were dependent on the exploitation system, with different targets for grazed land and land cut for hay or silage. The target grass dry matter yield ( $t\ DM\ ha^{-1}\ year^{-1}$ ) on improved grazing land varied between regions ( $X^2=55.987$ ,  $df=15$ ,  $P < 0.001$ ). Atlantic region farmers had the highest expectations for their improved PG, with 60% targeting a yield of 10–15  $t\ ha^{-1}$ , and 1% targeting 15  $t\ DM\ ha^{-1}\ year^{-1}$ . The target grass dry matter yield on PG improved for cutting also varied between biogeographic region ( $X^2=29.227$ ,  $df=15$ ,  $P < 0.05$ ). Most farmers in the Alpine, Atlantic and Continental networks targeted 5–10  $t\ DM\ ha^{-1}\ year^{-1}$ , while most farmers in the Pannonian network targeted up to 5  $t\ DM\ ha^{-1}\ year^{-1}$ . In the Boreal and Mediterranean networks, most respondents reported a target of 5–10  $t\ DM\ ha^{-1}\ year^{-1}$  (37.5% and 40%). Some farmers in the Atlantic (10%), Mediterranean (10%), Alpine (6%), and Continental (1%) networks targeted more than 15  $t\ DM\ ha^{-1}\ year^{-1}$  on their cutting land. These higher targets are reflected in the organic fertiliser use with the Atlantic region applying 57.2  $t\ ha^{-1}$  to improved PG silage ground; significantly more than Alpine (39.0  $t\ ha^{-1}$ ), Boreal (20.8  $t\ ha^{-1}$ ) or Continental (29.6  $t\ ha^{-1}$ ) respondents. This increased use of organic fertiliser on silage ground could be reflective of the increased target, or the higher stocking rate in this region leading to a greater availability of organic fertiliser. The Atlantic region farms had the highest stocking rate of dairy cattle (1.03 livestock units  $ha^{-1}$ ;  $P < 0.05$ ), and a significantly higher stocking rate of sheep (0.16) than all regions other than Alpine and a higher stocking rate of beef cattle (0.54) than all regions but the Mediterranean ( $P < 0.05$ ).

Grazing yields from improved PG varied between biogeographic region ( $X^2=19.64$ ,  $df=10$ ,  $P < 0.05$ ). Grazing yields from improved PG of 0–5  $t\ DM\ ha^{-1}\ year^{-1}$  were reported by the majority of all respondents in all regions other than the Atlantic networks where 55% of respondents reported yields of 5–10  $t\ DM\ ha^{-1}\ year^{-1}$ . A small proportion (3%) of farmers in the Continental networks reported yields of 10–15  $t\ DM\ ha^{-1}\ year^{-1}$ . Reported cutting yields also varied between biogeographic regions ( $X^2=25.35$ ,  $df=10$ ,  $P < 0.001$ ), with most farmers in Alpine, Boreal, Continental, Mediterranean and Pannonian networks reporting yields of 0–5  $t\ DM\ ha^{-1}\ year^{-1}$ . By contrast, in the Atlantic networks, 82% of respondents reported cutting yields of 5–10  $t\ DM\ ha^{-1}\ year^{-1}$ . The results confirm the higher grass yield potential from improved PG in Atlantic regions and indicate that many farmers are not achieving their target grazing and cutting yields from their improved PG platform.

The most important factors considered by farmers for improving PG performance varied according to the biogeographic region that the networks were in ( $X^2=29.39$ ,  $df=10$ ,  $P=0.001$ ). The majority (64–84%) of farmers in Atlantic, Boreal, Continental and Mediterranean networks considered 'soil compaction' and

'drainage' to be important factors affecting PG performance, whereas in Alpine regions 72% and 67% of farmers considered 'soil compaction' and 'drainage' to be 'not applicable' and in the Pannonian network 86% and 57% thought they were 'not important'. This is representative of the different land conditions in the different regions. This was further underlined when respondents from the different biogeographic regions reported the top three challenges to improving PG performance. Views were significantly different between regions for both improved and unimproved PG ( $X^2=233.6$ ,  $df=50$ ,  $P<0.001$  and  $X^2=81.409$ ,  $df=50$ ,  $P=0.01$ , respectively). 'Weather' was the most significant challenge in all regions apart from the Mediterranean. Respondents from the farm networks in the Atlantic and Continental regions reported 'weather' to be the most important constraint for improved and unimproved PG. 'Access to capital' was the next most frequently reported challenge for improved PG; placed in the top three challenges in farm networks in the Alpine, Boreal, Continental and Mediterranean regions. 'Environmental legislation' was also recognized as a significant constraint for improved (top three in Continental and Pannonian networks) and unimproved grassland (Atlantic, Boreal and Pannonian). Infrastructure was reported as one of the three most challenging aspects to improving performance in the Atlantic and Mediterranean networks; however, it was not cited by respondents from any other region.

## Conclusions

Farmers across Europe have varying expectations of their PG and will accept different levels of performance. These differences can be attributed to geographical differences between the farm locations. Despite these differences, there are challenges such as weather and access to capital, which are common to multiple biogeographic regions.

## Acknowledgements

The authors would like to thank all the extension officers who carried out the survey and the respondents who took time to fill it in. The SUPER-G project (Grant Agreement No. 774124) has received funding from the European Union Horizon 2020 Research and Innovation Programme.

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# Landolt indicator values changes as result of fertilization in *Danthonia alpina* grassland

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## Abstract

On natural grasslands, application of mineral fertilizers increases dry matter yields and also changes the botanical composition of plant communities. The objective of this study is to examine how the *Danthonia alpina* Vest type grassland changed as a result of fertilization in the hilly Balkan region. Unfertilized control and four fertilized treatments P60K60 (PK), N20P60K60 (N20), N80P60K60 (N80) and N140P60K60 (N140), were applied annually during a four-year period were examined. Mean Landolt's ecological indicator values (moisture (F), nutrients (N) and temperature (T)), were calculated for each treatment. The fertilized plots showed higher F and N, but lower T value, and the changes became more evident at the fourth year of application. During the research period, the NxPK treatments had a greater effect than PK treatments. The F was highest in treatments N140 (2,97) four years after fertilizers were applied; Nevertheless, T decreased greatly in the fourth year and ranged from 3,40 (control) to 3,03 (N140). In our experiment, short-term fertilizer application changed Landolt's ecological indicator values, which is the opposite of ongoing climate change effects.

**Keywords:** grassland, *Danthonia alpina*, Landolt indicator values, nitrogen

## Introduction

The plant species that comprise a grassland community are the product of habitat conditions and management practices. Each change in the management methods (e.g. frequency of cutting or fertilization level) has an impact on botanical composition of the sward and habitat itself. Indicator values according Ellenberg or Landolt express plant preferences for temperature, light, continentality, soil moisture, pH, and soil nutrients, and have been largely used to deduce plant communities' environmental characteristics. The environmental indicator values of plant communities indicate mean conditions of the realized niche of every species. On the basis of these indicators, it is possible to make conclusions about habitat changes over time, influenced by fertilizing, frequency of cutting etc. An important question is how the changes connect to climatic changes which are characterized by extreme weather events such as heat waves, droughts, heavy rainfall, hailstorms, and storms. According to current climate research, it can be assumed that by 2050 the annual average air temperature in highland region will rise about +2°C (Gobiet *et al.*, 2014). The primary objective of our experiment was to assess the impacts of different fertilization treatments on Landolt ecological indicator values (F, N, T) during four years in the hilly Balkan region.

## Materials and methods

### *Field experiment site*

This was located at Mitrovo Polje in the central part of Serbia (43°30' N, 20°52' E) on acid soil (pH<sub>KCl</sub> 4.09). Prior to the trial establishment the soil phosphorus content was 2.65 mg kg<sup>-1</sup> and potassium content was 7.96 mg kg<sup>-1</sup>. There was a high level of organic matter (8.96%) in the soil. The community was *Danthonietum alpinae* type grassland.

## Experiment

The experimental plots were established in an area with homogeneous vegetation and they were arranged as randomized block system. The plots were 10 m<sup>2</sup> in size, and each treatment replicated four times. The experiment consisted of five treatments: control, N20P60K60 (N20PK), N80P60K60 (N80PK), N140P60K60 (N140PK). Phosphorus and potassium were applied in autumn. Nitrogen was applied in spring, every years in mid-March, as ammonium nitrate (33% nitrogen).

## Analyses

Cover-abundance values of individual species was estimated right before first cut, using the 6 classes of Braun–Blanquet scale (+=<1%, 1=1–10%, 2=10–25%, 3=25–50%, 4=50–75%, 5=75–100%). According to Braun–Blanquet, cover values were converted to percentages: +=0.5%, 1=5%, 2=17.5%, 3=37.5%, 4=62.5%, 5=87.5%). The Landolt indicator value (Landolt *et al.*, 2010) for each plot was calculated as the mean of indicator values weighted with cover of each species present in the plot. We calculated the following Landolt indicator values for: nutrients (N), humidity (F) and temperature (T).

## Results

Significant dependence of N, F, T values of fertilization treatments on *Danthonia alpina* type grassland was found (Figure 1).

During the first year, no dependence was found between N and the fertilization treatment. The analysis of habitat conditions based on Landolt indicator values shows a considerable fertilizer effect on N in the second year, and the changes became more evident at fourth year of application. The mean ecological indicator values of nutrients gradually rose as applied nitrogen increased. The N was highest in treatments

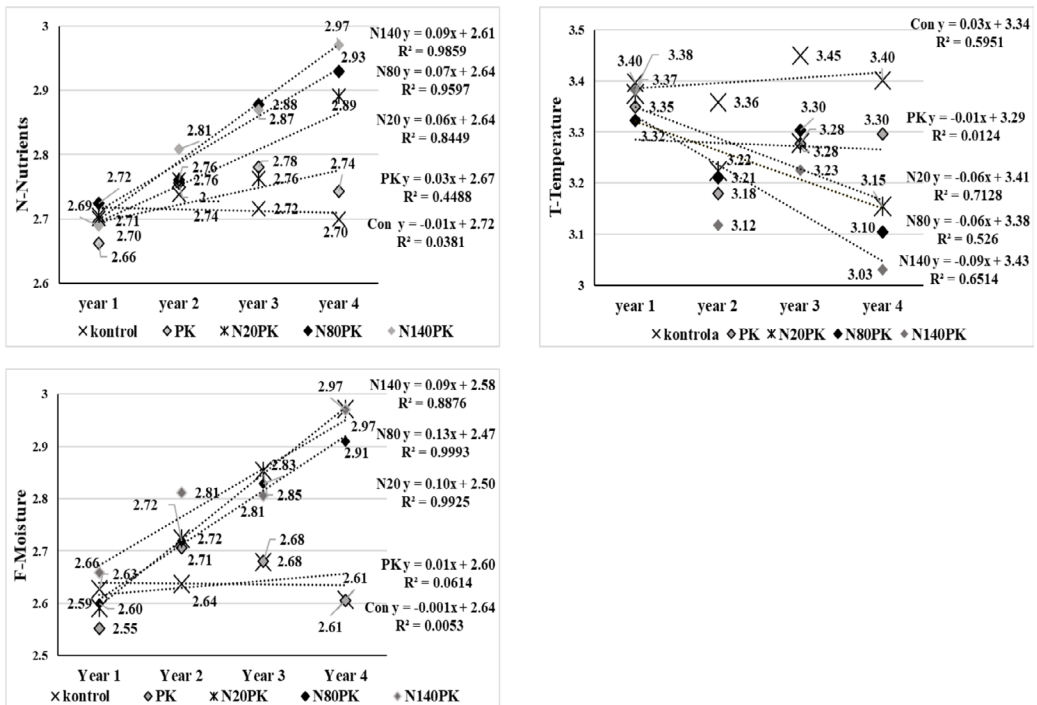


Figure 1. Mean Landolt indicator value for nutrients, temperature and moisture, in fertilized treatment during four years of addition in *Danthonia alpina* grassland (the coefficient of determination is significant at a value of  $R^2 > 0.6$ ).

N140 (2.97) compared to the control (2.7) four years after fertilizers were applied. Galka, (2005) discovered similar, that nitrogen addition had a positive impact on N (2005). The N was increased in PK treatments. According to Tiller *et al.* (2021), adding phosphorus to systems where it is the limiting factor leads the nutrients indicator value increase.

T decreased with increasing nitrogen addition. During the four-year period, indicator values for temperature in treatment N140 declined the most ( $R^2 > 0.6$ ). The decrease is most expressed in the fourth year, and ranged from 3.40 (control) to 3.03 (N140).

The impact of fertilizer application on F change was similar to that on N. There were no differences among treatments in F during the first year. Changes were more evident in the second, third and fourth years, and the changes became more expressed in the fourth year of application. During research period, treatments with nitrogen had a greater effect than PK treatment. Similar influence of fertilizers on ecological indicator value are detected by Chitry *et al.* (2009).

## Conclusion

Mean Landolt ecological value was changed in all fertilized plots and the NxPK treatments had a greater effect than PK treatments. Short-term fertilizers application on *Danthonia alpina* grassland increase N, F, while T decreased. These changes were most pronounced in the treatments where the highest amount of nitrogen was applied.

## Acknowledgement

The work was supported by Ministry of Education, Science and Technological Development (Serbia number 47/2023) -01/ 200217.

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# Impact of leaf area index on the grassland yield prediction

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## Abstract

The aim of our study was to evaluate the suitability of LAI calculated from satellite data (LAI-sat) for grassland yield prediction based on relations between in-situ ground measured yield indicators and LAI computed from in-situ values (LAI-cept) compared to relations between those indicators and LAI-sat data. The research was carried out in the years 2020–2023 on permanent grasslands located in dairy farms in central-western Poland. In each grassland, ground measurements were carried out in a representative 30 m×30 m plot every 2–3 weeks during the growing season. Fresh and dry matter yield was determined from biomass samples collected using a quadrat frame. Additionally, compressed sward height was measured using a Jenquip EC20 plate meter. LAI-cept was measured using AccuPAR LP-80 ceptometer and LAI-sat was obtained from platform Weekeeo based on Sentinel-2 satellite images at 10 m pixel resolution. Statistical analysis has shown that all the tested relations had high correlation coefficients. The accuracy between LAI and FM or DM was slightly higher for LAI-sat than for LAI-cept. We conclude that LAI delivered from satellite data can be used to support grassland farmers to make proper management decisions.

**Keywords:** leaf area index, grassland, yield prediction, remote sensing

## Introduction

The leaf area index (LAI) is one of the key biophysical metrics to characterize grassland vegetation growth. LAI can be measured using ground-based methods, but these approaches are time-consuming, labor-intensive, and difficult to apply at a regional scale. In the last few decades, remote sensing-based approaches, which are endowed with high temporal resolution and the capacity for large-scale observation, are increasingly used to estimate LAI. As reported by Reinermann *et al.* (2020), LAI is one of the most widely used indices within the studies investigating grassland management with remote sensing data, like NDVI and band reflectance values. Therefore, research towards practical applications of remote sensing-based LAI is needed to support appropriate grassland management decisions. The aim of our study was to evaluate the suitability of LAI obtained from satellite data for the grassland yield prediction based on relations between LAI in-situ and ground measured yield indicators.

## Materials and methods

The research was carried out in the years 2020–2023 as part of the project GrasSAT ([www.grassat.eu](http://www.grassat.eu)). Reference data were collected on 22 permanent grasslands selected in 10 medium and large dairy farms in the region of central-western Poland (Wielkopolskie voivodship). As suggested by Crabbe *et al.* (2019), on each site, a 30 m×30 m plot was randomly selected for in-situ ground measurements to encapsulate the resampled 10 m × 10 m spatial resolution of the Sentinel-2 imagery, allowing for a 10 m radius buffer around the ‘central pixel’ location for uncertainty in spatial registration of the image pixels. Field measurements were carried out every 2–3 weeks throughout the growing season. In this paper we report our investigations of the hypothesis that the correlation between grassland yield and different LAI obtained in-situ and from satellite is similar. The yield was represented by three different indicators: aboveground fresh biomass (FM), dry biomass (DM) and compressed sward height (CSH). On each site, the FM and DM yields were determined using the quadrat frame method from the area of 0.5×0.5 m with

four replications. The CSH was measured using Jenquip EC20 alu plate meter. LAI at the ground level (LAI-cept) was determined with AccuPAR LP-80 ceptometer (using effective plant area index  $L_e = \Omega L$  where  $\Omega$  refers to a clumping index resulting from the non-random distribution of canopy elements). The remote sensing-based LAI (LAI-sat) was obtained from platform Weekee based on a neural network that utilizes the surface reflectance of Sentinel-2A bands. The relationships between the in situ LAI-cept and biomass and the LAI-sat data were determined. The correlations were tested using the Pearson's  $r$  coefficient in the R statistical environment and modelled using simple linear regression with confidence interval displayed around the regression line (Wickham, 2016).

## Results and discussion

The analysis has shown that there is a high correlation between all the indicators of grassland yield and the optical indicators of LAI ( $r > 0.85$ ). The correlation between LAI and FM is closer for LAI-sat than for LAI-cept ( $r = 0.947$  and  $0.865$ , respectively). Similarly, the correlation between LAI and DM is slightly better for LAI-sat than for LAI-cept ( $r = 0.929$  and  $0.885$ , respectively). The strength of correlation between CSH and LAI-sat or LAI-cept is at a similar level ( $r = 0.856$  and  $0.856$ , respectively). In general, our results indicate that linear relationships between LAI-sat or LAI-cept and the studied grassland yield indicators are high. However, the scatter plots illustrating these relationships suggest that yield estimation using optical LAI indicators is most precise before the accumulation of grassland biomass reaches ca.  $550 \text{ g FM m}^{-2}$ , or  $150 \text{ g DM m}^{-2}$ , or before the average CSH is around  $13 \text{ cm}$  (Figure 1). All these threshold values are consistent with one another. Above these thresholds, the studied LAI indicators seem to be less responsive to the accumulation of grassland biomass or the CSH increase, which is indicated by the wider points dispersion and the weaker trend of increase in the plots. This is related to the change in the structure of aboveground biomass due to the transition from the vegetative to the generative growth stage in grasses and other plants. Another reason is foliage overlapping that makes some leaves invisible to the optical sensors.

Our study has shown that LAI-sat can be used to predict yield in decision support systems for grassland management, but the precision of this prediction can be further improved in future research. However, as reported by Reddersen *et al.* (2014), models for predicting biomass of extensively managed grassland using exclusive LAI were barely suited to predict biomass accurately, but can be improved significantly when combined with waveband selected common vegetation indices. We further propose that for tall and dense swards, the relationship between the yield and LAI-sat is modelled using a two-segment regression line (Muggeo, 2008), with the first segment steeper than the second one, and the breakpoint (yield indicator value where the two segments are connected) located near the above-mentioned threshold values.

## Conclusions

The results shows that there is a high correlation between optically assessed LAI and FM, as well DM yields or CLS. The strongest correlation coefficient values were obtained for LAI from satellite, with slightly weaker correlation values received for LAI *in situ*. We concluded that remote sensing-based LAI is suitable to predict grassland yields and support grassland management decisions.

## Acknowledgement

The authors would like to thank the Polish-Norwegian Research Programme for co-financing the GrasSAT project (grant agreement no. NOR/POLNOR/GrasSAT/0031/2019-00).

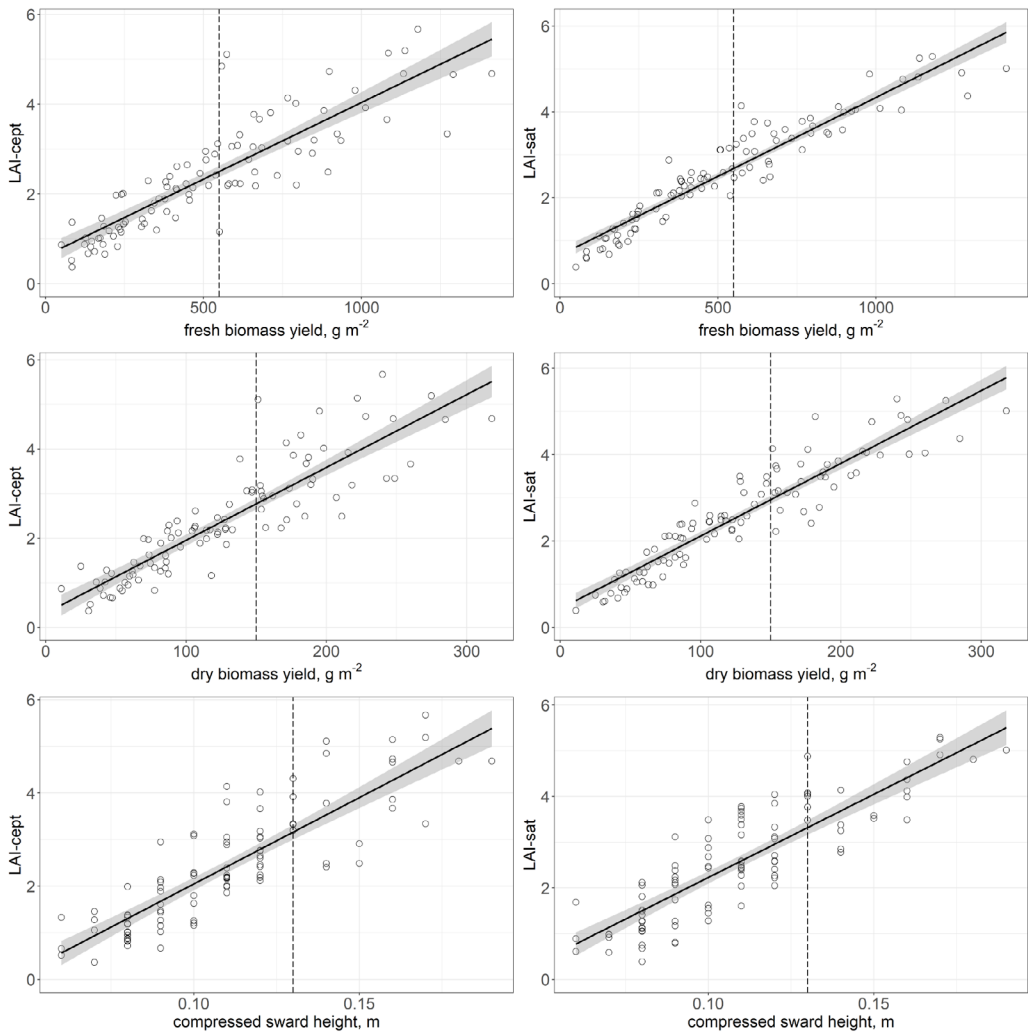


Figure 1. Correlation between in-situ measured LAI (left) and sensing-based LAI (right) and fresh biomass yield, dry biomass yield and compressed sward height on grassland (vertical long-dashed lines illustrate threshold values).

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# How does seed production in southern part of Norway affect the winter hardiness of varieties adapted to Northern Norway?

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## Abstract

Norway stretches from latitude 58° to 71° north. Thus, the climate is very different in the south compared to the north. Since seed production in the north is unpredictable due to the short growing season, commercial seed production of perennial forage grass cultivars has been located in the south-eastern part of Norway. We tested freezing and ice-encasement tolerances of three seed lots of different age of each of the northern-adapted cultivars 'Engmo' and 'Noreng'. The seed lots were prebasic (original), intermediate (mid), and current commercial (late). The results showed that both cultivars had reduced freezing tolerance when comparing plants from the original seed lots with plants from the current commercial seed lots, which originate from several generations of seed multiplication in the south. Regarding tolerance toward ice-encasement, there were no significant differences between seed lots or cultivars. This indicates phenotypic and genetic shifts within the cultivars towards less frost-tolerant populations. It is therefore important to implement seed production regimes of northern-adapted cultivars that reduce the risk of shifts and preserve the cultivar characteristics.

**Keywords:** timothy, cv. Engmo, cv. Noreng, seed lots, freezing tolerance, ice encasement

## Introduction

Grassland based forage production is the cornerstone of agriculture in northern Norway and timothy is the most important grass species. Production of commercial seeds of timothy cultivars bred for northern Norway is challenging because of the short growing season in the north, therefore the production is located in the south-eastern part of Norway. This can lead to risks of genetic shifts in the cultivars and thus change in key traits that are important in the north such as winter survival. Previous observations in field studies showed an almost linear decrease of winter survival from about 78% to about 53% for 0 to 6 generations of seed propagation of cv. 'Engmo' in south-east Norway (Andersen, 1971). Farmers in the north have in the recent years been complaining about reduced winter survival in the field. The synthetic cv. 'Noreng' replaced the old winter hardy landrace cv. 'Engmo' in 2005. In this study we tested the winter hardiness of the original seed lots and compared with newer seed lots of 'Engmo' and 'Noreng' to find out whether seed propagation in the south has changed the adaptive traits in the cultivars.

## Materials and methods

We tested the original seed lots of Engmo original (seeds produced in Malangen (69° N, 18° E) in 1988) and Noreng original (pre-basic seed from 1991) against seed lots now available on the market: Engmo late (commercial seeds from 2019) and Noreng late (certified seed from 2019), and intermediate seed lots: Engmo mid (commercial seeds from Iceland 2010, propagated in Canada) and Noreng mid (basic seed harvested at Björke (60° N, 11° E) in 2010). Seedlings were established in the greenhouse and cold acclimated for 2 weeks at 9°C, 1 week at 6°C, and then 2 weeks at 2°C at 12 h light. Then the seedlings were tested for both freezing and ice-encasement tolerance. Freezing tolerance was tested as described in Dalmanndottir *et al.* (2016). Seedlings for ice-encasement tests were prepared as for the freezing tolerance test, placed into boxes with icy water and were frozen to solid blocks at -2°C in the dark. Seedlings were kept encapsulated in ice for a certain number of days (from 0-80) before regrowth/survival was estimated. Survival of the plants was analysed in R using a probit regression model (GLM

model with probit link function) with both cultivars analysed together and the probability of survival as outcome. The freezing temperature was included as a base variable in all models and the factor variables origin, cultivar and their interaction were consecutively added, with the significance assessed using a likelihood ratio test.

## Results and discussion

There were significant differences between the freezing tolerance of the different seed lots ( $P \leq 0.001$ ) where the original seed lot was most hardy, the intermediate seed lot less hardy, and the late seed lot (current commercial seed) was the least hardy (Figure 1). This is also reflected in the  $LT_{50}$  values (Table 1), which is the temperature where 50% of the plants are dying. There was no significant difference between the two cultivars Engmo and Noreng ( $P=0.41$ ). These results show that the cultivars did undergo genetic shifts with time resulting in reduced freezing tolerance.

The results from the studies on ice-encasement are not as clear as those from the freezing tolerance (Figure 2). The difference between seed lots was not significant nor the difference between cultivars. This shows that freezing tolerance and tolerance against prolonged ice-encasement are not based on the same physiological mechanisms. Thus, a selection pressure towards one mechanism is not necessarily affecting the other mechanism.

The loss of freezing tolerance, when seeds are produced in the south of Norway over time is of major concern for farmers in northern parts of the country where the climatic conditions are very different. To minimize the risk of genetic shifts, the number of seed generations outside the area of origin and utilization should be kept to a minimum.

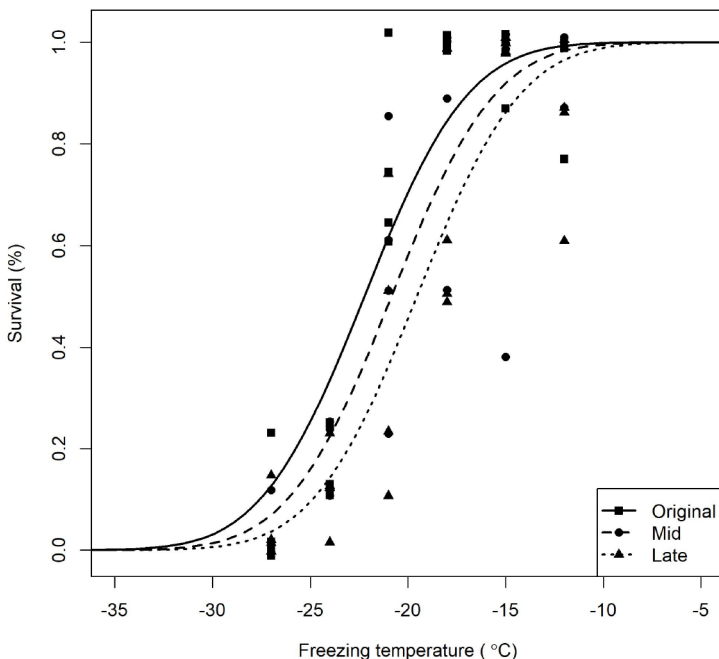


Figure 1. Survival (%) of seedlings of different seed lots as a mean of the two cultivars at lowering freezing temperatures.

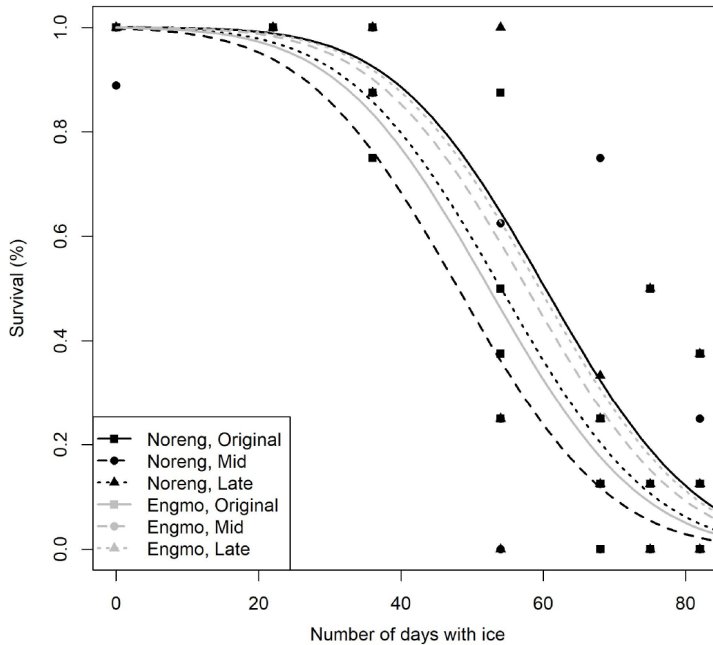


Figure 2. Survival (%) after prolonged treatment of ice-encasement. Different seed lots of Noreng (whole line) and Engmo (dotted line).

Table 1. Results from freezing test (LT<sub>50</sub> values) and ice encasement test (LD<sub>50</sub> values).

	Original	Mid	Late	Cultivar
LT50	-22.4	-21.1	-19.8	Noreng
	-22.0	-20.6	-19.3	Engmo
LD50	55.3	51.7	55.5	Noreng
	57.6	54.0	57.8	Engmo

## Conclusion

The freezing tolerance of the northern-adapted cultivars was reduced in the seed lots which are on the market today compared to the original seed lots. This shows that the seed production in south-east Norway may reduce the winter hardiness of seeds intended for use in northern Norway. Ice-encasement studies did not show significant differences between seed lots.

## Acknowledgement

We thank the Norwegian Research council (project number 303258, NeXTim) for support.

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# Risk of nitrate leaching at grassland renovation in spring versus autumn in the Netherlands

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## Abstract

In the Netherlands farmers renovate grassland when the botanical composition has deteriorated. This is done by destroying the sward followed by tillage and reseeding. Farmers prefer renovation in August over renovation in spring due to a higher success rate and reduced loss of production. The risk of nitrogen (N) loss by leaching, however, is expected to be higher in August because of high N mineralization from the old sward. In the Netherlands grassland renovation in the period 1 June–31 August is only allowed with a reduction on the N application standard of 50 kg ha<sup>-1</sup>. To assess how much this and other measures reduce the risk of N leaching, a field experiment was laid out. The treatments were reduction of tillage or N fertilization, mechanical destruction, and use of a nitrification inhibitor, compared with regular renovation in August, renovation in spring, and cultivation of maize. Observations were soil-mineral N in autumn, and nitrate concentration in groundwater the following February. All measures reduced the risk of nitrate leaching. The risk of nitrate leaching with grassland renovation in August was, however, still higher than no renovation, renovation in spring, or cultivation of maize. The highest risk of N leaching was found on the treatment with renovation in August by mechanical destruction of the sward.

**Keywords:** grassland, maize, renovation, N leaching, soil mineral N, nitrate concentration,

## Introduction

In the Netherlands farmers renovate grassland when the botanical composition has deteriorated. This is done by destroying the sward followed by tillage and reseeding. Farmers prefer renovation in August or September over renovation in spring due to a higher success rate and reduced loss of production. In spring the risk of drought occurrence before the grass is well developed is relatively high. Furthermore, missing the first and (part of) the second cut generally results in a higher production loss than missing cuts in autumn. The risk of nitrogen (N) loss by leaching, however, is expected to be higher at renovation in August because the development of the new sward is not rapid enough to take up all the N that is mineralized from the old sward (Velthof *et al.*, 2010). Therefore, regular grassland renovation by chemical destruction followed by tillage and N fertilization is only allowed in spring. If grassland is renovated in the period 1 June–31 August the farmer has to reduce the N application by 50 kg ha<sup>-1</sup>. It is, however, not yet assessed if this reduction of N fertilization results in a comparable risk of N leaching as grassland renovation in spring. To assess the effect of this reduction and other measures, field experiments with grassland renovation were laid out.

## Materials and methods

A single-year field experiment on grassland was laid out six times in three years: in 2019 one on loess soil and one on sandy soil, and in both 2020 and 2021 two on sandy soil. Every year new locations were chosen. The grass sward was at least three years old at the start of each experiment. The treatments were reduction of tillage or N fertilization, mechanical destruction, and use of a nitrification inhibitor, compared with standard renovation in August, renovation in spring, and cultivation of maize after chemical sward destruction (Table 1). The design was a complete randomized block design with four replications.

Table 1. Treatments, fertilization with cattle slurry, mineral fertilizer and total N applied, averaged over years and locations.

Treatment	Slurry (m <sup>3</sup> ha <sup>-1</sup> )	Mineral fertilizer (kg N ha <sup>-1</sup> )	Total N applied (kg N ha <sup>-1</sup> )
No grassland renovation (permanent grassland, standard)	45	215	397
Grassland renovation in May (standard before 2018)	20	155	234
Grassland destruction and sowing forage maize (standard)	15	30	91
Harvest of one cut, grassland destruction and sowing forage maize (sowing 3 to 4 weeks later than d, standard, choice of farmer)	40	110	276
Grassland renovation in August, chemical destruction plus ploughing (standard from 2018 on)	65	230	409
Grassland renovation in August, reduced tillage*	65	230	409
Grassland renovation in August, minimal tillage*	65	230	409
Grassland renovation in August, mechanical destruction	65	230	409
Grassland renovation in August, reduction of 50 kg N ha <sup>-1</sup> *	65	175	351
Grassland renovation in August, reduction of 100 kg N ha <sup>-1</sup> *	65	125	308
Grassland renovation in August, nitrification inhibitor (Vizura) *	65	230	409

Grassland renovation: destruction of the sward, tillage, preparation of seed bed, reseeding grass.

\* Measure to reduce N leaching.

In the experiment the measurements were soil mineral N in the soil layer of 0 to 90 cm below the surface in winter after grassland renovation or harvest of maize, and N concentration (nitrate and ammonium) in the upper metre of groundwater in February after renovation, all per plot. Data were statistically analysed with a mixed linear model with treatment as fixed part of the model, and year, location and replication as random part. Model components were estimated with Reduced maximum likelihood (Reml).

## Results and discussion

The differences in soil mineral N in 0–90 cm, averaged over all six year–location combinations (Figure 1) and N in groundwater (Figure 2), were as expected, although not always significant. Soil mineral N was lower on the treatment without renovation (A) and renovation in spring (B) than on the treatments with maize (C and D) and on the treatments with renovation in August (E to L). Reduction of tillage (F), minimal tillage (G) and reduction of N fertilization (J and K) reduced soil mineral N. Mechanical sward destruction (H) stood out from all other treatments by a higher soil mineral N. The use of nitrification inhibitor (L) had no effect on soil mineral N. For the N in groundwater (Figure 2) in general the same pattern was found, though a fewer differences between treatments were significant. The reduction of N fertilization (J and K), however, showed an opposite pattern to that expected: the N in groundwater was higher at the treatment with a lower N fertilizer (K) rate, but slightly lower than the treatment with standard fertilization (E). This output was the same for most individual year–location combinations; therefore, this was not the result of an outlier.

## Conclusion

Reduction of tillage and reduction of N fertilization reduced the risk of nitrate leaching. Soil mineral N directly after the growing season, and the N concentration in groundwater in following spring were lower when tillage and N fertilization were reduced. Nitrification inhibitor had no effect. Even with the appropriate measures, the risk of nitrate leaching at grassland renovation in August was, however, still higher than grassland renovation in spring or the cultivation of maize after sward destruction in spring. The highest risk of N leaching was found with mechanical destruction of the sward.



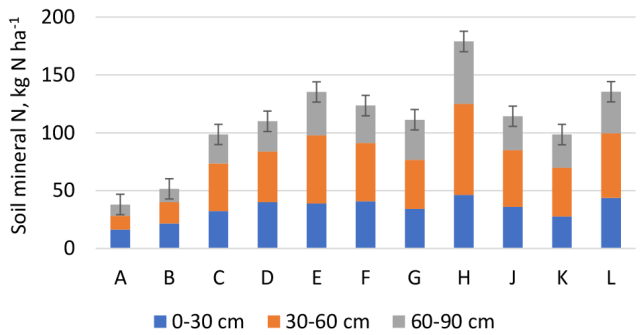


Figure 1. Soil mineral N, averaged over two measurements (November and December) and over all locations and years.

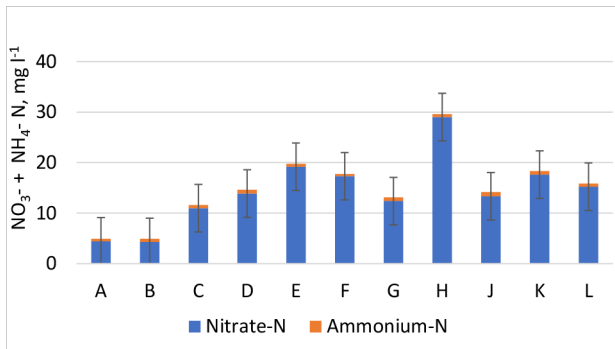


Figure 2. Nitrate- and ammonium N concentration in groundwater, averaged over two measurements (February and March) and all locations and years.

## Acknowledgement

The project was financed by the Ministry of Agriculture, Nature and Food quality.

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**Theme 4.**

**WHERE?**

**Where should we focus on  
which ecosystem services?**



# Spatial differences of ecosystem services provided by grasslands in Europe

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## Abstract

Grasslands, up until recent years, were considered as land suitable only for forage production, and received limited scientific and media attention. Since the beginning of 2000, however, they have been recognised for their role in providing a wide range of ecosystem services. These include habitats for wildlife, maintenance of biodiversity, carbon sequestration, prevention of soil erosion, nutrient regulation, ecotourism, wildfire prevention, production of medicinal, aromatic and edible plants and honey, while they still are essential lands for the production of high-quality livestock products. The variability in climate due to altitude and latitude, as well as different soil types and grassland vegetation communities that exist across Europe affect the ecosystem services grasslands provide. Additionally, the number and quality of ecosystem services provided by the grasslands depend on the management practices employed. Grazing management is a major factor affecting ecosystem services with grazing intensity, from abandonment to overgrazing being the main factors, while urbanization and over-tourism have also negative impacts on grasslands and their services. The objective of this paper was, through a literature review, to identify the spatial differences of ecosystem services provided by grasslands in Europe as well as to link those differences to variation in management practices. The findings can provide a scientific basis for optimal allocation of grassland resources and their sustainable management.

**Keywords:** altitude, latitude, management, multifunctionality, provisioning, cultural

## Introduction

The concept of ecosystem services has its origins in the late 1960s. However, it was not until the publication of the Millennium Ecosystem Assessment (2005), that the number of scientific papers analysing various ecosystem services of grasslands increased worldwide (Fisher *et al.*, 2009), especially in the last decade (Zhu *et al.*, 2023).

Grassland ecosystems in Europe cover more than one third of the total terrestrial area (Eurostat, 2020). These ecosystems, considered until the early 2000s as land suitable only for forage production, received limited scientific and media attention (Porqueddu *et al.*, 2016). However, since then, they are recognised for providing a wide range of other ecosystem services including habitat for wildlife, carbon sequestration, prevention of soil erosion, nutrient regulation, ecotourism, wildfire prevention, production of medicinal, aromatic and edible plants and honey (Bengtsson *et al.*; 2019; Egoh *et al.*, 2016; Porqueddu *et al.*, 2016), while they are still essential lands for the production of high-quality livestock products.

Ecosystem services are spatial-scale dependent (D'Ottavio *et al.*, 2018). The variability in climate due to altitude and latitude, as well as different soil types that exist across Europe, affect the ecosystem services grasslands provide. Additionally, the number and quality of ecosystem services provided by the grasslands depends on the management practices employed and their intensity (Laliberte, 2010). The three main factors affecting ecosystem services in most European grasslands are: grazing management, depending on the stocking rate and the grazing system applied; mowing frequency; and fertilization (Allan *et al.*, 2014; Bluthgen *et al.*, 2012). Intensive land-use modifies soil characteristics, plant-cover and biodiversity, and decreases multifunctionality in grasslands, and thus it can have negative impacts on the quantity

and quality of ecosystem services (Schils *et al.*, 2022; Teague and Kreuter, 2020). The abandonment of livestock grazing, mostly in mountainous areas, alters the vegetation structure and composition of grasslands, and subsequently alters the ecosystem services provided (Plieninger *et al.*, 2014).

The objective of this paper was, through a literature review, to identify the spatial differences of the non-feed ecosystem services provided by grasslands in Europe as well as to link those differences to variation in management practices. The results of the review are mainly addressed to researchers, experts, and policy makers who work with grassland ecosystems of Europe.

## Materials and methods

Europe is divided into five biogeographic zones (Mediterranean, Continental, Alpine, Atlantic and Boreal). For this analysis, in order to gain a sufficient number of eligible publications, the Alpine zone was combined with the Boreal zone, and the Atlantic with the Continental zone. These combinations were opted for, considering similarities in climate, vegetation, and management. A systematic review process was used to identify and characterise the existing knowledge in the specialized literature on the relationship between grasslands and ecosystem services in Europe. The literature was analysed with respect to the application of the Millennium Ecosystem Assessment (2005) elements which provided a categorisation of ecosystem services into four distinct areas: a) provisioning (products obtained from ecosystems such as food and fresh water), b) regulating (benefits obtained from regulation of ecosystem processes such as climate regulation and pollination), c) cultural (non-material benefits obtained from ecosystems services such as recreation and cultural heritage), and d) supporting services necessary for the production of all other ecosystem services such as nutrient cycling and primary production.

The Scopus® search engine was used to identify potential literature for a systematic review. Scopus is a database that indexes many more peer-reviewed journals compared to WoS, including a sufficient number of unique sources not covered by WoS. Additionally, there is a high level of overlap in the content indexed in WoS and Scopus (Pranckutė, 2021).

A custom operator string was created on the 11/01/2024 to identify academic articles between 1999 and 2023:

TITLE-ABS-KEY = (“ecosystem service” AND (pasture OR grassland OR rangeland OR shrubland)) for each one of the 50 countries with territory located within the common definition of Europe and/or membership in international European organisations.

From this search, 793 scientific papers were obtained. These papers were then screened against the selected inclusion criteria:

- Articles written in English
- Articles published in journals (not book chapters or conference papers)
- Articles that contained experimental data or case studies, not literature reviews or policy papers
- Articles studying ecosystem services other than forage production and nutritive value
- Articles focused on grassland ecosystems
- Articles specifically studying ecosystem services as such.

Through this process and according to the review criteria, 654 papers were excluded from the analysis. Some extracted papers included literature reviews, papers that were only descriptive and papers that did not address the relationship between grasslands in Europe and ecosystem services. Other papers were focused on forage production of grassland or specific plant species. The remaining 139 eligible papers

were analysed (Figure 1). Relevant information from included papers was organised in a database with five broad categories:

1. Article information (authors, year of publication, title, journal);
2. Geographic location (one of the three zones: Boreal and Alpine, Continental and Atlantic, Mediterranean);
3. Altitude;
4. Type of grassland management (Grazing, Mowing, Abandonment, Tourism);
5. Analysed Ecosystem services belonging to the four main groups (provisioning, supporting, regulating, and cultural services).

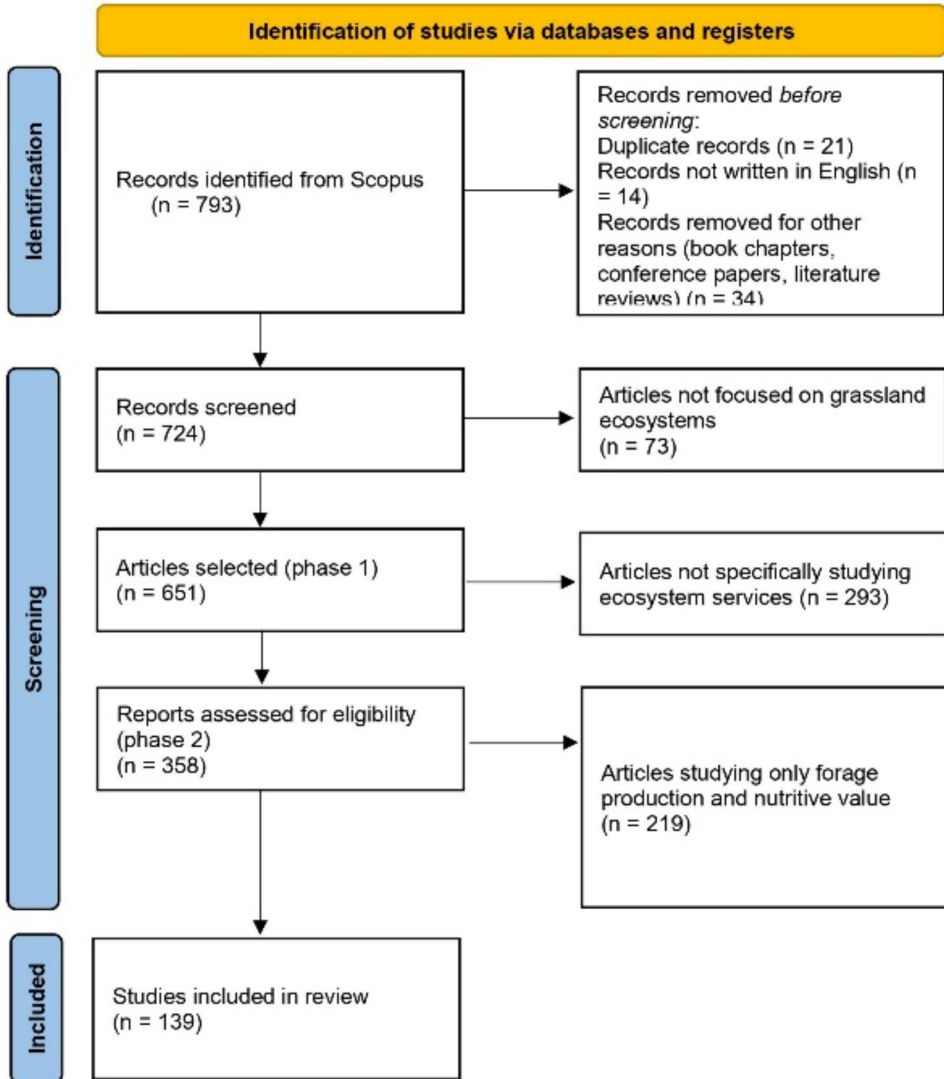


Figure 1. PRISMA flow diagram for selection of studies considered in the review.

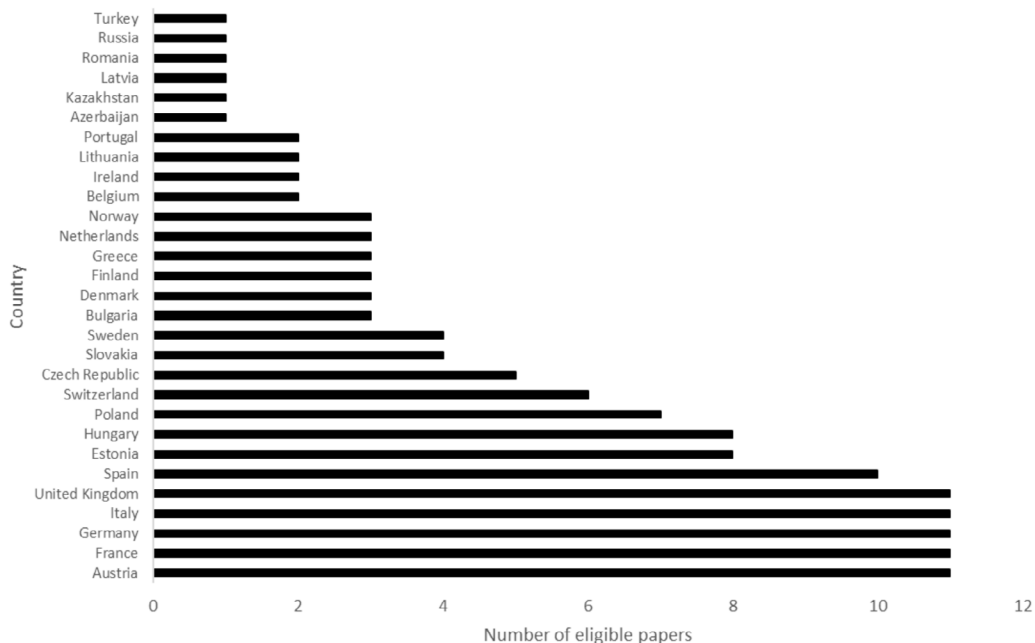


Figure 2. Scientific production addressing the topic of ecosystem services in European grasslands by country, as obtained from the search in Scopus ( $n=139$ ). The countries not included are those in which no relative document has been published.

## Results

Most of the eligible papers (Figure 2) are from Austria (11), France (11), Germany (11), Italy (11), United Kingdom (11) and Spain (10). It is noteworthy that there were no eligible papers from 21 European countries; small countries like Malta, Luxemburg, and Cyprus; countries in the Balkan peninsula including Serbia, Albania, North Macedonia, Croatia, Bosnia and Herzegovina; and countries in the eastern Europe (Armenia, Belarus, Moldova, Ukraine).

Although the use of the concept of ecosystem services in academic papers has become common since the early 2000s, the first eligible papers were published in 2009. Since then, the number of eligible papers studying ecosystem services in grasslands of Europe increased (Figure 3). This trend was recorded for the three zones. Most of the eligible papers were from the Continental (47) and Atlantic (18) zone, followed by the Boreal (20) and Alpine (28) zone, while 26 publications from the Mediterranean zone met the criteria.

The selected 139 papers resulted in a total of 272 ES being analysed as multiple ES categories could be studied within single papers. The ES categories were grouped in four groups: Supporting, Provisioning, Regulating, Cultural (Table 1). In all the zones, most papers dealt with Regulating services.

Regulating services were the most frequently analysed in all zones, and for the Mediterranean zone these services accounted for 53% of the total number of the papers. For papers from the Boreal and Alpine zones, Cultural (25%) and Supporting (24%) ecosystem services were mostly analysed, while Provisioning services were most frequently analysed in the Continental and Atlantic zone, in 22% of the eligible papers (Figure 4).

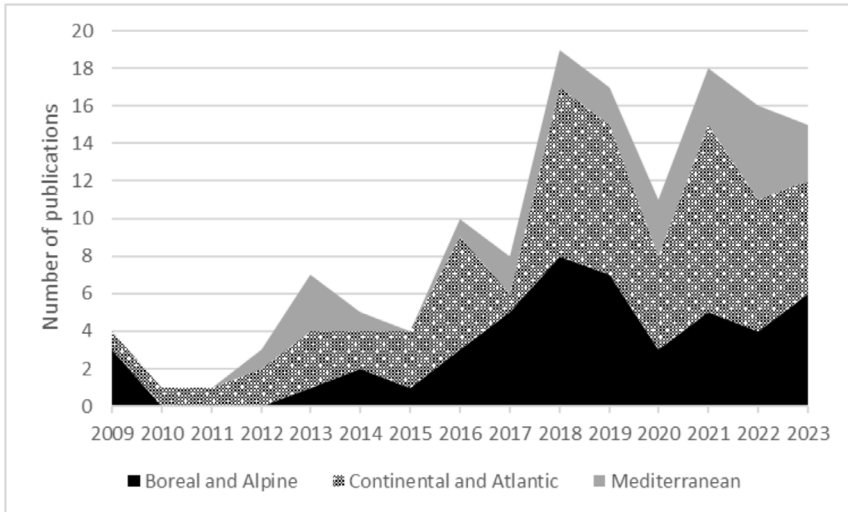


Figure 3. Number of eligible publications ( $n = 139$ ) per year in the period 2009–2023 for the three zones: Boreal and Alpine, Continental and Atlantic, Mediterranean.

Table 1. Ecosystem services (ES) found in the eligible papers ( $n = 139$ ).

ES Group	Number of ES			
	Total	Alpine and Boreal	Continental and Atlantic	Mediterranean
Regulating	110	42	45	23
Supporting	55	29	19	7
Provisioning	50	21	23	6
Cultural	57	31	19	7
Total	272	123	106	43

Each paper can deal with more than one ecosystem service category.

Most of the analysed papers dealt with only one or two ecosystem services groups simultaneously (Figure 5). Less than 25% of the papers in both the Mediterranean and the Continental and Atlantic zones analysed three or four ecosystem services groups provided by grasslands. In contrast, 48% papers from the Boreal and Alpine zone studied ecosystem services belonging to three or all four categories.

The effects of grassland management on ecosystem services were investigated in 54 eligible papers (data not shown). Grazing intensity effects were studied in all zones, but mainly in the Mediterranean (8 papers). Abandonment of grazing was mainly studied in the Boreal and Alpine zone (13 papers), and in three studies from mountainous regions from Spain and Italy in the Mediterranean zone. There were no eligible papers from the Continental and Atlantic zone examining pasture abandonment effects on ecosystem services. In contrast, mowing, fertilizer application, and seeding effects on grassland ecosystem services was investigated exclusively in the Continental and Atlantic zone (16 papers). Tourism effects on grassland ecosystem services were studied only in three manuscripts across all zones.



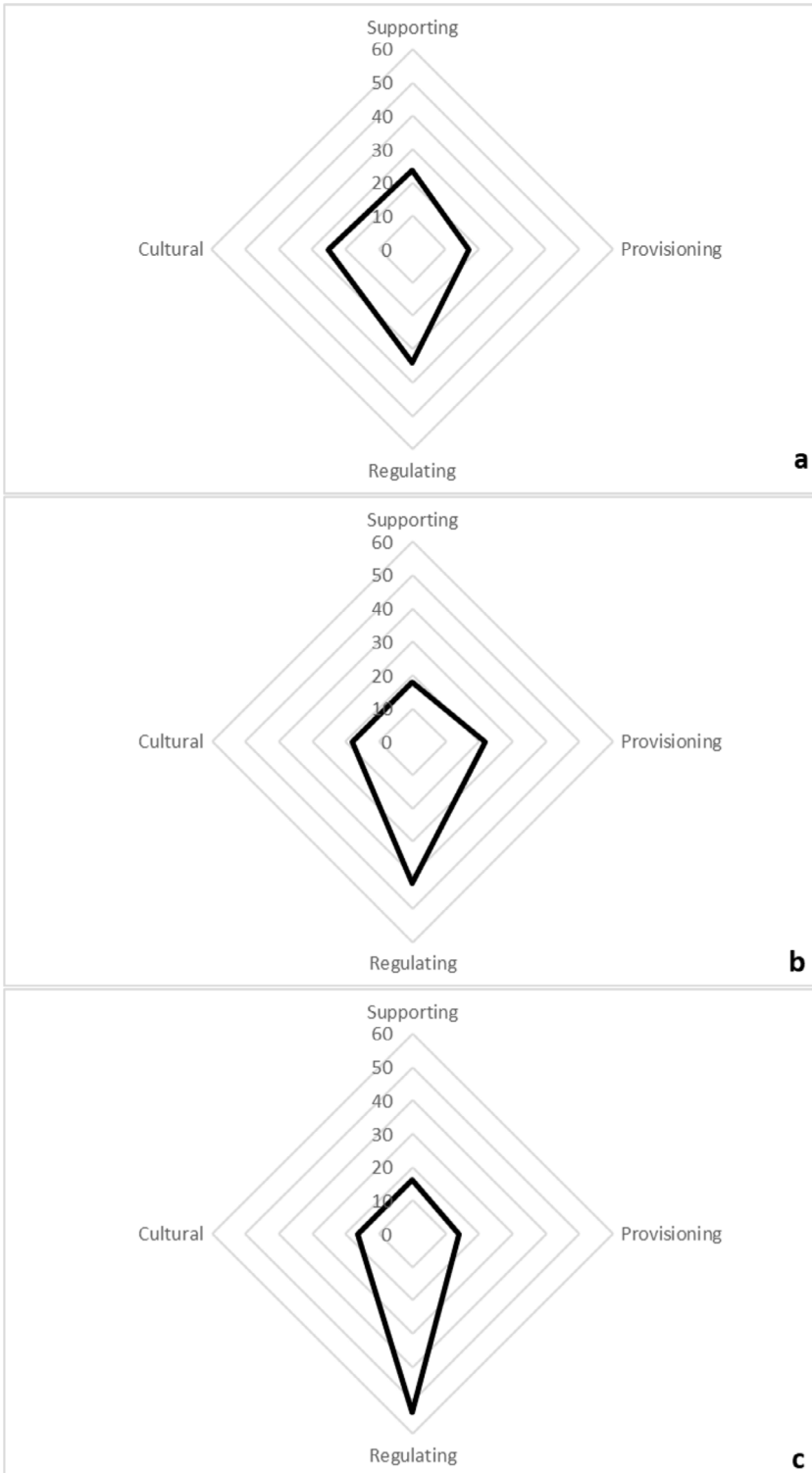


Figure 4. Percentage of findings of the four ecosystem service groups for each zone: (a) Boreal and Alpine, (b) Continental and Atlantic, (c) Mediterranean.

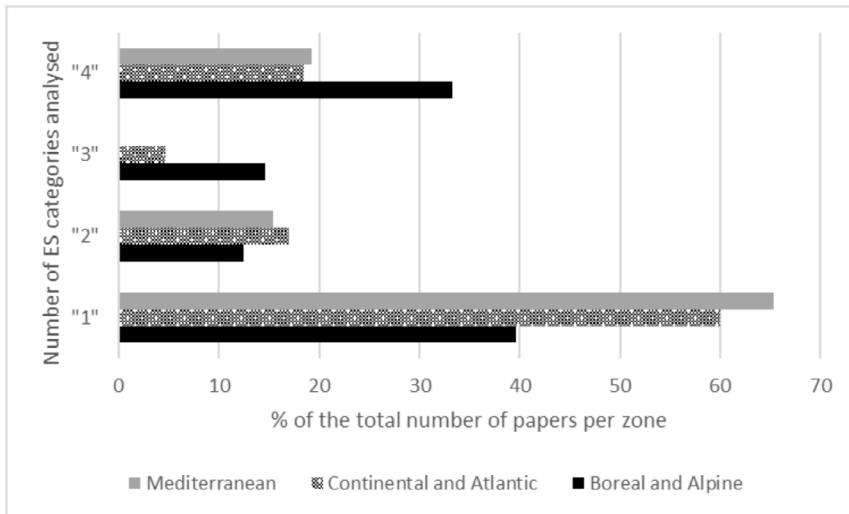


Figure 5. Number of ecosystem services analysed in the eligible papers ( $n=139$ ) in the three different zones.

## Discussion

The criteria used to include publications in this bibliographic review resulted in a small number of eligible papers. The keyword “ecosystem service” was a major dividing term between publications dealing with grasslands from a biophysical, a management, and a socio-economic point of view, and the eligible papers in this analysis that used the ecosystem service concept. Although the Millennium Ecosystem Assessment (2005) is widely accepted, the analysis of several extracted papers revealed misinterpretations concerning the concept of ecosystem services. The main one is regarding biodiversity. Biodiversity is considered as an ecosystem service when in fact it is not. D’Ottavio *et al.* (2018) who used the same keyword in their review about the ecosystem services of grazing systems reported similar findings.

The results of the present study confirm the findings of the recent bibliometric analyses conducted by Mancilla-Leytón *et al.* (2022) and Zhu *et al.* (2023) who reported that France, Germany, Italy, United Kingdom and Spain are among the leading countries worldwide in grassland science. Although all the eligible publications from Spain and the six from Italy were from the Mediterranean zone, the total number of papers from this zone that dealt with ecosystem services of grasslands was much lower than those of the other two zones, as this zone was not combined with others. This result could also be related to the small number of eligible papers from Greece, Portugal and Turkey, and that there are no studies from countries in the Balkan peninsula (i.e., Croatia, Bosnia and Herzegovina, Montenegro and Albania) and Cyprus. Perhaps, the public attitudes and perceptions regarding environmental issues, the limited funding for ecological research in some non-EU countries, as well as the opportunities and conditions for conducting research in those countries, could explain the lack of focus on grassland research and production of relevant studies.

Regulating services were the most frequently analysed in all zones. Carbon sequestration (e.g., Gret-Regamey *et al.*, 2014; Horrillo *et al.*, 2021; Van Vooren *et al.*, 2018) and pollination (e.g., Bagella *et al.*, 2013; Kallioniemi *et al.*, 2017; Noordijk *et al.*, 2009) were studied in many cases. The result confirms the increasing interest of the grassland scientific community across Europe on climate regulation issues as well as on pollination as a vital ecological service for many purposes.

As papers investigating solely forage production and quality were not included in this analysis according to the chosen criteria, the number of papers related to provisioning services of grasslands was comparatively low in all the zones, with the highest number in the Continental and the Atlantic zone. Bioenergy production in grasslands was investigated only in this zone (e.g., French, 2019; Kizeková *et al.*, 2018; Meserszmit *et al.*, 2022; von Cossel *et al.*, 2019) indicating a spatial difference in the interest for this service amongst the three zones.

Cultural ecosystem services were most frequently analyzed in papers from the Boreal and Alpine zone. This result is related to the aesthetic value of the landscape and recreational activities in the mountain regions (Schirpke *et al.*, 2016). In many Alpine regions income from tourism is much higher than that from livestock farming (Schirpke *et al.*, 2019), and a similar trend has been reported for other mountain regions worldwide (Price, 2013). The low number of papers in the Mediterranean zone reflects a lack of attention to cultural services even though traditional farming practices in grasslands are essential in maintaining biodiversity-rich landscapes (Simoncini *et al.*, 2019).

Multisectoral approaches in grassland ecosystem services research is limited in the Mediterranean and in the Continental and Atlantic zones. Ecosystem service multifunctionality, the simultaneous supply of multiple ecosystem services (Linders *et al.*, 2021) has not been studied in these zones, probably due to the lack of available data. In contrast, in the Alpine and Boreal zone almost half of the eligible papers followed this approach. The higher number of papers from these zones studying cultural services is indicating a more holistic approach to grassland ecosystems.

Overgrazing has negative effects on the vegetation structure and ecosystem functioning of grasslands worldwide (Dlamini *et al.*, 2016). Thus, an interest on the effects of grazing intensity on various ecosystem services was found in all the zones. Abandonment of extensive livestock farming occurs over the last decades (Cocca *et al.*, 2012; Zimmermann *et al.*, 2010), in many regions of Europe mainly in mountainous grasslands due to socio-economic reasons (Lasanta *et al.*, 2006). Grazing abandonment also occurs in lower-elevation grasslands in Scandinavia (Johansen *et al.*, 2019). Thus, more eligible papers from the Alpine and Boreal zone than from the other zones focused on the effects of grassland abandonment on their ecosystem function and services found. As mowing is more commonly practiced in central Europe (Tälle *et al.*, 2018), investigation of this management factor on grassland ecosystem services was done in the Continental and Atlantic zone. Koncz *et al.* (2020) compared the effects of grazing vs. mowing on carbon uptake in a study conducted in Hungary. Limited attention has been paid to tourism effects on mountainous grassland ecosystem services. In a study conducted in Greece, Kyriazopoulos *et al.* (2022) investigated the effects of ski-resort activities and livestock grazing on soil erosion.

A limitation of this literature review is the choice of language. Limiting the eligible papers to only those in English has undoubtedly excluded a number of papers published in other European languages. In addition, using a single database might not include all journals (Falagas *et al.*, 2008). Including papers that explicitly identify the term “ecosystem service” and thus indicate awareness of perspectives of the specific frame of research regarding the ecosystem services, i.e., the synergies and trade-offs between human beings and their natural environment, it limits the search as it excludes papers studying an ecosystem service but do not specifically refer to it as such.

## Conclusion

This study has confirmed the importance of grasslands in providing diverse ecosystem services across Europe. The division of Europe into three zones, confirmed the spatial differences of ecosystem services provided by grasslands, mainly for the provisioning and the cultural services. These differences are related to the variety of climatic condition, vegetation structure and composition, and management practices

applied among the three zones. The limited attention to the cultural services provided by grasslands especially in the Mediterranean and the Continental and Atlantic zone suggests that there is not a holistic, interdisciplinary approach regarding these ecosystems. Multisectoral approaches are essential to study grassland ecosystems because they can highlight the diverse range of ecosystem services provided, resulting in, appropriate policies and management. Grassland ecosystems are complex and should not be managed focusing only in one specific service. Researchers need to apply a holistic rather than isolating approach and provide a multisectoral analysis of grassland ecosystems in Europe.

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# Diverse grassland and diverse benefits: the different roles and purposes of grassland

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## Abstract

Grasslands serve specific purposes in different regions and in various ways, and their ecological and economic importance can vary depending on the specific type of grassland and the region in question. Grasslands can be highly multifunctional on a local-scale and across scales through various interactions of management and site conditions. The present study spans from the northern Arctic region, over central and eastern to the southern Mediterranean parts of Europe. It gives an overview of the diversity of grassland systems, in terms of management types and grassland products, as a starting point to present use cases of specific roles typical for distinct regions. It then discusses roles and adaptation possibilities under the conditions of prospected climate change in chosen regions.

**Keywords:** case study, grassland systems, climate change

## What is grassland and why is it a protectable resource?

Unlike steppes, savannas or pampas, most grasslands in Europe represent non-natural secondary ecosystems that replace forests through human management (Dengler *et al.*, 2014). Europe's grassland area can be roughly categorized into permanent and temporary, with the latter being rotated with (forage) crops on arable land (Allen *et al.*, 2011). The extent of sown and permanent grassland varies across countries. Permanent grassland covers about 13% of the EU area, ranging from near zero to >50% of the land area per country (EC, 2019). The main benefits of grassland are the provision of herbage biomass, the protection of natural resources, carbon sequestration, the support of biodiversity and the security of rural livelihoods or cultural heritage (Isselstein, 2021). The support and preservation of these functions requires recognition of the value for natural resources, humankind and economy (FAO, 2024). Grassland and semi-natural grassland account for large parts of protected areas of High Nature Value (HNV) within the European Habitats Directive (Natura 2000). Grassland functions often conflict with one another (Schils *et al.*, 2022). For instance, where intensive forage production is the primary aim, phytodiversity is small (Tallowin *et al.*, 2005; Klimek *et al.*, 2008). In North-Western parts of Europe forage production for cattle dominates – especially for dairy cows with high intensity (Reheul *et al.*, 2015). In southern parts of Europe, on the other hand, diverse extensive grassland systems have emerged as an adaptation strategy to the environment to diversify the uses of extensive systems that provide much higher biodiversity (Zamora *et al.*, 2007). However, certain regions in Southern, Eastern and Central Europe are characterized by livestock densities (EUROSTAT, 2020; Figure 1) too low to prevent grassland from succession and are thus at risk of losing the biodiversity preservation function.

## Where to follow which role?

A particular land use is the result of the interaction of anthropogenic and natural site factors. The former include political, legal or general institutional framework conditions, available technologies, economic structures and consumer preferences (Gömann and Weingarten, 2018). The availability of resources

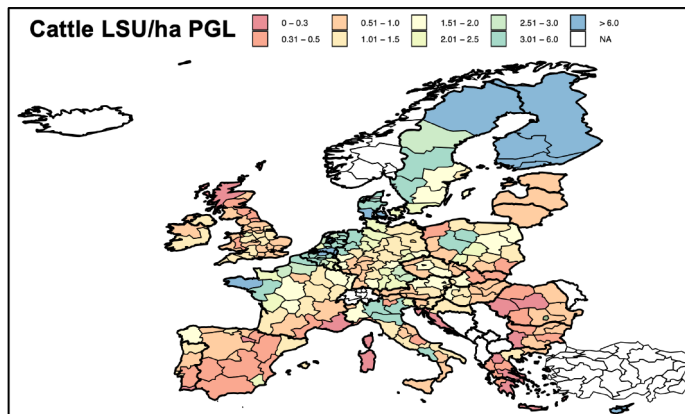


Figure 1. Cattle livestock unit density (1 LU=500 kg live weight) per ha permanent grassland (PGL) in EU obtained using data from FAOSTAT (year 2016).

and natural conditions (soil type, relief, climate) that determine plant biomass production (light, water, nutrients) are decisive for the choice of the grassland system. Furthermore, in certain regions, government regulations limit the options for use such as in nature reserves, and overall, the sales potential of products must also be considered. Competition with other land uses and the possibility of providing cultural leisure activities for people are also important. The aim of this paper is to highlight the diversity of grassland roles in terms of management types and grassland products in distinct regions. It then discusses roles and adaptation possibilities under prospected climate change in chosen regions.

### Diversity of grassland roles in chosen countries

For the purpose of this analysis, grassland roles are evaluated in terms of products and management systems obtained from grassland over a huge climatic gradient (from North to South, Figure 1). For this, we use expert knowledge from Norway, Poland, Germany and Spain (Table 1). Management types describe the nature of defoliation, whereas products result from this defoliation management - sometimes through further processing (e.g. fresh, wilting, drying etc.). Consequently, a cut grassland can produce several products such as silage or hay. The number of different grassland habitat types listed according to Natura 2000 habitats is used to express the extent of nature protection value within countries.

Of the 1.1 million hectares (Mha) of agricultural land (AL) in Norway (<https://arealbarometer.nibio.no/norge/>), 44% are used for grass production (silage and hay), 30% used for grain production, 16% for grazing and 2% for potatoes, vegetables, and fruits/berries. In the west and north, up to 95% of the area is grassland (Figure 2). The area of grassland in Poland is 3.2 Mha, which constitutes 21.4% of AL. Cut grasslands cover 2.8 Mha (18.6% of AL), and grazed pastures 0.4 Mha (2.8% of AL). The largest shares of grassland (between 40.3 and 32.8%) are recorded in the east and the south (Figure 2). Permanent grassland accounts for around 4.7 Mha in Germany. In addition, approximately 0.6 Mha of sown grassland consisting of annual or biennial grasses or legume-grass mixtures are used (permanent + sown about 31% of AL). Grassland accounts for >60% of the agricultural land in North-West Germany along the North-Sea coastline and in the region north of the Alps (Figure 2). In the high mountain range of Central Germany between 40 and 10% of the agricultural land is grassland (Figure 2). Grasslands and Dehesas (open woodlands) account for 25% of Spanish territory (about 43% of AL) and are found on 9.6 and 2.8 Mha, respectively. Larger shares of >60% are found in the north-west and the south-west of the country (Figure 2). Milk from dairy cows plays a major role in all countries (Table 1). In Spain a certain percentage of milk is produced from small ruminants. In the south of Spain dairy milk production relies



Table 1. Chosen countries, areas of permanent (PGL), temporary (TGL) and other (OGL) grassland (Mha), the percentage contribution on agricultural land (% AL), the herbage dry-matter yield potential (DMY, t DM ha<sup>-1</sup>) as based on Smit *et al.* (2008), the number of dairy cows and the number of sheep as based on national census data.

Country	PGL	TGL	OGL	% AL	DMY	Dairy cows (×10 <sup>6</sup> )	Sheep (×10 <sup>6</sup> )
Norway	0.44	0	0	44	2.0–6.0	0.2	2.0
Poland	3.2	0	0	21	3.6–6.0	2	0.3
Germany	4.7	0.55	0	31	5.3–8.8	3.8	1.6
Spain	9.6	0	2.8	43	1.0–7.0	0.8	15.0

TGL includes mixed grass-legume grasslands.

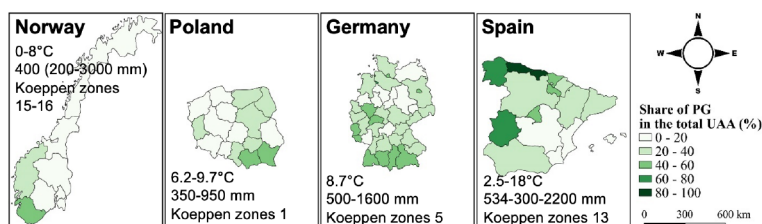


Figure 2. Share of permanent grasslands (PG %) in Norway, Poland, Germany and Spain of the total utilized agricultural area (UAA) at NUTS2 level. Data from 2016 except for Norway (2013) (Eurostat, 2024). Climatic data refer to means  $\pm$  spans.

heavily on imported feed from arable land. Dairy flocks of sheep are located in the country's north and centre, accounting for 37% of the total sheep population. Meat sheep play a role in Norway, Germany and Spain.

Across the countries considered, the number of main grassland management types was six, with variation between three (Norway) to five (Poland, Germany) unique types (Table 2). The number of grassland products varied between four (Norway) and nine (Germany) although clearly two (Poland) to four (Germany, Spain) products are obtained, and here grazing, silage, hay and mostly nature conservation were found. Special cases were found in Spain where the Dehesa ecosystem, although listed as forest land, represents a major grassland-like 'other' grassland type on 2.8 Mha. Here, nature conservation and the raising of livestock (e.g., pigs) is a unique product. Bioenergy refers to the production of biomass for energy provision. For instance, in Germany, there is interest in using re-wetted grassland in this manner. The category of sport products refers to leisure and snow sport in mountain areas although a combined utilization of sport and forage production is typical.

### Use case from Norway: Climate-friendly sheep systems for human food production

In Norway, sheep are grazing around 5–6 months of the year. Dairy cows graze part-time during the summer, and cows have access to feed both on pasture and indoors (grass silage and concentrate). The focus on environmental pressure and greenhouse gas (GHG) emissions from the agricultural sector has a high priority in Norway. Carbon sequestration and enteric methane emission during grazing are the main questions asked. Therefore, focus is put on measurements of emissions from grazing livestock. Grazing experiments conducted in Northern Scandinavia (Sweden, Norway) aimed at measuring feed intake and GHG emissions from individual sheep and dairy cows in order to design a more climate-friendly livestock system. The main findings from these experiments were that methane emissions were reduced during grazing compared to indoor feeding (Lardy *et al.*, 2023) and that NDF content of a pasture was a

Table 2. Overview of grassland management and products in chosen European countries. Grassland products refer to the types of different grass states that are harvested.

Management/product types	Norway	Poland	Germany	Spain
<b>Management types</b>				
Cut only	Yes	Yes	Yes	Yes
Grazing only	Yes	(Yes)	Yes	Yes
Mown pasture	Yes	(Yes)	Yes	Yes
Silvopasture	(No)	(No)	(No)	Yes
Nature conservation grassland	No	Yes	(Yes)	(Yes)
Others	(No)	(Yes)	(Yes)	No
<b>Grassland products</b>				
Grazing	Yes	(Yes)	Yes	Yes
Silage	Yes	(Yes)	Yes	Yes
Hay	Yes	Yes	Yes	Yes
Grass cobs/pellets	No	No	(Yes)	(Yes)
Sports	No	(Yes)	Yes	(No)
Bioenergy	(No)	(No)	(Yes)	No
Building material	(Yes)	(No)	(No)	No
Nature conservation	(No)	Yes	(Yes)	(Yes)
Game	No	No	(Yes)	Yes
Others	No	(Yes)	(Yes)	(Yes)
Sum of management types	3	5	5	4
Sum of grassland products	4	6	9	7
Sum of protected grassland and habitats (Natura 2000)	–	10	13	13

Results as based on expert knowledge. Yes = more than 20% of grassland area, No = not available, (No) rather <5% of area, (Yes) rather >5% and <20% of area.

driver for the emissions (Lind, pers. commun.). In general, a total annual production of, on average, 5 to 6 t dry matter ha<sup>-1</sup> can be produced in Norway (Bakken and Steinshamm, 2022).

## Use case from Poland: Multifunctional grasslands and their productive potential

In North-eastern Poland, grasslands are dominated by intensive management with yields reaching values of 8 to 10 t dry matter ha<sup>-1</sup> and host most of the dairy cattle. Southern Poland, including the foothill regions (Lesser Poland and Podkarpackie), is famous for sheep grazing and, less frequently, cattle with more extensive management and traditional cultural grazing. In Poland, the importance of grasslands in maintaining biodiversity is constantly increasing due to the decreasing demand for grassland feed for livestock and, consequently, lower intensity of grassland management. In order to protect valuable natural habitats, native breeds of farm animals are often used. The positive impact of sheep grazing on the protection of valuable dry grassland habitats in eastern Poland has been confirmed in several studies (e.g. Kulik *et al.*, 2016, 2019; Warda *et al.*, 2016). Grazing has a positive impact on the vegetation of grasslands in general, and traditional breeds are often used for this purpose (Kulik *et al.*, 2023; Rysiak *et al.*, 2021). In both cases, an important factor was extensive grazing with a stocking density not exceeding 1 LU ha<sup>-1</sup> — an intensity not available on many grasslands in Europe (Figure 1). In some regions of Poland, grasslands as valuable natural habitats are more important in maintaining biodiversity. For this reason, they are supported by payments under agri-environment-climate interventions. One of such regions is the Lublin Voivodeship, which is characterized by great natural values and a diverse environment. It is home to the largest number of Natura 2000 areas in the country (23 special protection areas for birds and 100

special habitat protection areas, a total of 123 areas out of 999 in Poland). Their total area in the Lublin Voivodeship is 0.7 Mha, which constitutes 29% of the voivodeship's area. Some of them also overlap with other forms of nature protection (Stanicka *et al.*, 2013). The grassland production ranges from as low as 1 t dry matter ha<sup>-1</sup> to 5 t ha<sup>-1</sup> under these nature conservation conditions.

### **Use case from Germany: Extensive climate-friendly grassland**

In central Germany, grasslands play a subordinate role in the agricultural land use (Figure 1), but they are of outstanding importance for the local biodiversity (Gossner *et al.*, 2016). The historically predominant use of grassland by dairy cattle has mostly disappeared (Teuteberg, 1981). This loss of livestock jeopardizes the future preservation of grassland, which is therefore an important task. This task is now primarily ensured by horses, beef cattle and small ruminants. Studies have shown that long-term extensive grazing under low-input conditions (without fertilizer input) at a moderate stocking rate (1.1 vs. 0.7 or 0.5 LU ha<sup>-1</sup>) maximizes grassland performance (Grinnell *et al.*, 2023), thus securing income from the sale of livestock and at the same time showing no trade-off for soil carbon storage (Komainda *et al.*, 2023). The net grassland productivity ranges between 1.5 and 4 t ha<sup>-1</sup> year<sup>-1</sup> (according to data of Grinnell *et al.*, 2023) under these conditions. In addition, the grazing-induced heterogeneity within pastures promotes phytodiversity (Tonn *et al.*, 2019) and leads to >11 plant species 0.25 m<sup>-2</sup> (Perotti *et al.*, 2018). However, grazing with 1.1 LU ha<sup>-1</sup> is difficult in many parts of Europe (Figure 1).

### **Use case from Spain: Dehesa system**

Traditional farming systems in Mediterranean areas have evolved to cope with harsh climate, seasonality, and limiting soil properties by taking advantage of the different farm resources (e.g., grass, acorn, browse, crops) through mixed extensive systems (sheep, beef cattle and Iberian pigs) with high added value. One of the most representative examples are Dehesas (6310 Habitat Directive), which cover 2.8 million ha, 5.5% of the Spanish territory, and extend across the southwest of the Iberian Peninsula (Miteco, 2021). Dehesa is an example of HNV farming system because of its integration of land use and biodiversity conservation, which leads to a unique potential to provide multiple ecosystem services (Plieninger *et al.*, 2021). At the landscape level, Dehesas results in a mosaic of grasslands, low-intensity crops, shrub, and woodlands that may support greater overall biodiversity than the Mediterranean woodland from which it originated. A Dehesa pasture may contain 135 plant species in 0.1 ha<sup>-1</sup> and 45 plant species m<sup>-2</sup>, mainly annuals (Marañón, 1985) of low annual production 1-3 t ha<sup>-1</sup> with marked seasonality (Olea and San Miguel-Ayanz, 2006). Dehesa is also the habitat of some of the most emblematic and endangered animal species such as the Iberian lynx (*Lynx pardinus*) and the Iberian imperial eagle (*Aquila adalberti*) (Olea and San Miguel-Ayanz, 2006). In addition, the agro-silvopastoral mosaic resulting from the management of the Dehesa makes these areas more resistant to forest fires, which are of concern in the Mediterranean basin, given the global increase in the number of forest fires and the occurrence of large-scale devastating fires (Varela *et al.*, 2020). Therefore, this system allows the provision of meat in limited climatic and edaphic conditions with multiple associated functions. In general, the meat produced in the Dehesa is characterized by its high organoleptic quality. Dry-cured meat products from Iberian pigs fed on acorns and pasture during their final fattening phase are the most outstanding example and have their own legal national legislation (BOE, 2014). Recent studies assessing the life cycle of Dehesa products have shown that this system can offset GHG emissions of cattle and Iberian pig production leading to negative net emissions in some cases (Reyes-Palomo *et al.*, 2022, 2023).

### **Constraints for grassland arising from climate change**

An answer to the question of “where to focus on which role of grassland in future” is not easy to find, since the ongoing climate change will require changes in the management of farms and protected grasslands in order to secure grassland functioning. The need for alteration will depend on the location, because projected impacts associated with climate change vary across regions (see Ergon *et al.*, 2018 for details).



until the 19<sup>th</sup> century but nowadays is symbolic (Oteros-Rozas *et al.*, 2012). Among the multiple reasons for the decline of these systems are: economic constraints, decoupling with global markets, higher cost than intensive systems, lack of vocational training and young herders, low prestige of pastoral work and lack of support by policies (namely CAP) (Liechti and Biber, 2016). The future of these systems will be key to preserving the role of grasslands in places where other systems are not viable.

## Diverse grassland for forage production under climate change

Multi-species mixtures are a vital tool to increase the biodiversity of sown grassland under both temperate and Mediterranean climate while at the same time supporting production (Hearn *et al.*, 2023; Ribas *et al.*, 2023) under current and future dry climate (e.g. Finn *et al.*, 2018). In a study by Glowacki *et al.* (2023) in Germany, lucerne was grown and combined with cocksfoot (*Dactylis glomerata* L.) and tall fescue (*Festuca arundinacea* L.) which are two potentially drought tolerant forage grasses. Lucerne pure stands were the best performing swards in that study because of low-input conditions without N fertilization. In a study by Komainda *et al.* (2021), including the century dry year 2018, herbage production was up to 87% higher in a lucerne-dominant sward compared to a simple sward reference and this effect was transferred to the subsequent crop with a difference of 55% in yield when compared to the previous simple sward. However, soil factors such as soil pH < 6 limit the use of lucerne in many parts (Figure 4). This limitation is not currently known for chicory (*Cichorium intybus* L.), a deep-rooting non-leguminous forage herb. The study by Nölke *et al.* (2021) tested its use over five years on one shallow and one deep soil-site in Central Germany. The study showed improved yield and forage quality stability over five years from inclusion of chicory especially in dry years. The stabilizing effect reported in Nölke *et al.* (2021) was strongest and significant on the deep soil-site, whereas the stabilizing effect was small at the shallow site possibly through varied rooting depth potential of the soil. Consequently, site conditions should be considered when choosing deep rooting forage species. Where soil depth is too limited for deep rooting, forage species with higher stomata control might be an effective way of targeting drought tolerance. In Komainda *et al.* (2019) alternative forage legumes were compared against white clover using artificial drought treatments. During drought phases white clover had 43% relative losses in herbage production while the alternative species (especially *M. lupulina* and *L. corniculatus*) lost only 26% compared to their well-watered controls. This tolerance to drought was related to a high degree of stomata control. The Mediterranean Basin holds a rich plant diversity source of species adapted to drought and grazing (e.g. *Trifolium subterraneum*). Although the potential of most of these species is known, their study and use are still limited; these species could play a fundamental role in securing production in Europe by providing resilience and resistance.

Sowing of multi-species grassland combined with agroforestry systems may serve as another adaptation strategy. Integration of trees in a treeless landscape causes disturbance and generates microclimates with modification in temperature, light, relative humidity or evapotranspiration (Mosquera-Losada *et al.*, 2023). In the study by Sutterlütti *et al.* (2023), a positive response of grassland to trees was expected under drought conditions, because of a potential for saving water through shading or an anticipated hydraulic lift. While the herbage biomass production was not reduced under drought compared to areas further away, the proportion of dead herbage was lower and consequently the herbage quality was higher near trees during drought periods. Stomatal conductance near trees was increased, which shows that the water supply of grassland in silvopastures is improved under drought conditions in temperate climate. On the other site, forage species can have negative influences on the trees especially under drought. For instance, while the nitrogen status of trees might improve through leguminous forages, competition for water increases with a resulting negative effect on tree growth (Dupraz *et al.*, 1999).

## Strengthening the role of grasslands in wildfire prevention under climate change

The increase of large-scale devastating forest fires especially in the Mediterranean Basin is of great concern. Rural depopulation has led to a significant increase in forest area with a growing trend towards larger forest stands, higher fuel loads and greater horizontal and vertical continuity of vegetation (Moreira *et al.*, 2011). This trend, coupled with climate change, has contributed to the emergence of the so-called sixth-generation wildfires. A shift in the strategy to combat wildfires is demanded, emphasizing the need to boost prevention investment and coordinate land-use regulations that encourage proactive self-defence in high-risk areas in order to establish fire-smart territories (FST) (Pulido *et al.*, 2023). In this context, grasslands and extensive livestock grazing featured by HNV farming systems contribute to shaping mosaics of land uses of low fuel load that can be implemented not only in woodland areas of high potential productivity, but also in disadvantaged and constrained areas where other agricultural activities are not feasible (Moreira *et al.*, 2011; Varela *et al.*, 2020). This key role in strategic areas must be addressed and recognised by public policies. In this sense, there are wildfire prevention programmes in Spain, supported by various regional and local administrations, which aim to promote FST or to use livestock grazing to remove vegetation from firebreaks in public forests.

## Conclusion

Defining roles of grassland will depend on the region in question and the future climatic or edaphic constraints. Exact data of grassland products per country are not available and this requires further studies. Studies will also be needed to strengthen the role of traditional practices in combination with multi-species grassland to achieve climate resilience.

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# Dairy Campus: Living Lab for biodiversity and precision agriculture

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## Abstract

This study shows how a modern Dutch dairy farm can gradually create room for biodiversity on 25% of the total farm grassland area and compensate feed production by using precision agriculture on the other 75%. In 2020, we applied a Living Lab strategy at experimental farm Dairy Campus (DC), a modern dairy farm with 500 dairy cows and a home plot of 125 hectares. DC staff, a contractor and researchers jointly developed space for biodiversity by iterative adjustments in business operations, which were based on observation, experience, and experimentation at both DC and the contracting company. In 2022, 31 hectares (25%) of the home plot for biodiversity could thus be freed up. The farm staff's mindset turned out to play a key role in realizing this. We conclude that productive species-rich grassland, natural biodiversity on corners, plot edges and ditch sides were relatively easy to incorporate. The necessary precision techniques, RTK-GPS and NIRS, had been adopted before. However, the increase of grass production through precision agriculture at 75% of the total farm plot was insufficient to compensate for the allocation of 25% of a total plot of 125 ha to biodiversity.

**Keywords:** dairy farm, biodiversity, precision agriculture, grassland

## Introduction

Dairy and grazing farms are the largest users of the Dutch grassland area (CBS, 2023) and may contribute significantly to the restoration of biodiversity. Farmers are mainly compensated by the government and to a limited extent by higher pricing of dairy products through sustainability quality marks on niche-markets. Since an adequate internal revenue model for biodiversity on dairy farms is lacking, the aim of this study was to show how a modern dairy farm can gradually create space for biodiversity on 25% of the total farm area and keep up its own feed production through the implementation of precision agriculture. In 2020 the approach of a Living Lab was applied at experimental farm Dairy Campus (DC), a modern dairy farm research centre with 500 dairy cows and a home plot of 125 hectares of grasslands. Co-creation was achieved in an experimental environment in which researchers and practical partners jointly developed and tried out knowledge and solutions for the complex issues of land use and productivity.

## Materials and methods

The starting point was an opportunity map of the DC plot showing where and which type of biodiversity and precision agriculture could be applied. Plots were selected on soil type, water level, infrastructure, influence of weather conditions, legislation, and practical applicability. The four types of biodiversity were taken from the conceptual framework for agricultural biodiversity (Erisman *et al.*, 2016). Planning, implementation, monitoring and adjustments in Living Lab DC were mainly aimed at realizing productive species-rich grassland (functional agrobiodiversity) and nutrient impoverishment of borders (ecological connecting zones). Machines and precision techniques were carefully selected and supervised for suitability, during adjustments of operations. Experiences with the new working method were exchanged in consultations between DC staff, contractor and researchers. Necessary adjustments in business operations at both DC and the contractor were taken step by step, jointly and iteratively.

Workshops, meetings, and demo days have been organised for knowledge development, sharing experiences and knowledge transfer to dairy farmers, contractors and education.

## Results and discussion

Initially, DC staff were reserved about allocating 25% of the home plot for biodiversity. They normally aim for the best possible use of all plots for manure placement and the production of roughage of consistent and high quality. By jointly exploring what could be realised and where, for both biodiversity and precision agriculture, DC staff started working on it. Productive species-rich grassland and nutrient impoverishment of borders were acceptable in combination with an expected yield increase through precision agriculture on high production grassland. That is because productive species-rich grassland does not necessarily mean lower grass yield (Høgh-Jensen *et al.*, 2006; Korevaar and Geerts, 2015; Wagenaar *et al.*, 2017), while edge management by impoverishment does lead to a decline in yield and nutritional value (Jansma *et al.*, 2021). The action strategy of formulating biodiversity in terms of production or performance fits the mindset of many farmers (Westerink *et al.*, 2019). DC staff themselves came with proposals for expansion after one year. In this way they succeeded in 2022 to free up 25% space on the home plot for biodiversity. Almost half of that space (15 hectares) consisted of edge management. This shows when the mindset is focused on more biodiversity, it can be achieved relatively quickly. This ties with the characteristics of the growth mindset (Dweck, 2006). Edge management and productive species-rich grassland can be easily incorporated in a modern dairy farm.

This study required techniques for precision fertilization, which already existed, namely Real Time Kinematic–Global Positioning System (RTK-GPS) and Near Infrared Spectroscopy (NIRS). Table 1 shows the effect on the dry matter yield of Controlled Traffic Farming (CTF) and Table 2 of Variable-rate application techniques (VRA). Results with VRA between years were opposite. In general, increase of grass production through precision agriculture on 75% of the farm area was insufficient to compensate for the loss on the unfertilized plot areas. Therefore, financial compensation for production losses from ecosystem services is currently still necessary. The plot balances a comparison of fertilization with yield, and made it easier to compare plots. Reduced fertilizing of edges and headlands is a first step in precision fertilization because higher yields of perennial ryegrass (*Lolium perenne*) cannot be achieved there, due to soil compaction. Less or no fertilization gives other grass species and herbs a better chance to grow and this may subsequently lead to improvements in soil structure, although herbs also need good (soil) conditions to grow. Once clover and the other herbs are well established, it is possible to achieve comparable yields and quality (Høgh-Jensen *et al.*, 2006; Korevaar and Geerts, 2015; Wagenaar *et al.*, 2017). In addition to the availability of machines and equipment, contractors have increasing knowledge and experience in precision agriculture and would like to help dairy farmers to further optimize grassland efficiency.

Table 1. Dry Matter yield per year (%) of Controlled Traffic Farming (CTF) compared with a conventional traffic system (100%).

	2021	2022
CTF	104	99
<i>P</i> value	0.203	0.725

Table 2. Relative N fertilization rate and DM yields in field experiments using Variable-rate Application techniques with more N (treatment A) and less N (treatment B) compared to the N application fertilization norm (ref) in 2021 and 2022.

	Ref <sup>1</sup>	2021		2022
		A	B	B
Fertilization	100	123	90	72
DM yield	100	99	102	88

<sup>1</sup>Application fertilization norm for grassland (without grazing) on clay soil; 385 kg available N ha<sup>-1</sup>.

## Conclusion

Depending on surface area, location and type of biodiversity, there may be consequences for quantity and quality of forage production. Productive species-rich grassland is, because of its comparable yield and quality, relatively easy to incorporate into a modern dairy farm. The same applies to biodiversity on poor corners, plot edges and ditch sides. With adequate silage making, this feed fits the requirements of young stock. For precision agriculture it is crucial to maintain the same management on each m<sup>2</sup> in the field. The necessary precision techniques, RTK-GPS and NIRS, are mainstream. Nevertheless, grass production increase by precision agriculture is still insufficient to compensate for the loss at unfertilized plot areas. Government policies remain important to compensate for yield loss due to edge management. We recommend further research on farmer mindset changes, species-rich grassland management, storage, and application of biodiverse crops in the cow's ration combined with knowledge sharing and education.

## Acknowledgements

We gratefully acknowledge Delphy, Westra, and Dairy Campus staff for their work in this Living Lab; the Dutch Ministry of LNV, Melkveefonds, Visscher Holland BV and Broekens BV for financially support; and Aeres Hogeschool for their contribution to the project.

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# The role of legume forage crop on nitrous oxide (N<sub>2</sub>O) emissions from a boreal grassland

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## Abstract

Nitrous oxide (N<sub>2</sub>O) emissions from agricultural soils account for 60% of the total global N<sub>2</sub>O emissions and it is essential to reduce these emissions for agriculture sustainability. Leguminous crops are a crucial source of nitrogen to the soil as they contain the N<sub>2</sub> fixing microbes in their root nodules. Recently, soyabean root nodules have been found to reduce atmospheric N<sub>2</sub>O, suggesting that leguminous crops can perform dual functions, N<sub>2</sub> fixation and N<sub>2</sub>O reduction. In our project ENSINK, we hypothesize that legumes, primarily via root-nodule-soil interaction, support soil N<sub>2</sub>O reduction, and thus lower the soil N<sub>2</sub>O emissions. To address our hypothesis, we conducted a mesocosm study with four treatments: bare, only red clover, only timothy, and mixed vegetation, and we measured the N<sub>2</sub>O fluxes for 37 days. Our results show that timothy as monoculture released the highest amount of N<sub>2</sub>O whereas red clover as monoculture released the lowest amount of N<sub>2</sub>O, supporting our hypothesis. The presence of red clover likely via soil-root interactions could have stimulated N<sub>2</sub>O reducing microbes, resulting in the lowest N<sub>2</sub>O emissions. We suggest that future studies should focus on studying the soil-root interactions to better understand the agricultural N<sub>2</sub>O emissions from grassland ecosystem.

**Keywords:** sustainable agriculture, nitrous oxide uptake, nitrogen cycle, denitrifiers

## Introduction

Nitrous oxide (N<sub>2</sub>O) is a strong greenhouse gas, ~300 times stronger than carbon dioxide, and agriculture is a major source of N<sub>2</sub>O globally. Agricultural soils are a considerable source of N<sub>2</sub>O because of the use of nitrogen (N) fertilizers — a primary substrate for the two main microbial N<sub>2</sub>O production pathways, nitrification and denitrification (Firestone and Davidson, 1989). Therefore, N<sub>2</sub>O emission reduction from global agricultural soils is considered to be an important step towards agricultural sustainability (Reay *et al.*, 2012). Several options (e.g., increasing nitrogen use efficiency, appropriate dietary choice and minimizing the food loss and waste) have been suggested to reduce the agricultural N<sub>2</sub>O footprint (Reay *et al.*, 2012). In addition to these options, the role of legumes in agricultural soil N dynamics has been considered to be a potential option to lower the soil N<sub>2</sub>O emissions (Senbayram *et al.*, 2015). A recent study has shown that soyabean (*Glycine max* L. Merr.) — a globally grown leguminous food crop, can substantially reduce atmospheric N<sub>2</sub>O to N<sub>2</sub> via the *nosZ* gene (Itakura *et al.*, 2013), thus opening a new research avenue for aiding agricultural N<sub>2</sub>O emission mitigation with other leguminous types of crop species, such as alfalfa and clovers, which are important leguminous forage crops globally. Here, we present our N<sub>2</sub>O emissions results from a mesocosm study conducted on a mineral soil using red clover (*Trifolium pratense* L. cv. Ilte) and timothy (*Phleum pratense* L. cv Nuutti) as crops of interest. Our objective in this study was to test the effect of red clover in N<sub>2</sub>O emissions dynamics from a grassland.

## Materials and methods

We conducted a mesocosm (id0.18 m, h, 0.12 m) experiment in a greenhouse (approx. 25°C) for 37 days. We included four treatments: no vegetation or bare (B, *n*=6), a mixture of timothy and red clover (*n*=6), only red clover (*n*=9), and only timothy (*n*=6) for assessing the effect of different vegetation

cover on N<sub>2</sub>O fluxes. The mesocosms were established using mineral soil (22% sand, 54% silt and 25% clay) collected from one of our research platforms, Anttila, located in Eastern Finland. The seeding and fertilization of the mesocosms were done according to the recommended practice for a legume grassland. For vegetated mesocosms, we seeded with 77% timothy (15 kg ha<sup>-1</sup>) and 23% red clover (4 kg ha<sup>-1</sup>) seeds and fertilized all the mesocosms at 75 kg N ha<sup>-1</sup>. In all mesocosms, we maintained the field bulk density (1.01 g cm<sup>-3</sup>) and moisture content to 50% of water filled pore space since the first day after establishing mesocosms. We measured N<sub>2</sub>O fluxes weekly using the static closed chamber method for 37 days in a greenhouse. A 25 ml gas sample from the chamber headspace was collected at intervals of 5, 10 and 20 minutes after chamber enclosure, and 20 ml of the gas samples was transferred immediately to 12 ml of preincubated vials for N<sub>2</sub>O concentrations measurement, which was measured by gas chromatography (Agilent, 7890A) at the Luke Joikoinen laboratory. Here, we present cumulative N<sub>2</sub>O fluxes from all treatments for 37 days. The cumulative N<sub>2</sub>O fluxes were calculated based on the assumption that there would be a linear increase in the fluxes because of N fertilization, and gap filling was done using the slope and intercept from linear interpolation of N<sub>2</sub>O fluxes that were measured.

## Results and discussion

Based on our mesocosm experiment we found that soil surfaces vegetated with timothy emitted the highest amount (114 g N<sub>2</sub>O-N m<sup>-2</sup> day<sup>-1</sup>) of N<sub>2</sub>O and, in contrast, surfaces vegetated with only red clover emitted the lowest amount of N<sub>2</sub>O (36.5 g N<sub>2</sub>O-N m<sup>-2</sup> day<sup>-1</sup>). Interestingly, bare soil surfaces (66.4 g N<sub>2</sub>O-N m<sup>-2</sup> day<sup>-1</sup>) and surfaces covered with mixed vegetation, i.e., timothy and red clover (63.8 g N<sub>2</sub>O-N m<sup>-2</sup> day<sup>-1</sup>) emitted almost the same amount of N<sub>2</sub>O. Our results indicate that the presence of timothy considerably promotes the activity of N<sub>2</sub>O producing microbes in soil compared to mixed vegetation and red clover alone. On the other hand, red clover seems to be effective in lowering N<sub>2</sub>O emissions when cultivated alone and when comparing timothy vs. mixed, red clover also seems effective lowering the impact of timothy-induced N<sub>2</sub>O emissions. Although at this stage our data cannot show explicitly the role of the red clover root system in lowering N<sub>2</sub>O emission, we hypothesize that legume root-soil systems could have promoted the activity of microbes containing nitrous oxide reductase gene, *nosZ* —the only known biological sink of N<sub>2</sub>O. While legumes, especially decaying or root remains, have been shown to increase the N<sub>2</sub>O emissions from soil (Inaba *et al.*, 2012; Uchida and Akiyama, 2013; Yang and Cai, 2005), there have also been reports showing the potential of legumes as an option to mitigate agricultural N<sub>2</sub>O emissions (Senbayram *et al.*, 2015). For example, soil microbes (e.g. *Bradyrhizobium* spp.) residing in the root system of soyabean have been known to reduce the soil produced N<sub>2</sub>O (Itakura *et al.*, 2013). Therefore, based on our study it could be expected that red clover as a forage legume in grassland systems could be a potential option to mitigate N<sub>2</sub>O emissions.

## Conclusion

Based on our study, we conclude that red clover could play an important role in lowering agricultural N<sub>2</sub>O emissions, likely via affecting the soil-root-microbe continuum. We suggest that future studies should focus on a better understanding of the soil-root interactions and its role in N<sub>2</sub>O flux dynamics. A holistic approach studying the flow of nutrients (C and N) and their transformation, soil microbial communities, especially microbes harbouring *nosZ* gene, and their ecological relevance could help us to better understand the N<sub>2</sub>O exchange dynamics from grassland ecosystems.

## Acknowledgement

This study is funded by the Academy of Finland in the project, “Mechanism of nitrous oxide (N<sub>2</sub>O) uptake in different cropping systems in different climate zones (ENSINK)”.

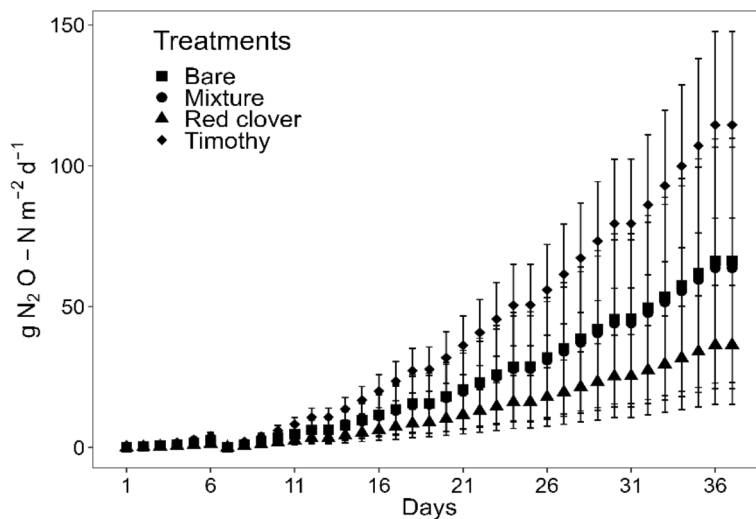


Figure 1. Cumulative emissions of N<sub>2</sub>O from the mesocosms study. The data points represent the mean ± SEM of six replicates, except for red clover which has nine replicates.

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# Agro-ecological indicators to assess the effect of grazing at farm level

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## Abstract

The Grazing4AgroEcology (G4AE) project is an EU network to promote grazing and support grazing-based farms on their economic and ecologic performances as well on animal welfare across eight European countries (France, Germany, Ireland, Italy, the Netherlands, Portugal, Romania, Sweden). Five principles of agroecology were adopted by the project and used as the base to determine agro-ecological indicators for the assessment of the grazing capacities at farm level: (1) Adopting management practices that aim to improve animal health, (2) reducing the inputs required for production, (3) reducing the risk of pollution by optimising the biogeochemical functioning of farming systems, (4) enhancing diversity within production systems to strengthen their resilience, and (5) preserving biodiversity in agro-ecosystems by adapting management practices. These indicators were implemented into a self-assessment tool that allows farmers to assess their own farm performance in order to make targeted improvements. The tool was trialled on eight farms in France and four farms in Ireland. The results have provided the farmers with a quantified assessment on the agro-ecological performance of their farms. It provides a basis for the dissemination and implementation of best practices within each farm and gives an insight into the potential to improve on-farm performance.

**Keywords:** agroecology, grazing, indicators, self-assessment, participatory methods

## Introduction

The benefits of grazing have been acknowledged in numerous studies: animals can express their natural behaviours, there is a positive impact on farmer income, preservation of biodiversity and landscape conservation. The Grazing4AgroEcology (G4AE) EU Horizon Europe project ([www.grazing4agroecology.eu](http://www.grazing4agroecology.eu)) aims to promote grazing and to support grazing-based farms on their economic and ecologic performances as well as on animal welfare. The G4AE project used the five principles of agroecology (Dumont *et al.*, 2013) and consolidated a list of on-farm indicators that point out strengths and weaknesses of a system, help farmers and other relevant actors, like advisers, to reflect on the farm's performance and identify a plan of action for further improvement (Trabelsi *et al.*, 2016). From these indicators a self-assessment tool was developed. The current research aimed to explore the implementation

of the tool for improved agro-ecological grazing practices. The results can be used to empower farmers' transitions to a sustainable pasture-based grazing system, following the five principles of agroecology.

## Materials and methods

A list of on-farm agro-ecological indicators was determined as questions and given weighted scores by the G4AE project consortium, with input from all project partners across the eight member states. This consultation improved the indicators viability across all countries and ensured all project partners were able to provide input on the relevant indicators for agroecology, specifically across the five principles of agroecology (Table 1). The number of indicators for each principle and an example of each in the form of a question are shown in Table 1. The scores of each principle are a mean of the scores of all rated indicators (with 100 being the highest possible score of each indicator) and weighted on their relevance. Indicators were formatted and functionalised as a Microsoft Excel-based tool in late autumn 2023.

A subset of partner farmers, eight from France and four from Ireland, from within the partner farm network (a network of fifteen farmers per member state who are integrated into the G4AE project and will be the source of best-practices and innovative ideas for grazing) completed the self-assessment tool with guidance from project facilitator agents and provided feedback on the use of the tool.

## Results and discussion

The individual on-farm agro-ecological performance was assessed on French and Irish farms using the self-assessment tool. Results per farm are displayed per principle of agroecology and as a total score for the French and Irish Farmers (Table 2). This gives individual farmers a clear indication of the potential for improving agro-ecological grazing management on their farm. In Table 2 both French and Irish farmers scored approximately 50% of the maximum score on 'Enhancing diversity' and it could be identified by farmers as an area they need to improve. French and Irish farmers also performed quite well (>80%) in 'Animal Welfare', 'Reducing Inputs' and 'Reducing the risk of pollution' (Table 2).

It is expected that farmers repeat completion of the self-assessment annually and that, similar to Alvez *et al.* (2013), an improvement in farming practices will be seen between years on the majority of farms. This fits into the FAO programme aiming to improve the sustainability of agriculture by focusing the attention on agroecology (Mottet *et al.*, 2020). The self-assessment will be conducted by a total of 120 G4AE partner farms as part of the project.

Table 1. The five principles of agroecology, the number of indicators and an example of an indicator in their question form for the self-assessment tool.

Principle of agroecology	Number of indicators	Example indicators (in the form of questions)
Management practices to improve animal health	20	Do animals have access to fresh clean water at all times (including grazing)?
Reducing the inputs required for production	35	Do you have legumes in your grasslands?
Reducing the risk of pollution	17	Do you have adequate storage for manure and slurry?
Enhancing diversity to strengthen resilience of production system	22	Are your products sold with quality labels strongly linked to the territory (PDO)?
Preserving biodiversity	28	Do you have ponds/wetlands on your farm?



Table 2. Results of the self-assessment tool, as percentage of the maximum score for each principle for agroecology and the average score for eight French (FR) and four Irish (IE) farms.

Country	Farmer	Animal welfare (%)	Bio-diversity (%)	Enhancing diversity (%)	Reducing inputs (%)	Reducing pollution (%)	Total score (%)
FR	1	84	86	55	89	82	79
FR	2	95	76	64	84	98	83
FR	3	88	53	47	84	90	72
FR	4	93	61	39	66	76	67
FR	5	90	68	43	82	69	70
FR	6	82	79	46	82	80	74
FR	7	95	79	61	83	84	80
FR	8	89	80	48	81	78	75
Average		89	73	50	81	82	75
IE	1	88	75	54	67	91	76
IE	2	79	57	46	89	100	78
IE	3	85	39	54	80	78	71
IE	4	88	57	54	96	74	79
Average		85	57	52	83	86	76

## Conclusion

Results feed into the formulation of context-specific limitations to and possibilities for agro-ecological improvement on farm level. The tool will become available for use on all pasture-based systems in the future and can be used to empower farmers' transitions to a sustainable grazing system while implementing improved agro-ecological grazing practices.

## Acknowledgements

This project (Grazing4AgroEcology) received funding from the European Union's Horizon Europe Research and Innovation Programme under Grant Agreement No 101059626.

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# Mapping wet grasslands to consider ecosystem services at the landscape scale

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## Abstract

The development of the concept of ecosystem services (ES) has highlighted the interest of grasslands and particularly wet grasslands. In addition to their biodiversity and their role in fodder production, they are of great interest for water regulation and carbon storage. The management of wet grasslands is often questioned but most often with the aim of improving one or a few services which are sometimes antagonistic. A simple method to evaluate indices of a set of ES has been used to characterize 162 grasslands among five marshes in Normandy, France, and then to map them at the landscape scale. No wet grassland achieved the highest scores for all services, but at the marsh scale, they appear complementary. This study underlines the importance of studying a set of ES simultaneously in order to reconcile livestock farming and environmental objectives. It also underlines that the scale of the marshes is more relevant than that of each of the grasslands they contain to evaluate the level of services provided. Therefore, that management, when possible, must be considered by involving all stakeholders. The heterogeneity of the marshes, partly resulting from the diversity of management, achieves to provide multiple services at this landscape scale.

**Keywords:** wet grassland, plant diversity, heritage value, soil C stocks, forage quality

## Introduction

Grasslands are now largely recognized as ecosystems which provide important ecosystem services (Bengtsson *et al.*, 2019). Among them, wet grasslands deserve particular attention as they are threatened with abandonment due to the management difficulties linked to their winter flooding encountered by farmers (Tasset *et al.*, 2019). They host a high biodiversity (Hayes *et al.*, 2015) including numerous habitats and species of community interest (Habitats Directive 92/43/EEC). They also represent a lever for climate regulation since their soil stores large quantities of carbon (C) (Adhikari *et al.*, 2009). Nevertheless, the conservation of these services is closely linked to the choice of farmers to continue to use them for animal feed. The quality of the forages from these grasslands is therefore a service that is crucial to take into account (Tasset *et al.*, 2019).

This study is part of a larger project led by the Regional Natural Park of the Marais du Cotentin et du Bessin (PnrMCB, Normandy, France) aiming to maintain agricultural practices on wet grasslands in a context of decreasing number of farmers in the territory, while promoting the environmental properties of wet grasslands. We analysed a set of services and we will focus here on the plant diversity, patrimonial value of habitats, C stocks and fodder quality of the grasslands of five marshes located in the PnrMCB.

## Material and methods

The PnrMCB is a territory of 1200 km<sup>2</sup> including 300 km<sup>2</sup> of marshes. The marshes flood every year in winter up to early spring. Agricultural practices, mostly based on dairy production, are extensive and consist mainly of late mowing and to a lesser extent cattle grazing. The absence of fertilization has been largely encouraged by “Agri-Environmental Schemes”. Five marshes, made up of depression zones in bocage landscapes, and therefore isolated from each other, were selected.

A simple method, “AgriZH”, was developed to easily assess the indices of a set of ES in the wet grasslands of Normandy. The method was verified by comparing these indices with measurements on a sampling of 30 plots. (1) We evaluated plant diversity through a numeric index computed by assigning points by visual assessment of three criteria: the diversity of flower colours, the number of different species observed during a walk of around ten steps, and the presence of heritage species. This index appears correlated with the species richness measured in four quadrats per plot ( $r=0.76$ ,  $P<0.001$ ). (2) The heritage value results from the classification of the plant population after using an identification key for the heritage habitats characteristic of the Cotentin and Bessin marshes. This index was correlated to a heritage value index (HV) calculated from vegetation surveys (4 quadrats) by weighting the species with their regional rarity and their specificity to wetlands ( $r=0.670$ ,  $p<0.001$ ). (3) Organic matter content was assessed by a soil colour method which was found to be correlated with soil organic matter content (SOM) ( $r=0.599$ ,  $P<0.001$ ) ( $r=0.599$ ,  $p<0.001$ ). The SOM contents were obtained on samples of four cores per plot (0–15 cm) by loss of mass by ignition. (4) Forage quality was assessed by the presence/absence of species from a list of high forage value species. This index is correlated with a forage energy index (NEM net energy for milk production, 1 NEM=1760 kcal) (Baumont *et al.*, 2007) measured on aerial biomass cut to 5 cm in mid-June or mid-July depending on the farming practices ( $r=0.580$ ,  $P<0.001$ ). This ES evaluation method was applied to characterize 162 grasslands among the five marshes and then map them.

## Results and discussion

Figure 1 presents the maps concerning one of the marshes, St Hilaire, as an example illustrating the results we obtained. This marsh corresponded to the largest sector of this study with an area of 4.05 km<sup>2</sup>. Mowing after July 25 was the dominant practice. The plots located in the centre of the marsh, on the lowest topographical levels, had peat soils with the highest carbon stocks (SOM around 35%). The heritage value was higher than in the rest of this marsh with the presence of species such as *Scorzonera humilis*, *Schoenus nigricans*, *Hydrocotyle vulgaris*, *Trocdaris verticillatum*. These plots presented a lower species richness than the rest of the sector. Likewise, the forage quality was lower there with NEM values close to 0.70. These grasslands were dominated mainly by *Molinia caerulea* and *Juncus* species which do not offer much interest in terms of fodder quality. The plots to the East, also at fairly low topographic levels, were distinguished by greater plant species richness (>15 species/m<sup>2</sup>) and better forage quality with NEM values close to 0.78. These plots had very variable heritage values ranging from low, for habitats close to common mesophilous grasslands, to high, for more oligotrophic ones with, for example, the presence of *Succisa pratensis*. Soil carbon stocks were also variable, even between two adjacent plots, with these two services revealing different management histories that have led to different soil properties and therefore fertility. Finally, the plots located to the west of the marsh, at higher topographic levels, offered a plant biodiversity service greater than that of the plots in the centre with a specific richness greater than 15 species per m<sup>2</sup>. Forage quality was higher there with NEM values of around 0.78. On the other hand, the level of heritage value of the habitats and the carbon stock of the soils were lower with SOM varying between 10 and 25%.

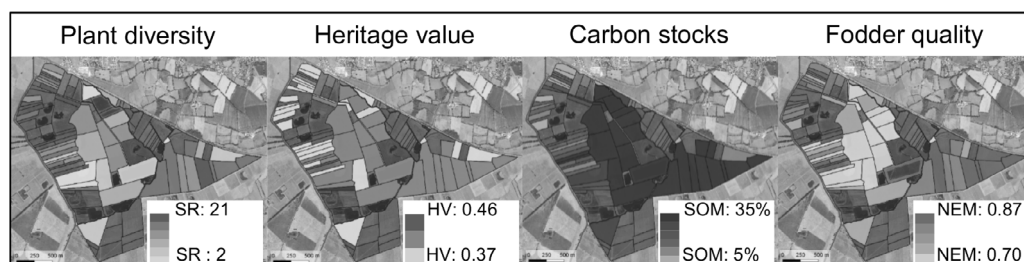


Figure 1. Maps of four ecosystem services produced on the grasslands of the Saint-Hilaire marsh. (SR, Species Richness (number of species m<sup>-2</sup>); HV, Heritage Value index; SOM, Soil Organic Matter content (%); NEM, Net Energy for Milk production, 1 NEM=1760 kcal).

These maps highlight that no grassland obtained the maximum score for all services. Some of them are particularly relevant for C storage but not fodder quality, others are interesting for the farmer with high quality but host a low diversity. Such trade-offs between forage production and diversity or heritage value (Raudsepp-Hearne *et al.*, 2010) or soil carbon storage (Kohler *et al.*, 2020) are often reported in grasslands. Because of trade-off between ES, and as previously shown on these same marshes, no habitat is the support for an optimized bundle of services (Lemauiel-Lavenant *et al.*, 2022). Nevertheless, at the marsh scale, the different grasslands appear complementary. Each habitat results from edaphic conditions but also its management history and provides its own bundle of services. The heterogeneity of the marshes, partly resulting from the diversity of management, achieves to provide multiple services at this landscape scale. This study underlines the importance of studying a set of ES simultaneously, in order to reconcile livestock farming and environmental objectives.

## Conclusion

This study highlights the importance of taking into account many services simultaneously. Indeed, when focusing on one of the ES and seek the improvement of this only ES, others may be harmed. The landscape appears to be an interesting scale for such studies since heterogeneity between grasslands ensures heterogeneity between ES and contributes to ensuring the delivery of a high level of ES at this scale.

## Acknowledgements

We are very grateful to AESN (Agence de l'Eau Seine-Normandie) which funded the project and to M. Deville, J-B. Wetton and N. Fillol, from PnrMCB, for the friendly partnership.

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# Effects of distance and orientation of hedgerows on grass production and quality in permanent grasslands

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## Abstract

Grass biomass production and quality were recorded in ungrazed paddocks located in Modave (Belgium) in order to study the influence of distance and orientation of hedgerows. The studied distances were 5, 10, 20 and 30 m from hedgerows. The paddocks were located to the East, West and South of the hedgerows. Biomass production and grass quality were measured twice: on July 11<sup>th</sup> and on August 9<sup>th</sup>. Weather conditions were favourable for grass growth during the first period (13 June –11 July) whereas the weather was dry during the second period (11 July–9 August). Biomass production was significantly higher during the first period compared to the second one ( $100.8 \pm 44.7$  vs  $27.8 \pm 15.8$  g m<sup>-2</sup>;  $P < 0.001$ ). East and West orientations allowed higher biomass production than the South orientation,  $P < 0.05$  irrespective of the period). During the second period, biomass increased progressively away from the hedgerows and became higher for East orientation at 20 m and 30 m compared to other orientations ( $P < 0.01$ ). Grass quality was affected by distances and orientations but remained high and sufficient to meet animal requirements. Our results show that hedgerows have influenced grass production, with different results depending on the orientation and growing conditions of the grass.

**Keywords:** agroforestry, grass biomass, grass quality, hedge, orientation, distance

## Introduction

Agroforestry is encouraged in agroecological systems and allows farmers to benefit from CAP payments. However, some livestock farmers are reluctant to plant hedges despite the numerous advantages they offer (such as improvement of biodiversity, protection against erosion and against drying out, capacity to store carbon and contribution to offer forages and to improve animal welfare by offering them shelter). These farmers fear a reduction in grass production due to competition between the growth of the hedge and that of the meadow. The present trial aims to provide data related to the influence of hedges on grass production and quality.

## Materials and methods

The trial was carried in Modave (Belgium), between 13 June and 9 August 2022 in 3 pastures grazed rotationally by dairy cows. Fences were erected in 6 paddocks along double hedges to protect grass from grazing. There were 2 paddocks by orientation: the hedgerows were located at East, West and South of the paddocks. Biomass production and grass quality were measured twice: on 11 July and 9 August at 5 plots per distance on each paddock. The grass was cut from a length of 2 meters in each plot. The width of the mower was 42 cm and the cut height was 5 cm. The grass was dried to obtain dry matter percentage. For the first period (cut), the grass swards were also sampled by hand plucking, with a sample was taken at each distance in each paddock, to provided samples for determination of chemical composition. The Dutch evaluation system was used to express energy content (VEM) and protein (digestible protein in the small intestine (DVE), rumen-degradable protein balance (OEB)). The grass composition in water soluble carbohydrate (WSCH) and crude protein (CP), crude fibre (CF), VEM, DVE, OEB in the

dry matter and digestibility were determined. The grass swards between the measured plots were also mown on 11 July to avoid shading which could have affected the grass growth as measured on 9 August. Statistical analyses were carried out with R4.2.2 ANOVA models, setup for biomass production and for grass chemical composition, considering orientation and distance as fixed effect. T tests, for data with normal distribution, Wilcoxon tests and Kruskal-Wallis tests for non-parametric set data, were used to compare the means.

## Results and discussion

Weather conditions were favourable for grass growth during the first period whereas the weather was dry during the second period. Logically, grass biomass was significantly higher for cut 1 ( $100.8 \pm 44.7$  vs  $27.8 \pm 15.8$  g m<sup>-2</sup>;  $P < 0.001$ ). Grass biomass values are shown in Figure 1 for each cut date, orientation and distance. Orientation had a significant effect: East and West orientations allowed greater biomass production than the South one, probably due to conditions of growth with a better solar irradiation ( $P < 0.05$ ). Significant effects were observed between distance and orientation ( $P < 0.01$ ), and cut and orientation ( $P < 0.01$ ). For cut 1, with weather conditions favourable for grass growth, the south orientation biomass increased at further distance of hedgerows and were lower for each distance compared to other orientations. East orientation seems to favour grass growth overall at 20 m ( $P < 0.01$ ). Grass biomass at West were highest than other orientations at 5 m distance and remained constant until 20 m, and increased at 30 m to reach East biomass. At cut 2, for East orientation, biomass increased progressively away from the hedgerows and values were significantly higher at 20 m and 30 m compared to other orientations at the same distances ( $P < 0.01$ ). South orientation biomass values were the lowest at every distance, except at 5 m where they were higher than other distances ( $P < 0.05$ ) and similar to the West orientation biomass at 5 m. At the opposite, for West orientations, biomass decreased away from the hedgerows to become similar to those of South orientation at the same distances (20 m and 30 m). Few data are available on this subject, but Van Vooren *et al.* (2018) and Heimsch *et al.* (2023) did not record a negative effect on hedgerow or tree row on winter wheat or oat crop beyond 2 metres. In our trial, we did not measure data under 5 metres because the grass at that distance had been trampled by the animals.

The grass chemical composition (cut 1) shows it was of good quality and compatible with the requirements of dairy cows (Table 1). The distances influenced CF content, which was significantly higher at 5 m than at 20 m and 30 m ( $211$  g vs  $196$  g kg<sup>-1</sup>;  $P < 0.05$ ). There was a tendency to have lower DVE value at 30 m compared to 10 m and 20 m ( $P < 0.10$ ). CF, WSCH, DVE, VEM and digestibility were lower for grass located East of the hedgerows ( $P < 0.05$ ). This could be attributed to a lower levels of the solar luminosity.

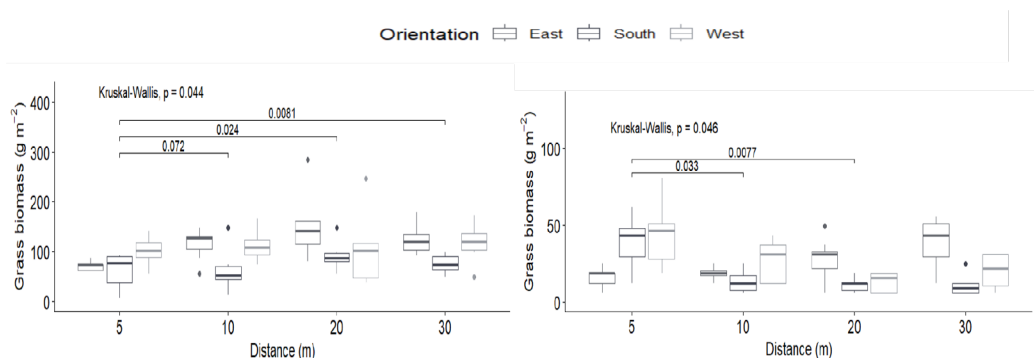


Figure 1. Grass biomass for each orientation and each distance of the hedgerows for cuts 1 and 2. For each cut, P-values are given for Kruskal-Wallis test and when there are significant differences between the distances.

Table 1. Grass chemical composition and feeding values at different orientation of the hedgerows (cut 1).

	Orientation of the hedgerow			SEM	P value
	East	West	South		
CP (g kg <sup>-1</sup> )	202.1	211.4	204.8	6.41	0.60
CF (g kg <sup>-1</sup> )	208.0 <sup>b</sup>	198.6 <sup>a</sup>	195.1 <sup>a</sup>	4.39	0.09
WSCH (g kg <sup>-1</sup> )	78.6 <sup>a</sup>	87.0 <sup>ab</sup>	97.1 <sup>b</sup>	8.60	0.31
DVE (g kg <sup>-1</sup> )	96.8 <sup>a</sup>	98.4 <sup>ab</sup>	99.4 <sup>b</sup>	1.10	0.12
OEB (g kg <sup>-1</sup> )	43.3	51.4	43.0	5.58	0.55
VEM	968.5 <sup>a</sup>	978.1 <sup>ab</sup>	992.9 <sup>b</sup>	6.17	0.06
Digestibility (%)	79.1 <sup>b</sup>	80.8 <sup>a</sup>	81.3 <sup>a</sup>	0.61	0.05

CP, crude protein; CF, crude fibre; WSCH, water soluble carbohydrate expressed in the dry matter; VEM, energy value; DVE.; digestible protein in the small intestine; OEB, dietary rumen-degradable protein balance.

## Conclusion

The South orientation had a negative impact when the weather was favourable, probably due to the shading effect, but this can protect grass in warm and dry conditions. Hedgerows were unfavourable for grass growth at 10 m and 20 m, compared to 5 m, when the weather was dry. From these first results, it can be concluded that the hedgerows influenced grass production but with different results depending on the orientation and growing conditions of the grass. Grass quality was high and sufficient to meet animal requirements, regardless of the distance from the hedgerow and the orientation. However, at East orientation grass quality seems to be lower.

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# Animal health service provided by grassland diversity: farmers' perceptions and strategies in four French regions

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## Abstract

Animal health provision from grassland is a potential response to animal health management. We aimed to identify how farmers perceive and use the health service of grassland in relation to the floristic diversity. To this end, comprehensive interviews were carried out with 30 farmers located in four regions characterized by different types of grasslands: plain area mainly with temporary grasslands; plain area mainly with permanent grasslands; mountain area mainly with permanent grasslands. A typology of farmers according to their management strategies of grassland diversity has been built. Three groups were identified: (1) one grassland service, where farmers have several grasslands, each providing a specific service ( $n=10$ ); (2) one grassland for multiple services, where farmers look for multiple services through one diversified grassland ( $n=10$ ); and (3) single service grassland where farmers use grassland only for the feed service ( $n=10$ ). We have shown that management strategies are consistent with the link they perceived between grassland diversity and animal health (totally convinced ( $n=14$ ), looking for evidence ( $n=8$ ) or not convinced at all ( $n=8$ )).

**Keywords:** grassland diversity, animal health, farmer's perception

## Introduction

Through their diversity, grasslands provide a wide range of services that meets multiple challenges faced by agriculture: limiting erosion, regulating water flows, filtering pollutants, preserving floristic, faunal and microbial biodiversity, carbon sequestration, and providing landscape benefits (Bengtsson *et al.*, 2019). Beyond these services, grasslands can be useful for animal health management. They provide balanced forage, particularly when grazed. The flora, through its diversity, can be a source of metabolites, such as carotenoids, phenolic compounds and vitamins, which are produced by grassland plants (Poutaraud *et al.*, 2017; Maxin *et al.*, 2020). Polyphenols, through their scavenging capacity, can contribute to reduce oxidative stress and inflammation in several animal species. Condensed tannins enhance antiparasitic activity (Hoste *et al.*, 2005). Despite references that suggest potential roles of grasslands to manage animal health, there is little knowledge about how farmers are using grassland and plant diversity for animal health management. We aimed to study farmers' perceptions of the link between grassland diversity and animal health, and the link with the forage system. Our hypothesis is that farmers who connect grassland diversity and animal health manage the forage system in a way that makes use of this service of health provision.

## Materials and methods

In 2023, we interviewed farmers from 30 farms (12 dairy cattle farms, 7 beef cattle farms, 3 ovine farms and 8 farms with multiple ruminant productions) selected in four areas in France: Pays de la Loire (PDL,  $n=7$ ), Indre ( $n=9$ ), Auvergne-Rhône-Alpes (AURA,  $n=5$ ) and Haute-Saône (HS,  $n=9$ ). These areas have a high proportion of grasslands in the utilized agricultural area (UAA), a diverse range of production systems, and a range of climates (continental, oceanic, and continental to mountain) and agronomic potentials. The average UAA of the sample was 137 ha (SD  $\pm 58$  ha) with 89% of forage area (SD  $\pm 12\%$ ) and 58% of grassland (SD  $\pm 29\%$ ). Comprehensive interviews were carried out with open-



ended questions to: i) collect data about the farm, the animals, the grassland diversity management; and ii) to access to the farmers' perceptions about the link between grassland diversity and animal health. The discourses have been recorded, transcribed then analysed to qualify the farmers on their grassland use for animal health services. Next, the discourse information was analysed using comprehensive sociology. The lexical fields used by farmers regarding grassland management and animal health were identified. Differences in lexical fields made possible to classify the farmers according to their perceptions and the spontaneity with which they talk about the use of grassland for animal health and their knowledge or expectations on this subject.

## Results and discussion

The analysis of the expected services and the management of the diversity at farm scale led to three profiles showing that farmers' management strategies are consistent with the link they perceived between grassland diversity and animal health (Table 1). The first one, "One grassland by service" ( $n=10$ ), groups farmers that are looking for multiple services through grassland diversity. They have several grasslands, each providing a specific service. Health service is provided by a "pharmacy grassland" used at specific moments. This group gathers "convinced farmers" ( $n=6$ ), speaking spontaneously about the health services provided by grassland and giving examples of health effects of plants, molecules or phenological stages, and farmers "looking for evidence" ( $n=4$ ), thinking that diversity can be useful for animal health but lacking knowledge and trying to explain it by comparison to human health. The second one, "One grassland for multiple services" ( $n=10$ ), gathers farmers searching for multiple services of grassland through one diversified grassland. Animals benefit from health service of this grassland through rotational grazing and stored forage. This group gathers "convinced farmers" ( $n=8$ ) and farmers "looking for evidence" ( $n=2$ ). The third one, "Single service grassland" ( $n=10$ ) gathers farmers using grassland only for the feed service, given by one type of grassland. This group gathers mainly farmers that are "not convinced" and hardly speak of the link between grassland diversity ( $n=8$ ) and few farmers "looking for evidence" ( $n=2$ ).

Farmers managing diversity as "One grassland for multiple services" work with permanent grasslands (70% of the UAA) to benefit from its multiple services. It can be explained locally by the existence of tools or advice developed to manage grassland diversity. For example, mountain farmers, who use natural grassland typologies to address the provision of multiple services (Schils *et al.*, 2022), are among those who are convinced that their practices enhance the health service of grasslands (e.g. AURA region). Conversely, the use of temporary grassland is more important in "One grassland by service" management (52% of the UAA). It can be explained by the hilly context observed in HS, Indre and valley area in AURA that force them to deal with various agronomical potentials. Diversified permanent grasslands are considered as less productive and poor but ideal for dry cows, young animals or for "medicinal" service. Highly productive multispecies temporary grasslands are sown for a protein-rich forage production. Finally, farmers in the "Single service grassland" are not characterised by the type of grassland they use, nor by the region. They adapt their forage system to their local conditions and focusing only on feed production. By doing that they pass by the other services that grasslands can provide at farm scale. As shown by Di Blasi *et al.* (2023), our study has not shown any effect, either with regard to the size of the livestock or the level of animal intensification, on the perception of health services of grasslands.

## Conclusion

While studies highlight that species could be useful to deal with animal health issue, part of the farmers are waiting for health service from the diversity of their grasslands. Those perceptions are associated with grassland diversity management at farm scale, partly impacted by the agronomic potential and the tools and advice available for farmers. To promote the health service provided by grasslands, researchers and advisers would have to produce local references according to the different ways to manage grassland diversity at farm scale.

Table 1. Grassland diversity management strategies and perceived link between grassland diversity and animal health.

		Convinced	In search of evidence	Not convinced	Total
One grassland by service	n	6	4	–	10
	AURA	1/5	2/5	–	3/5
	HS	3/9	–	–	3/9
	Indre	2/9	1/9	–	3/9
	PDL	–	1/7	–	1/7
	PG	41%	58%	–	48%
One grassland for multiple services	n	8	2	–	10
	AURA	2/5	–	–	2/5
	HS	2/9	1/9	–	3/9
	Indre	2/9	1/9	–	3/9
	PDL	2/7	–	–	2/7
	PG	69%	73	–	70%
Single service grassland	n	–	2	8	10
	AURA	–	–	–	–
	HS	–	–	3/9	3/9
	Indre	–	1/9	2/9	3/9
	PDL	–	1/7	3/7	4/7
	PG	–	34%	61%	56%
Total	n	14	8	8	30
	AURA	3/5	2/5	–	5
	HS	5/9	1/9	3/9	9
	Indre	4/9	3/9	2/9	9
	PDL	2/7	2/7	3/7	7
	PG	57%	56%	61%	58%

PG, average part of permanent grasslands.

## Acknowledgement

This study is part of the PRAIDIV project, financed by a special “account for agricultural and rural development” (CASDAR) allocation from the Ministry of Agriculture, which cannot be held liable.

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# Integrating arable and dairy farms through legume-based leys in the Netherlands

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## Abstract

Regionally integrated arable crop-livestock systems are expected to have lower environmental impacts compared to separate specialized systems. A potential strategy for integrated crop-dairy systems (ICDS) is the establishment of ley pastures, particularly legume-based swards, on arable fields. The aim of this study was evaluate the impact of ICDS on farm structure, environmental impacts and economic performance compared to a separate specialized dairy production system. To this end a whole-farm linear programming (LP) model of a dairy farm on sandy soil in the Netherlands was used, with low (7,209 kg milk cow<sup>-1</sup> year<sup>-1</sup>) and high (9,209 kg cow<sup>-1</sup> year<sup>-1</sup>) producing cows. Available land in the model could be used for perennial ryegrass, grass-white clover, grass red-white clover ley and forage maize. The model was extended with arable crops (potatoes, sugar beet and spring barley) to represent an ICDS. Land included in the rotation of the ICDS could be used for the arable crops, grass-red-white clover ley and forage maize. The ICDS was optimized by maximizing the joint income from dairy and crop production. Results of the study indicate the number of cows and gross margin per hectare were higher in the ICDS than in its specialized system counterpart, irrespective of the milk production levels. In contrast, the N surplus decreased after integration for the farm with low producing cows and increased for a farm with high producing cows. Hence, different milk production levels seem to impact the economic and environmental potential of ICDS.

**Keywords:** integrated crop-dairy system, leys, linear programming

## Introduction

The specialization of arable crop and livestock production systems is associated with high resource inputs and environmental challenges, such as nutrient surpluses. Regionally integrated arable crop-livestock systems have been proposed as a potential solution to reduce environmental impacts. A potential strategy to implement integrated crop-dairy systems (ICDS) is the establishment of ley pastures, particularly legume-based swards, on arable fields. Ley pastures with legumes could provide multiple ecosystem services, including nutrient provisioning and recycling by symbiotic nitrogen (N) fixation and the N transfer to subsequent crops in the rotation. Little is known about the implications of ICDS beyond field level, which highlights the need to get a better insight in the effects of ICDS on farm structure (e.g. livestock density and land use management), environmental (e.g. N surplus) and economic (e.g. farm income) performance. Potential outcomes of ICDS also seem to depend on milk production levels (Reinsch *et al.*, 2021) and could vary across farms with different levels of production intensity. Therefore, the objective of this study was to explore these effects for a farm with relatively low (7209 kg milk cow<sup>-1</sup> year<sup>-1</sup>) and high (9209 kg milk cow<sup>-1</sup> year<sup>-1</sup>) milk production levels on sandy soils in the Netherlands.

## Materials and methods

A whole-farm linear programming (LP) model was used to represent a dairy production system on a sandy soil in the Netherlands with all relevant activities and with the constraints that apply in practice (Alderkamp *et al.*, 2024) and was originally validated by Berentsen and Giesen (1995). The relevant activities of the dairy farm include on-farm feed production (maize silage, grass silage and grass for

grazing), related field operations and animal production. Other activities are purchasing maize silage, concentrates and mineral fertilizers. Constraints include fixed resources available to the farm (e.g. land area of 54.7 ha), environmental policies (e.g. phosphate ( $P_2O_5$ ) quota) and links between the different activities. Holstein-Friesian dairy cows with a yearly national average milk production of 9209 kg (DAIRY-HP; CRV, 2022) or a hypothetically reduced milk production level of 7209 kg (DAIRY-LP) as is given, e.g., for Jersey cows by Reinsch *et al.* (2021). The model distinguishes a summer and winter period, considers dietary requirements of the dairy cows and accounts for extra variable labour. A farm level N balance was linked with the LP model. A detailed description of the model and all assumptions can be found in Klootwijk *et al.* (2016). Costs of farm inputs and revenues were updated according to long-term expected market prices and national statistics. The model was extended with an arable crop production system of 65 ha including starch potatoes, seed potatoes, sugar beet and spring barley. Within the ICDS, forage maize and grass-red white clover were added to the arable crop rotation. Grass-red white clover could be selected with seed potato as a subsequent crop, which reduced the annual N fertilization requirements by 100 kg N ha<sup>-1</sup>. Furthermore, 40% of the available land on the dairy farm could be used for either perennial ryegrass or a grass-white clover mixture, and not for the arable crop rotation. We used economic optimization for four scenarios to determine the optimal farm plan and to evaluate changes in farm structure, labour income and N surpluses before (DAIRY-LP, DAIRY-HP) and after adding the arable crop production system to the dairy farm (ICDS-LP, ICDS-HP).

## Results and discussion

The number of cows, gross margin ha<sup>-1</sup>, and N surplus was lower for the low producing scenarios (DAIRY-LP and ICDS-LP) compared to the high producing scenarios (DAIRY-HP, ICDS-HP) (Table 1). Comparing the specialized production systems (DAIRY-LP, DAIRY-HP) with their respective integrated counterparts (ICDS-LP and ICDS-HP) showed that cow number and gross margin ha<sup>-1</sup> increased after integration. However, the increase in gross margin was higher (+€222 ha<sup>-1</sup>) for the high producing system compared to the low producing system (+€84 ha<sup>-1</sup>). Post-integration, farm management changes included a decrease in fresh grass intake per cow due to grass-red white clover swards used exclusively for silage. Furthermore, the use of mineral N fertilizer for the dairy production system increased for the ICDS because part of available manure was used on the arable farm. ICDS-LP had a lower N surplus (-6 kg ha<sup>-1</sup>) compared to DAIRY-LP, attributed to savings in high-protein concentrates, unlike the high production system (+8 kg ha<sup>-1</sup>). For the arable crops, the model selected the maximum share of the high value crops of potatoes and sugar beets. In reality, also other aspects such as the provisioning of various ecosystem services by ley-based systems should be accounted for to improve the comparison between ICDS and specialized systems. Finally, various reasons (e.g. imperfect data) could explain differences between outcomes in practice and model results. However, differences between scenarios could provide insight in the trend of certain changes.

## Conclusion

In conclusion, the ICDS resulted in an increase in the number of cows and gross margin ha<sup>-1</sup> for both low and high producing farms compared to their respective specialized counterpart. In addition, fresh grass intake reduced because the grass-red-white clover leys could only be used for silage. In contrast to the low producing farm, the N surplus increased for the high producing farm when integrating arable crops into the production system. Ley-based ICDS requires further research to be able to assess its full potential on sandy soils in the Netherlands.

Table 1. Farm structure, labour income and environmental performance of a typical Dutch dairy farm with a Holstein-Friesian dairy cow with a yearly milk production of 7209 (DAIRY-LP) or 9209 kg milk cow<sup>-1</sup> year<sup>-1</sup> (DAIRY-HP) and within the integrated crop-dairy system (ICDS-HP and ICDS-LP).

Item	Unit	DAIRY-LP	ICDS-LP	DAIRY-HP	ICDS-HP
Farm structure					
Dairy cows	No.	93	103	98	106
Total farmland	ha	54.7	119.7	54.7	119.7
Perennial ryegrass	ha	6.2	17.6	16.8	17.1
Grass-white clover	ha	21.5	4.3	10.9	4.8
Grass-red white clover	ha	9.6	13.4	9.0	12.9
Maize land	ha	17.5	19.4	18.0	19.9
Seed potatoes	ha	–	24.5	–	24.5
Starch potatoes	ha	–	8.2	–	8.2
Sugar beets	ha	–	24.5	–	24.5
Spring barley	ha	–	7.9	–	7.9
N <sub>min</sub> application perennial ryegrass	kg N ha <sup>-1</sup> year <sup>-1</sup>	275	350	300	325
Farm intensity	kg milk ha <sup>-1</sup> year <sup>-1</sup>	12,245	13,590	16,429	17,808
Diet dairy cows: summer					
	kg DM cow <sup>-1</sup> day <sup>-1</sup>				
Grass		10	7.1	10.0	6.7
Grass silage		0	1.4	0	1.5
Maize silage		6.4	6.4	6.4	6.4
Concentrates		1.9	3.5	5.1	7.0
Diet restricted by		E,R,T,G	E,R,T	E,R,T,G	E,T
Diet dairy cows: winter					
	kg DM cow <sup>-1</sup> day <sup>-1</sup>				
Grass silage		5.4	5.4	4.8	4.9
Maize silage		6.7	6.7	6.3	6.7
Concentrates		3.6	3.6	6.9	6.5
Diet restricted by		E,R,T	E,R,T	E,R,T	E,R,T
External inputs					
Purchased concentrates DP	Mg DM year <sup>-1</sup>	103	144	226	276
Purchased mineral N fertilizer DP	kg year <sup>-1</sup>	1,346	5,888	3,947	5,572
Purchased mineral P <sub>2</sub> O <sub>5</sub> fertilizer DP	kg year <sup>-1</sup>	739	921	650	638
Import organic fertilizer CP	Mg year <sup>-1</sup>	–	2,094	–	1,778
Gross margin DP	€ ha <sup>-1</sup>	2,771	2,855	3,451	3,673
Gross margin CP	€ ha <sup>-1</sup>	–	2,689	–	2,673
N surplus DP	kg ha <sup>-1</sup> year <sup>-1</sup>	110	104	132	140
N surplus CP	kg ha <sup>-1</sup> year <sup>-1</sup>	–	119	–	119

Abbreviations: N<sub>min</sub>, N mineral; E, energy requirements; R, rumen degradable protein balance; T, true protein digested in the small intestine; G, maximum fresh grass intake; DP, dairy production system; CP, crop production system.

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# Adaptive responses of meadow melliferous plants to low soil pH and change in soil structure

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## Abstract

Fragmented meadow habitats near arable land may be prone to increases in acidity due to excessive fertilization and may contain more sand in the meadow soil. Such soil changes disrupt the development of melliferous plants, changing their ecological niches. The aim of the study was to investigate the adaptation responses of melliferous plants grown on acidic and sandy soils. Pot experiment simulated the effect of acidity and sand content in the soil on germination and vegetative growth and development of four pollinator-dependent plants. Seeds of *Centaurea jacea*, *Knautia arvensis*, *Lychmis flos-cuculi*, and *Succisa pratensis* were collected from fragmented meadows in central Poland. These species were sown into pots filled with soil (pH 6.5–7; control), soil + sand, and acidic soil (pH 4.5–5). Only the germination capacity of *K. arvensis* was significantly suppressed by acidic soil. However, soil acidity inhibited seedling height in all species. Meanwhile, sand-mixed soil had a positive effect on the development of *L. flos-cuculi* and *C. jacea* which was expressed by the highest height and width of seedlings. Most species developed more leaves in the control, except for *L. flos-cuculi*. Neutral soil pH enhanced the ramet abundance of *L. flos-cuculi* compared to *K. arvensis* and *S. pratensis*.

**Keywords:** melliferous plants, acidic soil, sandy soil, germination capacity, vegetative growth and development

## Introduction

The progressive decrease in the biodiversity of meadow communities is a consequence of fragmentation and degradation of habitats, resulting primarily from irrational human activities, including intensification, especially excessive nitrogen fertilization, and cessation of use (Schils *et al.*, 2022). This has resulted in the acidification of soils, especially light, highly permeable soils, which dominate in Poland. Meanwhile, this type of soil may be favourable for some grassland species including grasses and forbs. Melliferous species are among the most important forbs for biodiversity, and are widely distributed in European semi-natural grasslands, including Poland (Klarzyńska and Kryszak, 2016). However, human-created isolated patches of meadow habitats (Van der Walt *et al.*, 2015) lead to a decrease in the size of populations of melliferous species and thus their growth patterns also become periodically limited. From this background it is assumed that declining pH and changing soil structure and composition may alter the adaptation strategy of melliferous plants. As a result, this may interrupt and delay in plant growth and development before entering the generative phase and seed setting. The aim of the study was to investigate the seed germination and vegetative efficiency of four melliferous plants as their adaptation responses, which affect their establishment in different soil pH and soil structure.

## Materials and methods

The studies covered four pollinator-dependent plant species of the meadow habitats: *Centaurea jacea* L., *Knautia arvensis* L., *Lychmis flos-cuculi* (*Silene flos-cuculi* (L.) Greuter et Burdet) and *Succisa pratensis* Moench. The most important characteristics of those species are listed in Table 1. Their seeds were collected from fragmented meadows in the Rządza Valley (central Poland) in July and October 2022 and were sown into pots in the following year. Soil conditions were simulated as in the existing fragmented meadows with three different substrates as soil (pH 6.5–7.0; control), soil+sand, and acidic soil (pH

4.5–5.0). The experiment had a completely randomized design with three replications. The plants were watered twice a week (full water capacity). The measured parameters included: germination capacity, seedling height, width, leaf number and ramet abundance. One-way ANOVA was performed, and significant results were analysed by LSD post hoc analysis. The statistical analysis was conducted by R Studio Program 4.3.0.

## Results and discussion

The germination capacity was significantly affected by acidity and sand content only for *K. arvensis* (Table 2). This relationship was not found for *L. flos-cuculi*, a species with similar requirements regarding soil pH (Zarzycki *et al.*, 2002). All tested species had smaller seedlings in acidic soil as the adaptive response. The height and width of *L. flos-cuculi* and *C. jacea* seedlings were the highest in the soil mixed with sand (Table 3). These results indicate that these species tolerate both clay and sandy soils with a neutral or slightly acidic reaction. Most species produced noticeably numerous leaves in soil with neutral pH. All studied species are perennial plants, hemicryptophytes, with the ability to produce new shoots or rosettes (ramets). In soil with pH close to neutral, *L. flos-cuculi* adapted faster by spreading more ramets than others (*K. arvensis* and *S. pratensis*) (Table 3).

Conversely, *C. jacea* did not produce ramets during the experiment. The shift of reproductive strategy from generative to vegetative ramet indicates a saving in energy needed by plants to survive (Muir,

Table 1. Characteristics of species.

Specification	Species			
	<i>C. jacea</i>	<i>K. arvensis</i>	<i>L. flos-cuculi</i>	<i>S. pratensis</i>
Botanical family	Asteraceae	Dipsacaceae	Caryophyllaceae	Dipsacaceae
Biological stability (durability)	Perennial	Perennial	Perennial	Perennial
Phytosociological unit	Mol–Arr Cl	Arrhen All	Mol caer O	Mol caer All Suc prat Ass
Edaphic value				
Soil moisture value	3	3	4	4
Soil acidity (pH) value	3–4	4–5	4–5	4–3
Flowering period (months)	VI–X	V–IX	V–VII	VII–IX

Phytosociological units: Cl, Class; O, Order; All, Alliance; Ass, Association; Mol-Arr, *Molinio-Arrhenatheretea*; Arrhen, *Arrhenatherion*; Mol caer, *Molinietalia caeruleae*; Suc prat, *Succisetum pratensis*. Soil moisture value: 3, moderately moist; 4, moist. Soil acidity (pH) value: 3, moderately acidic; 4, neutral; 5, alkaline.

Table 2. One-way ANOVA parameters in relation to the effect of different soil substrates on important features of four melliferous plants.

Parameter	Substrate df	F ratio			
		<i>C. jacea</i>	<i>K. arvensis</i>	<i>L. flos-cuculi</i>	<i>S. pratensis</i>
GC	2	2.26 ns	6.33 *	0.77 ns	3.03 ns
Height	2	2262 *	2.51 ns	185.19 *	4.37 *
Width	2	43.23 *	2.73 ns	21.37 *	3.24 ns
Leaf number	2	24 *	4.92 ns	10.62 *	4.82 *
Ramet	2	–	0.48 ns	93 *	1.04 ns

GC, Germination Capacity (%); df, degree of freedom; –, did not produce ramet; ns, non-significant.

\* Significant at  $P < 0.05$  or  $F \text{ ratio} > F$  table with  $\alpha = 0.05$ .



Table 3. The influence of soil substrate on important features for seedling. Post hoc LSD analysis was performed only for species with statistically significant results for tested parameters.

Parameter	Species	Soil substrate		
		Control	Soil+Sand	Acid soil
Germination capacity (%)	<i>K. arvensis</i>	23.33 a	13.33 ab	6.67 b
Seedling height (cm)	<i>C. jacea</i>	4.68 a	4.87 a	0.30 b
	<i>L. flos-cuculi</i>	7.27 a	7.63 a	0.90 b
	<i>S. pratensis</i>	2.63 a	1.33 ab	0.67 b
Seedling width (cm)	<i>C. jacea</i>	16.00 b	27.80 a	4.83 c
	<i>L. flos-cuculi</i>	14.37 a	17.07 a	4.50 b
Number of leaves	<i>C. jacea</i>	8.67 a	6.00 b	3.33 c
	<i>K. arvensis</i>	13.33 a	3.67 b	4.33 b
	<i>L. flos-cuculi</i>	37.33 a	33.67 a	6.00 b
	<i>S. pratensis</i>	14.67 a	5.67 b	4.33 b
Ramet abundance (no.)	<i>L. flos-cuculi</i>	6.00 a	5.00 a	0.00 b

Means in rows marked with the same letter indicate a non-significant difference, according to the LSD post-hoc test ( $P < 0.05$  or  $F \text{ ratio} > F \text{ table}$ , with  $\alpha = 0.05$ ).

1995). However, this strategy may harm pollinator activity by triggering pollinator loss and causing agamospermy for plants then reducing seed set, and germination capacity of plant progeny. Lack of progeny establishment may change the pollinator niche in the habitat (Vergeer *et al.*, 2003). Consequently, this is contrary to the role of grasslands in maintaining biodiversity.

## Conclusions

Soil acidification has a decisive influence on seed germination only for *K. arvensis* and on vegetative performance for all tested melliferous species. Sand-mixed soil has a positive effect on the development of *L. flos-cuculi* and *C. jacea*. These results provide scientific information about adaptation responses and strategies of melliferous plants under soil disturbance conditions to support restoration of their biodiversity in meadows.

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# Bite item diversity and bite item quality in an extensive grazing system with suckler cows

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## Abstract

Extensively managed grassland offers grazing livestock a diverse range of different bite items (BI). To investigate how realised BIs (selected by cows) are related to actual BIs (measured pre-grazing), and why they are selected, a study was conducted in the long-term extensive cattle grazing experiment 'Forbioben' with its randomised block design under two stocking intensities (moderate; lenient; in total six 1 ha paddocks). During two grazing periods in 2022 (spring, autumn) actual BIs were assessed pre-grazing using a modified sward stick (10×10 cm steel frame) to simulate adult cattle's bite size area. The realised BIs were assessed by video recording and observation of each cow in the morning and afternoon (4×2 minute intervals; in total 16 min cow<sup>-1</sup> and period) using a mobile phone app and subsequent video analysis. For each BI hand-plucked samples were taken for the determination of the crude protein (CP) concentration and these BIs were labelled as grazed and non-grazed subsequently. In total 51 unique BIs were found and more BIs were selected in pastures with greater actual BI diversity. The grazed BIs showed a greater CP concentration than the non-grazed BIs.

**Keywords:** extensive grassland, selective grazing, cattle, observation

## Introduction

Extensively managed grassland offers grazing livestock a diverse range of different bite items (BI). The BI selection of grazing cattle is a central component of the interaction between herbivores and the grass sward (Soder *et al.*, 2007). The selection depends on various interacting factors including BI diversity and availability as a consequence of grazing management intensity (Orr *et al.*, 2014). The availability of different BIs is termed the actual BI diversity in the following (for details, see Zanon *et al.*, 2022). From these actual BIs, cattle select a range of BIs that they prefer. These selected BIs are named the realised BIs (Zanon *et al.*, 2022). Understanding the BI selection in grassland is valuable for ensuring the sustainability, productivity and biodiversity conservation of extensive grazing systems. A better understanding would further allow to predict impacts of grazing species on the grassland ecosystem. Therefore, we investigated how realised BIs (selected by cows) are related to actual BIs (measured pre-grazing) in a long-term, extensive cattle grazing experiment under continuous stocking management.

## Materials and methods

The study was conducted during spring and autumn of 2022 as part of the long-term extensive cattle grazing experiment 'Forbioben' with its randomised block design ( $n=3$  replicates). It is located at the experimental farm of the University of Göttingen in Relliehausen, Solling Uplands, Lower Saxony, Germany. Two stocking intensities (moderate, target compressed sward height (CSH) 6 cm; and lenient, target CSH 12 cm; in total six 1 ha paddocks) were selected for this study. It is an extensive grassland system with no application of fertilisers, pesticides or maintenance measures. Regular CSH measurements were used to control the grazing pressure (for details, see Obermeyer *et al.*, 2022). All cows were non-lactating Fleckvieh suckler cows and, before each grazing period, the cows were weighed and randomly distributed to the paddocks according to their live weight. The resulting stocking densities in livestock units (LU=500 kg LM ha<sup>-1</sup>) were in spring  $3.9\pm 0.06$  LU for both treatments and  $4.5\pm 0.03$  and  $3.0\pm 0.05$  in autumn for moderate and lenient stocking, respectively. The actual available BIs were

assessed pre-grazing in two transects per paddock (200 points per paddock) using a modified sward stick (10×10 cm frame) to simulate the cattle bite size. At each point the botanical composition in terms of the dominating plant functional group (grass, legumes, non-legume herbs and all possible combinations of them (7 in total)), the phenology (vegetative, generative), the colour (green, brown, mixed) and the extended height at first contact point of the frame with the vegetation (short, tall) were measured. The BIs were consequently structured as, e.g. grass\_vegetative\_green\_short. A total number of potentially 84 unique BI combinations was possible in this way. All available BIs were sampled thereafter by hand plucking, subsequently dried (60°C, 48h) and analysed for the concentration of crude protein (CP) using NIRS. They were labelled as grazed or non-grazed Bis, as based on the realised BIs. These realised BIs were assessed by video recording and observation of each cow for 4×2 minute intervals each in the morning and afternoon over one full light day. In total 16 min cow<sup>-1</sup> and period (spring and autumn) were recorded for subsequent video-based determination of realised BIs. The diversity of unique actual and realised BIs per pasture and period were calculated subsequently by accumulating the number of different BIs per paddock, stocking intensity and period. Statistical analyses were performed with Rstudio (R Version 1.4.1717, 2021, The R Foundation for Statistical Computing, Vienna, Austria) using linear-mixed effects modelling to test for the effects of stocking intensity, period and their interaction for the actual and realised BIs. For the CP concentration the model included stocking intensity, period, grazed/non-grazed and their interaction. The paddock nested in block was used as a random term. Posthoc comparisons of means were done using Tukey's HSD test.

## Results and discussion

Extended sward heights were slightly higher in autumn than in spring and also slightly higher in lenient compared to moderate grazing (Table 1). A total of 266.1±4.06 livestock unit grazing days ha<sup>-1</sup> were conducted in the moderate and 194.5±1.23 in the lenient stocking intensity during 2022. The actual BI diversity was greater ( $p<0.001$ ) for both stocking intensities in spring (Table 1). This can be explained by the fact that more generative BIs are found in spring compared to autumn (38.9% vs 11.3%). In spring 50% of the actual BI measurements are made up by 28 Bis, while in autumn 50% were represented by 21.5 different BIs. The difference between the two stocking intensities in terms of actual BI diversity was minimal and irrespective of period (Table 1).

Different letters indicate significant differences between means within period, and letters in parentheses indicate significant differences between means within stocking intensities ( $p<0.05$ ). Extended sward height refers to arithmetic mean±SD.

The realised BI diversity was smaller than the actual BI diversity and likewise greater in spring than in autumn ( $p<0.001$ ). In both periods, the realised BIs were greater under lenient grazing compared to moderate grazing (Table 1), potentially as a consequence of a more heterogeneous grass sward structure

Table 1. Estimated means±se for of the number of actual and realised bite items for the interaction of period and stocking intensity.

Period	Stocking intensity	Extended sward height (cm)	Actual (number)	Realised (number)
Spring	Moderate	18.1±8.45	23.3±2.21 a (a)	12.07±0.077 a (a)
	Lenient	20.1±8.77	21.2±2.21 a (a)	14.91±0.085 b (a)
Autumn	Moderate	19.4±18.64	13.5±2.21 a (b)	1.36±0.038 a (b)
	Lenient	21.3±16.63	17.5±2.21 a (b)	4.54±0.046 b (b)

Different letters indicate significant differences between means within period, and letters in parentheses indicate significant differences between means within stocking intensities ( $p<0.05$ ). Extended sward height refers to arithmetic mean±SD.

(Obermeyer *et al.*, 2022) hosting different plant species at varied phenological development in different patch types. The CP concentration of the grazed BIs was greater both in spring ( $169\pm 6.1$  vs  $152\pm 6.3$  g (kg DM)<sup>-1</sup>) and in autumn ( $204\pm 8.1$  vs  $136\pm 6.1$  g (kg DM)<sup>-1</sup>) compared to the non-grazed BIs ( $p < 0.01$ ). This indicates that the bites of the cows had a greater CP concentration irrespective of stocking intensity.

## Conclusion

On pastures with greater actual bite item diversity, the cows realised more bite items in their diets. This was not affected by grazing intensity. Diets also had a greater herbage quality in terms of CP concentration than the non-grazed bite items.

## Acknowledgements

We gratefully acknowledge all the members of the EU H2020 SUPER-G project under the project ID 774124. We thank Barbara Hohlmann for supporting the field experiment with the handling of grazing livestock and for assistance in sample processing. Thanks to Knut Salzmann for supervising the livestock management.

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# Climate impact and energy return of reed canary grass or tall fescue on marginal land for biogas

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## Abstract

Emerging fuels, such as biogas, can be produced from grasses. However, in order not to compete with food production, use of marginal land is preferred. This study compares three systems for biogas production on poorly drained fields in northern Sweden. In the first scenario, reed canary grass (RCG) (*Phalaris arundinacea* L.) first harvest is used for biogas production while its second, delayed, spring harvest is used for briquette production. In the second and third scenarios, RCG and tall fescue (TF) (*Lolium arundinacea* Schreb.) respectively were harvested twice per year for biogas production. Energy return on investment (EROI) was highest for RCG to biogas and briquettes, 8.5, while RCG to biogas gave 7.8 and TF to biogas 8.3. The higher EROI for TF to biogas compared to RCG to biogas is caused by a higher specific methane production per kg organic matter. Net climate impact was lowest for RCG to biogas, 6.9 g CO<sub>2</sub> eq. MJ<sup>-1</sup> energy output, and highest for TF to biogas, 7.6 g CO<sub>2</sub> eq. MJ<sup>-1</sup>. This was largely due to longer duration of the RCG swards (10 years) than TF swards (3 years). All systems had quite similar climate impact and EROI so farmers can choose the system most suitable for their conditions.

**Keywords:** life cycle analysis, biogas, briquettes, reed canary grass, tall fescue

## Introduction

Both reed canary grass (*Phalaris arundinacea* L. (RCG)) and tall fescue (*Lolium arundinacea* Schreb. formerly *Festuca arundinacea* (TF)) have been proposed for biogas production on marginal land (Meehan *et al.*, 2017). RCG is a wetland plant that benefits from good water availability during the growing season. It is a potentially good crop on wetter marginal soils. In the second harvest, RCG produces an infertile straw with many leaves, while TF leaves form a tussock, without straw. Leaf-dominated biomass is favourable for biogas production, but more straw is favourable for spring harvest for incineration or for the fibre industry. Because of this, briquetting of the second harvest was investigated for RCG only. The aim of this study was to compare different scenarios using RCG or TF for biogas and briquette production using life cycle analysis (LCA) methodology.

## Materials and methods

Calculations of Energy return on investment (EROI) and LCA of climate impact were made for three scenarios. The first harvest in all scenarios was taken early, before the heading of RCG, and the biomass was round-baled and wrapped in plastic and used for biogas production (Figure 1; 10A–12A). Scenario 1: The second harvest was delayed until spring. The biomass was cut in late autumn, left in the field during winter, then round-baled and wrapped in plastic the following spring and used for briquette production (Figure 1; 10B–12B). Scenario 2: RCG harvested twice per year for biogas production and then treated as in the first harvest. Scenario 3: This was the same as scenario 2, but with TF.

No harvest is taken in the establishment year for any of the grasses, and the establishment energy costs are divided over ten harvest years for RCG and three harvest years for TF, according to present recommendations. The LCA was made according to ISO 14040 (ISO, 2006) and focused on the activities in Figure 1. The input data were collected partly from entrepreneurs. Yield data and data on chemical

## Establishment Two harvests per year Biogas plant Briquette plant

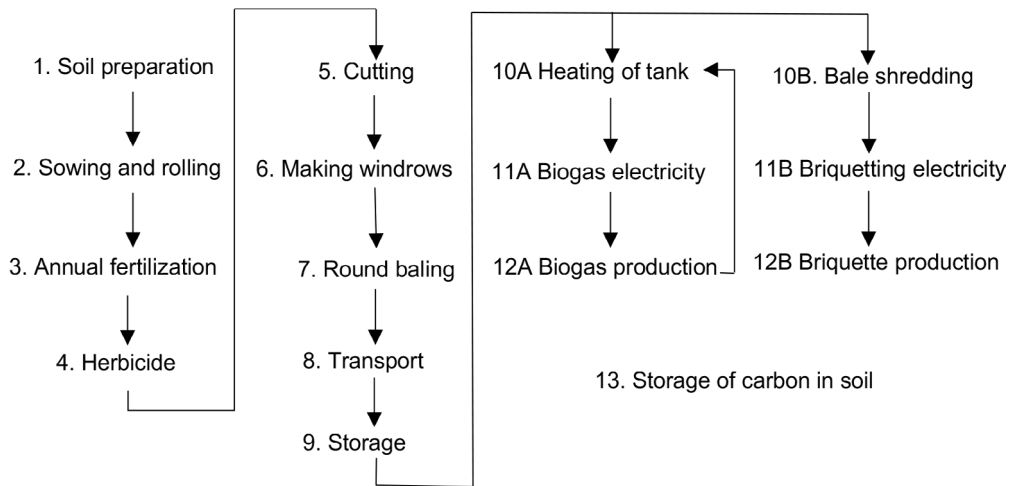


Figure 1. Flowchart of the EROI and LCA calculations.

composition for RCG and TF were collected from a field experiment with early first harvest and autumn or spring harvest in Umeå, Northern Sweden (Palmberg and Finell, 2022). Other data sources were literature data, official data, and Ecoinvent v3.8 (2021) accessed through SimaPro software. Only direct energy use was calculated, not production of machines and buildings. Digestate fertilizer from a dairy factory was used ( $25 \text{ Mg ha}^{-1}$  before establishment and  $15 \text{ Mg ha}^{-1}$  after the first harvests). The biomass was preserved by round baling and then wrapped in plastic. Soil carbon storage data were taken from Nilsson *et al.* (2020) and  $\text{N}_2\text{O}$  emissions were calculated as 1% of the N in fertilizer.

## Results and discussion

Calculated EROI was quite similar for the three scenarios. EROI was highest for RCG to biogas and briquettes, 8.5, while RCG to biogas gave 7.8 and TF to biogas 8.3. The briquette production of spring harvested material is energy efficient since the material is dried efficiently in the field, leading to low transport costs. In addition, all material is used for the briquettes, while biogas production also provides a residue, the digestate. This is a valuable fertilization resource, but for simplicity, we did not include it in the system analysis. The higher EROI for TF to biogas compared to RCG to only biogas is caused by a higher specific methane production per kg organic matter for TF.

The climate impact of the three scenarios is shown in Figure 2. The longer duration of the RGG swards leads to low establishment costs per energy output. However, RCG has a lower biogas production per kg organic matter than TF (Zhang *et al.*, 2021). This is partly compensated for by higher yield where RCG is thriving. However, higher yield also leads to higher transport costs, especially in the second harvests to biogas because the biomass dries poorly in late autumn, leading to more water transport. Soil carbon storage contributes to the low climate impact of the production for both grasses.

In our field experiment, RCG yields had a tendency to increase over the years, while TF yields decreased, probably because harvest timing was adapted to RCG. The second harvest was made late in the autumn or in spring to allow RCG to allocate nutrients and carbohydrates to the rhizomes. TF, on the other hand, should not be harvested late in autumn since it could weaken the sward. Thus, we might underestimate the production potential of TF to biogas.

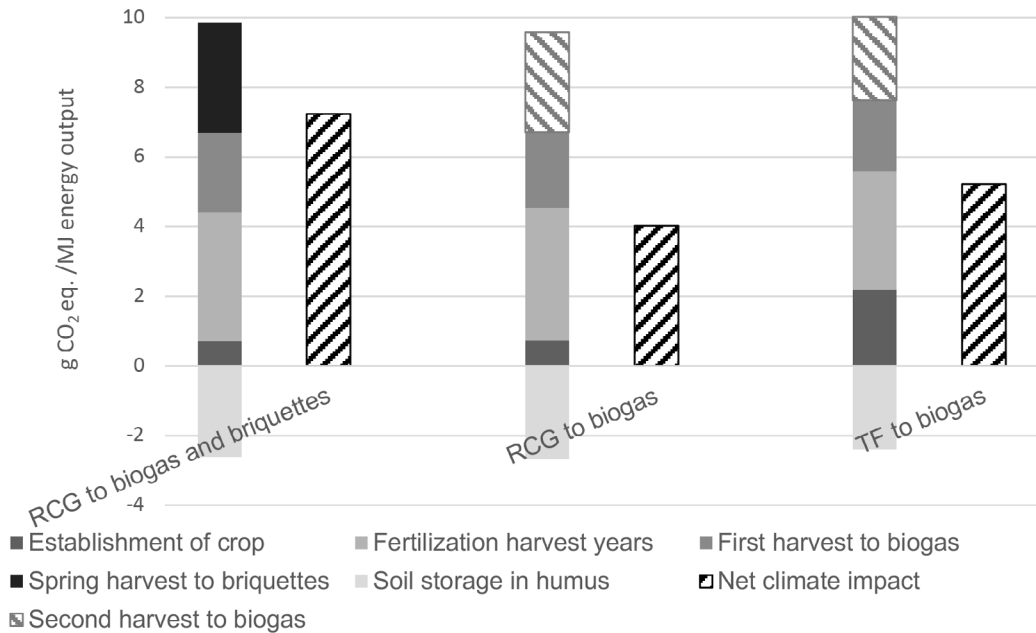


Figure 2. Climate impact of the establishment, fertilization, different harvests and soil carbon sequestration of the different scenarios. The net effect of all factors is shown by the right bar in each group. The second harvest was a delayed harvest in spring for briquettes (Scenario 1) or made in October for biogas (Scenarios 2 and 3).

## Conclusions

All systems had quite similar climate impact and EROI, so farmers can choose the system most suitable for their conditions.

## Acknowledgement

This study was financed by the Swedish Energy Agency, grant number 45912-2.

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# Where do we find permanent grasslands? Approaches and shortcomings of existing European-scale maps

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## Abstract

Knowing the area and distribution of permanent grassland (PG) is crucial for agricultural and environmental policy-making, quantifying ecosystem service delivery, or modelling effects of global change. The Copernicus High Resolution Layer Grassland (HRL) and the Ecosystem Types of Europe map (ETE) are the two most detailed European-scale maps of permanent grasslands available today. We compared the regional PG area predicted by the two mapping approaches and found considerable differences between the two, with direction and extent of these differences varying greatly between European regions. We related the ratio between the PG area predicted by ETE and HRL to the dominance of other land use classes in each region. ETE appeared to be more likely to classify heterogeneous agricultural areas as well as sparsely vegetated areas as PG than HRL, while the opposite was true for wetlands and urban green areas. However, these relationships explained only a small proportion of the differences between ETE and HRL. PG areas mapped by either approach were found to be mostly larger, but in some regions smaller, than PG area reported in agricultural statistics. Applications of these datasets need to take these limitations into account.

**Keywords:** permanent grassland, Europe, mapping, land use, remote sensing, inventories

## Introduction

Knowing the area and distribution of different types of permanent grassland (PG) is crucial for agricultural and environmental policy-making, quantifying ecosystem service delivery, or modelling the effects of global change. The Copernicus High Resolution Layer Grassland (HRL) and the Ecosystem Types of Europe map (ETE) are the two most detailed European-scale maps of PG available today. The ETE maps habitats following the European Nature Information System (EUNIS) classification, and distinguishes seven PG habitat classes. It has a 100-m resolution and is based on the Corine Land Cover 2012 map (CLC), with crosswalks informed by auxiliary datasets. The Copernicus High Resolution Layer Grassland 2018 maps PG at a 10-m resolution, based on time series data of the Sentinel-1 and Sentinel-2 satellites.

The definition of PG within both ETE and HRL includes agriculturally managed and unmanaged PG, but excludes areas with extended periods of inundation ('wetlands'), with >10% woody plant cover ('heathland') or vegetation cover <30% ('sparse vegetation'). These habitats, however, often occur in mosaics with grasslands, and may be difficult to distinguish from these. The same is true for complex mosaics of agricultural land use that include PG together with arable land and other land uses. In the CLC, which uses a minimum mapping unit of 25 ha, the latter are captured in three classes of 'heterogeneous agricultural areas'. As the ETE is based on the CLC, we expected its PG mapping to be less accurate in these areas than that of the HRL. Lastly, urban grasslands are included as PG in the HRL, but not in the ETE, all leading to potential differences between the two resulting PG maps.

Our aim was to assess the differences between ETE and HRL mapping of PG. We firstly tested if these are related to the importance of 'heathland', 'wetlands', 'sparsely vegetated areas', 'urban green areas' and



'heterogeneous agricultural areas' in the mapped area. Secondly, we mapped the spatial distribution of the observed differences, with statistical data on agriculturally used PG as a third point of comparison.

## Materials and methods

For our analyses, we used 100-m resolution raster of ETE (EEA, 2019), HRL (EEA, 2020b) and CLC 2012 (EEA, 2020a) over 33 European countries (Fig. 1a). While a pixel-by-pixel comparison would permit more detailed assessments, it risks compounding errors due to positional accuracy issues. Accordingly, we aggregated the raster data to quantify the PG area over statistical regions ('ETE-PG' and 'HRL-PG') and calculated their quotient ('ETE/HRL ratio'). We tested the relationship between that ratio and the relative area of the following CLC land cover classes: 'urban green areas' (CLC codes 112, 124, 141, 142), 'heathland' (322, 323, 324), 'wetlands' (411, 412, 421), 'sparse vegetation' (333) and 'heterogeneous agricultural areas' (241, 242, 243). We obtained the relative area of each class by dividing its area by the mean value of ETE-PG and HRL-PG per NUTS-3 region. We then fitted a linear model predicting the ETE/HRL ratio as a response to the relative areas of these five classes. The variance inflation factor of the five variables was 1.03–1.89, indicating only limited multicollinearity. We selected the minimum adequate model based on the model AICc and tested the significance of each variable when it was fitted after all other explanatory variables. In a second step, we used data aggregated at the level of NUTS-2 regions to map the ETE/HRL ratio. In addition, we related ETE-PG and HRL-PG to the PG area data from the Eurostat farm structure dataset of 2016 (Eurostat-PG; Eurostat, 2023).

## Results and discussion

The most parsimonious model explaining the ETE/HRL ratio at NUTS-3 level included all explanatory variables except 'heathland/scrub'. 'Heterogeneous agricultural areas' (regression coefficient  $\beta=0.063$ ,  $p=0.02$ ) and 'sparse vegetation' ( $\beta=0.143$ ,  $p=0.02$ ) increased the ETE/HRL ratio, while 'wetlands' ( $\beta=-0.258$ ,  $p<0.001$ ) and 'urban green areas' decreased it ( $\beta=-0.022$ ,  $p<0.001$ ). However, with an adjusted  $R^2$  of 0.036, the model only explained a small proportion of the variance of ETE/HRL ratios at NUTS-3 level.

At NUTS-2 level, the ETE/HRL ratio ranged from 0 to 5.24 (mean=1.14, SD=0.55,  $n=285$ ). Averaged over all NUTS-2 regions, HRL thus underestimated PG area compared to ETE, albeit with substantial geographical variation (Fig. 1a). In most NUTS-2 regions, the PG area reported in Eurostat was smaller than the area of either ETE-PG or HRL-PG (Fig. 1b, 1c). This can be explained by the scope of the Eurostat dataset, which only encompasses PG on farms above a certain size threshold. However, in 38 and 47 out of 278 NUTS-2 regions, the PG area reported in Eurostat was larger than that mapped by ETE and HRL, respectively. Some of this discrepancy may be due to certain countries using wider definitions of PG in their agricultural statistics than the two mapping approaches, e.g. by including areas with >10% shrub or tree cover, such as in the dehesa and montado areas of Spain and Portugal, or inundated areas such as peat bogs, as in the upland areas of the British Isles. By contrast, ETE and HRL definitions of PG are very similar, except for the inclusion or exclusion of urban green areas. Nevertheless, the two mapping approaches appear to treat wetlands, sparsely vegetated areas and heterogeneous agricultural areas differently, even after effects of urban green areas are accounted for. Heterogeneous agricultural and sparsely vegetated areas appear to be more likely to be classified as PG by ETE than HRL, while the opposite is true for wetlands. The low values of the regression coefficients and the adjusted  $R^2$  indicate, however, that this is only a minor contributing factor to the observed differences between ETE and HRL.

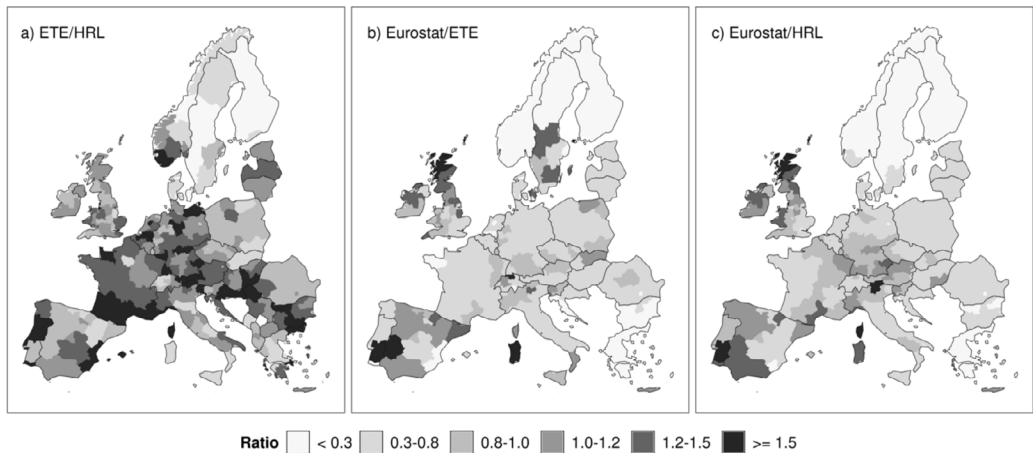


Figure 1. Pairwise ratios between the permanent grassland area per NUTS-2 region, according to the Ecosystem Types of Europe Map (ETE), the Copernicus High-resolution Grassland Layer (HRL) and the Eurostat agricultural crop area statistics (Eurostat).

As ETE and HRL have different reference periods (2012 and 2018, respectively), land use changes may have contributed to the differences. However, the ETE/HRL ratio was not correlated with the relative change of PG area between 2010 and 2016 for the 262 out of 285 NUTS-2 regions where the relevant Eurostat data were available ( $r=0.049$ ,  $p=0.43$ ).

## Conclusion

The two most detailed pan-European maps of PG differ considerably in the regional PG areas they predict. The direction and extent of these differences varies between regions and could only partly be related to the presence of land cover classes that might affect prediction accuracy of the two mapping approaches. Ongoing progress in remote sensing technology and application is likely to lead to increasing accuracy and consistency in ongoing EU mapping programmes. In the meantime, applications based on the spatial distribution of PG across Europe need to take into account the limitations of the currently available datasets.

## Acknowledgement

This work was funded by the European Union Horizon 2020 Research and Innovation programme (Grant Agreement 774124, 'Developing Sustainable PERmanent Grassland systems and policies, SUPER-G').

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# Grassland and grazing: scrutiny of benefits and side-effects

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## Abstract

Rearing livestock provides food and other products and is deeply integrated into environmental and social systems. The evaluation of livestock systems in terms of goods and services provided, as well as trade-offs and co-benefits, is complex and shapes the public discourse between science, producers, and consumers. Gaps in knowledge or selective presentation of facts may lead to opposing interpretations and case-specific relationships are sometimes generalized, ignoring their limited applicability. Being convinced that scientific studies provide information for informed policy decisions, we see the main shortcoming in a literature-based knowledge transfer. Here, we illustrate this complex exemplarily for the aspect of soil carbon accumulation associated with livestock rearing, using pasture-based systems. Subsequently, we propose a conceptual approach for a transparent and comprehensive evaluation of livestock production systems and their social and environmental impacts as a basis for public debate. We collect the information needed for a fact check that combines different aspects and accounts for case-specific conditions. We invite the scientific community to help making this approach as comprehensive as possible.

**Keywords:** public debate, conceptual approach, impacts, livestock

## Introduction

The assessment of agricultural practices and their environmental and social impacts is an important field of research using LCAs (van Zanten *et al.*, 2018), modelling studies (Weindl *et al.*, 2017) and field experiments (De Los Rios *et al.*, 2022) which are integrated in frameworks such as the “Food Systems Approach” (FSA, proposed by Wageningen University and Research and OECD). Studies focus on specific aspects and gain insights into management effects, e.g. by fertilization or grazing intensities under experimental conditions. From these, single aspects may be picked up by lobby organisations and enter the public debate and subsequently policy relevant decision processes. Sometimes, the role of livestock is discussed based on different positions rather than on evidence, culminating in mutually exclusive perspectives such as “Cows are climate saviours versus cows are main greenhouse gas emitters”. Here, arguments and emotions are exchanged not necessarily founded on science or considering specific contexts. Despite overarching frameworks like FSA that include the relevant aspects such as agricultural production, rural livelihoods, biodiversity, nutrient pollution, climate change, air pollution, or the carbon budget, details are often omitted to defend simple truths. The science-public-policy interface is therefore in need of targeted information to facilitate facts-based debates.

Evidence suggests that improved management in ruminant grazing systems can enhance carbon sequestration (Byrnes *et al.*, 2018; Conant *et al.*, 2003) but on a global scale this effect is compensated by the methane and N<sub>2</sub>O emissions of current livestock (Wang *et al.* 2023). Portraying cows as “climate saviours” might be overly optimistic (Idel, 2019). Soil carbon accumulation in pastures and grasslands is not only complex, but intimately linked to other nature’s contribution to people. Carbon sequestration

can contribute to climate change mitigation; however, an evaluation that informs public debate has to include a wider context and relevant GHG emissions from enteric fermentation, N<sub>2</sub>O emissions, and considering alternative land uses (Wang *et al.*, 2023). Similar complex trade-offs and co-benefits exist for other aspects, such as biodiversity protection or reduction of nitrogen pollution.

## Materials and methods

We focus on one prominent example with respect to societal perceptions of pasture-based livestock production: “Cows contribute to soil carbon accumulation”. For the complex carbon dynamics in livestock production systems, we propose a literature-based structured approach for identifying the relevant elements for evaluating the above statement: Step 1 is a reduction into key components that determine the net effect and help to define the system as simple as possible; Step 2 identifies all processes and interactions of management and natural conditions that influence the key components, in Step 3, a fact-check is conducted of directions and robustness of the driving processes. By structuring the information in this way, the complexity becomes transparent, while non-ambiguous cases can be separated from ambiguous ones, where case-specific information is needed to determine the overall net effect.

## Results and discussion

The statement “Cows contribute to soil carbon accumulation” requires exploration of how soil carbon stocks can be increased in pasture-based production systems, identifying the processes that can contribute as well as the boundary conditions.

Step 1: From a balance perspective, two key ways which can achieve a net increase in soil carbon are either increase the carbon inputs and/or reduce losses.

Step 2: Factors that are connected to livestock and influence these key components.

Carbon inputs into the soil can be increased by: 1. increased net primary production (NPP), which again can be achieved through: 1a. slight biomass removal that reduces maintenance but does not impede light interception, 1b. reduced abiotic stresses/limitations, such as water, nutrients, root-zone oxygen, acidity and salinity, 1c. reduced biotic stresses, such as pests, or reduced physical damage from trampling or fires; 2. reduced removal of net primary production from the system.

Carbon losses can be reduced by enhancing turnover times in the soil through: 1. enhancing mineral-organic compounds, 2. changed degradability of organic material (e.g. higher lignin content), 3. reduced oxygen supply.

Considering the range of drivers and key components, emphasizing the net effects is crucial. Oxygen stress in soils can reduce NPP (C inputs) but simultaneously slow down the decomposition of soil organic material. Other processes depend on their intensity and the environmental conditions; thus, require case-specific evaluation. Regular removal of grass biomass can stimulate plant growth and NPP, if it does not overly impede light interception through too strongly reduced leaf biomass. How much removal is too much depends on the overall productivity of the site and other co-limitations, such as nutrient deficiencies or drought. Thus, all processes and drivers in livestock systems have to be assessed whether they amplify (plus) or dampen (minus) or both. Here, we give three examples.

1. Grazing animals remove grass biomass and return it to the ground as manure. A portion of the carbon is kept within the animal or emitted via respiration (ca. 75%; Soussana *et al.*, 2014), so that the net carbon balance of grazing is always negative.

2. NPP may indeed increase by grazing, when the leaf removal is reducing maintenance respiration more than photosynthesis. The conditions under which a positive net effect can be realized require low animal densities within rotational grazing regimes or ley systems.
3. Turnover times of soil carbon depend on the temperature and moisture in the soil, the microbial soil community, the land-use history, and management practices such as tillage, fertilization or irrigation. Processes that are connected to the presence of livestock include nitrogen-related transformations within the soil because of a change in the carbon to nitrogen ratio of the organic input into the soil. The transformation of organic to mineral nitrogen components would shift and alters the nitrogen availability for plant growth and NPP.

These processes and interactions are to be depicted as a conceptual model which allows to identify positive or negative feedback pathways. The complexity of interactions may result in non-strictly monotonic outcomes (e.g. livestock density on NPP) and may show context-dependent positive or negative effects. Thus, pathways include relations with ambiguous leading signs, and have to be evaluated separately for different value intervals.

In a third step, a thorough review will underpin the conceptual model with values and uncertainty estimates. Scientific studies, e.g. comparing different grazing systems (Byrnes *et al.*, 2018) provide data for the influence of grazing on the target mechanisms and may inform on conditions for generalizing relationships. Reports and data from national statistics (e.g. from the German Federal Ministry of Food and Agriculture, BMEL) and databases with farm-specific information (e.g. Farm Accountancy Data Network (FADN), [https://agriculture.ec.europa.eu/data-and-analysis/farm-structures-and-economics/fadn\\_en](https://agriculture.ec.europa.eu/data-and-analysis/farm-structures-and-economics/fadn_en)) could improve the knowledge on a lot of interactions.

## Conclusion

We have shown that we can derive fact-checks by decreasing the complexity with this simple approach to summarize available scientific literature in a way that can be used in public debate and is understandable by the general public. The example of the connection of livestock to soil carbon is used as proof-of-concept and has to be discussed, broadened and tested in scientific as well as public debate.

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# Provision of biodiversity and ecosystem services from permanent grassland types

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## Abstract

Permanent grasslands (PG) provide a range of important ecosystem services (ES), including supporting biodiversity, regulating climate, mitigating risks of erosion and flooding, and providing clean water, animal feed, and recreational and aesthetic values. The provision of ES varies considerably between PG types. Here, we used an expert elicitation among 25 grassland academics across Europe to determine the effect of PG type on ES delivery. We distinguished between 18 PG types based on the presence of management, presence of succession, presence of woody plants, type of woody plants, renewal frequency, management intensity, presence of climatic limitations and defoliation type. ES delivery was scored for 19 ES indicators. The outcomes of the expert elicitation allowed us to identify five comparable groups of PG types with similar patterns of ES delivery, mainly along an intensity gradient. We conclude that the PG types in the PG Atlas are able to discriminate between different patterns of ES delivery which is an import prerequisite for communication to farmers, citizens, consumers, policy makers and scientists.

**Keywords:** ecosystem services, expert elicitation, grassland typology, management intensity

## Introduction

Permanent grasslands (PG) occupy around 34% of the utilized agricultural area in the European Union. They are important for supporting biodiversity and providing a range of ecosystem services (ES) (Schils *et al.*, 2022). The level of provision varies significantly between different PG types across Europe, which can be an obstacle for effective knowledge transfer and policy making. Identifying PG types across Europe that are similar in terms of ES delivery would improve communication between stakeholders and contribute to effective policy making. Previously, we have developed a PG typology consisting of 18 PG types based on management, i.e. defoliation, fertilization and renewal, as well as other factors like climate restrictions or the presence of woody plants (Tonn *et al.*, 2020). It is applicable at field and regional scales and is cross-referenced with existing classification schemes such as the EUNIS and Natura 2000 habitats classes. The typology is the backbone of a PG Atlas (<https://www.super-g.eu/>) which comprises maps, portraits and illustrative cases for each of the 18 PG types. The PG portraits present the explanation of a PG type and include a dedicated section on its ES delivery. Here, we present how we obtained expert opinions on ES delivery from PG types.

## Materials and methods

We carried out a two-step expert elicitation Delph-type process (Crime and Wright, 2006) among 25 grassland academics from 13 countries representing the Atlantic (9), Alpine (3), Boreal (2), Continental (7) and Mediterranean (4) biogeographic regions. The experts were presented with a set of questions to rate the effect of eight relevant factors, which distinguish PG-types from one another, on a specific ES indicator. The eight distinguishing factors were: presence of management, presence of succession, presence of woody plants, type of woody plants, renewal frequency, management intensity, presence of climatic limitations and defoliation type. The questions were answered separately for each of 19 ES indicators for biodiversity (pollinators, threatened species, soil biodiversity, plant diversity), climate regulation (nitrous oxide, methane-soil, methane-enteric, carbon sequestration), water quality (nitrate,

phosphate, pesticide), erosion and flood control (bulk density, runoff, soil loss), recreation and aesthetics (recreation, aesthetics) and animal feed (DM yield, energy content, protein content). We used a five-point scoring scale from very unfavourable to very favourable, which was transformed to a five-point scale from -2 to +2. For each ES indicator, the responses to the eight questions were used to calculate a preliminary score for each PG-type on a scale from 1 to 10. It is important to note here that there might be interactions between the effects of the distinguishing factors that make adjustments of scores necessary. Therefore, the experts were allowed to check the preliminary score and revise it into a final score. The outcomes of all first-round scores were discussed with all participants. In the second round, experts had the opportunity to adjust their first-round scores. Finally, the scores for the ES indicators were weighted and aggregated to scores for each of the six main ES.

## Results and discussion

The average number of responses per ES indicator was 15, but with considerable variation. In general, the indicators on biodiversity and animal feed had higher returns than the indicators on erosion and flood control, climate regulation and water quality. The most scored indicator was plant diversity, with 20 out of a maximum of 25 experts returning scores. At the other end of the scale was methane emission from soil with only 9 returned scores.

For the different ES indicators, there was also a considerable variation in agreement between experts. In general, agreement was relatively high for the indicators on animal feed and water quality, whereas agreement was relatively low for the indicators on climate regulation. The highest agreement was for DM yield and the lowest agreement was for methane emissions from soil and carbon sequestration.

Within a specific ES, the average scores of the individual ES indicators generally showed high correlations. The only exception was climate regulation, where carbon sequestration was less correlated to the other indicators. Therefore, we present the average aggregated scores per ES.

In the outcomes of the expert elicitation we identified five comparable groups of PG types (Figure 1). Within each of the five groups, the ES delivery showed a consistent pattern. The frequently renewed and high-intensity PG scored very high on the provision of animal feed at the cost of other ES (Figure 1a). Within this group, frequent renewal amplifies the contrast between animal feed and other ES, while the defoliation type (cutting vs. grazing) had mixed effects. For the medium- (Figure 1b) and low-intensity PG (Figure 1c), the pattern was more balanced, with relatively lower scores for animal feed, and higher scores for other ES. Within the medium and low intensity groups, the effect of climate limitations was mixed and rather small. Effects of defoliation type were also less pronounced compared to the frequently renewed and high intensity group. The group of woody PG types (Figure 1d) had an almost similar pattern as the low intensity PG, but are presented separately for clarity. Within this group, a higher intensity amplified the contrast between provision of animal feed and other ES. The difference between PG with trees or shrubs was quite limited, except for the higher value for aesthetics and recreation for PG with trees compared to PG with shrubs. The unmanaged PG types (Figure 1e) show the highest contrast, with almost no provision of animal feed and near-maximal scores for erosion and flood control, water quality and climate regulation. The contribution to biodiversity, and aesthetics and recreation, was similar or even lower than the low intensity PG types or woody PG types.

## Conclusions

Using expert elicitation, we found clear contrasts in ES delivery by PG-type, mainly along an intensity gradient. The PG types in the PG Atlas are able to discriminate between different patterns of ES delivery which is an import prerequisite for communication to farmers, citizens, consumers, policy makers and scientists.

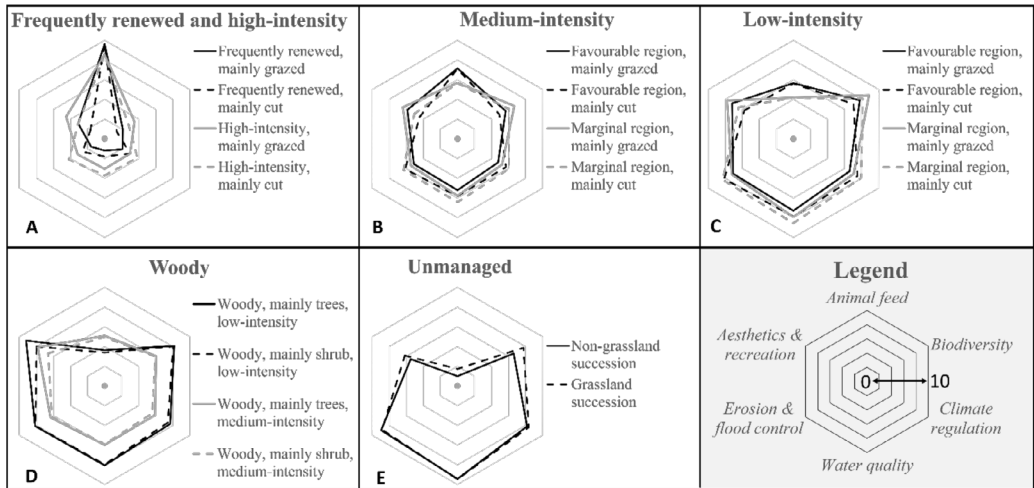


Figure 1. Average scores for the provision of ES for five comparable groups of PG types. The higher the score on a scale of 1 to 10, the higher the contribution to animal feed, biodiversity, climate regulation, water quality, erosion and flood control, or aesthetics and recreation. Within each group, two to four different PG types are presented with varying contrasts in renewal frequency, intensity, defoliation type, woody type and presence of succession.

## Acknowledgement

This work was funded by the European Union Horizon 2020 Research and Innovation programme (Grant Agreement 774124, 'Developing Sustainable PERmanent Grassland systems and policies, SUPER-G').

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# Yield potential and forage quality on free rangeland pastures in Northern Norway

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## Abstract

Only approximately 2% of the land area in Northern Norway is suitable for agricultural purposes. The short growing season and cold climate impose limitations for what can be produced. Agriculture still takes place here, with forage crops for livestock being the most important. On free rangeland areas, including both semi-natural and natural habitats, livestock grazing is common. The biomass production on some of these rangelands is presumed to be high, although little is known about the actual fodder potential. In 2022 a preliminary study was performed to determine abundance and variety of wild pasture plants, dry matter yield (DM) and feed quality in the (presumed) highest yielding vegetation types. Results showed an average of 1520 kg DM ha<sup>-1</sup> in spring and 5380 kg DM ha<sup>-1</sup> in autumn. Early season feed quality was high, but with rapidly decreasing trends. The number of pasture plants was also high. Our results show that in sub-arctic Northern Norway grazing animals can harvest substantial amounts of 'free' fodder of good quality, yet the official statistics show that only 14% of this resource is utilised. Continuous grazing is needed to maintain production and fodder quality in these areas.

**Keywords:** grazing, rangeland pasture, biomass production, ecosystem services, feed quality

## Introduction

The landscape in Arctic Northern Norway is dominated by narrow fiords, steep mountains and large rangeland areas with forest- and mountain pastures. Here livestock feed on free fodder throughout the growing season. Grazing on rangelands is important for the farm economy, since it is associated with low costs for both fertilizers and feed concentrates.

The cold climate limits the growing season, which typically ranges from late May to late August with mean July temperatures barely reaching 13°C in some places. However, the seasonal 24-h daylight above the Arctic circle, combined with good water supply, provides favourable growth conditions for pasture plants in this birch forest dominated Northern Boreal biome.

There is, however, a decreasing trend in the use of rangeland pastures, both because the number of farms and livestock in the region is declining (Norwegian Agriculture Agency, 2023), and also because there is a perception that rangeland grazing can result in a production loss, especially for high-yielding milking cows, compared to feeding close to or in the barn. Grazing is, however, crucial for maintaining openness, species composition, and biomass production in these natural and semi-natural landscapes (Artsdatabanken, 2023), which have been formed by hundreds of years of grazing and extensive human use. As livestock numbers decrease, the landscape becomes exposed to shrub overgrowth, with deterioration of both the species composition and feed quality.

During recent years, vegetation maps that display estimated feed quality in rangeland pastures, have become an important basis for farmers to prioritize areas for grazing (Nibio.no, Kilden, 2023). New technology, such as virtual fences, can also help farmers to apply better management of grazing livestock towards areas with higher biomass production and presumed better feed quality.

There is, however, a knowledge gap regarding the feed quality of fodder harvested by livestock on free rangeland, both in Northern Norway and in Norway generally. Existing quality estimates are based mainly on animal performance studies from the southern part of Norway. Taking the unique growth conditions in the north into consideration, we believe that both biomass production and feed quality might be higher than previously estimated. This study is a first step in quantifying the actual production potential and feed quality of fodder harvested in North-Norwegian rangeland areas.

## Materials and methods

In 2022 an introductory study was performed in the two richest vegetation types identified on feed quality maps, namely Tall Forb Meadow (TFM) and Meadow Birch Forest (MBF). The study area chosen was in the Lofoten Archipelago (68°11'59" N, 13°52'31" E) for TFM and Malangen outside Tromsø city (69°26'15" N, 18°33'13" E) for MBF. In both vegetation types, three localities were chosen, displaying some varieties within the vegetation type. At three different times during the growing season (spring, summer, autumn) the vegetation was cut on previously non-grazed or non-cut plots (0.25 m<sup>2</sup>), i.e. the plant growth was older at each cut. Several plots were cut in each locality if this was necessary to obtain enough material for further analysis. The plants on each plot were cut to a stubble height of 5 cm, regardless of whether the plants were considered edible or not. Before cutting, a thorough species determination was performed in all plots at all cutting points, and percentage ground cover visually estimated (Figure 1). At each cutting time the harvested plant material was dried at 60°C for 48 h, weighed and then analysed for its nutritional value by chemical analysis at the laboratory Oforlab in Norway (Table 1).

## Results and discussion

The botanical analysis showed large abundance of well-known forage plants in the material. The dominant grass species in surface cover were *Deschampsia cespitosa* and *Dactylis glomerata* (in TFM), whereas *Geranium sylvaticum* and *Alchemilla* sp. dominated among the herbs. All these species are known to be preferred by grazing animals; however, *D. cespitosa* is preferred mostly in its early phenological stages whereas it is rejected in later stages.

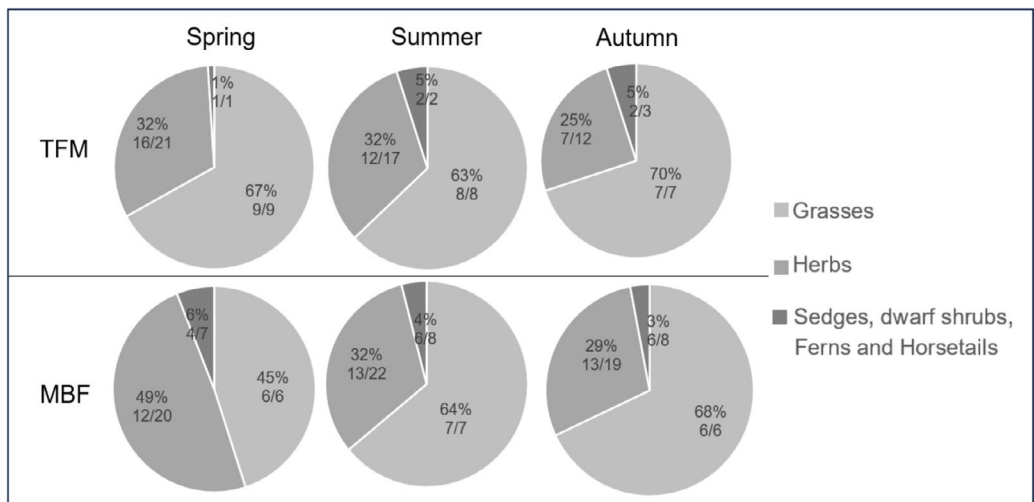


Figure 1. Mean botanical composition (%) in plant material investigated for the two vegetation types at each cutting time. AA/BB denotes the number of well-known edible forage plants (AA), according to knowledge and literature to total number of species (BB) in each functional group, identified in the plots.

Results show that already in early spring, at approximately 10 days after estimated start of growth, the measured yield in both TFM and MBF was over 1000 kg DM ha<sup>-1</sup>, with good nutritional levels. At the summer cut the yield was ca. 3600 kg DM ha<sup>-1</sup> in TFM and had risen with ca. 400 kg in the forested areas of MBF. In both vegetation types both energy and protein level were markedly reduced from spring to summer. The summer cut was performed a few days after an extraordinary heat period with temperatures rising to above 30 °C, which probably accelerated the plant development. At the last cut in autumn, the yield was over 5000 kg DM ha<sup>-1</sup> for the open areas of TFM, and ca. 1900 kg DM ha<sup>-1</sup> for MBF. However, the nutritional values were low.

Table 1. Yield (kg DM ha<sup>-1</sup>) and parameters for feed quality chemically analysed in spring, summer and autumn, in non-grazed/non-cut Tall Forb Meadow and Meadow Birch Forest in Northern Norway.

	Tall Forb Meadow (TFM)			Meadow Birch Forest (MBF)		
	Spring	Summer	Autumn	Spring	Summer	Autumn
Appr. days after growth start	10	35	95	10	40	95
Yield (kg DM ha <sup>-1</sup> )	1523	3640	5377	1077	1493	1883
Dry matter DM (%)	23.1	22.6	23.8	18.9	21.3	31.7
Feed unit milk (FUM (kg DM) <sup>-1</sup> )	0.85	0.73	0.65	0.83	0.67	0.68
Crude protein CP (g (kg DM) <sup>-1</sup> )	153	117	97.3	158	99.3	84.0
WS protein (g (kg CP) <sup>-1</sup> )	393	280	263	297	267	257
NDF (g (kg DM) <sup>-1</sup> )	389	493	557	344	490	525
iNDF (g (kg DM) <sup>-1</sup> )	50.1	104	189	55.5	122	141
Total sugar (g (kg DM) <sup>-1</sup> )	223	132	99.0	202	149	151

1.0 FUM=6900 kJ net energy; NDF, neutral detergent fibre; iNDF, indigestible neutral detergent fibre.

## Conclusion

This preliminary study shows that in sub-arctic northern Norway there is a substantial yield potential in rangeland areas. The abundance of forage plants is high and feed quality in spring is also high, after which it decreases rapidly. To maintain a good feed quality throughout the season, continuous grazing of new regrowth is probably needed. The actual feed potential might, however, be underestimated or overestimated, because grazing animals preferentially select the most nutritional parts of the available herbage, and also because of the regrowth of grazed vegetation, neither of which were accounted for in this study.

## Acknowledgement

This preliminary study was funded by the Norwegian governor of Troms and Finnmark county.

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# Can perennial grain crops combine the soil ecosystem services of grassland with starch production on sandy soils?

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## Abstract

Perennial crops, like permanent grassland, provide soil ecosystem services such as carbon storage and water regulation. Novel perennial grain crops, like Kernza (Intermediate wheatgrass; *Thinopyrum intermedium*) combine these services with the production of grain, providing a more sustainable alternative for starch production compared to annual crops such as corn and cereals. A trial was established in 2020–2022 on a sandy soil in The Netherlands. Yields of Kernza were compared to triticale grain and grass-clover. Kernza generally yielded less than triticale grain and grass-clover, and the yields of Kernza decreased over three years. Other studies have shown that Kernza is able to form a deep and extensive root system which is capable of extending the depth of nutrient and water uptake, therefore needing fewer external inputs. However, in the current trial the root zone was only 85 cm deep, and deeper layers consisted of compacted sand that were impenetrable to the roots. We conclude that Kernza did not develop its deep-rooting potential on this shallow sandy soil, which likely resulted in lower-than-expected crop yields from Kernza during our trial.

**Keywords:** Kernza, perennial, annual fodder crops, grain, ecosystem services

## Introduction

In addition to fodder production, grasslands provide valuable soil ecosystem services such as water regulation and carbon storage (Lindborg *et al.*, 2022). Due to disturbance of the soil and the absence of a permanent soil-cover, annual (fodder) crops like maize and other cereals often provide fewer soil ecosystem services (Schils *et al.*, 2022), but have a high production potential that is rich in starch. There has been an advocacy for annual cereal crops to move toward perennial systems in order to combine the production of grain with the benefits of perennial crops, such as grassland (Asbjornsen *et al.*, 2014). Ongoing breeding of Intermediate wheatgrass (*Thinopyrum intermedium*) led to the creation of the perennial grain Kernza. Although grain yields of Kernza are currently lacking behind those of annual grains, the total biomass production is comparable to grassland (Culman *et al.*, 2013; Jungers *et al.*, 2019) and it can be used as fodder. The objective of this trial was to compare Kernza with triticale cereal and grass-clover, for grain and biomass yields, and to study the effect of these crops on soil organic matter (SOM), soil structure and rooting.

## Materials and methods

A three-year trial (2020–2022) was setup as a randomised block design with four replicates comparing Kernza, triticale and grass-clover. The trial was performed on a sandy soil in the south of the Netherlands. Kernza and grass-clover were sown in September 2020, triticale was sown yearly in October. Seeding rates were 42, 120 and 34 kg ha<sup>-1</sup> seeds for Kernza, triticale and grass-clover respectively. Kernza was sown in rows with 30 cm distance. At the start of the trial, all crops received a one-time amount of 30 Mg ha<sup>-1</sup> of compost that provided 139 kg N ha<sup>-1</sup>. Each spring, the crops were fertilized with 50 kg N ha<sup>-1</sup> and other elements from an organic plant feed granulate. Ample K<sub>2</sub>O was applied to all plots. In the third year, all plots received an extra 50 kg N ha<sup>-1</sup> of the organic plant feed granulate. All crops were cut, weighed and analysed to determine dry matter yields. Grass-clover plots were harvested 2–4 times per year, depending on growth. Kernza and triticale were harvested once per year and were threshed to separate grain and straw. Soil organic matter was analysed in soil samples from the 0–10 and 0–30 cm

soil layer. The maximum rooting depth was determined from a soil profile pit. At the depths of 0–25 and 25–50 cm, the root intensity was visually scored on a scale of 1–10 and the soil structure was visually assessed and categorized as percentages of crumb, sub-angular, and angular particles. ANOVA statistical analyses were performed using SPSS Statistics.

## Results and discussion

Good stands of Kernza and grass-clover were established. However, the yields of all three crop types varied largely over the three-year trial period. Management of the crops was extensive, with small inputs of fertilizer and no irrigation. Therefore, seasonal and weather influences were an important factor, resulting in a large variation in yields. Grain yields of Kernza decreased over the three-year period, with the highest production occurring in the first year (Table 1). It is generally expected that the highest grain yields occur in the first two years (Culman *et al.*, 2013) but then decrease. Kernza grain yields were also low compared to triticale, as was expected. In the third year both Kernza and triticale failed to produce any notable amount of grain as both suffered losses, likely due to excessive rain and competition from weeds in spring, followed by a drought in summer. The good stand of Kernza in the first and second year had almost disappeared at the end of the third year. The total biomass yield of Kernza was comparable to grass-clover in the second and third year, but lower in the first year, likely because Kernza invests a lot of growth in its rooting system in the first year.

There were no significant differences in the amount of SOM between crops. Although SOM in the 0–10 cm soil layer appeared slightly lower for the triticale crop, as would be expected with the yearly tillage. Due to the slow process of building up or losing SOM, it is likely that the trial would need to be extended over

Table 1. Above- and below-ground parameters of grass-clover, Kernza and triticale.

Parameters	Unit	Grass-clover	Kernza	Triticale	<i>P</i> crop	<i>P</i> year	<i>P</i> C*Y
Above ground							
Mean total biomass yield year <sup>-1</sup>	Mg ha <sup>-1</sup>	6.3	4.1	4.9	<0.001	0.117	<0.001
2021	Mg ha <sup>-1</sup>	9.8 <sup>a</sup>	3.4 <sup>b</sup>	3.7 <sup>b</sup>			
2022	Mg ha <sup>-1</sup>	3.4 <sup>b</sup>	4.5 <sup>b</sup>	7.4 <sup>a</sup>			
2023	Mg ha <sup>-1</sup>	5.5 <sup>a</sup>	4.5 <sup>ab</sup>	3.6 <sup>b</sup>			
Mean grain yield year <sup>-1</sup>	Mg ha <sup>-1</sup>	N.A.	0.1	1.5	<0.001	<0.001	<0.001
2021	Mg ha <sup>-1</sup>	N.A.	0.3 <sup>a</sup>	1.2 <sup>b</sup>			
2022	Mg ha <sup>-1</sup>	N.A.	0.1 <sup>a</sup>	3.3 <sup>b</sup>			
2023	Mg ha <sup>-1</sup>	N.A.	0.007	0.001			
Below ground							
SOM 0–10 cm	%	4.1	4.0	3.8	0.510		
SOM 0–30 cm	%	3.6	3.6	3.5	0.765		
Crumbs 0–25 cm	%	55	70	75	0.325		
Sub-angular 0–25 cm	%	34	26	21	0.509		
Angular 0–25 cm	%	11	4	4	0.085		
Crumbs 25–50 cm	%	14	31	21	0.078		
Sub-angular 25–50 cm	%	42	51	44	0.807		
Angular 25–50 cm	%	44	18	35	0.189		
Root score 0–25 cm	1–10 scale	5.9	6.0	5.4	0.583		
Root score 25–50 cm	1–10 scale	4.3	3.9	3.9	0.274		
Max. root dept	cm	82.8	78.3	85.5	0.860		

Crop yields with different superscript are significantly different ( $P < 0.05$ ).

more years for larger amounts of SOM to build-up in the Kernza and grass-clover treatments. Sprunger *et al.* (2018) suggest that it takes more than four years to accumulate a 15% difference in SOM between intermittent wheatgrass and an annual wheat crop.

There were no significant differences in soil structure between the crops. There was a trend ( $P=0.078$ ) that soil in the Kernza treatment had more crumb structure at 25–50 cm depth.

No differences in root biomass scores or root depth were found between crops. Kernza has been reported to be able to reach rooting depths of up to 3 metres (DeHaan and Ismail, 2017) and a larger root biomass in the topsoil, compared to wheat (Sprunger *et al.*, 2018). However, the fertile soil layer in the current trial reached only 85 cm of depth, with a compacted layer beneath. This prevented all crops from developing a deeper root system. The inability to form deeper roots may also have stunted the growth of the above-ground biomass and affected production, as deep-root development is an important trait of Kernza.

## Conclusion

Kernza can establish and produce grain on a sandy soil in The Netherlands. However, in this trial the crop could not reach its full potential, likely due to the relatively shallow sandy soil which did not allow for deep rooting. Both the grain and biomass yields were lower than expected and the crop did not persist into a fourth year. Parameters for SOM, soil structure and rooting were not significantly improved for Kernza, compared to triticale. This is likely due to the relatively short duration of the trial. However, it is possible that Kernza could improve soil quality when grown for a longer period of time. In future experiments Kernza should also be investigated on sandy and clayey soils with deeper soil profiles.

## Acknowledgement

This project was part of the Public-Private Partnership KLIMAP.

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# Changes of sward characteristics in cut meadow after introduction of intensive sheep grazing

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## Abstract

The type of management and its intensity is the main driver of sward structure and plant community characteristics in temperate grasslands. The aim of the study was to find changes in plant community characteristics after introducing intensive sheep grazing on previously cut meadow. Therefore, a grazing experiment was established in 2019 on a meadow, previously cut once or twice per year. The meadow was intensively grazed by sheep through the whole year except during periods with snow cover, and sward height was maintained at 2–3 cm. The percentage cover of all vascular plant species was visually estimated in each plot in the years 2019–2023. Mean vegetation cover increased during the first three years of the experiment until it reached 100%. Mean cover of *Lolium perenne* and the prostrate legume *Trifolium repens* increased, whereas mean cover of forbs *Galium album*, *Hypericum maculatum* and *Veronica chamaedrys* decreased. The changes in plant species composition were found already in the second year of the study; however, species richness was not affected. After five years of intensive sheep grazing there was a rapid increase in cover of some grazing-tolerant species; however, typical pasture sward with dominance of species adapted to frequent defoliation has not yet been created.

**Keywords:** intensity, management, plant species composition, species richness

## Introduction

The type of management is one of the key drivers affecting grassland communities. Hay-making and grazing, are two basic defoliation options which can also be used in combination (Van Diggelen and Marrs, 2003). On meadows during hay-making the above-ground biomass is non-selectively cut and removed at the same time, while factors affecting vegetation under grazing management on pastures include stocking rate, selective grazing, trampling and nutrient enrichment (WallisDeVries, 1998). This results in different plant communities with different plant species composition on meadows (*Arrhenaterion*) and pastures (*Cynosurion*) (Chytrý *et al.*, 2010). However, little is known about how long it can take for changes from meadow to pasture, and *vice versa*, to occur. Therefore, this study addressed the related research question: What are the changes in plant community characteristics after introducing intensive sheep grazing on a previously cut meadow?

## Materials and methods

The experiment was established in 2019 on a previously long-term meadow cut once or twice per year, in Oldřichov v Hájích, Czechia (50°51'6" N, 15°5'18" E; 425 m a.s.l.). The area of the experiment has a 30-year mean annual rainfall of approximately 805 mm and a mean annual temperature of 7.2°C. The bedrock is granite and the soil is cambisol. The experimental site was a meadow which was cut once or twice per year for at least 20 years until 2018. The experiment is arranged in three randomized blocks with four replications (12 plots, each plot is 1 m<sup>2</sup>). Intensive grazing with sheep (Suffolk breed) was introduced on this traditionally managed meadow in May 2019. Continuous grazing was applied for the whole year with the exception of periods with snow cover, and the sward height was maintained at about 2–3 cm in the years 2019–2023. The percentage cover of all vascular plant species was visually estimated in each plot in the years 2019–2023. Nomenclature of vascular plant species follows the regional flora

(Kaplan *et al.*, 2019). ANOVA was used to analyse univariate data and redundancy analysis (RDA) in the CANOCO 5.0 program (RDA; ter Braak and Šmilauer, 2012).

## Results and discussion

Mean total (%) vegetation cover of all presented plant species increased during the first three years of the experiment until it reached 100% (Figure 1a). This increase is connected with the higher sward density after intensive grazing, which supports tillering of grasses and increase of stolon growing points of white clover; that is why pastures commonly have denser swards than meadows (Pavlů *et al.*, 2006). Based on RDA analysis there was a significant effect of year on plant species composition, which explained 20.3% of the variability ( $F=3.4$ ,  $P=0.002$ ) on all constrained axes (Figure 2). However, there were no significant changes in species richness in the years 2019–2023 (Figure 1b). After five years of intensive sheep grazing the presence of recorded species remained similar, but their proportions had changed considerably. For example, the mean cover of grazing-tolerant species such as *Lolium perenne* and *Trifolium repens* increased whereas the mean cover of forbs *Galium album*, *Hypericum maculatum* and *Veronica chamaedrys* decreased. Some typical prostrate pasture species such as *Hypochoeris radicata* and *Leontodon autumnalis* started to occur during the five years of the study. Further, there was no observed reduction in the number of forbs even though their total cover decreased over the same time. This means that the majority of forb species were still able to survive under grazing pressure by decreasing their height. However, it is not clear for how long these forb species can be resilient to the long-term selective grazing of sheep, because long-term continuous sheep grazing usually results in a reduction of forbs (Pavlů *et al.*, 2021). Besides *L. perenne* and *T. repens*, other typical plant species belonging to mesophile pastures (Chytrý *et al.*, 2010) have not yet been recorded.

## Conclusion

Although changes in plant species composition were already found in the second year of intensive whole-year grazing, the presence data of recorded species remained similar. After five years of this management there was found to have been a rapid increase in cover of some grazing-tolerant species, which started processes to change the meadow community to a pasture community. However, this process will take more years as the presence of other typical pasture species has not yet been recorded.

## Acknowledgements

The data collection was supported by the Technical University of Liberec and paper writing and finalisation by DiGraSO project No.100693117.

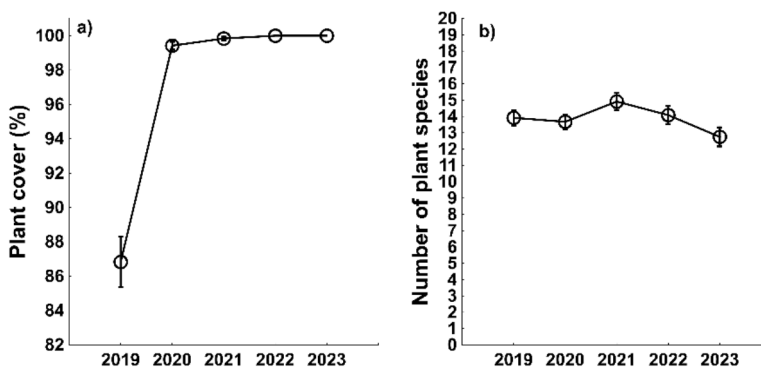


Figure 1. (a) The mean plant cover (%) and (b) the mean number of plant species in the years 2019–2023.



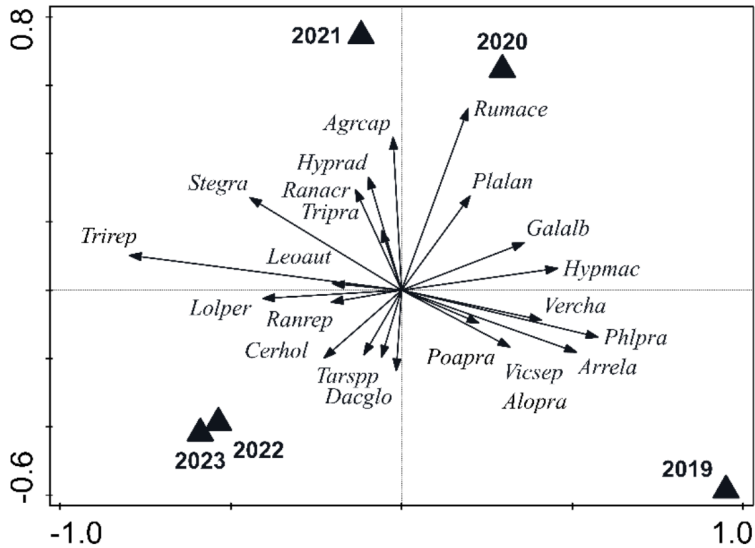


Figure 2. Redundancy analysis (RDA) ordination diagram. Results of RDA of plant species composition data collected in the years 2019–2023. Species abbreviations are based on the first three letters of the genus name and the first three letters of the species name: *Agrcap*=*Agrostis capillaris*, *Alopra*=*Alopecurus pratensis*, *Arrela*=*Arrhenatherum elatius*, *Cerhol*=*Cerastium holosteoides*, *Dacglo*=*Dactylis glomerata*, *Galalb*=*Galium album*, *Hypmac*=*Hypericum maculatum*, *Hyprac*=*Hypochaeris radicata*, *Leoaut*=*Leontodon autumnalis*, *Lolper*=*Lolium perenne*, *Phlpra*=*Phleum pratense*, *Plalan*=*Plantago lanceolata*, *Poapra*=*Poa pratensis*, *Ranacr*=*Ranunculus acris*, *Ranrep*=*Ranunculus repens*, *Rumace*=*Rumex acetosa*, *Stelgra*=*Stelaria graminea*, *Tarspp*=*Taraxacum spp.*, *Trifpra*=*Trifolium pratense*, *Trirep*=*Trifolium repens*, *Vercha*=*Veronica chamaedrys*, *Vicsep*=*Vicia sepium*.

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# Growth potential and yields of chicory and ribwort plantain

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## Abstract

Herbal mixtures are receiving increasing attention among dairy farming in the context of biodiversity or because they may contribute to reducing methane emissions (Wilson *et al.*, 2020). Yield determinations were made in these fields in 2023 to monitor the growth potential (growing stage trial), annual yield and nutrient value (in progress) of chicory (*Cichorium intybus*) and ribwort plantain (*Plantago lanceolata*). Chicory, in particular, had many problems in the second year. The persistency was low, which created many open spaces, resulting in high weed pressure and the plants quickly entered the generative state. On the other hand, the regrowth of chicory is high. Plantain has good persistence and a good yield under full mowing. The yields are certainly comparable to perennial ryegrass (*Lolium perenne*), but with a lower N input. However, chicory is a very sensitive crop. Grazing causes a lot of losses and mechanical treatment is almost impossible. Plantain offers more options, but the question is how resilient this crop is under grazing conditions.

**Keywords:** chicory, ribwort plantain, yield, biodiversity, growing trend

## Introduction

In the context of biodiversity and considering the increasing frequency of dry, warm, as well as very wet periods due to climate change, there is a growing emphasis on incorporating herbs into grassland mixtures. New Zealand has already accumulated significant experience with herbal mixtures in both research and practical applications. Literature indicates favourable outcomes in terms of yield, intake, and milk production (Wilson *et al.*, 2020). However, in the Netherlands, the utilization of mixtures with herbs, along with practical and research experiences, remains quite limited. To gain more knowledge of the use of herbs under Dutch conditions about the growth, nutritional value and effect on milk production, a study was initiated in 2022 to investigate different aspects of the use of herbs. In the spring of 2022, two herb mixtures (chicory and ribwort plantain) were sown on two separate paddocks of 1.5 ha each. In 2023, yield measurements were conducted on both paddocks to assess plant growth and total annual plant production. This article focuses on the yield component of the study.

## Materials and methods

The study was conducted on two experimental fields sown in spring of 2022, situated on heavy marine clay at the Dairy Campus (Leeuwarden, the Netherlands). The soil organic matter content was 9.8%, with a C/N ratio of 10, a total nitrogen soil reserve of 4180 mg N kg<sup>-1</sup>, and a phosphorus soil reserve of 26 mg P<sub>2</sub>O<sub>5</sub> kg<sup>-1</sup>. The plant-available phosphorus was low at 0.6 mg P kg<sup>-1</sup>. Yield determinations were carried out throughout the entire growing season of 2023. Strips were mowed approximately weekly from spring and after each harvest using a hand mower. The strips had dimensions of 2.0×0.6 m (1.6 m<sup>2</sup>). Three random chosen strips were mowed at each sampling moment (3 repetitions). This approach resulted in 4–6 sampling moments during the growing period of a cut, establishing a growth curve per cut. In total, six cuts were harvested. All samples were weighed and dried at 70°C to facilitate subsequent chemical analysis for nutritional value determination, which is still in progress. The nitrogen input remained low, with one application of 50 kg N ha<sup>-1</sup> applied in March and a second application of 50 kg N ha<sup>-1</sup> after

the second cut. No animal manure was applied. Differences in DM yield between the two herbs were analysed with ANOVA, with herb and cut as treatment and replicate as block.

## Results and discussion

The DM yield per cut is presented in Table 1. The chicory field exhibited numerous open areas where weeds proliferated (density of 60% on average in spring). For yield determinations, locations were sought where some chicory plants were still present. After the first cut, chicory rapidly went to the heading stage, leading to a reduced presence of leaf mass and lower yield compared to ribwort plantain. Plant recovery and increased leaf formation, with fewer stems, again occurred after the third cut. During the growing season the only a dry period occurred in June. The annual yield of ribwort plantain was 13.7 t DM ha<sup>-1</sup>, which was approximately twice as high as that of chicory (6 t DM ha<sup>-1</sup>). Figure 1 illustrates the growth trends of chicory and ribwort plantain for all six cuts. During the first and second cuts, ribwort plantain clearly grew faster than chicory. Only from the fourth cut onwards was the growth of both herbs comparable. The highest growth for both crops was achieved in the spring. The initial results of a ribwort plantain monoculture are promising. With a nitrogen input of 100 kg N ha<sup>-1</sup>, the annual yield of 13.7 t DM ha<sup>-1</sup> is comparable to that of a predominantly perennial ryegrass pasture receiving a nitrogen input of 250 kg ha<sup>-1</sup> on the same soil type (though not in this trial, but 13.5 t DM was harvested on grassland of the Dairy Campus).

Ribwort plantain exhibited resilience to drought, showcased by its performance during a dry period in June, and ongoing research suggests favorable cow intake. However, growth diminishes in the second

Table 1. DM yield (kg DM ha<sup>-1</sup>) per cut and total for plantain and chicory in 2023.

	Cut and date						Total
	1 (4 May 2023)	2 (31 May 2023)	3 (26 June 2023)	4 (9 August 2023)	5 (5 September 2023)	6 (25 October 2023)	
Chicory	502 <sup>ab</sup>	1 241 <sup>b</sup>	949 <sup>b</sup>	2 276 <sup>c</sup>	949 <sup>b</sup>	372 <sup>a</sup>	5 980 <sup>f</sup>
Plantain	3 357 <sup>d</sup>	3 933 <sup>e</sup>	2 090 <sup>c</sup>	2 247 <sup>c</sup>	1 302 <sup>b</sup>	812 <sup>ab</sup>	13 711 <sup>g</sup>

Different letters indicate a significant difference (LSD=543, SED=262).

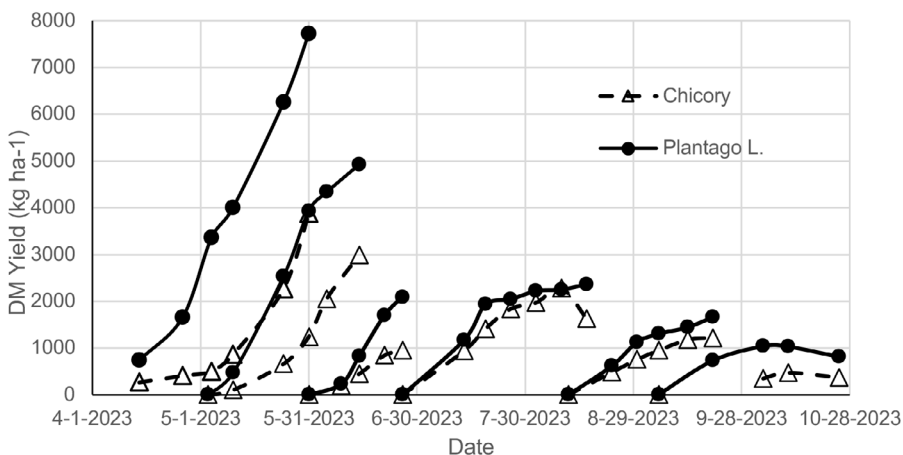


Figure 1. Growing trends of 6 cuts of ribwort plantain (*Plantago L.*) and chicory in 2023.

half of the season, with an optimal growth duration of 3 weeks. Beyond this period growth rate decreases significantly and in autumn, mortality surpasses growth. Robust leaves minimize losses during fresh material harvest. In contrast, chicory leaves are fragile, susceptible to mechanical harvest losses. Chicory, in its second year, faced challenges due to winterkill, resulting in an open sward with unwanted weeds. Notably, there was recovery in the second half of the season. Although growth was lower than in spring, the yield from July onwards was comparable to ribwort plantain. However, ribwort plantain appears more persistent than chicory. A 2022–2023 feeding trial with both herbs showed a good intake and milk production by milking dairy, supporting the expectation of nutritional values similar to grass, consistent with previous findings and literature (Minneé *et al.*, 2019).

## Conclusion

Both ribwort plantain and chicory show promise as forage for Dutch dairy farming. The yield of ribwort plantain is comparable to grass but with significantly lower nitrogen input. Chicory cultivation requires extra attention, and the crop seems less persistent, potentially requiring more frequent reseeding. Both herbs are expected to contribute to greater biodiversity.

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# Carbon sequestration practices in Eastern Netherlands: a grass and arable fields case study

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## Abstract

This study examines carbon sequestration practices among farmers in Eastern Netherlands, focusing on arable lands and grasslands within the Vruchtbare Kringloop Oost (VKO) region. We analysed a dataset of 15 220 soil samples (11 372 grassland at 10 cm depth and 3848 arable at 25 cm depth) over a decade, and used Near-Infrared Spectroscopy (NIRS) and engaged 311 farmers to assess the efficacy of carbon capture. Results indicate a positive shift in carbon sequestration on both arable lands and grasslands, with average values increasing from 47.95 to 50.0 tons per hectare and 49.85 to 50.75 tons per hectare, respectively. However, nuanced variations among individual farmers reveal mixed outcomes, necessitating tailored interventions based on specific practices and challenges. The study highlights the importance of sustainable farming practices in enhancing carbon sequestration, contributing to environmental goals. The observed increases underscore the potential for positive strides in land management, but comprehensive research is imperative to understand the factors influencing variations and guide precise recommendations for sustainable agricultural practices. These findings serve as a foundation for future assessments, informing strategies to optimize carbon sequestration and foster sustainable agriculture in the face of climate change.

**Keywords:** carbon sequestration, sustainable farming, climate change, soil testing, soil health

## Introduction

Farmers play a crucial role in mitigating climate change by adopting carbon sequestration practices. This introduction highlights their significance and focuses on the evolving landscape of global agriculture shaped by carbon sequestration. With climate change accelerating, the imperative for carbon sequestration becomes more pressing, underscoring farmers' unique position to make a tangible impact. Sustainable practices, such as cover cropping and reduced tillage, transform the agricultural landscape into a carbon sink. The amount of C stored in the aboveground is twice the amount of C as CO<sub>2</sub> in the atmosphere (Batjes, 1996; Janzen, 2006). This paradigm shift not only addresses climate change but also fosters soil health (Bonanomi *et al.*, 2010). Farmers adopting carbon sequestration practices initiate a ripple effect, creating awareness and action within communities. Beyond environmental benefits, these practices enhance soil fertility, increase productivity, and contribute to long-term resilience (Leinweber *et al.*, 1993; Tipping *et al.*, 2016). The introduction also touches upon the emerging market of carbon credits, aligning economic interests with environmental stewardship. Frequent analysis on carbon sequestration helps farmers to exploit this new economic opportunity (Reijneveld *et al.*, 2023). This study endeavours to lay the groundwork for subsequent assessments by providing insights into the carbon sequestration dynamics within VK-Oost farms. The primary objective is to furnish information regarding the present status of carbon sequestration in VK-Oost farms, facilitating the formulation of strategies aimed at optimizing carbon sequestration and improving sustainable agricultural practices in response to challenges posed by climate change.

## Materials and methods

The study utilized an extensive dataset of 15 220 production soil samples collected over a decade in collaboration with 311 farmers. To assess comprehensively the present state of carbon sequestration within VK-Oost farms, an inclusive approach was adopted, incorporating all farms without employing selective indicators. In total 11 372 grassland and 3848 arable soil samples were used, grassland samples were sampled at 10 cm, arable fields were sampled at 25 cm. The majority (89%) of the studied fields were sandy soils (>50% sand). Two sampling methods, house-method and GPS-based stratified, were employed to ensure representativeness in the study region's diverse agricultural practices and soil types. The systematic W-shape collection in each field (up to 5 ha) involved 40 subsamples, providing a holistic representation of soil properties. For fields larger than 5 ha, 20–25 subsamples were strategically selected based on GPS coordinates to ensure a geographically dispersed and statistically representative subset of samples. Since production samples were analysed, only the topsoil has been studied (grassland samples at a depth of 10 cm, arable samples at a depth of 25 cm). Near-Infrared Spectroscopy (NIRS) analysis was conducted on the 15 220 soil samples for a non-destructive, rapid assessment of multiple soil properties over ten years. The statistical analysis for this study was conducted using Microsoft Excel, with a focus on calculating and interpreting averages and quartiles. Relevant functions and tools in Excel were employed to derive mean values, providing a central measure for the analysed data. Additionally, boxplots were generated to visually represent the distribution and key statistical characteristics of the dataset.

## Results and discussion

The significant variability observed in the third quartile underscores the considerable room for enhancement in overall carbon sequestration, suggesting ample opportunities for interventions across the examined agricultural landscapes. Tailored solutions addressing sandy soil types, climatic conditions, and farming techniques are crucial for optimizing carbon sequestration on both arable lands and grasslands. Further, the study emphasizes the need for in-depth research to understand the reasons behind the observed variations and guide the development of precise recommendations for sustainable land management practices.

Carbon sequestration across arable lands and grasslands revealed substantial changes over the study period (Figure 1). On arable lands (Figure 2), a positive shift from an initial average of 47.95 to 50.0 t ha<sup>-1</sup> was observed. However, a breakdown among 300 farmers with more than one year of data showcased mixed outcomes, with 130 farms experiencing an increase in sequestration and 170 facing a decline. The analysis of carbon sequestration in grasslands demonstrated an encouraging positive trend, with the initial average of 49.85 t SOC ha<sup>-1</sup> increasing to 50.75 t SOC ha<sup>-1</sup>. Yet, among the 304 farmers with more than one year of data, 144 farms experienced increased sequestration, while 160 faced a decline. Figure 1 shows an increase in the spread of results in grasslands among farmers in 2023, causing the average to rise, where the median hardly changes from 44.33 t SOC ha<sup>-1</sup> to 43.45 t SOC ha<sup>-1</sup>. The large variation in the third quartile especially indicates great possibilities for the potential sequestration of carbon in both arable and grasslands.

## Conclusions

The observed increases in carbon sequestration on both arable lands and grasslands signify a positive trajectory, indicating the potential of sustainable practices to contribute to environmental objectives. However, the variations among individual farmers underscore the complexity of factors influencing carbon sequestration and highlight the need for targeted interventions. In the absence of prior data weighting, it is observed that the mass equivalence between 5-hectare and 15-hectare fields exists. It is important to note that discrepancies in mean and average values may arise depending on the utilization of actual hectare measurements.

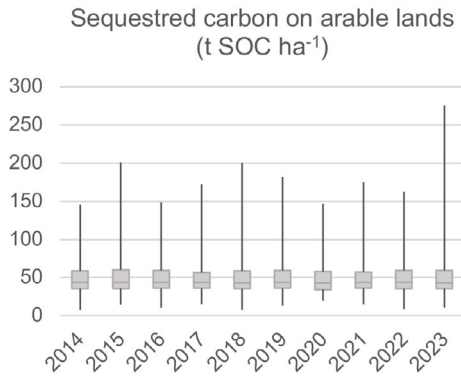


Figure 1. Sequestered carbon on grasslands per year.

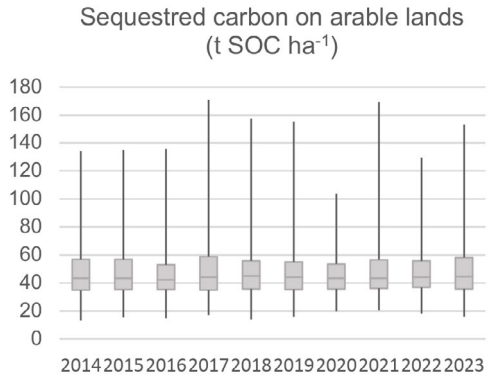


Figure 2. Sequestered carbon on arable lands per year.

## Acknowledgements

We thank the VK-Oost for allowing us to work with data originated by affiliated farmers. We thank Martijn van Oostrum for (re-)analysing over 15 000 samples by using the old spectra.

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# Balanced phosphorus fertilization in a mixed grazing and mowing system on grassland; results after 26 years

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## Abstract

In many affluent countries the excessive use of phosphorus (P) in agriculture has led to a high P content in the soil, thus threatening surface water quality by run-off and leaching. In the Netherlands P fertilization is limited to the net offtake from the land, so called balanced P fertilization, for soils with a sufficient soil P status. Balanced P fertilization, however, might affect grassland yield and quality negatively. In the short term a decrease in herbage P content is expected. In the longer term decreases in herbage yield can be foreseen. The objective of a long-term field experiment, initiated in 1997, was to examine the effects of balanced P fertilization compared with two levels of positive P surpluses in a mixed grazing and mowing system. Herbage yield, P content and soil P status were measured. In the last ten years DM yield did not respond to P fertilization. Herbage P content and soil test P responded positively to P fertilization. At balanced P fertilization the P content of herbage and soil test P decreased implying that the system was not in a steady state. Due to this it cannot be ruled out that DM yield will be affected negatively to balanced P fertilization eventually. On relatively P-rich soils, however, the DM yield seems not to be affected by balanced P fertilization in the first 15 to 25 years.

**Keywords:** grassland, grazing, phosphorus, balanced fertilization, dry matter yield, P content

## Introduction

In many affluent countries the excessive use of phosphorus (P) in agriculture has led to a high P content in the soil, thus threatening the surface water quality by run-off and leaching. On top of that, phosphate rock is a limited, non-renewable resource. In the Netherlands P fertilization is limited to the net offtake from the land, so-called balanced P fertilization, for soils with a sufficient soil P status, to protect surface water quality. Balanced P fertilization, however, might affect grassland yield and quality negatively due to conversion of plant available soil P into more resistant P fractions and leaching of small amounts of P. Decreases in P content of herbage are expected directly with decreasing P fertilization (Power *et al.*, 2005; Schulte and Herlihy, 2007). In the longer term decreases of herbage dry matter (DM) yield can be foreseen. Grazing is an important factor that determines P flows on grassland. On grazed grassland, herbage P is returned unevenly spread to the surface via excretion of faeces. At balanced P fertilization, at the parcel level, manure patches will have a positive P balance and the surrounding parts a negative P balance. So far, the implications of long-term balanced P fertilization on herbage yield and quality, and on soil P status, are not well quantified under grazing conditions. The objective of a long-term field experiment, initiated in 1997, was to examine the effects of balanced P fertilization and two levels of positive P balances, on herbage yield and quality, and on soil P status. This paper displays the results of herbage DM yield and P content in the 10 years from 2013 to 2022. The results until and including 2014 were published in proceedings of EGF 2016 (Van Middelkoop *et al.*, 2016a).

## Materials and methods

In 1997 an experiment was laid out on four grazed grasslands: two sandy soils, a marine clay and a peat soil. The two sandy sites stopped after 2012 and 2013, the experiments on young marine clay and peat site are still running (in 2022). At each location six plots were randomly assigned to a combination of P and N fertilizations. Fertilization levels (Table 1) were aimed to achieve soil surface surpluses of 180 and 300



kg N ha<sup>-1</sup> year<sup>-1</sup> (N180 and N300) and 0, 9 and 18 kg P ha<sup>-1</sup> year<sup>-1</sup> (P0 i.e. balanced P fertilization, P9 and P18). All treatments were aimed at balanced P fertilization with 40–50 m<sup>3</sup> ha<sup>-1</sup> cattle slurry. P9 and P18 surpluses were applied by adding superphosphate or triple-superphosphate. Cattle slurry and mineral P fertilizer were applied in spring and before the 4<sup>th</sup> cut. Mineral N fertilizer was applied throughout the whole season. At both locations the first and fourth cut were taken for silage, the other cuts were grazed by heifers or dry cows. DM yield was determined and herbage was sampled for analysis of P and N content on the day grazing started or cutting took place. The surpluses were calculated as fertilization minus output in silage cuts and in weight increase of heifers or dry cows. The consumption of grass and excreted nutrients during grazing were not accounted for as this was considered to be an internal cycle (Van Middelkoop *et al.*, 2016b). Differences for 2013 to 2022 between treatments in annual DM yields and P contents were statistically analysed in a linear model with a fixed and a random part with the Restricted Maximum Likelihood (Reml) method (Harville, 1977), using Genstat (17<sup>th</sup> edition). The fixed part comprised P and N fertilization, number of years, sites, and the interactions as explanatory variables. Interactions which were not significant were deleted to reach the final linear model.

## Results and discussion

In the last ten years DM yield at balanced P fertilization was not lower than at surpluses of 9 and 18 kg P ha<sup>-1</sup> (Figure 1a). In the analysis of the first 16 years, however, DM yield on peat was 8% higher at P18 compared to P0 (Van Middelkoop *et al.*, 2016a). The raw data show that the response to P on peat is small compared with the variability over time, although there is a difference on average. The response of DM yield to N fertilization was positive as could be expected at the applied N fertilization levels. In a long-term experiment residual effects of fertilization might occur and increase the DM yield response in time. This was not found for P or N fertilization. Herbage P content responded to P fertilization and not to N fertilization (Figure 1b). On average the herbage P content of P18 compared with P0 was 0.3 g P (kg DM)<sup>-1</sup> higher on clay and 0.6 g P (kg DM)<sup>-1</sup> on peat. Compared to the last ten years, the response for the first 15 years (Van Middelkoop *et al.*, 2016a) was lower: 0.1 g P (kg DM)<sup>-1</sup> on clay, and 0.4 g P (kg DM)<sup>-1</sup> on peat. The effect of the P surplus on herbage P content increased over time on both sites. At P0 the P content of herbage decreased. On peat the average P content of the last 10 years at P0 was 2.8 g P (kg DM)<sup>-1</sup>, which is below the standard for high productive dairy cows in the Netherlands. The lack of response in DM yield and the increasing response of P content to P surplus is in line with results found in other studies (Power *et al.*, 2005; Schulte and Herlihy, 2007). The decrease of P content of herbage and soil test P (data not shown) at balanced P fertilization in the last ten years implies that the system was not in a steady state. Due to this decrease, it cannot be ruled out that DM yield will be affected negatively to balanced P fertilization in the longer term. On relatively P-rich soils, however, the DM yield seems not to be affected by balanced P fertilization in the first 15 to 25 years.

Table 1. Soil organic matter (SOM), P-ammonium lactate (P-AL) at start, N fertilization and P fertilization on young marine clay and peat, mean over last ten years (2013–2022).

	Young marine clay	Peat
Soil organic matter (%)	7.7	52.3
P-AL-value, mg P <sub>2</sub> O <sub>5</sub> (100 g air dry soil) <sup>-1</sup> <sup>a</sup>	58	42
N fertilization N180-N300	205-327	158-302
P fertilization P0-P9-P18	27-35-44	18-27-34

<sup>a</sup>According to standards in the Netherlands: high on clay, amply sufficient on peat. 1 mg P<sub>2</sub>O<sub>5</sub> (100 g air dry soil)<sup>-1</sup> equals 4.37 mg P (kg air dry soil)<sup>-1</sup>.

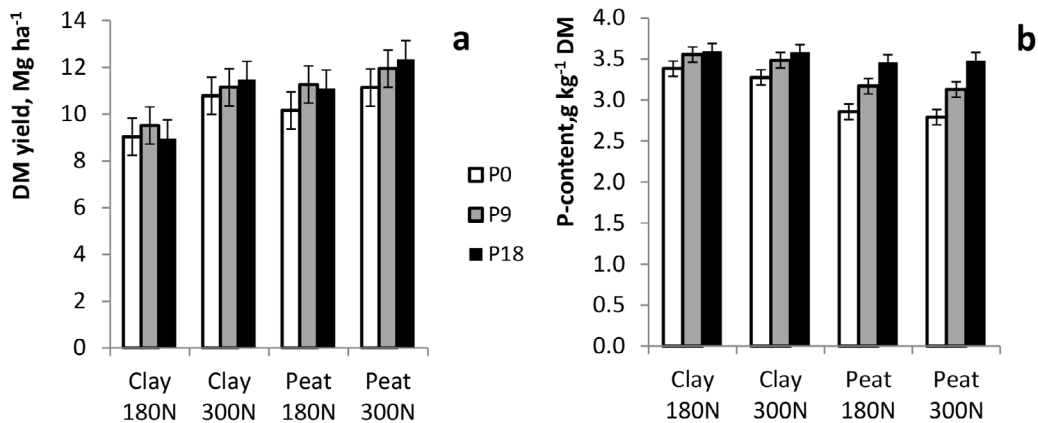


Figure 1. (a) Mean annual dry matter yields and (b) Mean P contents at the clay and peat sites, as a function of mean annual phosphorus surpluses in kg P ha<sup>-1</sup> (P0, P9 and P18), for two levels of nitrogen input, averaged over the period 2013–2022.

## Conclusions

On grassland that was mixed grazed and mown, P fertilization was aimed at soil surface surpluses of 0 (balanced P fertilization), 9 and 18 kg P ha<sup>-1</sup>. After 26 years DM yield was not lower at balanced P fertilization than at a surplus of 9 and 18 kg P ha<sup>-1</sup>. The response of the P content of the herbage to P surplus and soil test P still increased over time. The P content of the herbage at balanced fertilization decreased; on peat soil it was 2.8 g P (kg DM)<sup>-1</sup> averaged over the last 10 years, which is below the standard for high productive dairy cows in the Netherlands. The decrease of P content of herbage and soil test P at balanced P fertilization in the last ten years implies that the system was not in a steady state. Due to this, the possibility that DM yield will be affected negatively by balanced P fertilization in the longer term cannot be ruled out. On relatively P-rich soils, however, the DM yield seems not to be affected by balanced P fertilization in the first 15 to 25 years.

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# Inventory of the abundance of earthworm *Lumbricus terrestris* in grasslands on sandy soil

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## Abstract

The deep, vertical burrows of anecic earthworm *Lumbricus terrestris* contribute to the ecosystem service of water regulation in grasslands. They facilitate water flow and deeper rooting, thereby supporting the prevention of flooding and improving drought tolerance. In Europe, these earthworms occur in agricultural grasslands on various soil types. However, their distribution pattern is heterogeneous and not well-understood. Through characterisation of *L. terrestris* distribution patterns, we aim to grasp their potential for climate adaptive water regulation. In a field inventory ( $n=62$ ) we assessed the relationship between *L. terrestris* population density in grassland on sandy soils and: soil silt concentration; epigeic earthworm population density; and grassland age. Soil silt concentrations and *L. terrestris* population densities correlated positively. Population density of *L. terrestris* correlated negatively with *L. rubellus* abundance. Population density of *L. terrestris* was not significantly related to grassland age. Unexpectedly, we found *L. terrestris* in some very sandy soils. Our data were fitted into an existing predictive model, yielding 63% accuracy.

**Keywords:** deep-burrowing earthworms, grassland, water regulation, ecosystem functioning

## Introduction

Grasslands play a vital role in water regulation. Global climate changes cause prolonged dry periods and intensified peak rainfall (Pachauri *et al.*, 2014) both of which entail major impacts on plant growth, biogeochemical cycles and nutrient losses in agricultural grasslands. As soil ecosystem engineers, earthworms cause soil bioturbation and improve water regulation (Deru *et al.*, 2018). Deep-burrowing earthworms, e.g., *L. terrestris*, create vertical, semi-permanent burrows, reaching down to 2 m. The burrows can increase soil infiltration rate and infiltration capacity, helping to avoid waterlogging and flooding (Blouin *et al.*, 2013), while increasing rooting space, which can promote drought tolerance. It is known that *L. terrestris* distribution is heterogeneous at field and landscape scale, but we lack a set of parameters explaining their occurrence, especially on grasslands on sandy soils. Our objective was to improve our understanding of the factors that define *L. terrestris* presence and abundance in this habitat. A field inventory was executed, focussing on (1) soil texture, (2) groundwater level, (3) competitive interaction with resident earthworm species and (4) land use and management.

## Materials and methods

Thirty-one grasslands belonging to eleven farms on sandy soils in the Dutch province of Noord Brabant were sampled in the spring of 2021 (Van de Logt *et al.*, 2023). With geodata, we selected grasslands of varying geomorphology, interrelated with soil types, texture classes and ground water stages. Grasslands were categorised 'young' ( $\leq 3$  years) and 'old' ( $> 3$  years), by the number of years since renewal ( $n=11$  and  $n=20$  for young and old resp.), as *L. terrestris* is known to be sensitive to tillage. In each grassland, two plots were sampled ( $n=62$ ) on representative spots  $> 10$  m from the fence,  $> 40$  m between two plots. Per plot, a cube of soil,  $20 \times 20 \times 20$  cm, was excavated, hand-sorted, and all earthworms present were counted and identified to species. Three additional soil cubes were dug out to create a square pit of  $40 \times 40 \times 20$  cm, and 4 l of 0.01% allyl-isothiocyanate (AITC) solution was then applied to the pit to collect *L. terrestris* from deeper soil layers. All earthworms emerging within 20 minutes were collected, rinsed with water and stored in containers. Using an auger ( $\varnothing 10$  cm) the soil profile was assessed (0–120 cm). Gley depth

(cm) was used as a measure for temporary max. groundwater or pseudo-groundwater tables. Fifteen soil samples for chemical analysis were taken from the topsoil (0–10) and 30–40 cm soil layer with a gouge auger ( $\phi$  2.5 cm) within a 2 m radius from the earthworm sampling pit. SOM, pH and silt concentration were analysed (see Van de Logt *et al.*, 2023). Soils were categorised as loam-poor, light loamy or loamy sands, according to a Dutch texture classification (Van der Meulen *et al.*, 2007). R was used for correlative analysis of the data; data were also fitted into an existing model (Lindahl *et al.*, 2009) as this model predicts *L. terrestris* density  $m^{-2}$ , based on land-use type and soil texture.

## Results and discussion

Silt concentration at a depth of 30–40 cm was positively correlated with total ( $R^2=0.21$ ;  $p<0.001$ ), adult ( $R^2=0.33$ ;  $p<0.025$ ) and juvenile *L. terrestris* density for ( $R^2=0.15$ ;  $p<0.025$ ) (Figure 1). Surprisingly, a very loam-poor grassland hosted a high density of *L. terrestris*. Highest abundance was observed in soils with 20–40% silt (Figure 1). Higher *L. terrestris* densities in loamier soils were also reported in other studies (Decaëns *et al.*, 2003). Possibly, better moisture and nutrient retention in loamy sand provide a more favourable environment than loam-poor sand. Previous research suggested that earthworms suffer from the coarse texture and drought proneness of sandy soils (Hawkins *et al.*, 2008). However, *L. terrestris* has been reported to occur in coarsely textured soil, albeit in lower densities than in medium-textured soils. For 63% of the samples, the model by Lindahl *et al.* (2009) gave an accurate estimation of *L. terrestris* density (low, medium, high;  $<3$ ,  $3-10$ ,  $>10 m^{-2}$ , respectively). The accuracy of the classification tree for medium-textured soils and coarsely textured soils was 51% and 69%, respectively. Gley depth correlated positively with *L. terrestris* total densities in a model with the silt concentration predictor ( $R^2=0.25$ ;  $p<0.05$ ). Gley depth correlated negatively with both silt and clay concentrations at 10 and 40 cm depth ( $p<0.05$ ). Absence of compaction layers prone to waterlogging and associated formation of temporal shallow pseudo-groundwater levels indicates well-structured soils. Valckx *et al.* (2011) suggest that well-structured, porous and deep-drained soils are suitable for anecic earthworms. *Lumbricus rubellus* density and *L. terrestris* total and adult densities correlated negatively ( $R^2=0.10$ ;  $p<0.025$  and  $R^2=0.11$ ;  $p<0.025$ , respectively). No significant correlations were found between *L. rubellus* densities and *L. terrestris* juvenile densities. Negative interactions between the two species were also suggested in previous research under semi-controlled conditions (Lowe and Butt, 2002) but not yet in a field inventory.

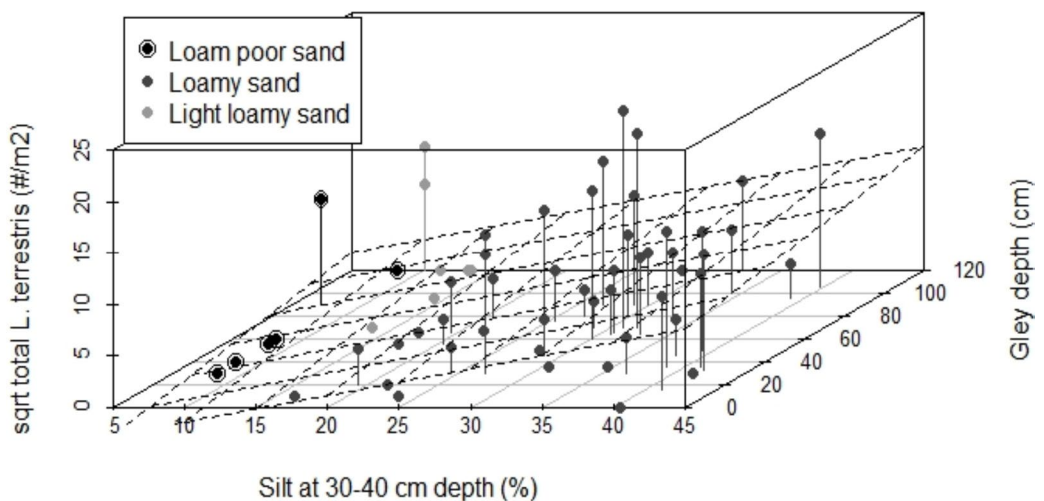


Figure 1. Correlation between soil silt concentration at 30–40 cm depth, gley depth and total *L. terrestris* population densities  $m^{-2}$ . The plane represents the related linear model. Total *L. terrestris* population densities are square root transformed.

A negative correlation could emerge from interspecific competition for limited food sources, both species feed on surface organic material. *L. rubellus* has a higher reproduction and growth rate than *L. terrestris* and may therefore outcompete the latter (Uvarov, 2009). Unexpected was that grassland age did not significantly correlate with *L. terrestris* population densities, possibly due to a slightly unbalanced dataset, with eleven young grasslands and twenty old grasslands, which was the result of limited availability of young grasslands in the area.

## Conclusion

*Lumbricus terrestris* was more abundant in soils with a higher silt percentage, likely because of positive relationships between loaminess and other soil factors that create favourable living conditions. Unexpectedly, *L. terrestris* was also abundantly present in a grassland on loam-poor sand. The model by Lindahl *et al.* (2009) correctly predicted the level of *L. terrestris* abundance based on land use and soil texture in 63% of the samples. A weak positive correlation was observed between *L. terrestris* density and gley depth; waterlogged layers could create an unfit environment. A negative correlation with *L. rubellus* abundance was shown, likely due to competition for food, perhaps combined with slightly diverging habitat preferences. The study did not reveal significant differences in *L. terrestris* abundance based on grassland age, possibly because the dataset was not sufficiently balanced for sward age. Overall, this correlative study provides further insights into *L. terrestris* habitat selection.

## Acknowledgement

This project was part of the Public-Private Partnership KLIMAP.

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**Theme 5.**  
**WHOM?**  
**For whom are grasslands**  
**important?**



# The intricate pathway for the future grasslands; who comes first, people or policy?

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## Abstract

Grasslands are important in the context of farming-nature relationships due to their interconnected ecosystem, as illustrated by the Montado silvo-pastoral system and its biodiverse Mediterranean pastures. These extensive land use systems serve not only as habitats for diverse wildlife but also contribute significantly to essential ecological functions. Biodiverse pastures foster water infiltration, shield the soil from erosion, and function as a carbon sink, highlighting their multifaceted benefits, particularly in an era of climate disturbances, such as water scarcity. The significance of these qualities is well-recognized by key stakeholders of the system, particularly farmers. Nutritive and long-lasting grasslands translate to a substantial reduction in the costs of livestock production. However, the ability to safeguard grasslands is diminishing due to intensification processes, declining soil health and an escalating frequency of droughts and floods. Farmers, as key decision-makers in grassland management, find their options dwindling. A pivotal aspect of the farmers' decision-making process revolves around the directives of the Common Agricultural Policy (CAP). A significant portion of their income is derived from subsidies, with farmers' associations playing a crucial role in guiding their members through the subsidy application process. However, these associations are evolving to focus more on the professional management of subsidy applications and less on assisting farmers in adopting management practices that ensure grassland resilience in the face of current climate conditions. Given that CAP encompasses a broad array of distinct interventions, it becomes imperative to articulate policies based on common desirable outcomes. Failure to do so may result in the achievements of one intervention hindering the goals of others. Therefore, a careful examination of policy influence on decisions shaping the future of grasslands is vital. Our goal is to describe and discuss the intricate relationship between policy and farmers' decision-making options that have the potential to compromise the resilience of grassland systems.

**Keywords:** stakeholders, governance, transdisciplinarity and policy

## Introduction

Grasslands are one of the most common ecosystems in Europe, covering about 34% of the total land area (Eurostat, 2020). Grassland types are highly diverse (Peeters, 2009). They include natural grasslands, semi-natural grasslands (extensively managed for grazing or forage production) and agricultural grasslands. Natural grasslands in Europe are classified by plant communities into seven main habitats according to EUNIS (Chytrý *et al.*, 2020): dry grasslands, mesic grasslands, seasonally wet and wet grasslands, alpine and subalpine grasslands, woodland fringes and clearings and tall forb stands, inland salt steppes and sparsely wooded grasslands. Semi-natural and agricultural grasslands can vary along a spectrum from low intensity of human management (semi-natural grasslands, which may have a mixture of non-native grass species with native species that reseed spontaneously) to management options such as fertilisation, irrigation, reseeding or treatment with amendments or herbicides (agricultural grasslands). Furthermore,



each grassland type can exhibit a wide range of diversity in terms of species composition and dominant plant functional groups (grasses, legumes or forbs). Some grasslands may host a high number of different plant species resulting in species-rich environments. Conversely, other grasslands may have relatively low species diversity, either because of environmental conditions or human activities such as agriculture or grazing.

Despite these very different compositions, grasslands share important common features (Zhao *et al.*, 2020). Grasslands support a wide range of plant and animal species (insects, birds, mammals and reptiles), many of which are specially adapted to their particular habitat. They often experience seasonal climate patterns, with distinct wet and dry seasons. These climatic variations influence the growth of vegetation and the behaviour of animals within the ecosystem, but the periodicity of seasons is vulnerable to climate change. Grasslands are soil builders in the sense that they play a crucial role in soil formation and maintenance. The deep root systems of grasses help to stabilise the soil and prevent erosion. They also support diverse communities of micro-organisms, which play a key role in decomposing organic matter and cycling nutrients through the soil. Grasslands can also act as important carbon sinks, storing carbon both in the soil and in plant biomass. Finally, grassland landscapes are important for improving the quality of life of human communities provided by their aesthetically and recreational value.

Nevertheless, grasslands have been significantly affected by human activities such as agriculture, grazing, urbanization, and habitat fragmentation (Schils *et al.*, 2022). These activities have led to the conversion of natural grasslands into croplands, pastures, and urban areas, resulting in habitat loss and fragmentation for many grassland species. The current CAP reveals inadequacy for biodiversity conservation in grassland ecosystems (Pardo *et al.*, 2020). Negative trends were reported for avian communities in hay meadows (Assandri *et al.*, 2019) and for grassland butterflies with a 39% decline since 1990 (Warren *et al.*, 2021) despite some natural and semi-natural grasslands and their wildlife are protected under EU Nature Directives (e.g. Nature Restoration Law).

Permanent grasslands have experienced a significant loss during the last decades. For example, losses have been estimated at about 30% between 1967 and 2007 for Belgium, Netherlands, Luxemburg, France, former West Germany and Italy (Huyghe *et al.*, 2014). Upper Normandy lost 200,000 ha of permanent grassland between 1970 and 2000 (Souchère *et al.*, 2003). In Portugal, in contrast, the area of permanent pasture has increased by 14% in the last decade (INE, 2019). These areas account for 31% of land use, equivalent to around 2.8 million hectares of pasture and scrubland (Onyango *et al.*, 2021). This increase is a result of the conversion of cereal cropping systems to forage-livestock systems in the less productive and more marginal areas. However, this conversion has been carried out with an attitude of near abandonment (Carvalho, 2018). One of these extensive grazing systems is the Montado, a silvo-pastoral system where livestock graze all the year round under an open tree cover of holm and cork oaks. As a combined result of CAP implementation in Portugal and market trends, the number of suckler cows in the region where the Montado is the dominant land use system, has increased 50% in the last two decades (GPP, 2020). The increased intensity and specialization of livestock grazing explained 52% of the recent Montado area loss, with an estimated annual regression rate of 0.14% year<sup>-1</sup> (Godinho *et al.*, 2016). Montado is a specific case where grasslands play a key role in a complex and multifunctional land use system. We will use this specific case to illustrate how a transdisciplinary approach can help the transition from linear to complex thinking. Our goal is to illustrate our own mindset evolution and how we are navigating uncertainty towards a co-constructed future. We hope that our case study can motivate other researchers to participate in wider and collective approaches towards a future where grasslands and all services they provide are not threatened.

## The importance of a social-ecological perspective

Several authors have illustrated the importance of a social-ecological perspective when dealing with management of natural resources. We focus on Elinor Ostrom’s work with the social-ecological systems (SES) framework, tightly linked to her Nobel Prize work on “Governing the Commons” (Ostrom, 1990). The SES framework aids in moving away from linear thinking (e.g. understanding the relation between two variables) and emphasizes the connections between subsystems (see Figure 1). The frameworks are constituted by multiple tiers, so within each subsystem identified in Figure 1 there are a set of variables that should be considered (details in McGinnis and Ostrom, 2014). The arrows in Figure 1 need to be understood while using the framework, and the variable in each subsystem provide a particular piece of the overall puzzle that is described in the central subsystem of Figure 1: Interaction (I) and Outcomes (O). Hence, the framework induces its users not only to understand a particular subsystem but the relationship between subsystems and the outcomes of these interactions.

Stakeholders are included in the governance system along with many other variables that can help understand their process of deliberation and decision making. How can grasslands be in decline when their importance is evident? As in many other natural resources management challenges, the answer to this question is spread across layers of decision-making involving farmers, the private and public sectors, administration, and decision makers. All these decisions are connected and iterative. The SES framework helps us map these connections. So, when embarking on the design and implementation of a transdisciplinary approach towards the sustainable management of the Montado system, we used the framework to start framing the problem (Guimarães *et al.*, 2018).

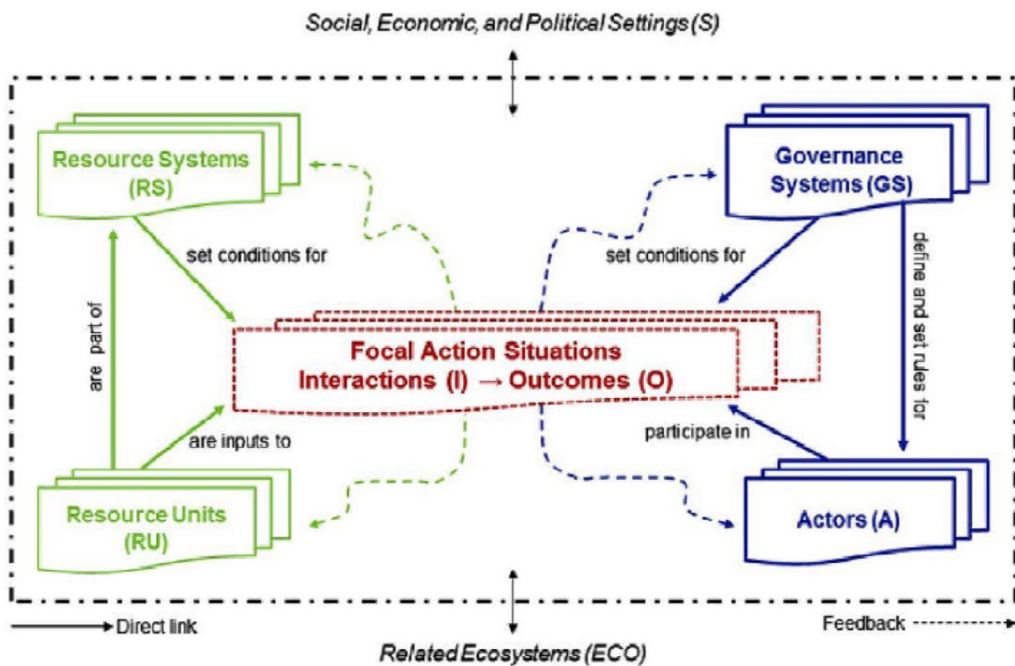


Figure 1. Revised social-ecological systems (SES) framework with multiple first-tier components. Source: McGinnis and Ostrom (2014)

The paper of Guimarães *et al.*, (2018) is a fragment of the extensive review and thinking put into this exercise. The paper explored who the stakeholders are, how they interact with each other and with the resource system. In this paper, we recognized the limited quantity of science about the human side of the Montado — how little science knows about the people who act upon the Montado. More than 70% of the literature reviewed focused on the ecological side of this SES. We ponder how this situation compares to others, but in the Montado case, we are missing up-to-date humanities and social science perspectives.

The SES framework was instrumental in our efforts to comprehend the complexity of the problem, and we encourage others to explore its applicability in their own contexts: How can the issue of grassland sustainability be understood in a holistic manner? Perhaps drawing from the legacy of Elinor Ostrom can offer valuable perspectives to address this question.

## Stakeholder engagement and transdisciplinarity

One aspect that elucidates decision-making is the capacity to communicate effectively. Elinor Ostrom stated that instead of the rationality depicted by *homo economicus*, as described in classical economics, we embody *homo cooperaticus*, making decisions with a perspective oriented towards the common good when communication channels exist, alongside a genuine capacity to influence decisions that affect us (Anderies and Janssen, 2012). What, then, accounts for the current general decline of natural resources (and common goods)? When communication channels are absent and decisions are made on our behalf, we revert to *homo economicus*, basing our decisions on individual interests regardless of their impact on others.

For this and many other reasons we have made several efforts in stakeholder engagement and the focus on transdisciplinary approaches. The concept of transdisciplinary (TD) research, as defined by Klein *et al.* (2001), involves the collaborative effort of various academic disciplines in conjunction with non-academic practitioners to address real-world problems. Pohl (2011) further elaborates on TD research, highlighting four key elements:

1. Comprehensive understanding of complexity: TD research aims to fully comprehend the complexity of the issue at hand.
2. Diverse perspectives considered: It considers a wide array of perspectives related to the issue.
3. Integration of abstract and case-specific knowledge: TD research combines theoretical knowledge with practical, case-specific information.
4. Generation of descriptive, normative, and practical knowledge: It creates knowledge that is descriptive, normative (involving ethical or value-based considerations), and practical, with the aim of promoting what is perceived as the common good.

In the TD research process, representatives from various disciplines, from both private and public sectors, as well as civil society, collaborate to develop knowledge on a specific issue while striving to align with these four key aspects.

A key aspect of our own approach to stakeholder engagement is the time perspective associated with a long-term commitment between our research institutions and the territories we work with. So, stakeholder engagement is not limited to the scope of the funded project within a 2–3 years' time frame. It's not about the number of workshops that we develop employing a multi-actor approach, and it's not a sign of success when we have a high number of diverse stakeholders in the room. Our approach to stakeholder engagement is the development of a long-term (at the moment we have an 8-year-long dialogue platform working), evolving dialogue between academia and all stakeholders implicated in the Montado sustainability. Our financed projects, even though with different specific agendas and goals,

contribute as they allow this dialogue to evolve where some questions are answered and others are posed in an iterative cycle as illustrated by Pohl *et al.* (2017), in Figure 2.

In Figure 2 the white arrows illustrate the iterative nature that resonates with our own approach. The large round arrows denote the intersection between scientific knowledge and a societal problem handling, while the smaller round arrows illustrate the dynamics occurring independently on each side. The main transdisciplinary steps are identified as 1<sup>st</sup> problem framing, 2<sup>nd</sup> problem analysis, and 3<sup>rd</sup> exploration of impact. As explained by Pohl *et al.* (2017), projects progress through the stages in varying sequences (thin straight and angled arrows in Figure 2). During these stages researchers of different disciplines collaborate and involve societal actors in joint research and learning experience. The intensity of collaboration and involvement is functional-dynamic, i.e., it varies depending on the purpose of the specific stage. This process involves balancing two rationalities (thought styles): the scientific pursuit of truth and the practical emphasis on workability.

An example of how we are implementing a long-term transdisciplinary process is through the Tertúlias do Montado initiative (Guimarães *et al.*, 2024), which has been running since 2016. The Mediterranean Institute for Agriculture, Environment and Development (MED) has been conducting multi- and interdisciplinary studies on the Montado for over 20 years. At a certain point, resources were in place to initiate a transdisciplinary approach, and our hypothesis is that transdisciplinarity implies a mindset that is not immediate and should be framed within a medium to long-term strategy. Tertúlias do Montado has evolved into a stable space for dialogue, functioning as a permanent problem-framing venue (see Figure 3; Guimarães *et al.*, 2024). Back in 2019, 45% of 100 of the participants of Tertúlias do Montado indicated that they changed practices because of their engagement in Tertúlias do Montado and 60% of these participants are farmers (Guimarães *et al.*, 2024).

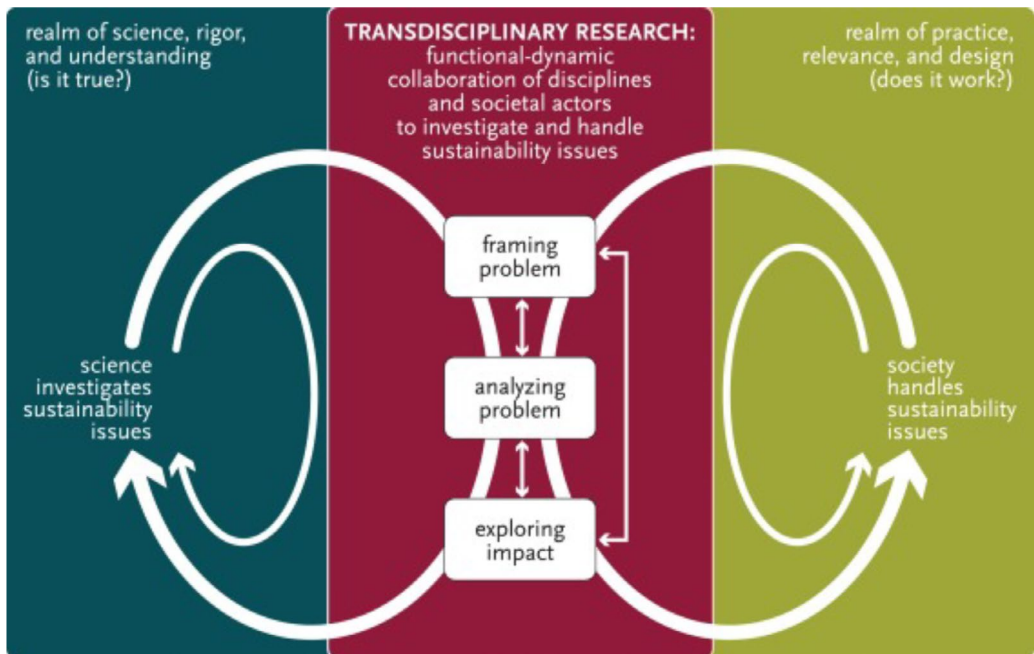


Figure 2. The transdisciplinary research process as described in Pohl *et al.* (2017).

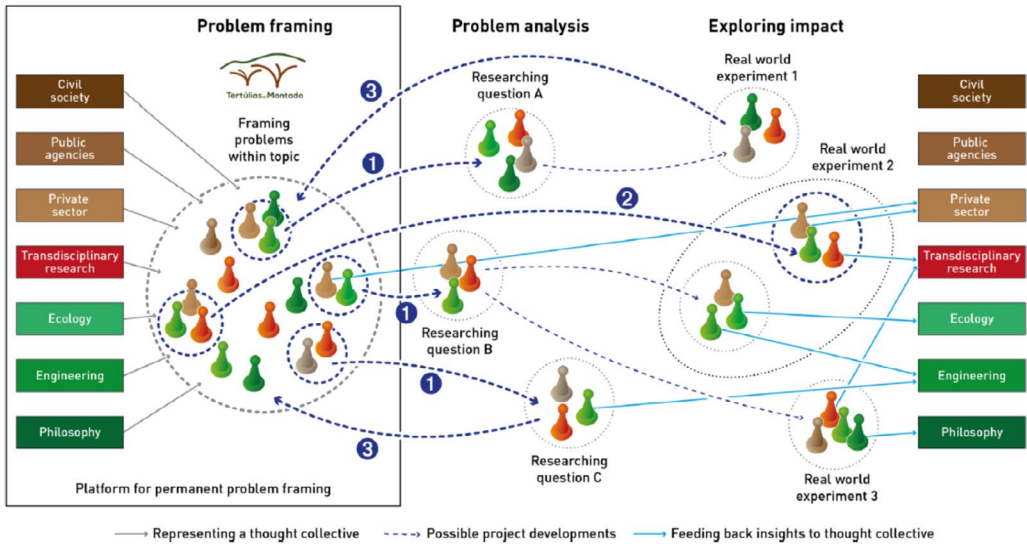


Figure 3. “Tertúlias do Montado” as a permanent problem framing platform where researchers of different disciplines or interdisciplinary fields and practitioners of different sectors related to the Montado jointly frame the Montado sustainability problems (from Guimarães *et al.*, 2024). From this 1<sup>st</sup> phase, different pathways can be developed, moving to problem analysis phase (1) or directly to exploring impacts (2). The inverse pathway is also possible as “Tertúlias do Montado” is an open platform, so groups working in the analysis or impact phases can also come back to problem framing (3) in “Tertúlias do Montado”.

How is stakeholder engagement understood in the readers context? We encourage you to adopt a long-term perspective for stakeholder engagement and to devise strategies to bridge the gaps between financed projects, fostering a collective discourse within the dialogue processes you initiate under specific projects. Rather than solely planning stakeholder engagement around project objectives, consider an alternative approach: contemplate how financed projects can contribute to addressing broader issues that are crucial within the context of your efforts to combat grassland decline in the territories you are focused on.

## Governance models

Creating communication channels is important but not sufficient. Understanding existing governance models and integrating new communication channels into current models is essential (Pinto-Correia *et al.*, 2021). Simple designs such as private property, government ownership, or community organizations are not adequate solutions for the governance of the complex problems we face today. Relying solely on governments, self-governing networks, or market relations does not appear to be the appropriate pathway. Learning to live with uncertainty and discovering new governance mechanisms and institutional arrangements may enhance resilience. Despite the importance of various combinations of networks and market relations in governance, a broader range of interactions aimed at securing collective interests needs to be considered, including interactions between public and private actors. From this perspective, attention must be given to the art of steering interactions and establishing the foundations for the complex set of relationships that emerge from governance models. Governance mechanisms can foster innovation and facilitate the processes of transition towards sustainability that are necessary (Bernard *et al.*, 2023; Luján Soto *et al.*, 2021). Therefore, the contribution from social sciences is of utmost importance (Pinto-Correia *et al.*, 2021).

In summary, it's not just about who the stakeholders are; it's also about how they interact and where interactions are needed but are not occurring. In the Montado case, we realized that policymaking at the

European and national levels was a clear and key leverage point (Guimarães *et al.*, 2018; Pinto-Correia, 2021). Therefore, our efforts have also focused on policymaking that influences the sustainability of Montado. Previous studies show that the implementation of CAP in Portugal has led to antagonistic dynamics within the system and is closely related to the declining trend of the Montado area and tree density (Azeda *et al.*, 2021; Guimarães *et al.*, 2018; Pinto-Correia and Azeda, 2017). We need policies that take a holistic approach to the Montado and a strong and integrated AKIS (Agricultural Knowledge Innovation Systems) in Portugal. In our country, the heavy bureaucratization of agricultural policy has induced specialization of farmers' associations in dealing with paperwork, and support to farmers is, in many situations, reduced to bureaucratic issues (Pinto-Correia *et al.*, 2019).

Back in 2017, we started the development of a results-based model (RBM) aimed at contributing to:

1. a better understanding in policy of the Montado as a SES that integrates agriculture, forestry and livestock production within the same space and time.
2. the co-responsibility between farmers, science, the administration, and farmers' associations, working together towards environmental results.
3. the development of tools that can be used by stakeholders to understand and track the ecological status of the Montado.

We started with a cross-fertilization visit to the Burren Programme in Ireland (Ferraz de Oliveira *et al.*, 2019). In the Burren, rural entrepreneurship builds on the unique characteristics of local landscape, which is internationally recognized for the richness and diversity of its heritage and flora. In this context, the producer plays the role of guardian of the existing natural and cultural values while maintaining an economically viable business model. In June 2018, a group of Montado farmers, researchers, and public administrators, a total of 20 people, visited the Burren in Ireland (Figure 4). The objective was to gain knowledge on the experience of locally co-constructing results-based agri-environmental measures through a multi-actor approach and to discuss possible problems and applicable solutions that could be transferred to the Montado. The expectations were to learn about the involved actors and institutions and to see in loco the implementation experience of a results-based approach supporting sustainable agriculture.

On the last day, we collectively decided to proceed with designing this model for the Montado case. To develop the necessary work, we defined a step-by-step procedure (Figure 5; Pinto-Correia *et al.*, 2022). Each step involved a sequence of interactions considering the roles of each type of stakeholder.



Figure 4. The group discussing the application in the Burren with local farmers.



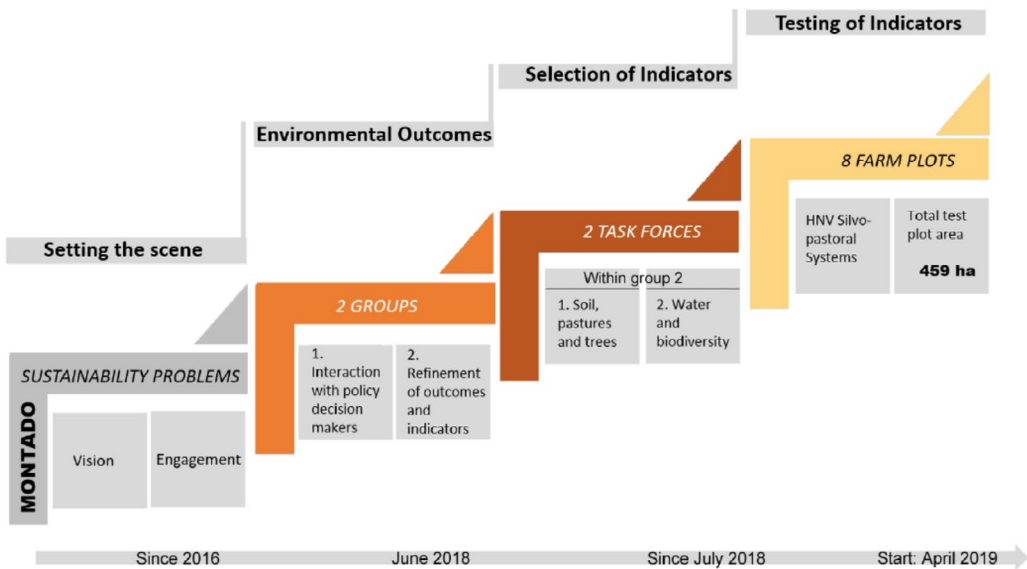


Figure 5. The stepwise approach used for the co-construction and testing of a RBM for the Montado (source: Pinto-Correia *et al.*, 2022).

Decisions and updates among the different working groups were undertaken in a TD arena developed for this specific purpose (Figure 6). The TD arena was designed from the beginning with multiple tiers and different responsibilities in mind (Figure 4); its structure proved vital in the process of constructing the RBM. The core research team, composed of scholars from various backgrounds working in an interdisciplinary research unit on the outskirts of the case study area, was responsible for coordinating the TD arena, including inviting stakeholders to participate. This core team also managed the work that enabled the identification of the environmental results. Other experts serving as project consultants intervened when the main research team needed to secure the scientific validity of proposals. The TD arena included a group of land managers and owners who presented their preferences, concerns, and practical experience. Public administration officers oversaw the setting of boundaries and addressed the administrative issues that such a program would bring to the current governance paradigm. All decisions were made collectively within the TD arena.

There might be many other combinations and ways of developing an RBM; the one we developed worked and it represents another effective transdisciplinary effort. Today, we are scientifically coordinating a pilot agri-environmental payment program under the current implementation of the CAP in Portugal. This is the first time in Portugal that a research unit has been given this responsibility. It represents a paradigm shift in policy design at the interface between science and policy. We are working with 184 landowners and managers to improve environmental results across 6500 ha of the Montado.

### Integration of experts and expertises

There is no question about the importance of discipline and interdisciplinary studies in the development of the examples we have referred to. Without the scientific knowledge accumulated so far, we could not have made the efforts and scientific contributions listed in the present article. However, it is important to highlight that within our team, we have what is currently being explicitly described in academia as Integration Experts and expertise. These experts lead, administer, manage, monitor, assess, accompany, and/or advise others on integration within inter- and transdisciplinary projects or programs (Hoffman *et al.*, 2022). The importance of this role is evident in many communities and their achievements. The lack of recognition and resources devoted to this role is also the reason why some efforts that attempt to

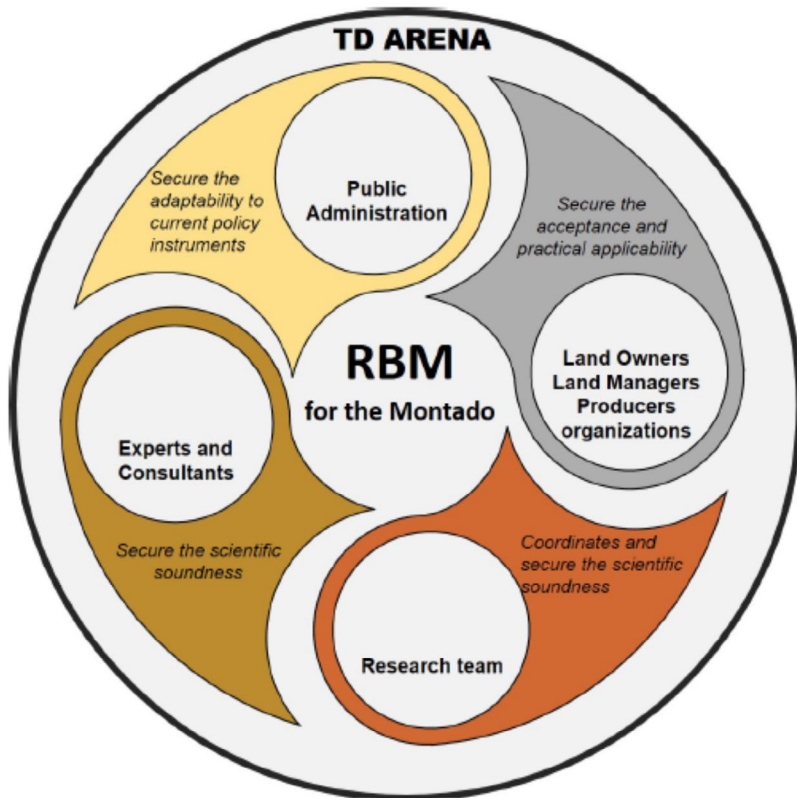


Figure 6. The design of the transdisciplinary arena around the development of a Result-Based Model (RBM) for the Montado. Source: Pinto-Correia *et al.* (2021).

develop these types of approaches fail (Guimarães *et al.*, 2018; Hoffman *et al.*, 2022; von Wehrden *et al.*, 2019). Hence, we take this opportunity to encourage colleagues who focus on the study of grassland from a natural science perspective to secure the allocation of resources for integration expertise.

As mentioned before, it's not just about gathering stakeholders and different disciplines. To address complex issues that require collaborative efforts from people with diverse backgrounds, values, interests, and perceptions, you need competences, tools, and methodologies that enable effective collaboration. Several organizations and networks around the world are advocating for the formalization of integration experts and expertise in academia (Hoffman *et al.*, 2022). They investigate the nature of cross-disciplinary integration, detail the challenges inherent in leading integration, explore the necessary expertise for addressing these challenges, and compile methods and tools that support the overall process of integration and implementation. Building on the work developed by Hoffman *et al.* (2022), we support the idea that Integration Experts and expertise are critical, in colloquial terms, for closing the gap between 'talking the talk but not walking the walk' (Åm, 2019: p. 171). We not only support the idea of Integration Experts, within our team we have integration experts and we are developing such type of expertise within our team members.

## Conclusions

Returning to the question posed in the title of this contribution: The intricate pathway for the future of grasslands, who comes first, people or policy?



Grasslands are socio-ecological systems that require holistic approaches to address current sustainability problems. Stakeholder engagement and transdisciplinary approaches should be explored. Engaging people needs to be coupled with a systemic understanding of the context that shapes their decisions and finding the leverage points that can induce change. This is a long-term process based on increasing mutual trust, between researchers and farmers. In our case study, policy plays a key role and there is some room for manoeuvre for more adaptive policy tools. In our future work, we aim to take advantage of the opportunity we have in working directly with public administration to gain an in-depth understanding of how policy implementations can be simplified and more goal oriented. This would allow more time to be spent working towards solving the decline of the Montado and less time on paperwork and ensuring that rules are respected.

## Acknowledgements

The authors would like to greatly thank the participants of Tertúlias do Montado, without their commitment and interest, this initiative would have never been possible. Special thanks to all land managers, experts, and administration officers that are actively participating in the construction process of the RBM. This study was also partially funded by National Funds through Foundation for Science and Technology (FCT) under Project No. UIDB/05183/2020 (MED).

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# Putting grassland at the heart of animal farming

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## Abstract

Grassland production is at the core of farming on Skogsgård farm in south-west Sweden. This organically certified 400 ha farm has been run by Anna and Anders Carlsson since 1995. Half the land is owned, half is rented. Rotational grazing is applied at least six months a year for the farm's cattle. The main feed during winter is silage from grass and whole crop cereals. The farm's 220 Holstein and Fleckvieh dairy cows have access to temporary grasslands close to the barn via paved pathways. Young stock graze more distant semi-natural grasslands, including three nature reserves. Two-year silage leys are included in the crop rotation and all crops are used on-farm as animal feed. Actions taken to increase resilience on Skogsgård include building irrigation ponds, changing to animal breeds more suited to grazing and forage-based rations, purchasing machinery to optimise harvesting and, most importantly, adapting grazing management for the cattle. Knowledge capital on the farm has been extended via different networks in Sweden and abroad, not least EGF meetings. Future plans include investments in milking robots and a cubicle house and, hopefully, a generation shift. Grasslands will increase in importance in future, e.g. for carbon sequestration.

**Keywords:** carbon sequestration, crop rotation, grazing management, knowledge and innovation systems, multi-species swards, resilience

## Introduction: Skogsgård farm

Our vision at Skogsgård is to maintain a family farm business producing large volumes of high-quality food in an efficient way. At the same time, we aim to support natural processes and enhance biodiversity, coping with and adapting to climate change.

Skogsgård (56°49'14.52" N, 12°44'37.1" E) is located in south-west Sweden, around 10 km from the coast, at average elevation of 28 m a.s.l. The conditions for grassland production are good, with on average 900 mm precipitation per year and 150 days per year with precipitation, of which 50 days are with snow. Mean annual temperature in the local region is 7°C (monthly maximum 20°C, monthly minimum -4°C) and the length of the growing season is 220 days (SMHI, 2024). In 1992, dairy cows were introduced again after a break of 30 years with other animals on the farm. Anders and Anna Carlsson have been running the farm since 1995, at which time they converted to organic production. Anders is a trained carpenter and has also several certificates from agricultural college. With the help of his family, he has employed these skills to build a free-stall barn. Anna has a Master's degree in agriculture specialising in crop cultivation and worked for some years as a farm adviser. The farm now has approximately 220 dairy cows, mostly Holstein and Fleckvieh breeds. Some are crossed with breeds such as Limousin, Charolais and Aubrac to make beef production more efficient. Some purebred Fleckvieh bull calves are kept for breeding and some are raised for slaughter. Changing from purebred Holstein to a variety of breeds with different purposes is part of the strategy for better utilisation of local conditions on the farm. Calving is restricted to two periods per year, October-December and March-May, in order to produce as much milk on grass as possible, but still use the buildings in an optimal way. The dry period of our cows is at the end of the grazing season or in winter, reducing our need for high-quality fodder.

Since 2020, the dairy coop Arla has been calculating our on-farm climate footprint. Through these calculations, we have made interesting observations. In 2021, when we had a high proportion of roughage in the feed ration, yield was 8200 kg energy-corrected milk (ECM) per cow. We used 1.6 m<sup>2</sup> of land to produce 1 kg of ECM; this corresponds to 6250 kg ECM ha<sup>-1</sup> year<sup>-1</sup>. In 2022, we increased the proportion of cereals and faba beans in the feed ration and the cows produced 9200 kg ECM. However, land use increased to 1.9 m<sup>2</sup> (kg ECM)<sup>-1</sup> and milk yield ha<sup>-1</sup> decreased to 5260 kg. Our conclusion is that it is more land-use efficient and economically viable to have a large proportion of forage in the feed ration than low proportions. We can achieve higher ECM yields per area with grazing and silage than with cereals and faba beans.

The farm comprises about 400 ha, half of which is owned and half rented. Of this, 35 ha are unploughed semi-natural grasslands. The soil type is very varied, from sand to heavy clay, but is mostly silty loam. The region is part of a nitrate-sensitive area near the Kattegat Sea, and special regulations apply (Swedish Board of Agriculture, 2023). Our goal is to have as much green cover as possible during the year. Spring ploughing is preferred if the soil is not too heavy. The farm has a lot of cover crops, preferably for grazing. Spring- and autumn-sown cereals and legumes, e.g. peas and vetches, are grown for feed on 100 ha. The crop sequence is under constant development. A typical five-year crop rotation is: ley undersown in spring barley, ley I, ley II, oats with peas/vetches and winter wheat. We keep some of our animals on deep litter and some in cubicles, producing slurry. The deep litter manure is applied to cereal crops or as compost on temporary grasslands in late summer, contributing to a rich soil biota. The slurry is mainly used for the leys and winter cereals. Under organic regulations, the farm is allowed to use potassium sulphate (K42:S18), which benefits grass and clover in both cut and grazed temporary grasslands. The farm also buys straw and receives horse manure for compost bedding in the barn. All soils are limed regularly with crushed limestone to increase the pH from 6.1-6.3 to 6.8, to increase the availability of soil nutrients. There are five full-time employees on the farm and some interns during the season, in addition to family members (three at the moment).

## Different grassland categories

Grassland production is believed to be the best option for providing the organic milk and beef animals on the farm with energy and protein. Grassland is also a safe crop in both dry and wet weather. The farm has four different categories of grassland, all requiring different inputs and different management approaches, but together they provide rations for the animals throughout the year. These categories are: leys on temporary grasslands, grazing on temporary grasslands, semi-natural grasslands and nature reserves (Peeters *et al.*, 2014).

### *Leys on temporary grasslands*

Ley is included in the crop rotation. The species included are red clover (*Trifolium pratense* L.), white clover (*Trifolium repens* L.), timothy (*Phleum pratense* L.), perennial ryegrass (*Lolium perenne* L.), chicory (*Cichorium intybus* L.), caraway (*Carum carvi* L.) and plantain (*Plantago lanceolata* L.); i.e. a multi-species sward. The aim is to include a large proportion of red clover to achieve a large protein yield but the ley is not allowed to remain in place for more than two years, due to problems with root rot (Wallenhammar *et al.*, 2008). It is normally harvested four times a year, yielding 8-9 tonnes ha<sup>-1</sup> year<sup>-1</sup>. White clover is included as a complement to red clover and is good at closing gaps in the sward. Last year, we introduced two seed mix strategies to better exploit the potential of red clover by optimising fertilisation: (i) 28% red clover, 5% white clover, 28% timothy, 32% intermediate and late tetraploid perennial ryegrass, 3% chicory, 2% caraway and 2% plantain (total seed rate 21 kg ha<sup>-1</sup>) and (ii) 18% white clover, 37% timothy, 37% perennial ryegrass, 5% caraway and 3% plantain (total seed rate 19 kg ha<sup>-1</sup>). Legume proportion at harvest ranges between 300 and 700 g (kg DM)<sup>-1</sup>.

The late developmental rate in both timothy and intermediate diploid and tetraploid perennial ryegrass makes them similar to red clover. The sward is permitted to grow until early heading of perennial ryegrass and early flowering of red clover before the first cut, which is in early June. Timothy gives the largest yield at the first cut, while the perennial ryegrass produces a more even yield over the season and dominates over timothy by the fourth cut, which is taken on 25 September, approximately. Herbs (chicory and caraway) have been included in the ley for many years, and also plantain in recent years. These have different rooting depths and thus give more efficient nutrient uptake, soil structure improvements, increased mineral content in forage and supplementary growth over the season. The chosen herbs are easy to establish. Chicory performs similarly to red clover in the sward with respect to persistence. Caraway is a biennial species which thrives in cold conditions in spring. Plantain is drought-tolerant and seems to persist for at least two years when cut and even more when grazed. The ley is often undersown in spring, in spring-sown barley or oats. Whole-crop silage is used as a safe system to establish the ley, followed by one or two ley cuts in autumn. In recent years, the farm has also tried sowing pure stands in spring, followed by mowing, which gives very good sward establishment even in dry years.

The farm has its own ley harvesting machinery, in order to secure access and to be flexible to changes in weather. The mower is 6 m wide, while tedder and rake are 12 m. A self-propelled chopper to which wagons are connected is used, in order to reduce soil compaction. The contracted slurry tanker is also 12 m and for the past year slurry injection has been performed.

#### *Grazing on temporary grasslands*

The cows graze close to the barn, mostly on temporary grasslands. New roadways now provide access to 98 ha for grazing, with 1.8 km to the farthest pasture. These paddocks are reseeded when needed, i.e. after 4–10 years when the total grass growth per season is declining. In recent years, these pastures have been established as pure stands without cover crop for a dense sward, allowing grazing as soon as possible. The seed mixture consists of 12% white clover, 24% timothy, 58% intermediate and late tetraploid perennial ryegrass, 2% chicory, 2% caraway and 2% plantain (total seed rate 29 kg ha<sup>-1</sup>), so that the temporary grasslands for grazing provide a ‘mixed salad’ for the cows, for good palatability, supplementary growth over the season, good mineral content and good soil structure (Figure 1).

The cows are let out in mid-April and the ‘magic day’ is around 8 May, which coincides with dandelion (*Taraxacum* sec. *Ruderalia*, Kirschner, *Ollgaard* and *Štěpánek*) bloom. After this ‘magic day’ the pasture produces more than the cows can consume. During mid-season, the goal is to supply only a few kilograms of concentrate in the milking parlour and to consume the rest of the dietary requirement on pasture. In dry periods, the amount of concentrate is increased and supplemented with silage. In very wet grazing periods, the grain supply is increased to ‘dry up’ the rumen. The cows are given a new paddock after each or every second milking occasion, depending on the size of the paddock. Paddock size is adjusted with plastic-coated electric fencing on reels, which is easy to erect. The grazing is also managed so that the cows only have to walk far to a paddock once a day. Biomass yield is, on average, 8 Mg ha<sup>-1</sup> from the eight rotations per season.

All fields are walked once a week and pasture height is measured with a plate meter (‘Grasshopper’; True North Technologies, 2023), a service from Ireland to which the farm subscribes because a similar system is not available in Sweden. Grass samples are cut, dried and weighed to calibrate the plate meter and achieve the correct amount of dry matter yield. As the farm has multispecies leys the sward can be somewhat uneven, especially in early summer, when the species are heading/blooming. However, the plate meter has been found to be sufficiently precise to give a grass wedge showing expected grass growth (Figure 2). The goal is to graze when the perennial ryegrass has 2.5–3.0 leaves (Agriseeds, 2017), depending on season (Figure 3). This method works well even though there are several species in the sward.



Figure 1. A grazed multi-species sward at Skogsgård in late summer. Photo: Anders Carlsson.

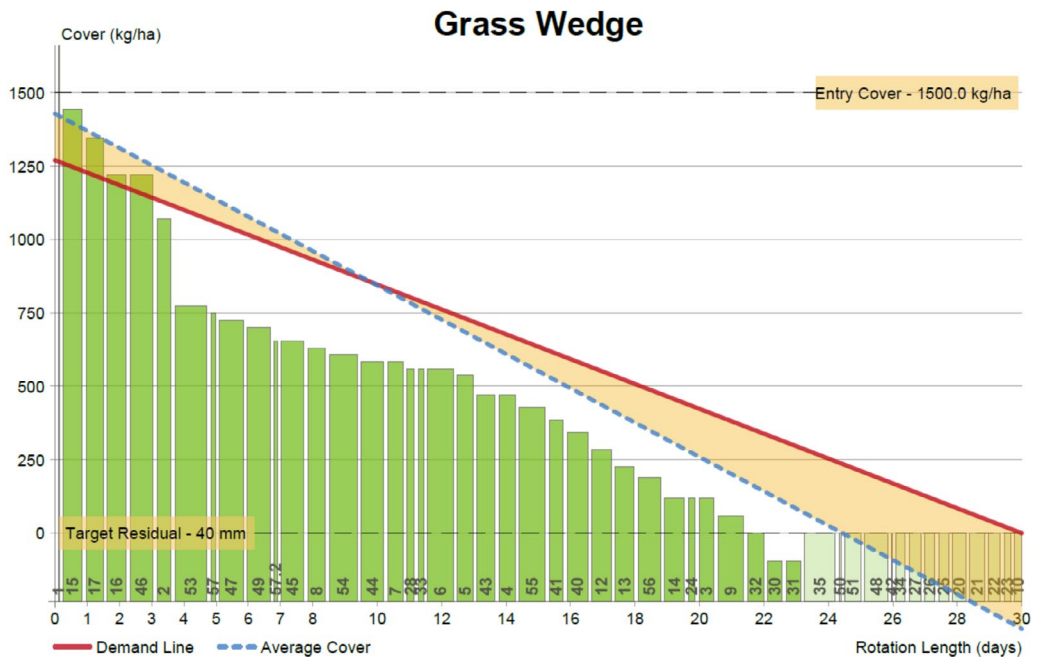


Figure 2. Grass wedge from 24 July 2023 at Skogsgård farm (True North Technologies, 2023). The image shows the deficit of dry matter yield in many paddocks after a long period of drought. Paddock identification number is included in the bottom of each bar.



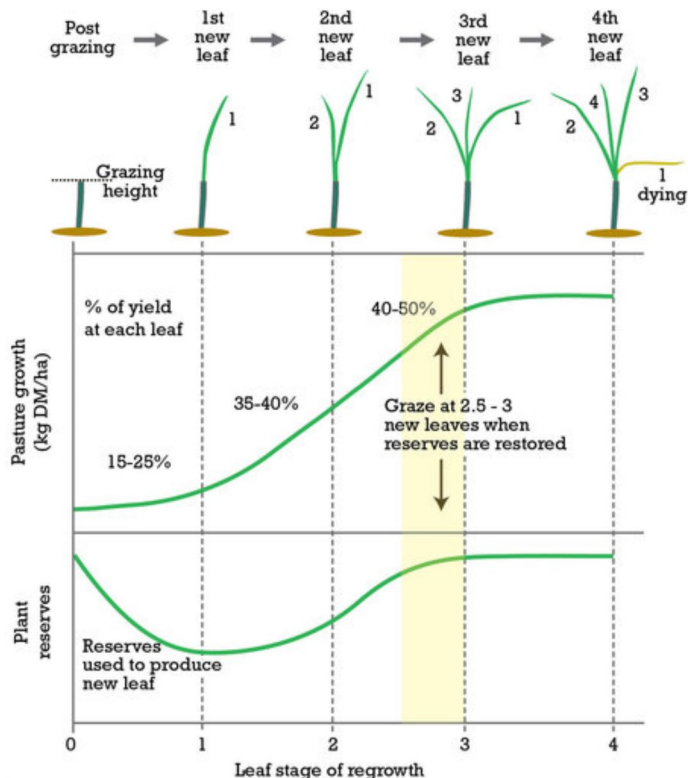


Figure 3. Regrowth, energy reserves and yield per leaf in perennial ryegrass (Agriseeds, 2017).

### *Semi-natural grasslands*

In south-west Sweden, many farmers have given up cows and thus semi-natural grasslands that cannot be ploughed are becoming abundant. It is often difficult and expensive to rent/buy arable land, but pasture is cheaper. However, it usually requires some restoration, as many pastures are overgrown with scrub. In-calf heifers on Skogsgård are usually driven to leased pasture 30 km from the farm, with daily stock supervision provided by the owners of the land. The lot comprises 30 ha with silvo-pastures and both semi-natural and temporary grasslands and the farm's 100 heifers have access to nine paddocks with 3–4 days per paddock, a system that has been developed over time. Twenty-five years earlier there were only two paddocks, but while improving grazing management for the farm's dairy cows, the heifer grazing systems were also refined. If the sward grows too fast for the heifers to graze, one or more paddocks may be harvested, where possible, but the goal is to avoid this. Therefore, the heifers are let out gradually in the spring, preferably before growth starts, giving the time to graze better. The rotation system has led to better growth in the heifers, while at the same time making it possible to have more animals on the same area. It also resulted in better grass growth and grass quality. Through hard grazing in each rotation, the forage plants are revitalised, with fewer stems and less rejected patch of grass, and weeds are kept in check. The paddock is then grazed again when three leaves have emerged (Agriseeds, 2017) (Figure 3). This system has resulted in better grass growth and grass quality.

In recent years, wild boar and fallow deer have damaged and grazed the pastures. In 2023, which was a dry year, it was only possible to graze 70 heifers at best, instead of the normal 100, and around mid-summer only 40 heifers. The land owners have been consulted about hunting to control numbers, but this is not an easy task.

### *Nature reserves*

There are nature reserves on some land which the state bought from the farm, because it had valuable fauna or flora or high cultural and historical values. At the moment, three different nature reserves are grazed. Traditional semi-natural grasslands in Sweden are among the most species-rich in the world, but are now often overgrown with scrub. Shade by pioneer trees and bushes means that the grasslands are depleted of diversity. When the scrub is cleared, the species richness of flora and fauna increases. Grass production also increases, presumably increasing carbon sequestration in the soil.

Under organic farming regulations, the farm is not allowed to deworm animals as a preventative measure. First-time grazers therefore need to be let out onto ungrazed land for at least one year, to avoid getting parasites. The strategy on the farm is to let out dairy and suckler cows with two to three calves each on semi-natural grasslands. Foster calves can usually cope with parasites because they gradually acquire immunity. The calves are reared for replacement, breeding or fattening. The cows and calves are moved around in a paddock system, even on the semi-natural grasslands, in order to obtain good-quality pasture and to allow the pasture to grow between the grazing periods. The cows and calves are let out early in the spring, after accepting each other in the barn (Figure 4). More animals are let out as the sward grows. The calves follow the cows and thereby learn to graze and follow the routine of moving between paddocks. At weaning in autumn, the cows are gradually removed and the calves remain in their familiar environment. When grass growth declines, they are moved together with other young stock onto new parasite-free land. In addition to displaying good growth during the summer, these calves also grow well in the following winter while in the barn. The farmers enjoy performing these daily stock-handling routines and have also received a very positive response from neighbours, who appreciate this form of calf rearing.



Figure 4. Dairy cows with foster calves grazing semi-natural grassland. Photo: Anna Carlsson.



## Increased resilience

Being a dairy farmer is very complex. Apart from the challenges of running a business, the farmer also needs to consider the economic, ecological and social sustainability perspectives. A farm is like a spider's web in which everything is connected (animals, machines, buildings, work) and when one thread is pulled it has an effect somewhere else. It can take time to implement change of a more comprehensive nature. A farm needs to provide sufficient turnover, not only for daily expenses such as staff, concentrate, diesel, interest rate, but also for large future investments.

In recent years, Skogsgård farm has found that it is becoming increasingly important to create resilience on various levels. This includes adapting the stock to have animals that are able to grow and produce milk on pasture and forage. Having enough own land for fodder production is another important measure, as in some years it is difficult to buy organic fodder because of shortages. Even though the south-west is the rainiest part of Sweden, prolonged dry periods can occur, so the farm has built ponds to collect water in winter and has invested in an irrigation system, especially for cow pasture. Good roadways to the pasture support cow traffic even during very wet periods, enabling good grazing management.

## Knowledge transfer and innovations

Over the years, the Carlsson family has been involved in various trust assignments. Among other things, Anna has been a board member of Halländska Vallföreningen, a regional forage and grazing association, and chairman of the Swedish Grassland Society. This has provided good insights into current issues in the grass production area and into research being conducted around Sweden. It has also led to study tours to other countries and participation in a number of EGF conferences. Being able to meet people from other countries with different grassland conditions has been very rewarding in terms of exchange of experience and take-home messages. As 'Facilitator Agent' in Inno4Grass, a Horizon 2020 project in which the Swedish Grassland Society was a partner, Anna had the task of mediating and promoting innovations by farmers within and between the participating countries (Inno4Grass, 2019). Trips and study visits have provided many experiences and it is good to be curious and open-minded. However, it is often best for a farmer to find their own new knowledge and technology, as it is difficult for others to know the specific needs on a farm. Care is needed when copying a method seen elsewhere, as it is easy to miss a small but essential aspect.

A strength of Skogsgård farm is that it can quickly adapt an idea or action to its own production, and invest time, money or work if the idea appears good and realistic to implement. Researchers need to find funding to test and implement an innovative idea, but if a farmer develops an innovation it will not be verified or recognised until confirmed by research.

Interest in grassland production and grazing has led to many study visits to Skogsgård farm by agricultural colleges, advisers, authorities and researchers. During such visits, Anders and Anna consider it important to show some simple examples of how to improve production, but also to highlight that much underlying knowledge and timing are required. They often receive positive feedback for thinking 'outside the box' and believe that adapting old and proven knowledge can create new systems. The best visits are those from members of the public, who often have a positive view of dairy farmers but are so distanced from production that they do not know how food is produced. Seeing their eureka moment when they understand that there is a connection between earthworms in the soil, grass, the cow and milk is very satisfying. The most important lesson for them is that farmers want to produce good food and that they care deeply about their animals and the environment.

## Future perspectives

Anders and Anna Carlsson have four children between the ages of 19 and 26, three of whom have chosen to study agriculture. They are involved in farm operations when possible, together with studies and jobs on other farms or internships abroad. One daughter works as an adviser in dairy production and has moved to her partner's family farm 100 km away. If the others want to take over the farm in future, it is important to start this generational shift in time. It is a complicated matter because much capital is tied up in the farm and the children who do not take over must also be compensated. When the next generation takes over, they must be given the opportunity to run the farm in their own interest and with their own ideas. Being able to create their own production solutions will give them the energy and the will to persevere with animal farming.

There are plans to install milking robots on Skogsgård. In Sweden, milking robots are generally placed indoors and it is difficult to combine robotic milking with keeping dairy cows on pasture. However, innovative solutions have been developed in other parts of Europe (Samsom, 2021). For example, in the ABC system three paddocks per day are provided so that new pasture attracts cows to the robot (Lely Center Mullingar, 2024). Logistically, it is beneficial to have a robot unit out on pasture and thus reduce the distance between milking point and paddock. In winter, the same robot can be used in the barn, enabling robot milking all year round and ensuring that milking is done in the same way every day. Robot milking decreases the need to find trained and experienced staff, which is becoming increasingly difficult. Today, the farm often needs to train staff in milking and they often come from other countries, so it is difficult to know how long they will stay. For several years, the farm has recruited some young people from the area, who come for a few years and work extra on weekends and holidays, as a useful complement to the regular employees.

The future ambition for Skogsgård farm is to continue developing the grazing management system. One possibility is to introduce virtual fences, which would eliminate the need to erect temporary fences. If the system proves fully trustworthy, it may be possible to avoid physical fences altogether. It would also provide greater opportunities to graze catch crops on fields that are not normally grazed. Monitoring grazing animals and assessing grass growth with drones is another possibility, as it would provide instant information on whether the animals need to be moved without disturbing their grazing. However, in order to promote such developments and innovations in agriculture, laws and regulations need to be adapted and simplified to reduce the fear of making mistakes and receiving sanctions. Letting farmers, advisers, researchers and other stakeholders co-create guidelines may be a good way forward. It is also important that farmers have the time and opportunity to participate.

For several years, Skogsgård farm has made climate calculations and has mapped emissions from production. However, there is still a need for a more well-developed and accurate system demonstrating the benefits in carbon storage in a grassland-based system.

## Conclusions

Grassland can be successfully managed in many different ways. Knowledge and innovation can be captured in the neighbourhood, but also worldwide. This paper covers some of the ecosystem services provided by Skogsgård farm's grassland-based integrated animal and crop production system. Better understanding is needed of the system's other values and its role in the landscape and in society. The example of Skogsgård shows that it is possible to produce high-quality food while at the same time coping with economic, ecological and social challenges in order to create a more resilient system.

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# Stakeholder perception of nutrient-poor meadows in the Trudner Horn Nature Park (South Tyrol, Italy)

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## Abstract

The management of nutrient-poor meadows (NPM) in the Trudner Horn Nature Park (South Tyrol, Italy) has a long tradition. Typically, this ecologically very valuable grassland is unfertilised and mown once a year or every second year. The aim of our study was to find out how different population groups evaluate NPM visually in comparison to other, more intensively managed meadows (IMM). For this purpose, a structured online questionnaire was used to interview grassland farmers, apple and wine growers and people not employed in the agricultural sector. The respondents were asked to evaluate the aesthetic value of landscape pictures of both grasslands at 3 different phenological vegetation stages: start of the growing season, growing season before flowering, and flowering. Higher ratings for NPM were observed for all groups at the flowering season, with the grassland farmers rating IMM lower than the other groups. The farmers also expressed a higher appreciation for pictures at the start of the growing season than the other groups. This indicates that grassland farmers have a higher aesthetic preference for grasslands when elements like flowers are missing and grassland biomass is low. Overall, they differentiate more strongly between NPM and IMM.

**Keywords:** aesthetic value, landscape appreciation, occupation, farmers, vegetation phenology

## Introduction

Nutrient-poor meadows (NPM) developed at grassland sites with low soil nutrients content due to a lack of fertilisation and are only mown once a year or every two years. A characteristic and rich flora and fauna, adapted to these conditions, has developed over the centuries. For nature conservation reasons it is important that NPM are recognised and appreciated by the farmers and also by the general public, so that that citizens agree with their protection in the long term through financial support. A positive public attitude towards species-rich swards can be achieved through visual stimuli, among other things (Fischer *et al.*, 2020). In this study, we addressed the question about the visual preference of NPM in comparison to more intensively managed meadows (IMM) depending on the professional background and on the phenological stage of the meadows.

## Materials and methods

A questionnaire was created with Microsoft Forms, within the frame of which the respondents were asked to rate on a 5-point Likert scale (from 1=I don't like it at all, to 5=I like it very much) 14 pictures of meadow landscapes (for the complete questionnaire, see <https://data.mendeley.com/datasets/kzt2x3s273/1>). Three pictures depicted IMM and two NPM at the start of the growing season (SGS). Pictures of the same five meadows were also taken later in the growing season before flowering (GBF) and two pictures each for IMM and NPM (different from those taken at SGS and IMM) at the full flowering stage (FLO).

The questionnaire was distributed by the South Tyrolean Farmers' Association (Südtiroler Bauernbund) and the South Tyrolean Mountain Farming Extension Service (BRING). Moreover, the link was shared in

Facebook groups belonging to 13 municipalities, of which five partially fell within the Nature Park Trudner Horn (South Tyrol, NE Italy) and the others border on these municipalities. Furthermore, the link to the survey was shared via WhatsApp with the social network within the abovementioned municipalities.

The answers from three professional groups (GR=grassland farmers, FW=fruit and wine growing farmers, NA=non-farmers) were collected and used for the analysis. The appreciation of different pictures was analysed via a linear mixed model. The dependent variable, which is in principle ordinally scaled, was handled as a metric to account for the complexity of the design. The dependent variable was the appreciation rating (5-point Likert scale) given to each image. Three factors were investigated: the vegetation phenology stage, the type of meadows, and the occupation of the respondents. The statistical model included the main effects and all their possible interactions as fixed terms. Type III sum of squares, estimates by Restricted Maximum Likelihood and Satterthwaite approximation of the denominator degrees of freedom were used. Moreover, the respondent was included as a random term, in order to account for the correlation between ratings of different pictures provided by the same person. Multiple comparisons between estimated marginal means were performed by Least Significant Difference (LSD). Normal distribution of the residuals and variance homogeneity were visually checked by means of diagnostic plots. A significance level of 0.05 was used for all tests. All statistical analyses were performed by IBM SPSS Statistics 29.1.1.0 (171).

## Results and discussion

The respondents provided 245 valid responses (GR 56, FW 55, NA 134). All main terms of the three factors (phenology stage, type of meadows, occupation), their two-way interactions and their three-way interaction ( $P=0.009$ ) were found to affect the landscape perception (Table 1).

The landscape appreciation always increased from a sub-neutral or neutral level at SGS to an intermediate level of perception at GBF (Figure 1). GR was the group that generally rated the vegetation at SGS better, and the only one that rated IMM and NPM differently at this stage (IMM=3.3, NPM=2.9). At the GBF stage, all respondents' groups consistently rated IMM slightly better than NPM (about +0.2 on average). Whilst the perception of NPM further increased among all groups and approached its highest level, that of IMM slightly increased for NA, remained unchanged for FW, and it even decreased for GR. Therefore, the difference in the perception between IMM and NPM was highest for GR. As already shown by Junge *et al.* (2015), the level of appreciation of the different pictures in this study also generally increased with the progress of the phenological development up to flowering. This increase is likely to be linked to the increasing green tones in the vegetation and then to the appearance of different coloured flowers. However, the results suggest that grassland farmers took into account to a larger extent the ecological value of the meadows in their assessment.

Table 1. Effect of the investigated factors on the landscape appreciation.

Source	df	F	P-value
Phenological stage (PHE)	2	1318.8	<0.001
Meadow type (VEG)	1	67.1	<0.001
Occupation of the respondents (OCC)	2	4.0	0.020
PHE*VEG	2	113.0	<0.001
PHE*OCC	4	28.9	<0.001
VEG*OCC	2	16.1	<0.001
PHE*VEG*OCC	4	3.4	0.009

df, degrees of freedom; F, Fisher's F; P, probability.

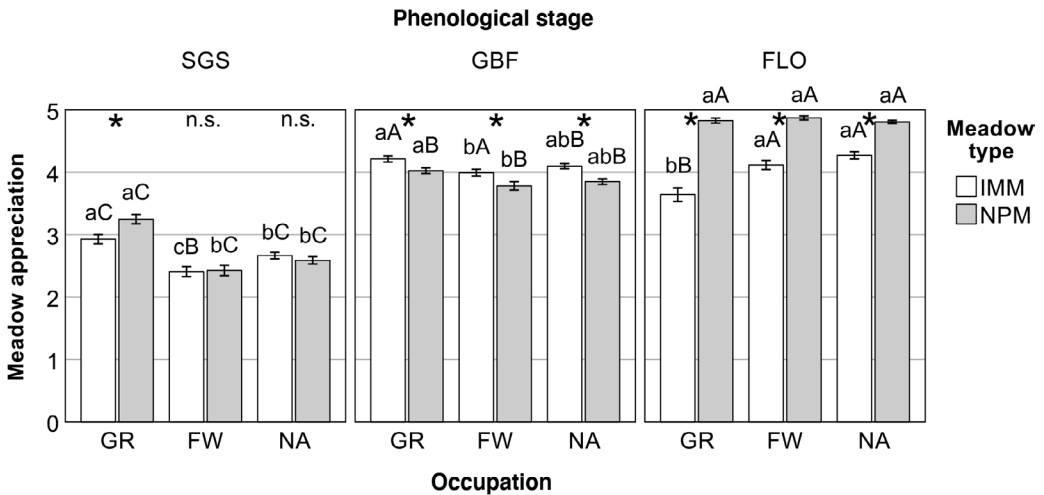


Figure 1. Landscape appreciation of the respondents (mean  $\pm$  standard error) on a 5-point Likert scale (from 1=I don't like it at all, to 5=I like it very much) depending on phenological stage, meadow type and occupation of the respondents. Abbreviations are explained in the main text. Post-hoc comparisons by LSD. \*Significant differences depending on the meadow type (n.s., not significant). Means depending on the profession within the vegetation phenological stage and the meadow type sharing no common lower-case letter, and means depending on the phenological stage within occupational group and vegetation type sharing no common upper-case letters, significantly differ from each other.

## Conclusion

Besides the expected increase of the appreciation level of NPM and differences between NPM and IMM with progressing phenology up to the flowering stage, the study also provided evidence about differences in the perception due to the occupational background. Grassland farmers are the professional group that more strongly differentiate in their appreciation between NPM and IMM at the beginning of the growing season and at the flowering stage.

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# Multi-actor approach to explore information sharing opportunities to promote emission reduction on grazing dairy farms

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## Abstract

There is increased pressure for the agricultural sector to reduce greenhouse gas emissions and reach climate targets. ClieNFarms aims to guide farmers across Europe to reach climate neutrality using a multi-actor approach. In 2023, a structured workshop, with 31 participants, was held in Ireland using a multi-actor, living lab type approach to establish knowledge on solutions to improve the sustainability of pasture-based milk production systems. Forty solutions to reduce GHG emissions and increase carbon sequestration within pasture-based dairy farms were discussed in terms of likelihood of implementation and impact. Further discussions were held to gain knowledge on the prerequisites and systemic barriers of high impact, low likelihood of implementation solutions identified by the actors, and on what support farmers require and what other stakeholders can offer farmers to implement these solutions. Solutions related to slurry spreading and fertiliser application were identified as the solutions with the highest likelihood of implementation. Cooperation between actors is required to implement high impact, low likelihood of implementation solutions. Overall, farmers were receptive to implementing solutions, however, they require concrete guidelines with no mixed messages.

**Keywords:** carbon sequestration, GHG emissions, sustainability, grassland, living lab

## Introduction

The European agricultural sector is being challenged with targets to reach climate neutrality by 2050. A multi-actor approach, defined as the co-creation and sharing of complementary expertise (Feo *et al.*, 2022), is required to implement sustainable solutions on-farm whilst retaining a productive business. A living lab approach has previously been defined for increasing sustainability and resilience within the agricultural sector (McPhee *et al.*, 2021). In the context of agriculture, living labs involve a range of stakeholders with the farmer playing a central role, encouraging actions to be implemented on-farm (McPhee *et al.*, 2021). The aim of this living lab workshop was to initiate discussions on solutions to decrease greenhouse gas (GHG) emissions and increase carbon (C) sequestration within pasture-based dairy farms, and to gather knowledge on empowering farmers to get these solutions onto farms.

## Materials and methods

A one-day structured workshop focussed on implementation of solutions to help dairy farmers transition towards climate neutrality took place in Ireland in January 2023. A total of 31 stakeholders attended, including farmers, advisers, researchers, policymakers and industry (dairy co-operatives, processors, bankers, agricultural input suppliers (e.g. feed and fertiliser companies)). Participants were firstly split into four groups and facilitators guided discussion and captured information. Forty solutions (10 solution cards per table) that reduce GHG emissions and increase C sequestration within pasture-based dairy farms were ranked by each participant on assumed likelihood of implementation (1=low, 10=high). Each participant subsequently scored the solutions from 1 (low) to 5 (high) based on impact (ability to reduce GHG emissions and increase C sequestration). Impact was further compared to a

score previously determined by four researchers involved in dairy production, GHG emissions and C sequestration research for each solution, and discussed. After the workshop, a *t*-test was used to compare the impact scores between the participants and the experts. To close the workshop discussions were then held to gain knowledge on the prerequisites and systemic barriers of four high-impact, low-likelihood of implementation solutions identified by the actors in the previous activity (this paper will focus on grass-legume mixtures), and on what farmers require and what other stakeholders can offer to get these solutions implemented.

## Results and discussion

Figure 1 reports the average ranking for likelihood of implementation for each solution. The two solutions with the lowest likelihood of implementation were both related to soil management. The four solutions with the highest average likelihood of implementation were related to slurry spreading and fertilizer application. This aligns with data on the increased adoption of low emission slurry spreading in Ireland, with 75% of dairy farmers using low emission slurry spreading in 2022 (Buckley and Donnellan, 2023). Figure 1 also reports the average impact for each solution given by the workshop participants and experts in the field. Solutions related to trees were rated in the top five for impact by participants (Forest/nature, Agroforestry, and Hedgerows/trees). Additional solutions in the top five were 'Low-emission synthetic fertiliser' and '(Re)wetting of organic soils.' Overall there was no significant difference between the average impact reported by the participants and the experts ( $P=0.270$ ). All solutions had a difference in score between the participants and the experts of  $<2$ , with the exception of 'Reduce herd size', in which the experts rated the impact higher than the participants by 2.3. This highlights that generally stakeholders are knowledgeable on the impact of solutions, but there is a need to educate stakeholders on the benefits and implications of a reduction in herd size.

Generally, the prerequisites and barriers identified for implementing 'Grass-legume mixtures' on grazing dairy farms were common between stakeholder groups. Poor grassland management and a lack of knowledge on the establishment, management, and benefits of clover were identified as major barriers, with farmers specifically asking for clear advice with no mixed messages. Utilising farmer knowledge and peer-to-peer learning in conjunction with formal knowledge from academia and industry is important for enhancing sustainability within agriculture. Participating farmers reported that attitude and the relatively slow process (3-5 years+) required to convert the farm from grass-only to grass-clover may be a barrier to incorporating clover. Buckley *et al.* (2015) reported that multi-functional benefits increased the motivation for farmers in Ireland to adopt a solution. Time and cost of establishing and managing clover was seen as a barrier by all, with advisers suggesting grants are a prerequisite for implementation. All stakeholders discussed the need for good soil fertility, with advisers specifically highlighting the lack of soil sampling on farms, as well as the implementation of recommendations where sampling occurs, as barriers. Farmers should be targeting animal manures on fields with P and K indexes of one and two, and applying lime to fields to achieve an optimum pH of 6.3. Fear of bloat from incorporating clover in the sward was also seen a barrier by all stakeholders. Farmers wanted clear advice and simple solutions to managing bloat. Researchers also suggested that GHG emission factors for grass-clover need to be quantified.

## Conclusion

The living lab type workshop successfully encouraged multi-stakeholder participation for aiding farmers to implement solutions to reduce GHG emissions and increase carbon sequestration. Utilising a multi-actor approach was successful at ensuring challenges were looked at from all angles. Focus is required on implementing high impact, low likelihood of implementation solutions. There was a general consensus across all stakeholders on the prerequisites and barriers to incorporating clover on-farm. Overall, farmers were receptive to implementing solutions; however, they would like clear concrete guidelines. More



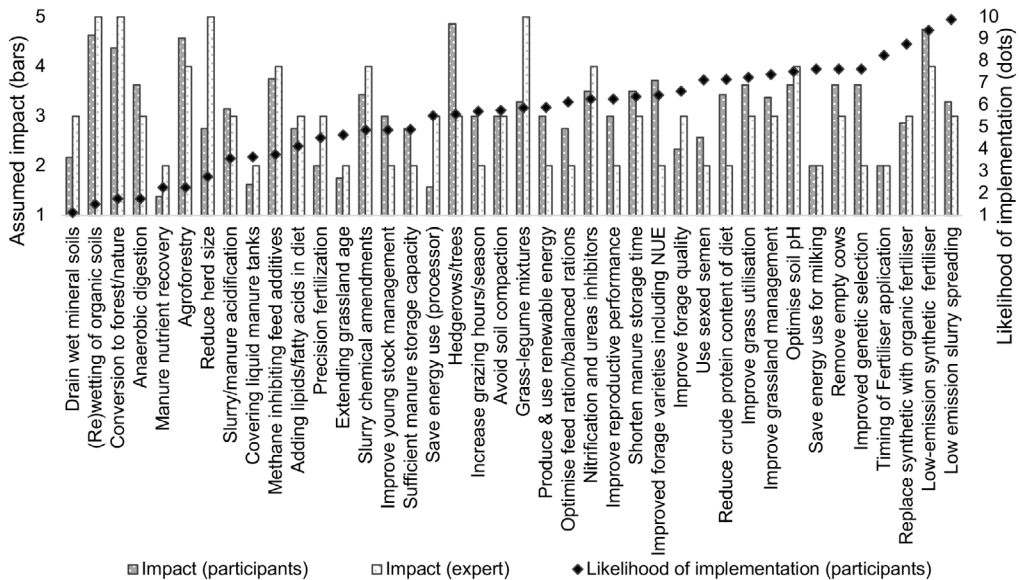


Figure 1. Average likelihood of implementation for each solution from participants (1=low, 10=high). Average assumed impact for each solution (1=low, 5=high), for participants (grey bar) compared to the experts.

Figure 1. Average likelihood of implementation for each solution from participants (1=low, 10=high). Average assumed impact for each solution (1=low, 5=high), for participants (grey bar) compared to the experts.

detailed analysis will be completed within a future scientific publication to get a better understanding of the interactions and implications of the observed differences between stakeholders.

## Acknowledgements

We thank all the participants and facilitators for contributing to the workshop. The workshop was undertaken as part of the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement No. 101036822 (ClieNFarms).

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# Understanding drivers of farmers' intention to implement livestock protection measures against wolves in Bavaria, Germany

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## Abstract

Wolves (*Canis lupus*) are recolonizing Germany and confront pastoral farmers with the potential threat of livestock depredation. Protection measures such as wolf-deterrent fences are considered indispensable for preventing carnivore attacks on livestock but require pro-active implementation by farmers. Drivers of farmers' intention to adopt protection measures for livestock are not well understood. We employed the Theory of Planned Behaviour to explain farmers' intention to implement livestock protection measures based on attitude, subjective norm, perceived behavioural control and worry, as an additional emotional component. In 2022, we conducted an online survey among livestock owners in Bavaria, Germany, receiving 353 responses. Using structural equation models, we explored farmers' intention to implement (i) anti-wolf protection measures in general, and (ii) wolf-deterrent fences in particular. The results point out that subjective norm is most important, i.e., farmers care about the views of their peers regarding livestock protection measures. Perceived behavioural control, i.e., farmers' perception to be able to implement measures, was notably low and moderated the effect of attitude on intention. We conclude that targeted policy measures should enhance feasibility and financial viability of preventive measures and support successful farms in acting as disseminators for effective livestock protection.

**Keywords:** fencing, grazing, livestock depredation, human-carnivore conflict, online survey

## Introduction

Facilitated by international conservation regimes, populations of large carnivores are recovering in many parts of Europe. In particular, wolves have been successfully recolonising Germany since the beginning of the 21<sup>st</sup> century. This confronts pastoral farmers with the new threat of livestock depredation by wolves. Sustainable co-existence with wolves requires effective livestock protection measures (van Eeden *et al.*, 2018) but implementation of such measures depends on acceptance by farmers (Petridou *et al.*, 2023) threatening wolf conservation and impacting human livelihoods. Most countries implement relevant compensation programs, which are however rarely accompanied by proactive husbandry practices vetted with scientific research. We investigated the influence of husbandry practices on wolf depredation losses for 70 sheep/goat and 68 cattle herds with quantitative modeling of data from semi-structured interviews of livestock farmers along a livestock damage gradient in NW Greece. Sheep/goat herds were better protected than cattle herds in seven preventive measures and annual losses of sheep/goat livestock units were three times lower than losses of cattle livestock units in our study area. Furthermore, according to national compensation data from Greece, costs paid for cattle have doubled in recent years, whereas

they have been cut in half for sheep/goats. Our modeling identified three core preventive measures that significantly reduced wolf depredation risk for both herd types, namely increased shepherd surveillance, systematic night confinement, and an adequate number of livestock guardian dogs (optimal ratio was 3 Greek guardian dogs per 100 sheep/goats and 7 guardian dogs per 100 cattle. Wolf-detering fences are widely recommended as an effective means to reduce livestock losses. To investigate drivers of German farmers' intentions to implement livestock protection measures in general, and wolf deterring-fences in particular, we applied the Theory of Planned Behaviour (TPB; Ajzen, 1991), which explains behavioural intention based on an individual's attitude, subjective norm and perceived behavioural control. Building on recent research in human-wildlife coexistence, we extended the TPB by the emotional factor of worry (Eklund *et al.*, 2020).

## Materials and methods

In late 2022 we conducted an online survey among pastoral farmers in Bavaria, southern Germany, where the first recolonizing wolf settled in 2016. The link to our survey was sent to eight regional associations of pastoral farmers and livestock breeders. The core of our survey were questions guided by the TPB, which we asked for protection measures in general and for different specific measures, such as wolf-detering fences. To assess the latent construct of attitude, we asked the respondents to indicate if they thought measures were rather reasonable, good, and advantageous or the opposite. Regarding subjective norm, we asked if respondents perceived that other livestock owners apply protection measures and if they feel that their neighbours and people important to them think that they should do so. Perceived behavioural control was assessed in terms of the preconditions and everyday life on the farm, which can make it easy or difficult to implement measures, and the costs and time required for the measures. All items were rated on a 7-point Likert-type scale. We calculated structural equation models (SEMs) in the R package lavaan (Rosseel, 2012) to evaluate the drivers of farmers' intention to implement livestock protection measures in the next three years.

## Results and discussion

The 353 participants completing our survey ranged from age 19 to 91 years; the majority were male (72.0%). Part-time farming was most common (62.5%), whereas fewer respondents kept grazing livestock as their main business (20.5%) or hobby (17.0%). The total pasture area varied between <1 ha and 600 ha (mean±SD 24.6±52.9 ha). The largest share of the pasture area was in most cases used for cattle ( $n=162$ ) and sheep ( $n=134$ ), followed by goats ( $n=42$ ) and horses ( $n=12$ ). One respondent each declared deer, poultry, and alpaca as the main grazing animal species. Most respondents (86.4%) had already implemented at least one anti-wolf measure. Our SEMs (Figure 1) explained 26% and 45% of the variance in intention to implement anti-wolf measures in general, and wolf-detering fences in particular. A study among Swedish animal owners (Eklund *et al.*, 2020) explained 27% of variance in the intention to use unspecific interventions to prevent carnivore attacks; thus, specifying the type of protection measure seems to benefit the explanatory power of models. As postulated by the TPB, attitude, subjective norm and perceived behavioural control affected the intention positively, with subjective norm as the strongest driver. This indicates that the opinions and behaviours in their peer group play an important role when livestock owners decide how to protect their animals against wolves. Eklund *et al.* (2020) also found a strong effect of subjective norm on the intention to use interventions to prevent carnivore attacks. Lacking experience with livestock protection measures (Hill *et al.*, 1996) as well as strong identification with local traditions and livelihoods related to pasture-based livestock farming (Sjolander-Lindqvist, 2009) might predispose farmers to give particular weight to what their peers think and do.

Perceived behavioural control had a direct positive effect on intention in the model for wolf-detering fences but not for anti-wolf measures in general. Referring specifically to installing fences, it seems reasonable that perceived effort influences intention, because respondents were probably able to imagine

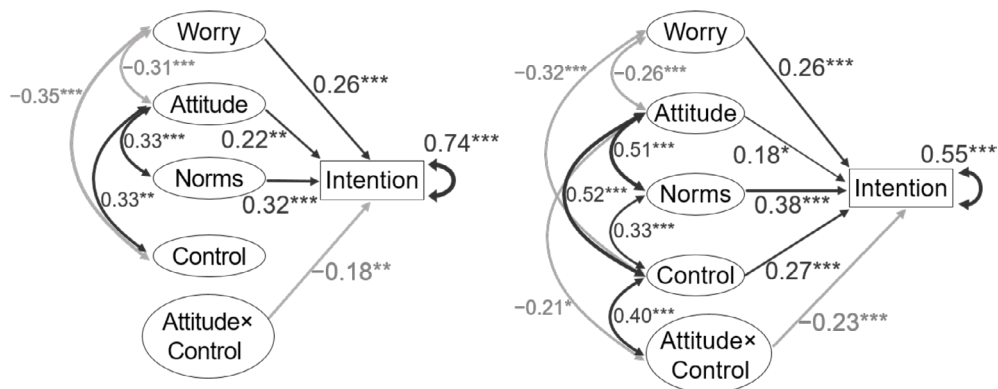


Figure 1. Drivers of livestock owners' intention to implement protection measures against wolves as represented by structural equation models. Asterisks indicate the significance level of the standardised estimates (\* $p \leq 0.05$ ; \*\* $p \leq 0.01$ ; \*\*\* $p \leq 0.001$ ).

the workload for this task (respondents indicated a current mean time requirement of  $24.1 \pm 50.1$  h ha<sup>-1</sup> per grazing season for all fencing-related work). According to the TPB, perceived control reinforces the effects of norm and attitude on intention (La Barbera and Ajzen, 2021), but we found a negative interaction of attitude and control in both SEMs. With increasing control, the positive relationship between attitude and intention became weaker, even insignificant at high levels of control. Hence, implementing livestock protection despite low sense of control seems to require conviction (high attitude).

This result should be seen in light of comparatively few respondents reporting high control (only 3–16% of respondents chose scores 6 or 7 for control items).

In addition, our models show that higher levels of worry about wolf attacks on grazing livestock increased the intention to use preventive measures. Thus, our results support the Swedish study's finding that addressing emotions is important for understanding human behaviour with regard to the challenges of human-wildlife coexistence (Eklund *et al.*, 2020).

## Conclusion

Attitudes, subjective norms and perceived behavioural control contributed to explaining pastoral farmers' intention to implement livestock protection measures, in particular for wolf-deterrence fences. Given low levels of control, practical and financial support for preventive measures seems expedient. However, addressing farmers' subjective norm might be essential to enhance the intention to adopt livestock protection measures, e.g. via support for successful farms as role models of effective livestock protection in areas where wolves return.

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# How German grazing dairy farmers perceive feed intake of cows on pasture and dietary effects

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## Abstract

Grazing practices in Germany vary greatly and range from exercise grazing where cows are fed almost completely indoors, to grazing with little or no additional feeding. Farmers often do not know the feed intake and the related uptake of energy, protein and fibre of their cows on pasture. This is not only important for adequate nutrition but also for animal health. We interviewed 39 dairy farmers in Germany in 2022 and collected data about their grazing practices, herds, and indoor feeding, for calculation of total feed intake and intake on pasture to find out how farmers perceive the feed intake on pasture. Farms were categorised into three grazing categories based on daily dry matter intake from pasture: exercise, partial, and full pasture. Exercise and partial pasture farmers overestimated the feed intake from pasture. We found that exercise and partial pasture farmers were more concerned about malnutrition and related animal health issues caused by pasture than were graziers who rely entirely on pasture based dairy farming. Such interactions between grazing practices and the perception of dietary effects should be taken into consideration for research on grazing and extension projects.

**Keywords:** attitude, perception, bias, management, nutrition

## Introduction

In Germany, grazing is not the predominant form of dairy husbandry, as the majority of cows do not have pasture access (van den Pol-van Dasselaar *et al.*, 2020). Grazing practices can range from grazing with very little or no additional feed, to exercise grazing where cows are mainly fed indoors. In exercise grazing, cows visit a paddock for a few hours a day with only marginal grass intake at pasture. The impacts of grazing and of pasture on animal nutrition depend on the amount of time spent in the paddock and the proportion of pasture herbage in the diet (Pérez-Ramírez *et al.*, 2008). The objective of the study presented here was the assessment of the beliefs grazing dairy farmers in Germany hold regarding potential dietary effects related to grazing cows on pasture. This paper only reports on farmers' views of potential detrimental effects, such as nutrient deficiency, digestive issues, or high milk urea values.

## Material and methods

In 2022, 39 farm managers that graze their dairy cows were interviewed about their grazing management. The farms were located mainly in Northwest and South Germany, the major dairy and grassland areas of Germany. However, this selection of farms does not necessarily represent a complete cross-section of all grazing systems in Germany. A questionnaire was used to establish farm structure, indoor feeding, grazing management, animal welfare, and the estimated feed intake of the cows on pasture, focusing on the grazing season. The milk yield was obtained from herd test results. The pastures were dominated by perennial ryegrass. The feed intake from pasture was calculated by subtracting the supplements from the total intake which was calculated according to Gruber *et al.* (2004). The farms were divided into three grazing categories based on the calculated daily dry matter intake (dDMI) from pasture: Exercise pasture (EP, intake <30%), partial pasture (PP, intake 30–84%), and full pasture (FP, intake ≥85%). Farmers' attitudes and perceptions were assessed in the context of their respective grazing category. Statistical

analysis was conducted in R 4.3.2. The difference between calculated and estimated pasture proportion of total feed was compared with the Wilcoxon-Test for each grazing category. The Chi-Squared test was used to determine independence between the grazing categories in binary questions (BQ). Likert scaled answers were analysed with the Kruskal–Wallis test.

## Results and discussion

Table 1 gives an overview on farm structure data of the interviewed farms grouped by the three grazing categories. There were fewer FP farms compared to PP and EP, as the FP system is not commonly used in Germany; to some extent this confirms a representation of the grazing dairy sector by the interviewed farms (van den Pol-van Dasselaar *et al.*, 2020). The cows on PP and EP farms spent a similar amount of time in the paddock even though the feed intake from pasture can be considerably lower in the EP group. A further differentiation of pasture-access time and actual time on pasture would be beneficial for an appropriate definition of the time on pasture and the calculation of feed intake per hour on pasture. A disparity between farmer-perceived feed intake and calculated feed intake on pasture could be observed mainly for EP farms (overestimate mean±SD +27±25%,  $p<0.001$ ), less so for PP farms (overestimate mean±SD +10±22%,  $p=0.096$ ), but not for the FP farms ( $p=0.158$ ). This indicates a lack of knowledge of the farmer about realistic estimates for feed intake on pasture, as well as potential dietary effects on animal health and welfare.

Farmers' views on feed intake on pasture, including dietary effects of energy, fibre, and protein supply from pasture, differed among the three grazing categories. Farmers from the three categories also differed in their view on potential health issues related to grazing. When asked whether energy supply from pasture can be a problem on their farm (BQ), 50% of FP, 53% of PP, and 56% of EP farmers agreed ( $p=0.971$ ). Cows on PP and EP farms have less than 84% feed intake of the total dDMI from pasture. On EP and PP farms in this sample, the dDMI from pasture was only 26 ±21%. These farms could balance potential dietary issues related to grazing by indoor feeding. FP farmers feed little to no supplements. Therefore, it was expected that farmers from the EP and PP category were less likely to consider the energy content of their pasture an issue. However, it was the FP farmers who were the most positive regarding the energy supply from pasture. A similar pattern was observed concerning the supply of fibre and protein for grazing cows from pasture. When asked whether a lack of supply of crude fibre from grazing is relevant on their farm (BQ), this was not an issue for any FP farmer, while 33.3% of PP and 41.2% of EP farmers replied yes ( $p=0.172$ ). The fibre content of fresh grass is high enough to ensure sufficient fibre supply (McEvoy *et al.*, 2010). Even if this was not the case, on PP and EP farms this could be balanced by indoor feeding. However, this perception might prevent PP and EP farmers from extending grazing on their farms. FP farmers generally had fewer concerns regarding pasture as sufficient

Table 1. Comparison of farm data between the three grazing categories.

	Exercise pasture (EP)	Partial pasture (PP)	Full pasture (FP)
Number of farms	18	15	6
Number of cows	118±103	88±378	101±74
Milk yield 305d-lactation (ECM kg cow <sup>-1</sup> )	8998±1756	7762±1835	7489±1178
Stocking rate (SLU ha <sup>-1</sup> )	9.9±5.8	4.9±1.9	3.7±1.4
Grazing time (h day <sup>-1</sup> )	14.0±6.6	13.1±4.6	21.5±2.0
Calculated feed intake on pasture (%)	10±10	45±12	97±5
Farmer estimated feed intake on pasture (% of total DMI)	36.2±28.3	54.9±18.49	93.3±12.1

Values given are means±SD. ECM, energy corrected milk from herd testing (Spiekers and Potthast, 2004); SLU, standard livestock unit; DMI, dry matter intake.

feed for dairy cows, which is consistent with the findings of Becker *et al.* (2018). A possible reason for the FP farmers' fewer concerns is that their perception of the FP grazing system, as well as their expectations regarding milk yield or animal health concerns, are influenced by the grazing system they already use on their farm. This bias towards a familiar system causes a self-reporting error that was also described by Becker *et al.* (2018). All FP farms mentioned that milk urea levels exceeded 300 mg l<sup>-1</sup> at least in autumn, whereas 60% of PP and 44% of EP farmers reported this as well ( $p=0.0563$ ). On a 1-7 Likert scale, FP farmers on average neither agreed nor disagreed that high crude protein content in the sward, indicated by high milk urea levels, can lead to problems on their farm (mean $\pm$ SD=4.0 $\pm$ 2.00). The PP and EP group agreed slightly more with the statement (5.0 $\pm$ 1.62 and 4.8 $\pm$ 1.2, respectively) ( $p=0.327$ ). Similar to the perception of energy or fibre content, this result was contrary to expectations. More extensive grazing, as applied on FP farms, is often combined with seasonal calving systems: 83% of FP farms used seasonal calving compared to 13% of PP and 17% of EP. Seasonal calving in autumn or winter can circumvent potential grazing related issues as the cows are in later lactation and more metabolically stable with the start of the grazing season.

## Conclusions

EP and PP farmers overestimated the negative impact of grazing on animal nutrition and health. The relationship between grazing category and farmer perception bias should be considered when designing programmes to promote grazing in Germany. This is also relevant for setting up extension services or future research. A proper classification of grazing systems and related topics is needed to assure precise communication and avoid self-report errors.

## Acknowledgement

This work is supported by the Federal Ministry of Food and Agriculture; grant 2819MDT100.

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# Honey bees pollen collection in a grasslands bocage territory

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## Abstract

Society has become increasingly disconnected from the territory in which they live. This is particularly true in bocage which has been transformed in the last decades resulting in a decline in grassland areas and the length of hedgerows. In this action research project “Melli-Faire-territoire”, inhabitants are invited to discover their territory from the angle of foraging honey bees. At a collective historic cob apiary located in a grassland bocage landscape, volunteers collected samples of pollen from the hives. Pupils have been associated in the assessment of pollen diversity. Microscopic analyses revealed that ligneous vegetation was foraged by bees in spring, while white and red clovers developing in grasslands were the major contributors to summer pollen supplies. White clover in particular played a critical role in supplying bee pollen all through the season, even though it originates from several habitats. We confirm here the importance of the grassland-hedges association to preserve a pollination ecosystem service. In addition to the scientific results, this project made it possible to involve citizens who appeared to be very motivated by this action.

**Keywords:** sustainable farming, bocage, grassland, melliferous flora, honey bee

## Introduction

In Europe, in the countryside, there are now few relations between farmers and other inhabitants. Landscapes have been transformed into areas with larger fields, with fewer grasslands and hedges in the case of bocage. Rural areas also host honey bees, and their colony behaviour and vitality are largely driven by the farming systems which influence agricultural landscapes (Allier *et al.*, 2017). Bees' foraging choices depend on the availability of floral resources (Odoux *et al.*, 2012), but many rural environments no longer provide food continuity for insects, creating periods of scarcity (Requier *et al.*, 2017). The agricultural activity of a territory is directly linked to the dominant economic model in terms of production and income, but this model is closely directed by the consumption patterns and purchasing practices of consumers, and their distance from the place of production. However, agricultural intensification leads to a regional imbalance in the region's food supply. Our study took place within the framework of an action research project located in Normandy, France, in a bocage landscape with high proportions of grasslands. Inhabitants were invited to discover their territory from the angle of the area foraged by honey bees. Since 2020, some volunteers have learned beekeeping to manage together some traditional beehives, housed in a recently restored historic cob apiary. Bees thus create a link between everyone involved in the foraging area. The part of the study described here presents only the identification of collected bee-pollen and fieldwork for botanical inventories, which allows identification of the spatial landscape components supplying the insects' diet.

## Material and methods

The study is located in Tessy-Bocage (Manche, Normandy) in a rural territory with about 30 farms, mostly dairy producers, of which a third are engaged in organic production. The total area considered was within a 3 km radius around the beehive (2827 ha), as a theoretical home range of the honeybees along a season (Requier *et al.*, 2017). Melliferous flora inventories have been carried out in April, May, July and October 2023 by botanical experts. Farmers and inhabitants also joined some of these tours. Graphic parcel register (RPG Géoservices) was used to map the agricultural parcel uses of the area,

using QGIS software. Plant species potentially visited by bees were recorded within the study area on 17 survey sessions during 2023 (11 transects of 200 to 1150 m length each). Beekeepers managed three bee colonies in the centre of the area during 2022 and 2023 in order to collect bee pollen at the entrance of the hives every two weeks during the apicultural season from April to October, with pollen-traps fitted at the entrances. Thirty-one samples were obtained, cleaned and weighed. According to the assumption that pollen colour diversity is linked to floral diversity, the number of different colours of pollen were counted by middle-school pupils or beekeepers, according to the CSI protocol (Brodschneider *et al.* 2021). All the samples from both years were sent to the EVA laboratory for identification by microscopic analysis according to Louveaux method. They were mounted onto a slide dressed with stained glycerol gelatine and we determined at least 1200 pollen grains at 400× magnification on a slide transect. Pollen type quantifications were expressed as percentages of grain number grains and in relative mass (Tamic *et al.*, 2016).

## Results and discussion

The agricultural landscape covered 84% of the area, consisting mainly of permanent grasslands (49%) and forage maize (30%). Temporary grasslands represented 6% of the area, forage crops 4%, cereals 2%, orchards 1%, rapeseed, peas and others less than 1% each. The hedgerows length reached 124 m ha<sup>-1</sup>, while woody land represented 16% of the area. Floral inventories highlighted 174 plant species potentially attractive for bees. Flora was classified according to the major habitat observed for each species, namely grasslands and pathways (54 species, including *Trifolium*), hedges and woods (38 species), crops (13 species) and riparian habitats (11 species). Taxa richness ranged from 5 to 21 per sample. We didn't find any significant link between the number of pollen colours and taxa richness observed by the microscopic method but pupils were very motivated to be involved in this scientific study. Palynological determination for 2022–2023 showed for the most dominant species the following distribution in proportions within the samples: *Trifolium repens* (20%), *Hedera helix* (11%), *Crataegus monogyna* (9%), *Trifolium pratense* (8%), *Sinapis alba* (5%), *Prunus* sp. (4%), *Zea mays* (4%), *Rubus fruticosus* (4%) and *Salix* sp. (3%). Five species accounted for more than 50% of the collected grains and 12 species for more than 75%. The occurrence of some taxa was higher, i.e. *Trifolium repens*, *Taraxacum officinale*, *Trifolium pratense*, *Plantago* sp. and *Zea mays*, which were encountered 26 to 8 times out of 31 samples. The bee colonies collected between less than 1 g in autumn to 250 g in April. The percentage compiled with the mass of the samples recorded in 2023 highlighted the importance of certain taxa harvested in high quantities such as *Prunus* sp. and *Crataegus monogyna*. In terms of amounts, Figure 1 shows the importance of ligneous flora of hedgerows and woods in spring for the bee diet, (*Prunus*, *Crataegus*, *Hedera*, *Rubus*, etc.), while in summer crops are important especially in August (*Zea*, *Phacelia*, *Sinapis*, etc.). Grasslands seem mainly foraged in spring but their contribution in honeybee resource is lower in terms of pollen diet than that of ligneous species. Our study confirms the outcome of other European studies (Brodschneider *et al.*, 2021) that the highest diversity for honeybee pollen was found in spring, even in a bocage landscape. The permanent grasslands contribution could not be established precisely due to the massive flowering of clover in summer in temporary grasslands.

## Conclusion and perspectives

Hedgerows and woody areas are the major landscape components for the honeybee pollen diet in a bocage landscape. However, white clover in particular played a critical role in supplying bee pollen all along the season. We confirm here the importance of the grassland-hedges association to preserve a pollination ecosystem service at the landscape scale. The results are now presented to the territorial actors, stakeholders and families.

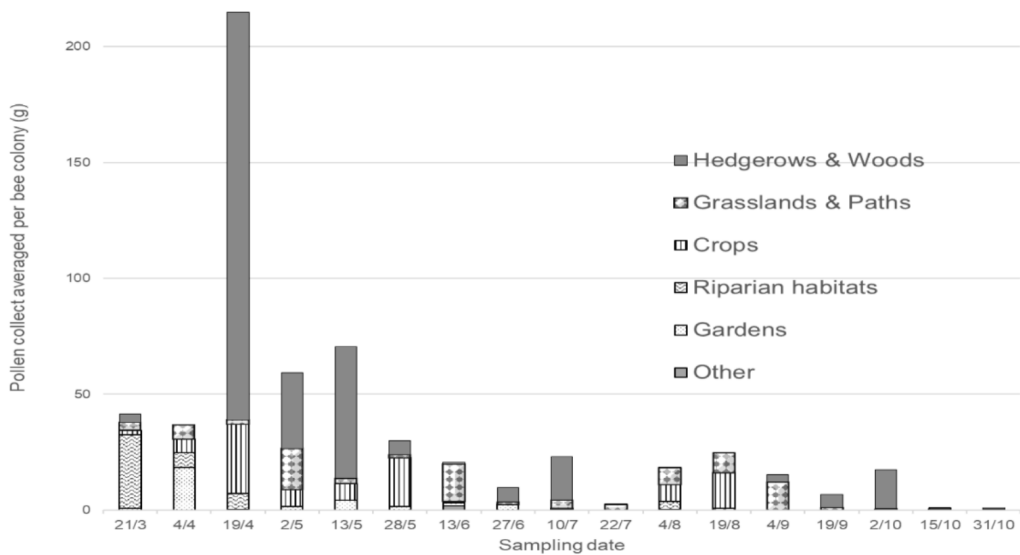


Figure 1. Pollen amount (g colony<sup>-1</sup>) in different habitats per sampling date (2023).

## Acknowledgements

We thank the Fondation de France for supporting this programme encouraged by Tussy-Bocage municipality. We also thank the Pistil volunteers, as well as the inhabitants, college pupils, beekeepers and farmers for their active participation.

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# Bibliometric analysis of the literature focusing on ecosystem services provided by grasslands

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## Abstract

We propose an original approach to reviewing the literature on the ecosystem services (ES) provided by grasslands. This review consists of a bibliometric analysis of the thematic fields of 1086 scientific articles published since 1995 and listed in ScienceDirect on the basis of a search for the keywords 'Ecosystem services' AND 'grasslands'. The textual analysis of the abstracts was carried out using Iramuteq, a software interface using R to study thematic fields in text corpora. We carried out a hierarchical descendant classification based on the proximity of words in the abstracts of the articles studied. This analysis revealed 5 recurring themes: (1) soil quality and life (nitrogen, phosphorus, biomass, fungi, bacteria, fertilisation); (2) water quality and regulation (retention, precipitation, plateau, etc.); (3) Biodiversity (species richness, pollinator, insect, habitat, flower, etc.); (4) dynamics of grassland areas (land, area, decline, crops, scenario, etc.); (5) perception of services by stakeholders (stakeholders, perception, interview, citizen, experts, etc.). These fields are often covered by thematic journals. The measures to support ES such as payments for environmental services still seem little studied. A better understanding of grassland ES, especially from the perspective of cross-disciplinary approach mixing ecology, agronomy, zootechnics and economics, is necessary for better operationally.

**Keywords:** grasslands, ecosystem services, bibliometric analysis, payment for services

## Introduction

The concept of Ecosystem Services (ES) first appeared in the 1970s, at the time of awareness of the impact of human activities on the environment and entered the scientific literature in the 1990s (Gomez-Baggethun *et al.*, 2009). Many texts introduce ES from a biodiversity perspective (e.g. Ehrlich and Wilson, 1991). Today, this concept is used more generally by economists, decision-makers and the private sector in the context of payments for environmental services (Capodaglio and Callegari, 2018). Bibliometric analyses give assess to research trends (Zhang *et al.*, 2016). Such analyses highlight unexplored fields of research that still have to be investigated (Mori and Nakayama, 2013). This bibliometric study aims to find out when, how and to what extent researchers have focused on ES provided by grasslands.

## Materials and methods

The bibliographical search was carried out *via* the ScienceDirect database (DB), taking into account titles, abstracts and keywords by using four sets of keywords: (i) 'Ecosystem services' AND 'grassland', (ii) 'Ecosystem services' AND 'grassland' AND 'payment', (iii) 'Ecosystem services' AND 'meadow', (iv) 'Ecosystem services' AND 'prairie'. A DB was then created in Zotero from a BibTeX file including the title, the year, the abstract and keywords. The data were formatted for analysis in IRaMuTeQ, a software package designed to mobilise R for multidimensional text analysis.

Only abstracts were used for the next analyses in which the text corpus was lemmatized, i.e. words were reduced to their dictionary forms. Only adjectives, adverbs, common names and verbs were taken into account in the analyses. A classification of the abstracts was then achieved on the basis of the frequency of association between words in the abstracts, in order to identify groups of articles according to topics.

The chosen keywords are common to all abstracts, so they weren't discriminants for the classification's construction.

## Results and discussion

Although the field of ES research is relatively young, there is a substantial and rapidly growing body of literature on the topic (Zhang *et al.*, 2019). The first DB contained 1086 articles and books. The first article was published in 1995 (Gren *et al.*, 1995) and it would be another 10 years before subsequent papers were published. However, the first works to mention term 'ES' appeared systematically in the 1980s, notably in the works of Ehrlich and Ehrlich (1981), Ehrlich and Mooney (1983) and Costanza *et al.* (2017). In 2005 interest for ES begins its expansion.

The hierarchic classification has highlighted five main abstract thematic areas (Figure 1). Class 1 which brings together the greatest number of articles (24.1%) is represented by words on indices of ES linked to biodiversity (species, diversity, pollinator, forage). Then, in decreasing percentage of papers, class 2 corresponds to public policy, class 3 to water quality and climatic regulation (retention, precipitation, fixation), class 4 to grassland area dynamics and class 5 is devoted to soil.

The bibliometric analysis makes it possible to identify the main ES associated with grasslands. They are linked to biodiversity, soil and water quality, but production (fodder, meat, milk and their quality), animal welfare and health are less addressed. Cultural services, more difficult to evaluate, are also largely

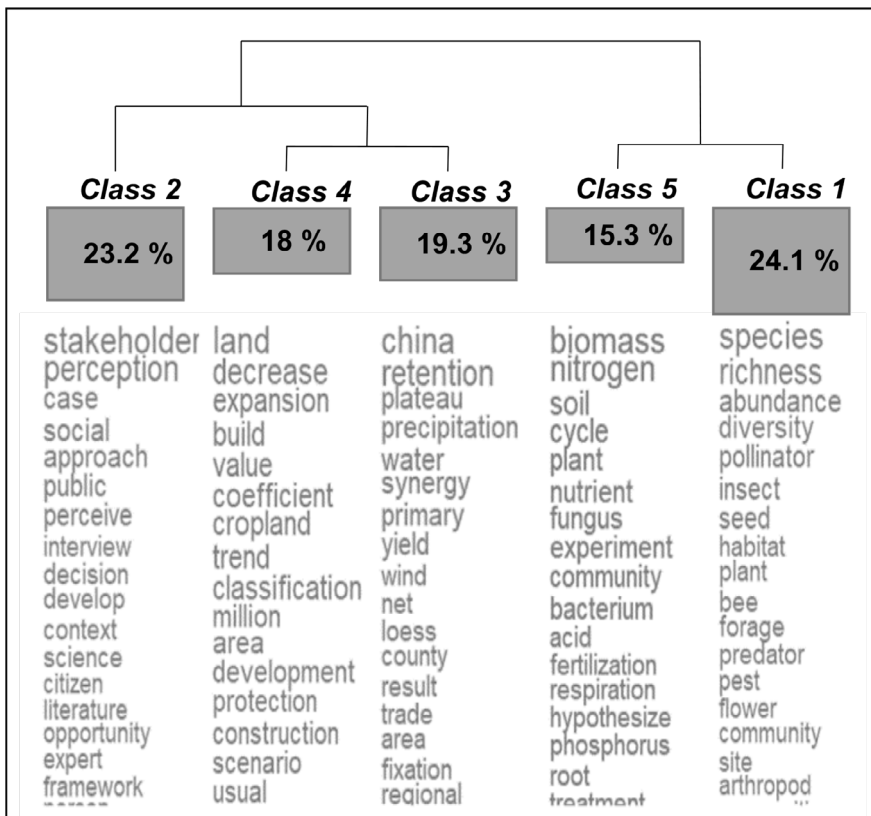


Figure 1. Classification of abstracts based on word proximity to identify thematic groups of articles. The percentages in the grey boxes indicate the percentage of articles involved in each class.

missing in these studies (Hirons *et al.*, 2016). Payments for services, currently at the forefront of public policies (Capodaglio and Callegari, 2018), does not yet appear as a major theme in the literature focusing on grasslands. However, our ScienceDirect DB does not cover all scientific literature and should be completed with other scientific publishing website searches.

## Conclusion

Even if the scientific literature on the ES provided by grasslands is abundant, it is recent and doesn't integrate all the ES. This bibliometric analysis allows us to realise that the services of water quality, biodiversity and soil are majoritarian studied, while other equally important services exist, such as fodder production and its quality. To have a better understanding about the ES provided by grasslands, it is important to conciliate approaches in ecology, zootechnics, agronomy and economy, in order not only to integrate all the ES into the scientific literature, but also to balance the ESs with each other and reconcile economic profitability and environmental preservation.

## Acknowledgement

The authors thank the French network 'Avenirs Prairies' for its financial support.

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# Sharing grass related innovations to enhance the resilience of European dairy farms

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## Abstract

European dairy farmers face major challenges, putting pressure on their resilience. The hurdles they encounter are diverse and ever evolving, ranging from market volatility and environmental pressures to shifting consumer demands and regulatory complexities. This also leads to succession insecurity as the future for young dairy farmers is uncertain. A lot of knowledge is already available that could strengthen the resilience of dairy farmers; however, this knowledge does not often reach them. The R4D (Resilience for Dairy) project aims to disseminate the most promising solutions to dairy farmers to become more resilient. This paper describes how the most urgent needs and practical solutions were collected. In total, 100 ready-to-use best practices were selected. Among those, nine grass-related innovations were ranked highly by farmers and other stakeholders: improving protein self-sufficiency thanks to a better grassland management, multispecies swards to enhance forage uptake and biodiversity, intercrops to reduce nitrate leaching, practices to capture carbon in soil, management of hedges and marginal areas to improve biodiversity, agroforestry, increasing grazing vs indoor feeding to meet customer desires, new grazing systems to increase market value, and virtual fencing.

**Keywords:** dairy production, resilience, innovation, grasslands, best practices

## Introduction

Resilience in the European dairy farming sector encapsulates a multifaceted approach that extends beyond mere economic viability. It encompasses mental fortitude, economic adaptability, technical efficiency, animal welfare, and the development of socially responsible production systems. In the final report of the EIP-AGRI Focus Group “Robust and Resilient dairy production systems” (2018) it is stated that to achieve more resilient and robust dairy farms, there is a pressing need for updated education for both farmers and advisers. The challenges faced by dairy farmers in Europe are diverse and ever-evolving, ranging from market volatility and environmental pressures to shifting societal demands (Delanoue *et al.*, 2015) and regulatory complexities. In navigating these challenges, the concept of resilience emerges as a guiding principle, reflecting the industry’s capacity to withstand, adapt, and thrive in the face of adversity.

Three key areas in the dairy sector to face challenges are: economic and social resilience, technical efficiency and environment, animal welfare and society-friendly production systems. Those issues are interconnected and depend on the livestock farming system, rearing management, people involved in the production process, feeding and material resources, and level of use of innovation (Fagon *et al.*, 2017). A lot of knowledge to be more resilient in those three key areas is already available but does not reach the farmers and advisers. Hence a platform that shares all that knowledge in an easy and accessible way is needed. This paper describes how the R4D (Resilience for Dairy) project, which aims to improve the European dairy sector’s sustainability and resilience, widely disseminates innovations, facilitating knowledge exchange between farmers. The best practices related with grasslands or grass-based systems are put to the fore.

## Materials and methods

### *Collection of the farmers most urgent needs and solutions*

An inventory of needs for dairy farmers to be resilient was created through an online questionnaire distributed (online and on paper) across 15 EU countries (BE-Wallonia, BE-Flanders, DK, FI, FR, DE, HU, IE, IT, LT, LU, PL, SI, ES, NL, UK). Due to the survey being distributed online, the reach and response rate cannot be determined. In the survey, a list of 183 needs were proposed to stakeholders, asking them to assign a score from 0 (not applicable) to 5 (highly applicable) to each of them according to the potential to improve farm resilience. These needs were allocated to one of ten predefined domains (1, animal nutrition; 2, animal management; 3, health; 4, welfare; 5, ecological and environmental footprint; 6, social issues; 7, financial needs; 8, budget management; 9, information sources; 10, labour conditions) to clearly identify the topics that farmers consider as pivotal. To create this inventory, two main steps were taken: a literature review and a consultation among partners.

### *Evaluation of the collected solutions and creation of national workplans*

At the same time, already existing practical solutions to face challenges were collected during local meetings among farmers, researchers and advisers. An assessment scheme was developed based on 5-scale questions related to the following sub-categories: social resilience (less to more), economic resilience, technical efficiency, environment, animal welfare, societal perception items, readiness and acceptability (low to high). This scheme was applied to 185 practices, techniques and tools (named solutions) collected in the 15 countries. Sixty-two experts from universities and research institutes scored these solutions, completing a total of 3300 assessments. The solutions were also scored by farmers and stakeholder in local workshops and some on the field in all 15 countries on the same sub-categories, with focus on readiness and acceptability. The scoring took place with, in mind, farm types or systems where the solution is applicable and attractive. When answering the question about the impact of the solution, the average dairy farm in the region was taken as a reference. This collection of potential solutions also led to the creation of national workplans focusing on local most urgent challenges and the ranking of the most promising solutions from the global inventory. At the end, the 100 most promising solutions were selected for dissemination with factsheets, videos, webinars or on farm demos ([www.resilience4dairy.eu](http://www.resilience4dairy.eu)).

## Results

### *EU dairy farmers most urgent needs*

From the 535 surveys completed (average 33 per country, IQR 14–37), 379 answers came from farmers and 156 from advisers, vets, researchers, and others. Regardless of regional differences, the improvement of work-life balance and the necessity of a transparent and effective communication with civil society are in the top 10 issues that farmers must face to be resilient in the future, just on the same level of other more technical challenges, like animal health/welfare and energy self-sufficiency (Table 1). Work-life balance is always in the top 3 position and often in 1st position regardless of cluster (farmers/non farmers, men/women, under 40/over 40 years of age etc.).

### *Farmers, advisers and researchers proposed solutions.*

Practical management issues related to dairy cow care, nutrition and feed production were emphasized as existing solutions, in relation to actions that can be controlled at farm level. The domain with the highest number of proposed solutions was “animal nutrition”. Table 2 lists the solutions for which farmers and advisers can share existing knowledge, but also the ones for which they want to receive more information.



Table 1. Results of the collection of most urgent needs by the online survey (535 answers).

Rank	Domain	Need
1	Quality of life	Work-life balance
2	Animal welfare	Improvement of welfare conditions of cows
3	Quality of life	Salary/returns
4	Communication	Effective communication and transparency to the public on agricultural practices
5	Prevention	Innovative testing/analysis for early detection of diseases
6	Animal welfare	Improvement of welfare conditions of calves
7	Quality of life	Flexibility
8	Environment	Energy efficiency and use of renewable energy sources
9	Prevention	Innovative detectors/devices for metabolic disease, pathologies
10	Animal welfare	Innovative and animal-friendly housing grazing behaviour, calving time detectors

Table 2. Domains with the highest number of proposed solutions (online survey and NDA meetings) and examples of ready-to-use knowledge and most promising solutions.

Domain	Ready-to-use knowledge (farmers can share knowledge)	Most promising solutions (farmers want more information and training)
Feeding	Protein self-sufficiency, optimizing /reducing protein feeding, novel feeds	Improving protein self-sufficiency thanks to a better grassland management
Grass/Forage	Forage quality, analyses, platemeter measuring, drones; Reduce silage storage losses	Multi-species swards to enhance forage uptake and biodiversity
Grazing	Improving grazing management, new grazing systems	Virtual fencing. Increasing grazing vs indoor feeding to meet customer desires, new grazing systems to increase market value
Other	Slurry technologies, agroforestry	Intercrops to reduce nitrate leaching, Practices to capture carbon in soil, Management of hedges and marginal areas to improve biodiversity, Agroforestry on farms

## Conclusion

The improvement of work-life balance is the most urgent issue that farmers must face to be resilient in the future. Solutions related to grassland management and grazing are already well known by the farmers, advisers and researchers involved in the R4D project in the 15 participating EU countries. Although, some topics still need to be explained, demonstrated, and disseminated at farm level. These technical ready-to-use best practices will only be implemented if they are financially feasible and if they help European dairy farmers face the three main challenges identified: improving their work/life balance, improving animal welfare and facilitating communication towards the society.

## Acknowledgement

Resilience for Dairy (R4D) has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No. 101000770.

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# What policies are needed in Europe to protect grasslands and support their sustainable management?

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## Abstract

Permanent grasslands (PG) are vital in supporting many local rural economies and can deliver a range of important ecosystem services (ES). However, multiple socioeconomic and climatic threats threaten their existence. This paper summarises feedback from online stakeholder workshops in Sweden, Czechia, Spain and the United Kingdom to discuss how policy and implementation could be improved to ensure better protection of PG and its sustainable management. There was some concern that PG that delivers multiple ES is undervalued in many schemes and that there is a need for increased clarity, communication and advice to help land managers protect PG and be rewarded for sustainable management.

**Keywords:** grassland, ecosystem services, sustainable, policy

## Introduction

Permanent grasslands (PG) occupy around 30% of the utilised agricultural area in Europe, and they deliver a range of important ecosystem services (ES) such as supporting biodiversity, storing carbon, providing clean water, reducing flooding risk, creating cultural landscapes and producing meat and dairy products. However, their existence and the services they provide are under threat from abandonment, afforestation, climate change, cultivation, intensification and extensification (Prangel *et al.*, 2023). PG management intensity varies greatly across Europe and there is, therefore, great variety in the degree of multifunctionality and overall benefit to society (Schils *et al.*, 2022). Policies are needed to protect PG and support PG management systems that are sustainable; producing positive social, economic and environmental outcomes and do not negatively impact the lives of future generations. This paper outlines the current agri-environment policy trajectories in Sweden, Czechia, Spain and the United Kingdom (UK) and summarises feedback from stakeholder groups in these countries in terms of how to improve the formulation and implementation of policy to ensure better protection of PG and its sustainable management.

## Materials and methods

National-level online workshops were carried out with PG stakeholders, representing farmers, policy makers, researchers and nature conservation groups in Sweden, Czechia, Spain and the UK in autumn 2023 using a standard format, including an introductory session to outline current regulations and policies to protect PG and support land managers. Stakeholders were asked a series of questions to elicit feedback on whether current policies are successful in delivering sustainable PG systems: (i) What policies are needed to support sustainable PG?; (ii) Are regulations and penalties working?; (iii) Are enough incentives provided?; and (iv) If not, what should be changed? The workshop transcriptions were assessed for feedback, and the key responses mentioned by at least three stakeholders at each workshop were summarised.

## Results and discussion

Workshop participants represented policy makers, farmers, researchers and citizen groups who discussed current policies, schemes and associated issues. Through the Green Deal Farm to Fork and Biodiversity Strategies, the EU has set ambitious 2030 targets to reduce the use of manufactured fertilisers, to increase organic farming coverage, and introduce measures to restore natural ecosystems (including grasslands). This will be achieved through the implementation of national Common Agricultural Policy (CAP) Strategic Plans (EU, 2023) with member states supporting farmers through direct payments, rural development interventions, eco-schemes and agri-environmental-climate commitment, and the implementation of national action plans to protect habitats under the Nature Restoration Law.

In Sweden (11 attendees), eco-schemes have been introduced alongside the continuation of cattle headage payments. Nature conservation groups felt that the cattle subsidy should be conditional on grazing animals, since supporting cattle production does not necessarily support the preservation of ecologically valuable grasslands. There were discussions around what constitutes PG and the type of support needed for lower-intensity ‘semi-natural’ grasslands as opposed to more ‘intensive’ PG of lower biodiversity value. Different support systems or management options may be required for different types of grasslands such that all types of farmers can apply for eco-schemes. There was general consensus that a higher level of support for PG should be linked to sustainable stocking rates. The CAP Strategic Plan must more clearly reflect the fact that long-term productive agriculture is dependent on functioning and resilient ecosystems, to increase the competitiveness of forage-based livestock systems, maintain farm numbers and provide multiple public goods. There is a market for meat and milk from ‘natural pastures’ that is currently underexploited. However, predators (e.g. the wolf, *Canis lupus*) are a significant concern to livestock farmers and ultimately are a threat to open grasslands due to the risk of land abandonment.

In Czechia (34 attendees), a significant challenge is implementing policy that suits large enterprises focused on production and smaller farms focused on sustainability and public goods provision. Greening payments have been replaced by eco-schemes and a ‘whole-farm approach’, with rules on when and what proportion of PG can be cut and grazed through the year. A strict limit to stocking rates on PG has been removed (although a stocking rate limit at the farm level remains), but it was felt that some of the rules (such as minimum and maximum uncut areas) are still too prescriptive and need greater flexibility to achieve eco-scheme aims. Delays to decisions on subsidy conditions, payment levels (income foregone or societal value) and farm plan requirements have also resulted in uncertainty and a lack of clarity. For many farmers it is difficult to justify maintaining and managing PG as the returns from production and subsidy do not cover the management costs. There is a need for higher payments that reflect societal value, and greater flexibility in targets and management requirements.

In Spain (19 attendees), the workshop focused on the dehesa, a silvopastoral system prominent in the Mediterranean region, which is characterised by a combination of trees, livestock and pasture, offering a range of products and ES. However, the dehesa ecosystem is vulnerable to low economic profitability and environmental and social challenges (Parra-López *et al.*, 2023). In response to these challenges, Spain’s CAP Strategic Plan for 2023–2027 incorporates specific measures for the sustainable management of dehesas, including an eco-scheme for Mediterranean Grassland Areas, with measures to increase soil carbon and biodiversity, and a strategy to transform a significant portion of dehesas into organic production (MAPA, 2023). All attendees agreed on the uniqueness of the dehesa and its associated ES, and acknowledged the challenges of creating and coordinating policy for a landscape that sits between agricultural and forestry administrations. There was some concern that stocking rate requirements could lead to intensification, and a consensus that payments should be linked to the provision of ES. There was strong demand for simplification of administrative processes, and greater financial, technical and

administrative support for dehesa farmers. A single list of eco-scheme (agriculture and forestry) measures for dehesa farmers would be welcomed.

In the UK (18 attendees), agri-environment policy is a devolved matter, with differing rates of policy change in each nation. In England, direct payments are being phased out and replaced by 'public money for public goods' through a three-tier Environment Land Management (ELM) scheme, including a 'menu' of PG management options. To protect PG, due account must be taken of the Environmental Impact Assessment (EIA) Regulations. A farmer or land manager must not begin or carry out a project on PG (that has not been cultivated for 15 years) or 'semi-natural grassland' without first obtaining a screening decision or consent from the authorities. UK stakeholders felt that regulations were sufficient to protect PG, but that more resource is needed for implementation and enforcement, including more advice and greater scrutiny of applications to cultivate PG. The discussion around agri-environment schemes reflected uncertainty among farmers regarding what has been a very rapid policy transition. There were concerns about regular changes to schemes, payment levels and inconsistencies between lowland and upland support. There was an expressed preference for a return to direct payments and from a cultural ES perspective, there was concern that 'traditional' PG landscapes could be lost if tree planting policies were followed.

## Conclusions

There was some consensus that sustainable PG systems that deliver multiple ES may be undervalued in many schemes and that low payment rates and overly prescriptive schemes (e.g. in Czechia) could lead to intensification and abandonment. Stakeholders expressed a need for greater clarity, flexibility, improved levels of communication and advice, and a single support system that caters for land managers of grasslands with and without trees.

## Acknowledgements

European Union Horizon 2020 Research and Innovation Programme funding of the SUPER-G project (Grant Agreement no. 774124) is gratefully acknowledged.

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# The perspectives of young European farmers and students on grazing

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## Abstract

Grazing of livestock has many benefits. The magnitude of grazing and how grazing is conducted determines how successfully these benefits are achieved, and this is affected by the attitude of farmers towards grazing. The current study aims to explore the perspectives of young farmers and students in the livestock sector on grazing in eight European countries (France, Germany, Ireland, Italy, the Netherlands, Portugal, Romania and Sweden). For this purpose, an extensive online survey was conducted across these countries to study barriers for grazing and drivers to grazing, as perceived by farmers and students. We collected 1410 valid responses. There were clear differences in perceived barriers and drivers related to the local contexts. The most often mentioned barriers were climate (no grass in dry periods, heat stress), land fragmentation (not enough grazing land surrounding the farm), and lack of knowledge/education. The most often mentioned drivers were animal welfare, animal health, and 'I like grazing/grazing is my preferred production system'. The results have significant implications for policymakers, educators, and other agricultural stakeholders.

**Keywords:** barriers, drivers, grazing, mind-set, next generation farmers

## Introduction

Grazing allows animals to be managed as naturally as possible. Numerous studies have shown that grazing can have positive effects on the income of farmers, the preservation of biodiversity, the conservation of cultural landscapes and enhancement of animal welfare. How successfully these positive effects are harnessed, or put into practice, depends on the magnitude of grazing and how grazing is conducted in relation to the local context in which grazing is applied. The attitude of farmers and the perception of the advantages and disadvantages has changed over the years, going from positive to less positive. This influences decision-making processes among farmers as their mindset affects management decisions (e.g. Reijs *et al.*, 2013; Van den Pol-van Dasselaar *et al.*, 2021). The current research aims to explore the perceived barriers to grazing and drivers for grazing of young farmers and students in animal husbandry. The results can be used to remove obstacles and promote the adoption of grazing.

## Materials and methods

An online survey for young farmers and students was developed using SurveyMonkey ([www.surveymonkey.com](http://www.surveymonkey.com)). Young farmers were defined as persons in aged 16–40, either active as a livestock farmer or currently in the animal husbandry education system (future professionals). Students were accepted if they had animal husbandry or livestock in their educational programme. The project partners translated the survey into local languages and actively spread the survey via social media, farm events and the educational system in eight countries: France, Germany, Ireland, Italy, the Netherlands, Portugal, Romania and Sweden. In Italy, the survey was spread in two distinctive regions (South Tyrol

and Sardinia). These regions are representative of contrasting climatic and socioeconomic conditions (alpine *vs.* Mediterranean environment). Responses were collected from spring 2023 to autumn 2023. Respondents were asked to choose five potential barriers to grazing and five potential drivers for grazing from a predefined list of 21 barriers and 17 drivers. This list was determined in the consortium with people from different countries taking into account the insights from each individual country. If a topic was considered important in one country, it would become part of the list, also if other countries would not consider it important. Since the project partners consisted of an array of people from practice (e.g. farmers unions, extension services) and science, the list is expected to encompass the most relevant issues and to be of good quality.

## Results and discussion

In total, the survey yielded 1410 valid responses of young farmers and students spread over the different countries. The number of valid responses per country varied considerably. France and the Netherlands contributed to about two-thirds of the total number with 458 and 456 valid responses, respectively. The other countries contributed with 43 to 116 valid responses. The most often mentioned barriers to grazing are given in Table 1. As expected, there were differences between countries related to the local contexts. Climate is a major and understandable concern, especially in regions with hot, dry summers. Lack of grass during dry periods and heat stress on animals can directly affect the viability of grazing systems. The presence of predators such as wolves in South Tyrol is particularly relevant for remote locations at higher altitudes, where wolves are known to roam. There were also institutional barriers, like land fragmentation and lack of knowledge/education.

The most often mentioned drivers for grazing are given in Table 2. Animal welfare was by far the most important driver, often linked to animal health. The importance of animal welfare and animal health reflects a global trend in which farmers, consumers, and regulators are becoming increasingly aware of the importance of animal welfare on farms and in food production (Alonso *et al.*, 2020). Other high-ranking drivers were: for Germany and Ireland, 'I like grazing/grazing is my preferred production system'; for Ireland, less costs and/or higher revenues; for the Netherlands and Sweden, the image of livestock farming; and for Sweden, biodiversity. The Swedish natural grasslands are probably the main reason for the importance of biodiversity.

Table 1. Barriers to grazing in France (FR), Germany (DE), Ireland (IE), Italy (IT, Sardinia=SA, South Tyrol=ST), the Netherlands (NL), Portugal (PT), Romania (RO) and Sweden (SE) (% of respondents, only barriers with at least 40% are given for each country/region).

Barrier	FR	DE	IE	IT		NL	PT	RO	SE
				SA	ST				
Climate (no grass in dry periods, heat stress)	71	48	53	58		52	65	47	
Land fragmentation (not enough grazing land surrounding the farm)	62		70		41	42		42	50
Lack of knowledge/education	41		77	58			40	59	
Variability in grass quantity and quality	56		62			58			47
Predators, like wolves		64			79				42
Consumer demands for low prices		61					40		67
Time/available labour		52				47	43		
Not enough grass available/low grass production when grazing	41							45	
Money, i.e. costs too high and/or benefit too low				42			43		
Unfavourable topography (e.g. the pastures are too steep)					50				

Table 2. Drivers for grazing in France (FR), Germany (DE), Ireland (IE), Italy (IT, Sardinia=SA, South Tyrol=ST), the Netherlands (NL), Portugal (PT), Romania (RO) and Sweden (SE) (% of respondents, only drivers with at least 40% are given for each country/region).

Driver	FR	DE	IE	IT		NL	PT	RO	SE
				SA	ST				
Animal welfare	76	90	58	67	90	70	75	80	66
Animal health		65	61	48	76	62		76	44
I like grazing/grazing is my preferred production system	41	71	67			41			44
Biodiversity	42	48					46	44	74
Less costs and/or higher revenues	49		89				46	51	
Image of livestock farming	44	51				64			66
Less need to buy protein	43			60	48	49			
Quality of animal products, for example improved fatty acid composition	41			48			51	48	
Labour reduction			43		41				
Premiums/subsidies						50			
Carbon sequestration							49		
Consumer demands							41		

## Conclusions

There were clear differences between regions in perceived barriers to grazing and drivers for grazing among young farmers and students related to the local contexts. The most often mentioned barriers were climate (no grass in dry periods, heat stress), land fragmentation (not enough grazing land surrounding the farm), and lack of knowledge/education. The most often mentioned drivers were animal welfare, animal health, and 'I like grazing/grazing is my preferred production system'. Understanding the perspectives of key stakeholders, such as young farmers and the next generation of agricultural professionals, is essential for informed policy and educational interventions at early ages for promoting the adoption of grazing.

## Acknowledgements

This research has received funding from the European Union's Horizon Europe Research and Innovation Programme under Grant Agreement No 101059626 (Grazing4AgroEcology; [www.grazing4agroecology.eu](http://www.grazing4agroecology.eu)) and has been co-funded by the Taskforce for Applied Research SIA, part of the Dutch Research Council (NWO), and the Dutch Ministry of Agriculture, Nature and Food Quality (LNV) (Kennis voor Grasland en Beweiding).

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