The future role of ley-farming in cropping systems

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The OECD Co-operative Research Programme Sustainable Agricultural and Food Systems sponsored this symposium through financial support for several of the key note speakers and these proceedings.

Foreword

The sustainability of agroecosystems is a significant pathway for the availability of healthy and nutritious human food. However, achieving and maintaining this feasible status in agro-ecosystems is not so easy. As stated in the Farm to Fork and Biodiversity Strategies, there is a need to transform management for increasing carbon capture and storage, and for regulating and reducing GHG emissions, while also enhancing biodiversity. Furthermore, it remains important to avoid loss of other ecosystem services related to plant production, to improve nutrient cycling in various cropping systems, and to broaden the range of production value chains in the circular bioeconomy. Leys in cropping systems comprise a significant component of agroecosystems as they provide multiple and diverse ecological services. Stockless farming is gradually growing in many countries, hence the contribution of ley-farming to ecosystem services for improving sustainability of cropping systems is becoming increasingly important in all different farming systems.

The 22nd symposium of the European Grassland Federation (EGF) hosted by Lithuania marks the return of EGF to a Baltic country, 18 years after the 13th EGF Symposium was held in Estonia. This year's symposium coincides with the 700th anniversary of Vilnius, the capital of Lithuania.

The symposium has three themes: (1) Eco-efficiency of leys in farming systems; (2) Biodiversity and other ecosystem services; (3) Multi-species swards and intercrops for crop rotations benefits. Support in grazing and cutting regimes are introduced and evaluated in plenary and poster sessions.

During the mid-symposium tour, we invite the delegates to visit experimental fields at the Lithuanian Research Centre for Agriculture and Forestry (LAMMC) and innovative cropping of sustainable farming companies. The post-symposium tour presents the situation in western Lithuania where high precipitation amounts through spring to summer ensure high quality grass production.

We would like to thank all authors for their contributions and reviewers on assessing the suitability of a manuscript for publication, the EGF secretary, our sponsors and supporters, and all delegates attending the 22nd EGF symposium 'The future role of ley-farming in cropping systems'.

Lina Šarūnaitė

Chair of the Organising Committee

Žydrė Kadžiulienė

Chair of the Scientific Committee

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Leys in sustainable farming systems

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Abstract

A grass ley is defined as a temporary grassland that is integrated in a crop rotation. It has its origin in the 16th century in Brabant and Flanders, where red clover replaced the fallow period in the crop rotation. Today, the main reasons for the use of leys have remained very similar; next to weed and pest control, leys are used for improving soil quality, fertilisation and feed for livestock. Where leys originally consisted of mainly red clover, they have evolved to leys with lucerne, grasses and mixtures with grass-clover and grass-clover with forbs. *Grosso modo*, three types of farming systems in which leys are used can be distinguished: (1) dairy/beef farming systems with leys in rotation with fodder crops; (2) mixed dairy/beef and arable farming systems (on one farm or regionally cooperating farms) using leys in the arable rotation for forage or biomass for energy; and (3) arable farming systems using leys for internal input as cut and carry fertilizers (C&C) or 'green fertilizer'. In this paper these three types of farming systems are described and illustrated with case studies. The role of a ley, consequences for species choice, ley duration and percentage of leys in the system are discussed.

Keywords: temporary grassland, crop rotation, integrated crop-livestock systems, cut and carry fertilizers, ecosystem services

Introduction

In the dictionary, the term ley, lea or lay is described as 'arable land laid down for grassland'. Allen *et al.* (2011), define a grass ley as a temporary grassland that is integrated in a crop rotation. A temporary grassland is composed of annual, biennial, or perennial forage species kept for a short period of time (usually only a few years) (Allen *et al.*, 2011). Temporary grassland in the EU is defined as grassland included as a part of a normal crop rotation, lasting at least one crop year and for less than five years (Lesschen *et al.*, 2014). Therefore, according to EU definitions, all temporary grasslands are leys.

In this paper we first go into the history of leys. Subsequently we describe the main motivations for the use of leys and which ecosystem services they provide and how this affects the choice of species. *Grosso modo*, three types of farming systems in which leys are used can be distinguished: (1) dairy/beef farming systems with leys in rotation with fodder crops; (2) mixed dairy/beef and arable farming systems (on one farm or regionally cooperating farms) using leys in the arable rotation for forage or biomass for energy; and (3) arable farming systems using the harvest of leys directly or harvested as silage for internal input as fertilizer the so called cut and carry fertilizers (C&C). In this paper these three types of farming systems are described and illustrated with case studies. The role of a ley, consequences for species choice, ley duration and percentage of leys in the system are discussed.

Historical perspective

A first record of a ley as an integral part of the crop rotation is from the 16th century in the Low Countries, Brabant and Flanders, now situated in Belgium. In the Low Countries, red clover (*Trifolium pratense*) structurally replaced the fallow period in the three-course crop rotation and changed it to a four-course rotation (Blom *et al.*, 2006; Peeters, 1980). This was also observed by the English writer Sir Richard Weston during his exile in the Low Countries in 1644-45, who wrote this in his account 'A Discours of Husbandrie used in Brabant and Flanders: shewing the wonderful Improvement of Land there; and serving as a Pattern for our Practice in this Common-Wealth' (Weston, 1652). This account is one of the first descriptions of the use of a farming rotation including (red) clover. The Norfolk four-course rotation with a red clover ley became the cornerstone of the 'agricultural revolution' in the 18th century, very well described in Kjærgaard (1994, 2003).

After the second world war the combination of the use of mineral fertilizers, pesticides, high-yield crop varieties, mechanisation, and government policies, subsidies and grants improved productivity. A ley crop rotation in a mixed farming system became of less importance and further agricultural specialisation and intensification was a fact (Knox *et al.*, 2011; Robinson and Sutherland, 2002). In Belgium, 4.6% of the agricultural area was covered with different species of clovers in 1929 (Atlas). The area started to decline quickly from 1935 onwards, to reach about 0.1% of the agricultural area in 2000. In the Netherlands, the area of clover and lucerne (*Medicago sativa*) on arable land reduced from 9% just after the second world war to 1% at present (CBS Statline). At the same time there was an increase in the area of temporary grassland on specialised dairy and beef farms in The Netherlands from 4% in 1970 to 11% in 2000, which peaked at 20% and 26% in 2003 and 2015, respectively, when EU regulations on permanent grassland were formalised (CBS Statline). Probably it was the formalisation of the regulations itself, which made farmers more aware about the difference between permanent and temporary grassland, which made both categories to be more carefully registered. Temporary grassland covered 6% of the agricultural land in Belgium in the period 2015-2019; permanent grassland covered 34% and silage maize (*Zea mays*) 12% (Statbel, 2008). On Danish dairy farms, in 2015-2019, 28% of the agricultural land was used for temporary grassland (predominantly grass-clover), 7% was permanent grassland and 24% was silage maize (Kristensen *et al.*, 2020). The average duration of the temporary grasslands was short, only three years on average due to a general yield and quality decline with grassland age (Eriksen *et al.*, 2012; Kristensen *et al.*, 2022).

At present, the 'From Farm to Fork' policy of the EU with, among others, plans for reductions in the use of pesticides (-50%) and fertilizers (-20%), combined also with an increase in fertilizer prices due to the Ukraine war, call for management practices that can support this. The use of leys is one of these management practices. In the recent decades, there is already a revival of leys as a management practice in sustainable farming systems including organic, agro-ecological and regenerative farming (Erisman *et al.*, 2016; Schreefel *et al.*, 2020).

Motivations and ecosystem services

Initially, the use of leys in the 16th century was aimed at improving soil fertility specifically and soil quality in general, with reduction of weeds and pests, and providing feed for livestock for recycling manure, but also as feed for horses and oxen for animal traction. Today, motivations for the use of leys remain essentially the same. However, in addition to the aims listed above, new motivations have arisen. For example, food for pollinators and other insects has now become an explicit objective of leys, whereas in the past the role of flowering leys of clover and lucerne in the crop rotation was merely a side-effect. Also, functions like climate regulation, water purification and phyto-extraction of residues of pesticides in the soil were not necessary or valued at that time (Martin *et al.*, 2020; Van Eekeren *et al.*, 2022).

The motivations of the past can be translated into ecosystems services in the present. Ecosystem services are the benefits that people derive from ecosystems to meet physiological, economic and non-material needs (MEA, 2005). Recently, Coolidge *et al.* (2022) and Martin *et al.* (2020) have provided an extensive review of the benefits and ecosystem services of leys in future farming systems. Martin *et al.* (2020) distinguish services flowing to crop production (input services more or less equivalent to regulating and maintenance services) and those flowing from crop production (output services more or less equivalent to provisioning services). Among input services they consider; soil conservation, nutrient provision and recycling, soil water retention, and biological control of pests and weeds. The main output services identified are: water purification, climate regulation, habitat provision for biodiversity conservation, and production of forage for livestock (both quantity and quality) or biomass to produce energy or C&C fertilizers. These services can be on field, farms, landscape, watershed and global level. In addition to these services, Martin *et al.* (2020) discuss the potential negative impact of leys including the competition for land with food crops and the increase in GHG emissions when leys are fed to ruminants.

Species and mixtures used

The composition of leys varies depending on region and the main motivation(s) or ecosystem service(s). They can consist of monocultures or mixtures of different grass, legume and forb species. For grasses, often grasses like perennial (*Lolium perenne*) and Italian ryegrass (*Lolium multiflorum*) are used because of their wide tolerance to different conditions, versatility of use (silage, hay and grazing), and high digestibility for livestock (Kingston-Smith *et al.*, 2013), but also their positive effect on weed suppression and soil structure in monoculture or in mixture with legumes (Connolly *et al.*, 2018; De Haas *et al.*, 2019; Van Eekeren *et al.*, 2008). Legumes grown in monoculture or in mixture have the benefit of reducing the need for mineral nitrogen (N) fertilizers as a result of their N-fixing abilities (Anglade *et al.*, 2015; Hoekstra *et al.*, 2017; Nyfeler *et al.*, 2009, 2011) but can also have positive effects on aboveground and belowground biodiversity (De Haas *et al.*, 2019; Van Eekeren *et al.*, 2008). For a further increase in resilience and ecosystem services forbs can be included in mixtures with grass and legumes (Cong *et al.*, 2017, 2018).

In an experiment in the Netherlands, the potential effects of different grass-clover mixtures and monocultures in an (organic) arable crop rotation on below- and aboveground traits and services were investigated. The experiment included two grasses (perennial and Italian rye grass) and two clovers (red and white clover (*Trifolium repens*)) in monocultures and mixtures. The grass monocultures showed high root density, good weed suppression and perennial ryegrass especially had a positive effect on soil structure (Table 1). Clover on the other hand had a positive effect on the soil mineral N, and earthworm abundance tended to be higher in the clover monocultures. Moreover, clover showed high herbage dry matter yield (particularly red clover) and N yield, and white clover showed high digestibility. When (some of) the four species were combined in grass-clover mixtures, they combined the positive effects of the species and often even outperformed the (best) monocultures (De Haas *et al.*, 2019). These results are in line with the findings of Finn *et al.* (2012, 2013).

The integration of leys in farming systems

We define three types of farming systems in which leys can be used. In the following sections we will give a general description of leys in each of these three farming systems, followed by a case study.

1. Dairy/beef farming systems with leys in rotation with fodder crops

Most specialised dairy and beef farms in North-West Europe combine grassland and arable land for cultivation of silage maize and, to a lesser extent, cereals for whole crop silage and fodder beet. In most cases, silage maize is permanently cultivated (year after year on the same field). Permanent cultivation of silage maize on the same production area leads to soil fertility problems, nitrate leaching and a possible decline in production. Long term research on the effect of ley-arable rotations compared with permanent grassland and arable land in the Woburn experiment in Rothamsted (Johnston *et al.*, 2017) and on the M66-1 experiment on a sandy loam soil in Melle (Nevens and Reheul, 2001, 2002, 2003; Van Eekeren *et al.*, 2008) have shown that the rotation of three years grass white clover, succeeded by three years of silage maize is optimal both for productivity as well as for N-use efficiency and soil quality. In the ley-arable rotation in Melle, soil parameters including soil organic matter (SOM) and N-total showed values

Table 1. Relative score (worst performing (--), best performing (++)) of the indicators used to assess ecosystem services for different grass and clover monocultures and mixtures (Lp = *Lolium perenne L.*, Lm = *Lolium multiflorum Lam*, Tp = *Trifolium pratense L.*, Tr = *Trifolium repens L.*) (adapted from De Haas *et al.*, (2019).[1]

Ecosystem service	Fallow	Monocultures				Mixtures					Range
		Lp	Lm	Tp	Tr	Lp:Tp	Lm:Tp	Lp:Tr	Lp:Tp:Tr	4s	
Soil conservation and water retention											
Soil structure (% crumbs)	--	+/-	--	+/-	--	+	+/-	++	++	++	10-50
Root score[2]	--	++	+	+/-	+/-	++	++	++	++	++	0-8
Nutrient provision and cycling											
Soil mineral N (kg N ha^{-1})	-	--	-	++	++	-	+/-	+	+/-	+/-	57-136
Biological control of pests and weeds											
Weed suppression (% weed cover)	n.d.	+/-	++	--	--	+/-	++	+	+	+	0-11
Habitat provision for conserving associated biodiversity											
DMY in spring[3] (t ha^{-1})	n.d.	+/-	--	++	++	+/-	--	+/-	+/-	--	1.5-8.4
Soil biota 0-25 cm[1]	+/-	--	-	++	++	+	++	++	+	++	3.0-8.5
Soil biota 25-45 cm[1]	--	+	+	+	++	++	++	+	++	++	0.0-7.0
Earthworm density (n m^{-2})	--	-	+/-	+	++	+	-	++	++	+	113-994
Forage production and quality											
Herbage DMY (t ha^{-1} yr^{-1})	n.d.	--	+/-	+	+/-	++	++	+	++	++	5.9-15.9
N conc. (g N kg^{-1} DM)	n.d.	--	--	++	++	+/-	--	+/-	+/-	-	11.4-37.9
N yield (kg N ha^{-1})	n.d.	--	--	++	+	++	+/-	+	+	+/-	77-475
Digestibility (g kg^{-1} DM)	n.d.	+/-	--	-	++	-	--	+/-	-	-	633-801
DOM yield (t ha^{-1})	n.d.	--	+/-	+	+	++	+	+	++	++	4.2-10.8

[1] DM = dry matter; DMY = dry matter yield; DOM = digestible organic matter.

[2] Score out of 10.

[3] Relatively low herbage mass in spring is important for meadowbird chick development and survival. n.d. = not determined.

that were in between the values of permanent grassland and permanent arable land (Table 2). There were more roots and a greater root biomass up to a depth of 20 cm in temporary grassland compared to permanent grassland, which improves the drought tolerance of temporary grassland above permanent grassland (Reheul *et al.*, 2007). Indicators for soil structure were comparable in permanent and temporary grassland. Soil structure and earthworm biomass declined in the arable stage, but recovered when rotated with temporary grassland (Van Eekeren *et al.*, 2008).

Case study 1: Dairy systems with 60% permanent grassland and 20% three-year lasting leys with grass-clover in rotation with 20% arable land (60%-20%-20% system)

Due to EU-legislation for derogation, until 2022 the land use of most conventional dairy farms on sandy soils in the Netherlands consisted of 80% grassland and 20% arable land, with the latter mainly consisting of silage maize cultivated year after year on the same area. Based on the results of the experiments at Rothamsted and Melle, and taking into account all the challenges of the dairy industry such as the need for improving soil quality, reducing the use of artificial fertilizer and soya, reducing the emissions of greenhouse gasses (GHG), ammonia and nitrate, increasing biodiversity and keeping up the EU legislation of permanent grassland, a land use of 60% permanent grassland, and 20% three-year lasting leys with grass-clover (red and white) in rotation with 20% arable land was proposed (60%-20%-20% system).

Table 2. Mean soil chemical, physical and biological characteristics of 36-39 years of permanent grassland (PG), temporary grassland (TG, grass white clover ley), temporary arable land (TA) and permanent arable land (PA) (Van Eekeren *et al.*, 2008).[1]

	Units	Treatments					Year	Treat.×year
		PG	TG	TA	PA	*P*-value	*P*-value	*P*-value
SOM[2]	g kg dry soil^{-1}	60.7 a	33.2 b	34.9 b	21.1 c	<0.001	0.004	0.003
N-total[2]	g N kg dry soil^{-1}	2.95 a	1.52 b	1.61 b	0.95 c	<0.001	<0.001	NS
Soil structure 0-10 cm[1]								
Crumb	%	33 a	32 a	8 b	8 b	<0.001	-	-
Earthworms[2]								
Total biomass	g m^{-2}	166 a	52 b	14 bc	5 c	<0.001	NS	NS
Grass roots[3]								
20 cm depth	n m^{-2}	1,081 b	1,813 a	-	-	<0.008	-	-

[1] Values followed by the same letter within a row are not statistically different at the 5% error level for the main treatment effect. NS = not significant.

[2] Means of 2002, 2003 and 2004. SOM = soil organic matter.

[3] Measured in 2004 only.

The impact on economics and environmental parameters of the change of the common land use (80% permanent grassland and 20% permanent arable land) to the 60%-20%-20% system was calculated with the model BBPR (De Boer, 2007). Calculations were based on an average farm in the east of The Netherlands on sandy soils: 100 dairy cows with accompanying young stock, 850,000 kg of milk on 47.2 ha of land. The land is structured as 60% (28.3 ha) permanent grassland close to the farm and a field plot of 18.8 ha at some distance from the farm buildings. The permanent grassland is used for mixed grazing/mowing management. On the field plot, 50% (9.4 ha) of the area (which is permanent grassland in the current situation), is sown with a mixture of perennial ryegrass, red and white clover in a three-year rotation with silage maize (instead of permanent silage maize in the current situation). On this ley, the nitrogen application rate is reduced from 242 kg N ha^{-1} (current rate on permanent grassland for mowing) to 54 kg available N ha^{-1}. Taking advantage of the N-mineralisation of the ploughed ley, the maize in rotation with the ley receives no fertilizer nitrogen in the first year of the rotation, half of the currently recommended 147 kg N ha^{-1} for permanent silage maize in the second year and full fertilizer application in the third year. Due to the halving of the N-application from slurry, an extra potassium application is necessary. The model showed that this change in land-use resulted in an increase in labour revenue by €7,400 with low fertilizer prices and by €12,175 with high fertilizer prices (Table 3). Because the gross herbage yield of the ley increases by more than 2 Mg DM ha^{-1}, less silage maize is needed and the roughage costs fall by more than €4,700. Due to the higher proportion of grass in the diet, the input of soya can be reduced, resulting in a reduction in concentrate costs of almost €4,000. Due to a higher crude protein (CP) content in the ration, the costs for slurry export of the farm increase by €2,100. Due to the nitrogen fixation, the costs for nitrogen fertilizer decrease by approximately €2,500 and €7,500 for low and high fertiliser prices respectively. However, the costs for potash fertilizer increase because less slurry is used. The costs for seed increase by almost €300 because grass-clover seed is €70 ha^{-1} more expensive than grassland and more than 3 ha grass-clover has to be sown every year. By spreading less nitrogen fertilizer, the nitrate leaching at farm level is lower than in the current situation (from 24.0 to 22.2 mg $NO3^{-}$ l^{-1}). The increase in ammonia emissions remains limited, although more CP in the ration (and therefore more N in the slurry) still leads to slightly higher ammonia emissions compared to the baseline situation (+1.6 kg $NH3^{+}$ ha^{-1}) (Van Eekeren *et al.*, 2016).

Table 3. Economy of the current average dairy farm with 80% permanent grassland and 20% permanent arable land with silage maize, and the difference when the land use is changed towards 60% permanent grassland, and 20% three-year grass-clover ley in rotation with 20% arable land with silage maize (adapted from Van Eekeren *et al.*, 2016).

	Current situation	**Difference with current situation when changed to 20% grass-clover leys**	
Fertilizer N price	Low	Low	High
Revenue from milk, etc. (A)	€342,865	+€0	+€0
Allocated costs (B)	€124,231	-€10,297	-€15,029
- Concentrate	€54,340	-€3,950	-€3,950
- Roughage and other feed	€21,536	-€4,686	-€4,866
- Nitrogen fertilizer	€6,515	-€2,565	-€7,624
- Potassium fertilizer	€1,442	+€646	+€974
- Seed	€2,961	+€278	+€278
Non allocated costs (C)	€228,401	+€2,854	+€2,854
- Contract work	€36,840	+€583	+€583
- Slurry export	€7,688	+€2,145	+€2,145
- General	€17,300	+€0	+€0
Labour revenue (A – B – C)	€-9,767	+€7,443	+€12,175

[1] Calculations with a low and high fertilizer N price are shown (€1.10 and €3.27 per kg N-fertilizer, respectively).

2. Mixed dairy/beef and arable farming systems using leys in the arable rotation

Although mixed livestock-crop systems at farm level have decreased in Europe, in practice examples can still be found, and leys have an important function in these systems. For example, the leys on an arable farm may be grazed by livestock owned by arable or livestock farmers. On a larger scale, arable farms may sell the harvested herbage for fodder or biomass, or leys may be contract-grown by companies that look after the growing and harvest. This is practised for example with lucerne, which is artificially dried and used as concentrates or other livestock feeds. On the other hand, also new mixed farming systems, also referred to as integrated crop-livestock systems (ICLS), have been reintroduced to promote more climate-resilient, sustainable and economically viable farming systems, compared to specialized and intensive systems (Sekaran *et al.*, 2021). Interesting are the ICLSs in which the cooperation affects the constituent farms, in order to produce more efficiently and economically, given the natural, economic and social constraints (Schut *et al.*, 2021).

Case study 2: Partnership farms in organic farming

In the Netherlands, more and more organic arable and dairy farmers work together in a so called partner farm concept (Nauta *et al.*, 1999; De Wit *et al*, 2006): mixed farming beyond farm level with one arable farm and one to five dairy farms (on average 1.9 dairy farms) at some distance of each other (average 34 km, up to 90 km) (De Wit *et al.*, 2022). In this system, most arable farms grow short term grass-clover leys for one to two years, as fodder for dairy farms in exchange for, e.g. manure, as a way to overcome some of the major disadvantages of specialisation for organic farmers: it widens the crop rotation of the arable farms, mainly facilitating N-fixation and weed suppression, while simultaneously it secures the supply of fodder with a high protein content and digestibility to the livestock farms, both at relatively low costs given the organic preconditions. The organic arable farms are mainly interested in grass-clover mixtures rather than in wheat or maize production to be used as concentrate because of the need of N-fixation and weed suppression in the rotation related to labour shortages. Therefore, the cooperation is often with organic dairy/beef farms and not with specialised organic chicken or pig farms. Major attention is given to, e.g. appropriate grass-clover mixtures, indicating the importance of high yielding red clover, both in terms of production and weed-suppression, above white clover. The most appropriate grass component in these mixtures is less clear, as e.g. hybrid and Italian rye grass might be suitable for weed suppression but at the risk of suppressing the share of clover and hence N-fixation (De Haas *et al.*, 2019; De Wit *et al.*, 2015).

The best way to include leys in an arable cropping system remains an ongoing process, because of the continuous changes of crop proportions in a cropping system in line with dynamic demands. However, an evaluation after 15 years showed that most arable farms cooperating in an ICLS, had devoted 25-40% of their agricultural area to grass-clover leys. This was in line with impact calculations with the model FARMDesign (Groot *et al.*, 2012) that indicate that high economic returns are still possible if 1/3rd of the crop rotation consists of grass-clover, while lower shares of grass-clover in the crop rotation might be hampered by labour constraints (e.g. weed control) or too low N-surpluses to support high yielding cash crops (Figure 1) (De Wit *et al.*, 2022). With such ICLS beyond farm level, decision making on livestock and crops is split into separate entities with different persons, incurring many socio-psychological issues. In their evaluation, participating farmers ranked the ease of communication as most important, followed by perceived reliability and potential for a long-term cooperation (De Wit *et al.*, 2022). In conclusion, the evaluation showed that partner farms can be characterized as informal but market-oriented 'business partnerships', based on the exchange of high valued materials as well as enhanced ecosystem services of leys.

3. Arable farming systems using leys on their own farm as C&C fertilizers

The objective of arable farming systems using leys as C&C fertilizers is to develop intensive cropping systems that facilitate more effective use of on-farm N-fixation (Antichi *et al.*, 2008). This was achieved by developing cropping systems based on leys of grass-clover or lucerne used as fertilizer (Van der Burgt *et al.*, 2013). Nutrients accumulated by these leys can be used as C&C fertilizer without being sold off-farm as forage and passing through an animal. This is desirable because the revenues from these crops are rather limited, whereas the on-farm nutrient-use efficiency on these organic arable farms can be improved. On top of this, the nutrients in these crops are better balanced for crop growth than the nutrients available in animal manure. In experiments, C&C fertilizers based on lucerne or clover have shown to be at least as efficient as animal manure in terms of N supplying capacity (Sorensen and Thorup-Kristensen 2011; Van der Burgt *et al.*, 2013). Although this form of use of leys started out as an extra way to use the product of leys next to forage, it is now also developing as a practice for certified vegan products cultivated without animal manure.

Case study 3: Experimental farm Kollumerwaard without external fertilizer

On the experimental farm Kollumerwaard in the North-East of the Netherlands, a field trial with an organic 6-year arable rotation based on 100% biologically fixed nitrogen without animal manure was established in 2012. The location has very fertile soil, reclaimed from the sea 50 years before, with 12% clay and 1.0% organic carbon (Van der Burgt *et al.*, 2021). The expectation was that a system relying on its own N-fixation will be strongly nitrogen-limited. To get a clear answer on the nitrogen fixation potential on the farm, no other inputs from outside the farm were used. The crop rotation and fertiliser strategy are presented in Figure 2. Each of the six crop fields is 0.8 ha, resulting in a total trial surface of 4.8 ha. The herbage produced in the ley, is a mixture of legumes (lucerne, red, white and Alexandrian clover), and harvested and conserved as silage until the next year as if it were fodder for ruminants. However, instead of feeding it to animals and using the resulting manure as fertilizer, the harvested product is directly used as C&C fertilizer. Therefore, the fluctuating yearly nitrogen production of the ley determines the N fertilizer input of the winter carrot, oats and seed potato in the following year.

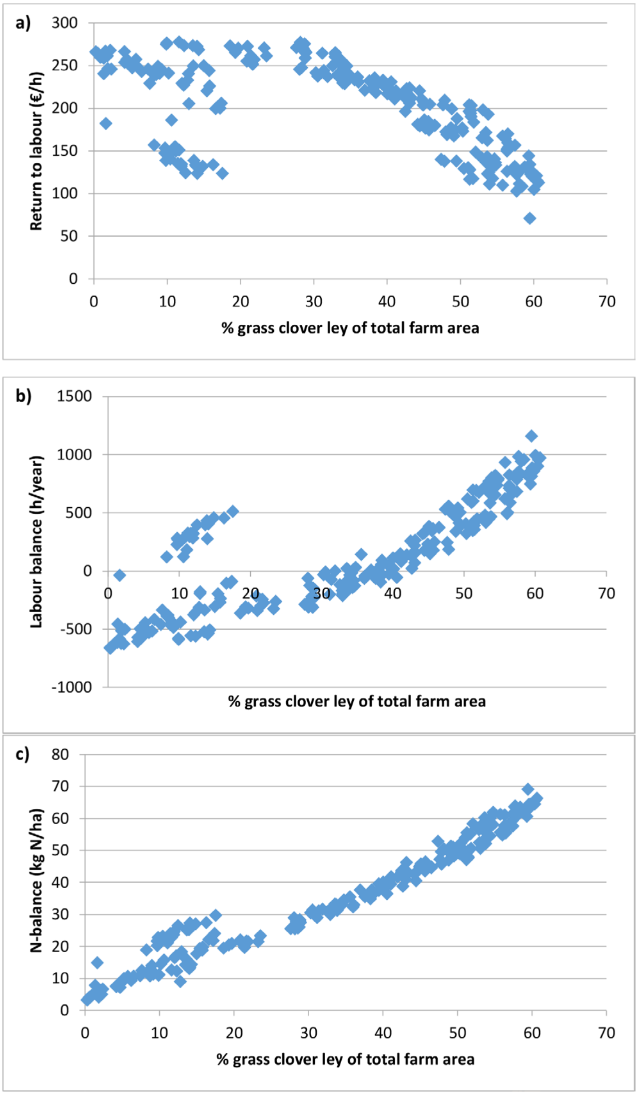

Figure 1. The effect of % grass-clover leys of the total area in the arable crop rotation on the return to labour (A), labour balance (B) and N balance (C) on the arable farm (adapted from De Wit *et al.*, 2022).

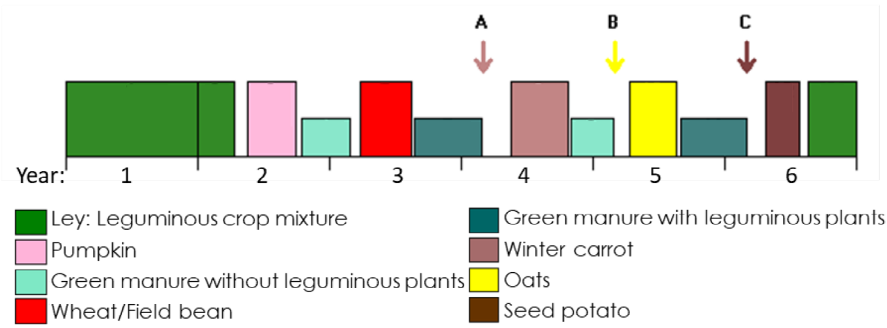

Figure 2. Six year crop rotation (see legend) and fertilizer scheme of C&C (Cut and Carry) fertilizer with arrows: (A) C&C fertilizer, 20% of C&C harvested in the year before; (B) C&C fertilizer 30%; (C) C&C fertilizer 50% (Van der Burgt *et al.*, 2021).

The yields of the different crops over nine experimental years (2012-2020) are shown in Table 4. Considered on a per hectare basis, the crop yield was not much lower (0% cereals, up to 10% seed potatoes) compared to manure-based organic production at this farm and in the region. In organic farming with manure, some crops might also be nitrogen-limited, others not (potato: *Phytophthora*-limited). However, taking into account that 1/6th of the area is needed for the C&C fertilizer, crop production on the whole farm level was at least 17% lower. The production of the C&C fertilizer was stable except for one year with an extreme low production, possibly due to drought. The wheat/field bean showed a strong trend to increased yield, as a result of the learning process on how to cultivate this mixed crop (variety choice, row distance, seed depth, mixed rows or separate rows). Carrot production was influenced by germination success (drought, weeds, soil structure) and weed pressure. No explanation is found for the strong increase in oats yield. Seed potato yield level was dominantly influenced by *Phytophthora*.

The nitrogen performance of the system has been evaluated by means of the NDICEA model (Van der Burgt *et al.*, 2006), which confirmed that the system is highly nitrogen-limited. There were no signs of other nutrient deficiencies. Soil nutrient stock was abundant, and during nine years only a small decrease was measured in P stock (P-AL, mg P_2O_5/100 g, -0.776 per year, P<0.01) and a considerable decrease in P availability (P-water soluble, mg P_2O_5 l^{-1}, -2.325 per year, P<0.001). Soil organic C and organic N showed no statistically significant change during the experiment, both measured and modelled. The system was highly N-efficient: N-efficiency defined as N-output/(N-fixation + N-deposition) was 76%. This high efficiency was due to very low losses: 17 kg N ha^{-1} yr^{-1} was lost due to leaching and 7 kg N ha^{-1} yr^{-1} was lost due to denitrification in the topsoil. There were no losses by volatilization since no manure or artificial fertilizer was used. N-losses in the silage process were minimal. In this system, 17% of the surface is used for C&C production, occupying space in which no cash crop can be cultivated. A higher C&C intensity could result in higher N-available levels and hence in higher potential yields of the cash crops, but the effect on the financial benefit is uncertain. This will highly depend on the availability and price of organically certified manure. If that price will go up, which is expected to occur, the C&C pathway will become more and more attractive as an additional source of nitrogen. Also, the C&C system resulted in additional costs associated with contract labour for the harvest of the C&C fertiliser (4 cuts per year). The next years' distribution was realized with own labour and equipment. Both activities result in additional costs compared to the purchase and application of manure.

Table 4. Yield level[1] of the different crops during the first nine experimental years (2012-2020) of a field trial with an organic 6-year arable rotation based on 100% biologically fixed nitrogen without animal manure (Van der Burgt *et al.*, 2021). The trend shows if the production has increased (+) over the years and if it has decreased (-) over the years measured.

	# years	Average	Minimum	Maximum	Trend
C&C fertilizer (in dry matter)	9	9,222	4,511	11,434	0
Pumpkin	7	20,665	14,400	27,181	+
Wheat/Field bean (85% d.m.)	6	4,572	2,545	8,308	++
Carrots	9	67,694	46,566	82,800	-
Oats	7	5,636	3,600	7,836	++
Seed potatoes	9	35,413	27,550	42,381	+

[1] Yield is expressed in kg fresh material ha^{-1}, except for the ley for the C&C fertilizer, which is expressed in kg dry matter ha^{-1}.

Results of this field trial show that this type of ley-based nitrogen-limited system can only perform adequately if every link in the crop rotation chain is dedicated to adding nitrogen into the system and preventing losses due to leaching, denitrification and volatilization. In the current experiment, 87% of the time a growing crop was present on the fields, and 51% of the time a legume or legume-including crop mixture was present on the field. Moreover, in ley-based nitrogen-limited systems the pathway ley → animal → manure results in substantial inevitable N-losses, compared to the near-zero N-losses observed in this C&C fertilizer system (data not shown). If nitrogen is or becomes limiting in (organic) arable farming, the losses in the animal pathway should be seriously discussed.

Conclusions

Integrating leys in farming systems is an important measure when, in the near future, production levels are to be increased with more limitations on the use of agrochemicals, high costs for N-fertilizer and pressure to reduce GHG-emissions. We have shown that leys can play an important role in pure dairy/beef, mixed dairy/beef-arable, and pure arable farming systems. Although many ecosystems services are affected by leys, nutrient provision and recycling via N-fixation of legumes, and maintenance of soil quality are important drivers behind the use of leys in all three systems (Table 5). Pest and weed control is also very important for the organic arable farms (system 2 and 3), more so than for conventional and organic dairy/beef farms. The function and the ecosystem services of the ley will determine the species and variety choice and needs more research and development. Legumes should be part of all leys for their N-fixation ability but the relative importance may differ (for example more important in C&C systems compared to dairy/beef systems). Whereas grasses in monoculture or in mixture with legumes can play an important role in weed suppression, care must be taken that this does not occur at the expense of the percentage of leguminous species. Forbs in multispecies mixtures can have a positive effect on different ecosystem services (e.g. habitat for biodiversity, drought resistance and weed control). At the moment this is the subject of research in a multisite experiment in which the yield benefits of multispecies leys and their legacy effects on a follow-on crop is investigated (O'Malley *et al.*, 2023). Also, the different duration of the leys in the three systems will affect variety choice and management of establishment. For example, persistence of red clover and forb species will play a more important role on a dairy/beef farm than on an arable farm, but early establishment by variety choice or management will be more important on arable farms. The percentage of leys in a system will depend on legislation, balance with food production, N-balance, labour balance, return to labour, etcetera, but will be about 17-40% of the total farms area. This requires customization and adaptation of the different systems over time.

Table 5. Overview of the use of leys in three farming systems; pure dairy/beef, mixed dairy/beef-arable and pure arable.

Farming system	Pure dairy/beef	Mixed dairy/beef-arable	Pure arable
Following crop	Fodder crop, Arable	Arable	Arable
Ley product	Forage	Forage or biomass	Cut & carry fertilizer
Ley product on or off farm	On farm	Off farm	On farm
Ley species[1]	Grasses, legumes, forbs	Grasses, legumes, forbs?	Legumes, grasses?, forbs?
Ley duration	2-3 years	1-2 years	1-2 years
Percentage of leys in system[2]	20-40%	25-33%	17-25%

[1] ?=For future research.

[2] Estimation based on case studies, the depends on a lot of factors and can change over time.

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Functionality and perspectives of long-term and short-term grasslands in agroecosystems: the case of Lithuanian agriculture

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Abstract

Diversification of agricultural systems and regeneration of soil functions are of crucial importance for many farming systems and this could be achieved by including multipurpose leys. They can lead to increase in agricultural productivity; however, it is very important to optimise functional diversity by combining different species characteristics that are well-adapted to local conditions. The ecosystem services provided by herbaceous plants that are managed as either long- or short-term swards for use in conventional or organic farming systems in the nemoral environmental zone will be presented. Results are discussed about grasses and legumes as regards possible stability of productivity within the vegetation season and over several years, and additionally on the influence of swards on the quality of herbage and soil characteristics. Furthermore, the assessment of the use of nitrogen fixed by perennial legumes for successive crops, and the impacts of the legume-based organic fertilizers for the maintenance of soil fertility and subsequent crops productivity, will be considered. The feasibility of common and less common grasses for different end-uses will be delivered. Improving adaptability and resilience of perennial grasses and legumes by the breeding process is one of the pathways for enhancing sustainability of agroecosystems and the results achieved at the Lithuanian Research Centre for Agriculture and Forestry will also be discussed.

Keywords: cultivars, breeding, energy crops, legumes, grasses, ley farming

Introduction

Sustaining agricultural productivity, increasing biodiversity, restoring and maintaining soil health and ecosystem services, including carbon capture and storage in agricultural landscapes, is essential in the context of the food and energy system (Jordon *et al.*, 2021). It is important for various agricultural farming systems – conventional, organic, intensive, low input, carbon farming, regenerative agriculture etc. One of the main roles of grassland is increasing of biodiversity in the environment. Enhancement of biodiversity in cropping systems both above- and below-ground are prerequisites for sustainable farming (Tiainen *et al.*, 2020). Nevertheless, grass species diversity increases grassland resilience. Diversity effects in multi-species mixtures are enhanced by the inclusion of legumes and useful forbs (Brophy *et al.*, 2017; 2018; Lüscher *et al.*, 2014, 2022).

Legume-based rotations supply nutrition at lower environmental cost and provide the most benefit when crops go directly into human food chain (Costa *et al.*, 2021); however, we cannot eliminate the benefits for animal nutrition and the increasingly prominent function as sustainability of cropping systems. The regenerative approach on leys in cropping systems has increasingly emphasized the benefits of ley pastures in cropping systems and demonstrated that they can provide a wide range of ecosystem services (Bengtsson *et al.*, 2019; Martin *et al.*, 2020; Zhou *et al.*, 2019). Well-managed multispecies swards can enhance the crop productivity as well as environmental sustainability (Finn *et al.*, 2013; Jaramillo *et al.*, 2021). Leys within integrated crop livestock systems play an important role in soil fertility, soil C sequestration and soil N mineralization potentials, and indicate that this low-input system can still maintain high crop productivity while simultaneously reducing the negative environmental effects (Berdeny *et al.*, 2021; Smith *et al.*, 2021). The practice of feasible perennial herbaceous systems can mitigate environmental impacts of current crop production, while providing biomass for the bioeconomy (Englund *et al.*, 2020; Schaub *et al.*, 2020).

An understanding of the multifunctional ecosystem services provided by grasslands and an assessment of environmental and management factors are essential to influence the processes driving change in multifunctionality of grassland ecosystems services (Richter *et al.*, 2021). Ecosystems services can vary strongly from location to location depending on the respective geographical or cultural circumstances; also, using various agri-environmental actions gives a positive effect on the processes linked to multiple ecosystem services (Bullock *et al.*, 2021). It must, however, be admitted that managing those multi-level processes is not easy. In the Nordic Europe region climate change may pose significant challenges to the growing conditions of perennial grasses, but may also provide opportunities to increase their productivity (Ergon *et al.*, 2017). In the face of such terms, one of the solutions is to focus on the plant breeding of new, more diverse varieties, that have characteristics to enable them to react as mildly as possible to the increased environmental stresses.

Improving the quality of herbaceous plants, especially legumes, herbage production, and mitigating the impact of environmental stress factors on plants and agroecosystems continue to be important multiple objectives. This paper reviews the research findings over the last decades developed by researchers from the Lithuanian Research Centre for Agriculture and Forestry.

A brief history of agriculture and grass husbandry in Lithuania

Though Lithuania is small country in the European context and its contribution in global food and feed production system is rather minor, agriculture is an important sector for Lithuania itself, supplying production not only for the country's residents, but also for export to other countries. Agriculture has been very important in Lithuania for a long time with approximately 75% of its territory dominated by rural landscapes and more than 50% is dedicated for agricultural purposes. Lithuania is in a nemoral climatic zone, where the mean annual air temperature is 7.4 °C and growing season lasts about 170-200 days (April-October), precipitation is very variable, but on average is 675 mm y^{-1}. Lithuania's natural conditions are favourable for agriculture, but they vary considerably within the country; different soils, uneven relief of the fields, and even differences in climate, which have implications for the selection of the most suitable plant and crop structure in individual locations.

Grasses were sown in Lithuania already back in the second half of the 18th century, while the scientific research on grasslands was initiated just at the beginning of the 20th century when Dotnuva Research station was established. The first studies focused on the fertilization of meadows, followed by research on forage legumes with a particular focus on red and white clover. Since the 1950s and later, all sectors of agriculture, including grassland management and husbandry, started to develop more intensive and targeted ways resulting in a significant increase of the production (Kadžiulis, 1972). Scientific research intensified after the establishment of the Institute of Agriculture in 1956.

Animal and grassland husbandry, as the main suppliers of the fodder chain, expanded throughout Lithuania, both on more fertile loam and less fertile sandy loam soils, as well as on flat and hilly terrain. From the establishment of the Institute until the restoration of Lithuania's independence (1990), many research studies on fertilization were carried out, covering the diversity of soils, grasses and their use. Grass production included research on grassland, pasture, perennial grasses for forage and seed and forage production in a variety of soils and climates. Establishment of different earliness of grasslands, consisting of perennial forage crop species of different biological characteristics, ensured a stable supply of fodder. According to recommendations of that time, the dominating species in early grasslands were cocksfoot, smooth bromegrass, Kentucky bluegrass, and red and white clovers. The composition of mid-early grasslands was more diverse and consisted of meadow fescue, timothy, red and white clover, alfalfa and Kentucky bluegrass, while late pasture swards included timothy, perennial ryegrass, Kentucky bluegrass and white clover as inclusion of white clover in grasslands resulted in increased production of high-quality fodder. Along with the botanical composition of grasslands numerous of research has focused on their management and application in the regions of Lithuania in different soils (Daugėlienė, 2010; Tonkūnas and Kadžiulis, 1977).

The research at that time was also directed towards botanical composition and its effect on grassland productivity, its chemical composition, NPK fertilization regimes and ratios in relation to the soil physicochemical properties. The studies concluded that changing the optimal ratio reduces the yield, greatly deteriorates its quality, the botanical composition of grasses, and the chemical composition of fodder. Part of the research was devoted to the basics of the establishment of grasslands, their management and renovation (Grassland Farming, 1995; Grassland Husbandry in Lithuania, 1995). However, the research included not only perennial pastures but meadow as well, which are the main source of winter fodder for ruminants; thus the topics on mixture composition, establishment, management of the meadows were of high importance. In order to have a winter forage the forage grasses and legumes were included in crop rotation. For a long time, red clover (*Trifolium pratense* L.) was widely grown in Lithuania and in many cases it was the most productive, only when alfalfa was grown on non-acidic soils was it superior to clovers. The alsike clover (*Trifolium hybridum* L.) and common corniculatus trefoil (*Lotus corniculatus* L.) were cultivated less often. Among the grasses, the most popular were timothy, meadow fescues, cocksfoot, perennial ryegrass, red fescues etc. *Festulolium* began to be popularized (Grassland Farming, 1995; Grassland Husbandry in Lithuania, 1995; Kadžiulis, 1972). The establishment of perennial pastures, meadows or herbaceous crop rotations also required the provision of grass seeds, which was one of the more difficult tasks in the northern climate. Various studies on seed production have been carried out. The latest research works carried out in the last three decades at our institution are reviewed in the subsequent sections of this paper.

We came to the period of the restoration of Lithuanian independence with these characteristics of land use: 700,000 ha perennial grasslands, and perennial grasses 600,000 ha in arable land, so the total of grasslands was 1928,000 ha, or 44.5% of the agricultural land (3,431,800 ha). At that time animal husbandry dominated (in 1990 there were 232,000 cattle and 842,000 cows). Large numbers of meadows and pastures were established and used for grazing and as the basis for winter forage; however, in practice the productivity was quite low.

After re-establishment of independence structural changes were made in the agricultural sector: the former collective and state farming was replaced as property restoration passed to private farms and agricultural companies. Private ownership was re-established and remarkably changed the management in the agricultural sector. In last decades not only has agricultural policy changed, but also the environmental policy. When Lithuania started preparing for the accession to the European Union, a number of financial mechanisms were dedicated in the agricultural sector. The macroeconomic indicators of agriculture, together with the food processing industry, have improved. In recent years, the production strategy focused on increasing production volumes and exports. Not only did this not help to improve the economic results of farms, but it also increased the operational risk of excess supply of agricultural products in the global market and the challenges of climate change. The structure of agriculture has also changed a lot. Grasslands now make up a much smaller part of total agroecosystems. Over the past decade, the areas of perennial pastures and meadows (aged 5 years and more) have been decreasing. In 2015, the declared area of arable land was 2,140 ha, perennial grasslands were 665,803 ha, and grasslands 278,033 ha. Meanwhile, in 2022 the areas were 2,280 ha, 565,821 ha and 167,701 ha, respectively (https://osp.stat.gov.lt/). The livestock sector is declining. In 2022, cattle (including beef cattle) numbered

629,000 and dairy cows only 225,000. Grasslands are still important in ruminant livestock production, but they are no less important for various crop production systems due to their ability to contribute to the reduction of pesticides and fertilizers. In recent decade, the crop production farms increased in both number and area, taking about 52% of arable land; however, their impact on sustainability of the agriculture was very limited or even opposite because of the insufficient inclusion of leys in crop rotations. Only at the beginning in the 21st century was more attention paid to the contribution of grasslands and their services of agroecosystems, such as bioenergy, biodiversity and agroecology.

Currently, Lithuania exports agricultural and food products to more than 140 countries of the world, which accounts for 20% of the country's total exports. New, modern, information systems-based agro-technologies are successfully used and implemented in modern agriculture. Farmers also successfully use the recommendations of scientists, which help to solve modern challenges related to climate change, environmental protection, and economic and social problems.

Legume-grass swards for value of herbage and diversity

The development of grasslands depends on various factors and circumstances: natural conditions, land use system, economic and changing farming conditions. Grassland-use systems can be very different: grasslands can be mowed, grazed or combined (both mowed and grazed). The composition of swards and utilization intensity affects the productivity, quality and stability of the herbage under influence of climate factors, stress and interspecies composition of swards. The intensity of plant growth varies and depends on different types of stress, which may affect the condition of swards. In our country, a number of different species of grasses have been studied in various soils and in our climate conditions and by applying different methods of using grasses (mowing, grazing) (Daugėlienė, 2010). It is difficult to review all the studies in detail in the relatively short scope of this paper, but the most important ones are worth noting on the functionality of legume-grass swards.

The intensity of grassland production depends on local circumstances and the use of the produced forage, although it might be flexible over time. The best sward composition, stable balance between legumes and grasses in the swards, sustainable forage production within season and over several years are relevant objectives in grassland management. The research of different legume-grass mixtures allows us to point out that white clover competes well with grasses without N fertilization with 4-5 grazings per season in the first 3 years of sward use (Kadziuliene, 2000; Skuodiene *et al.*, 2000). White clovers could also ensure a long-term stable productivity (5-6 t DM ha^{-1} on average) of swards without nitrogen fertilization (Daugėlienė, 2010; Gutauskas, 2003; Skuodiene, 2003;). In long-term swards (after more than 50 years) with regular application of K and P it is possible to obtain a sufficient white clover content (16.9-25.5% of DM yield) and a stable DM yield (6.0-6.9 t ha^{-1} y^{-1}) (Sarunaite and Kadziuliene, 2018).

So far studies have focused on the overall productivity of swards and their forage value as well as how different plant species can help each other in a mixture. The meadow fescue (*Festuca pratensis* L.)-white clover mixture without mineral nitrogen gave higher yields compared with mixtures of *Festuliolium*-clover. The results demonstrated that *Festuca* and *Festuliolium* mixtures with white clover were more sensitive to frequent utilization ((5-6 times per season) than the *Lolium perenne* mixture (Petraityte and Gutauskas, 2003). Frequency of grazing can have a major influence on the pattern of diverse swards productivity and it need to exploit for the complementarity of growth and species compatibility. In the studies of swards consisting of *Trifolium repens* L., *Medicago sativa* L., *Lolium perenne* L., *Poa pratensis* L., *Festulolium* hybrid under frequent (6 times per season) and less frequent (4-5 times per season) grazing management on a gleyic loamy soil (*Cambisol*) a positive effect of legumes on the distribution of yield over the whole grazing season was obtained. Swards containing lucerne exhibited the highest sustainability over five years under both grazing frequencies. The lucerne-based swards were more productive than grass swards, with and without nitrogen application (240 kg N ha^{-1}). Frequent grazing was more suitable for more even distribution of yield over season; however, higher and more stable DM yields were obtained under more extensive grazing, the crude protein content also reflecting the effects of sward composition and grazing frequency on DM yield (Kadžiuliene and Kadžiulis, 2005; Kadziulis and Kadziuliene, 2006). Swards with a different composition, but also with the forage legumes in the mixture, showed that legumes improve the productivity of the grasslands, but are better maintained when less frequent cutting is applied (Slepetys and Slepetiene, 2013). The stability and quality of the yield (about 6 t DM ha^{-1}) of swards with alfalfa under less frequent use can be maintained for about 10 years, although the species composition will have changed significantly. In the first years of swards use the swards were dominated by sown grasses, and there were only traces of forbs. In the tenth year of use of the grasslands, sown grasses still prevailed in the swards (55-65%), and already in the twelfth year accounted 60-70% of various forbs (Kadžiulienė *et al.*, 2013).

Forage legumes and grasses in mixtures, or swards of pure grasses are both important for supplying winter fodder. Often, the first cut of grassland is used for producing winter fodder, and second cut and later is use for grazing or is taken for green fodder for dairy cows in housed systems. Studies show that in Lithuania, grasses with different biological properties, such as timothy, meadow fescue, perennial ryegrass and one of the newer species as *Festulolium*, are among the most potential species for such swards. Timothy is one of the most common types of grasses, and has been grown in Lithuania for a long time, but it must be recognized that a small proportion is found in swards and input to production is rather small. This grass are more sensitive to frequent grazing or cutting, although some studies show that plants of this species are one of the late ones, but, compared to other grasses, the most productive in terms of the dry matter yield of the first cut. Meadow fescue is a long-lived, fairly productive, good wintering, moderately disease-resistant grass with a strong root system, suitable for haying and grazing, and is also more tolerant of lack of moisture and has better regrowth after cutting (Lemežienė *et al.*, 2004). Perennial ryegrass is one of the most valuable grasses in Europe because of its high yield and good forage quality; however, in the nemoral zone, ryegrass has disadvantages in terms of its persistence in the swards (Lemežienė *et al.*, 2004; Nekrošas and Kemešytė, 2007). *Festulolium* is more suitable for short-term grasslands, as a good harvest is obtained only in the first year of use (Kemešytė *et al.,* 2020). Breeders are focusing on improving the productivity and nutritional value of these grasses in new varieties.

Grass species in monocultures usually give high forage yields when supplied with high inputs of fertilizer nitrogen. Therefore, it is very important to find out the compatibility within grasses and in mixtures with legumes for swards used for mixed management. One of the suitable options is the use of mixtures of red clover with diverse grasses (timothy, perennial ryegrass or reed canary grass and tall fescue) (Skuodienė *et al.,* 2000). Alfalfa in mixtures with grasses is also very feasible for the mixed-use swards (the first cut for winter fodder, the last cut for grazing) (Šidlauskatė *et al.*, 2022). Red clover is suitable for mixtures grown in various soils; however, alfalfa reacts to soil acidity and there are not many soils suitable for alfalfa growing in our country. Therefore, the search for opportunities for growing alfalfa in less favourable conditions is very valuable (Liatukienė *et al.*, 2020; Skuodienė *et al.,* 2023).

Nutrient supplies are important limiting factor for grasslands growth; however, this is often requiring the use of synthetic fertilizers. The combination of grasses with legumes is a possible solution not only for maintaining a high level of production, but also to reduce the amount of mineral nitrogen fertilizers and obtain good quality grass production. There are uncertainties around the abilities of recent cultivars of perennial ryegrass, fescue and other grasses in interspecific competition with forage legumes in more northern climates. In the study of single-species and multi-species swards with three, four, six, and eight plant species in the mixtures including grasses and legumes, and with two backgrounds of N fertilisation (N0 and N150 kg ha^{-1} yr^{-1}), higher yields and forage quality were found in legume-grass swards without

N fertilisers than with the use of 150 kg N ha^{-1} The mineral N fertilisers reduced legume DM yields in the multi-species swards (Šidlauskaite *et al.*, 2022).

In a recent study of several forage legumes and grasses, the effects of sown plant diversity on total yield showed that some agronomic mixtures under relatively low nitrogen levels may perform better than grass monocultures under high nitrogen levels and at different levels of management intensity. The diversity effects were substantially reduced in the 3rd year, most probably due to reduced levels of legumes in sward (Šidlauskaitė *et al.*, 2022).

Results of studies show that perennial ryegrass cultivars differ in tolerance of stressful conditions such as drought and cold. Tetraploid genotypes are more tolerant of stressful growth conditions than diploid ones. Tetraploids are also characterized by better spring growth, higher dry matter yield and better regrowth after cutting (Kemesyte *et al.*, 2017; Nekrošas and Kemešytė, 2007). In our experiments we used several cultivars of ryegrass and their mixtures to understand how they affect competitive ability in swards. However, there are few results and the cultivars mixture productivity is always higher than that of separate cultivars (Šidlauskaitė *et al.*, 2021).

Grasslands with a diverse species composition not only lead to an increase in biodiversity, but can also ensure a more stable performance. The soils of Western and Southeastern Lithuania are naturally acidic. Where plant nutrition conditions are limited perennial grasses could grow and provide reasonable productivity and anti-erosion protection (Skuodienė *et al.*, 2020) and it is important to note that under these conditions different types of clover-grass swards promoted organic carbon immobilization in microbial biomass (Skuodienė *et al.*, 2017).

At present interest is being stimulated in carbon sequestration and grasslands represent one of the agricultural practices for storage of carbon. The total average soil organic carbon (SOC) stock in mineral topsoil of grasslands was documented at 74 t ha^{-1} (83.2 t in *Cambisols*), and although it is a smaller amount than in forests, it is somewhat more than in arable land (Armolaitis *et al.*, 2022). The trend was observed that a higher content of organic carbon is accumulated in the soil under legume-grass swards. Humic acids that apparently conserve carbon in the soil tended to accumulate in the lower content under grass swards applied with mineral nitrogen than under grass-legume swards (Kadziuliene and Slepetiene, 2004). This is important not only for total SOC, but also for distribution of various forms of lability. Studies show that using digestate for fertilization in grasslands can be observed to promote accumulation of SOC and C of mobile humic acids (Šlepetienė *et al.*, 2020). In our country, not many studies have been conducted on SOC accumulation or C sequestration in grasslands.

The contribution of legume-based systems in cropping systems

Legume-based leys are main drivers in diverse cropping systems, but they are even more important in stockless cropping systems. The incorporation of legumes into cropping rotations can be provided as main crops or pre-crop, catch crops or as intercrops in different environmental conditions and farm characteristics. The successful establishment of forage legumes is one of the most important factors influencing the latest effects of ecological services. Sowing legumes into spring cereals often delays the development and yield formation of legumes. During the first growth year of the sward, forage legumes produce low yield, and usually swards start to be used only in the second year of growth. Especially, if growth of the undersown cereal is rapid, the legumes suffer and swards produce lower yields in the following year (Grassland Farming, 1995; Grassland Husbandry, 1995). Red clover grows more successfully when sown into the cover crop, whereas alfalfa is more sensitive to the cover crop (Grassland Farming, 1995). In long-term experiments it has been established that in order to avoid fungal diseases and damage caused by nematodes of forage legumes, it is best to sow by maintaining intervals of 3-4 years without legumes. In order to grow legumes frequently, it is necessary to alternate legume species, e.g. clover of various species or lucerne. In such cases, the legume share in the crop rotation can be increased up 45-55% (Kadziulis, 2001).

Even if the main concern is feed, is still an effort to balance several ecosystem services, which are often interrelated. In a comparison of swards of diverse composition (variously composed of white clover, red clover, alfalfa, perennial ryegrass, timothy), the best yield was given by pure alfalfa and mixture of alfalfa with grasses. Their dry matter yield was 8.31-9.78 t in the first and 7.41-9.61 t ha^{-1} in the second year of the swards' use. The yield potential of pure red clover and mixed with grass was slightly lower in the first year of use (6.82-8.76 t ha^{-1}). Such a tendency was also found when evaluating the amount of crude protein or nitrogen accumulation in herbage (Kadziuliene, 2004; Kadziuliene and Kadziulis, 2007).

Sustainable agriculture obliges us to find ways to use nitrogen stored by legumes as efficiently as possible, avoiding nitrogen leaching and losses to the environment. Legume and grass mixtures were sown with and without a cover crop of barley or peas for whole crop, and barley for grain, to ascertain yield formation and contents of biological nitrogen fixed during the first two years of sward age. The nitrogen accumulated in herbage and biologically fixed over the two years of the sward's age depended on legume species and competitive plant (cover crop) species. The highest amounts were obtained in lucerne and ryegrass swards that grew without a cover crop. The amounts of biologically fixed nitrogen by red clover and ryegrass swards were significantly lower than that fixed by lucerne and ryegrass swards (Šarūnaitė *et al.*, 2008, 2012).

Crop rotations, crop mixtures and cover crops are tools used to increase yield stability and soil fertility that are able to support biodiversity at local and regional levels. Ploughed in residues of forage legumes incorporate large amounts of biological nitrogen in the soil (Kadziuliene, 2004; Arlauskienė and Maikštėnienė, 2006) and enrich the nutrition of subsequent crops. Especially in organic farming, legumes have a positive effect on growing winter or spring wheat (Kadziuliene and Kadziulis, 2006). The effect of red clover on spring cereal was investigated in organic farming ecosystems. Red clover was undersown in oats or without cover crop. The incorporation of red clover biomass into a cropping system showed the beneficial impact on grain yield of subsequent spring cereals. The incorporation red clover with low input of organic fertilizers can serve as a short-term management tool to improve the regulation of ecosystem services in organic cropping systems (Kadžiulienė *et al.*, 2018).

In experiments, winter wheat was directly drilled into pea stubble, white and red clovers. The control plots conventionally drilled after ploughing barley and pea stubble, white and red clover, and perennial ryegrass. The winter wheat yield in direct drilling system was reduced by 50% or more compared with that sown conventionally. A significant positive effect of nitrogen amount in winter wheat grain yield was observed in the direct drilling. A significantly higher total Nmin content was observed in the soil in direct drilling system clover-winter wheat as compared with that sown conventionally (Šarūnaitė *et al.*, 2013).

Cereal and legume intercropping is important in many low input agricultural systems. Interactions were investigated between combinations of different intercropping of oat (*Avena sativa* L.) and black medick (*Medicago lupulina* L.), oat and white clover (*Trifolium repens* L.), and oat and Egyptian clover (*T. alexandrinum* L.) under organic farming conditions. The results showed that oats dominated in the intercropping systems and forage legumes aboveground biomass decreased, compared to that of monocrops. However, in oat–forage legume intercropping systems, the legumes had no adverse effect on oat grain yield and helped in reducing weed competition (Gecaite *et al.*, 2021). Forage legumes (*Medicago lupulina* L., *Trifolium repens* L., *T. alexandrinum* L.) grown in pure and forage legume–winter wheat (*Triticum aestivum* L.) strip tillage intercrops reduced the yield of winter grain intercropped with black medic and white clover. The winter wheat sown by strip tillage produced the highest grain yield after a dead mulch of Egyptian clover; however, mulching of the aboveground mass of forage legumes did not significantly increase the yield of winter wheat grain and supress weeds (Arlauskienė *et al.*, 2021a,b).

The assessment of technologically processed plant based organic fertilisers (fresh mass of red clover, fermented red clover mass, fermented pea and wheat mass, composted red clover and wheat straw mass and granulated cattle manure) showed that in the 1st year, the grain yield of spring wheat was significantly increased using fresh red clover mass and granulated cattle manure. In the 2nd year of cropping the fermented red clover mass enhanced the yield of spring barley grain the most notably. The highest content of nitrogen in cereal grains and straw was accumulated using fresh red clover, fermented red clover and granulated cattle manure fertilisers. The effect of fermented red clover and granulated cattle manure was comparable; however, a greater improving effect was exhibited by the fermented red clover in the loam soil and by granulated cattle manure in the clay loam soil (Toleikienė *et al.*, 2020). Green manure produced from red clover herbage mass by ensiling red clover had the highest C:N ratio and most probably reduced plant available nitrogen (Arlauskiene *et al.*, 2019).

Herbaceous crops for bioenergy

The increase in global energy demand and the decrease in available fossil fuels, as well as concern about the effects of greenhouse gases on global climate change have pushed humanity towards the search for alternative energy sources. Perennial herbaceous crops could contribute to the biomass supply for bioenergy (Povilaitis *et al.*, 2016; Šiaudinis *et al.*, 2015; Tilvikienė *et al.*, 2016). In 1997, the Institute of Agriculture, LAMMC, started initial research aimed at the search for energy crops, evaluation of their cultivation technologies and evaluation of their use potential (Kryževičienė *et al.,* 2001). First, attention was given to the research of growing local species of grasses: cocksfoot (*Dactylis glomerata*), tall fescue (*Festuca arundinacea*), reed canary grass (*Phalaris arundinacea*), awnless brome (*Bromus inermis*) (Lemežienė *et al.,* 2009; Tilvikiene *et al*., 2012).

In order to expand the range of energy crops in the country, non-native, introduced perennial herbaceous species were also studied: miscanthus (*Miscanthus × giganteus*), cup plant (*Silphium perfoliatum*), mugwort (*Artemisia dubia*), virginia mallow (*Sida hermaphrodita*), switchgrass (*Panicum virgatum*), lupin (*Lupinus polyphyllus*), fodder galega (*Galega orientalis*), alfalfa (*Medicago sativa*), Japanese bamboo and Sakhalin knotweed (*Reynoutria japonica, R. sachalinensis*) (Butkutė *et al.*, 2015; Kadžiulienė *et al.*, 2014; Norkevičienė *et al.*, 2016a; Šiaudinis *et al.*, 2015, 2017; Slepetiene *et al.,* 2016; Slepetys *et al.*, 2013; Tilvikienė *et al.*, 2012; Titova and Bakšienė, 2015). The productivity of energy crops depends on the species and varies from year to year, but for most non-traditional plants it is higher compared to traditional grasses. The most productive and potentially promising for bioenergy in our country are miscanthus, mugwort, virginia mallow and tall fescue, especially for the use of biomass in combustion (Kadžiulienė *et al.*, 2017; Tilvikienė *et al.*, 2018).

The biomass quality varied significantly with the plant species and fertilization rate (90 or 170 kg N ha^{-1}). The highest productivity of evaluated energy crops was achieved in *M. giganteus* (21.54 t ha^{-1}) and *A. dubia* (17.86 t ha^{-1}) swards fertilized with 170 kg N ha^{-1}. *Sida hermaphrodita* (12.30 t ha^{-1}) and the traditional crop *F. arundinacea* (10.99 t ha^{-1}) produced the lowest biomass DM yield. The biomass quality of those crops was in line with the results obtained by other researchers and may be compared to woody short-rotation forests (Tilvikiene *et al.*, 2020).

Studies have shown that grasses are also suitable for biogas production (Tilvikienė *et al.*, 2016) and are more efficient in releasing biogas, compared to forage legumes (Šleptienė *et al.*, 2016), but in terms of biomass productivity none can be matched widely in the biogas sector to maize (Povilaitis *et al.*, 2016). The production, quality and energy potential of herbaceous plants are affected by the stages of cultivation, harvesting, transportation, processing and storage. One of the ways to optimize the yield and quality of biomass can be the regulation frequency and time of cutting during the growing season (Norkevičienė *et al.*, 2016b; Pocienė and Kadžulienė, 2016; Šiaudinis *et al.*, 2021; Tilvikienė *et al.*, 2012).

The results of the study of perennial grasses tall fescue (*Festuca arundinacea* Schreb.), cocksfoot (*Dactylis glomerata* L.) and reed canary grass (*Phalaris arundinacea* L.) for biogas production suggest that the biomass yield and energy potential depend on grass species and growing technology. The highest biomass yield and energy potential was achieved for tall fescue harvested twice per season and for the first time at the flowering stage. Fertilization with a higher level of nitrogen had a positive effect on all of the swards. The variation in cellulose, hemicellulose and lignin was most influenced by the time of the cut. The concentration of lignin did not exceed 7%. The carbon to nitrogen ratio of biomass was suitable for biogas production in all of the swards. It was determined that in northern climate conditions tall fescue cut twice per vegetation season and fertilized with 180 kg N ha^{-1} may be promising energy crop for biogas production (Tilvikienė *et al.*, 2016). Perennial grasses are important for biogas production not only because of the possible optimal energy potential per hectare, but also because of favourable environmental parameters. Researchers have found that the cumulative balance of greenhouse gases showed a decrease in emissions of these gases (Nekrošius *et al.*, 2014).

Breeding of perennial grasses for adaptation to environmental stress and production demands

Plant breeding provides a way for increasing the performance of plants by targeting and improving the plant genome. Crop breeding activities started back in 1922 with the establishment of Dotnuva Crop Breeding Station, where crop breeders applied traditional breeding methods and focused on crop quality and yield as well as disease resistance. One of the first developed Lithuanian-bred forage crop varieties was 'Liepsna' – an early diploid red clover variety, registered in 1957. Two years later in 1959, the late variety of red clover 'Kamaniai' was developed and distributed (Lazauskas and Dapkus, 1992). However, a huge step forward in forage crop breeding was taken after the establishment of the Forage Crop Breeding Centre in 1972 where innovative breeding methods and techniques, such as polyploidy, mutagenesis, *in vitro* culture and double haploids, as well as interspecific and intergeneric hybridisation, were applied in forage crop breeding programmes and resulted in thirty-seven varieties of legumes and grasses developed before 1990. After Lithuania restored its independence, for the last three decades, in the breeding programmes the main focus was put on *Trifolium pratense, Trifolium repens, Phleum pratense, Lolium perenne, Festuca pratensis,* ×*Festulolium* and *Dactylis glomerata* as the most promising species for animal feed production (Kanapeckas *et al.*, 2011; Lemežienė *et al.*, 2004). Genomic *in situ* hybridization (GISH) was used to determine and characterize the interaction between *Lolium* and *Festuca* genomes on a chromosome level (Pašakinskienė *et al.*, 1997, 2000). Later, the study began of the patterns of interaction and stabilization of fescue genomes, which would be related to their wintering and longevity (Jones and Pašakinskienė, 2005; Lideikytė and Pašakinskienė, 2007; Lideikytė *et al.*, 2006). In addition to the varieties registered after restoration of independence in 1990: 'Lina DS' (*L. multiflorum* subsp. *multiflorum* × *F. pratensis*), 'Punia DS' (*F. pratensis* × *L. multiflorum* subsp. *italicum*), 'Puga' (*L. multiflorum* subsp. *italicum* × *F. pratensis*), and 'Vėtra' (*L. muliflorum* subsp. *italicum* × *F. arundinacea*) are productive and good quality (Nekrošas and Kemešytė, 2007). All Lithuanian varieties are of the *Lolium* type. When developing new varieties, not only yield, but also dry matter yield stability indicators are taken into account, on the basis of which it is possible to predict the reaction to changes in growing conditions and recommend the necessary agrotechnology to obtain an optimal yield (Kemešytė, 2022). After conducting research in the Baltic countries and Sweden, it was found that 'Punia DS' is one of the most plastic varieties (Aavola *et al.*, 2013), so its range of use can be very wide. All created varieties were entered in the National List of Plant Varieties and in EU Common Catalogue of Varieties of Agricultural Plant Species.

It is no coincidence that a lot of effort is put into the selection process of *Lolium perenne*, as in many European and world countries, it is one of the most desirable species in grasslands nowadays. However, the species has some disadvantages in the northern climate. Perennial ryegrass is short-lived, suffers from spring frosts, and its dry matter yield is significantly reduced in the second year of use (Lemežienė *et al.*, 2001; Nekrošas, 1999; Tarakanovas *et al.*, 2004). Research is carried out in the field and in the laboratory to develop promising breeding material (Aleliūnas *et al.*, 2015; Dabkevičienė et.al., 2017; Kemešyte *et al.*, 2017). A considerable amount of genetic work has been devoted to improving various characteristics of perennial ryegrass, using the latest methods (AFLP, GWAS) (Aleleliūnas *et al.*, 2015; Brazauskas *et al.*, 2011, 2010, 2013; Jonavičienė *et al.*, 2012, 2009, 2014; Statkevičiūtė *et al.*., 2015, 2018). It is important to assess the genetic diversity, phenotypic plasticity and adaptability of the initial breeding material to adverse environmental conditions. Phenotyping in regard of biotic and abiotic factors is also an important part of genetic research (Helgadóttir *et al.*, 2018; Yates *et al.*, 2019). Climate change is driving an increased focus on improving the drought and disease resistance of ryegrass and other grass varieties (Akinroluyo *et al.*, 2020, 2021; Jaškūnė *et al.*, 2020, 2021; Kemešytė *et al.*, 2019). The new project EditGras4Food aims to improve perennial ryegrass for winter hardiness, persistence and biomass formation on water-limited conditions. This will enable us to utilize the information gained in future genomic selection programmes which are prescribed to develop ryegrass varieties with improved freezing and drought tolerance and persistence. Recently, 6 varieties of perennial ryegrass ('Sodrė', 'Elena DS', 'Veja DS', 'Verseka', 'Alduva', 'Raminta') and one Italian ryegrass varieties ('Ugnė') have been entered into the National List of Plant Varieties and the EU Common Catalogue of Varieties of Agricultural Plant Species.

Forage legumes are in high demand because of their ability to form a symbiosis with atmospheric nitrogen-fixing bacteria of the genus *Rhizobium*, and, primarily because of this characteristic, they are very important in implementing greening programmes or organic farming and for implementing biodiversity or other strategies. Alfalfa, and both red and white clovers are the most valued and widely used in practice among all forage legumes in Lithuania. However, in unfavourable growing conditions the registered varieties do not always reveal their species potential, and therefore the better performing varieties adapted to variable stresses are desirable.

Of all the species in the *Trifolium* genus, red clover is the most widely cultivated and is an extremely important agricultural crop suitable for sustainable farming. Four varieties of red clover created in the last three decades are included in the National List of Plant Varieties and in the EU Common Catalogue of Varieties of Agricultural Plant Species: 'Arimaičiai', 'Vytis', 'Sadūnai 'Radviliai'. Currently, breeding programmes at LAMMC Institute of Agriculture focus on increasing plant productivity as well as on developing disease-resistant varieties. The selection of red clover varieties and breeding lines resistance to *S. trifoliorum* and *Fusarium* spp. have been done *in vitro* and also *in situ* (Mikaliūnienė *et al.*, 2015). Great attention is paid to collecting and conserving the wild genotypes of red clover as well. The wild populations of red clover were collected across Lithuania territory and currently are used in pre-breeding process (Petrauskas, 2021). Mass and individual selection have been done based on high number of flowers per plant and earliness for flowering. White clovers are valued for high fodder quality, quick regrowth and resistance to grazing. Currently, the three registered varieties of white clover 'Sūduviai', 'Nemuniai' and 'Dotnuviai' are characterized by disease resistance, a good yield of biomass, dry matter and seeds, as well as they are suitable for growing in mixtures with grasses. According to studies conducted in Lithuania it was found that these varieties provided higher plasticity under various growing conditions (Sprainaitis *et al.*, 2003; Tarakanovas *et al.*, 2007). *Medicago sativa* plants are characterized by high productivity, rich protein content and tolerance to drought. The latest four varieties of alfalfa 'Žydrūnė', 'Antanė', 'Malvina' and 'Birutė' are described as productive, as well as performing good overwintering and regrowth after harvesting (Svirskis, 2002). One of the current breeding aims is to reduce alfalfa sensitivity to aluminium

(Liatukienė *et al.*, 2020, 2021). In Lithuania the minor species *Trifolium hybridum* grows well in moist and acid soils and is suitable for fodder production. The varieties ‘Lomiai’ and ‘Poliai’ are included in the National and EU Common Catalogue of Varieties of Agricultural Plant Species. During recent decades, special attention was paid to wild and semi-natural ecotypes of legumes and grasess that have been regularly collected during field expeditions in Lithuania, Latvia, Estonia, Kaliningrad region, and Ukraine since 1994. Collected material is preserved in the Lithuanian Plant Gene Bank. Native ecotypes are adapted to the local climatic and growing conditions because thousands of years of natural selection have left only genes that encode the most necessary features and characteristics. Some of them are valuable for their feed quality or biological properties, others for their aesthetics.

The challenges of a rapidly changing environment and new agricultural needs are encouraging farmers to grow new, non-traditional varieties, not only for feed, but also for landscaping, or with exceptional qualitative functional properties and suitable for the development of nutritional added products. Maintaining species diversity on farm can bring benefits, for instance it helps to optimize production under heterogeneous agro-ecological conditions, allows production for a wider variety of products suitable for different uses, provide commercial opportunities in multiple localities, as well as reduce vulnerability to market and climate variability. In Lithuania the search for promising legume species for non-traditional uses (for food, suitability for ecological revegetation, for landscaping project) is carried out on the species: *Onobrychis viciifolia*, *Lotus corniculatus, Trifolium hybridum, T. medium, T. incarnatum, T. montanum, T. rubens, T. arvense, Medicago falcata, M. lupulina, Astragalus glycyphyllos, A. cicer, Melilotus officinalis,* etc.

Some grass species that are bred for fodder, such as cocksfoot, tall fescue and reed canary grass, may also be suitable for biofuel production. Therefore, in recent years, the direction of developing energy grass varieties has been included in the breeding programmes. New productive varieties were bred and registered including reed canary grass ‘Pievys DS’, and tall fescue varieties ‘Monas’ and ‘Medainis’. Recently the populations of non-native switchgrass were tested for their biomass chemical composition, yield potential and suitability for the biofuel production (Norkevičienė *et al.*, 2016). Perennial grass species have a high energy potential and therefore plant biomass could at least partially replace fossil biofuels. High yet very realistic hopes are placed on the progress of technologies used for biomass-energy conversion which could reduce the cost of lignocellulose processing and consequently increase the demand for high yielding energetic plants. With the changing European Union and Lithuanian agricultural policy, the diversification of crops, the maintenance of perennial meadows and the preservation of biological diversity are announced. It is obvious that grasses could be used not only for fodder production, but also to help meet other needs such as recultivation of abandoned territories, energy production, anti-erosion purposes, etc. Seldom-cultivated native species, such as *Phleum nodosum*, *Arrhenatherum elatius, Phalaris arundinacea, Agrostis capillaris*, and *Deschampsia cespitosa* may be suitable for achieving new eco goals.

Future perspectives

The projected increase in temperature, together with increased CO_2 levels in the atmosphere, indicate likely yield increase in all agricultural crops that are now being cultivated in Lithuania. However, at the same time there is the challenge of increasing stockless agricultural systems concurrent with new problems arising in plant nutrition and soil health, and in threats from pests and diseases. Improving the management of nutrients in stockless conventional and organic farming systems in the long-term will need more research results, need elaborate innovative use of legume biomass in cropping systems.

More research is also needed on grasslands in diverse farming systems and the interaction of biodiversity in creating ecosystem functions. More attention should be paid to the above-ground and below-ground biomass potential of multispecies grasses and integration into ecosystem functions and agricultural practices. Ley farming as one of the important components of farming systems (organic farming, carbon farming, regenerative farming etc.) has high potential to underpin the transition to sustainable ecosystems services and healthy diets targeted by the European Green New Deal Farm to Fork strategy. Future research should focus on improving forage legume–cereal intercrop management in order to optimize plant residue mineralization and to obtain satisfactory and stabile subsequent cereal yields.

Contemporary agriculture needs new varieties of grasses and legumes with a full range of plastic properties, adapted to rapidly changing environmental conditions, allowing the formation of heterogeneous grasslands. The new varieties must not only be high yielding, but also have good forage qualities, longevity, resistance to frost, drought, diseases and pests, as well as rapid regrowth after cutting.

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Theme 1.
Eco-efficiency of leys in farming systems

Novel rural circular solutions for grass-based agri-food chains

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Abstract

New technological and business solutions for alternative uses of grassland biomass can contribute to circular economies and sustainable agroecosystems. We discuss integrated innovations for circular agro-food systems using novel technologies with surplus biomass from grassland in replicable business solutions. In doing so, we investigate what are the key characteristics and necessary conditions for the adoption of novel circular solutions for grass-based agri-food chains. Results show how grass types with different characteristics can be processed cost-efficiently providing novel and high-value products and services. Case studies indicate that grass-based innovations depend on robust business models embedded in local innovation structures. The studied business models in areas successfully serve niche markets and take advantage of pull factors to attract complementary activities. Such pull-factors include besides competitive pricing, a growing demand, shorter value chains and the organisational capacity to integrate components of technical and social innovations. The findings show that the valorisation of grass and the emergence of alternative grass-based business models are fostered by the readiness of complementary activities, the alignment of the business environments and the coordination of actions between key actors and resource uses along the value chain. Enabling factors for innovative business models include, inter alia, availability of financial resources, supportive rules and regulations, adequate infrastructure for production and mobilization, opportunities for cooperation, capacity building of local actors and organisations as well as consumer demand for bio-based products. The potential use of grass for different side-products suggests additional cascading and added-value potential for different grass-based solutions. These solutions provide opportunities to close the circular loop, contribute to sustainability and create alternative revenues for farmers. Policy-makers and stakeholders in rural development should consider the readiness of innovation drivers, particularly consumers, manufacturing, and regulations, in addition to technological readiness as a necessary condition for innovation.

Keywords: grassland biomass, novel products, business model, business environment, circular agrifood system, case study

1. Introduction

Bioeconomy strategies on European, national and regional levels call for an efficient use of renewable biological resources. The development of new business models from underutilised biomass as source material has the potential to strengthen rural areas. Value chains for alternative end-products might require new biotechnologies, but also have the chance to provide new ecosystem services such as carbon sequestration. In the bioeconomy, the focus of terrestrial source material lies, so far, on forest and agricultural crop residues. Yet, grassland and shrubland cover 28% of the EU land area (Eurostat, 2018). In some of these areas, large parts of the biomass produced currently rots and decays before and after mowing, incurring costs and depriving individuals and society of benefits. There are diverse reasons why grass biomass is underutilised in certain areas. For example, grass may have a low nutritional value due to late harvest times in nature conservation areas, or as roadside grass, making the grass unsuitable as animal feed. Another reason is the difficult access to grass biomass located in remote areas.

Alternative business models need to be developed for increasing the biomass use-efficiency and to profit from the ecosystem services provided. As grass biomass quality differs depending on the reason for its underutilisation and as local preconditions are highly variable in space, different business models are necessary for different places. In doing so, we contribute to the question of what are the key characteristics and necessary conditions for the adoption of novel rural circular solutions for grass-based agri-food chains.

2. Methods and material

We discuss four farm-scale demonstration cases of circular integrated agri-food chains in Denmark, Germany, Sweden and The Netherlands. The case studies are part of the EU-funded GO-GRASS project, which aims to develop cost-effective and circular agri-food chains exploiting the underused potential of grass resources. Case studies can be used to explain and characterize occurrences or phenomena in their natural environments, which could aid in outlining the causal relationships and pathways that come from the development of new policies or services (Yin, 2009). In this paper, we aim to describe these four innovative grass-based business cases, and to understand their business models and environment respectively. The findings and conclusions are based on a collaborative and open approach using qualitative analysis and interviews with relevant stakeholders. Lessons learned from the GO-GRASS cases and from desk research are also included (Grundmann *et al.*, 2022).

The grass-based agri-food chain studied in Germany produces biochar via carbonisation of grassland-cuttings from wetlands as supplement for soil improvement. In the Netherlands it is using digester and fermentation technology to produce paper and carton products from roadside grass and from nature or fauna grass. In Sweden it aims to establish briquetting technology at local and small-scale to produce climate-friendly and heat-treated animal bedding from reed canary grass. Finally, the grass-based agri-food chain studied in Denmark operates a farm-scale green biorefinery to extract protein concentrates for monogastric animals from grassland situated in nitrate sensitive areas.

3. Results

The successful implementation of novel circular agri-food chains based on grasslands depends on enabling factors identified at the level of the business models and of the business environments. The factors include the availability of unused feedstock, multi-faceted local benefits and beneficiaries, added

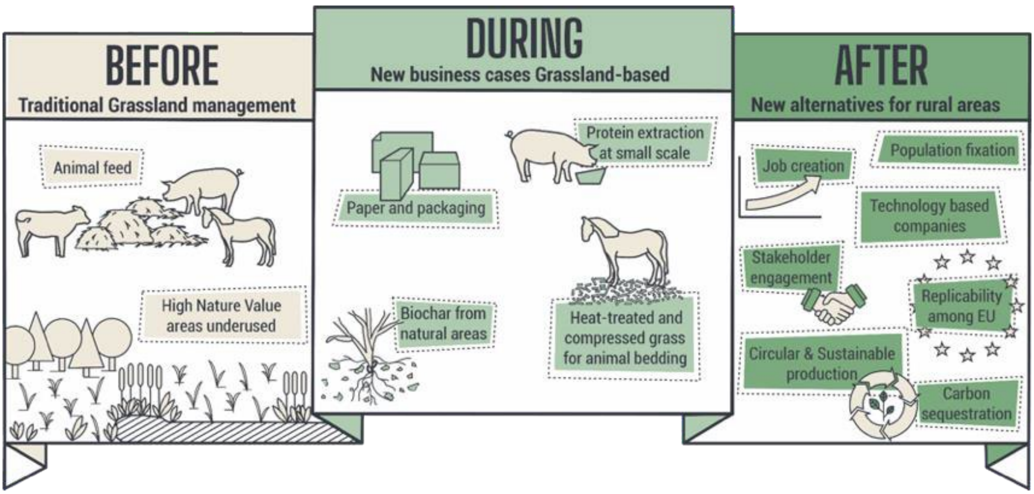

Figure 1. Grass-based business cases developed in the EU-funded GO-GRASS project.

value from the provision of ecosystem services, financial resources, supportive rules and regulations, adequate infrastructures for production and mobilisation, cooperation structures, training and education of local actors as well as consumer demand for bio-based products (Adamseged and Grundmann, 2020).

One of the strengths of the 'new' business models in the four studied cases is the availability of unused grass as the key resource to produce local-based products and services. The end-users of most of the primary products are farmers or local manufacturers, and this enables the creation of backward and forward linkages with other economic and social activities at the local or regional levels, resulting in further opportunities for diversifying business models such as biogas or fertilizer production to close the circular loop process.

The Swedish case combines better use of the locally produced animal bedding from reed canary grass. The mixture with straw and manure can be used for higher efficiency of biogas production and the residual can further be reused in agricultural farms and in gardens. Therefore, this technology and business model developed by researchers, farmers and business entrepreneurs from Research Institutes of Sweden (RISE), Glommers Miljö Energi, and Västakra Gard particularly strengthens circularity. There are big areas in Sweden that are abandoned, and there are earlier drained peatlands which are often a source of carbon emission. Swedish authorities have so far only suggested rewetting of these areas to stop the CO_2 emissions, but research shows that a crop like reed canary grass with its deep root system and viable growth can establish several benefits on these organic fields: as a carbon sink, production of biomass to replace fossil and/or other alternatives, and providing biodiversity particularly in forest regions. In the Swedish case, the reed canary grass is shredded, pressed into briquettes with screw presses and then shredded to flakes – an innovative material for animal bedding, which afterwards can easily be used as fertiliser, as well as for biogas and energy production. The screw press with temperatures high enough to reach hygiene quality, and the shredding of briquettes are key components in the process. Reed canary grass is a much more suitable source for animal bedding than the materials used so far – sawdust and wood shavings – which hold more potential for the use in biorefinery processes or material development. Furthermore, reed canary grass bedding with manure will result in higher efficiency of the biogas process and contribute to increase circularity. The value proposition is based on the process step of converting the agricultural crop into uniform shapes, facilitating its handling and storage. Depending on the customer needs, the bedding material is delivered in different size packages, from a 20 kg bag to bulk deliveries. Therefore, the focus is the optimization of the briquetting technology along with other technologies such as grass shredding, briquette shredding and packaging.

In the case of the Netherlands, digester and fermentation technology is used to produce paper and carton products from roadside grass and from nature or fauna grass. The case is based on a process to extract fibres from grass to produce high-quality packaging and paper, besides biogas. Researchers, farmers, consultants and business entrepreneurs from the Application Centre for Renewable Resources (ACCRESS – Wageningen University), HIEMSTRA, Vereniging Noardlike Fryske Wâlden and Papierfabriek Schut develop the technological processes including a cleaning system, which will separate unwanted components from the harvested grass. They also optimise the technology of reducing sugars by digestion and separating and cleaning fibres from the grass. The grass-fibres are separated and isolated through a digestion process and washing process and then used to produce paper and cartons. The final grass-fibre used to fabricate the end products reduces transport costs and less chemical usage for preparation than fibre produced out of wood. The main value proposition is the high value product made from the low quality and waste grass. Paper and carton manufacturers buy the grass fibre, while farmers can draw additional benefits from the liquid fraction. This has enabled a more sustainable production of high-quality paper and packaging, and it creates new revenue streams and circular valorisation of the liquid fraction for farmers and landowners. Additionally, the business of turning a low value resource into paper generates value and revenues for (regional) governments. The solution reduces the costs previously needed for disposing of mown roadside grass. The environmental benefits are also clear, as fewer trees have to be cut for the production of paper. The small-scale production of paper, where a small portion of grass (8%) is added, is a process that already exists. However, liberating the cellulose from the grass and almost completely substituting all the wood-based cellulose is a breakthrough innovation in the paper industry. Some preliminary results obtained on the environmental assessment of the grass-based paper imply there is a lower carbon footprint when the whole value chain is considered (from the cultivation until the product fabrication) even when considering that the energy needs for grass dewatering are higher than for wood dewatering. However, there are some other components of the value chain, such as lower requirements for heating or chemicals for the extraction of vegetal fibres from grass than from wood, which reduce the environmental impact of grass-based paper. The customer segments indicate the multi-faceted local benefits and beneficiaries of the business model for grass delivery by the landowners, local/regional governments, natural park management organizations and society as a whole.

The value proposition of the Danish case is the co-production of high value products from green biorefining and reduced nitrate leaching to support farmers in their continued license to produce. Danish agriculture is intensive, and 87% of the agricultural area is in crop rotation. Researchers, farmers, cooperatives, consultants and business partners from Aarhus University, Food and Biocluster Denmark, and Velas are developing a farm-scale bio-refining technology to extract protein concentrates for monogastric animals from grassland situated in nitrate sensitive areas. The extracted organic protein concentrate can be fed to pigs and poultry to enrich their diet and substitute soy meal. Other product streams are a fibrous pulp, which is used for ruminant feed, biogas or biomaterials as well as brown juice that is used for biogas and subsequently as fertiliser. The case partners are working on the optimisation of biorefining processes to provide high yields and high purity of the protein product as well as quality co-products in the form of renewable bioenergy and recycled nutrients. The biorefining products include organic protein concentrates, high quality roughage for ruminant feed, biochemicals and specific high value compounds and biogas recycled fertiliser from treated biomass. Denmark's leading position in developing technologies within green biorefinery has provided the opportunity for the development of the business model. The main customers are cooperatives, farmers and other local community actors. The players are testing and optimizing new integrated technology and demonstrating it in an industrially scalable facility. The main innovation of the process revolves around using different grasses and legume mixes, harvest and logistics, and mechanical wet fractionation to increase yields of protein at scale. The increasing quality of protein concentrate is tested in feed trials with pigs and the fibre pulp is tested in large farm-scale feeding trials with dairy cows. The site is cooperating with new commercial biorefineries in Denmark to develop and implement the technology for processing grass and legumes. These biorefineries will produce first a commercial protein concentrate to substitute soy, a fibre fraction for cattle feeding and a brown juice that can be used for biogas production. This will open a new market and contribute to the required reductions in nitrate leaching due to the Water Framework Directive by converting annual cropland into more or less permanent grassland. The establishment of grass-derived protein for organic farming in Denmark would also contribute to the reduction of soybean imports and derived emissions from the long transport and destruction of ecosystems in the local area exploited for soybean production.

In the German case, an important area of grassland is underutilised due to low quality of the grass either for feed or as inputs for biogas production. The value proposition of the German case business model is to increase the water holding capacity, nutrient retention and carbon storage capacity of the soils through the production and application of biochar from surplus grass. At the German case site researchers, farmers and civil society associations from the Leibniz-Institute for Agricultural Engineering and Bioeconomy, and the Nationalparkverein Unteres Odertal process low nutritional quality grass from wetlands into biochar. By implementing a first complete processing line, the grass is converted into biochar via pyrolysis (i.e. thermal decomposition in an inert atmosphere). The final product can be used on agricultural farmland, where it may provide a multitude of benefits, such as increasing water holding capacity and nutrient retention of the soil. Farmers are particularly interested in the biochar to be applied site-specifically as a soil amendment to agricultural fields outside the national park. This practice increases the fertility and water holding capacity of the soil. The biochar can be mixed with compost, biogas digestate or manure to enrich the char particles with nutrients before it is applied. During the conversion process, large amounts of heat energy are being released and can serve as heat supply for industrial and housing uses. A large fraction of carbon from the grass remains in the biochar and is returned to the soil. Therefore, the overall process may be viewed as a decentralised carbon sequestering technology that releases energy and produces biochar, thereby contributing to negative carbon emissions. Once implemented, this innovation can also be used to valorise other types of lignified biomasses e.g. from urban parks, nutrient-poor grasslands and even roadsides.

The case-study findings show that grass-based agri-food chain innovations require supportive business environments and enabling institutional settings to materialise. We identified the availability of sufficient direct funds as one of the important necessary factors for scaling-up novel grass-based products in the studied cases. Funding mechanisms designed to incorporate and promote the specific benefits generated by grass producing and processing companies are major enablers from the enterprise's perspective. The cases presented in this study are financially supported by the research and innovation project, and the availability of financial resources is a precondition for upscaling or replicating the demo. Therefore, the availability of financial resources for the actors in the value chain is a precondition, which might be supported by private or public funding.

Innovative grass-based businesses benefit from regularly adjusted policy regulations and administrative procedures to develop more sustainable products. Practitioners welcome clear and dedicated regulations that specifically support the developing of grass-based agri-food chains. Implementation of the EU water framework directive provides a favourable environment for grass cultivation in Denmark and opens opportunities for grass-based products. On the other side, the misalignment between EU regulation on fertilising products ((EU) 2009/1009) and the German Fertilizer Ordinance makes marketing of grass-based biochar as a soil amendment highly challenging in Germany.

Consumers play a decisive role in the development of innovative grass-based business models. For example, consumer perception is a strong driver in the Danish demo, where there is also an increasing demand by livestock farmers for regional protein, as the environmental awareness of end-costumers has recently been raised. Our findings suggest that building a high level of confidence and trust with regard to the quality of the grass-based products has a positive impact on consumers' willingness to choose grass-based products. Product certification and clear information on the verified quality, usability, production methods and raw materials used in the production of grass-based goods contribute to the consumers' choice of grass-based products. Creating an enabling environment for innovative, emerging grass-based business models through raising consumers' perception and opening market opportunities is a long-term endeavour. Unless coupled with responsible consumption patterns, sustainable production cannot contribute to the overall goals of sustainable development.

Sufficient capacity in the manufacturing of inputs along the value chain proves to be a necessary condition for completing the technical and social innovation process successfully (Orozco and Grundmann, 2022). Delayed innovations in the technology and process implementation were often caused by a lack of capacity in manufacturing and provision of equipment and services as, for example, the need to modify and test a cleaning system that can ensure an efficient separation of unwanted components from the harvested grass. This underlines the importance of readiness and capacity in manufacturing for successful innovation.

Supportive conditions for creating enabling business environments for novel circular agri-food chains are the results of coordinated efforts from stakeholders, such as public agencies, research institutes, cooperatives, networks, or associations interacting with the enterprises. Existence of opportunities for cooperation counts as an important enabling factor. This is true regarding local stakeholders (e.g. farmers, researchers, business owners, etc.), but as the GO-GRASS project has revealed, it is also true for the four demonstration sites. Intensive collaboration between cases generated new ideas for problem solving as shown, for example, with the same briquetting technique that can be used in the Swedish and German demo.

4. Discussion

The GO-GRASS project explores how grass types with different characteristics can be processed into novel and high-value products. It is a key learning from GO-GRASS project that the success of new grass-based technological innovations depends on their local development potential for a business model and their commercial potential in finding their niche markets. This implies that the business models for new grass-based product should consider actual pull-effects of markets that draw grass-based businesses into a region. Such pull-effects include, besides competitive pricing, a demand for the product itself and characteristics such as sustainability, regionality and others.

Competitive pricing of the end-product and profitability of the business model are a precondition for successful technical innovation implementation. The studied cases show that by-products such as heat from biochar production may bring in additional revenues that are crucial for the profitability of the businesses. The German and Danish demos involve nutrient cycling by returning nutrients from processed biomass back to the soil. This may reduce the costs for farmers due to reduced fertilizer purchases. In addition, prices for fertilizers are expected to increase in the near future (and they have already) due to expected shortages (phosphorus) or a high energy demand for fertilizer production (nitrogen). Biochar production in the German demo releases large amounts of energy during the conversion process and this can be used for heat generation, but the heat amount produced would exceed the need of a normal farm. If this additional heat is, however, needed by the operator of the biochar plant, then it will save related costs. Avoiding long-distance transportation also reduces costs, as is the case for locally produced proteins instead of imported soy-based ones in the Danish demo or grass-fibre from local biomass instead of imported fibre produced from wood for paper production in the Dutch demo. In the latter case, the farmers and landowners also save costs occurring for disposal of roadside grass. The profitability of a business model can also be increased through additional income from side products. This is the case for the aforementioned fertilizer and heat if the innovative technology operator has no need for them and sells these side products.

Such cost savings and additional profit from side products, from or efficiency increase, can counterbalance other higher production costs linked to the innovative technology and, hence, enable competitive pricing.

Another pull-effect for demand creation of innovative grass-based products is its sustainability. The long-term carbon storage linked to biochar added to agricultural land leads to a positive climate impact for this business model and, hence, increases the sustainability of crops grown on this land. If the biochar is mixed with compost, biogas digestate or manure before it is applied to the soil, it may contribute to a better retention and slower release of nutrient. This improves nutrient availability for plants and reduces nutrient leaching to the groundwater. The required reduction of nitrate leaching in the Dutch and Danish case is also achieved by converting annual cropland into permanent grassland when new market outlets are opened, and demand increases for grass-based products. Reducing the need for long-distance transportation leads to a lower level of carbon emission of local value chains compared to the use of imported bio-based raw materials or end-products.

Regional products and services rely on resources that are locally available. This provides new opportunities for local stakeholders and has the potential to strengthen rural areas. An example of such opportunity is given by the Dutch case that produces grass fibres. Grass delivery by the landowners, local/regional governments or natural park management organizations profit as they can sell the grass instead of paying for its disposal. Customers profiting from it are the paper and carton manufactures that buy the grass fibres, and farmers can profit as they can use the liquid fraction for biogas production and/or as fertilizer. The growing need for paper-derived products for packaging to replace plastics and using grass wastes to replace allochthonous species makes this local business attractive.

A key learning outcome from the case studies is that the success of new grass-based products depends on their commercial potential. This implies that the new grass-based product should target a market experiencing a pull-effect, meaning that market demand criteria include other conditions besides competitive pricing. We therefore recommend to look deeper into the mechanisms that facilitate the shaping of markets for grass-based products and to investigate the demand patterns for them. It is essential that these new products demonstrate attributes that distinguish them from existing solutions, for example by emphasizing sustainability, local production, and organic and circular qualities. Grass-based products are still niche products demanding only a few per cent of the market in their respective segments (existing products include e.g. cutlery and cups, paper, energy, packaging), and a deeper examination of customer preferences, supply chain characteristics and market conditions is needed.

In addition, lock-ins relate to investments in farm machinery, facilities and labour for handling grass as a feed source or, the procedures, machines and public resources and actions deployed for managing roadsides verges. However, with the abundance of grasslands and great variation in grass typologies across Europe and a strategic focus at European level on the bioeconomy, Green Deal, rural revitalisation and innovation make it clear that market development is central to enhancing innovative grass valorisation.

Current policies support land use to ensure the agroecosystem sustainability within the Pillar I of the Common Agricultural Policy. However, neither the direct payments nor the eco-schemes are thought to support the value chains in the post-2020 CAP. In the 2014-2020 CAP, support to the value chains is associated with some Pillar II measures, which in the case of livestock-derived products is poorly implemented.

The importance of developing new and alternative grass-based products for the valorisation of grassland systems seems unquestionable. However, it is also important to optimise the new value chains to reduce the number of generated by-products together with the development of new technologies aimed at the recovery of potential high-value products from the residues. Carbon efficiency of value chains could be considered as a new evaluation parameter alongside carbon footprint and circular aspects.

Development of markets and value chains for grass-based products are inter-related with the demand and supply for conventional and currently used non-grass-based products, thus pointing at highly diverse markets functioning across several sectors (e.g. agriculture, energy, materials, etc.). Due to the abundant volume of grasses and great variation in properties of grass types, the potential for shaping new value chains is important and covers different scales.

5. Conclusions

Novel grass-based agri-food chains can create backward and forward linkages with different sectors and local actors. These provide a big potential to close the circular loop, contribute to sustainability and create different revenue systems in the business models. New sources of revenues such as payments for storing carbon and for the liquid fraction as fertilizer are yet to be exploited to establish new revenue streams to achieve the large potential of grassland, and there is a need to create awareness with potential customers about the products and services. The potential use of grass for different side products reveals the cascading potential of the biomass for different grass-based solutions.

The case studies show that the implementation of successful business models requires not only technical innovations but also institutional, organisational and social innovations that contribute to more cooperation, jointly strategy setting, shared governance structures and learning at business and other levels. Enabling factors for innovative business models include, inter alia, availability of financial resources, supportive rules and regulations, adequate infrastructure for production and mobilization, opportunities for cooperation, training and education of local actors as well as consumer demand for bio-based products. Regulations need to be well aligned with each other to be supportive for the novel grass-based agri-food chains. A thorough and comprehensive understanding of the innovation processes and interdependencies between grass-based agri-food chains and their business environment can significantly help to adequately address any misalignments that may hinder their development.

Recommendations for the grass-based sector emphasise regulations and the creation of market structures for a secure supply of raw materials at stable prices. Policy makers should further consider measures for sufficient and fair market competition, secure and transparent sustainability profiles of bio-based inputs and due diligence of all actors along the value chain. Finally, sufficient capacities for regulation and innovation at all levels, and rewarding multifunctional uses of grassland resources are essential to exploit opportunities to close the circular loop, contribute to sustainability and create alternative revenues for farmers.

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Eco-efficiency of leys in dairy farming systems

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Abstract

Specialization of agricultural systems in western Europe and intensification of production in livestock and cropping systems are associated with high resource inputs. Intensive production often results in nutrient surpluses which are associated with adverse effects on the environment. A transformation of agriculture is needed to achieve the targets set for climate and environmental protection. The eco-efficient pasture-based milk production project at Lindhof, which commenced in 2016, demonstrates a productive and profitable dairy system within a ley-based integrated crop-livestock system (ICLS). At the organically managed Lindhof farm, a herd of spring-calving Jersey cows rotationally graze grass-clover leys that are part of a cash crop rotation. The dairy cows benefit from highly productive swards with excellent nutritive value, while the crops benefit from nutrient transfer from grazing excrements and crop residues. Compared with specialized systems, the ley-based ICLS demonstrates an alternative system for dairy production in conjunction with the provision of several ecosystem services; low nutrient losses, low carbon footprint and positive effects on agro-biodiversity at an overall high level of land use efficiency. Thus, it aligns with the principles of ecological intensification and contributes significantly to more functional diversity in agriculture, reduces social costs considerably and works towards fulfilling the EU-Farm to Fork Strategy.

Keywords: eco-efficiency, grass clover, integrated crop-livestock systems, rotational grazing

Introduction

More than 80% of the world's arable lands are devoted to monocultures to produce grains for humans and animals, and the amount of crop diversity per unit of arable land continues to decrease (Altieri *et al.*, 2015). This is attributable to the increasing expansion and intensification of the livestock sector in many regions (Godfray *et al.*, 2010). Accordingly, this phenomenon has brought changes in land use and has reduced agro-diversity within landscapes as crop-livestock systems, forage crops and grassland areas have diminished. In high-income countries, for instance, specialized dairy farms are typical in the livestock sector, where highly productive dairy breeds are fed all year indoors. These animals require high-energy and protein-rich diets, usually imported from external sources, resulting in land importation. In some regions, cereal crops and silage maize for ruminant feeding are cultivated on former grasslands. Consequently, depending on the type of feed and feeding regimes adopted, there is competition between arable land used for feeding farmed animals and land used for food produced directly for human consumption. However, alternative animal production concepts must be explored for the 'Farm-to-Fork' strategy of the European Union (EU) to be successfully implemented.

The concept of agricultural circularity considers the role that animal-sourced foods can play in feeding the world, taking advantage of the inherent trade-offs in the food system. For example, some suggest that livestock should only exist in our future food system when feed-food competition is minimal (Mottet *et al.*, 2017; Springmann *et al.*, 2018; van Zanten *et al.*, 2018). Farmed animals can convert by-products, which humans cannot or do not want to consume (for example, crop residues, co-products and wastes from the food chain) into valuable products (meat, milk, eggs, manure) while providing various ecosystem services. In their study, van Zanten *et al.* (2018) showed that producing food from livestock that recycles these leftovers and using existing grassland resources for animal production could meet 14-23% of the protein demand per capita per day from animal-sourced foods. This offers an effective and efficient way of using arable land for food production and it is estimated to reduce N and P losses by 40% and 46%, respectively, and greenhouse gas (GHG) emissions by 19-50%, compared with a business-as-usual scenario (Röös *et al.*, 2017). In the debate, it has to be specifically considered that ruminant livestock production results in relatively higher GHG emissions compared with non-ruminants; however, the latter exacerbates the above-mentioned feed-food competition on agricultural land.

Biodiversity, climate and water quality are threatened in many European regions due to the high intensity of agricultural production. There is a need to find innovative ways to ensure, the multifunctionality of dairy: producing milk in ways that have lower risks for biodiversity, climate change, water quality, and animal welfare, particularly in Europe. Sustainable intensification (SI) of agricultural systems has been proposed to navigate through competing food system priorities to deal with the planetary boundaries defined for agricultural production (Garnett and Godfray, 2012). However, recently SI has been taken as a justification for worldwide intensification of agricultural production and, hence, increased use of resources. Instead, concepts should be developed in a local context to ensure clean production and provision of other ecosystem functions (Ecological Intensification; Tintonell, 2014). This might involve applying input factors in such a way that the relationship between economic output (product, service, activity) and environmental impact caused by production, consumption and disposal are optimized.

This *eco-efficiency* can be increased by altering the management of individual crop and livestock enterprises or the land-use system (Wilkins, 2008). When there is an increasing quantity of resource inputs, the output level typically shows diminishing marginal productivity; however, the environmental load increases disproportionally with increasing resource input (Figure 1). The highest eco-efficiency can be achieved at the point where the quotient of environmental load and yield is lowest, and the associated reduction of production levels of agricultural commodities is accepted within a society. To ensure high overall eco-efficiency of agricultural systems, it is imperative that high-input farming systems, characterized by high environmental load (Point 'a'; Figure 1), de-intensify the production of agricultural commodities in order to increase the provision of ecosystem services for common goods. This might not necessarily mean a complete adoption of organic agricultural practices but a mix of strategies. In addition, the production of specific agricultural commodities could be shifted to regions/locations where ecological efficiencies are highest. On the other hand, many smallholder farms in developing countries are located at a lower point of the production function (Point 'b'). Such farms are characterized by low productivity, efficiency and environmental load, primarily due to low input levels. For such farms, some level of SI will be necessary to increase food production and land use efficiency (Brandt *et al.,* 2020) without compromising ecosystem services. In this context, ecological intensification should aim at complementing or replacing artificial inputs with natural resources to reduce environmental costs without negatively impacting productivity.

Benefits of including leys in cropping systems

Integrated crop-livestock systems (ICLS) maximize the interaction between system components, i.e. soils, plants, and animals and, therefore, have the potential to decrease environmental impacts while maintaining production levels (Ryschawy *et al.*, 2017). Such systems were essential in recycling nutrients in the past, but the advent of synthetic fertilizers have rendered them less important (Schut *et al.*, 2021). One possibility to reduce the high environmental loads associated with the highly specialized farming practices of our modern production system (i.e. a separation into arable crops farms and dairy farms) is to promote cooperation by developing joint crop rotations to facilitate nutrient cycling. Where grass-clover leys are a component of the crop rotation, nitrogen fertilizer and plant protection inputs can be lowered while additional carbon is sequestered in the soil (Goudriaan, 1992). This integration minimizes nutrient leaching and promotes biodiversity via species mixtures in forage production.

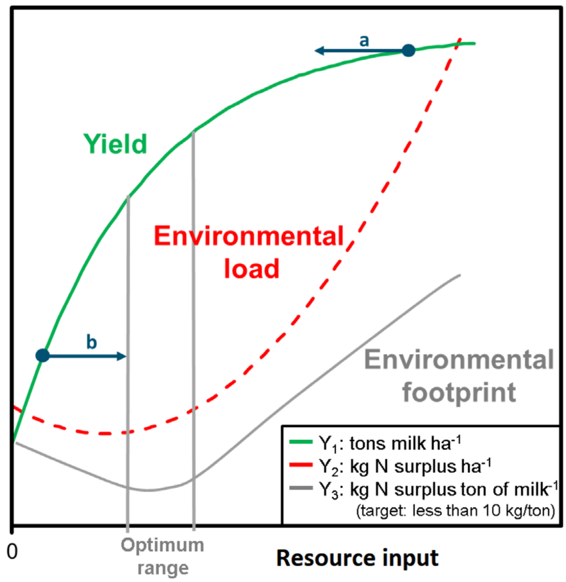

Figure 1. The principle of eco-efficiency illustrated by the relationship between resource input (e.g. N input (x)) and yield (e.g. milk production (y_1)), environmental load (e.g. N surplus per ha (y_2)) and environmental footprint (e.g. N surplus per unit milk produced (y_3)).

Perennial grass crops have a longer growing season, a dense, fibrous root system, and substantially larger below-ground biomass productivity compared to annual cash crops (Loges *et al.*, 2018). Mixed grass-clover ley-arable systems (temporary grass of 2-5 years) can capture the benefits of grass swards temporally in combination with periods of intensive cropping and ensure higher protein self-sufficiency of the livestock farm (Peyraud *et al.*, 2014). Also, the termination of grasslands results in a large quantity of mineral N available to subsequent crops, thus reducing N fertilizer requirements in the arable phase of the rotation with greater yields of the following arable crop (Alderkamp *et al.*, 2022; Lemaire *et al.*, 2015). Furthermore, ley-crop rotations can provide indirect ecosystem services, such as improvement of soil fertility and biodiversity, control of weed and pest populations, and saving energy due to the economy of tillage and N fertilizers (Lemaire *et al.*, 2014). Multispecies swards in leys, including tannin-rich species, may have additional benefits like lowering N_2O emission from soils via biological nitrification inhibition (de Klein *et al.*, 2019) and mitigating enteric methane emissions when fed to livestock (Beauchemin *et al.*, 2020).

Multispecies leys might potentially conserve and improve biodiversity by supporting a range of insect species (Badenhausser *et al.*, 2009), thus contributing to the food chain for birds and other species (Lemaire *et al.*, 2015). Compared to continuous arable cropping, leys are reported to improve soil structure through several biological processes, e.g. larger litter inputs, high C and N returns from grazing animals, constant high food or C supply, reduced tillage disturbance and a larger population of earthworms (Yeates *et al.,* 1998) and herbivorous nematodes (Bouwman and Arts, 2000).

'Eco-efficient pasture-based milk production' at Lindhof farm

The Lindhof research farm demonstrates an ICLS, where a spring-calving Jersey herd is managed in a 'low-input system', grazing on grass-clover leys (2 to 3 years of ley phase). The project was designed to demonstrate high eco-efficiency of pasture-based milk production, providing multiple ecosystem services (food production, clean water, climate change mitigation, biodiversity, animal welfare, attractive landscapes, etc.) while maximizing milk production per ha. It was hypothesized that combining N selfsufficient high-yielding grass-clover leys with low N_2O emissions and significant carbon sequestration, and with the benefits of low input pasture-based milk production, leads to high land use efficiency and low environmental footprints per kg of energy corrected milk (ECM) (product carbon footprint [PCF] and product nitrogen footprint [PNF]). Moreover, it may provide additional benefits for biodiversity and significantly reduce the social costs per kg of ECM produced.

The Lindhof farm

The Lindhof research farm is located in the state of Schleswig-Holstein on the Eckernförde Bay of the Baltic Sea (N 54°27 E 9°57; mean air temperature 8.7 °C; mean annual precipitation 785 mm) (www.lindhof.uni-kiel.de). The dairy herd was established in the autumn of 2015, with 80 cows (now increased to 100 lactating dairy cows). The milk at Lindhof is produced according to the guidelines of the German organic farming associations (Bioland and Naturland). The main focus is to achieve maximum utilization of the grass-clover leys instead of permanent grassland in the context of ICLS and N_2 fixation in organic farming.

Cows calve from mid-January to March to maximize utilization of grazed pasture. The start of the grazing season is typically in March and continues for 260 days (155 days of full-day grazing). The animals receive over 90% of their energy demand from pastures from the 60th day of lactation. During the first 60 days of lactation, cows are fed concentrates and high-energy grass-clover silage. The grazing management is characterized by intensive rotational grazing with daily allocation and some strip grazing (twice-a-day allocation) from mid-April to late July. The pastures are highly productive grass-clover-herb mixtures, grazed at the 3-leaf stage of perennial ryegrass, achieving energy contents of 7.6 to 7.8 MJ NEL kg^{-1} DM (NEL = Net Energy for Lactation) in May/June, with an average of >7 MJ NEL kg^{-1} DM over the grazing season. The silage cuts (baled silage) reach energy contents of around 7 MJ NEL kg^{-1} DM with high usable crude protein values of 182.5 g kg^{-1} DM in June. Herbage allocation is based on weekly sward height measurements with a GPS-based rising plate meter. The measurements are analysed using simulation models and a mobile app currently under development, optimizing the amount and nutritive value of the daily herbage allowance.

Paddocks are primarily grazed and defoliated 8 to 10 times a year. All paddocks are cut for harvesting silage bales at least once during the grazing season. From September onwards, the decreasing daily growth rate of grass-clover in the second half of the grazing season is compensated by expanding the grazing area using cover crop swards (annual ryegrass) and grass-clover swards that are newly established each year by undersowing into winter cereals. Thus, there is an even supply of available herbage during the entire grazing season. After the third winter, the final grazing of the paddocks is completed in early spring before fields are ploughed and sown for oat production, followed by two other cash crops before a new ley is established in the same manner as described above.

Productivity and production costs compared to conventional indoor dairy systems

A comparison between key performance indicators of the Lindhof system and some 350 typical conventional indoor dairy farms in the state (Table 1) demonstrates that the combination of a spring-calving Jersey-based herd with an intensive pasture-based system is highly productive and profitable. The Lindhof system is superior in milk composition, the proportion of milk produced from forage, a low need for purchased concentrate feed and a low adjusted reproduction rate (replenishment without stock changes). The comparatively low age at first calving is reflected in the low rearing costs.

The amount of milk produced from forage at Lindhof (5,284 kg ECM cow^{-1}, Table 1) is based on calculations used by the German extension service. It is assumed that each kg of concentrate feed is converted to a net milk yield of over 2 kg ECM. That means that the energy demand for maintenance

Table 1. Production parameters, economic results and nitrogen balance (2019/20) of the experimental farm Lindhof compared to the average of 356 dairy farms fully evaluated by the chamber of agriculture (extension service) of Schleswig-Holstein (Branch accounting of milk production (BZA)).[1]

	Unit	Lindhof	Average of 356 by BZA
Dairy herd	number of cows	94	166
Body weight	kg cow^{-1}	470	670[2]
Milk yield	kg ECM cow^{-1}	7,007	9,433
Milk yield natural	kg cow^{-1}	5,728	9,257
Milk yield per kg body weight	kg ECM kg^{-1} BW	14.90	14.08
Milk solids production (fat plus protein)	kg cow^{-1}	592	702
Fat	%	5.59	4.2
Protein	%	3.99	3.45
Concentrate feeding	dt cow^{-1} year^{-1}	8.00	28.10
Concentrate feeding efficiency	g kg^{-1} ECM	120	295
Milk production per ha MFA on farm[3]	kg ECM ha^{-1} MFA	10,946	14,866
Milk produced from forage[4]	kg ECM cow^{-1}	5,284	3,767
Proportion of milk produced from forage[4]	%	75.41	39.93
Adjusted reproduction rate	%	18.20	33.40
First calving age (LKV-SH, 2021)	months	24.6	28.4[6]
Calving interval (LKV-SH, 2021)	days	362	400[6]
Costs for veterinary, medicines + hoof care	ct kg^{-1} ECM	1.48	1.64
Total feed costs[5]	ct kg^{-1} ECM	16.81	22.12
Costs of producing forage	ct kg^{-1} ECM	12.17	13.35
Concentrated feed costs	ct kg^{-1} ECM	3.83[8]	8.77
Mineral N fertilizer input	kg N ha^{-1} MFA	0	99
N balance[7]	kg N ha^{-1} MFA	88	149

[1] SH = Schleswig-Holstein; ECM = energy-corrected milk; MFA = main forage area; BZA = branch accounting, source: LK-SH (2021).

[2] Estimated value based on the average of the breeds.

[3] Without area requirements for imported feed.

[4] Milk from concentrates excluded according to LK-SH (2021) calculation.

[5] Rearing replacement heifers included.

[6] Farms in the same region.

[7] Farm-gate N balance of only the dairy operation.

[8] From organic production at a 63% higher price.

of the cows is calculated to be provided by forages. However, if the energy demand for maintenance is distributed evenly over the total energy consumed by the animal (as the sum of forage and concentrated feed), which is more realistic, the calculated milk produced from forage at Lindhof would increase to 5,865 kg ECM cow^{-1} and the milk gain from concentrates is close to 1 kg ECM per kg of concentrate feeding. Applying this alternative calculation method to the data supplied by the German extension service for the typical conventional indoor dairy farms in the state (Table 1), the calculated milk produced from forage would increase from 3,767 to 5,519 kg ECM cow^{-1}. This means that in both systems the proportion of milk produced from forage is underestimated and milk produced from concentrates is overestimated. This has implications for the feed cost calculations: the realistic concentrate feed costs per kg ECM are thus significantly higher than what is reported by the German extension service. About 48% of the total annual energy demand of the dairy cows in the Lindhof system came from grazed pasture, 35% from grass-silage and 16% from concentrates. Based on the amount of crude protein consumed by the cows, 47% of the milk was produced from grazed pastures, 41% from the protein-rich grass-silage and only 11% from grain-based concentrates.

The full cost of forage production based on grass-clover managed in a mixed mowing/grazing system was, in the farm accounting year 2019/20, 16.47 cents per 10 MJ NEL and 0.74 cents per kg of crude protein. This means that at Lindhof, forage was produced much cheaper than in comparative farms (Table 2). The veterinary/hoof care cost per cow per year at Lindhof is 11% lower than those of the comparative farms. The key factor here is the low hoof care cost of grazing animals. The age at first calving of 24.6 months and a calving interval of 362 days demonstrates the positive economic effect of good fertility management.

Labour costs have a significant impact on the economics of production. At the Lindhof farm, extensive documentation enables the allocation of working hours to the individual branches of the business. The Lindhof is yet another demonstrator of labour-efficient pasture-based milk production with few hours required per cow per year, contrary to the long-standing belief that pasture management increases labour requirements on farms. The farm's infrastructure (good accessibility of all pastures from the milking parlour) plays a key role here. There are also considerable savings for manure handling in comparison to indoor farms.

Ecological metrics compared to conventional indoor dairy systems

Dairy production significantly contributes to NO_3 pollution of surface and ground water. This growing concern has led to the establishment of many regulations (e.g. the European Union Nitrate Directive). Results from field measurements over three years at Lindhof (Smit *et al.*, 2021) showed low (<10 kg N ha^{-1}) and medium (10-28 kg N ha^{-1}) nitrate leaching from fertilized permanent grasslands and grazed grass-clover swards, with an annual ryegrass sward grown as a catch crop, respectively (Figure 2). As expected, grazing resulted in higher NO_3-N losses compared to cutting. Within grazed leys, leaching losses increased with the age of the swards. On average, there were no significant differences between permanent grasslands and leys. The relatively low nitrogen losses from grazed swards at Lindhof can be explained with: (1) lower sward clover contents on grazed compared to cut swards; and (2) removal of N via at least one silage cut taken during summer also from grazed paddocks.

NO_3-N leaching depended on the amount of mineral nitrogen access, land use, soil type, prevailing weather conditions and length of the growing period. The mean cumulative nitrate leachate over the experimental period was 8.5 ± 1.8 kg NO_3-N ha^{-1}. Cover crops under grazing and fertilization had the highest N-leaching losses (~20 kg NO_3-N ha^{-1}). However, the values observed at Lindhof are below values reported in the literature (10-80 kg NO_3-N ha^{-1}), placing Lindhof at the lower end (Biernat *et al.*, 2020; Eriksen *et al.*, 2015; Askegaard *et al.*, 2011). This might be due to a highly effective rooting system, with root length density exceeding 100 km per m^2 per year measured on grasslands at Lindhof (Chen *et al.*, 2016). Overall, the tested treatments registered nitrate concentrations well below 25 ppm and thus contributed a dilution effect in the crop rotations. Also, a high N carry-over from grass-clover swards to

Table 2. Full costs analysis of forages in the 2019/2020 financial year.

	Lindhof grass-clover-silage	**BZA 2019/20[1] grass-silage**	**BZA 2019/20[1] maize-silage**
Energy yield, MJ NEL ha^{-1}	57,228	57,593[1]	84,746[1]
Crude protein yield, kg CP ha^{-1}	1, 275	1, 456	907
Total costs, € ha^{-1}	943.75	1,865.98[1]	2,039.44[1]
Total cost, ct 10 MJ^{-1} NEL	16.47	32.40[1]	24.07[1]
Total cost, ct kg^{-1} CP	0.74	1.28	2.25

[1] Source: LK-SH (2021), all including land cost; BZA= Branch accounting of milk production; NEL = net energy for lactation.

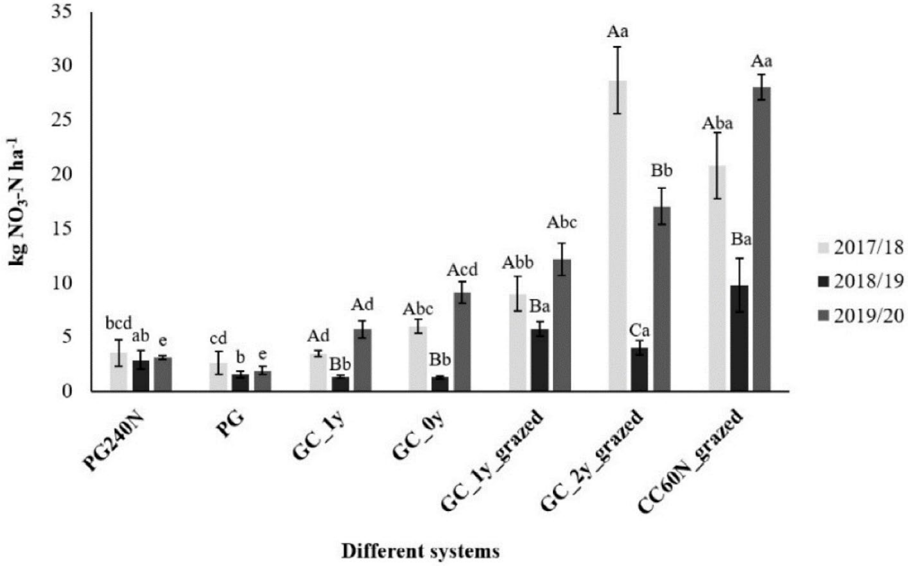

Figure 2. Average nitrate leaching losses (kg NO_3-N ha^{-1}) of the different systems (permanent grassland (PG), PG240N is PG receiving 240 kg N ha yr^{-1}; GC is grass-clover swards; 0,1,2 years old; CC60N is catch crop fertilized with 60 kg slurry N ha^{-1}); (PG-Permanent Grassland, GC = Grass clover, CC cover crop (annual ryegrass); (Smit *et al.*, 2021). [Note: N load of 30 kg N ha^{-1} is equivalent to the threshold exceeding 50 ppm nitrate concentration in the leachate (275 mm of percolated water) – EU limit in drinking water is 50 mg l^{-1}].

the next cash crop unit was observed (Smit *et al.*, 2021), reducing the risk of groundwater contamination from grazed leys.

Flower-visiting insects have an essential function in plant pollination, and pollination by bumblebees, for instance, increases the yield of many crops (Orford, 2016). However, pollinator populations are in decline (Bommarco *et al.*, 2012). A study at Lindhof (Beye *et al.*, 2022) showed that legume mixtures incorporating *Trifolium* spp. could enhance bumblebee abundance. Within a variety of mixtures tested, the effect was clearly observed in the grazed binary and tertiary mixtures of the grass-clover leys (perennial ryegrass (*Lolium perenne* L.), white clover (*Trifolium repens* L.) and red clover (*Trifolium pratense* L.), Figure 3. This demonstrates that species richness and legume inclusion in grass-clover leys increases agro-biodiversity compared with conventionally managed grasslands not containing flowering legumes. Whereas 541 wild bees (bumblebees and one solitary bee) belonging to two genera and ten species were recorded in plant-species-enriched grass-clover swards, none was recorded on conventionally managed grasslands for silage making (4-5 cuts per year). However, the potential of the species-enriched mixtures to promote wild bees was reduced by intense grazing, and more long-tongued bumblebees were found on strips of the binary (*Lolium perenne and Trifolium repens*) and multispecies mixtures (*L. perenne, T. repens, T. pratense; Lotus corniculatus, Cichorium intybus, Plantago lanceolata, Carum carvi* and *Sanguisorba minor*) when grazing was excluded. Although the results from this study were modest, the authors suggested that the inclusion of more forage species with open flowers in successive phenologies, and the adjustment of grazing to these phenologies, could provide more continuous diverse floral resources for insects.

In an experiment at Lindhof, adding herbs to the grass-clover swards did not provide additional benefits in terms of enteric methane reduction (Loza *et al.*, 2021), nitrification and denitrification losses (Nyameasem *et al.*, 2021) or dry matter yields (Lorenz *et al.*, 2021) as the herbs were maintained at low-

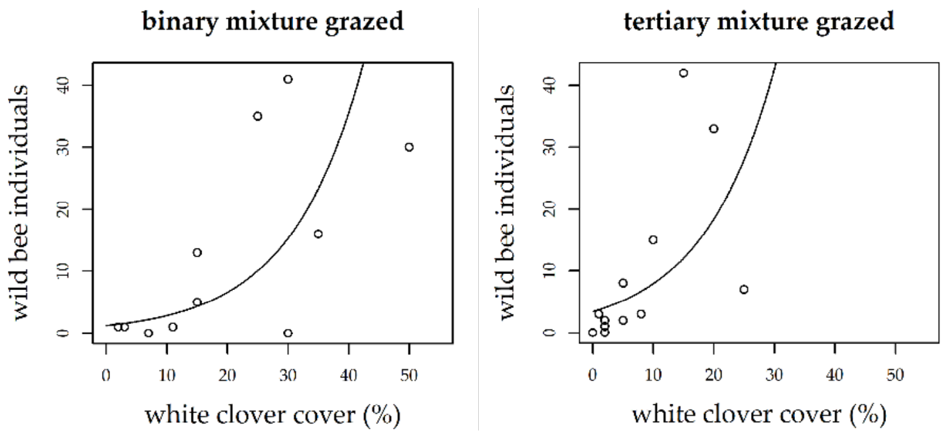

Figure 3. Effect of white clover cover on wild bee abundance in grazed grass-clover swards (binary mixture of perennial ryegrass and white clover; tertiary mixture of perennial ryegrass, white clover and red clover) (Beye *et al*., 2022).

yield proportions throughout the grazing season. Nevertheless, enteric methane emissions per unit of milk were very low (8.8 g CH_4 kg ECM and 9.8 g CH_4 cow^{-1} day^{-1}, on average, for spring and summer measurements, respectively). These values were much lower than the previously reported 17.9 and 17.4 g CH_4 kg ECM^{-1} for Jersey cows fed 0 and 4 kg of concentrate, respectively (van Wyngaard *et al.*, 2018), and 13.4 g CH_4 kg ECM^{-1} reported for Jersey cows fed 61% of the concentrate (Olijhoek *et al.*, 2018). The highest N_2O-N emission factor for cow urine and dung, measured using static chambers, was less than half compared with the IPCC default (0.3 vs 0.77%). Whereas the low CH_4 emissions and milk output were attributed to the high quality of feed (Loza *et al.*, 2021), the high ability of the pastures to utilize nitrogen inputs from excreta patches was attributed to high N uptake (Nyameasem *et al.*, 2021).

The long-term use of grass-clover in crop rotations leads to a substantial accumulation of soil carbon (Reinsch *et al.*, 2019; De Los Rios *et al.*, 2022), significantly reducing the PCF of milk production at Lindhof. The PCF of milk from the Lindhof grazing system is around 0.6 kg CO_2eq kg^{-1} ECM compared to the >1 kg CO_2eq kg^{-1} ECM for standard milk from year-round indoor farming (Figure 4). Such additional services from the ley system are not yet monetarized, but this will mean that, with a milk yield of 750,000 to 900,000 kg ECM per farm per year and a planned CO_2 price of 100 € t^{-1} of milk scheduled for 2030, the advantage for the grazing system in terms of avoided GHG emissions is around 5 ct per kg ECM, with comparable land use efficiency.

In a previous study, Reinsch *et al.* (2021) highlighted the difficulty for farm systems exclusively specialized in dairy production to achieve farm N surpluses below 100 kg N ha^{-1} (Figure 4). A clear relationship between N balance and GHG emissions showed that the farms with higher N surplus also had higher GHG emissions per unit of milk and unit area. A decrease from an average farm N balance of +150 kg N ha^{-1} down to about +50 kg N ha^{-1}, as realized in the Lindhof system, would result in a substantial mitigation potential for social costs caused by reduced environmental N pollution. We estimated the avoided environmental costs (Table 3; ct kg^{-1} ECM) using available prices from the literature to have a rough estimate regarding social cost avoided by the Lindhof system (IFG) relative to the indoor system (IC), according to Reinsch *et al.* (2021). The social cost avoided by adopting the Lindhof system was estimated at 25 ct kg^{-1} ECM, and the highest cost component was 13 ct kg^{-1} ECM from P pollution. We envisage that comparable calculation methods will become more critical regarding greenhouse gases and nitrogen loads.

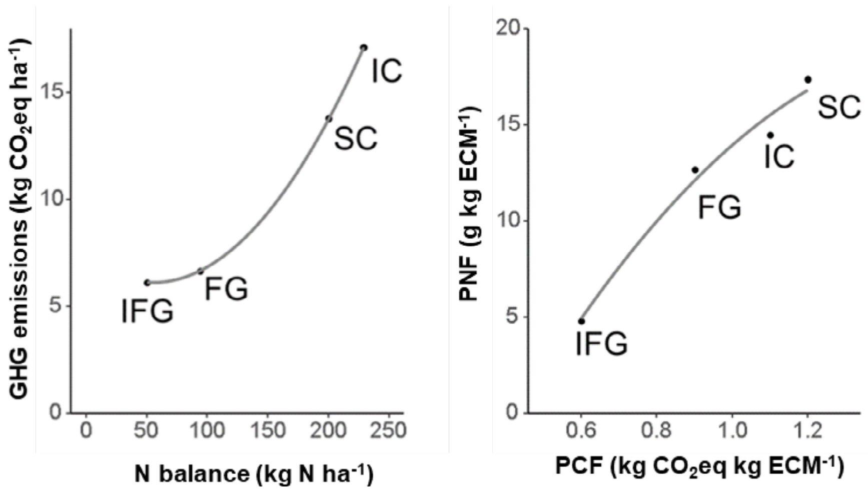

Figure 4. Relationship between Farm N balance and greenhouse gas (GHG) emissions per ha (A) and Product Carbon Footprint (PCF) and Product Nitrogen Footprint given as N surplus per kg ECM (PNF) (B) among the different systems (IC, intensive-confinement; SC, semi-confinement; FG, full-grazing; IFG, integrated full-grazing) according to Reinsch *et al*. (2021).

Table 3. Avoided abiotic environmental costs per kg ECM compared between the Lindhof system and intensive indoor system.

	Indoor dairy	Lindhof	Difference	Unit cost	Social cost avoided by Lindhof system (€ kg^{-1} ECM)
GHG, kg CO_2eq kg^{-1} ECM	1.10	0.60	0.50	100 € t^{-1} CO_2[1]	0.05
Surplus N, g N kg^{-1} ECM	12.0	5.0	7.0	10 € kg^{-1} N[2]	0.07
Surplus P, g P kg^{-1} ECM	1.20	0.01	1.10	120 € kg^{-1} P[3]	0.13
Total					0.25

[1] www.boerse.de/rohstoffe/CO2-Emission rights price (Sept. 2022).

[2] European Nitrogen Assessment Report (2013).

[3] UBA (2021). https://www.umweltbundesamt.de/tags/phosphor.

The 2.5-year grass-clover ley phase used for milk production provides additional services to the downstream cash crop part of the farm. N-rich grass-clover roots and stubble stored in the soil, protected from leaching, ultimately supply the direct succeeding crop (edible oats) with almost 150 kg N ha^{-1}. All manures of the farm are applied to the following winter cereals to increase yields. This means that the cereal crops practically compensate for the excess N from the milk production (88 kg N ha^{-1} per ha of grass-clover forage). The sum of the areas for crops and pastures results in an N balance of +18 kg N ha^{-1}. At the same time, this makes it possible to minimize unavoidable N losses and contributes to a positive humus balance.

Conclusions

Milk output per ha in specialized dairy systems in Western Europe is high, and with the advantages of economies of scale, specialized dairy systems have a greater potential to generate high incomes and compete well in the milk market as long as social costs are not accounted for. This competition, however, depends on milk price and the extent of the competitive advantage of low feed costs generated from grazed systems. The study confirmed that the ongoing specialization and the intensification of dairy production do not adhere to the need for mitigating GHG and nitrogen emissions at the regional level. These systems, thus, do not follow the principles of ecological intensification. On the other hand, the Lindhof case study demonstrates the benefits of a grass-clover ley-based ICLS compared to specialized systems for a range of ecosystem services, including water and air quality protection, climate change mitigation and agro-biodiversity, while operating at an overall high level of land use efficiency. Thus, the system highlights a 'resilient ICLS narrative' (ley systems + pasture-based dairy) that contributes significantly to more functional diversity in agriculture, reduces social costs considerably and works towards fulfilling the Farm to Fork Strategy. Even under the assumption of similar producer prices as in conventional systems, the Lindhof approach was economically competitive. Further advances in research, development, and, above all, advisory services are now required to facilitate the implementation of ley-based ICLS into the dairy production sector and utilize the benefits for the environment, as it was demonstrated at the Lindhof farm.

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Timothy cultivars differ in uptake of macroelements

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Abstract

Uptake of nitrogen (N), phosphorus (P), potassium (K) and magnesium (Mg) by timothy (*Phleum pratense* L.) was summed over the years of 2019-2021. We hypothesized that six cultivars diverge in herbage yields and thus would require different nutrient inputs in order to enhance their cultivation efficiency. We followed the YARA crop nutrition programme with annual mineral fertilization rate of N 156, P 10, K 91, Mg 13 kg ha^{-1}. In the first harvest year, cultivars removed significantly different amounts of N, P, K and Mg with their herbage yields. In the second year, the cultivars also varied in their K and Mg uptake, whereas in the third, all uptake differences disappeared. Yearly growing conditions of the plants affected their harvestable biomass production, which altered the quantities of unused N and Mg or deficiencies of P and K. With harvested biomass, timothy cultivars removed 70% of N and 47% of Mg that was applied, whereas only 57% of P and 48% of K was covered by YARA mineral fertilizers altogether during 2019-2021. The yearly nutritional imbalance led to enrichment of the soil with Mg, impoverishment from P and K and reduction in its pH.

Keywords: nitrogen, phosphorus, potassium, magnesium, nutrient balance

Introduction

Timothy is a widely cultivated forage grass in northern latitudes. The species has superior tolerance to adverse wintering conditions but has poor regrowth capacity. Østrem *et al.* (2014) stated that northern-adapted forage grasses seem to have a specific mechanism for growth inhibition during autumn. In Estonia, annual herbage yield is often collected with two conservation cuts, and a third harvest yields only a very scant crop, especially after prolonged drought in summer. Signs of global warming expressed by dry and hot periods have become more evident in Estonia. We have observed that mineral fertilizers spread after grass harvesting in June often dissolve only partly, hence regrowth will utilize them insufficiently. Plant nutrition below its utilization capacity does not allow realization of crop production potential and leads to depletion of soil nutrient reserves. Conversely, excessive fertilization is to be avoided for both economic and ecological reasons. Quantification of nutrient uptake potential of widely cultivated grass species and prospective variety candidates would make it feasible to differentiate their fertilizer input rates according to their actual demands, thereby enhancing crop cultivation efficiency. The aim of this research was to compare the quantities of main nutritional elements that were applied to timothy, to those removed by the crop.

Materials and methods

In June 2018, two Estonian (Tika and Tia) and four Finnish timothy cultivars (Dorothy, Rhonia, Tuure and Uula) were sown in a field trial at the Centre of Estonian Rural Research and Knowledge in Jõgeva. The seeding rate was 20 kg ha^{-1}. Based on the YARA crop nutrition programme, 20 m^3 ha^{-1} of cattle slurry was incorporated into the soil just before establishment. Applied slurry contained 28 kg N, 6 kg P and 22 kg K but was excluded from further calculations. Steady fertilization regime implemented in the grass harvest years comprised spring applications of Yara Mila fertilizer equivalent to N 90, P 10, K 56 kg ha^{-1}, followed by an application of N 66, K 35, Mg 13 kg ha^{-1} for regrowth. Total annual nutrient rates were N 156, P 10, K 91, Mg 13 kg ha^{-1}. A Hege 212 harvester was used to cut and weigh the plots at heading stage of control cv. Tika. At this time the cultivars had not reached the same phenological stage. Instead, we were aiming at identical growth periods and conditions for all cultivars. On average, the second harvest took place 55 days after the first. Grass samples were dried at 45 °C for 48 h and macroelements (N, P, K, Mg) were measured in the laboratory of plant biochemistry of Estonian University of Life Sciences. Air temperatures, averaged between 1 May and 20 August, i.e. until harvesting of regrowth, were similar in 2019 and 2020 to long-term average (2000-2021) or higher by 2.2 °C in 2021. Accumulated rainfall over the above-mentioned period amounted to 178 mm in 2019 and 225 mm in 2021, which were below the long-term average (251 mm). Water deficit limited plant growth and uptake of nutrients from fertilizers. The only favourable year was 2020 with abundant precipitation (305 mm) and cooler temperatures. Statistical analyses (ANOVA and Fisher's LSD test, $P<0.05$) were carried out by Agrobase 20™.

Results and discussion

We detected significant differences in nutrient amounts removed by herbage yields at two-harvest regime in the first harvest year (Table 1 and 2). In the second year, cultivars differed in K and Mg removal, but in the third year all differences became non-significant. Applied N and Mg were efficiently utilized by the young timothy stand in the first harvest year, which removed maximum quantity of P. The top-yielding cv. Tuure utilized the applied amounts of N and Mg to the utmost, whereas shortages of P and K became evident. Dorothy produced maximum dry matter yield of 11.27 Mg ha^{-1} in 2020. This depleted the soil from P and K and unused Mg remained minimal. Application rates of P and K were insufficient even for the least productive cv. Tika, an Estonian selection for improved grazing resistance.

Table 1. Yearly herbage production of timothy cultivars and quantities of excessively applied nutritional elements at annual mineral fertilizer application rate N 156, P 10, K 91, Mg 13 kg ha^{-1}.[1]

Cultivar	Dry matter yield, Mg ha^{-1}			Nitrogen (N), kg ha^{-1}			Magnesium (Mg), kg ha^{-1}		
	2019	2020	2021	2019	2020	2021	2019	2020	2021
Tika, control	7.57	9.49	4.68	47	44	78	6.1	8.6	8.2
Tia	8.37	9.66	5.19	13*	34	72	5.1	8.2	7.4
Dorothy	9.64*	11.27*	5.63	22	43	74	4.0*	7.2*	7.4
Rhonia	8.69*	10.77*	5.53	22	39	70	5.8	8.7	7.5
Tuure	10.15*	9.76	5.73	6*	49	69	3.3*	8.1	7.1
Uula	8.38	9.58	5.09	30	46	78	5.4	8.4	7.5
Mean	8.80	10.09	5.31	23	42	74	5.0	8.2	7.5
LSD 5%	0.84	0.94	1.22	26	20	20	1.9	1.1	1.9

[1] An asterisk indicates significantly different ($P<0.05$, Fisher's LSD test) from control cultivar Tika.

Table 2. Yearly deficiency of nutritional elements (kg ha^{-1}) measured for timothy cultivars at annual fertilizer application rate N 156, P 10, K 91, Mg 13 kg ha^{-1}.[1]

Cultivar	Potassium (K)			Phosphorus (P)		
	2019	2020	2021	2019	2020	2021
Tika, control	83	128	20	7.9	8.5	0.6
Tia	109*	141	30	11.9*	10.2	1.9
Dorothy	122*	162*	37	12.8*	10.8	2.1
Rhonia	114*	159*	39	11.4*	10.6	2.6
Tuure	147*	147	41	15.3*	9.7	2.8
Uula	108*	141	27	10.3	9.4	1.6
Mean	114	146	32	11.6	9.9	1.9
LSD 5%	21	29	22	2.7	2.9	2.4

[1] An asterisk indicates significantly different ($P<0.05$, Fisher's LSD test) from control cultivar Tika.

Herbage production, as well as K concentration within (not presented) and uptake by the plants attained maximum in 2020. Magnesium surplus also peaked in the same year. Hence, K led to antagonistic effect on the uptake of Mg, referred by Jakobsen (1993). N surplus increased yearly meanwhile the P deficiency became negligible. Thus, the use of macroelements, except K, did not follow the herbage yield curve during three years. Stressed stands revealed the minimal shortage of P and K in 2021. From excessive N and Mg fertilization, cv. Tia with highest crude protein content, left least, with 119 kg ha^{-1} N unused (Figure 1). Tuure – a second-most productive cultivar left 18 kg ha^{-1} Mg unused. Tia and Tuure were the most effective in using N and Mg as their herbage yields contained 75% of applied quantities of N and 54% of Mg, respectively. In view of the deficiencies, the fertilizers covered at most 64% of P and 54% of K from the amounts contained in herbage yield of Tika. On average, the herbage yields of timothy removed 70% of N and 47% of Mg applied to the field, whereas the recommended fertilization rate covered merely 57% of P and 48% of K removed with herbage.

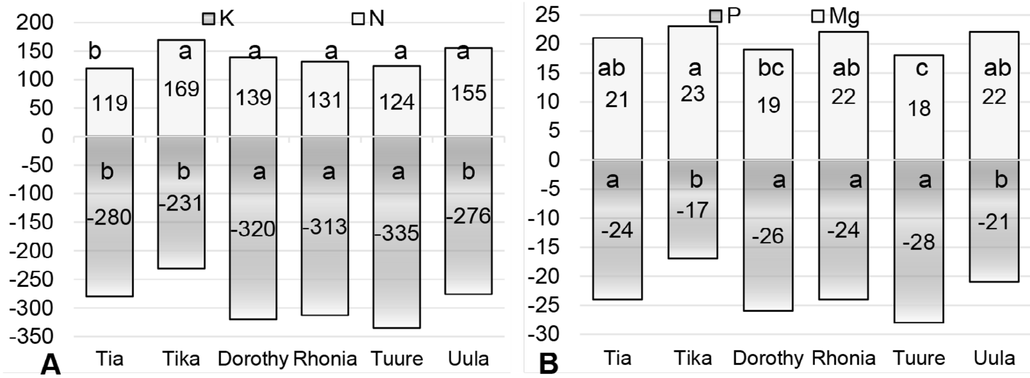

Figure 1. Total nitrogen excess vs potassium deficiency (A) and magnesium excess vs phosphorus deficiency (B) in kg ha^{-1} summed during the years of 2019-2021. Different lower case letters in rows are significantly different ($P<0.05$; ANOVA, Fisher's LSD test).

We measured soil nutrient contents and pH before and after the trial. The measurements revealed that P content was decreased from 35 to 33 mg kg^{-1} and K from 154 to 142 mg kg^{-1}. The pH of soil reduced from 7.6 to 6.7, and Mg content increased from 129 to 159 mg kg^{-1}.

Conclusions

The tested mineral fertilizer application rates did not attain nutrient balance in timothy cultivation in Estonian edaphic and climatic conditions. Smaller N and Mg fertilization rates should be applied, whereas P and K rates should be increased, at least when yearly drought periods occur.

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The effects of an innovative digestate and wood ash mixture fertilizer on the productivity of alfalfa

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Abstract

Field trials with alfalfa (*Medicago sativa* L.) were carried out on a sod stagnogley soil. The objective of the research was to study the influence of different rates of digestate and wood ash mixture fertilizer on the quality of alfalfa yield. Soil agrochemical parameters were: pH_{KCl} 6.0, organic matter content – 2.2%, phosphorus (P_2O_5) content – 50-80 mg kg^{-1}, and potassium (K_2O) content – 160-190 mg kg^{-1}. Fertilizers of the mixtures consisting of pig and cattle manure digestate and woodchip ash in different ratios (digestate to wood ash = 3:1 and 4:1) were used for alfalfa fertilization. The amounts of the innovative mixed fertilizer for alfalfa were 15 and 30 t ha^{-1}. Both norms of the pure digestate from pig and cattle manure were used as control. Trials were conducted randomized in three replications. No significant differences between the values of soil acidity were observed at the beginning of the experiment. In the course of the trial, pH_{KCl} increased and varied from 6.6 to 6.8, which was optimal for alfalfa growing. On average, the use of the innovative fertilizer contributed to a significant increase in crude protein (CP) content for all variants. A significant (P<0.05) fertilization influence on alfalfa yield was also observed.

Keywords: digestate, wood ash, fertilizer mixture, alfalfa, yield quality

Introduction

As a result of the operation of biogas and biomass co-generation plants, production by-products – digestate and ash – are obtained. These are good sources of plant nutrients, as they contain many microelements and macroelements important for plant growth; therefore, digestate can be used as an effective fertilizer for crops (Koszel and Lorencowicz, 2015; Risberg *et al.*, 2017). The use of digestate for fertilization of herbs has a positive effect on their productivity (Tilvikiene *et al.*, 2020; Barbosa *et al.*, 2014). Also, studies have found the positive effect of ash on soil properties, structure and water regime in the soil (Demeyer *et al.,* 2001). Ash fertilizer increased the amount of phosphorus, potassium, calcium and magnesium available to plants in the soil (Fuzesi *et al.,* 2015). However, the use of both these products separately may cause certain ecological problems. In order to prevent this, at least partially, an idea arose to mix digestate and ash together in certain proportions and use the obtained mixture for fertilizing different crops. In the Baltic countries, the expansion of areas under alfalfa cultivation is limited by a large proportion of acidic soils and the absence of winter-hardy varieties (Bender, 2000).

The objective of the research was to study the influence of different rates of the digestate and wood ash mixture fertilizer on the quality of alfalfa yield.

Materials and methods

Field trials with the alfalfa (*Medicago sativa* L) variety 'Birute' were carried out on sod stagnogley soil. Soil agrochemical parameters were: pH_{KCl} 6.0, organic matter content – 2.2%, phosphorus (P_2O_5) content – 50-80 mg kg^{-1}, and potassium (K_2O) content – 160-190 mg kg^{-1}. Fertilizers of the mixtures consisting of cattle manure digestate and woodchip ash in different ratios (digestate to wood ash = 1:0, 3:1, and 4:1) were used for alfalfa fertilization. The norms of the innovative mixed fertilizer for alfalfa were 15 and 30 t ha^{-1}. Both norms of the digestate from pure cattle manure were used as control. The weighed fertilizer was spread evenly by hand before sowing and incorporated into the soil with a compactor. The chemical composition of the new fertilizer varied depending on its type and the ratio of components, but the pH ranged between 9.27 and 11.22.

Randomized trials were conducted in three replications. The harvest recording area in each replicate was 10 m^2. The experimental plot was sown with a Hēge-80 seed-drill on 10 May 2019 at a seed rate of 20 kg germinating seeds per 1 ha.

Swards were cut three times during the vegetation season. The first mowing was carried out in the first decade of June (at the beginning of budding-flowering), and subsequent mowings were caried out after approximately 40 days – in mid-July and at the end of August. Qualitative indicators were determined in the Biotechnology Scientific Laboratory (BSL) of the Latvia University of Life Sciences and Technologies. The chemical composition of plants for the first cut was determined using the following methods: dry matter (DM) – dried; N – by modified Kjeldahl. The mineral elements P and K were analysed by atomic absorption spectrometry. The data were statistically analysed using the three-way analysis of variance with the type of fertilizer, fertilizer rate, and the ratio of digestate and wood ash in the mixture as factors, and the difference among means was detected by LSD at the P<0.05 probability level (Excel for Windows, 2003).

Results and discussion

No significant differences in soil acidity were observed at the beginning of the experiment. The analysis of test results revealed an increase in soil pH response from 6.0 to 6.44-6.52, depending on the fertilizer rate and the ratio of the mixture of components in the first year of alfalfa swards use. At the end of the research, pH_{KCl} had increased and varied from 6.64 to 6.83, which was optimal for alfalfa growing. The development of a high-quality alfalfa crop is a complex process of plant interaction with plant growing systems and environmental conditions, which affect the rate of photosynthesis, plant metabolism and growth. The use of both fertilizer types contributed to high alfalfa yield. Using the mixtures of pig manure digestate and wood ash in different ratios, average alfalfa dry matter yield was 12.66 Mg ha^{-1}, while using the mixtures of cattle manure digestate and wood ash, the average yield was 12.40 Mg ha^{-1}. The applied fertilizer rate and the ratio of the mixture of components (F-factor> F-criterion) demonstrated a significant positive effect on the yield. Innovative mixed fertilizer with wood ash contributed a significant yield increase, in comparison with the pure digestate application. The type of fertilizer and the ash and digestate ratio did not have any significant effect on alfalfa yield (Table 1). A one-time application of new fertilizer rates and mixtures ensured a high-quality alfalfa yield in two years. Protein content in the dry matter yield of the first alfalfa harvest averaged 178 to 196 g kg^{-1} DM over two years. The average NDF content varied from 389 to 436 g kg^{-1} DM, and ADF content 225 to 262 g kg^{-1} DM. Average NEL content in mixed stands was 6.58-7.63 MJ kg^{-1} DM.

Table 1. Influence of the digestate and wood ash mixtures on the DM yield of alfalfa (average of two years of use in three cuts).

Type of fertilizer (F_A)	Fertilizer rate, t ha^{-1} (F_B)	The ratio of digestate and wood ash in the mixture (F_C)	Average DM yield, Mg ha^{-1} (F_C) LSD $_{0.05}$=1.44	(F_B) LSD $_{0.05}$ =1.35	(F_A) LSD $_{0.05}$ =0.93
Pig manure digestate		1:0	9.53		
	15	3:1	13.95	12.18	
		4:1	13.04		
		1:0	10.95		12.66
	30	3:1	14.58	13.14	
		4:1	13.88		
Cattle manure digestate		1:0	9.23		
	15	3:1	13.42	11.61	
		4:1	12.17		12.4
		1:0	11.41		
	30	3:1	15.54	13.19	
		4:1	12.62		

Conclusions

The use of digestate and wood ash mixtures had a positive effect on soil fertility, alfalfa productivity, and yield quality. The use of the innovative soil fertility enhancer can be an effective way of recycling both products, and can also be an environmentally friendly alternative to mineral fertilizers.

Acknowledgements

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Grass clover swards: key for Dutch dairy farms under legislative pressure?

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Abstract

Dutch dairy farms are facing further strengthening of legislation to minimize the environmental impact of production. One of these recent policy measures is the planned abolition of derogation. Derogation allowed dairy farmers to go beyond the 170 kg N ha^{-1} of animal manure under specific conditions. Establishing grass-clover swards might be a strategy towards more sustainable production and to deal with the uncertainty imposed by expected policy changes and other external factors such as feed and mineral fertilizer prices. The aim of this study was to quantify and evaluate the environmental and economic implications of implementing a perennial ryegrass-white clover or a perennial ryegrass-red+white clover sward on a representative dairy farm with sandy soils in the Netherlands in a post-derogation era. The changes in environmental impacts (e.g. nitrogen losses), economic performance (e.g. labour income) and farm configuration (e.g. diet composition) were assessed. We combined a whole-farm dairy model based on linear programming with a nutrient balance to quantify and evaluate the environmental and economic implications. Results show that the use of clover swards after derogation abolition could be an economically viable strategy and can result in a lower N surplus depending on the sward type.

Keywords: dairy farm, derogation, *Trifolium pratense* L., *Trifolium repens* L.

Introduction

Pure perennial ryegrass (*Lolium perenne* L.; PRG) swards are commonly used in Dutch dairy production systems. The use of white clover (*Trifolium repens* L.) and/or red clover (*Trifolium pratense L.*) in perennial ryegrass mixtures might, however, have a positive effect on feed quality, animal performance and environmental impacts (Lüscher *et al.*, 2014; Reinsch *et al.*, 2021). In addition, expected policy changes impose uncertainty of external factors such as feed and fertilizer prices, which is further driving the interest to improve grassland management on Dutch dairy farms. One of these recent policy measures is the planned abolition of derogation. Derogation allowed dairy farmers to go beyond the 170 kg nitrogen (N) ha^{-1} of animal manure under country specific conditions. For the Netherlands these conditions included having at least 80% grassland, and the use of synthetic phosphate (P_2O_5) fertilizer was not allowed. Establishing perennial ryegrass-clover swards can be a strategy towards more sustainable production and to deal with the uncertainty imposed by policy changes and fluctuating farm input prices. Ryegrass-white clover mixtures (GWc) are commonly used in a permanent system due to their high persistence and resistance to grazing. Ryegrass-red+white clover mixtures (GRWc) are typically used in cutting-only ley systems. Little is known about the potential of using these ryegrass-clover mixtures after derogation abolition, which highlights the need to get a better insight in their environmental and economic performance. The objective of this study was therefore to quantify and evaluate the environmental and economic implications of implementing a GRWc sward or a GRWc ley on a representative dairy farm in a post-derogation era with sandy soils in the Netherlands.

Materials and methods

A whole-farm dairy model based on linear programming was used to model an average dairy farm on a sandy soil with all relevant activities and constraints according to Dutch practice (Klootwijk *et al.*, 2016). The relevant activities of the 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 synthetic fertilizers. Constraints include fixed resources available on the farm (e.g. land area), environmental policies (e.g. phosphate (P_2O_5) quota) and links between the different activities. The model is based on a Holstein-Friesian dairy cow herd with a yearly milk production of 9,209 kg with 3.61% protein and 4.43% fat (CRV, 2022). The model distinguishes a summer and winter period, considers dietary requirements of the dairy cows and accounts for extra variable labour. A farm level nutrient balance (N and P_2O_5) 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, revenues and milk production levels were updated according to long-term expected market prices and national statistics. The reference scenario was a farm in a post-derogation era and the option to choose maize land and grassland with PRG. The maximum farm average annual amount of N_{min} was 200 kg ha^{-1} for grassland. Forage maize was assumed to be cultivated in a ley-arable system. Therefore, average N fertilization requirements were set to 55 kg N_{min} ha^{-1} yr^{-1}. Thereafter, we added the option of GRWc swards used for cutting only. The GRWc swards were fertilized with 85 kg N_{min} ha^{-1}. The percentage of clover was assumed to be on average 40% in GRWc with a symbiotic N fixation of 45 kg N t DM^{-1} (van Dijk *et al.*, 2022). The yield of the GWRc ley was 74 GJ of NE_L yr^{-1}. We then replaced PRG with only an option for GWc, for which the level of N fertilization was also set to 85 kg N_{min} ha^{-1}. The percentage of clover was assumed to be on average 30%, with N fixation of 45 kg N t DM^{-1} (van Dijk *et al.*, 2022). The yield of GWc swards was 65 GJ of NE_L yr^{-1}. All other input values were assumed to be similar to the PRG scenario and available farmland was kept constant for the different scenarios. We used economic optimization to determine the optimal farm plan and to evaluate changes in farm structure, labour income and N and P_2O_5 surpluses.

Results and discussion

Comparisons show that both the number of cows and farm intensity increased for the GWc and GRWc scenario compared with the PRG scenario (Table 1). For the reference scenario, 69% of the total farmland was used for PRG. This share was 79% (50% PRG and 21% GRWc) for the GRWc scenario and 66% of grass white clover for the GWc scenario. For all three scenarios this was lower compared with the prescribed minimum of 80% for a farm using derogation. In summer, the maximum amount of fresh grass was fed for the PRG scenario. For the GRWc and GWc scenarios, this maximum was not used and the share of concentrates in the diet increased, but with a lower protein content. The nutritional value is different for PRG, GWc and GRWc swards; this partly explains why the diet composition changes across the three scenarios. Synthetic fertilizer use was lowest for the GWc scenario. In all three scenarios, a maximum application of 170 kg of N ha^{-1} yr^{-1} from animal manure was allowed, which restricted manure application. In the GRWc scenario the N_{min} application on PRG was higher due to higher allowance on total grassland in combination with the lower N application for GRWc. The highest labour income was found for GWc. This was the result from a higher cow number compared with the PRG strategy but the relatively low mineral fertilizer use. N surplus was with 106 kg ha^{-1} lowest for the GWc scenario. In reality, other aspects such as the farm situation or personal motives are being considered in grassland management decisions. For example, the use of GRWc might be easier to implement compared with transitioning to GWc swards only.

Table 1. Farm structure, labour income and environmental performance of an average Dutch dairy farm with three different grassland management systems.[1]

Item	Unit	PRG	PRG + GRWc	GWc
Farm structure				
Dairy cows	no.	95	96	99
Youngstock	no.	55	56	58
Total farmland	Ha	54.7	54.7	54.7
Grassland	% total farmland	69	50 PRG; 21 GRWc	66
Maize land	% total farmland	31	29	34
N_{min} application grassland	kg of N ha^{-1} yr^{-1}	200	250 PRG; 85 GRWc	85
Farm intensity	kg of milk ha^{-1} yr^{-1}	15,672	16,156	16,600
Diet dairy cows: summer	kg of DM cow^{-1} day^{-1}			
Grass		10	9.8	8.0
Grass silage		0	1.4	0
Maize silage		5.8	5.0	6.4
Concentrates		5.6	5.4	6.9
Diet restricted by[2]		E,R,G	E, R,T	E, T
Diet dairy cows: winter	kg of DM cow^{-1} day^{-1}			
Grass silage		4.2	4.8	4.7
Maize silage		6.7	6.2	6.7
Concentrates		7.0	7.0	6.5
Diet restricted by[2]		E,R	E, R, T	E,R,T
External inputs[3]				
Purchased maize silage	t of DM yr^{-1}	0	0	0
Purchased concentrates	t of DM yr^{-1}	228	230	256
Purchased mineral N fertilizer	kg yr^{-1}	4,711	4,833	746
Purchased mineral P_2O_5 fertilizer	kg yr^{-1}	115	560	243
Manure management				
Manure application restricted by[4]		aN, tN	aN, tN	aN
Total excretion	kg of phosphate yr^{-1}	4,921	4,629	4,751
Applied on own land	kg of phosphate yr^{-1}	3,894	4,143	3,852
Labour income	€ yr^{-1}	14,047	23,684	26,491
N surplus	kg ha^{-1} yr^{-1}	119	135	106
P_2O_5 surplus	kg ha^{-1} yr^{-1}	1.2	0	0

[1] GRWc = perennial ryegrass-red+white clover ley; GWc = perennial ryegrass-white clover mixture; Nmin = N mineral; PRG = pure perennial ryegrass sward; DM = dry matter.

[2] E = energy requirements, R = rumen degradable protein balance, G = maximum fresh grass intake, T = true protein digested in the small intestine.

[3] 177 € KVEM^{-1} maize silage. 225, 260, 325 € kg^{-1} for the standard, medium and high protein concentrates, respectively. 0,95 € kg^{-1} N, 0,87 € kg^{-1} P_2O_5.

[4] Can be restricted by: tN = total mineral N; aN = N from animal manure (170 kg N ha^{-1}); P = total P_2O_5.

Conclusions

The use of grass-clover swards could be an economically interesting strategy. However, it does not necessarily reduce the quantity of external inputs like purchased mineral fertilizers and concentrates. The quality and corresponding environmental impact of these external inputs may vary due to, for example, lower protein concentrates. For the GWc scenario the N surplus decreased relative to the PRG scenario while for the GRWc scenario it increased.

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The effect of red clover-based management on the organic matter mineralization intensity in arable soils

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Abstract

Legumes have advantages in the ecosystem and in the food chain. Legumes can be included in the ecosystem for the different purposes, but their cultivation has some difficulties is to make benefits impact on the environment; therefore the management of legume growth is complex, and their use is usually insufficient in crop rotation. The multiple seasonal soil uses, a significantly strengthen or reduce the reserves of humus. This is also directly linked to climate change. There is a need to understand more about the processes of carbon decomposition and stabilization occurring in the soil. Two field trials were carried out on loamy and clay loam *Cambisol* sites to test the effect of oat straw mass, fresh and fermented red clovers mass, and granulated cattle manure on the spring wheat yield productivity, soil viability and nutrient cycle. Fresh red clover mass used for fertilizer in combination with oat straw did not give up on efficiency to granulated cattle manure in terms of N amount inserted. It has been found that introduced swards and straw residues or organic fertilizers in the autumn decompose and release nutrients. Introduced fresh red clover mass in the autumn due to more intensive decomposition and N-release significantly increased the yield of cereals.

Keywords: ecosystem, crop rotation, organic matter, soil, yield of cereals

Introduction

Cultivation of cereal crops together with intercrops is one strategy for simulating a natural ecosystem that can improve soil ecosystem functions (Traveira *et al.*, 2020). Nitrogen is one of the most yield-limiting nutrients. As fertilizers become more expensive, and environmental regulations become more stringent, the sustainability of agroecosystems becomes a top priority. Therefore, to implement this strategy, it is important to include nitrogen-fixing legumes to ensure the nutritional processes of companion plants (Sarunaite *et al.*, 2022). Cover crops, such as red clover, accumulate nitrogen collected from the atmosphere, reduce soil erosion, increase the amount of organic matter, suppress weeds and are favourable for increasing biodiversity (Gaudin *et al.*, 2013). The process of mineralization is one of the most important biological processes in which humus is formed from organic material. In sustainable agricultural systems, much emphasis is placed on ensuring the sustainability of agricultural nutrient cycling in ecosystems. The aim of this study was to evaluate the intensity of mineralization of organic material depending on the granulometric composition of the soil and green manuring during the non-vegetation period in a cool temperate climate biome.

Materials and methods

Two experiments were conducted in 2015-2016. The field experiment in Joniškėlis (55°23'49''N, 23°51'40''E) was conducted on a drained heavy clay soil. In the soil, clay particles (<0.002 mm) comprised 27%, followed by smooth clay with >50% clay particles. The field experiment in Dotnuva (56°03'69''N, 24°16'44''E) was conducted in a deeper carbonate loamy medium-heavy clay. Soil pH_{KCl} was 6.5-7.0, humus content was 2.5-4.0%, mobile P content was 50-80 mg kg^{-1}, and K content was 100-150 mg kg^{-1}. Decomposition rate was measured by using the standard Tea Bag Index method (Keuskamp *et al.* 2013). The method involved two types of commercially available teas: green tea consisting of 89% green tea, and rooibos tea consisting of 93% rooibos incorporated into the soil in early December. Two background soils were used: (1) cultivated cereals and straw incorporated into the soil by scraping, later when ploughing, and (2) cultivated crops with clover mulch, straw and green matter incorporated into the soil when ploughing. Teas were incorporated at two depths: 4-7 cm and 14-17 cm. The studies were conducted in 4 replicates. Tea bags were dug 4 times at 30-day intervals, i.e. in January, February, March, and early April. The intensity of mineralization of the organic matter was estimated from the mass loss.

Results and discussion

Decomposition of more labile green tea was affected by soil type, green manuring and incorporation depth, but the effect was uneven in all 120 days of non-vegetation period (Table 1). The effect of soil type was significant ($P<0.05$) for three months, depth of tea incorporation for two months, the type of green manure for one month.

Table 2 shows that organic matter (teas) starts to decompose immediately after insertion. Firstly, decomposition begins with water-soluble organic compounds leached from organic matter. Green tea contains a quarter of them, red tea an eighth. Another important factor is the favourable meteorological conditions in December for mineralization of organic matter.

Table 1. Significant effects of soil type, green manure, incorporation depth and their interaction on the decomposition of green tea material.[1]

Organic fertilizers and field characteristics	Degree of decomposition (%) (days)/mean of both experimental locations			
	30	60	90	120
Soil type (S)	*	*	*	ns
Green manure (M)	**	ns	ns	ns
Depth (D)	**	**	ns	ns
S × M	**	ns	ns	ns
S × D	ns	ns	ns	ns
M × D	*	**	ns	ns
S × M × D	ns	ns	ns	ns

[1] *, **significant at P=0.05, P=0.01 level; ns = not significant.

Table 2. Intensity of organic matter mineralization in soil (Joniškėlis and Dotnuva)[1]

Organic fertilizers and field characteristics	Insertion depth cm	Degree of decomposition (%) during period of decomposition (days)/experimental location							
		30		60		90		120	
		Joniš-kėlis	Dotnu-va	Joniš-kėlis	Dotnu-va	Joniš-kėlis	Dotnu-va	Joniš-kėlis	Dotnu-va
Green tea as affected by winter wheat	4-7	22.4	21.25	26.7	31.07	37.0	38.39	37.3	41.70
	14-17	20.8	-	25.4	33.79	29.3	40.20	36.7	47.81
Green tea as affected by w. wheat with red clover	4-7	17.9	14.82	18.3	23.90	34.3	39.19	34.1	43.29
	14-17	22.8	21.55	26.9	37.20	32.4	39.35	24.3	43.27
Average	4-7	20.2	17.87	22.5	27.49	35.7	38.79	35.7	42.50
	14-17	21.8	21.55	26.2	35.50	30.9	39.78	30.5	45.54
	Average	21.0	19.71	24.4	31.50	33.3	39.29	33.1	44.02
Red tea as affected by winter wheat	4-7	10.4	4.48	x	6.53	10.3	13.24	10.3	16.15
	14-17	7.8	4.38	x	8.66	14.5	14.23	14.5	16.05
Red tea as affected by w. wheat with red clover	4-7	10.1	3.58	x	5.40	10.9	15.68	10.9	16.72
	14-17	8.1	7.93	x	8.70	10.3	17.23	10.3	17.88
Average	4-7	10.3	4.03	x	5.97	10.6	14.46	10.6	16.44
	14-17	8.0	6.16	x	8.68	12.4	15.73	12.4	16.97
	Average	9.2	5.10	x	7.33	11.5	15.10	11.5	16.71

During the research period, the green tea mass decomposed by 44.02% in the light loam clay (Dotnuva) (Figure 1). This shows that the mass of plant residues and organic fertilizers applied in the autumn is not mineralized during the vegetation period. Its intensity depended on the chemical composition of the incorporated organic substances, the granulometric composition of the soil and meteorological conditions. The data were more consistently distributed here: the higher intensity of mineralization was found in the deeper soil layer and in the soil where cereal straw was incorporated.

Conducted studies with organic material of different decomposition intensity (according to C:N) showed that in December an average of 20.4% of the easily decomposable organic material (green tea) and an average of 7.2% of the more difficult to decompose organic material (red tea) were decomposed. In the later study periods (after 60, 90, and 120 days), both of the tested organic materials (tea types) decomposed more intensively in the soil that had lighter rather than heavier granulometric composition.

Conclusions

This study has shown that mineralization of organic matter is most active in a light granulometric composition soil. This suggests that plant residues or organic fertilizers applied in the autumn are decomposing and releasing nutrients. In soils without cover crops, these nutrients may be leached. Therefore, introduced fresh red clover mass in the autumn due to more intensive decomposition and N-release can significantly increase the yield of cereals.

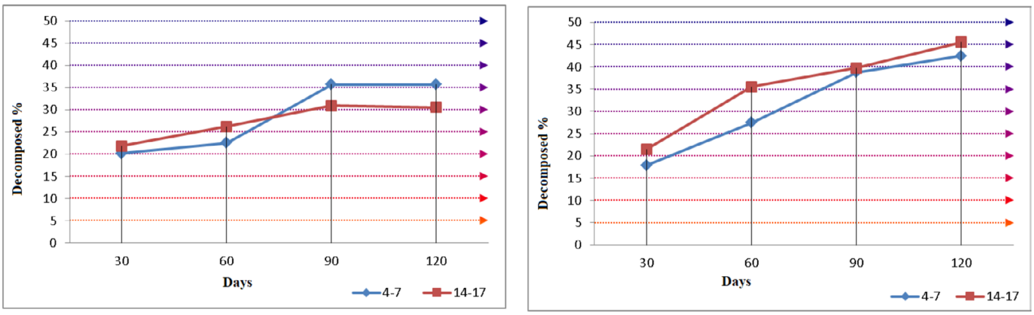

Figure 1. The intensity of green tea decomposition includes granulometric composition in soils (December-March) in 2015-2016. (A) In heavy loam clay (average data), (B) in light loam clay (average data).

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Effect of cessation of fertilization and reduction of mowing frequency of grass-legume mixtures on temporary grassland

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Abstract

Grass-legume mixtures on arable land are important as an additional feed source for ruminants, and their efficiency depends on the management. The aim of the study was to assess changes in the botanical composition, value and yield of the temporary meadow sward after cessation of fertilization and a reduction of mowing frequency. Two grass-legume mixtures (M1, M2) and a mixture of grasses (M3) were sown in 2014: M1 and M2 – *Dactylis glomerata*, *Festulolium braunii* and *Trifolium pratense* or *Medicago* × *varia* (M1 and M2, respectively); M3 – *D. glomerata*, *F. braunii* and *Lolium perenne*. For the next three years (2015-2017) appropriate NPK fertilization was applied and sward was mowed three times. Then for five years (until 2022) fertilization was stopped and mowing was reduced to two times. The botanical composition, utilization value and yield of the sward of mixtures were assessed. It was found that the share of sown species in mixtures noticeably decreased (*D. glomerata* to 15%), while that of herbs (especially *Taraxacum officinale* approx. 45%) and weeds increased. Unsown *Trifolium repens* was also noticed (10%). Changes in botanical composition caused by reduction of management intensity resulted in lower utility value of sward and a reduction in yield.

Keywords: botanical composition, extensive management, grasses, legumes

Introduction

The use of mixtures of grasses with legumes on arable land is important for an additional source of feed for ruminants. The cultivation of simple grass-legume mixtures (2-4 components) becomes more important in conditions of intensive, short-term utilization and the demand for fodder with a higher content of nutrients (Bélanger *et al.,* 2014). The components of temporary grass-legume mixtures in temperate climate are *Medicago* × varia, *Trifolium pratense* and highly productive grass species, including *Festulolium braunii*, *Lolium perenne* and *Dactylis glomerata* (Olszewska *et al.,* 2019). Compared to pure-sown swards, simple grass-legume mixtures sown on temporary grasslands are characterized by higher and more stable yields, as well as a weaker response to adverse environmental conditions than monocultures, especially in the case of more frequent periods of drought (Janicka *et al.,* 2012, Staniak and Harasim, 2018). The cultivation of grass-legume mixtures is also of great importance for the protection and improvement of the environment. They enrich the soil with organic matter and nutrients and improve the soil structure (Hajduk *et al.,* 2015). Complete cessation of fertilization and utilization results in the disappearance of valuable species from the sward and a decrease in yield and quality (Fisher and Rahman, 1997). The aim of the study was to evaluate the effect of discontinuing the fertilization and reducing of mowing frequency during 5 years on changes in the botanical composition, value and yielding of grass-legume mixtures used on temporary grasslands.

Materials and methods

The research was conducted at the Experimental Field in Miedniewice (central Poland). The experiment was established in 2014 on Luvisol soils of the texture of loamy sand (slightly acidic, $pH_{KCl} = 5.8$). Two grass-legume mixtures and a mixture consisting of grasses were sown: M1 – *Dactylis glomerata* L. (cv. Berta), *Festulolium braunii* (Richt.) A. Camus, cv. Sulino, *Trifolium pratense* L., cv. Rozeta (55%, 30% and 15%, respectively). M2 – *Dactylis glomerata* L., cv. Berta, *Festulolium braunii* (Richt.) A. Camus, cv.

Sulino, *Medicago x* varia T. Martyn, cv. Radius (55%, 30%, 15%) and M3 – *Dactylis glomerata* L., cv. Berta, *Festulolium braunii* (Richt.) A. Camus, cv. Sulino, *Lolium perenne* L., cv. Gagat (45%, 30%, 25%). For the next three years (2015-2017) NPK fertilization was applied (kg ha^{-1}): N-90, P-35, K-100 and the sward was mown three times. Then for five years (until 2022) fertilization ceased and mowing was reduced to two times per year. The botanical composition (by botanical-weight method), dry mass of yield and soil cover by plants were assessed. The utilization value of the sward (UVS) using the Filipek method (1973) was also evaluated. In this method, plant species have a value from -3 (harmful or poisonous plants) to 10 (the best quality plants). The UVS of the sward was assessed on the basis of the average share of a given species in the sward and its value. Sward quality based on the average UVS can be categorized as very good (10-8.1 points), good (8.0-6.1), poor (6.0-3.1) and very poor (below 3.0). Statistical analyses of the obtained data from 2017 and 2022 were done (two-way ANOVA). The differences between means were determined using Tukey HSD test ($P \leq 0.05$).

Results and discussion

It was found that discontinuing of NPK fertilization for five years and reduction of mowing frequency from three to two per year caused a significant decrease in soil cover by plants from mean 63.6% (2017) to 42.0% (2022). However, there were no differences in sodding between the mixtures in the corresponding years. Moreover, the cessation of fertilization and limiting mowing to two cuts influenced not only changes in the botanical composition, but also significantly reduced yields (Table1). Similar regularities were found by Fisher and Rahmann (1997). The yields of individual mixtures decreased in 2022 compared to 2017 (2-3 times). The yield of the mixture containing grasses was only 2.56 Mg ha^{-1}, while the mixtures with legumes yielded slightly better (especially M2 with *Medicago × varia*). This could also be due to unfavourable weather conditions (especially the summer droughts repeated in recent years) and the durability of the sown species. The average USV value before limiting the intensity of management was 8.76 and was classified as very good, while after five years – specified as good (6.77). This was probably due to the large share of herbs, especially *Taraxacum officinale* and unsown *Trifolium repens* (10%). Changes in the botanical composition caused a decrease or disappearance of species sown in mixtures, and an increase in the share of rosette plants in the sward (Figure 1).

Conclusions

Cessation of fertilization and limiting the frequency of mowing resulted in a decrease in the share of species sown in mixtures on temporary grasslands. At the same time, the floristic diversity of the sward was greater due to the presence of herb and weeds species. Changes in botanical composition caused by desistance of fertilization and reducing the mowing frequency, age of the sward, and unfavourable weather conditions resulted in a lower UVS and a decrease in the yield of mixtures.

Table 1. The influence of cessation of fertilization and mowing limitation on soil cover (%), DM (Mg ha^{-1}) and UVS values of grass-legume mixtures (2017 – the last year of fertilization and three-time mowing, 2022 – after 5 years of fertilization cessation and reducing mowing).[1]

Year	Mixture	Cover	DM Yield	UVS
2017	M1	64.17b	8.16c	8.33bcd
	M2	62.17b	10.26d	9.27d
	M3	64.50b	7.85c	8.66d
2022	M1	47.00a	3.06ab	7.56abc
	M2	42.92a	3.64b	6.01a
	M3	43.67a	2.86a	6.76ab

[1] Means marked with the same letters in columns do not differ significantly at $P \leq 0.05$. DM = dry matter.

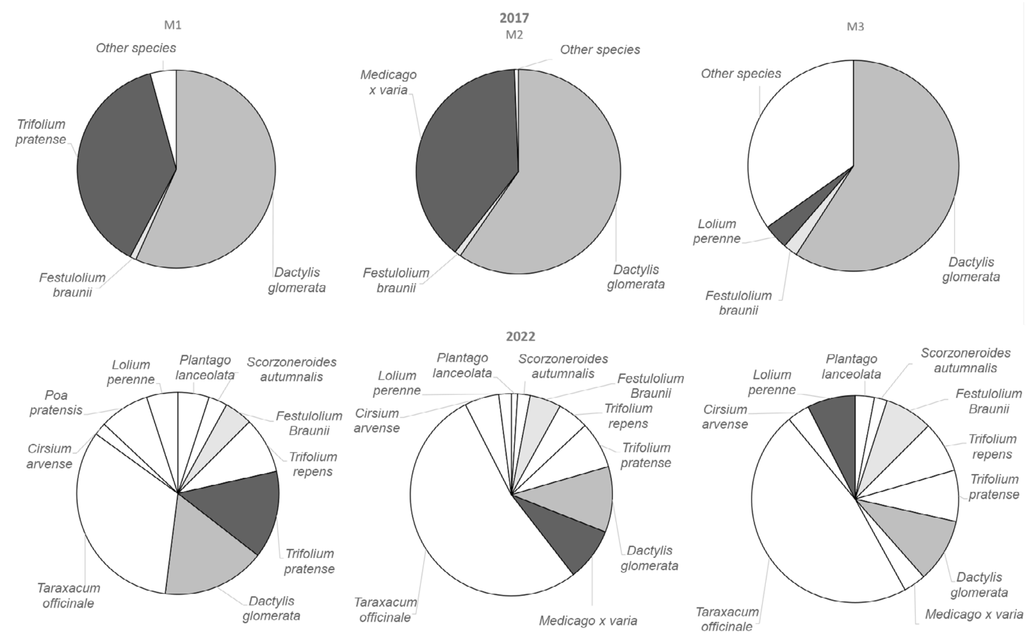

Figure 1. Botanical composition (%) of the sward of grass-legume mixtures (M1, M2, M3) in the years of study (2017 and 2022).

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Nitrogen fertilization and utilization of sorghum as an extra forage crop on dairy farms

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Abstract

Sorghum may have potential as an extra forage crop on dairy farms. It can be an addition to the crop rotation with maize and grass-clover leys, in order to prevent disadvantages of continuous maize cropping. However, concerning the cultivation of sorghum, questions arise around fertilization and nitrate leaching caused by residual mineral nitrogen (N) after harvest. Therefore, in 2018 and 2019, we performed experiments to quantify N utilization of sorghum and to estimate optimal N fertilization, including the risk of nitrate leaching. Two types of sorghum (a type high in starch (ST) with a potential for high starch content and type high in fibre (FT) with high cell wall content) and maize (reference) were compared, each with three levels of N fertilization (0, 70, 140 kg N ha^{-1}). In both years, dry matter (DM) and N yield were highest in maize (P<0.001; up to 6 t ha^{-1} more DM yield for maize than for ST or FT). The two sorghum types differed in N response: FT showed an N response, whereas ST did not. The type high in fibre had the lowest residual N in the soil after harvest (P<0.001; up to 40 kg ha^{-1} less residual N on FT plots), indicating a possible reduction of nitrate leaching. Optimal level of fertilization was expected to be lower for sorghum than for maize. A relatively high yield of ST under N limitation indicates that sorghum cultivation is possible on relatively poor soils. In conclusion, because of the low required N inputs and the potentially reduced risk on nitrate leaching, sorghum is a promising crop to extend crops rotations on dairy farms.

Keywords: sorghum, mineral nitrogen, fertilization, yield, nitrate leaching

Introduction

Sorghum, a crop originating from the African continent, may have potential as an extra forage crop in dairy farming in temperate regions of western Europe. It can be added in crop rotations with maize and grass clover leys and in that way prevent the disadvantages of continuous maize cultivation, such as a decrease of organic matter in soils, soil compaction, plant-related diseases and high water and nutrient inputs. Sorghum has been shown to be more tolerant to water shortages (Lemaire *et al.*, 2006), has a high nitrogen (N) usage efficiency (Thievierge *et al.*, 2015) and it may positively affect organic matter accumulation in soils (as compared to maize) (Schittenhelm and Schroetter, 2014). For dairy farming, two types of sorghum are available, a type with potential for high starch content (ST); *Sorghum bicolor*), and a type rich in fibre (FT), with high cell wall content; *Sorghum sudanense*). The type with high starch content has a plant height of 100-140 cm and a relatively high grain yield, whereas FT has a plant height of 200-300 cm and a lower grain yield. From nutritional point of view, sorghum silage can (partly) replace maize silage in the diet of ruminants.

Even though the cultivation, harvest and ensiling of sorghum have similarities with the cultivation of maize, many questions remain concerning fertilization and nutrient leaching of sorghum cultivated in the temperate regions of western Europe. Therefore, in 2018 and 2019 we performed experiments to quantify the nitrogen (N) utilization of sorghum and to estimate the optimal N fertilization, including the risk of nitrate leaching. Results were analysed with a two-way ANOVA with the statistical package R.

Material and methods

A field experiment was implemented in Moergestel on a field with a sandy soil and a history of long-term arable land use. Two types of sorghum were compared with maize: ST (cultivar C7) and FT (cultivar NutriHoney in 2018 and cultivar Suzy in 2019). Three levels of N fertilization (0, 70 and 140 kg N ha^{-1}, applied as calcium ammonium nitrate, CAN) were tested in four randomised replicates, adding up to 36 experimental field plots. Each of the 36 experimental plots was 3.5 m wide and 5 m long and had 7 rows of sorghum or maize with a distance of 50 cm between the rows. In both years, plants were sown in May. Plant density was 110,000 plants ha^{-1} for maize and 225,000 plants ha^{-1} for sorghum.

In both years the crops were harvested in September. On every plot, 3 rows of 3 metres were harvested. Subsamples of the biomass yield of each plot were sent to a laboratory (Eurofins) to analyse dry matter (ISO 6496), ash content (ISO 5984), nitrogen (Kjeldahl method, ISO 5983), sugars (Luff-Schoorl method, NEN3571: 1947nl), starch (amyloglucosidase method, ISO 15914) and digestibility of organic matter (based on Tilley and Terry, 1963). Furthermore, directly after harvest, soil samples (0-90 cm) were taken to analyse residual mineral N as an indication of potential nitrate leaching.

Results and discussion

In both years, dry matter and N yield was highest in the fertilized maize plots. Without fertilization, in 2018 ST had the highest N-yield (Table 1). The two sorghum types differed in N response: ST did not, or hardly, result in extra yield with higher N fertilization, but FT did show an N response (Table 1). Maize and FT both had a higher N yield with higher N fertilization, but for ST this response was observed only in 2018. As a result, residual N after harvest was higher in ST, especially in 2019 (Table 1). The fibre type had the lowest values for residual mineral N in the soil after harvest, despite a lower N yield.

Digestibility of organic matter and starch concentration were highest in maize and lowest in FT (Table 2). Sugar concentration was highest in the FT, and in 2018 the N concentration was higher in the ST (Table 2). In 2019 no differences in N concentrations were observed. Differences between years might have been caused by weather conditions. For FT, different varieties were used in 2018 and in 2019, which may also have caused differences, for example in starch content.

Table 1. Yield, N-mineral in the soil and N yield with different fertilization levels in 2018 and 2019.

Crop	Maize			Sorghum C7 (ST)			Sorghum Nutrihoney / Suzy (FT)			*P*-values		
N application (kg ha^{-1})	0	70	140	0	70	140	0	70	140	Crop	N appl.	Crop×N
2018												
Yield (t DM ha^{-1})	13.4	19.0	21.1	12.4	13.2	14.6	10.8	15.7	15.3	<0.001	<0.001	0.1
Residual N (kg N ha^{-1})	19	21	31	18	26	59	10	12	29	<0.001	<0.001	0.018
AEN[1] (kg DM kg N^{-1})	n.a.	80	29	n.a.	11	20	n.a.	69	0	n.a.	n.a.	n.a.
N yield (kg N ha^{-1})	114	184	239	126	151	194	92	159	192	0.015	<0.001	ns
2019												
N application (kg ha^{-1})	0	60	120	0	60	120	0	60	120	Crop	N appl.	Crop×N
Yield (t DM ha^{-1})	14.6	17.7	16.9	10.8	9.8	10.2	10.7	11.3	13.7	<0.001	<0.03	<0.03
Residual N (kg N ha^{-1})	34	47	53	32	56	76	27	38	34	<0.001	<0.001	<0.01
AEN[1] (kg DM kg N^{-1})	n.a.	52	0	n.a.	0	0	n.a.	10	40	n.a.	n.a.	n.a.
N yield (kg N ha^{-1})	147	251	196	118	97	101	80	97	167	<0.01	<0.001	ns

[1] AEN = agromic efficiency of applied nitrogen; DM = dry matter.

Table 2. Nutritional value with different fertilization levels in 2018 and 2019.[1]

	Maize			Sorghum C7 (ST)			Sorghum Nutrihoney / Suzy (FT)			*P*-values		
2018												
N application (kg ha^{-1})	0	70	140	0	70	140	0	70	140	Crop	N-appl.	Crop×N
Digestibility OM (%)	81	80	80	71	71	70	65	65	65	<0.001	ns	ns
Starch (g kg ds^{-1})	339	369	388	326	311	313	198	268	242	<0.001	ns	ns
Sugars (g kg DM^{-1})	97	74	77	51	60	68	119	85	77	<0.001	ns	0.037
N (g kg DM^{-1})	8	10	11	10	11	13	8	10	13	<0.001	<0.001	ns
2019												
N application (kg ha^{-1})	0	60	120	0	60	120	0	60	120	Crop	N-appl.	Crop×N
Digestibility OM (%)	77	77	77	66	68	68	60	58	60	<0.001	ns	ns
Starch (g kg ds^{-1})	432	457	449	296	310	317	133	98	145	<0.001	ns	ns
Sugars (g kg dm^{-1})	35	27	33	56	56	56	71	81	68	<0.001	ns	ns
N (g kg dm^{-1})	10	14	12	11	10	10	8	9	12	ns	ns	ns

[1] OM = organic matter; DM = dry matter.

The optimal level of N-fertilization was found to be lower for sorghum than for maize. The lower residual N in the soil after harvest on the FT plots indicates a possible reduction of nitrate leaching. The lack of response to N fertilization of ST coincided with a relatively high yield under N limitation, leading to the conclusion that sorghum cultivation is possible on relatively poor soils. The nutritional value of sorghum may be lower than the nutritional value of maize, but thanks to the low N inputs and low residual mineral N in the soil, sorghum has potential as an extra crop in dairy farming.

Conclusions

This study has shown that the N requirements of sorghum differ from the N requirements of maize, and that N fertilization of sorghum should thus be lower than of maize. The lack of response to N fertilization coincided with a relatively high yield under N limitation, leading to the conclusion that sorghum cultivation is possible on relatively poor soils. In conclusion, because of the low required N inputs and the reduced risk on nitrate leaching, sorghum is a promising crop to extend crops rotations.

Acknowledgements

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Effect of grazing on soil physical properties and primary productivity in a crop-livestock system

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Abstract

The aim of this work was to evaluate the impact of grazing on the physical properties of the soil and the productivity of an annual pasture (ryegrass: *Lolium multiflorum* L.) and their successive crop (maize: *Zea mays*). The ryegrass was mechanically harvested for haylage production or grazed when the pre-defoliation sward height was between 20 and 25 cm. The mechanical harvesting was carried out at 5 cm above the ground level, while the post-grazing sward height target was 50% of pre-defoliation sward height. Soil density and porosity were determined in samples collected annually at different depths. The whole maize plants were cut when DM content was nearly 35% of the fresh plant. The physical properties of the soil were not affected by the different pasture defoliation methods. The pasture DM accumulation and DM production tended to be higher in grazed compared with non-grazed pastures. Crude protein content increased, while neutral detergent fibre decreased in grazed compared with non-grazed pastures. The maize chemical composition and DM production were similar for plants from previously grazed and non-grazed areas. In conclusion, four consecutive years of grazing did not impair the physical quality of the soil or the DM production of either the pasture or the silage maize.

Keywords: grazing, *Lolium multiflorum*, silage, *Zea mays*

Introduction

Most forage species used as cover crops have good nutritional value, digestibility and dry matter (DM) productivity. Thus, crop-livestock systems may be a tool to improve land use and food security. However, there is a concern that grazing could impair soil physical proprieties and decrease the DM yield of subsequent crops. Additionally, trampling, animal excretion and selective grazing may decrease DM herbage productivity (Jing *et al.*, 2017). The aim of this work was to assess the effect of grazing on soil physical properties and DM production of herbage and subsequent crop throughout four subsequent years in a dairy system.

Materials and methods

The experiment was conducted in Lages, SC, Brazil (50.18°W, 27.47°S; 920 m above sea level). The treatments were annual ryegrass (*Lolium multiflorum* L.) with or without grazing followed by maize crop (*Zea mays*), which were planted in plots of 150 m^2 distributed in a randomized block design with four replications. Pasture evaluations were carried out throughout four years (2017, 2018, 2019 and 2021). In 2020, due to the COVID pandemic, data were not collected but experimental plots received their respective treatments. Both grazed and non-grazed pastures were defoliated when swards reached a pre-defoliation height between 20 and 25 cm. Grazed pastures were defoliated by lactating dairy cows with a post-grazing target height between 50 and 60% of pre-defoliation height, which may be considered as lenient grazing. Non-grazed pastures were mechanically harvested at 5 cm above the ground level.

Actual pre- and post-defoliation sward heights were measured using a 0.5-m sward stick by averaging the first contact of 50 readings taken randomly throughout each experimental unit. At pre- and post-defoliation events pasture biomass was measured from three 0.1 m^2 quadrats per experimental unit. The quadrats were cut at ground level using scissors, and individual samples were dried in an oven for 72 h at

60 °C. Dry matter accumulation rate was calculated by the difference between pre-defoliation forage mass and previously post-grazing defoliation mass divided by the number of days between each defoliation event. Each year, total forage DM production was measured by adding the first pre-defoliation forage mass plus DM accumulation rate throughout the forage growing season. Chemical composition was determined throughout the grazing season in pasture samples collected by hand plucked or mechanically harvest in grazed or non-grazed pastures, respectively. Maize evaluations were carried out in 2021 and 2022. When the whole plants reached 35% of DM content, two rows of 10 linear metres per plot were cut at 20 cm above ground level. Plants were weighed and crushed in a mill with a 5 cm sieve. One sample per plot was dried in an oven for 72 h at 60 °C to determine the DM content and chemical composition of whole maize plants. Soil for determination of physical properties were sampled once a year from 2017 to 2021. Two sampled points were collected per plot, in layers of 0-5, 5-10, 10-20 and 20-30 cm. Density and porosity analyses were performed based on the principles of the volumetric ring (Embrapa, 2011). The dependent variables were analysed using analysis of variance. The least square means were considered as significantly different if $P<0.05$, and P-values between 0.05 and 0.10 were considered trends; standard error of the mean were reported to describe variations.

Results and discussion

Annual forage DM yield tended to increase ($P<0.08$) by approximately 500 kg ha^{-1} and DM accumulation rate was 25% greater ($P<0.01$) in grazed compared to mechanically harvested plots (Table 1). These results may be explained because grazed plots showed greater ($P<0.01$) post-defoliation sward height, supporting greater leaf area index, light interception and photoassimilates production after defoliation events (Carvalho *et al.*, 2018). Crude protein (CP) content increased ($P<0.01$) and NDF content decreased ($P<0.01$) in forage sampled from grazed compared with non-grazed treatment, which is in agreement with Simon *et al.* (2021), where the nutritional value of grazed herbage was greater than the same forage harvest for hay production. The DM yield of whole maize plants was similar (average= 14,611 kg ha^{-1}), but OM and CP content were greater ($P<0.01$) in previously grazed compared to non-grazed areas. These results are in agreement with other authors (Santos *et al.*, 2018), who concluded that in a medium-term experiment grazing throughout the previous season did not impair either DM yield or chemical composition of maize for silage production. Finally, the lack of difference in maize DM yield between treatments in the current work may be explained, at last partially, because grazing did not impair soil density and porosity, except for a small increase in density between 10 to 20 cm depth.

Conclusions

After four consecutive years of a lenient grazing throughout the cool season, grazing did not impair the physical quality of the soil and DM production of both pasture and maize silage.

Table 1. Physical properties of the soil and productivity of annual ryegrass (*Lolium multiflorum* L.) and the successive crop (maize: *Zea mays*) in areas grazed or not throughout four years during the cool season.

	Treatments		SEM	*P*<
	Control[1]	Grazing		
Ryegrass				
DM yield (kg ha^{-1})	4010	4496	184	0.08
DM accumulation rate (kg ha^{-1})	25.7	32.4	1.49	0.01
Pre-grazing sward height (cm)	23.1	23.7	0.28	0.16
Post-grazing sward height (cm)	8.69	12.1	0.23	0.01
Chemical composition (g (kg $DM)^{-1}$)				
Organic matter	894	904	15.9	0.01
Crude protein	199	248	28.3	0.01
Neutral detergent fibre	485	474	21.8	0.01
Acid detergent fibre	249	226	29.4	0.01
Maize				
DM yield (kg DM ha^{-1})	14,967	14,255	811	0.55
Chemical composition (g (kg $DM)^{-1}$)				
Organic matter	967	963	8.3	0.01
Crude protein	77.9	84.5	12.8	0.01
Neutral detergent fibre	614	599	11.0	0.37
Acid detergent fibre	293	288	65.0	0.60
Density (g cm^{-3})				
0-5 cm	1.35	1.32	0.026	0.56
5-10 cm	1.33	1.41	0.055	0.40
10-20 cm	1.29	1.39	0.022	0.05
20-30 cm	1.26	1.28	0.035	0.71
Porosity (m $(m^3)^{-1}$)				
0-5 cm	0.538	0.524	0.010	0.41
5-10 cm	0.495	0.511	0.009	0.31
10-20 cm	0.525	0.509	0.020	0.61
20-30 cm	0.539	0.516	0.012	0.27

[1] Without grazing.

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Overview of dry matter contents and drying rates of forage species to assess the drying abilty of leys

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Abstract

The drying speed is a pivotal factor for successful conservation of high-quality forage. The drying process can be described by an exponential function driven by a species-specific drying rate, the water content at harvest time and the time elapsed since harvesting. Drying rates are provided by the literature, but there is no available study providing an overview over the whole investigated species range. To this aim, we selected nine papers providing the dry matter content and/or the drying rate (or the necessary variables to compute it) of forage species and expressed their dry matter content and drying rate as the difference to that of *Lolium perenne*. In general, legumes were found to have lower initial dry matter contents and to dry at slower rates than grasses, but differences between species, especially within grasses, were apparent. Among the legumes, *Trifolium pratense*, often playing a relevant role in grass-legume leys, has one of the lowest drying rates. Under sub-optimal weather conditions, grass-legume leys with relevant yield proportions of legumes should, therefore, be professionally dried in barn drying facilities to produce high-quality forage.

Keywords: dry matter content, drying rate, grasses, legumes

Introduction

Leys with a relevant yield proportion of legumes have a high-quality potential. However, to exploit this potential, a successful forage conservation is required. The drying speed is a pivotal factor for achieving high-quality forage, as it contributes to minimise field losses and to achieve forage stability within a short time. The progress of the drying process in terms of forage water content (M, g water g^{-1} DM) can be mathematically described by an exponential function driven by a species-specific drying rate (k, $hour^{-1}$), by the water content at the time of mowing (M_0, g water g^{-1} DM) and by the time (in hours) elapsed since harvesting (t-t_0) (Rotz and Sprott, 1984):

$$\boldsymbol{M = M_0 e^{-k(t-t_0)}}, \text{ so that } \boldsymbol{k = -\ln\left(\frac{M}{M_0}\right)/(t - t_0)}$$

The expected dry matter (DM) content at the time of mowing and the drying rates of forage species are therefore pivotal information to evaluate the suitability of seed mixtures, depending on the expected drying conditions and/or drying facilities. This information is provided in the literature for a narrow range of selected species, and usually only for few species within a single literature source. To the best of our knowledge, there is no study providing an overview over the whole investigated species range, enabling a quantitative comparison between species investigated in different studies. Therefore, we searched available data in the literature to provide an overview of (1) how the main grassland species differ regarding their expected initial dry matter (DM) content, i.e. the DM content at the time of mowing, and (2) how they differ in terms of drying rate.

Materials and methods

We selected eight papers providing both the initial DM content at the time of mowing and the drying rate of forage species or the necessary variables (M, M_0 and t-t_0) to compute it, and addressing at least *Lolium perenne*, *Dactylis glomerata* or *Medicago sativa* besides other species (Sorenson and Person, 1967; Morris, 1972; Tetlow and Fenlon, 1978; Jones and Prickett, 1981; Owen and Wilman, 1983; Alli *et al.,* 1985;

Clark *et al.,* 1989; Brink *et al.,* 2014). An additional source (Jeangros *et al.* 2001) provided DM values for one forb (*Taraxacum officinale*) besides *Lolium perenne*. Fourteen grasses, seven legumes and one forb species could be considered: *Agrostis stolonifera* (Agr sto), *Alopecurus pratensis* (Alo pra), *Anthoxanthum odoratum* (Ant odo), *Cynodon dactylon* (Cyn dac), *Cynosurus cristatus* (Cyn cri), *Dactylis glomerata* (Dac glo), *Festuca arundinacea* (Fes aru), *Festuca ovina* (Fes ovi), *Festuca pratensis* (Fes pra), *Festulolium* (Festul), *Lolium multiflorum* (Lol mul), *Lolium perenne* (Lol per), *Panicum coloratum* (Pan col), *Phalaris arundinacea* (Pha aru), *Phleum pratense* (Phl pra), *Medicago sativa* (Med sat), *Melilotus albus* (Mel alb), *Onobrychis viciifolia* (Ono vic), *Trifolium hybridum* (Tri hyb), *Trifolium pratense* (Tri pra), *Trifolium repens* (Tri rep), *Taraxacum officinale* (Tar off). The DM content and the drying rates of the species addressed by each paper were expressed as the difference to those of *Lolium perenne*. For papers in which *Lolium perenne* was not addressed, the difference was computed with reference to *Dactylis glomerata* or *Medicago sativa*, which could be linked in turn to *Lolium perenne* thanks to other sources addressing at the same time *Lolium perenne* and these species.

Results and discussion

Legume species were found to have, in general, a lower expected initial DM content than grasses (Figure 1). Legumes exhibited a DM content range of 150 g kg^{-1} to 300 g kg^{-1} lower than that of *Lolium perenne*. For legumes, the highest DM content was found for *Trifolium repens* and the lowest for *Trifolium hybridum*. Within the grasses, the values ranged from a negative value of around 120 g kg^{-1} to a positive one around 60 g kg^{-1} in comparison to *Lolium perenne*. The highest DM content was found for *Festuca ovina* and the lowest for *Cynodon dactylon*. *Taraxacum officinale* showed a DM content similar to that of *Trifolium repens*.

The overview of the drying rates resembles, in terms of species rank, that of the expected initial DM content and shows that the legumes have in general a lower drying rate compared to the grasses (Figure 2). *Festuca arundinacea* showed the highest drying rate (+0.04 $hour^{-1}$ higher than that of *Lolium perenne*), whilst *Phleum pratense* possesses a lower drying rate. Among the legume species *Trifolium hybridum* had the highest drying rate and *Trifolium pratense* and *Melilotus albus* the lowest. Concerning the functional groups, we can assume in general that an increasing yield proportion of legumes slows down the drying process.

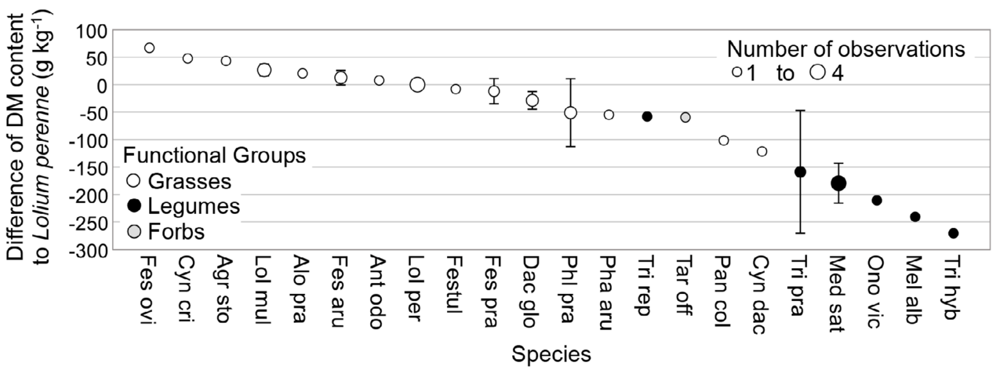

Figure 1. Expected difference in initial dry matter (DM) content (mean ± standard error if replicates were available) with respect to the reference species *Lolium perenne*. The dot size reflects the number of observations.

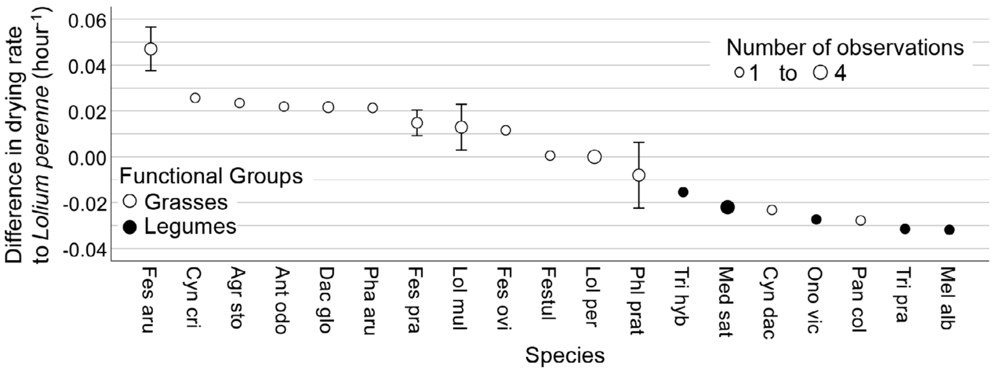

Figure 2. Expected difference in drying rate (mean ± standard error if replicates are available) with respect to the reference species *Lolium perenne*. The dot size reflects the number of observations.

Conclusions

In general, grasses show a higher DM content at the time of mowing and a higher drying rate than legumes. This hampers the field drying of leys, as they usually exhibit a high yield proportion of legumes, at least in the initial phase. Moreover, weather conditions for field drying are worsening towards the end of the growing season (low vapour deficit pressure), exacerbating the problem. Therefore, under sub-optimal weather conditions, grassland rich in legumes should be professionally dried in barn drying facilities to produce a high-quality forage.

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Improvement of temporary grassland by *Dactylis glomerata* L. overseeding at different dates

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Abstract

Overseeding, without disturbing the existing sward, is one of the methods for improving grassland and extending its period of use. The aim of this study was to evaluate the effectiveness of temporary grassland renovation by cocksfoot (*Dactylis glomerata* L.) overseeding depending on the date of its implementation. The study was carried out on a temporary grassland, on a mineral soil, in north-eastern Poland. Overseeding was performed with a Vredo slot seeder, on four dates: early spring, after the first cut, after second cut and after third cut. The overseeding effect was assessed based on the plant development in the sowing year as well as the yield and species composition of the first re-growth in the following year. It was found that *D. glomerata* is a species useful for overseeding of temporary grasslands in eastern Poland. The best time for overseeding temporary grasslands is late summer (end of August), and the riskiest period after the first cut, when there is a high risk of drought.

Keywords: temporary grassland, overdrilling, *Dactylis glomerata*, sowing date, drought

Introduction

In north-eastern Poland, a region dominated by milk production, temporary grassland plays an important role, especially on farms with a small area of permanent grassland or with low productivity. Maintaining high yields and suitable species composition of grassland is difficult due to the increasingly frequent extreme weather conditions associated with global climate change, especially long-term droughts (Borawska-Jarmułowicz *et al.*, 2022). In order to improve the condition of the grassland and extend the period of its use, it is recommended, among other things, to introduce seeds of grass and legume species by overseeding, without disturbing the existing sward. This method is beneficial to the environment compared to traditional reseeding after ploughing. The issue of the date of overseeding is not fully clarified, as it was found that habitat conditions, including the weather, have a greater impact on the development of seedlings than the date of overseeding (Janicka, 2007). One of the components of grass-legume mixtures used for temporary grassland is *D. glomerata*; it is a drought-tolerant species and better suited than many other grasses to drier locations and longer periods of drought. The aim of the study was to evaluate the effectiveness of temporary grassland renovation by *D. glomerata* overseeding depending on the date of its implementation.

Materials and methods

The study was carried out on a temporary grassland, located on mineral, medium compacted soil, with regulated water relations, in north-eastern Poland. The soil was characterized by a neutral reaction (pH in KCl 6.8), medium content of potassium, high content of phosphorus and magnesium. The temporary grassland was 5 years of age and had low yields. Grassland cover density was less than 60%. Short-lived species such as *Lolium multiflorum* and *Trifolium pratense*, sensitive to unfavourable weather conditions, had completely disappeared. The grassland had a high proportion of low-growing grass species (over 60%), a low proportion of high-growing grass species (less than 20%), legumes were less than 20%, and dicotyledonous herbs and weeds (mainly *Taraxacum officinale*) less than 5%. The species present in the grassland were species of good nutritional value; it was decided to carry out overseeding in order to improve the plant cover density and yield. The experiment had a randomised complete block design with four replications. Each plot was 50 m^2 in area. Overseeding was performed with a seeder with a disc sowing system (Vredo slot seeder) four times in 2018: early spring (13 April), after harvesting the 1st regrowth (10 June), after 2nd cut (21 August) and after 3rd cut (16 September). Seed rate of *D. glomerata* cv. Amera was 20 kg ha^{-1}. Mineral fertilization, with the following rates in kg ha^{-1}: 107 N (in three rates), 12 P (in spring) and 90 K (in two rates – in spring and after the 1st cut) were applied. The overseeding effect was assessed based on the plant development in the sowing year: number of days from sowing to emergence and tillering, number of seedlings per 1 m^2 and their growth dynamics. As well as the yield and species composition of the first re-growth, in the following year when *D. glomerata* was in the earing stage, 4 June, all treatments were cut on the same date. The feed quality was identified using the fodder value score (FVS) according to Filipek (1973). This method based on 14-point scale (from -3 to 10), the highest values (10 and 9) are given to plants that are the best in terms of utility, and the lowest (-1 to -3) to poisonous plants. For each treatment, the average FVS weighted by species yield was calculated. The results were statistically processed using the one-factor analysis of variance (ANOVA), the significance of the differences was verified with the Tuckey's multiple comparison test ($P<0.05$).

Results and discussion

The rate of initial development of *D. glomerata* depended on the date of overseeding. Spring was late and cold, the average daily temperature in March -3.3 °C and it was about 4 °C lower than the multi-annual temperature, it resulted in later than usual heating of the soil and delayed emergence. After sowing in spring, plants emerged after 20 days. But they emerged after 9-11 days after sowing in other periods (Table 1). Quick emergence in June was the result of good weather conditions; unfortunately, high temperature and lack of rainfall in the next period caused the seedlings to wither completely.

The beginning of the tillering phase occurred a week earlier after sowing in August (35 days after sowing) compared to spring and September (42 and 41 days after sowing, respectively). Four weeks after overseeding, a significantly higher number of seedlings was found on the treatment sown in August (Table 1). However, the number of seedlings after spring sowing was significantly lower than after August sowing, but higher than the number of seedlings on the treatment sown in September. Six weeks after overseeding, seedling density was lower than four weeks after overseeding. This difference was the smallest in spring by 14% and the largest in September by 25%. Favourable weather conditions after August sowing caused that *D. glomerata* seedlings grew the fastest and six weeks after sowing they were significantly higher. After evaluating the increase in dry matter yields after the renovation, it was found that the best results were obtained after overseeding in August, an increase of 44%, compared to the yield of original grassland (Table 2). Based on this finding, early spring and September dates should therefore be considered. The effectiveness of the August term is also evidenced by the largest share of *D.*

Table 1. Influence of overseeding date on growth and development of *Dactylis glomerata* seedlings 4 and 6 weeks after sowing.[1,2]

Specification	Overseeding dates			
	13 April	10 June	21 August	16 September
Hydrothermic index (∑ mm·∑ $°C^{-1}$)	0.105 very dry	0.025 extremely dry	0.304 very wet	0.087 extremely dry
Number of days from sowing to emergence	20^{a}	9^{c}	10^{bc}	11^{b}
Number of days from sowing to tillering	42^{a}	-	35^{b}	41^{a}
Number of seedlings per 1 m^{-2} 4 weeks after sowing	485^{b}	-	525^{a}	415^{c}
Number of seedlings per 1 m^{-2} 6 weeks after sowing	417^{a}	-	412^{a}	310^{b}
Height of seedlings [mm] 6 weeks after sowing	46.5^{c}	-	64.5^{a}	62.5^{b}

[1] Hydrothermic index was calculated taking into account the sum of precipitation and the sum of air temperatures in the period of two weeks before sowing and two weeks after sowing.
[2] Means in rows with different superscript letters indicate a significant difference at $P<0.05$.

Table 2. Influence of overseeding date on plant cover density, yielding and botanical composition of the first regrowth in the next year in relation to the original grassland.[1]

Specification	Original grassland	Overseeding dates			
		13 April	10 June	21 August	16 September
Yield (kg DM·ha^{-1})	$3,680^{c}$	$5,080^{b}$	$3,390^{d}$	$5,290^{a}$	$5,190^{ab}$
Plant cover density (%)	63^{c}	75^{a}	65^{c}	74^{ab}	72^{b}
Botanical composition (%):					
Dactylis glomerata L.	-	32.0	-	36.0	32.0
Phleum pratense L.	13.0	9.0	14.0	8.0	8.0
Lolium perenne L.	27.0	16.0	24.0	19.0	20.0
Poa pratensis L.	38.0	28.0	38.0	24.0	25.0
Medicago sativa L.	18.0	12.0	19.0	10.0	12.0
Herbs and weeds	4.0	3.0	5.0	3.0	3.0
FVS (Fodder value score)	9.48	9.32	9.42	9.32	9.32

[1] Means in rows with different superscript letters indicate a significant difference at $P<0.05$.

glomerata in the botanical composition of the grassland. This confirms previous reports that in this period there are usually more favourable habitat and weather conditions for seedling development, including less competition from old sward (Janicka, 2007). It should be emphasized that the value of the fodder, regardless of the date of overseeding, was very good – FVS>9.

Conclusions

The best time for overseeding of temporary grasslands is late summer (end of August) and the riskiest period is after the first cut, when there is a high risk of drought. Our result showed that the weather had a decisive influence on the effect of overseeding, especially the distribution of precipitation, which determines the availability of moisture in the soil. *Dactylis glomerata* was found to be a useful species for overseeding temporary grasslands in eastern Poland.

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Effect of mowing frequency reduction on utility value of grass-clover sward in organic cultivation

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Abstract

Temporary grasslands in organic cultivation with a high share of legume plants allow the obtaining of fodder of better quality and to have a significant positive impact on the environment. However, one problem in organic farming is the variable and unpredictable increase in the share of herbs and weeds in the sward. The aim of this research was to assess the impact of reducing the frequency of mowing on the species composition, value and yielding of meadow grassland located on a certified organic field. In 2012, a grass-clover mixture, composed of *Lolium perenne*, *Trifolium pratense* and *Trifolium repens* was sown on arable land. Until 2017, the sward was mowed three times a year, and then the number of cuts was reduced to two. The botanical composition of the sward, utilization value scores and yield were determined. Results showed that the limited mowing frequency reduced the share of sown species and increased the presence of herbs and weeds, especially *Taraxacum officinale*. Reduction of utility value of the sward and of yielding were also noted. The obtained results indicate that limited management of temporary meadow with dominance of clover after a few years may cause a gradual deterioration of sward condition under organic cultivation.

Keywords: legumes, *Lolium perenne*, mowing frequency, organic cultivation

Introduction

Methods of organic agricultural are more environmentally friendly than intensive agriculture, which depends on the use of pesticides and synthetic fertilizers in the production of crops and animals. Organic farming has positive effects on the species richness and abundance (Bengtsson *et al.*, 2005; Gomiero *et al.*, 2011). Temporary grasslands in organic cultivation with a high share of legume plants allow farmers to obtain fodder and animal products of better quality and with significantly positive environmental impact. The inclusion of legumes also reduces the requirement for nitrogen fertilizers and can improve dry matter (DM) production compared to perennial ryegrass or other grass monocultures. However, a problem in organic farming is the variable and unpredictable increase in the share of herbs and weeds in the sward, often related to weather conditions (Staniak *et al.*, 2011). The objective of the research was to analyse the influence of reduction of mowing frequency from three to two times a year on changes in the species composition, value and yield of a meadow sward.

Materials and methods

The research was conducted at the certified organic field at the Experimental Station of the Institute of Agriculture of Warsaw University of Life Sciences – SGGW (51 570 N, 20 90 E). In autumn 2012, an arable land grass-clover mixture was sown with the following composition: *Lolium perenne* L. (cv. Solen; 10.3 kg ha^{-1}), *Trifolium pratense* L. (cv. Nike; 7.0 kg ha^{-1}) and *Trifolium repens* L. (cv. Grasslands Huia; 6.3 kg ha^{-1}) in equal share (33.33%). Until 2017, the sward was mowed three times per year, and then during the next 5 years the number of cuts was reduced to two per year (2018-2022). No fertilization was applied. The botanical composition by the botanical-weight method, DM yields, and soil cover with plants were determined. The utilization value score of the sward (UVS) was also assessed according to Filipek's method (1973), where a number is assigned on a 14-point scale: from -3 (the worst utility, poisonous plants) to +10 (the best utility). The elaborated UVS of each plant species result from both the yield and feed value. The UVS of the sward was determined on the basis of the average weighted cover of individual species in the community and their number of UVS. Quality of sward can be classified as: very good (10-8.1 points), good (8.0-6.1), poor (6.0-3.1) and very poor (<3.0). The studies analysed averages from 2017 (last year of 3 cuts per year) and 2022 (last year of 2 cuts per year).The experimental data were analysed by one-way ANOVA. The significance of differences between means was determined using the Tukey HSD test at the significance level of 0.05.

Results and discussion

The studies showed changes in the botanical composition of the sward (Figure 1). Under the three-times mowing regime *L. perenne* dominated in the first regrowth (from approx. 33% in 2012, through 80% in 2015 to approx. 50% in 2017). In subsequent two regrowths, the share of this species decreased and ranged from 32% (2nd) to 28% (3rd). Legume plants (*T. pratense* and *T. repens*) dominated in the second and third regrowths (approx. 65% in total in 2017). The share of herbs and weeds was approximately 10%. *Taraxacum officinale* was the dominant herb (approximately 5%). Moreover, the favourable botanical composition of the sward in 2017 could have resulted from the sufficient amount of precipitation (535 mm during the growing season, also the highest in the research period) (Figure 2A).

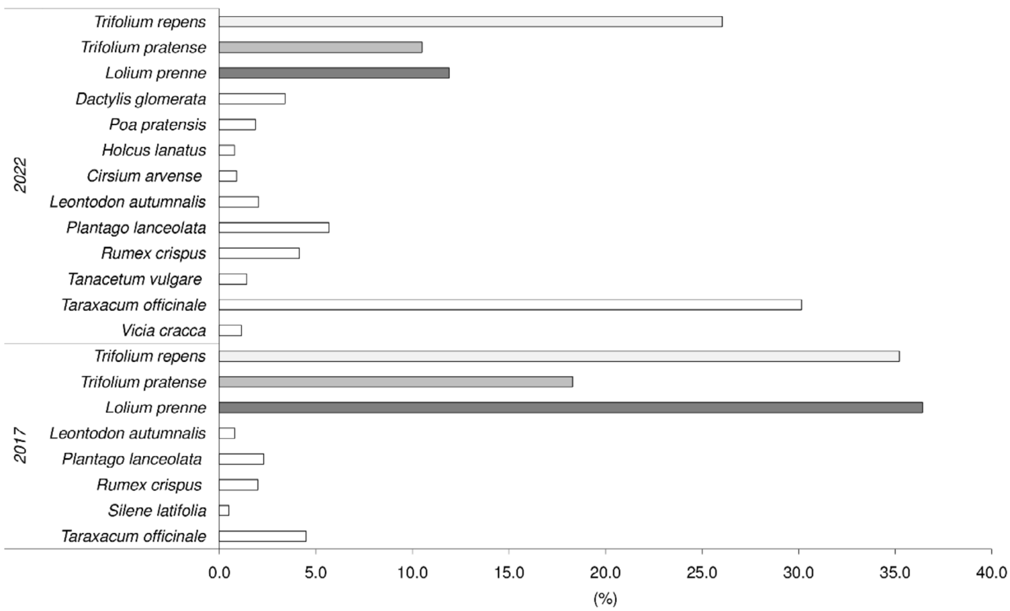

Figure 1. Floristic composition of grass-clover sward (%) in the compared years (2017, 2022) of the study (annual averages).

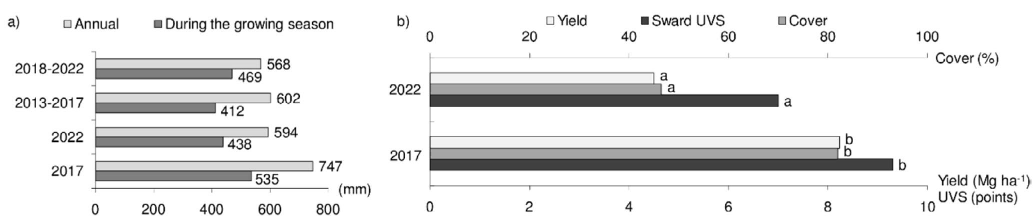

Figure 2. (A) Sum of precipitation in study period; (B) Soil cover with plants (%), annual DM yield (Mg ha^{-1}) and UVS values of the sward (points) in organic cultivation after a period of 2- (2017) and 3- (2022) cuts utilization; bars marked with the same letters do not differ significantly.

Reducing the number of mowings to two decreased the share of sown species in the sward, especially *L. perenne* and *T. pratense* (to 12 and 10%, respectively in 2022). In the studies of Gaweł (2011), the short durability of *T. pratense* was considered the reason for its rapid disappearance from the sward. *T. repens* was distinguished by a larger share in sward. According to Mastalerczuk *et al.* (2020) this species is characterized by higher durability than other sown species and tolerance to water deficiency. Simultaneously, the reduction of the share of sown species in the mixture promoted dicotyledonous invasion, especially *T. officinale* (approx. 30%) and *Plantago lanceolata* (5.7%). Among the weeds in the sward was *Rumex crispus* (4.1%). These changes were also related to the weather conditions during the growing seasons (especially repeated summer drought) and durability of sown plants.

The research showed a decrease in the UVS and the annual yield of the sward (Figure 2B). The sward UVS changed from very good (9.3 points) to good (7.0 points), while the yield decreased twice (from 8.2 to 4.5 t ha^{-1}). The increase in the share of rosette weeds (*T. officinale* and *P. lanceolata*) in the sward also decreased the degree of soil cover with plants (from 82 to 47%).

Conclusions

Limited utilization of organic meadow with a dominance of clovers, varied durability of species, as well as unfavourable weather conditions (especially lack of precipitation) caused changes in the botanical composition of the sward (an increase in the percentage of *T. officinale* and a decrease in *T. pratense* and *L. perenne*). These changes could have caused a gradual deterioration of the meadow condition, reducing the yield and the forage value of the sward.

Acknowledgements

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Effects of different P fertilizers on the forage value of clover-grass mixtures

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Abstract

The complex effects of P fertilization on the nutritive parameters of clover-grass stands are not yet understood satisfactorily. Apart from the different extent of P limitation on the various clover-grass sites, this may also be due to the diverging physiological reaction of the individual functional groups of a forage stand to P fertilization. We assume that the type of P fertilizer and the interactions of P fertilization with the type of legume also have effects on the parameters of the forage value of a mixture. This study aimed at uncovering the effects of P fertilization on the forage value of clover-grass stands. Plots of two field trials with the factors 'type of P fertilization' and 'type of leguminous mixing partner' were cut 3 times per main cropping year, sampled, analysed for the test characteristics of the nutritive forage value by near-infrared reflectance spectroscopy (NIRS). The evaluation of the influences of the factors on the quality characteristics was carried out by means of mixed linear models as well as the uncovering of the effect paths by means of structural equation modelling. The P fertilization increased the proportion of legumes in the mixtures, but this did not necessarily result in an increase in the crude protein content. The type of legume, but also the cut and the age of the stand play an important role for the expression of the feed value. The results contribute to a more comprehensive understanding of the P effects on the development of quality parameters in clover-grass mixtures.

Keywords: P fertilization, structural equation modelling, white clover, bird's-foot trefoil

Introduction

While the effects of nitrogen on the forage value of grass and clover-grass mixtures are well known (Clavin *et al.* 2017; Frame and Boyd 1987), the complex effects of P fertilization on the nutritive parameters of clover-grass stands cannot yet be explained and estimated satisfactorily. Apart from the different extent of P limitation on the various clover-grass sites, this may also be due to the diverging physiological responses of the individual functional groups of a forage stand to P fertilization (Stroia *et al.* 2010). During stand development, P fertilization can also influence the composition of the stand and thus indirectly influence the forage value. Furthermore, we assume that the type of P fertilizer and the interactions of P fertilization with the type of legume also have effects on the parameters of the forage value of a mixture. The aim of this study is to contribute to uncovering the complex effects of P fertilization on the forage value of clover-grass stands.

Materials and methods

Two 3-year field trials with the factors 'type of P fertilizer' and 'type of leguminous mixing partner' serve as the basis for our investigations. Different slow-release P fertilizers (bone char BC, TIMAC®) have been applied in addition to a 0-P control at an application rate of uniformly 40 kg P ha^{-1} in the spring of each trial year (for further details see Chapter 6.2. in Mahnke *et al.* 2017). The plots were cut 3 times per main cropping year, sampled, the samples dried at 55 °C, ground to 1 mm sieve width and analysed for the test characteristics of the nutritive forage value by near-infrared reflectance spectroscopy. The evaluation of the influences of the factors was carried out by means of mixed linear models and subsequent analysis of variance as well as the uncovering of the effect paths by means of structural equation modelling (SEM). All statistical analyses and figure creation were conducted in R (Version 4.1.1; R Core Team, 2021). The R-package 'lavaan' was used for SEM.

Results and discussion

Both the type of P fertilizer ($P<0.001$) and the legume species ($P<0.001$) had a significant influence on the proportion of legumes within the tested mixtures with identical grass partners (mix of *Lolium perenne, Festuca pratensis, Poa pratensis* and *Phleum pratense*). There was no interaction between the type of P fertilizer and the legume species ($P=0.839$, n.s.). Hence, there is no reason to assume that certain phosphorus fertilizer types are particularly suitable for individual legumes or should be rejected. Although the P fertilization demonstrably increased the legume content, this was not reflected in generally higher crude protein contents ($P=0.348$, n.s.), as is commonly postulated. While in the case of white clover there was at least a tendency for protein content to increase with increasing clover content in the 1st and 3rd growth (Figure 1), higher trefoil clover contents did not guarantee higher feed values.

While P fertilization has been shown to increase the proportion of legumes in the mixture, the soil content of plant-available P_{DL} has no such effect (Figure 2).

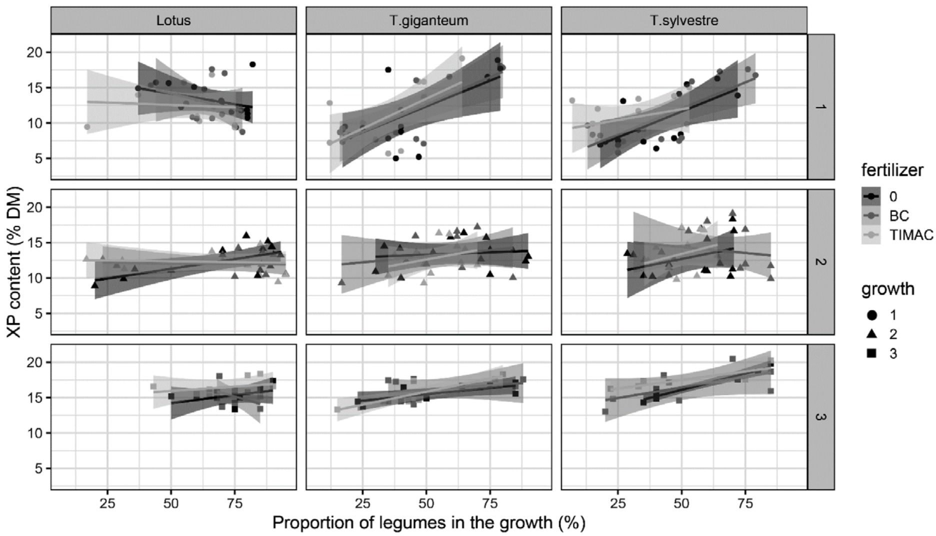

Figure 1. Relationship between legume content in the mixture and the resulting crude protein content (XP) in the three growths as a function of the leguminous mixture partner and the kind of P fertilizer.

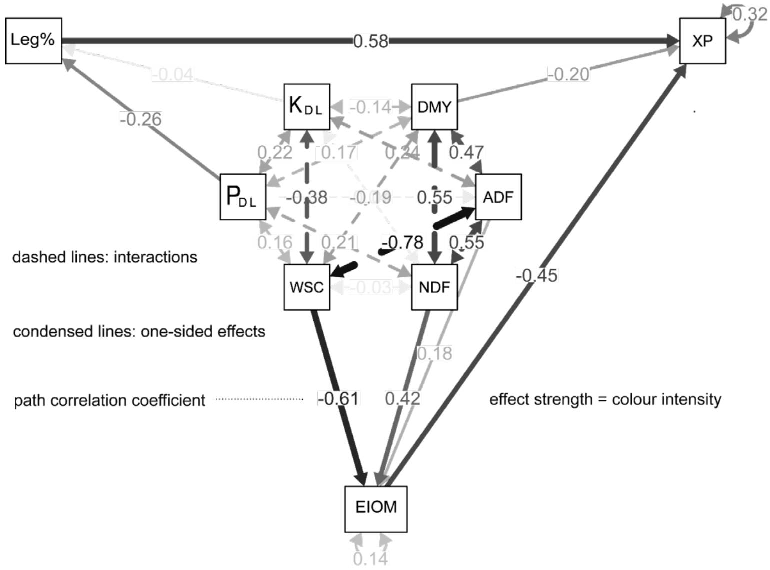

Figure 2. SEM plot of the target variable crude protein content (XP). Data originate from the organically managed site over two main cropping years. DMY = dry matter yield; EIOM = enzyme-insoluble organic matter; Leg% = legume percentage; XP = crude protein content; WSC = water soluble carbohydrates; ADF/NDF = Van Soest-fractions.

Conclusions

The effects of P fertilization on the forage quality of clover-grass mixtures are complex and mainly indirect, with legume content occupying a key position. However, the effects of the legume content cannot be generalized independently of the legume species.

Acknowledgements

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Effect of nitrogen application methods on nitrogen surplus and product carbon footprint of grassland

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Abstract

Efficient N management in agriculture is required to reduce the environmental footprint of agri-food systems to preserve biodiversity and ecological resources. Field experiments were conducted on meadows at four grassland sites across Germany with varying ecosystems to estimate the impact of different fertilization techniques on carbon footprint and nitrogen surplus. The fertilizer (135-270 kg N ha^{-1} yr^{-1}) was applied as calcium ammonium nitrate and/or untreated, acidified (target pH=6.0, adjusted with sulphuric acid) or nitrification inhibitor-treated (3,4-dimethyl pyrazole phosphate) cattle slurry. Cattle slurry was applied either using trailing shoes or by open slot injection to a depth of 5 cm in 2 to 3 splits. The product's carbon footprint was estimated by life cycle analysis. Nitrogen surplus, calculated as the difference between N applied and uptake in harvested biomass, ranged from -110-182 kg ha^{-1} yr^{-1} and was lower for mineral fertilizer than the slurry treatments in some instances. All tested slurry application technologies worked efficiently with no systematic advantages or disadvantages for climate protection. This study showed that using manure instead of mineral-fertilizer variants could reduce greenhouse gas emissions per hectare but not nitrogen surplus. However, N surpluses resulting from slurry application could be low when favourable climatic conditions occur and grasslands are productive.

Keywords: carbon footprint, trailing shoe, slurry injection, temperate

Introduction

Applied slurry on the field is associated with high losses and low nitrogen (N) use efficiency compared with chemical fertilizers, due to the high uptake of mineral fertilizers by plants (Nannen *et al.*, 2011; Nyameasem *et al.*, 2022). However, N stabilization techniques, such as slurry acidification, nitrification inhibitor (NI) use, and/or below-surface placement techniques, have been suggested to reduce N losses (Nyameasem *et al.*, 2022; Seidel *et al.*, 2017). Although the different N application/treatment methods involve variable input factors with an associated environmental cost, the environmental footprints of such techniques are seldomly investigated. Considering that only one impact factor of a technique can bring about negative consequences for other impact categories, we applied Life Cycle Assessment (LCA) to assess the effect of the different N delivery techniques on product C footprint and N surplus of temperate grassland soils.

Materials and methods

The trials were carried out on four grassland sites characterized by different soil properties and climatic conditions across Germany for two consecutive years, from spring 2019 until spring 2021, with a separate field at each location in each experimental year. Fertilizer N, ranging 135-270 (mean ± standard deviation = 168±81) kg N $ha^{-1}yr^{-1}$, was applied to the grasslands in 2-3 splits, depending on the N status of soils and local regulations. The application treatments comprised: untreated cattle slurry applied with trailing shoe (TS) or by slot injection (SI), acidified slurry applied with trailing shoe (TS +A), and NI-treated cattle slurry applied by slot injection (SI +NI). In addition, slurry application techniques were compared with a non-N-fertilized control (N0) and a mineral fertilizer treatment (calcium ammonium nitrate, CAN). The site characteristics, experimental design, greenhouse gas and NH_3 measurements, harvested biomass and N yields have been previously described (Nyameasem *et al.*, 2022). The difference between N applied and uptake in harvested biomass constituted N surplus, and the functional units considered for the LCA were 'per hectare', 'per GJ net energy lactation' and 'per dry matter'. For calculating the product C footprint (PCF), the important measured field-related GHG emissions, N_2O and NH_3, were considered (Table 1). The conversion to CO_2 equivalents (CO_2 eq.) was based on the IPCC methodological guidelines (IPCC, 2006). Mechanical field cultivation corresponded to the standard cultivation methods and working widths in north-western Europe. The CO_2 emissions per ha of the respective field operations were taken from the EcoInvent database 3.0 (Econinvent, 2016). Statistical evaluation was conducted in the programme interface (R Core Team, 2022). The data were evaluated using the R statistics interface, with the package 'nlme' in a mixed model where the application technique was considered a factor and the location and trial year as random effects. Multiple mean comparisons were made using the Tukey HSD test, and significant differences were declared at a P-value <0.05.

Results and discussion

N surplus ranged from -110-182 kg ha^{-1} yr^{-1} depending on treatment, location and experimental year and was lower (P<0.05) for mineral fertilizer than the slurry treatments but differences between the slurry application techniques were not significant (P>0.05; Figure 1A). The higher N surplus observed for slurry relative to CAN might be due to the lower N uptake efficiency of slurry N compared, as the organically bound N fraction (which makes up about 45% of total N) limits N availability (Nannen *et al.*, 2011). Higher precipitation and perennial ryegrass proportions in swards were significantly associated (P<0.05) with lower N surpluses and explained about half of the observed variations in N surplus across experimental sites and years. On the other hand, the slurry application methods had lower PCF compared with CAN, although this could only be statistically confirmed for the 'CO_2 emissions per hectare'. The determined product emissions are in the expected range (Ecoinvent, 2016; Reinsch *et al.*, 2018) for cut grassland stands. For efficiency advantages between the application techniques, the acidification method (TS+A) achieved the lowest PCF of about 0.2 t CO_2 eq ha^{-1} or 1.3 kg GJ NEL^{-1} yr^{-1} on average relative to TS. Considering that the slurry acidification method is associated with low ammonia emissions (Nyameasem *et al.*, 2022) this suggests there is a potential advantage for this method in other impact categories (e.g. eutrophication and acidification potential).

Conclusions

This study confirms that farm manure leads to greenhouse gas savings per hectare compared to mineral-fertilized treatments. All tested slurry techniques work efficiently with no systematic advantages or disadvantages for environmental or climate protection. Although N surpluses from slurry application were higher than for mineral fertilizer, low surpluses may result under favourable climatic and productive grassland conditions. In further examination, the potential of the technologies concerning their NH_3 savings must also be considered and used as a decision criterion.

Table 1. Mean (± standard error) N input, N and energy outputs per each application technique.

Treatments	N applied	NH_3-N volatilized	Direct N_2O-N	Indirect[1] N_2O-N	N yield	DM yield	ME	NEL
	kg N ha^{-1}					t ha^{-1}	GJ ha^{-1}	
N0	-	-	0.60±0.07	0.20±0.01	132.6±9.2	6.3±0.4	68.6±4.00	41.9±2.45
CAN	198±3.7	3.0±0.7	1.27±0.18	0.51±0.07	235.3±10.0	8.9±0.3	98.2±3.03	60.2±1.91
TS	203±6.4	23.6±2.8	0.94±0.16	2.6±0.28	182.0±8.6	7.7±0.3	83.7±3.30	51.0±2.03
TS+A	200±6.7	12.4±1.7	0.82±0.09	1.4±0.17	188.2±8.5	8.0±0.3	86.5±3.43	52.6±2.08
SI	203±6.4	18.3±2.3	0.97±0.14	2.0±0.23	184.9±10.2	7.9±0.3	85.4±3.40	52.2±2.08
SI+NI	203±6.4	20.0±2.5	0.92±0.13	2.2±0.25	193.3±9.7	8.1±0.3	87.7±3.41	53.6±2.07

[1] Estimated using IPCC default factor; DM = dry matter; ME = metabolizable energy; NEL = net energy of lactation.

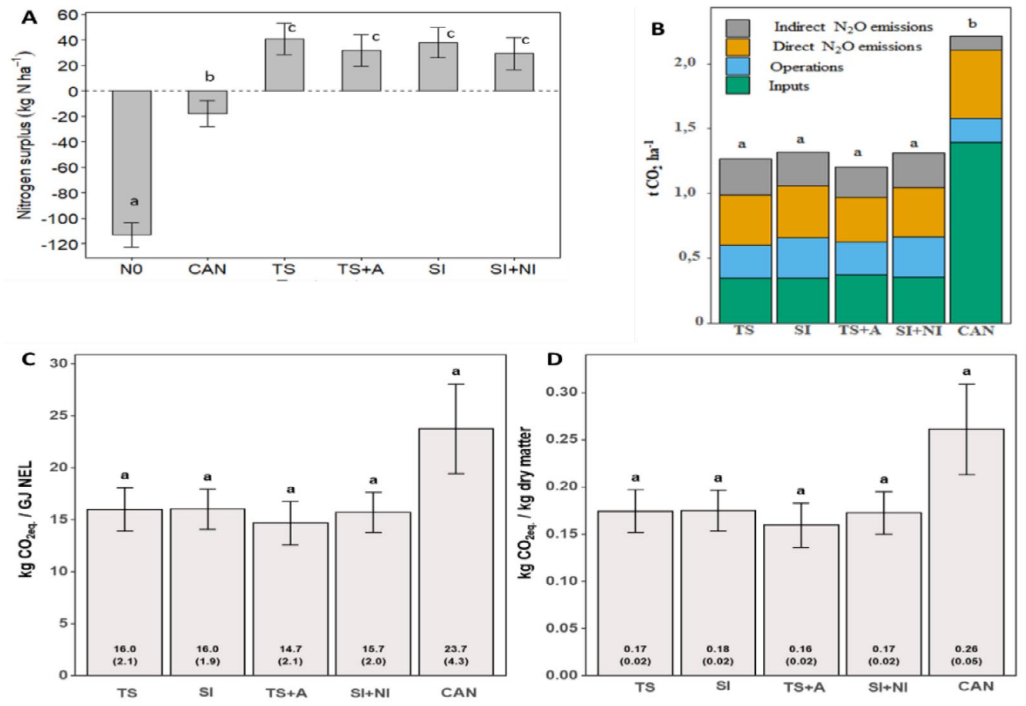

Figure 1. Bar graphs showing N annual N surplus (A), GHG emissions (B) and product GHG footprint expressed for NEL (C) and dry matter (D) for the temperate grasslands.

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Effect of the yield proportion of grasses on the drying process of forage from lucerne-based leys in a mountain environment

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Abstract

Due to recurrent drought spells during summer, the establishment of leys based on lucerne (*Medicago sativa*), which is known to be tolerant against drought, is gaining increasing interest also in mountain areas. However, legumes are known to dry more slowly than grasses in the field and this can represent a critical point concerning the field drying of forage from the growth cycles towards the end of the growing season. To this aim, lucerne is often combined with grasses in seed mixtures. Data from a four-year field experiment in a mountain environment were used to investigate the effect of the yield proportion of grasses in the sward on the drying rate of the forage and on the crumbling losses during field curing. Monocultures of three lucerne cultivars, as well as three seed mixtures combining 40% seed weight of lucerne and 60% of different grasses were compared in swards harvested four times per year, of which selected growth cycles were investigated for botanical composition and dry matter content just after mowing and after field curing. The results show that the increase in dry matter content is related to the vapour deficit sum and that the yield proportion of grasses positively affected the drying process.

Keywords: dry matter content, drying, vapor pressure deficit, *Medicago sativa*, grasses

Introduction

Lucerne (*Medicago sativa*) is known to be tolerant against drought. The recurrent drought spells increasingly affecting grassland in the mountain areas of the Alps suggest that a broader use of lucerne in leys may be advantageous, as long as the requirements of this species, especially concerning the soil pH, are met. However, legumes are expected to dry slower than grasses and this can be a critical aspect concerning field drying, especially for the growth cycles taking place in the late part of the growing season, in which the vapour pressure deficit sums are low, and the drying process progresses slowly. Moreover, the high crude protein content of lucerne forage poses a challenge to farmers' expertise if the forage is to be ensiled, and currently popular milk production specifications like haymilk prohibit including silage in cattle feed. A possible way to facilitate the field drying phase consists of using seed mixtures including grasses beside lucerne, as they are expected to have both higher initial dry matter (DM) contents as well as higher drying rates than legumes (Fundneider *et al.,* this volume). We analysed data of an experiment conducted in the past with the aim to quantify the effect of the yield proportion of grasses on the drying advance of leys based on lucerne.

Materials and methods

In order to investigate the effect of the botanical composition and of the weather conditions on the drying process of leys, we analysed the data of a field trial conducted over four years (2000 to 2003) in a mountain environment in the Alps (5187428 N, 725611 E; 892 m a.s.l. altitude, 7.1% slope, SW exposition, Teodone/Dietenheim, South Tyrol, NE Italy). In this experiment, six seed mixtures (SM) were investigated, three of them being monospecific with one cultivar each of *M. sativa* (EU = Europe/4C = 4-Cascine/SE = Selene) and the other three being a mixture of 40% seed weight of the lucerne cv. Europe in combination with 60% grasses (DP = 40% *Dactylis glomerata* Amba + 20% *Phleum pratense* Toro; DG = 60% *D. glomerata* Amba; FA = 60% *Festuca arundinacea* Barcel). A seed rate of 40 kg ha^{-1} was used. The trial was laid out as a randomized complete block design with four replicates and a plot size of 28.8 m^2. For three growth cycles (2001_2 = 2^{nd} cut in 2001, 2002_2 = 2^{nd} cut in 2022 and 2003_1 = 1st cut in 2003), the yield proportion of the functional groups grasses, legumes and forbs was visually estimated in each plot prior to mowing and forage samples were taken just after mowing and at the end of field drying phase. Until harvest, the mown biomass was manually turned with a fork at least once daily. For 2001_2, samples were additionally taken on the day before the end of the field drying phase. The DM content was determined for each forage sample by drying them at 60°C until weight constancy. The difference of DM content with reference to that of the mowing event was used as a measure of the drying attitude. Weather data were obtained from a weather station at the experimental field and the cumulated vapour pressure deficit was computed according to Allen *et al.* (1998). In case of precipitation during the field-drying phase, the rewetting effect resulting in a water content increase according to Gupta *et al.* (1989) was taken into account in computing the difference in DM content. The effect of the seed mixture and of the growth cycle, as well as of their interaction, on the yield proportion of grasses and legumes was investigated by means of linear mixed models. Measurements within the same plot over time were accounted for by means of repeated measures and the covariance structure was chosen using the Akaike Information Criterion (AIC). Multiple comparisons were performed by LSD test. The DM difference between mowing and sampling was analysed according to that described above, but the model additionally accounted for the respective vapour pressure deficit sum, the yield proportion of grasses at the time of mowing and the seed mixture, representing the effect of the lucerne cultivars used in the first three treatments and the grass species combined with lucerne in the three other seed mixtures. The adjustment of the polynomial degree of the metric variables, as well as the inclusion of interaction terms between the seed mixture and the metric variables were forward stepwise tested using AIC as an indicator of fit. ANOVA assumptions were checked by means of diagnostic plots using residuals and predicted values.

Results and discussion

Both the yield proportion of grasses and that of legumes were affected by the seed mixture, by the growth cycle and by their interaction (all $P<0.001$). The use of monospecific seed mixtures with lucerne alone resulted in very high legume proportions, which ranged approximately between 80 and 90% in the fourth observation year (Table 1). The yield proportion of unsown grasses at the end of the same period did not exceed 11%.

For the seed mixtures combining lucerne with grasses, the yield proportion of grasses decreased over time from 45 to 61% in 2001 to 6 to 20% in 2002, and to 17 to 25% in 2003 depending on the seed mixture, with the seed mixture including *F. arundinacea* (FA) exhibiting lower grass proportions than the other

Table 1. Effect of the cultivar of *M. sativa* (Eu = Europe, 4C = 4-Cascine, SE = Selene) or of the partner grasses (FA = *Festuca arundinacea*, DP = *Dactylis glomerata* + *Phleum pratense*, DG = *Dactylis glomerata*) included in the seed mixture on the yield proportion of the functional groups grasses and legumes in the investigated growth cycles.[1]

Seed mixture	Seed weight proportion of *M. sativa* (%)	Grasses (%)			Legumes (%)		
		Growth cycle			Growth cycle		
		2001_2	2002_2	2003_1	2001_2	2002_2	2003_1
EU	100	0.0^{c}	3.3^{b}	6.5^{c}	99.0^{a}	91.3^{a}	83.8^{a}
4C	100	0.0^{c}	3.2^{b}	5.0^{c}	99.0^{a}	87.8^{ab}	89.5^{a}
SE	100	5.0^{c}	7.7^{b}	10.8^{bc}	94.0^{a}	82.8^{bc}	81.3^{ab}
FA	40	45.0^{b}	5.8^{b}	17.5^{b}	53.0^{b}	87.0^{ab}	70.0^{bc}
DP	40	57.3^{a}	16.0^{a}	28.8^{a}	40.8^{c}	76.0^{cd}	58.8^{c}
DG	40	61.3^{a}	20.5^{a}	25.0^{a}	36.5^{c}	71.5^{d}	61.3^{c}

[1] Estimated marginal means sharing no common superscript letter within each growth cycle do not significantly differ from each other.

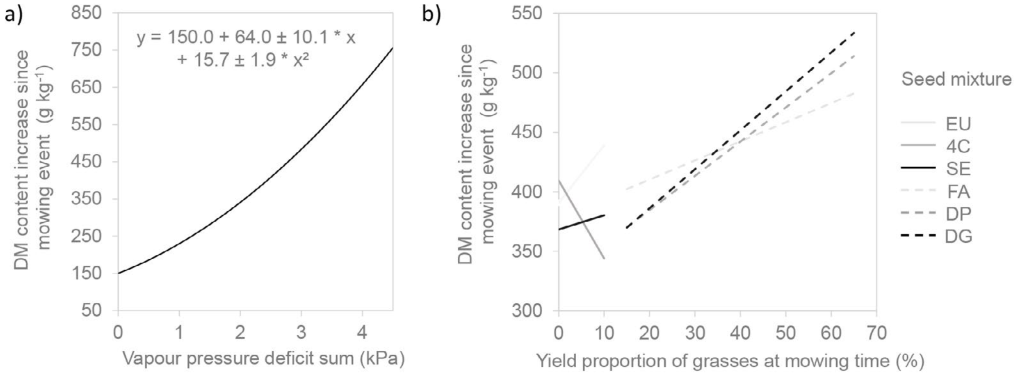

Figure 1. Predicted values of the increase in DM content since the time of mowing depending (A) on the vapour pressure deficit sum (taking also rewetting into account) and (B) on the interaction between the yield proportion of grasses at the mowing time and the cultivar of *M. sativa* or of the partner grasses in the seed mixture. Eu = Europe, 4C = 4-Cascine, SE = Selene) or of the partner grasses (FA = *Festuca arundinacea*, DP = *Dactylis glomerata* + *Phleum pratense*, DG = *Dactylis glomerata*). Predicted values shown refer to the observed range of the yield proportion of grasses for monospecific seed mixtures and those including grass species.

mixtures. This was compensated by higher legume yield proportions, which ranged between about 60 and 70% at the end of the observation period.

The final statistical predictive model accounted for the seed mixture (F=15.6, P<0.001), a second-degree polynomial for the vapour pressure deficit sum (F=39.9, P<0.001 for the linear term; F=68.7, P<0.001 for the quadratic term), the yield proportion of grasses at the time of mowing (F=5.8, P=0.025) and its interaction with the seed mixture (F=7.4, P<0.001). This confirms the already known primary role of the vapour pressure deficit in affecting the drying process in the field (Figure 1). Concerning the seed mixtures containing only lucerne and resulting in a very small proportion of unsown grasses along the whole investigation period, no clear general effect was observed, with a contrasting trend of the estimated parameter of the interaction term for EU (increasing by 3.2±1.9) and 4C (decreasing by -8.2±2.3). For the mixtures including grasses, a consistent positive effect of the grass proportion (ranging between 15 and 65%) was found, with an increase of DM content of 1.6 (for FA) to 3.3 g kg^{-1} for DP.

Conclusions

If no barn drying system is available and the expected reduction in potential forage quality due to the grasses is acceptable, including grasses in seed mixtures with lucerne allows to improve the potential for a quicker progress of the drying process in the field.

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Using innovative legume-based mixtures as cover crops in a multi-functional olive system

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Abstract

The main objective of this experiment is to provide useful indications on sustainable management of multi-functional olive systems under Mediterranean rainfed conditions by analysing the effects of different soil management on olive trees. Two field trials were established in 2022 in Italy and Lebanon, using a randomised block design and comparing four treatments: (1) cover crop with innovative mixture (IM); (2) cover crop with commercial mixture (CM); (3) natural cover + fertilization (NC); (4) traditional soil tillage + fertilization (ST). The preliminary results referred to the first year showed a better establishment of NC compared to both mixtures, regardless of whether it is below or outside the tree canopy. The higher grass-based biomass produced by NC below the tree canopy may have influenced the competition for available soil water between the sward and tree components. Regarding the water potential of olive trees, during the period of greatest water deficit, ST showed lower levels of water potential and NC showed a significantly lower photochemical efficiency. Moreover, NC promotes a smaller fruit size while no significant differences emerged between the other treatments.

Keywords: cover crop, soil management, olive grove, Mediterranean environment

Introduction

Among different soil management techniques with a low environmental impact, an important role is given to cover crops, not only for production purposes but also to ensure soil fertility year after year (Mercenaro *et al.*, 2014). Temporary or permanent cover crops can improve rhizosphere conditions and tree mineral nutrition, increase soil bearing capacity, reduce soil erosion and N-leaching, etc. (Arias-Giraldo *et al.*, 2021). However, cover cropping may cause a reduction in water availability for trees, with potential negative effects on yields, particularly under dry conditions in Mediterranean environments. On the other hand, cover cropping can provide additional forage production for sheep or horse grazing. The main objective of this experiment is to provide useful indications for the sustainable management of multi-functional olive systems under Mediterranean rainfall conditions by analysing the effects of cover crops on olive groves.

Materials and methods

Two field trials were established in January 2022 in Sardinia (Italy) and Lebanon, using a randomised block experimental design with three replications and plot of 1000 m^2. Four treatments were compared: (1) cover crop with sowing of innovative forage legume-based mixture (IM) (*Medicago polymorpha*, *Trifolium brachycalycinum*, *T. yanninicum*, *Lolium rigidum*); (2) cover crop with sowing of commercial complex forage mixture (CM) (*Dactylis glomerata, Festuca rubra, L. multiflorum, L. perenne, M. polymorpha, Ornithopus sativus, T. incarnatum, T. michelianum, T. resupinatum, T, subterraneum, T. yanninicum*); (3) natural cover + fertilization (NC); (4) traditional soil tillage + fertilization (ST), as reference treatment. Before sowing, we carried out a minimum soil tillage followed by fertilization (30 kg P ha^{-1}). The data collection conducted on the grassland sward, below and outside the tree canopy, considered the following parameters: soil cover percentage and seedling establishment in the first year (sampling area 25×25 cm), dry matter yield (DMY) and its botanical composition (sampling area 100×50 cm). Only one cutting date was applied at a height of 5 cm, at the end of May 2022. The effects on physiological response of olive trees were determined by monitoring: (1) water status as stem water potential (SWP), monitored through the Pressure bomb; (2) photosynthesis efficiency (Fv/Fm ratio); (3) olive production. One-way ANOVA and Tukey's test were applied to test differences between treatments for each variable (P-value <0.05). In this contribution only the results of the trial conducted in Sardinia are presented and discussed.

Results and discussion

Concerning the sward component, a higher percentage of soil cover and a higher presence of legume below the tree canopy than outside, during the establishment phase, were observed in CM (15 vs 10% and 5 vs 1%, respectively). Furthermore, IM showed a higher DMY below than outside the tree canopy 0.64 vs 0.41 Mg ha^{-1}, respectively). These results can be explained by the higher soil moisture conditions below the tree canopy. Outside the tree canopy, NC showed better establishment than both mixtures, with a higher number of seedlings m^{-2} and greater soil cover; furthermore, in NC we observed a significantly higher presence of grasses and other non-legume species (Table 1A). In both mixtures we observed a significantly greater presence of legume species. Below the tree canopy, NC showed similar performance; however, IM showed a higher presence of legume and lower presence of grasses than CM. Regarding DMY, in the NC plots outside the tree canopy we observed a lower biomass moisture compared to both mixtures and a higher contribution of grasses compared to CM (Table 1B). Below the tree canopy, NC showed higher DMY than the other two cover crops, with lower biomass moisture, higher contribution of grasses and lower contribution of other non-legume species.

Table 1. Comparison of the establishment parameters (A) and comparison of the dry matter yield (DMY) (B) between the three cover crop treatments in the first year of the experiment.[1]

A					
Treatment	**Soil cover (%)**	**N. seedlings m^{-2}**	**Grass (%)**	**Legume (%)**	**Other species (%)**
Outside the tree canopy					
Innovative mix	17^{b}	720^{b}	25^{b}	74^{a}	1^{b}
Commercial mix	10^{b}	652^{b}	32^{b}	67^{a}	1^{b}
Natural cover	86^{a}	2,305^{a}	86^{a}	3^{b}	11^{a}
Below the tree canopy					
Innovative mix	14^{b}	334^{b}	22^{c}	74^{a}	5
Commercial mix	15^{b}	496^{b}	43^{b}	53^{b}	5
Natural cover	84^{a}	2,443^{a}	78^{a}	0^{c}	22

B					
Treatment	**Yield (Mg DM ha^{-1})**	**Moisture (%)**	**Grass (%)**	**Legume (%)**	**Other species (%)**
Outside the tree canopy					
Innovative mix	0.41	25^{b}	36^{ab}	36	28
Commercial mix	0.45	25^{b}	5^{b}	51	44
Natural cover	1.09	41^{a}	91^{a}	0	9
Below the tree canopy					
Innovative mix	0.64^{b}	27^{b}	6^{b}	22	71^{a}
Commercial mix	0.38^{b}	25^{b}	14^{b}	26	60^{a}
Natural cover	1.07^{a}	40^{a}	81^{a}	0	19^{b}

[1] Different letters indicate significantly different means in different treatments (P<0.05).

With regard to the eco-physiological response of olive plants, SWP showed a similar trend among the four treatments under observation (Figure 1A). From June to the first decade of September, olive trees showed a gradual increase in SWP, in response to reduction in soil water content. Only in August and until the rainy event in late September, the SWP of ST showed statistically lower values than the other cover crop treatments. Moreover, Fv/Fm ratios in all treatments dropped below 0.70, suggesting significant photo-inhibition (Figure 1B), also conceivable by leaves showed occasionally high temperature values in the season (data not shown). During the driest and hottest period of the season, the olive plants subjected to ST showed the highest photosynthetic efficiencies. Even if only at one sampling date, NC promoted a greater decrease in photosynthetic efficiency. With regard to yield, the average weight of the olive in NC was statistically lower than IM, CM and ST (1.68 g, 1.97 g, 2.01 g and 2.18 g, respectively).

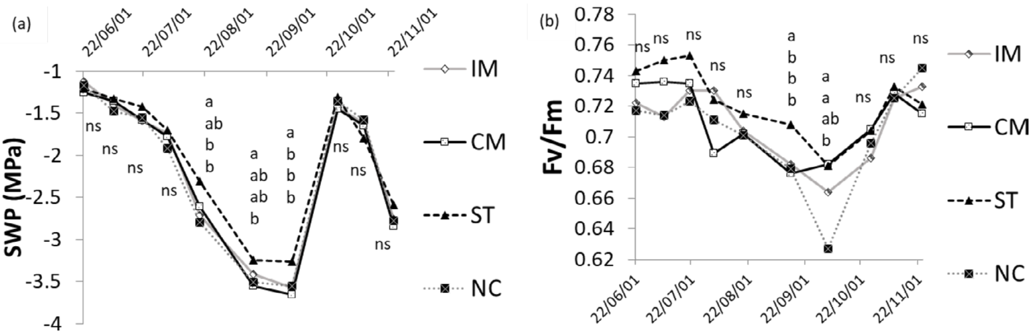

Figure 1. Stem Water Potential (SWP, megapascal = MPa) (A) and photosynthetic efficiency (Fv/Fm) (B) under four different soil management treatments (IM = innovative mixture; CM = commercial mixture; ST = soil tillage; NC = natural cover) during the study period (10 sampling dates). Different letters indicate significantly different means; ns = no significant difference (*P*<0.05).

Conclusions

The preliminary results of our study showed a better establishment of the natural cover compared to both mixtures, regardless of whether it is below or outside the tree canopy. The higher DMY produced by NC may have influenced the competition for available soil water between the sward and tree components. At least as far as yield aspects are concerned, the use of natural cover is not recommended in Mediterranean environments where irrigation cannot be used. It will be very important to monitor the long-term effects on competition for water, nitrogen and at the same time the provision of ecosystem services.

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Yield of drought-resistant summer leys under extreme climatic conditions

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Abstract

Climate change is putting a strain on forage production in Switzerland and large parts of Europe, in particular due to increasing climatic extremes, such as periods of drought alternating with heavy rainfall. In this study, five different drought-resistant summer leys were tested on three farms in the Swiss Jura: (1) Sudan grass (*Sorghum sudanense*); (2) hybrid sorghum (*Sorghum bicolor* × *Sorghum sudanense*) – Persian clover (*Trifolium resupinatum*) – Egyptian clover (*Trifolium alexandrinum*); (3) foxtail millet (*Setaria italica*); (4) pearl millet (*Pennisetum glaucum*); (5) black oat (*Avena strigosa*) – Crimson clover (*Trifolium incarnatum*); and compared with a commonly used ley (6) oat (*Avena sativa*) – pea (*Pisum sativum*) – common vetch (*Vicia sativa*). The trial was carried out during the summers of 2021 (characterized by heavy rainfall in July) and 2022 (characterized by drought in July and extreme temperatures in July and August). The yields and the costs of the six leys were assessed. The yield was significantly different among leys (P<0.001), while there were no significant differences between years. Overall, the leys providing the highest biomass production were (2) and (3), with 2.8±0.2 and 2.9±2.1 Mg DM ha^{-1}. Our results show that there is a range of summer leys that can be sown to produce forage as an alternative to (6), at comparable or often lower costs. Their nutritional quality will be analysed to assess possible adaptation strategies to climate change.

Keywords: climate change, drought, forage, millet, Sudan grass, sorghum

Introduction

One of the most important trends related to climate change is the increase in the frequency and intensity of extreme periods, such as severe droughts alternating with heavy rainfalls (Hänsel *et al.,* 2022). This phenomenon is putting a strain on forage production, with higher yield variability, especially during summer periods, which are increasingly characterised by drought and extreme temperatures. For this reason, the adoption of drought-resistant summer leys, sown after crop harvest, is considered as an adaptation strategy (Lambert *et al.,* 2020). In recent years, for example, the area sown with hybrid sorghum has increased in Switzerland (i.e. 300 ha in 2020) and Central Europe, as this forage species has lower water requirements and can withstand high temperatures (Maulana and Tesso, 2013). Moreover, other species, such as Sudan grass, Pearl Millet, Foxtail Millet, and Crimson clover can be well adapted to dry and hot climates (Lambert *et al.,* 2020). However, little is known about how these species respond under extreme and contrasting summer climatic conditions.

In this study, the yield of five different drought-resistant summer leys was assessed and compared during two climatically different years (2021 and 2022): (1) Sudan grass; (2) hybrid sorghum – Persian clover – Egyptian clover; (3) foxtail millet; (4) pearl millet; (5) black oat – Crimson clover; and compared with a commonly used ley (considered as control treatment), i.e. (6) oat – pea – common vetch.

Materials and methods

The study was conducted at three different farms in the Canton of Jura, Switzerland. They were located at 400-500 m a.s.l. in the municipalities of Courrendlin, Fontenais and Grandfontaine, respectively. The soils were sandy, loamy and clayey-loamy, from 45 to 60 cm deep, with a pH between 6.8 and 7.9, and organic matter between 4.3 and 9.1%. The mean annual precipitation and temperature in the region are 961 mm and 9.5 °C, respectively (1991-2020 data, average from the stations of Delémont and Fahy, www.meteosuisse.admin.ch).

At each farm, six drought-resistant summer leys were sown in July 2021 and 2022 (Table 1). The cultivars used were Piper (Sudan grass), Pacific Graze (hybrid Sorghum), Rusty (Persian clover), Blue gold (Egyptian clover), Rucerus (foxtail millet), Alberto (pearl millet), Cadence (black oat), and Contea (Crimson clover). The leys were sown in a completely randomized block design, within 40-60 × 5-6 m strips, with three replicates in each farm. The leys were sown after barley harvest and the sowing was carried out between 23 and 30 July in 2021 and between 7 and 13 July in 2022. Leys were fertilized a few days before sowing with 25-35 kg N ha^{-1} (25-35 m^3 ha^{-1} of slurry applied to all leys in both years) and in 2021 with 30-40 kg N ha^{-1} a month later (the mineral fertilization was not applied in 2022, due to the extreme dry conditions). The leys were harvested between 8 and 11 October 2021 and on 22 September 2022. Yields were measured with a motor mower in 2021 (within three areas of 3.3 m^2 per strip) and with a Haldrup harvester (within three areas of 7.5 m^2 per strip) in 2022. A sample of about 250 grams was taken at each measurement, weighed and dried for 72 hours in an oven at 60 °C to measure the dry matter content. The interactive effects of ley type and year were analysed using ANOVA, with site as error term, followed by Tukey test.

Results and discussion

The climatic conditions during the summers of 2021 and 2022 were very different. Indeed, July 2021 was characterized by extreme rainfalls, with an average of 300 mm, while the average precipitation in this month is 93.5 mm (1991-2020 data, average from the stations of Delémont and Fahy, www.meteosuisse.admin.ch). Contrarily, July 2022 was characterized by a severe drought, with just 35 mm of rainfall. The month of August was drier than average in both years, while the month of September was drier than average in 2021 and wetter in 2022. Moreover, the months of July and August were very different in terms of average temperatures, with 17.1 °C and 20.3 °C in 2021 and 2022 respectively, which corresponded to -1.2 and + 2 °C differences compared to the 1991-2020 average.

The yield was significantly different among leys, whereas there were no significant differences between years (Figure 1). The treatments providing the highest yields were foxtail millet and hybrid Sorghum, Persian clover, and Egyptian clover, with 2.9±2.1 and 2.8±0.2 Mg dry matter (DM) ha^{-1}, while the treatments producing less biomass were pearl millet and black oat and Crimson cover. The Sudan grass and the control treatment produced intermediate biomass levels and were not significantly different from the others.

A study from western Switzerland found comparable data for the hybrid Sorghum (Hayking cultivar), with yields ranging from 2.51 to 3.18 Mg DM ha^{-1}, under water-limited and not-water limited conditions, respectively (Mosimann *et al.,* 2013). However, under this study the Sorghum was cut twice, whereas

Table 1. Sowing characteristics and costs of the six summer leys tested.

Treatments	Seed density (kg ha^{-1})	Seed depth; interrow (cm)	Seed costs; sowing and rolling costs (CHF ha^{-1})[1]	Total costs (CHF ha^{-1})[1,2]
1. Sudan grass	15	2; 25	81; 329	746
2. Hybrid sorghum (Hs)-Persian clover (Pc)-Egyptian clover (Ec)	20 (Hs)-8 (Pc)-12 (Ec)	2; 25 (Hs) – 0.5; 0 (Pc, Ec)	228; 426	990
3. Foxtail millet	25	1; 12.5	120; 329	785
4. Pearl millet	25	1; 12.5	190; 329	855
5. Black oat (Bo)-Crimson clover (Cc)	80 (64 Bo: 16 Cc)	1; 12.5	237; 329	902
6. Oat (O)-pea (P)-common vetch (Cv)	175 (100 O-40 P-35 Cv)	2; 12.5	262; 329	927

[1] Costs have been calculated according to the Memento Agricole (Agridea, 2021).

[2] Total costs include 220 and 116 CHF ha^{-1} for slurry application and soil tillage, respectively.

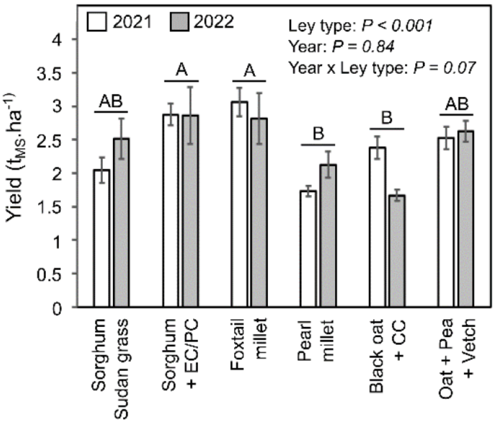

Figure 1. Yield of the summer leys tested in both years. Different letters indicate significantly different means ($P<0.05$). EC, PC, CC indicate Egyptian Clover, Persian Clover, and Crimson Clover, respectively.

in our study just once. In the same region, Mosimann *et al.* (2017) measured in 2015 very comparable data for the oat-pea-common vetch ley, while lower yields for hybrid Sorghum and foxtail millet (with mean values lower than 2 Mg ha^{-1}). In France, Meslier *et al.* (2014) found much higher yield values for pearl millet (4.8 Mg ha^{-1}) and foxtail millet (5.6 Mg ha^{-1}), but under good climatic conditions that did not cause stress to the plants. Finally, all treatments had lower costs than the control treatment, with the exception of the hybrid Sorghum, Persian clover, and Egyptian clover treatment.

Conclusions

The six summer leys tested in this experiment provided stable yields, even under extreme and contrasting climatic conditions. In particular, the foxtail millet and the hybrid Sorghum, Persian clover, and Egyptian clover leys provided the highest biomass production and they can be considered as a valid alternative to the leys commonly used in the region. Future analyses on their nutritive quality will provide useful information to assess possible adaptation strategies for forage production to climate change.

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Optimization of ley seed mixtures in a mountain environment concerning forage yield

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Abstract

In the mountain areas of South Tyrol (NE Italy) there is a shortage of arable fields and silage maize is often cultivated as a monoculture. Although maize is a self-tolerant crop, crop rotation would be advisable to reduce the pressure of arable crops weeds, to increase soil fertility and to prevent the spreading of maize rootworm. Grass-clover leys would be well suited as rotation crops in such intensive production areas. The optimization of seed mixtures for grass-clover leys in mountain areas was studied by simultaneously investigating the effect of three regionally recommended grass-clover mixtures, three red clover (*Trifolium pratense*) cultivars and the replacement in the seed mixtures of *Festuca pratensis* by *Festulolium* on the forage yield. The experiment was conducted over a period of three years with four cuts per season as randomized complete block design with three replicates. Our results show that testing red clover cultivars in specific target seed mixtures seems to be a relevant approach to get accurate results about their performance and that greater differences between cultivars are mainly expected in the first harvest years.

Keywords: grass-clover leys, crop rotation, red clover cultivar, *Festulolium*, meadow fescue

Introduction

Grass-clover leys provide high-protein feed due to their high proportion of clover in the seed mixture. This is even more relevant in the current times with rising prices for concentrates. When integrated into the crop rotation with silage maize, grass-clover leys would be beneficial for reducing the pressure of arable crop weeds, for increasing soil fertility and as the most effective preventive measure against the spread of maize rootworm (*Diabrotica virgifera virgifera*). The aim of this study was the optimization of seed mixtures for grass-clover leys in mountain areas concerning the forage dry matter yield (DMY), by simultaneously addressing three different aspects: (1) the suitability of different regionally recommended seed mixtures; (2) the use of different red clover (*Trifolium pratense*) cultivars having previously proved their suitability in monospecific cultivar test trials; and (3) the replacement in the seed mixtures of meadow fescue (*Festuca pratensis*, cv. Pardus) by *Festulolium* (× *Festulolium braunii*, cv. Hostyn).

Materials and methods

The field experiment was established in Dietenheim/Bruneck (South Tyrol, NE Italy, 46° 48' 8.064' N, 11° 57' 23.908' E, 891 m a.s.l.) in the late summer of 2019. The three-factorial experiment was designed as a randomized complete block design with three replicates, with a plot size of 1.2×7.2 m. The investigated factors were: (1) the grass-clover mixture (WW/IR/KG), differing, among others, in the proportion of red clover in the seed mixture (5%, 13.3% and 27.3% respectively) (Krautzer *et al.*, 2020); (2) the red clover cultivar used in the seed mixtures (Semperina/Spurt/Milvus); and (3) the replacement of *F. pratensis* in the original seed mixtures by *Festulolium*. The experiment was fertilized with mineral fertilizers to supply 40 kg N ha^{-1} and 60 kg K ha^{-1} in spring 2020, and then with 15 m^3 ha^{-1} of digested cow slurry after the first, second and third harvest in 2020 and 2021, and also in spring and after the first and second cut in 2022. Four cuts per season were performed. The yield proportion of red clover was determined in at least one third of the plots by manual sorting of 0.25 m^2-forage subsamples obtained within randomly placed metal frames and harvested by means of electric scissors, and dried at 60 °C until weight constancy after separation. The remaining plots were visually estimated using the results of manual sorting as a reference. The harvest was done at a stubble height of 5 cm with a forage harvester

(Haldrup F-55, HALDRUP GmbH, Ilshofen). Fresh subsamples of about 500 g were taken for each plot, weighed and dried at 60 °C until weight constancy to determine the dry matter content and the DMY. The annual DMY from 2020 to 2022 was analysed by a mixed model accounting for the seed mixture, the red clover cultivar, the replacement by *Festulolium*, the year and their interactions, all treated as fixed terms, whilst the block was treated as a random factor. Yield values observed in the same plot over years were treated as repeated measurements with a first-order autoregressive covariance structure. Multiple comparisons of estimated marginal means were performed at the highest interaction level by LSD. The significance level was set at P=0.05.

Results and discussion

We detected no significant main effect of seed mixture (SM), red clover cultivar (RC) and replacement of *F. pratensis* by *Festulolium* (FR), but there were a significant two-way interaction of RC × FR (F=4.8, P=0.014), a large effect of the year (YR) (F=38.4, P<0.001) as well as two two-way interactions of YR with SM (F=4.2, P=0.004) and with RC (F=7.3, P<0.001). Finally, a significant effect of the four-way interaction YR × SM × FR × RC (F=2.504, P=0.019) was found. Differences depending on the red clover cultivar occurred in the first and second harvest years, but these were no longer evident in the last year of the experiment (Figure 1). It was apparent that a yield improvement was mainly obtained by Spurt or Milvus, depending on the specific combination of seed mixture and the replacement of *F. pratensis* by *Festulolium*. This suggests that testing cultivars in specific seed mixtures would be a useful approach, as Semperina had been found to be the best yielding cultivar in the previous monospecific cultivar test trial at the same site. The decreasing occurrence of differences related to the red clover cultivar over time is probably due to the declining yield proportion of red clover on the total DMY over time, which decreased on average over the cultivars from 57.8 to 54.0 and 15.4% for KG, from 45.1 to 50.6 and

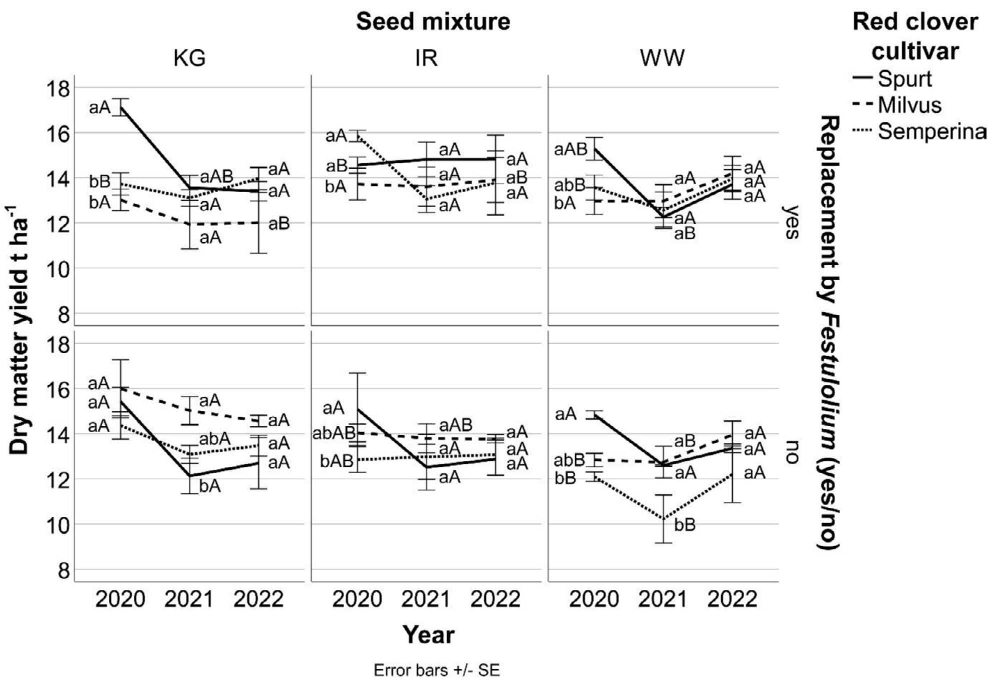

Figure 1. Mean annual dry matter yield depending on seed mixture, red clover cultivar and the replacement of meadow fescue by *Festulolium* over the years. Means of different red clover cultivars within year, seed mixture and replacement by *Festulolium* sharing no lower-case letter significantly differ from each other. Means of different seed mixtures within year, red clover cultivar and replacement by *Festulolium* sharing no upper-case letter significantly differ from each other. SE = standard error

11.8% for IR, and from 27.1 to 26.5 and 7.9% for WW. Differences between seed mixtures occurred as well mainly in the first two harvest years, with KG and IR providing in general higher yields then WW, depending on the red clover cultivars. Differently from that observed for a single growth cycle in the same experiment (Gamper *et al.*, 2022), testing of cultivars needs to be done in specific target seed mixtures, depending also on the duration of the sward. A replacement of meadow fescue by *Festulolium* was shown to be beneficial in terms of yield for some red clover cultivar x seed mixture-combinations, although no consistent advantage was shown under the given experimental conditions. The DMY obtained from the tested seed mixtures over the whole experiment duration averaged 13.1 (WW) and 13.8 t ha^{-1} yr^{-1} (KG and IR) and was therefore at a satisfactory, high level for all seed mixtures.

Conclusions

Testing of red clover cultivars in specific target seed mixtures for the establishment of leys in a mountain environment seems to be a relevant approach to get accurate results about their performance, especially concerning the first harvest years. Due to the inconsistent advantage provided by the replacement of *F. pratensis* by *Festulolium*, the application of this approach to the investigated seed mixtures seems not to be generally advisable under the given experimental conditions.

Acknowledgements

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The effect of botanical composition and its dynamics on yield and persistence of mixtures

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Abstract

Multispecies grasslands are available for a variety of climatic environments and for different purposes: grazing, silage and hay. This study aimed at quantifying the effect of species diversity and their compatibility on yield and persistence of mixtures over the season as well as ability to mitigate effects of climate change. Fourteen forage crop species – sainfoin, lucerne, alsike clover, red clover, tall fescue, red fescue, festulolium, Italian ryegrass, perennial ryegrass, meadow foxtail, red top, tall oatgrass, timothy and cocksfoot – were used to compose five different trinary-nonary mixtures. Grass:legume ratio dynamics and productivity of the mixtures were evaluated over the season and the mixture containing tall fescue produced the highest total dry matter yield (11.65 kg m^{-2}). Mixtures with cocksfoot were very productive in terms of first harvest, 6.23 and 5.76 kg m^{-2} respectively; however, summer drought determined productivity decrease and differences between these mixtures. The results obtained suggest that cultivars play an important role in mixtures composition and their productivity. Moreover, soil water deficit was the main factor affecting plant persistence, resulting in sward thinning for all mixtures and subsequent weed growth.

Keywords: legumes, grasses, multi-species, biomass

Introduction

Swards can reduce reliance on agrochemical inputs and increase agrobiodiversity and thus help to implement the EU Green Deal's 'Biodiversity' and 'Farm to Fork' strategy aiming at reducing fertilization by 20%, using 25% of agricultural land for organic farming, strengthening biodiversity-friendly farming practices, returning pollinators to agricultural land, etc. Sward seed mixtures are available for a variety of climatic environments and for different purposes: grazing, silage and hay. They are usually composed on the basis of knowledge about adaptation, performance and persistence of individual species and cultivars in pure stands. However, there is little information on the diversity effects in specific mixtures in specific environments (Helgadóttir *et al.*, 2018). It is often difficult to achieve the desired protein content in livestock diets with grass-based forages.

Forage grasses and their mixtures have been investigated in Lithuania for decades; however, the vast majority of research projects lacking the systemic view of species interaction in the mixtures, sward productivity and ecosystem benefits it might provide. Moreover, in most cases, the mixtures consisted of no more than 3 species, with the main focus on forage biomass (Vaičiulytė and Bakšienė 2011) or biomass and soil health without evaluation of species compatibility and persistence (Skuodienė *et al.,* 2017).

Materials and methods

The experiment was set up in Kalvarija distr., Lithuania in 2020 and conducted in 1.5 ha plots where samples were taken in 5 replicates at 1 m^2 microplots. The composition of the trinary-nonary mixtures is presented in Table 1.

The seeding rate of perennial (*Lolium perenne* L.), Italian ryegrass (*Lolium multiflorum* Lam. ssp. *italicum*) and Festulolium (*Festulolium*) was 25; tall fescue (*Festuca arundinacea* Schreb.) and tall oatgrass (*Arrhenatherum elatius* (L.) P.Beauv. ex J.Presl & C.Presl) was 20; meadow foxtail (*Alopecurus pratensis* L.),

Table 1. The composition of the grass:legume mixtures used in the experiment.

Species /cultivar / percentage in the mixture									
Mixture 1									
sainfoin	lucerne	alsike clover	Italian ryegrass	tall fescue	red fescue	meadow foxtail	tall oatgrass	red top	
Meduviai	Malvina	Lomiai	Ugnė	Medainis	Raudys	Valentas	breeding population	Violeta	
17%	10%	3%	17%	13%	13%	10%	10%	7%	
Mixture 2						**Mixture 3**			
lucerne	red clover	tall fescue	festulolium	timothy		red clover	perennial ryegrass		festulolium
Birutė	Radviliai	Monas	Punia DS	Obeliai		Vytis	Elena DS (4×)	Veja DS (2×)	Vėtra
23%	26%	29%	14%	8%		43%	23%	23%	11%
Mixture 4						**Mixture 5**			
red clover	cocksfoot	timothy				lucerne	cocksfoot	festulolium	
Sadūnai	Regenta DS	Dubingiai				Malvina	Luknė DS	Punia DS	
34%	56%	10%				40%	40%	20%	

timothy (*Phleum pratense* L.) and red clover (*Trifolium pratense* L.) was 12; red fescue (*Festuca rubra* L.) and lucerne (*Medicago sativa* L.) was 15; white (*Trifolium repens* L.) and alsike clover (*Trifolium hybridum* L.) was 8; red top (*Agrostis gigantea* Roth.) was 5 and sainfoin (*Onobrychis viciifolia* Scop.) was 75 kg pure live seed ha^{-1}. The first cut was performed when legumes were at the late-bud to first-flower stage of development and subsequent cuttings were taken at 40-50 day intervals.

The mixture swards in the microplots were cut, weighed, and separated into groups: grasses, legumes, weeds. For dry matter determination a sample of 0.5 kg was dried at 105 °C to a constant weight and weighed again. Mixture effect on DMY was determined by ANOVA and subsequent post hoc Tukey HSD test at the 5% probability level (P<0.05).

Results and discussion

The herbage and dry matter yield of the first cut comprised approximately 60% of the annual yield and the second and third cuts each contributed 20% (Figure 1). The exception was mixtures with cocksfoot; these produced 75% of the total yield at the 1st cut, and the 2nd cut represented 15% (Mix4) or 19% (Mix5), and the 3rd harvest 10 and 6% respectively.

No significant differences were found between the 1st cut herbage yield of the mixtures. In contrast, significant differences were found between Mix2 and Mix3 for dry matter yield (DMY, P<0.05).

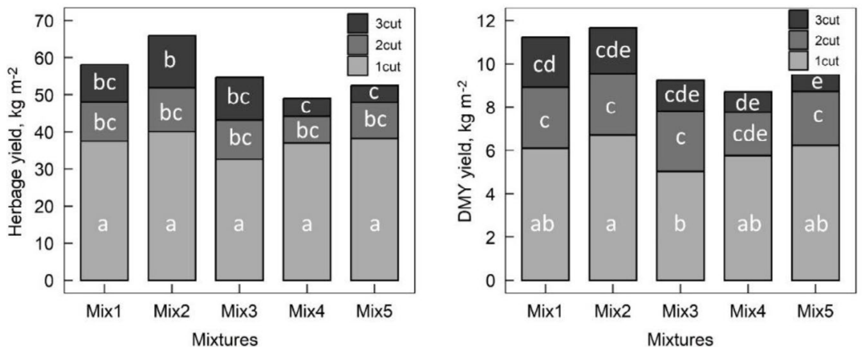

Figure 1. Herbage and dry matter yield of the grass:legume mixtures. Cuts denoted by the same letter do not differ significantly between mixtures (Tukey HSD, P<0.05).

Drought was the key factor determining low 2nd cut yield. Although mixtures did not differ significantly between each other, Mix5 containing the cocksfoot cultivar 'Luknė DS' produced 0.5 kg m^{-2} higher yield. Though mixtures with cocksfoot, namely Mix4 and Mix 5, were very productive in terms of 1st cut, 6.23 and 5.76 kg m^{-2} respectively, their last cut yield was the lowest. The lower DMY of Mix4 suggests that not only did species composition play an important role in mixture productivity but cultivars did so as well. The highest total herbage (65.97 kg m^{-2}) and DMY (11.65 kg m^{-2}) were found in Mix2, which was dominated by tall fescue.

The percentage of legumes before the 1st cut varied from 4 to 23% (Figure 2) where Mix2 and Mix3 had the highest and mixtures with cocksfoot, the lowest values. The percentage of legumes increased before 2nd cut and ranged from 11 to 35% while Mix4 and Mix5 were dominated by cocksfoot, 79 and 88% respectively. Composition of mixtures changed even more before the last harvest, when weeds were found in all mixtures except Mix4 and Mix5. Mix3 was the most protein-rich having almost 3-fold more legumes as before 2nd cut, while in Mix4 and 5 comprising cocksfoot, only single legumes plants were found. The plasticity and compatibility of species and varieties in mixtures is one of the most important indicators of yield, seasonal distribution, tolerance to stress and grass:legume ratio. Multispecies mixtures Mix1 and Mix2 produced higher DMY than Mix3, which is commonly used in intensive farms, suggesting that biodiverse mixtures can pay off under unfavourable conditions.

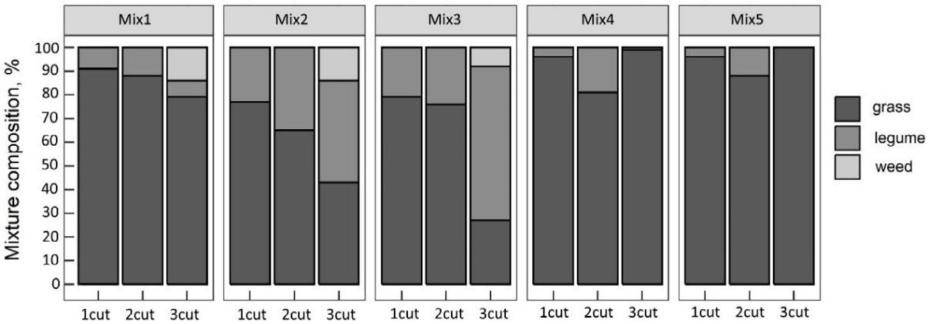

Figure 2. Mixture composition before 1st, 2nd and 3rd harvest.

Conclusions

Mixture dominated by tall fescue (Mix2: tall fescue (29%) + festulolium (14%) + timothy (8%) + lucerne (23%) + red clover (26%)) was the most productive under summer drought conditions. Steady legume ratio in the mixtures, and thus the quality, can be maintained or increased over the season by sowing more persistent species such as red clover and lucerne.

Acknowledgements

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Hybrid ryegrass can improve the botanical quality of multi species mixtures for leys

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Abstract

Perennial ryegrass (*Lolium perenne* L.) is a highly valuable species in mixtures for leys. However, under mowing it is soon replaced by companion grasses. In a three-year experiment, the effect of a partial replacement of perennial ryegrass by hybrid ryegrass (*Lolium* × *hybridum* Hausskn.) of the 'perennial' phenotype on ryegrass content was examined. Botanical composition and dry matter yield of four mixtures consisting of either pure perennial ryegrass or perennial and hybrid ryegrass of the 'perennial' phenotype, cocksfoot (*Dactylis glomerata* L.), meadow fescue *(Festuca pratensis* Hudson.), timothy (*Phleum pratense* L.), red clover (*Trifolium pratense* L.) and white clover (*Trifolium repens* L.) were assessed after the 1st and the 2nd winter. Adding hybrid ryegrass increased total ryegrass content 1.47 fold to 46% throughout the experiment. At the same time, hybrid ryegrass reduced the non-ryegrasses by about 30%. While hybrid ryegrass reduced clover content after the 1st winter by 33%, it did not affect the clover content after the second one. Dry matter yield was unaffected by hybrid ryegrass, neither in the first cuts after winter nor on an annual basis. Adding hybrid ryegrass of the 'perennial' phenotype may improve botanical quality of multi species mixtures with perennial ryegrass for three-year leys.

Keywords: grass-clover mixtures, leys, hybrid-ryegrass, *Lolium* × *hybridum* Hausskn., species proportion, botanical quality

Introduction

Perennial ryegrass (*Lolium perenne* L.) is a versatile forage species used in multi species mixtures for leys. In Swiss 'Standard Mixtures' for three-year leys for good conditions, perennial ryegrass is the basis for high yields of high-quality forage (Suter *et al.*, 2021). However, under mowing, it will be eventually replaced by the more persistent grass species that should help to maintain productivity of the mixture in the second main utilisation year (Nyfeler *et al.*, 2009; Suter *et al.*, 2012). Since forage quality of other grasses is lower than of perennial ryegrass (Davies and Morgan, 1982; Frame, 1991; Turner *et al.*, 2006), the replacement should happen as late as possible. This is partially achieved by the use of early varieties of perennial ryegrass with a good persistence (Suter *et al.*, 2012). However, further improvement is required to meet the needs of modern forage production. New varieties of the more competitive hybrid ryegrass (*Lolium x hybridum* Hausskn.) of the 'perennial' phenotype (Nay *et al.*, 2022) might help to maintain a high proportion of ryegrasses. Thus, a field experiment was established to examine the potential for improvement of botanical composition of the most important grass-clover mixture for three-year leys in Switzerland.

Materials and methods

Four mixtures were sown in the field at Ellighausen, Switzerland (47°36'41.3'N, 9°08'34.6'E; 520 m a.s.l.) in a randomised complete block design with four blocks in April 2016. All mixtures contained early perennial ryegrass, cocksfoot (*Dactylis glomerata* L.), meadow fescue (*Festuca pratensis* Hudson), red clover (*Trifolium pratense* L.) and white clover (*Trifolium repens* L.). Two mixtures contained one of two varieties of late perennial ryegrass while to the other two mixtures contained one of two varieties of hybrid ryegrass of the 'perennial' phenotype instead (Table 1). Prior to sowing and in spring the experiment was fertilized with 40 kg P ha^{-1} and 240 kg K ha^{-1}. Each of the 5 annual growth cycles received 25 kg N ha^{-1} in the form of ammonium nitrate. At harvest of the spring-growths of 2017 and

Table 1. Seed rates (kg ha^{-1}) of four experimental grass-clover mixtures containing one of two varieties of either late perennial ryegrass (LP) or of hybrid ryegrass of the 'perennial' phenotype (LH).

	LP		LH	
Species / cultivar	**LP1**	**LP2**	**LH1**	**LH2**
Red clover cv. *Global*	2.0	2.0	2.0	2.0
White clover, medium leaved cv. *Hebe*	1.5	1.5	1.5	1.5
White clover, large leaved cv. *Bombus*	2.5	2.5	2.5	2.5
Cocksfoot, early cv. *Beluga*	5.5	5.5	5.5	5.5
Meadow fescue cv. *Préval*	12.0	12.0	12.0	12.0
Timothy cv. *Rasant*	2.5	2.5	2.5	2.5
Perennial ryegrass, early cv. *Lacerta*	3.0	3.0	3.0	3.0
Perennial ryegrass, late cv. *Calibra*	4.0			
Perennial ryegrass, late cv. *Soraya*		4.0		
Hybrid ryegrass, 'perennial' phenotype cv. *Palio*			4.0	
Hybrid ryegrass, 'perennial' phenotype cv. *Palmata*				4.0
Total	33.0	33.0	33.0	33.0

2018, botanical composition was analysed. DMY of both the two spring growths and the annual totals of 2017 and 2018 were recorded. All statistical analyses were performed with the statistical software R's 'aov'-function (R Core Team, 2021).

Results and discussion

Even though ryegrass content in all mixtures decreased as expected (Suter *et al.*, 2012) between the two analysed harvests from 44 to 33% (P<0.05), the mixtures containing hybrid ryegrass exhibited a 1.47 fold total ryegrass content (46%) throughout the experiment, compared to the mixtures without hybrid ryegrass (P<0.01), irrespective of the varieties used (Figure 1). At the same time, hybrid ryegrass reduced the content of non-ryegrasses by about 30% from 23 to 16% (P<0.05). This may have a positive effect on forage quality (Davies and Morgan, 1982; Frame, 1991; Turner *et al.*, 2006).

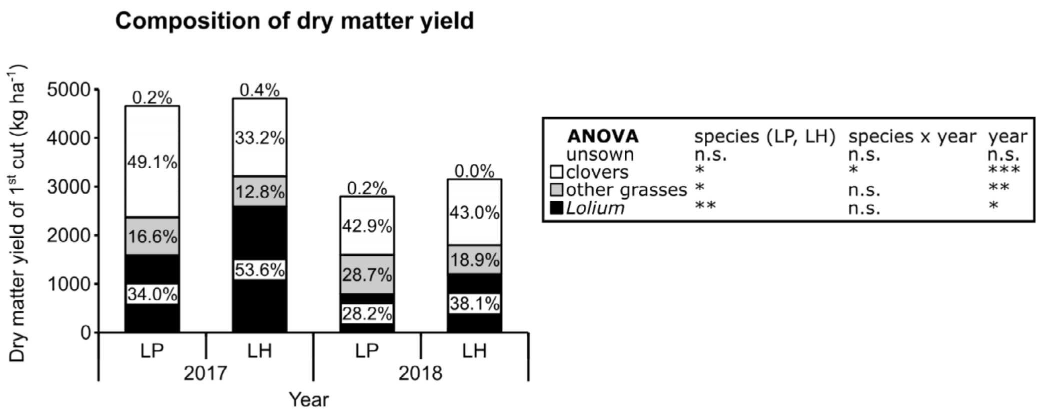

Figure 1. Dry matter yield and yield composition of the spring cuts of two consecutive years of grass-clover mixtures with red clover, white clover, meadow fescue and cocksfoot containing either *Lolium perenne* (LP) alone or *Lolium perenne* and *Lolium* × *Hybridum* (LH). Analysis of variance (ANOVA) for effects of species (LP, LH), year and species × year on yield composition: n.s. = not significant, * P<0.05, ** P<0.01 and *** P<0.001.

While hybrid ryegrass reduced clover content (mainly white clover, data not shown) after the 1st winter by 33% (P<0.05), it did not affect the clover content after the 2nd winter (interaction species [LP, LH] × year: P<0.05), showing that its higher proportion after the 2nd winter was due to a hybrid ryegrass induced reduction of non-ryegrasses. The fact that the increase of the proportion of ryegrasses was mainly due to a shift within the grass fraction shows that it may be possible to increase the quality of the mixture while maintaining the positive effects of the legumes on yield and forage quality (Nyfeler *et al.*, 2009) as well as on symbiotic N_2 fixation and the whole plant production system (Nyfeler *et al.*, 2011).

Dry matter yield, which decreased from 12,573 kg ha^{-1} in the 1st year to 7,213 kg ha^{-1} in the 2nd year (data not shown), was only affected by the year (P<0.001). The same picture (year: P<0.001) could be observed with the yield of the first cuts after winter, which decreased from 4,750 kg ha^{-1} in the first year to 2,979 kg ha^{-1} in the 2nd year (Figure 1). This shows that the benefit of adding hybrid ryegrass lies in an improvement of botanical quality rather than in an increase in yield.

Conclusions

Hybrid ryegrass of the 'perennial' phenotype has the potential to increase the content of ryegrasses in the yield also after the 2nd winter. Thus, replacing at least a part of perennial ryegrass in the seed mixture with hybrid ryegrass of the 'perennial' phenotype may improve botanical quality of multi species mixtures for three-year leys.

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The effect of overseeding and molybdenum fertilization on the proportion of clover in grasslands

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Abstract

The proportion of white clover in grasslands can decrease over time. Especially in organic grasslands, root nodules of clover are important to fix nitrogen in the soil to stimulate grass production. The number of root nodules is significantly higher in case of Molybdenum (Mo) deficiency than under good Mo supply. However, even though the number is higher the root nodules are unable to bind nitrogen from the air without sufficient Mo. To study the effect of Mo fertilization and overseeding, grasslands with perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens* L.) on clay soil at the experimental organic farm 'Aeres Farms' in Dronten in the Netherlands were monitored. The effect of overseeding and Mo on the proportion of white clover was examined using four treatments: neither overseeding nor Mo fertilization, only Mo fertilization, only overseeding, or a combination of Mo and overseeding. The treatments were carried out in the spring of 2022. Monitoring took place in the autumn of 2022. Results show an increase of white clover in grassland on clay soil when overseeding took place. Mo fertilization did not increase the proportion of white clover.

Keywords: white clover, grassland, overseeding, molybdenum

Introduction

Mo is a trace element found in the soil and is required for growth of most organisms including legumes (Graham and Stangoulis, 2005; Sardesai, 1993). Leguminous plants in partnership with Rhizobium bacteria have the ability to convert N_2 into reactive N by increasing the rate of biological N-fixation (Galloway *et al.*, 1995). N-fixation involving symbiotic association between Rhizobium bacteria and legumes is influenced by several factors including availability of Mo (Greenwood and Hallsworth, 1960). The symbiotic bacteria require about ten times more Mo for N_2-fixation than the host plants. For this reason, Mo deficiency will commonly occur in legumes before it does in other plants when grown in the same soil (Thibaund, 2005). In case of Mo deficiency, the dry matter yield and the amount of crude protein of clover increases with fertilization of 0-0.2 kg sodium molybdate ha^{-1} (Van Eekeren, 2000). Overseeding white clover can increase the amount of clover in grassland swards as well, but the results are variable (De Wit *et al.*, 2004). The aim of this study is to understand the effects of Mo fertilization and overseeding on the proportion of white clover in organic grassland.

Materials and methods

The effect of overseeding and Mo on the proportion of white clover was examined on grasslands with perennial ryegrass and white clover on calcareous marine clay soil at the experimental organic farm 'Aeres Farms' in Dronten in the Netherlands. The soil has pH of 7.1. The topsoil contains silt clay loam with an organic matter content of about 3%. There were four treatments:

0. neither overseeding nor Mo fertilization (2 plots);
1. only Mo fertilization (3 plots);
2. only overseeding (4 plots, of which 2 sprinkled);
3. a combination of Mo and overseeding (4 plots, of which 2 sprinkled).

Treatments 1 and 3 were fertilized with 140 g ha^{-1} of sodium molybdenum. Treatment 2 and 3 were overseeded with 2 kg ha^{-1} white clover, together in a mixture with perennial ryegrass and timothy grass (*Phleum pratense*). Overseeding and Mo fertilization were carried out in the spring of 2022. The monitoring took place with random points of inclusion in autumn 2022. On each plot, 12 measurements were taken using a steel frame measuring 50×50 cm. A photograph was taken of each frame and the clover proportion was determined using the 'clover indicator' (De Visser and Philipsen, 2002). The clover indicator shows five categories ranging between 10 and 75 percent clover proportion, with photos to visualize clover proportions in grasslands. The plots had the same management, except sprinkling or not. Half of the plots with treatment 2 and half of the plots with treatment 3 were sprinkled with 30 mm of water once in August. Since all treatments were sprinkled, a one way ANOVA had to be chosen, with both treatment method and sprinkling being a combined variable (e.g. 'Treatment2Sprinkled' and 'Treatment2NotSprinkled'). In addition, a linear regression was used to determine the effect of treatments 0 to 1. The data were analysed using JASP version 0.16.3.0 and an alpha of 0.05.

Results and discussion

Treatment method influenced the amount of white clover (P<0.01). The proportion of white clover was highest when only overseeding had taken place. Overseeding (treatment 2) gave a higher proportion of white clover compared to Mo fertilizing (treatment 1), with and without sprinkling (P<0.05). The linear regression predicted a higher proportion of white clover with overseeding (treatment 2) compared to neither overseeding or Mo fertilization (treatment 0) (P<0.05). When plots were sprinkled, the proportion of white clover was higher (P<0.05).

Management influences the proportion of white clover. Plots were grazed during the season. The time of grazing and its duration could vary slightly from plot to plot. Fertilization and mowing management were similar. The results of this study are not consistent with previous experiments in which an effect of Mo fertilization was found (Van Eekeren, 2000). In the current experiment, Mo fertilization did not lead to an increase in clover proportion.

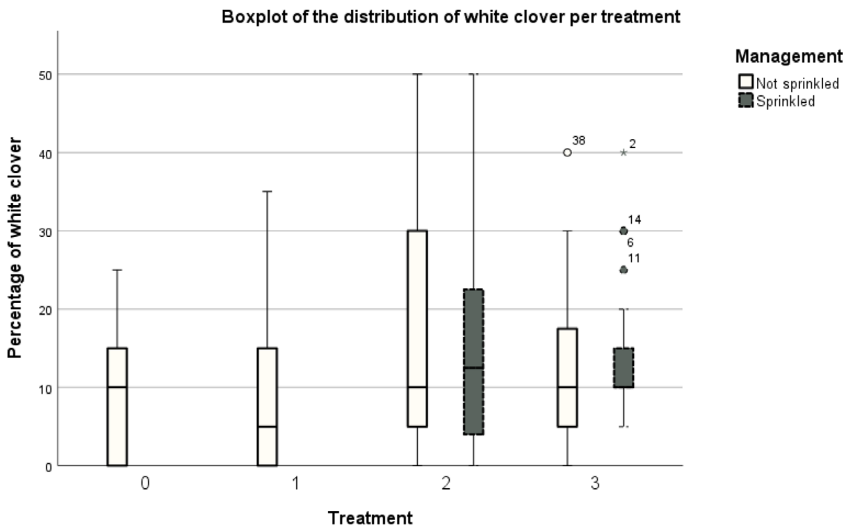

Figure 1. The proportion of white clover measured in the plots (in %). Treatment 0 = neither overseeding and Mo fertilization, 1 = Mo fertilization, 2 = overseeding and 3 = overseeding and Mo fertilization.

Figure 2. Example of a picture of a measurement of white clover.

Conclusions

The proportion of white clover increases in autumn when the land is overseeded during spring. Molybdenum, either when fertilized only or in combination with overseeding, did not increase the proportion of white clover in this study, although other studies show different results. When farmers want to increase the amount of white clover in grasslands, overseeding is an effective method.

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Theme 2.
Biodiversity and other ecosystem services

Grass leys promote soil carbon sequestration and improve nitrogen cycling and soil structure

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Abstract

Intensification and specialization of farming systems in Europe and elsewhere has resulted in poor crop rotations, with low plant and animal diversity. This has resulted in more uniform landscapes, soil carbon loss and low efficiency in nutrient cycling, particularly in regions dominated by annual crops. Inclusion of ley in crop rotations is expected to increase soil organic carbon (SOC) stocks, nitrogen availability and improve soil physical properties. The effect of ley-arable rotations versus continuous annual cropping on soil quality, soil organic carbon and soil biology was assessed by summarizing and discussing results from publications from long-term experiments in Norway and Sweden. These studies support the hypotheses that the inclusion of leys in crop rotations promotes soil fertility and carbon sequestration in Northern Europe, supplies nutrients to subsequent crops and improves soil physical properties. However, one or two years of ley in rotations may not be enough for maintaining SOC and good soil structure over time. For keeping the relatively high SOC concentrations occurring at many sites in Northern Europe, the proportion of ley in rotation should be at least 50%.

Keywords: biological diversity, grasses, legumes, nitrogen, soil fertility, soil organic matter

Introduction

Agricultural intensification and specialization of farming systems has led to fewer mixed farms in Europe and worldwide (Cooledge *et al.*, 2022; Knox *et al.*, 2011; Schut *et al.*, 2021). Between 2005 and 2016 the number of mixed farms declined by 10.9% and 3.7% in the eastern and western Europe Union (EU) countries (Eurostat, 2019). The considerable increase in monoculture cultivation has resulted in environmental problems, particularly soil degradation that includes soil erosion, organic matter decline, compaction, salinisation, landslides, contamination, surface sealing and biodiversity decline (Montanarella, 2007). Enhanced soil protection strategies are therefore necessary to counteract such processes.

Temporary grass-clover leys in crop rotations are usually included in organic farming, but they are also currently encouraged in conventional systems (Zani *et al.*, 2021). Leys have a positive impact on soil quality as they provide additional carbon (C) input. The fraction of C fixed by photosynthesis, and further converted into biomass, is the first step in capturing carbon from the atmosphere. The fixed carbon that enters the soil system through plant residues and rhizodeposition affects soil organic carbon (SOC) quality and quantity (Kätterer and Bolinder, 2022). Only a part of C in these residues is stabilized in soil through physical, chemical, and biological processes (Basile-Doelsch *et al.*, 2020). Several studies highlight the importance of plant roots for which a larger fraction of added C is stabilized than for above-ground residues (Kätterer *et al.*, 2011; Rasse *et al.*, 2005). This is not only connected to higher recalcitrance of root tissues but also to their higher physico-chemical protection, interactions with metal ions, and probably also to the diversity of species promoting spatial heterogeneity and molecular diversity of C inputs (Lehman *et al.*, 2020). Recent research has further stressed that when temporary grass-clover leys are grazed (i.e. if the farm includes livestock), there may be an additional benefit to soil C accumulation and enhanced nutrient cycling and utilization, and consequently improved sequestration in the agroecosystem (Assmann *et al.*, 2017; Chen *et al.*, 2015).

The inclusion of grass-clover leys in arable rotations not only contributes to soil C stocks, but also improves soil aggregate stability, biodiversity, fertility and nutrient cycling (Cooledge *et al.*, 2022). A satisfactory proportion of legumes in the mixture increases nutrient supply and soil fertility, via both symbiotic nitrogen (N_2) fixation by legumes (Nyfeler *et al.*, 2011; Suter *et al.*, 2015) and increases in soil organic matter (Paustian *et al.*, 1997). The strong wish to decrease the use of fossil fuel and industrial fertilizers indicates the increasing role of legumes in crop rotations. Thus, a potential for an increased utilization of biologically fixed N exists in all kinds of farming systems.

The proportion of N derived from the atmosphere through biological fixation differs between and within species, ranging from 0% to almost 100% (Büchi *et al.*, 2015). This huge variation reflects that successful N fixation in symbiosis with rhizobia is a complex process and depends on local conditions such as the quantity of N available from the soil (Høgh-Jensen and Schjoerring, 1994), soil pH, temperature and moisture (Liu *et al.*, 2011). The use of cover crops that often contain grass-legume-herb mixtures have recently attracted great attention in arable farming (King and Blesh, 2018; Martin *et al.*, 2020). Cover crops in crop rotations can reduce nutrient leaching and supply these nutrients to subsequent crops. However, there is a risk that some N can be lost during winter even in perennially based cropping systems (Sturite *et al.*, 2021). Therefore, good synchrony between N mineralization and N uptake by succeeding crops is a challenge in agricultural systems, particularly in regions with a long winter period.

Inclusion of leys in crop rotations affects many ecosystem services. Several studies show that well-managed grass leys in crop rotations reduce weed infestation (Schuster *et al.*, 2018, 2019). The greater competitiveness of grasses than of arable crops with weeds provides an efficient non-chemical control strategy (Dominschek *et al.*, 2021) and protects human health and the environment. Moreover, leys may also interrupt the cycle of crop diseases and provide habitats for insects and vertebrates (Kumar *et al.*, 2019).

Long-term experiments that include leys in crop rotations are valuable for evaluating long-term impacts on soil quality, carbon sequestration, soil fertility and nutrient availability. In this paper, results from long-term experiments from Norway and Sweden, with and without leys in the crop rotations, will be presented and discussed. More generally, we will also evaluate the role of legumes in ley and in crop rotation and implications on other ecosystem services.

Ley contribution to soil quality, SOC and soil biology

Experience from long-term field trials in Norway

In Norway, a considerable increase in arable cropping occurred after 1950 due to government policy to secure cereal growing in the climatically best suited areas. At the same time milk and meat production was supported in more marginal areas. Thus, mixed farming units declined in south-eastern and central Norway and farm specialization was prioritised. This has affected soil properties negatively and resulted in SOC decline, as recognised worldwide (Smith, 2008) and in experimental trials over several decades in Norway (Riley *et al.*, 2008, 2022; Uhlen 1991).

Uhlen (1991) studied how inclusion of ley in cereal rotation affects SOC in a clay soil in SE Norway. His study included either 2-years ley or 4-years ley in six-year crop rotations, with and without manure application and/or straw retention. The experiment lasted until 2014. After three decades the soil analyses showed a slight increase of SOC concentrations in a 2:4 year cereal:ley rotation with the use of cattle manure, whilst it remained unchanged in such rotation without the use of cattle manure. A decline of SOC was recorded in a 4:2 year cereal: ley rotation, indicating that a two-year ley period is too short to maintain stable SOC concentrations. Over the same period, the cultivation of cereals in monoculture led to the loss of about 10% of the topsoil SOC. Declines in SOC were found to have continued after six decades, even in the 2:4 year cereal:ley rotation, where it was expected to find a further SOC increase (Marina Bleken, pers.com.).

The oldest still-running trial in Norway celebrated 100 years in 2022. It is located at Møystad in SE Norway on a loam soil with moderately high humus content of between 4.5 and 6.5% (Riley, 2016). The trial comprises 72 fertilizer/manure treatment plots (size 30 m^2), of which 8 plots have received no fertilizer throughout, 20 plots receive various amounts of farmyard manure (FYM) and 44 plots receive various amounts and combinations of N, phosphorus (P) and potassium (K) in the form of mineral fertilizer. All treatments have either four or eight replicates. A 7-year crop rotation includes three years of ley and four years with annual crops. The original aim of the trial was to find out whether the long-term use of inorganic (mineral) fertilizer was equally as beneficial as the use of farmyard manure, for crop yields, economy, soil structure, animal feed and human health. The same practices throughout the decades allow us evaluate soil carbon concentration changes and soil health generally.

Riley and Kristoffersen (2022) found that the concentration of SOC in the topsoil declined markedly between the 1930s and 1960s, partly due to changes in ploughing depth (Figure 1). In recent years, SOC concentrations have remained relatively stable in treatments with FYM and in that which has received a moderately large amount of NPK-fertilizer each year since 1922. In other treatments, declining SOC has been recorded, despite the use of ley. Expressed as organic carbon, the concentrations in the period 1963-2019 were on average 0.74% higher in treatments with FYM than without fertilizer since 1922. In the treatment with a large amount of NPK since 1922, the corresponding difference was half as much (0.37%). The long-term monitoring indicates that topsoil's total-N concentration has declined slightly over time. It was on average 10% higher in treatments with FYM than without fertilizer, but differences between treatments were very small. The C:N ratio decreased markedly between 1935 and 1996, from ca. 15 to ca. 9, suggesting a strong degree of humification. This long field history reflects the complexity in carbon sequestration and storage. Regardless of having leys in three out of seven years and reasonably high application rates of N in FYM and NPK fertilizer each year, the initial level of C concentrations in the 1930s were significantly higher than those in 2019. There are other studies that show decline of SOC over time despite regular manuring (Christensen, 1990). The latter author found SOC increase in plots with low initial carbon and concluded that it is easier to raise carbon levels in soils with a low initial level than in soils with a high initial level. At the Møystad trial, the initial SOC concentration was above 5%

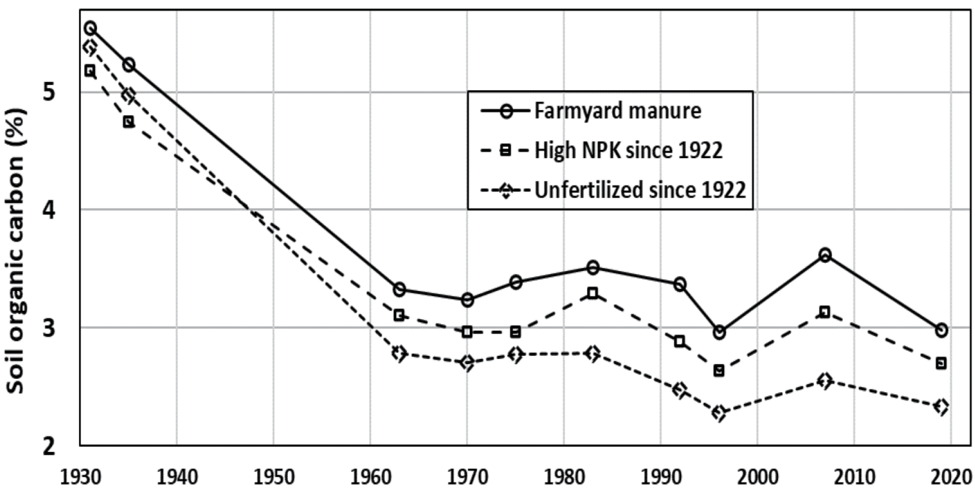

Figure 1. Topsoil organic carbon concentrations in a long-term fertilizer trial in SE Norway.

and dropped to about 3% after 40 years (Figure 1). Thereafter, SOC concentrations remained relatively stable. Börjesson *et al.* (2018) suggested that in soil with low SOC concentration (usually below 3%), SOC increases might be expected with regular manure applications and plant residue inputs.

In another ongoing experiment in SE Norway, established in 1988-1989 at Apelsvoll Research Centre, different production systems are compared. Both arable and mixed dairy systems are studied at a whole-system level, including both conventional and organic practices (Figure 2). Cropping systems represent arable production typical for the region including a reference system (CS1 in Figure 2) that reflects arable practices that are common when little attention is paid to non-point source losses of nutrients or to the maintenance of SOC levels. The trials with leys are typical of mixed dairy farming, with 50-75% ley in the rotation. More details about the trial set up are given by, amongst others, (Riley *et al.* 2008, 2022). The state of soil properties before the start of the experiment were characterised by Riley and Eltun (1994). In a later study Riley *et al.* (2008) reported that topsoil concentrations of SOC and the stability of soil aggregates had declined after 15 years of arable management with autumn ploughing and straw removal, whilst under shallow spring tillage with the use of catch crops and straw retention, both variables had been maintained at levels similar to those in mixed dairy systems including the use of FYM. Further, a positive relationship between soil porosity and incidents of leys within rotation was found (Table 1).

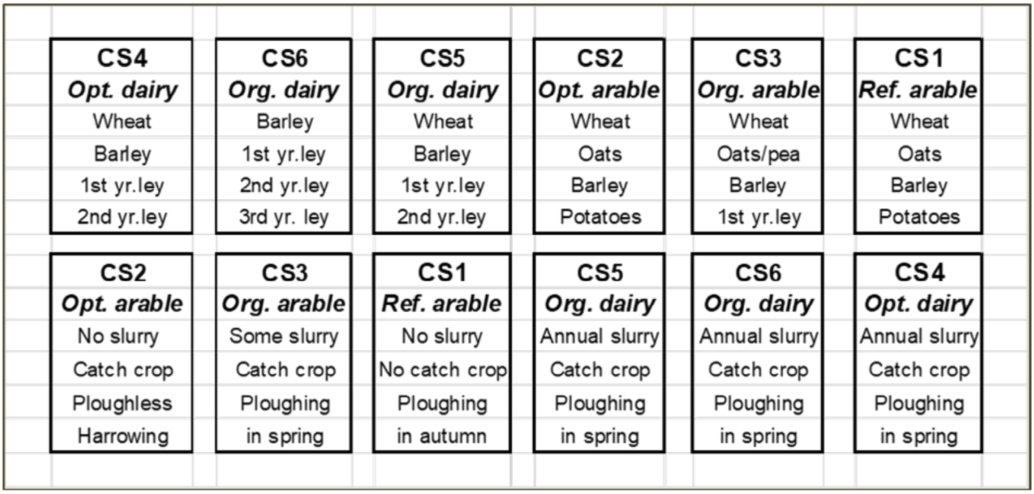

Figure 2. Distribution and main features of cropping systems CS1-CS6 on twelve 60 × 30 m model farms, with two per system. Crops grown every year since 1999 are shown in the upper row (Rep. I) and slurry use, catch cropping and tillage are shown in the lower row (Rep. II).

Table 1. Topsoil (0-30 cm) means of soil bulk density, total porosity and soil organic matter measured with cropping systems CS1-CS6 in 2003, fifteen years after the start the experiment.

System	CS1	CS2	CS3	CS4	CS5	CS6	s.e.
Ley % in rotation	0%	0%	25%	50%	50%	75%	
Bulk density (kg l^{-1})	1.43	1.40	1.38	1.34	1.36	1.39	0.04
Total porosity (%)	44.8	45.0	46.8	47.0	47.5	48.9	1.1
Organic matter (%)	3.1	4.1	3.9	4.0	3.8	3.9	0.3

The rotation with 75% ley showed the greatest volume of air-filled pores. The soil tended towards over-compactness in all arable systems, whereas it was in the optimum range in mixed dairy systems with a high proportion of leys and slurry applications (Figure 3). The systems with the highest soil aggregate stability (almost 90%) were those with either 75% ley or reduced tillage. However, all rotations that included ley had high aggregate stability, whereas the arable system with conventional tillage had very low stability. High earthworm density was also found in systems comprising leys. The organic dairy system with 75% ley contained 92 earthworm channels m^{-2}, whilst the arable reference system (CS1) had only 21 channel m^{-2}. Thus, the leys contributed to better soil structure and biological activity than pure arable crops. A conclusion from this study is that inclusion of 50% leys in crop rotation may be sufficient to maintain good soil structure.

Soil carbon measurements were repeated in 2016 (Riley *et al.* 2022). Despite the high proportion of leys in dairy systems, the SOC concentration in topsoil (0-30 cm) had declined significantly between 1988 and 2016. Similarly, the SOC mass at 0-45 cm depth declined significantly throughout the same period (Table 2). Mean SOC mass reduction for all treatments was from 89 Mg ha^{-1}, representing a loss of 0.3% yr^{-1}of the initial SOC. However, the carbon losses tended to be lower in dairy systems than in arable systems. Changes in bulk density (BD) were recorded for all cropping systems and BD declined significantly with increasing SOC concentration in both topsoil and subsoil. Calculations of carbon inputs to each of the cropping system from 1990 to 2016 are shown in Table 3. The inputs were based of crop yields, using the allocation coefficients described in Bolinder *et al.* (2007) and Bolinder *et al.* (2015). Carbon in manure, seeds and other additives was included. It was assumed that carbon concentrations were 45% of dry matter in all plant tissues, choosing the rather high estimate. Mean carbon input was 22% higher in dairy systems than in arable systems. It was assumed that the SOC might be maintained with an input of 4 Mg C ha^{-1} yr^{-1} whilst lower inputs are insufficient to maintain the SOC mass unaltered. A meta-analysis of 49 sites worldwide showed that 12% of cumulative carbon inputs are retained in SOC after an average of 18 years (Maillard and Angers, 2014), whilst in Norway, Uhlen (1991) calculated 17% after 30-50 years.

Experience from long-term field trials in Sweden

In Sweden, farming management practices have also led to bigger farm units and specialization. Kätterer *et al.* (2013) summarised data from several long-term experiments in Sweden that compared crop rotations with and without leys. Well known are the 'Swedish humus balance experiments', which were started between 1970 and 1980 at four sites in Sweden (latitudes between 56° and 64°N) at the experimental

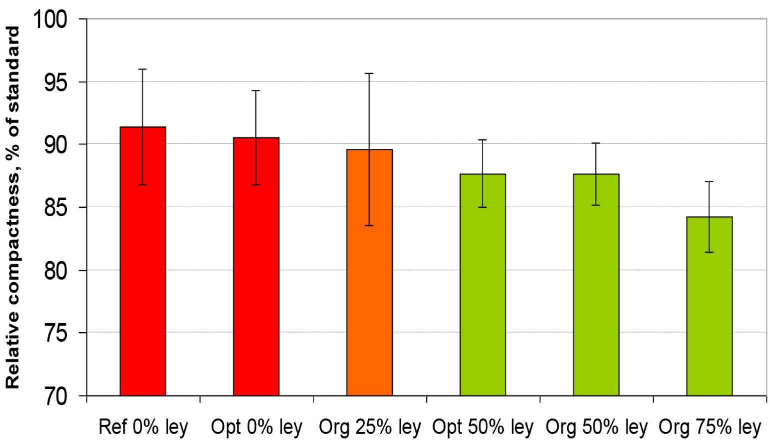

Figure 3. Relative degree of compactness (bulk density as percentage of compaction at 200 kPa) measured in the topsoil of six cropping systems (error bars are standard deviations).

Table 2. Total soil organic carbon (SOC) mass (Mg ha^{-1}) measured at 0-45 cm depth in 2016, 28 years after the start of the experiment, and changes since 1988. Changes at system level were not statistically significant, but the overall decline was significant at P=0.04.

System	CS1	CS2	CS3	CS4	CS5	CS6	
Ley % in rotation	0%	0%	25%	50%	50%	75%	Mean
SOC in 2016	82	88	84	98	83	97	89
Change since 1988	-13	-6	-23	+2	-5	-10	-9

Table 3. Estimated total inputs of C to soil (Mg ha^{-1}) from various sources from 1990 to 2016.

System	Aboveground	Roots	Exudates	Manure	Seeds	Total
CS1	29.3	18.9	12.4	0.0	3.4	64.0
CS2	47.1	17.9	11.7	0.0	3.4	80.1
CS3	47.7	25.3	16.5	2.6	2.1	94.2
CS4	34.7	27.6	31.1	15.9	1.8	111.1
CS5	27.1	22.6	25.1	11.0	1.8	87.7
CS6	23.1	21.5	32.2	14.0	1.4	92.2

stations Lanna, Lönnstorp, Röbäcksdalen (Röbäck II) and Säby. The study is still on-going. The trials were designed to investigate the effect of N fertilization on SOC in a forage-dominated rotation (one year of spring barley undersown with grasses and clovers followed by three years of a grass-clover ley) and an arable rotation within only cereals (mainly spring-sown barley, oats and wheat), where crop residues were either removed or incorporated into the soil. In both rotations, four N-fertilization levels were compared with four replicates. Topsoil (0-20 cm) carbon concentrations were determined in archived soil samples taken at the start of the experiments and in samples taken in autumn 2008. Using data from these four sites, SOC increased significantly with increasing rate of N fertilization in the cereal rotation. Inclusion of ley in crop rotation resulted in higher SOC stocks by 0.34-0.56 Mg C ha^{-1} yr^{-1} and 0.42-0.60 Mg C ha^{-1} yr^{-1} compared with the cereal rotations with or without residue removal, respectively. In contrast to the cereal rotation, N fertilization had no significant impact on SOC stocks in the ley rotations. This may be explained by an increasing proportion of clover in the leys at low N-fertilization rates. Kätterer *et al.* (2013) pointed out that changes in SOC are more likely to be negative when initial SOC stocks are high. Thus, site history before the start of the experiment determines whether a certain rotation or management will lead to increasing or decreasing SOC stocks in absolute terms. This means that, although leys will accumulate more C in soils than cereal-dominated rotations, even ley-dominated systems may result in decreasing SOC stocks, as has also been found in SE Norway (Uhlen, 1991; Riley *et al.*, 2022).

Later investigations at Lanna and Lönnstorp sites confirmed that stocks of SOC after 35 years were significantly higher in the ley rotation than in cereal monoculture (Börjesson *et al.*, 2018).

In northern Sweden, the long-term experiments were started in 1957 at three sites (60° to 65°N), in Offer, Ås and Röbäcksdalen (Röbäck I). Four 6-year rotations differing in proportions of annual and perennial crops were investigated (Bolinder *et al.*, 2010). The rotations start with undersown barley, followed by either 1, 2, 3 or 5 years of leys (i.e. a mixture of red clover, timothy and meadow fescue). The other crops in these rotations are mainly winter rye, fodder rape, green fodder and some root crops. The rotations with three and five years of ley received about 4.5 Mg C ha^{-1} through FYM, and the rotation with two years of ley received about 3 Mg FYM-C ha^{-1} over a complete 6-year period. Data on top soils (0-25 cm) after 30 years indicated that SOC changes were proportional to the frequency of leys in the rotation. Furthermore, the initial concentrations of SOC at the sites determined the direction of subsequent changes in SOC concentrations. In the silt loam soil at Röbäcksdalen, which had a high initial SOC concentration, there was an overall decline in SOC concentration. In the gravelly loam at Ås with an intermediate initial SOC concentration, SOC concentration was maintained only in the 5-year ley rotation, whilst in the silty clay loam at Offer, with the lowest initial SOC concentration, only the 1- and 2- year ley rotations showed a net loss. The Offer site was resampled in 2008 representing temporal changes over 52 years. Mean sequestration rate at Offer was +0.12 Mg C ha^{-1} yr^{-1} for the 5-year ley in rotation. The rotation with 3-years ley was approximately in balance (-0.04 Mg C ha^{-1} yr^{-1}), whereas the in rotations with only two or one years of leys, the ley period was too short to maintaining SOC (-0.18 and -0.24 Mg C ha^{-1} $yr^{-1,}$ respectively).

Based on the findings from these long-term studies in Sweden, we can repeatably conclude that leys have positive effects on SOC and should be included as much as possible in crop rotations.

Role of legumes in ley

Before the widespread use of fertilizers, it was typical to maintain 25-50% of a farm in a legume-rich pasture or cover crop (Crew and Peoples, 2004). Thus, the use of legumes in crop rotations was common practice, and soil fertility was maintained through biological fixation of atmospheric N. Through ploughing and decomposition, the nutrients accumulated in plant biomass were released to subsequent crops. In a field with white clover, N-fixation was estimated to account for 23 to 545 kg N ha^{-1} $year^{-1}$ (Carlsson and Huss-Danell, 2003). This wide range includes estimates from different sites and management regimes, measured with different methods and often without including stolons, rhizomes and roots. Average aboveground N-fixation rates reported for Norway range from 85 kg ha^{-1} y^{-1} in the North to 144 kg N ha^{-1} y^{-1} in the South (Lunnan, 2004). Tzanakakis *et al.*, (2017) reported that as little as 15% clover in seed mixtures contributed substantially to total N yield in ley. This study was carried out at Tjøtta (65°N, 12°Ø) in northern Norway. However, poor persistence of red clover and a marked reduction in the number of plants in the fourth ley year made this potential short-lived. The transfer of biologically fixed N from clover to companion grasses was estimated by comparing ^{15}N natural abundances in grasses and clovers between years. N transfer from clover to grass species was small in the first period (first to third production year) but increased markedly in the second period (third to fourth production year) (Figure 4A). In the fourth production year, N derived from clover accounted for 16 and 17% of harvested N in timothy and meadow fescue, respectively (Figure 4B). This illustrates that the N transfer rate between legumes and non-legumes increases gradually. Other beneficial services could include reduced N-leaching and reduced weed pressure (Thorup-Kristensen *et al.*, 2003). On the other hand, additional N input from legumes in the system could provoke nitrate and dissolved forms of organic N leaching and denitrification in periods when N availability is larger than demands for plant growth (Sturite *et al.*, 2021). This may happen in off-season periods when air temperatures are low and plant growth limited. Field experiments over two winters, conducted at two coastal locations in Western and Northern Norway, showed that pure grass leys emitted less N_2O than leys that contained clover (Sturite *et al.*, 2021). At the same time, it was found there was considerable net-increase of plant N in stubble and roots throughout winter. Therefore, species mixtures that optimize N below-ground uptake and storage should be developed and tested under field conditions.

Final remarks

Adoption of mineral N fertilizers has boosted the amount of food that farms can produce (Crews and Peoples, 2004). A war in Europe has essentially changed the situation worldwide and fuelled demand for food security in each country. Nitrogen sources based on biological N fixation are easily adoptable at the

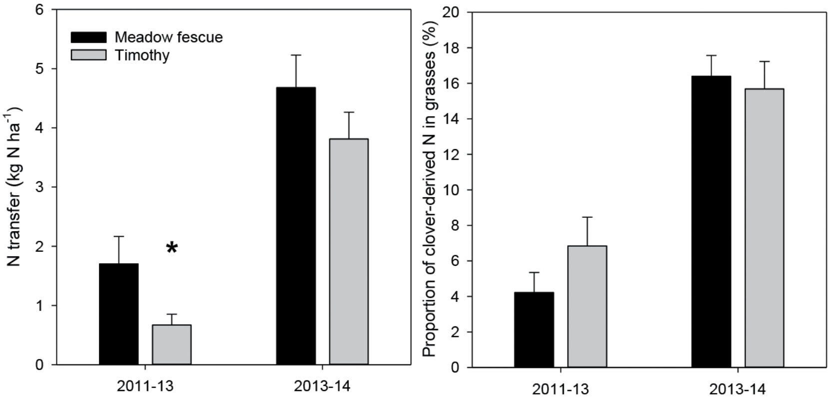

Figure 4. (A) Average N transfer from clover to meadow fescue and timothy estimated by ^{15}N natural abundance in first harvests for the periods 2011-2013 and 2013-2014 and (B) the proportion of grass N derived from clover (SE; n=18). Asterix denote significant difference within grass species.

farm level. Farmers should be convinced about the importance of ley inclusion in crop rotation and their role in sustaining soil fertility and SOC and most important cash crop yields. Introduction of financial instruments at the initiation phase can play an important role together with effective education of farmers (Costantini *et al.*, 2020). Adequate support should be considered to each specific pedo-climatic zone and socio-economic context. The results from long-term experiments in a Nordic climate summarized here show that inclusion of leys in crop rotations had a positive impact on soil structure, SOC and soil biology. SOC concentrations and stocks generally increase with the frequency of leys in rotations. However, 1 or 2 years of ley in rotations may not be sufficient to maintain SOC over time under Nordic conditions in soil with relative high SOC content.

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Characterization of biochar alone and mixed with compost or digestate as fertilizers in maize crop

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Abstract

The future of agriculture should address emerging challenges: reduction in nutrient losses of at least 50% by 2030, while ensuring no damage in soil fertility; reduction the environmental footprint of agricultural intensification; decrease in European heavy dependency on rock phosphate. Biochar, a charcoal produced by pyrolysis of different biomass sources, is being investigated as a means of carbon sequestration and may be useful to mitigate climate change. Several studies have shown that addition of biochar can improve soil fertility, increase crop yield, and reduce contaminations. This study investigated the effects of biochar (B) application, alone or in mixture with digestate (BD) or compost (BC), versus chemical fertilization (C) on maize crop (*Zea mays* L.) yields and quality. Treatments were evaluated in a completely randomized block design with three replications. No significant differences were observed between treatments. However, results show that B application can allow reduced chemical fertilization without affecting maize crop yield, and be a key management strategy for enhancing maize productivity and environmental safety. Based on these results we suggested that chemical fertilizers could be at least partially replaced by biochar.

Keywords: biochar, crop yield, inorganic fertilizer, agricultural management, sustainability

Introduction

Environmental care and climate-change mitigation are specific priorities of the European Common Agricultural Policy. In this situation, farmers are forced to implement new systems that meet these environmental requirements which involve the use of few inputs such as nitrogenous fertilizers. In addition of biochar ability to improve soil physical, chemical, and biological properties, the agronomic value of biochar mainly resides in it has been identified as a low-cost technology that could be an interesting tool to minimize adverse impacts of farming on the environment e.g. a reduction of agrochemicals (Duku *et al.*, 2011), as well as that to contribute to the recovery of nutrients from waste and increase crop yields, while abating climate change (Woolf *et al.*, 2010). Furthermore, owing to the stability of biochar carbon, applying biochar with other amendment as compost or digestate can enhance compost properties, leading to higher added value and a much better carbon sequestration potential compared to individual mixing of biochar or compost with soil (Ronga *et al.*, 2020; Schulz and Glaser, 2012). The aim of this study was investigated the effects of application biochar alone or in combination with digestate or compost versus chemical fertilization on maize crop yields and quality.

Materials and methods

The experiment was conducted on agricultural soil located in the North of Spain (43°34'32.9"N, 5°53'08.0"W; 100 m a.s.l.). The climate is classified as warm maritime according to Papadakis climate classification. Four different treatments were organized into a randomized complete block design with three repetitions (12 plots of agricultural land of 5×5 m each one) in spring 2021. Fertilizer to supply P (135 kg P_2O_5 ha^{-1}) and K (260 kg K_2O ha^{-1}) for maize crop was applied to all plots, the amounts based on previous soil analysis. The N application rates as basal fertilization dressing for each treatment are shown in Table 1. Biochar has a high microporosity and specific surface area. Therefore, the amount of biochar necessary to cover the nitrogen needs of maize is too high. For this reason, it was decided to replace only 50 fertilizer units with biochar. Moreover, all treatments were supplemented with 50 kg N ha^{-1} of calcium ammonium nitrate (27%) as topdressing fertilization when maize plants reached 25 cm height. The biochar used was produced by pyrolyzing pine wood and presented a concentration of 10.6 g N kg^{-1}. The mixtures of biochar with digestate and compost had N concentrations of 11.46 and 4.85 g N kg^{-1} respectively.

The maize variety FAO 300 was used in all plots with a planting density of 90,000 ha^{-1}. Maize was harvested for silage when plants were at grain maturity stage, between quarter and two-thirds milkline. The sampling was carried out taking the plants from two parallel rows with 0.60 m distance between them along 5 metres, constituting a sampling area of 6 m^2. Maize samples were chopped (Viking, GE355), homogenized and dried in an oven at 60 °C for 24 h to determine the dry matter content and thus calculate the dry matter yield per ha. The dried maize samples were ground at 0.75 mm and analysed by NIRS (Foss NIRSystem 5000, FOSS NIRSystems, Inc.) for dry matter (DM), crude protein (CP) and starch. The metabolizable energy (ME) was estimated according to NRC (2001). One-way analysis of variance was performed to assess the significant differences in maize yield between different treatments using R software (R Core Team, 2021).

Results and discussion

The effects of biochar applied alone or in combination with compost or digestate on maize yields are shown in Figure 1. The B fertilization showed better agronomic results than BD, BC, and C, although without significant differences (P>0.05). The highest yield in DM, starch and CP and high content in ME were obtained with B fertilization (9,638 kg DM ha^{-1}; 3,212 kg starch ha^{-1}; 815 kg CP ha^{-1} and 115 GJ ME ha^{-1} respectively). Several studies reported that biochar can act as a soil conditioner, which improves soil water holding capacity, enhances soil nutrient retention and boosts plant growth (Sohi *et al.*, 2009). These studies have been conducted in a wide range of climates, soils and crops. The results of this study show that under the agro-climatic conditions of the North of Spain, part of the mineral nitrogen required for maize crop development could be replaced by biochar (alone or in combination with compost or digestate) without affecting the yield or nutritive value. This finding represents an interesting potential key management strategy for maintaining maize productivity while reducing the use of mineral fertilizers. However, this is a preliminary finding and longer-term studies are required to examine the long-term effects of the treatments on soil physicochemical properties and yield and quality responses. It is not therefore possible at this stage to draw any quantitative conclusion, certainly not to project or compare the impact of a particular one-time addition of biochar on long-term crop yield.

Conclusions

The fertilizer treatments tested (biochar, digestate activated biochar and compost activated biochar) did not influence yields or energy contribution of maize crop compared to conventional fertilizer treatment (chemical fertilization). These findings suggest an interesting alternative for reducing amounts of inorganic fertilizers.

Table 1. Nitrogen application rates for basal dressing fertilization in each treatment.[1]

Treatment	Description
Conventional (C)	150 kg N ha^{-1} as basal dressing fertilization with CAN 27%
Biochar (B)	100 kg N ha^{-1} of CAN 27% + 50 kg N ha^{-1} of biochar
Biochar – Digestate (BD)	100 kg N ha^{-1} of CAN 27% + 50 kg N ha^{-1} of biochar mixture with digestate 50:50
Biochar – Compost (BC)	100 kg N ha^{-1} of CAN 27% + 50 kg N ha^{-1} of biochar mixture with compost 50:50

[1] CAN = calcium ammonium nitrate.

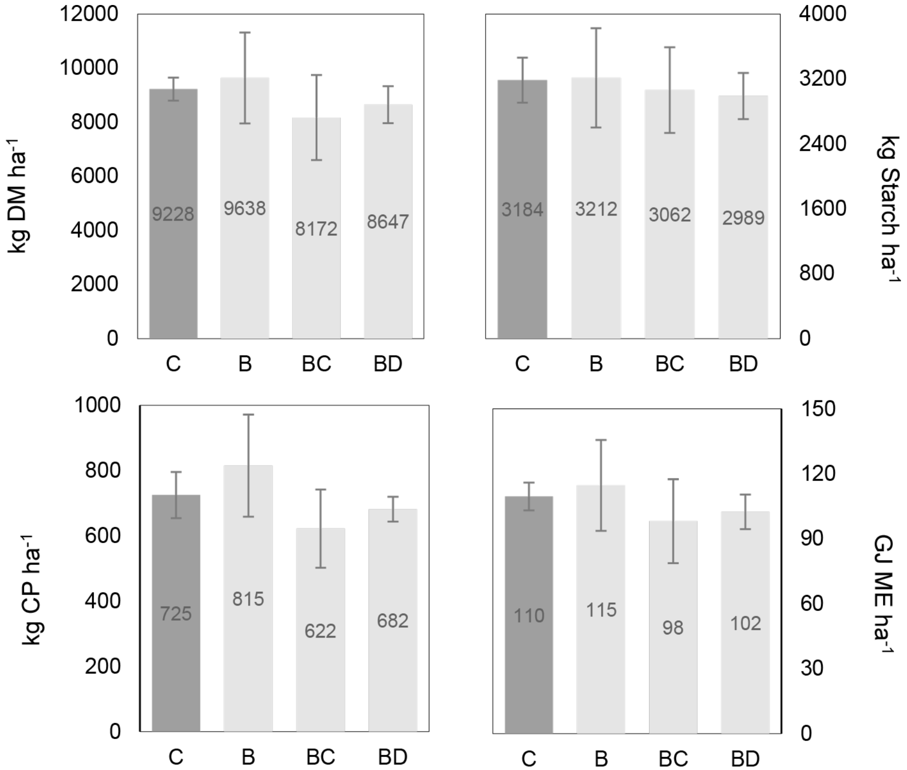

Figure 1. Dry matter (DM), starch, crude protein (CP), and metabolizable energy (ME) yields. Fertilizer treatments: conventional (C); biochar (B); biochar-compost (BC); biochar-digestate (BD). Error bars are standard deviation.

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A review on agricultural soils as a sink for nitrous oxide (N_2O)

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Abstract

Nitrous oxide (N_2O) emissions from agricultural soils account for 60% of the total global N_2O emissions and, for sustainable agriculture, it is essential to reduce these emissions. Since decades, agricultural soils have been viewed as a substantial N_2O source; however, an increasing number of studies also show negative fluxes of N_2O, indicating uptake of N_2O by agricultural soils. To better understand the underlying mechanisms of N_2O uptake in agricultural soils, we reviewed and collected data on soil N_2O uptake and associated soil physio-chemical properties from 135 scientific papers published between 1971 to 2020. We collected data from two dominant agricultural ecosystems, grasslands and croplands, across the globe. Here, we present the current knowledge of soil N_2O uptake and highlight its major drivers. Additionally, we discuss the current status regarding soil N_2O uptake, and future research lines needed to better understand the N_2O flux dynamics in agricultural ecosystems.

Keywords: denitrifiers, nitrous oxide uptake, nitrogen cycle, sustainable agriculture

Introduction

Nitrous oxide (N_2O) is a strong greenhouse gas, ~300 times stronger than carbon dioxide. Agriculture is a major source of N_2O globally because of the use of nitrogen (N) fertilizers – a primary substrate for the two main microbial N_2O production pathways, nitrification and denitrification (Firestone and Davidson, 1989). Therefore, N_2O emission reduction from global agricultural soils is considered as an important step towards agricultural sustainability (Reay *et al.*, 2012). Studies assessing N_2O uptake in agricultural ecosystems are scarce compared to those assessing N_2O emissions, given the general assumption that agricultural soils are net N_2O emitters instead of sinks because of fertilization and the inherent problem to validate negative N_2O fluxes. Nevertheless, previous reviews on soil N_2O uptake (Chapuis-Lardy *et al.*, 2007; Liu *et al.*, 2022) highlighted the importance of soils as an N_2O sink, including the regulatory role of soil properties and agricultural practices. Here, we present the current knowledge of N_2O uptake rates from global grasslands and croplands and highlight its drivers. Additionally, we will discuss the further research needed to improve our knowledge of N_2O uptake in agricultural systems.

Material and methods

The data in this study were collected from 135 published scientific papers searched in the Web of Science from years 1971-2020 with 'nitrous oxide uptake in soil' as the key search phrase. Nitrous oxide uptakes rates were extracted from text, tables and figures, including supplementary files. We used an online open-source tool, graphreader (http://www.graphreader.com/) to extract N_2O uptakes rates from figures, especially when the N_2O uptake rate was not evident in the text and when we found figures with more data than represented in the text. We collected minimum and maximum ranges of N_2O uptake rates. Where it was necessary, we converted given N_2O uptakes rates to $\mu g\ N_2O\text{-}N\ m^{-2}\ h^{-1}$ for better representation and allowing comparisons across studies. Finally, we categorized N_2O uptakes rates for the two ecosystem types, 'grasslands' and 'croplands', based on the information given in the papers. The grassland ecosystem also includes pastures, cultivated or growing wild, as per the definition provided by FAO.

Results and discussion

Our systematic review and synthesis represent all climatic zones, extending from the hot tropics to the cold boreal region, and shows that studies reporting N_2O uptake in global croplands and grasslands increased with time (1 study y^{-1} in 1981 vs 8 studies y^{-1} in 2020), especially after early 2000. We found higher N_2O uptake rates for grasslands (-0.1 to -1,066 μg N m^{-2} h^{-1}) compared to croplands (-0.02 to -660 μg N m^{-2} h^{-1}) and these N_2O uptake rates are higher than those reported by Chapuis-Lardy *et al.* (2007). We found N_2O uptake occurring under diverse soil conditions, down to freezing temperatures, at low soil pH (3.5) and high soil pH (8.7), in dry oxic conditions, soils with high mineral N and high enzymatic activities related to denitrification. Our data synthesis challenges the traditional view regarding agricultural soil N_2O uptake, i.e. that N_2O uptake can occur only in soils with low mineral N, low oxygen, and high moisture content.

While it is important to understand N_2O emissions as affected by agricultural management practices and land-use options, our data synthesis indicates that such effects can also be seen in soil N_2O uptake. For instance, we found higher N_2O uptake rates in croplands with reduced tillage or no-tillage compared to conventional tillage (Tellez-Rio *et al.*, 2015). Similarly, we also found uptake of N_2O from both unfertilized and fertilized (370 kg N ha^{-1} y^{-1}, grasslands) soils (Kim *et al.*, 2010). However, the underlying mechanisms behind N_2O uptake are still unknown. After the discovery of N_2O reduction by non-denitrifiers or *nosZ* clade II organisms (Jones *et al.*, 2013; Sanford *et al.*, 2012), there has been great scientific curiosity to know more about the potential of *nosZ* clade II organisms for N_2O reduction. This is because the *nosZ* clade II shows higher functional diversity, the organisms perform denitrification similar to exclusive denitrifiers and non-denitrifier respiration, they can act as electron sinks, and mediate detoxification processes in soils (Shan *et al.*, 2021). A novel finding has shown that soybean (*Glycine max* L. Merr.) – a globally grown leguminous food crop – can substantially reduce atmospheric N_2O by reduction to N_2 via *nosZ* gene transcripts (Itakura *et al.*, 2013). This opens a new avenue of research for promoting agricultural N_2O emission mitigation with other legume crop species, such as alfalfa and clover, which are important leguminous forage crops globally. Indeed, our review has also found evidence showing N_2O uptake in both grasslands (Maas *et al.*, 2013) and croplands (Koga *et al.*, 2004) cultivated with legumes. Based on our literature synthesis, we identify soil pH, soil water content, soil temperature and mineral N availability as important soil physicochemical properties that may affect soil N_2O uptake in croplands and grasslands, as suggested by Liu *et al.* (2022). Nevertheless, we also identified agricultural management practices and cultivated crop species affecting the net N_2O emissions and the N_2O sink strength of croplands and grasslands. We believe that data such as on soil microbial biomass, potential denitrification rates and expression levels of the *nosZ* gene, which were not studied in the available publications could aid in explaining the reported uptake phenomena.

To better understand the N_2O dynamics in agricultural ecosystems, future research should focus on deciphering why, how, where and when in agricultural soils N_2O uptake occurs in tandem with soil N_2O emissions. Future studies should consider the application of a suite of analytical approaches such as $^{15}N_2O$ pool dilution technique (laboratory and *in-situ*) (Yang *et al.*, 2011; Wen *et al.*, 2016), omics methodologies (Jones *et al.*, 2013; Sanford *et al.*, 2012) and static chamber to eddy covariance techniques (Shurpali *et al.*, 2016), along with the assessment of important soil physicochemical characteristics, in the presence and absence of crops of interest, while assessing agricultural soils for net N_2O uptake.

Conclusions

Given the increasing number of studies published annually, a better understanding of negative N_2O fluxes from agricultural ecosystems is urgently needed. Research should consider measuring key regulatory factors to better understand/explain any negative N_2O flux occurring, while until recently such fluxes were considered as chamber flux measurement errors. This will enable us to use these data in earth system models and thus better constrain the global and regional N_2O budgets. This will pave the way to improve N_2O mitigation strategies for agriculture.

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Changes in mineral concentrations during extended growth intervals in grass-legume mixtures

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Abstract

Herbage minerals affect performance of grazing and grass-fed cattle. In silages, knowledge of herbage minerals is desired to balance supplements in indoor feeding rations. Herbage P, Ca, Mg and K concentrations in 4 grasses and 4 legumes grown in binary grass-legume mixtures were investigated under a silage cutting regime. Data were collected during two harvest years in May in replicated plots. Herbage samples were hand-separated and individual species were analysed. The concentrations of Ca and Mg were higher in legumes than in grasses. The rates of change in P, Ca and Mg concentrations during 2-week intervals differed among species, and between the grass and legume functional groups, i.e. Mg and Ca concentrations declined during extended growth in grasses, but not in legumes. 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, 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 extended 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 and annual yields and nutritive values of these mixtures were determined (Elgersma and Søegaard, 2016). Changes in nutritive values of grass and legume components during extended growth were reported (Elgersma and Søegaard, 2018). The aim of this experiment was to study macromineral concentrations of grasses and legumes, grown in mixtures, during extended growth in spring. We hypothesized that mineral concentrations would differ among species. We expected that concentrations of minerals would be higher in legumes than in grasses. As a result of heading, we hypothesized that concentrations would decline at a faster rate in grasses than in legumes during prolonged growth.

Materials and methods

Perennial ryegrass (*Lolium perenne*,'PR') was sown with each of four forage legumes: red clover (*Trifolium pratense,* 'RC'), lucerne (*Medicago sativa,* 'LU'), birdsfoot trefoil (*Lotus corniculatus*'BT') and white clover (*Trifolium repens*'WC'); white clover was also sown with hybrid ryegrass (*Lolium boucheanum*, 'HR'), meadow fescue (*Festuca pratensis*, 'MF') and timothy (*Phleum pratense,* 'TI'), respectively. The 7 two-species grass-legume mixtures were sown in 2006 in a cutting trial with 4 replications in Denmark. Plots were fertilised with 300 kg total N ha^{-1} in cattle slurry. Plots were harvested five times in 2007 and four times in 2008 at a residual stubble height of 7 cm. Dry matter yield and botanical composition were determined at each harvest during two years. Results of all nine harvests were reported by Elgersma and Søegaard (2016). Following harvests of primary growth (14 May 2007 and 21 May 2008), hand-separated samples were analysed for each species in two replicates; for white clover and perennial ryegrass, samples were taken from the perennial ryegrass – white clover mixture. 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 × year as fixed factors (n=2), and of functional group was analysed using the GLM procedure of the SAS statistical package. Furthermore, values of the slopes of linear regression lines, 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 were found among the 8 species in concentrations of Mg, Ca and K in spring (Table 1). As a functional group, the legumes had higher Mg and Ca concentrations than the grasses (P<0.0001). All grass species had lower Mg and Ca concentrations than any legume species. Among legume species, red clover had the highest Mg concentration. Lucerne had the highest Ca concentration and white clover the lowest, and birdsfoot trefoil had the highest K concentration. Among grasses, only for Ca concentration a difference was found i.e. hybrid ryegrass had a lower concentration than meadow fescue and perennial ryegrass.

Table 1. Concentrations (g kg DM-[1]) of magnesium (Mg), phosphorus (P), calcium (Ca) and potassium (K) and their rate of change (Rate) (g kg DM-[1] week-[1]) during 2-week growth intervals in eight species (S) that were grown in two-species grass-legume mixtures. Data are derived from cuts in spring (May) in each of two harvest years.[1,2,3,4]

Species		Concentration				Rate			
		Mg	P	Ca	K	Mg	P	Ca	K
WC		1.87^{b}	2.8^{abc}	14.5^{b}	15.4^{bc}	0.02^{ab}	-0.40^{ab}	1.05^{b}	-3.71^{ab}
RC		2.58^{a}	2.98^{a}	12.6^{c}	18.6^{bc}	0.03^{ab}	-0.47^{ab}	0.56^{c}	-3.86^{ab}
LU		2.03^{b}	2.47^{bc}	16.0^{a}	15.1^{bc}	-0.05^{abc}	-0.37^{ab}	0.74^{bc}	-4.14^{ab}
BT		1.82^{b}	2.94^{ab}	12.0^{c}	27.5^{a}	0.04^{a}	-0.54^{b}	1.86^{a}	-6.32^{b}
PR		0.91^{c}	2.87^{abc}	3.7^{de}	12.5^{c}	-0.08^{bc}	-0.57^{b}	-0.54^{e}	-4.09^{ab}
HR		0.84^{c}	2.40^{c}	2.5^{f}	15.6^{bc}	-0.10^{c}	-0.57^{b}	-0.37^{de}	-4.79^{ab}
MF		1.04^{c}	2.80^{abc}	4.1^{d}	19.1^{b}	-0.07^{abc}	-0.36^{ab}	-0.34^{de}	-6.53^{b}
TI		0.94^{c}	2.88^{abc}	3.0^{ef}	21.0^{b}	-0.05^{abc}	-0.28^{a}	-0.08^{d}	-1.64^{a}
Sign.	S	<0.0001	NS	<0.0001	<0.01	<0.05	<0.05	<0.0001	NS
	Y	NS	<0.05	<0.0001	n.a.	<0.0001	<0.0001	<0.0001	n.a.
	S×Y	NS	<0.05	<0.0001	n.a.	<0.001	NS	<0.0001	n.a.
SE		0.09	0.15	0.30	1.82	0.03	0.06	0.14	1.20
Functional group									
Legumes		2.08^{a}	2.82	13.8^{a}	19.1	0.01	-0.44	1.05^{a}	-4.27
Grasses		0.93^{b}	2.74	3.3^{b}	17.0	-0.07	-0.45	-0.34^{b}	-4.51
Sign.	FG	<0.0001	NS	<0.0001	NS	<0.05	NS	<0.0001	NS
	Y	NS	NS	<0.0001	n.a.	<0.0001	<0.0001	<0.01	n.a.
	FG×Y	NS	<0.05	<0.0001	n.a.	<0.01	NS	<0.05	n.a.
SE		0.07	0.09	0.36	1.74	0.02	0.04	0.16	0.71
NRC[3]		2.2	3.5	6.2	6.0				

[1] K contents were only determined in year 2.

[2] Within a column, values without a common superscript are significantly different; significance of *P* values is shown.

[3] Dairy cow requirement (g kg DM-[1]) in rations (NRC, 2001).

[4] Species abbreviations: Legumes: white clover (WC), red clover (RC), lucerne (LU), birdsfoot trefoil (BT), and Grasses: perennial ryegrass (PR), hybrid ryegrass (HR), meadow fescue (MF), timothy (TI). Effects of functional group (FG) and year (Y) on concentrations and rates are also shown. SE = standard error, n.a. is not applicable, NS = not significant.

Differences were found among the 8 species in rates of change in concentration of Mg, P and Ca during extended growth. Mg and Ca concentrations, on average, did not decline in legumes, whereas they did decline in the grasses. P and K concentrations declined in all species. Among legumes, the Ca concentration increased fastest in birdsfoot trefoil and was slower in red clover than in white clover. Among grasses, the P concentration declined slower in timothy than in the ryegrasses, the Ca concentration declined faster in perennial ryegrass than in timothy, and the K concentration declined fastest in meadow fescue.

Lucerne and red clover had comparable herbage production levels in mixtures with perennial ryegrass, but red clover had a higher digestibility and protein concentration, and a lower NDF concentration than lucerne, while digestibility and protein concentration declined faster and NDF concentration increased faster in lucerne during extended growth than in red clover. The perennial ryegrass – red clover mixture had the most desirable Ca and Mg profile for dairy cows; moreover, this mixture was superior in terms of productivity, legume content, N concentration and N yield (Elgersma and Søegaard, 2016). Results of these studies can be used in practice when designing grass-legume mixtures to find a balance between the aims of high forage yield, nutritive value and mineral concentration, and resilience to changes during prolonged growth in spring.

Conclusions

As hypothesized, concentrations of Ca and Mg differed between the grasses and legumes functional groups, being higher in legumes. Concentrations also differed among species within each functional group. Red clover had the highest Mg concentration. Contrary to expectation, functional groups were not different for levels, nor for rates of change, in P and K concentrations. The results indicate a potential for optimizing dairy cow mineral intake from home-grown herbage through inclusion of legumes in the sward.

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Nitrate leaching from fertilized grass-clover leys and legacy effect following cultivation

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Abstract

Grassland cultivation is accompanied by a large increase in mineralization of N. Depending on fertilizer history and management in the arable phase of the crop rotation this may lead to considerable amounts of nitrate being leached. This study's goal was to determine nitrate leaching in fertilized grass-clover leys and in following cultivation in spring cereals. The experiment was carried out on-farm at two sites over two years of grassland with increasing applications of mineral fertilizer (0-480 kg available N ha^{-1}) alone or in combination with a basic application of cattle slurry, and two years after spring cultivation with spring cereals. During the grassland phase the type of fertilizer had no effect on nitrate leaching, which increased quadratically as a function of application rate, with a range of 3 to 117 kg N ha^{-1} (0.33-17 mg l^{-1}) in the first year and less in the second year, when clover proportion was lower due to the self-regulatory nature of grass-clover mixtures. Following cultivation, spring barley harvested green with Italian ryegrass, undersown efficiently, reduced nitrate leaching in the first season compared to spring barley without a cover crop. With fertilization in grass-clover leys below 200 kg N ha^{-1}, 0-5% of additional fertilizer-N was leached. Similarly, with an efficient cover crop strategy following cultivation, leaching was kept at low levels. Overall, it was possible to compose mixed ley-arable rotations that fulfilled nitrate leaching requirements even where restrictions on nitrogen load to aquifers are significant.

Keywords: fertilizer, grass-clover, legacy effect, nitrate leaching

Introduction

Grass-clover leys are important for dairy farming. The grassland area is predicted to expand for a variety of reasons, including carbon sequestration to counteract climate change, the need for raw materials for higher-value goods (green bio processing and biochemical manufacturing), and energy production. Herbage response to N application varies between years and sites but it is generally found that N application increases grass production partly at the cost of clover but with an overall productivity advantage (Nyfeler *et al.*, 2009). On dairy farms, slurry is collected and utilized as a fertilizer in systems where cows are fed partly or year-round indoors, and farmers typically apply slurry in the early season and mineral fertilizer later.

Estimating the increase in nitrate leaching for a crop by adding extra N – the 'marginal leaching' – is a good measure when evaluating crop N-use efficiency and associated losses. This study's goal was to determine nitrate leaching in fertilized grass-clover leys as well as the nitrate leaching in cereals following grassland cultivation.

Materials and methods

The experiment was carried out on-farm at two sites over three years. Grassland was supplied with increasing applications of mineral fertilizer (0-480 kg available N ha^{-1}) alone or in combination with a basic application of cattle slurry in the two years prior to spring cultivation and spring cereal cropping. Details on the experimental setup, parameters and management can be found in Kristensen *et al.* (2022). Briefly, the experiments started in 2018 at two farmers' fields in south-western Jutland, Denmark (hereafter 'SW') and in mid-western Jutland (hereafter 'MW'). The soils at both sites were classified as sandy soils. The ley at SW consisted of 10% white clover (*Trifolium repens* L.) and 90% of different varieties of perennial ryegrass (*Lolium perenne* L.). At the MW site, the ley consisted of a mixture of 11% red clover (*Trifolium pratense* L.), 7% white clover, 37% perennial ryegrass and 45% festulolium (*Festulolium braunii*, K.A.). At both sites, ten treatments in four replicates were arranged in a randomized plot design. For two consecutive years (2018-2019), plots (12×3 m) were fertilized with increasing N levels from 0 to 480 kg plant available N ha^{-1} yr^{-1}, either as mineral fertilizer or combined with a basic application of cattle slurry (120 kg available N ha^{-1}). Both were applied in spring and after each cut. In the following year (2020) after spring cultivation was established, spring barley (*Hordeum vulgare* L.) with Italian ryegrass (*Lolium multiflorum* L.) was undersown to estimate legacy effects. Before the experiment started, three suction cups were installed at a depth of 1 m in each plot. During the period September-March, soil water was sampled approximately every second week by exerting a negative pressure of 80 kPa about three days prior to sampling. Nitrate leaching was calculated as described by Eriksen *et al.* (2015).

Results and discussion

During the grassland phase, the type of fertilizer had no effect on nitrate leaching (Figure 1). Leaching increased quadratically as a function of application rate, with a range of 3 to 117 kg N ha^{-1} (0.33-17 mg l^{-1}) in the first year and less in the second year, when the clover proportion was lower due to the self-regulatory nature of grass-clover mixtures. Following cultivation, spring barley harvested green with Italian ryegrass undersown efficiently reduced nitrate leaching in the first season compared to spring barley without a cover crop (Figure 2). This was independent of application rate and type of fertilizer used in the previous years with grass-clover (Figure 2). These results illustrate the importance of efficient cover crops such as the fast-growing Italian ryegrass in the arable phase of mixed ley-arable rotations.

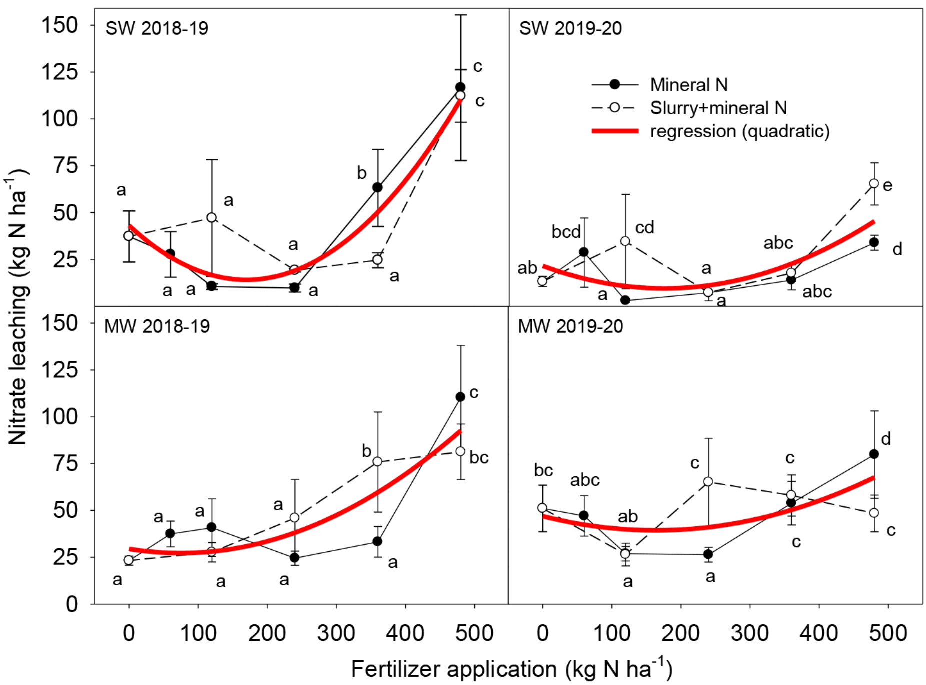

Figure 1. Annual nitrate leaching at both sites and two years as function of N fertilizer rate (sum of mineral N in fertilizer and slurry) and fitted regression. Error bars: SE (n=4). Different letters indicate significant differences between treatments (P<0.05).

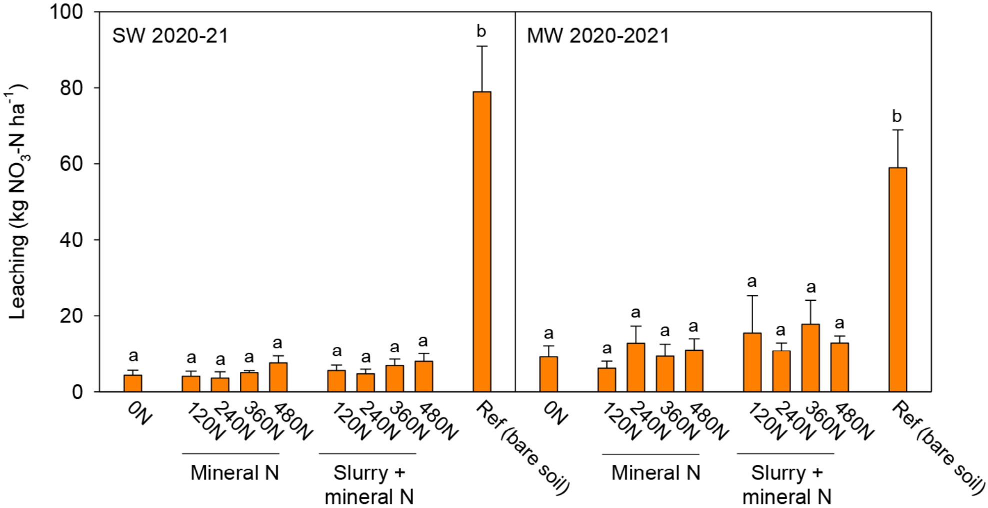

Figure 2. Annual nitrate leaching at both sites following grassland cultivation and seeding of unfertilized spring barley with undersown Italian ryegrass. Fertilizer rates during the grassland phase are indicated. 'Ref (bare soil)' refers to spring barley plots without cover crop. Error bars: SE (n=4). Different letters indicate significant differences between treatments ($P<0.05$).

Conclusions

With fertilization in grass-clover leys below 200 kg N ha^{-1}, 0-5% of additional fertilizer-N was leached (marginal leaching). Above 200 N ha^{-1} the marginal leaching increased dramatically. Similarly, with an efficient cover-crop strategy following cultivation, leaching was kept at low levels. Overall, it was possible to compose mixed ley-arable rotations that enabled nitrate-leaching requirements to be fulfilled even where restrictions on nitrogen load to aquifers are significant.

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Comparison of forage legumes for ecological intensification in sustainable agricultural systems

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Abstract

Two analogous field experiments were carried out in 2018 and 2019 at the Joniškėlis Experimental Station of the Lithuanian Research Centre for Agriculture and Forestry. The soil at the experimental site was classified as an *Endocalcari-Endohypogleyic Cambisol.* The study was aimed to determine the intensity of growth of white clover (*Trifolium repens* L.) black medick (*Medicago lupulina* L.) and Egyptian clover (*Trifolium alexandrinum* L.) and the quantities of nutrients accumulated in their biomass and transferred to winter wheat yield. The forage legumes were cultivated using conventional tillage system on an organic farm. The aboveground biomass of forage legumes was dependent on the year's meteorological conditions, plant growth stage and cultivation method. In both years, black medick had the lowest aboveground biomass and white and Egyptian clover had the highest. Annual Egyptian clover developed the fastest. The species differed in their intensity of absorption of nutrients. In the aboveground biomass white clover accumulated large amounts of nitrogen and potassium; for Egyptian clover, nitrogen and phosphorus; and black medic, potassium. All species of forage legumes had significant differences on the subsequent yield of winter wheat.

Keywords: aboveground mass, black medic, white and Egyptian clovers, nutrients, organic agriculture

Introduction

Forage legumes plants are one of the main sources of nitrogen (N) in agroecosystems (Raza *et al.*, 2020). Most crop farms are dominated by legume crops (peas, beans, lupins) that are sensitive to phytosanitary breaks (Arlauskienė *et al.*, 2020); however, forage legumes are much more beneficial (Gecaitė *et al.*, 2021). The modern use of perennial legumes is primarily for forage production, but integration into arable systems can enhance sustainability and preserve environmental integrity (McKenna *et al.*, 2018). The results clearly show that N fixation capability varies significantly with species of forage legume. N fixation also varies significantly with management and environmental factors that affect plant development and total N yield (Raza *et al.*, 2020). Furthermore, clover has a high potential for N fixation, has a low carbon to nitrogen ratio (C/N) and a fast rate of biomass decomposition and transfer to subsequent crop (Rittwika and Supatra, 2020). There is a need for as much research data as possible on the technological peculiarities of the production of legume crops. It is also important to study their biopotential and usefulness, ecosystem services provided, and to highlight their added value in the feed and food chains. The study was aimed to determine the intensity of growth of white clover (WC) (*Trifolium repens* L.), black medick (BM) (*Medicago lupulina* L.) and Egyptian clover (EC) (*Trifolium alexandrinum* L.) and the quantity of nutrients accumulated in their biomass and transferred to winter wheat (WW).

Materials and methods

Two analogous field experiments were established and carried out in 2018-2019 and 2019-2020 in separate areas. In the first year of the experiment in 2018 and 2019, the main crop was spring oats (O) and forage legumes, in second year – WW. The O (cv. Migla DS seed rate 180 kg ha^{-1}) was sown on 23 April 2018 and 16 April 2019 using narrow spacing a drill (with 0.125 m row spacing) at 3 cm depth. At the same time the forage legumes BM (cv. Arka 133 DS), WC (cv. Nemuniai) and EC (Cleopatra) (seeds rate 8 kg ha^{-1} in 2018; 10 kg ha^{-1} in 2019) were sown in monocrops at 2 cm depth using a narrow spacing drill. The oat grain yield harvested on 2 August 2018, and on 4 August 2019, the straw was chopped and spread. Forage legumes were mulched twice during the growing season (mid-July and late summer before ploughing). Using conventional tillage (used deep inversion tillage and a pre-sowing cultivation unit) winter wheat (cv. Gaja DS; seed rate 230 kg ha^{-1}) was sown with 0.125 m row spacing, at a depth of 2.5-3.0 cm (in 2018 on 27 September, in 2019 on 25 September 2019). Grains yield of winter wheat were harvested with a small combine harvester when the cereals were at full maturity stage (BBCH 89). Grain samples (1 kg) of WW were taken from each plot for determination of dry matter (DM) and nutrients content. The aboveground mass of forage legumes was determined twice, before the first mulching and at the cereal ripened grain (BBCH 87-89 growth stages). The aboveground mass of legumes was cut from four locations in each field (0.25 m^2 sites), weighed, dried to air-dry mass, crushed and the dry matter was determined (dried to constant mass at 105 ° C temperature). The total yield and amount of nutrients were determined by summing the data of both legumes cuttings. Plant samples for N determination were analysed using the Kjeldahl method with a Kjeltec system 1002 (Foss Tecator, Hoganas, Sweden). The concentration of phosphorus (P) was quantified spectrophotometrically by a coloured reaction with ammonium molybdate-vanadate at a wavelength of 430 nm on a Cary 50 UV-Vis spectrophotometer (Varian Inc., Palo Alto, CA, USA). Respective potassium (K) concentration was evaluated by atomic absorption spectrometry with an Analyst 200 (Perkin Elmer, Waltham, MA, USA) in accordance with the manufacturer's instructions. The data were statistically processed using one-factor analysis of variance (ANOVA). The significance of differences among the treatment means was estimated at the 0.05 probability level.

Research and discussion

First experimental year. Results showed that the yield was most affected by the meteorological conditions of the years. Assessing the different species of forage legumes, it was found that BM had the lowest yield of aboveground biomass and WC and EC had the highest. The yield of the aboveground mass of these forage legumes was not differ significantly from each other (data not shown). Significant differences in the content of N, P, K in the aboveground mass of forage legumes were found (Table 1).

According to two years of data, WC (in 2018) and EC (in 2018 and 2019) accumulated significantly higher N and P content in the aboveground mass, compared to BM species. The greatest aboveground mass K content was measured in WC. Of the forage legumes, BM accumulated the lowest levels of K as well as N and P.

Second experimental year. All species of forage legumes (as preceding crops) had a significant impact on the yield of winter wheat (Table 2).

Table 1. The amount of nutrients accumulated in the aboveground mass of forage legumes.

Years	Crops	Concentration of nutrients g kg^{-1} DM			Accumulated nutrients, kg ha^{-1} DM		
		N	P	K	N	P	K
2018	BM	28.10bc	2.84a	22.77a	65.13d	6.58b	52.32d
	WC	28.87c	2.95a	32.40abc	117.01c	10.22c	111.48c
	EC	28.50bc	3.29b	30.37ab	100.72c	11.65c	106.32c
	Average	28.49	3.03	28.51	94.29	9.48	90.04
2019	BM	30.9abc	2.97abc	27.93 b	82.51b	7.95b	74.51b
	WC	28.87a	2.75a	34.13d	96.36bcd	8.84bcd	110.63d
	EC	29.63abc	2.88abc	26.90b	115.74d	11.25d	105.12bcd
	Average	29.80	2.87	29.65	98.20	9.35	96.75

[1] BM = black medick; WC = white clover; EC = Egyptian clover; means followed by the same letters are not significantly different at $P<0.05$.

Table 2. Nutrients uptake intensity in winter wheat.[1]

Years	Pre-crops	Winter wheat grain yield kg ha^{-1}	Accumulated nutrients in yield (grain + straw)					
			N		P		K	
			kg ha^{-1} DM	change± %	kg ha^{-1} DM	change± %	kg ha^{-1} DM	change ± %
2019	0	2,808a	57.9a	0	13.7ab	0	27.7a	0
	BM	3,668b	84.6bc	+26.7	14.0ab	+0.3	38.9bc	+11.2
	WC	4,137d	99.0c	+41.1	16.0ab	+2.3	40.1c	+12.4
	EC	3,969bcd	88.9bc	+31.0	17.6b	+3.9	38.5bc	+10.8
	Mean	3,645	82.6	+32.9	15.3	+2.2	36.3	+11.5
2020	0	3,542a	69.0a	0	11.5a	0	47.1a	0
	BM	5,684bc	125.7b	+56.7	17.1bc	+5.6	85.7bcd	+38.6
	WC	5,920c	162.5d	+93.5	20.7bc	+9.2	105.2d	+58.1
	EC	5,418bc	140.1bcd	+71.1	21.9c	+10.4	82.6b	+35.5
	Mean	5,141	124.3	+73.8	17.8	+8.4	80.2	44.1

[1] BM = black medick; WC = white clover; EC = Egyptian clover; means followed by the same letters are not significantly different at $P<0.05$.

The WW grown after WC produced a yield of 4,137 and 5,920 kg ha^{-1} (in 2019 and 2020, respectively), or 47.3% and 67.1% significantly higher compared to the WW grown after O. The N, K (in 2019 and 2020) and P (in 2020) content accumulated in the WW (grain and straw) increased significantly when growing after forage legumes, compared to oats. Experimental data showed that WC as a preceding crop led to the greatest increase in N and K content in WW yield, compared to other legume species. The data indicated a tendency for EC to increase P content in the subsequent WW yield.

Conclusions

Contents of N, P, K in the aboveground mass of forage legumes varied depending on the meteorological conditions of the year and the aboveground mass. All forage legumes had a significant positive impact on the subsequent yield of WW and its accumulation of nutrients.

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Effect of farmyard manure application on renewed, high nature value grassland

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Abstract

Species-rich meadows are usually not fertilised as there is a general consensus among botanists that fertilization results in decline of biodiversity. Nevertheless, long term nutrients removal can lead not just to hay yield reduction but also to decline of biodiversity. The trial aiming to verify the effect of farmyard manure (FYM) on dry matter yield, forage quality and biodiversity was established in November 2019 on low yielding meadow re-established in 2000 after 25 years of arable period, in a protected area of the White Carpathians. Composted FYM at 20 Mg ha^{-1} was applied every year on the sward surface in late autumn to prevent N losses. In 2022, after three FYM applications, the hay yield increased (2.25 vs 1.83 t ha^{-1}) and crude protein content increased (74.2 vs 66.8 g kg^{-1} DM). Mineral content (ash, P, K, Ca and Mg) and other quality parameters (NDF, ADL and NEL) were not affected by FYM application. Yield assessment using rising plate meter showed linear relationship with measured values in 2022 (R^2=0.506).

Keywords: species-rich grasslands, White Carpathians, forage, yield, quality

Introduction

White Carpathians meadows are famous for high diversity of plants and other organisms. Species-rich meadows are usually not fertilised as there is a general consensus among botanists that fertilization results in decline of biodiversity. Nevertheless, long term nutrients removal can lead not just to hay yield reduction but also to decline of biodiversity. The aim of the experiment was to evaluate the effect of regular application of composted farmyard manure on hay yield and quality of species poor meadow established in 2000 on arable land, which was formed from flower-rich meadows in 1970s (Prach *et al.*, 2013).

Materials and methods

The trial was established in November 2019 as a Latin square (four treatments and four repetitions) with plot size 4×4 m. The treatments were as follows: 1. control, 2. composted manure application (20 Mg ha^{-1}), 3. overseeding by autochtonous legumes and a combination of 2 and 3. In this contribution only the effect of manure application was analysed. The plant species with the highest proportion in the forage were *Bromus erectus* Huds. (0-40%), *Festuca rubra* L. and *F. rupicola* Heuff. (both 8-77%), *Arrhenatherum elatius* (L.) Presl (0-15%) and *Fragaria viridis* Weston. The soil is very heavy silty clay loam (clay 36%, silt 51%, sand 13%) chernozem formed on flysh parent rock. Soil characteristics are shown in Table 1.

Coordinates of the site are 48.857°N, 17.434°E. Average long-term annual temperature is 6.9 °C, sum of precipitation is 613 mm and altitude 410 m a.s.l. Traditional management of the meadows was one cut

Table 1. Nutrient status (mg kg^{-1}, Mehlich III), pH, organic carbon and total nitrogen at the start of the experiment (topsoil 0-200 mm).

pH_{CaCl2}	P	K	Ca	Mg	C_{org} (g kg^{-1})	Nt (g kg^{-1})
4.88	20	280	3,074	266	29.6	3.6
acidic	low	good	good	high		

for hay in June and grazing of aftermath by cattle in late August and September. Plant growth is limited by regular summer droughts. Manure was applied manually in late November/early December in 2019, 2020 and 2021. Forage yield was evaluated by trimming the stand at the height of 30 mm from the area of 1 m^2 taken from the central part of each plot on 10 June 2022 after heading of main grass species. Before cutting the forage, botanical evaluation was made and compressed height was measured by rising plate meter on 9 subplots. Forage quality was analysed in an accredited laboratory by standard methods using wet chemistry. One-way ANOVA was employed to determine the differences between the treatments (unfertilised × fertilised).

Results and discussion

FYM increased the forage yield only in the third year after three manure applications (Table 2). Mineral content in the forage was not affected despite high rates of delivered nutrients (Table 2). The explanation can be strong binding of nutrients to clay minerals and to soil organic matter and low ability to uptake these nutrients in dry conditions. A similar slow reaction to FYM use was also recorded by Rodd *et al.* (2021).

When nutrients balance is calculated (Table 4) there are substantial differences between the nutrients applied in FYM and those exported in harvested forage. This probably results in increasing soil nutrient reserves. The highest disproportion is noted for potassium, where the fertilised sward exported just 6.5 kg ha^{-1} of K more than unfertilised one, but 251 kg ha^{-1} K was applied to the soil. Potassium content in the forage is, nevertheless, low. The effectiveness of FYM nutrients at this site is very low. It can be associated with low soil pH or with regular spring and summer drought. Low pH also probably leads to the low occurrence of legumes. The most common legumes in 2022 were *Viccia hirsuta* (L.) Gray and *Trifolium dubium* Sibth., with average proportions in forage 3.9% (0-17%), so their importance for nitrogen fixation was low.

Table 2. Forage yield and minerals content in the forage after 3 years of manure application (20 Mg ha^{-1}) in 2022.[1,2]

Treatment	**DM yield**	**P**	**K**	**Ca**	**Mg**
Unit	Mg ha^{-1}	g kg^{-1} DM	g kg^{-1} DM	g kg^{-1} DM	g kg^{-1} DM
Unfertilized	1.66^a	2.25	11.5	0.26	0.09
Fertilised	2.03^b	2.46	12.6	0.33	0.10
P-value	0.021	0.124	0.181	0.413	0.406

[1] Different superscript letters within the same parameter column mean a significant difference ($P<0.05$).

[2] DM (dry matter) yield was evaluated after sward trimming and weighing.

Ash, fibre fractions and net energy concentration (NEL) were also not changed by FYM application. Crude protein (CP) was the only parameter of forage quality which was increased by FYM (Table 3). Nevertheless, ash and CP content and NEL values were very low for feeding of dairy cows.

Table 3. Forage quality after 3 years of manure application (20 Mg ha^{-1}) in 2022.[1]

Treatment	**Ash**	**NDF**	**ADL**	**CP**	**NEL**
Unit	g kg^{-1} DM	MJ kg^{-1} DM	MJ kg^{-1} DM	MJ kg^{-1} DM	MJ kg^{-1} DM
Unfertilized	49.8	690	62.1	66.8^a	4.56
FYM	52.2	669	60.2	74.2^b	4.66
P-value	0.545	0.508	0.712	0.032	0.523

[1] Different superscript letters within the same parameter column mean a significant difference ($P<0.05$). NDF = neutral detergent fibre; ADL = acid detergent lignin; CP = crude protein; NEL = net energy concentration; FYM = farmyard manure.

Table 4. Manure quality and amount of elements applied and exported by the rate of 20 Mg ha^{-1}.

Parameter, unit	Content in fresh manure, %	Application rate, kg ha^{-1}	Export by forage, kg ha^{-1}	
			Unfertilised	Fertilised
Dry matter	43.1	8.620	1.660	2.030
Org. matter	11.5	2.300	1.577	1.929
N	0.427	85.4	17.7	24.1
P	0.267	53.4	3.7	5.0
K	1.255	251	19.1	25.6
Ca	1.111	222	4.3	6.7
Mg	0.266	53.2	1.5	2.0

The linear relationship between rising plate measurement (nine partial measurements were taken from each 1 m^2 immediately before sward trimming) and DM yield was quite low (R^2=0.506), probably due to uneven botanical composition in particular plots (patches of strawberries and fine-leaved fescue alternating with *Bromus erectus* and *Arrhenatherum elatius*).

Conclusions

There was an increase in forage production only after three years of composted farmyard manure application. Forage quality was not affected by FYM application (minerals, fibre fractions, NEL) with the exception of crude protein. The forage cannot satisfy the nutrients needs of cattle. Hay production is not profitable for farmers in this area and management of these meadows is strongly dependent on subsidies.

Acknowledgements

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Effect of mineral fertilization on root distribution in temperate grasslands

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Abstract

Root development has a major impact on productivity and ecological stability in semi-natural grasslands. There is a lack of information on how root distribution is affected by mineral fertilization over the growing period. We measured the total root dry matter biomass of the meadow stand at depths of 0-15 cm and 15-30 cm during the first and autumn cut. Three levels of fertilization were evaluated: unfertilized control, N50P40K100, and N200P40K100. In the first cut, the highest root biomass was found in N50P40K100 in the 0-15 cm layer. In the deeper layer, the highest root biomass was observed in the treatment N200P40K100. In the autumn cut, N200P40K100 showed the highest root biomass in both layers. Fertilized stands showed a tendency for higher root biomass, but they differed in the depth of their distribution in association with the season. This could have a significant effect on the adaptability of the vegetation to drought periods or the higher efficiency of carbon storage in the soil.

Keywords: *Arrhenatherion* meadow, dry root biomass, nitrogen uptake

Introduction

Grasslands are increasingly coming into a focus as providers of different ecosystem services, such as the carbon storage capacity of soil, water retention or reducing of soil erosion where below ground parts of vegetation and its spatial distribution in the soil play an important role. Applied fertilizers cause changes in botanical composition (e.g. Dindová *et al.*, 2019) and also effects on the distribution of roots (Głab and Kacorzyk, 2011). The coexistence of species in grasslands is possible when the spatial distribution of a plant's root system spreads into different rooting depths, because of strong belowground competition (Casper and Jackson 1997). Plant species with different rooting depths may increase total community nutrient uptake in grasslands, because of differential use of the soil resources (Cardinale *et al.*, 2007) obtained from different soil layers and may contribute to adaptation and resistance to drought stress (Hoekstra *et al.*, 2015). The aim of our study was to reveal how mineral fertilization affects root distribution of whole plant communities in different cuts and layers of the surface soil in an *Arrhenatherion* meadow.

Materials and methods

A long-term fertilization experiment is situated on an *Arrhenatherion elatioris* meadow near the village Senožaty (485 m a.s.l., Czech Republic). The soil is a well-drained Cambisol with a sandy-loamy texture. For more details see Dindová *et al.* (2019). Three treatments were evaluated: an unfertilized control, and two different levels of mineral fertilization, viz, N50 PK and N200 PK (50 kg N and 200 kg N, respectively, 40 kg P, 100 kg K ha^{-1}). The measurements were taken within the EJP Soil project MIXROOT-C in 2022, a year characterized by moderate water deficit in spring and above-average precipitation in the second half of the growing season. *Leontodon hispidus, Achillea millefolium* and *Arrhenatherum elatius* were dominant in control, N50 PK, and N200 PK, respectively. Two-cut management was applied and the yield was measured from an area of 4.8 m^2. Root biomass was measured using a soil core (8 cm diameter) in 0-15 cm and 15-30 cm depths. Before the spring and autumn cuts, root samples were taken from three soil cores from three replicates, resulting in nine samples for each treatment. 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^{-2}. The data were analysed by one-way analysis of variance (ANOVA) within each depth and for each sampling period. Significant differences between means were reported using the Tukey HSD test at α=0.05.

Results and discussion

In 2022, the annual forage yields of treatments were 2.33, 4.6, and 7.73 t ha^{-1} for unfertilized control, N50 PK, and N200 PK, respectively. The majority of root biomass was located in the 0-15 cm layer across all evaluated treatments and both periods (Figure 1). According to Jackson *et al.* (1996), the accumulation of grassland biome concentrates root biomass in the 30 cm topsoil, where our results showed that the 0-15 cm layer represents 97% of total roots up to 30 cm.

The fertilization showed a tendency to increase DRB in all depths and for both sampling periods, but there was no clear relationship between applied nutrient doses and root biomass (Figure 1). This was especially the case in May when the N200 PK treatment resulted in only significantly higher DRB for the deeper profile. In the same experiment, Dindová *et al.* (2019) reported higher ratios of legumes and forbs in N50 PK than in N200 PK in the first cut. It can be concluded that the root biomass was related more to a higher proportion of high-diversity dicotyledonous species groups (N50 PK) than total forage yield in the spring period. These results are in line with Mueller *et al.* (2013) who found a positive correlation between species richness and root biomass and increasing root accumulation in the presence of legumes.

However, the highest amount of DRB was located in both layers in N200 PK in October, indicating a positive relationship between forage and root biomass production. Similarly, Steinaker *et al.* (2008) documented a positive correlation between leaf and root production. In contrast to these findings, a negative effect of nitrogen fertilization was reported for root density (Głab and Kacorzyk, 2011) or root:shoot ratio for plants experiencing nitrogen deficiency as they invested in root formation (Poeplau, 2016).

It seems that the effect of fertilization on root biomass was not consistent within the growing season and could be modified by the proportions of different functional groups, grassland vegetation type or soil humidity (Fiala *et al.*, 2009; Titlyanova *et al.*, 1999). The effect of grassland management on root

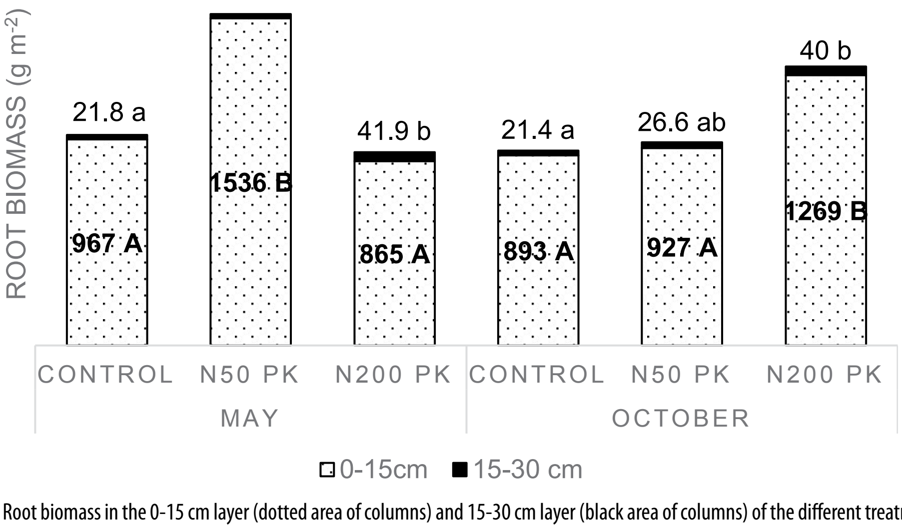

Figure 1. Root biomass in the 0-15 cm layer (dotted area of columns) and 15-30 cm layer (black area of columns) of the different treatments in May and October. Different letters indicate statistical differences between treatments in the 0-15 cm layer (capitals) and 15-30 cm layer (lower case letters) within each sampling period (Tukey HSD, α=0.05).

dry matter distribution is manifested especially in the 0-5 cm soil layer (Głab and Kacorzyk, 2011). Regarding seasonal fluctuation, variability was visible only in the top layer, 0-15 cm, whereas the deeper layer provided a stable amount of roots with clearly higher DRB at the N200 PK treatment.

Conclusions

Based on these one-year results, it can be concluded that fertilization has an obvious effect on root biomass distribution, but this influence could be modified within a season. Clarifying these relationships can help with the optimization of grassland management towards adaptability of grasslands to climate change as well as the improvement of carbon sequestration.

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Can *Equisetum palustre* L. be combatted using electrical weed control?

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Abstract

Marsh horsetail (*Equisetum palustre* L.) is harmful to livestock and it increases in grassland when fertilisation is reduced. The combination of being a toxic feed and having few options to reduce its population brings challenges, especially for organic dairy farming. In a field trial on grassland with marsh horsetail infestation, electrical weed control (Zasso Xpower) was compared to no control of marsh horsetail and aboveground control using a chain harrow. Three replicates were placed in a field with high infestation and three in low infestation. Before treatments were applied, ground cover of marsh horsetail was between 8 and 15% in the high infestation plots and between 0.5 and 4% in the low infestation plots. Chain harrowing showed an initial reduction between 17 and 21%, while electrical control showed a reduction between 42 and 94%. Although initial reduction through electrical control was promising, in the next year marsh horsetail levels returned to that of the control treatment. The reduction through use of the chain harrow was not significant. Therefore, we conclude that a Zasso Xpower, as a single application, to control of marsh horsetail did not result in the desired level of reduction in the longer term. More frequent applications could provide a desirable effect.

Keywords: grassland, herbicide, semi-natural

Introduction

Marsh horsetail (*Equisetum palustre* L.) is harmful to livestock when grazed and is known to increase its population in grassland when fertilisation is reduced (Müller *et al.*, 2020). In grassland with a high level of fertilisation it is unable to compete with surrounding grasses (Borg, 1971). Especially in grassland with a high groundwater table marsh horsetail is able to spread more quickly. Grasslands with marsh horsetail with contents of 5% or more cause issues for livestock. Therefore, it is of great importance to be able to reduce its content in the field without sacrificing yield (Hünsche, 2010). Besides draining and fertilisation there are few known methods to combat marsh horsetail (Mukula, 1963). Only rolling of grasslands is known to reduce marsh horsetail after frequent application (Timmermans *et al.*, 2016). The goal of this research is to study the effect of Electrical weed control (EWC) to combat Marsh horsetail in grassland. EWC could be a potential method to control marsh horsetail as the grass itself is not affected by the electricity (C. Thijssen, personal communication).

Materials and methods

In order to test its effectiveness in a semi-natural grassland with an existing marsh horsetail infestation, electrical weed control (Zasso Xpower) was compared with no control of marsh horsetail, and aboveground control using a chain harrow. The grassland used was a semi-natural grassland which had been managed this way for 15+ years. Three replicates were placed in a high infestation area and three in a low infestation area. All treatments were applied on 13 September 2021 in field area of 3m x 6m. Infestation of marsh horsetail was done by visual estimation by an expert on the basis of canopy cover and expressed in %. This is based on an estimation of standing aboveground biomass. Visual estimations were done one week before application of treatments and one, two, three, five and 35 weeks after.

Results and discussion

In the week before applications of the treatments there was no significant difference in the plots to which the treatments were applied. Marsh horsetail infestation ranged between 8 and 15% in the high infestation plots and between 0.5 and 4% in the low infestation plots. After application of the treatments a decline in marsh horsetail biomass was observed in both the electrical and chain harrow treatments. Use of the chain harrow resulted in a relative reduction of 21 and 17% in the high and low infestation respectively, as observed three weeks after application. In the same period EWC resulted in a relative reduction of 90 and 42% in the high and low infestation respectively. At 36 weeks after application there was only a significant difference between the chain harrow and the EWC observed in the high infestation (Table 1). No significant difference between the control treatment and the EWC was observed in both high and low infestation levels at 36 weeks after treatment. Therefore, one application of EWC for the control of marsh horsetail cannot be considered effective in the longer term. However, the results in the short term suggest the method is able to provide some control over marsh horsetail. It could possibly be effective when combined with other treatments or as multiple applications of EWC. Some drawbacks of this method are the cost per hectare, the possible reduction in other species in grasslands and a possible reduction in grass growth. The application of EWC can be considered effective in term of reducing the aboveground biomass and would therefore be suitable for reducing the harmfulness for livestock.

Table 1. Effect of electric weed control (EWC) treatments on amount of marsh horsetail (% aboveground cover).[1]

Treatment	Before treatment	1 week after	2 weeks after	3 weeks after	5 weeks after	36 weeks after
Absolute infestation high						
Control	10.7	11.3^{a}	11.3^{a}	11.3^{a}	13.0^{a}	13.3^{ab}
Chain	11.7	11.0^{a}	11.0^{a}	9.3^{a}	18.3^{b}	18.0^{a}
EWC	9.3	0.2^{b}	0.2^{b}	1.0^{b}	3.7^{c}	10.0^{b}
P-value	0.698	0.013	0.013	0.007	<0.001	0.012
Absolute infestation low						
Control	2.7	2.7	2.7^{a}	3.0^{a}	6.7	5.3
Chain	2.7	2.3	2.3^{ab}	2.3^{ab}	6.3	5.0
EWC	1.2	0.2	0.0^{b}	0.5^{b}	2.3	2.7
P-value	0.241	0.056	0.030	0.023	0.080	0.165

[1] Treatments were applied on 13 September 2021. Different superscript letters denote significant difference between treatments within measurement moment and infestation level.

Conclusions

Although EWC of marsh horsetail seemed to work in the short term there was no evidence for longer term control. Therefore it is not a viable option for the organic control of marsh horsetail using one application. A further possibility would be a repeated application.

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Grass-legume mixtures: a novel approach to forage production in Ethiopia

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Abstract

Ethiopia has the highest livestock numbers in Africa, and a large part of the population depends fully or partly on cattle for their livelihoods. The country experiences high rates of soil erosion due to degradation of cropland and rangelands, and overgrazing is a serious problem. In this paper, we report results from the first two harvests of two field experiments established in June 2021 at two different highland locations in Ethiopia: Hawassa in the south, and Bahir Dar in the north. Four species; two legumes (*Desmodium intortum* and *Stylosanthes guianensis*) and two grasses (*Brachiaria* hybrid 'Cayman' and *Panicum maximum* 'Mombasa') were sown in monocultures and various mixtures in a simplex design. Dry matter yields and botanical composition from each cut were recorded. The first harvest was taken around 100 days after establishment, while the second harvest was taken during the drought period, i.e. in January 2022 at Hawassa and in March 2022 at Bahir Dar. The difference between monoculture and mixture community performances varied in magnitude from site to site and across harvests; we found evidence of positive interactions between grasses and legumes at Hawassa. These preliminary results show that grass-legume mixtures using tropical species have some potential under Ethiopian conditions.

Keywords: grasses, legumes, monocultures, mixtures, productivity, Africa

Introduction

The livestock numbers in Ethiopia are among the highest in the world and almost all farmers hold some cattle or other livestock (Bachewe *et al.*, 2018). Most of the feed for the livestock is derived from free grazing on overgrazed rangeland and from crop residues. This aggravates the ever-rising problems with soil erosion and soil degradation. Cultivation of forage for livestock is still not very common or widespread in Ethiopia, and the number and variety of forage species used is limited. The adoption of forage legumes by farmers is also minimal (Muir *et al.*, 2014). A shift towards more intensive cut-and-carry feeding, using improved forage species for feed production, could counteract soil degradation. More yielding and better-quality livestock feed than communal grazing on crop residues or rangeland can alleviate the grazing pressure on land used for food crops. Positive mixing effects of grasses and legumes have been found in numerous studies under temperate conditions, e.g. Finn *et al.* (2013), but has not been studied extensively under tropical conditions (Muir *et al.*, 2014). In the current study, we examine the performance of improved grass and legume species. We report on results from the first two harvests of monocultures and various mixtures of grasses and legumes grown at two sites in Ethiopia.

Materials and methods

Field experiments were established at the end of June 2021 in Bahir Dar on a silty loam soil, pH 5.56 (10°30'N, 37°29'E, 1972 m a.s.l.) in the north and Hawassa on a loam, pH 6.63 (7°30'N, 38°28'E, 1660 m a.s.l.) in the south of Ethiopia. At each site, monocultures, and mixtures of two, three and four species were sown in a simplex design (Cornell, 2002) in a total of 60 plots (3×7 m^2). The species were the grasses *Brachiaria* hybrid 'Cayman' and *Panicum maximum* 'Mombasa', and the legumes *Desmodium intortum* 'Greenleaf 'and *Stylosanthes guianensis* 'Ubon'. In addition to monocultures, mixtures of two, three and four species varied to include both equal and unbalanced proportions, i.e. one species dominated the mixture. The plots were fertilised with 100 kg DAP ha^{-1}, i.e.19 kg N, 16.5 kg P and 7 kg S ha^{-1} at sowing. No fertilisers were distributed after establishment. The species *S. guianensis* did not establish well at either site. All plots were weeded manually after establishment. Plots were harvested manually after ca. 100 days in mid-October 2021 at both sites. The second cut was taken 17 January 2022 at Hawassa, and 28 March 2022 at Bahir Dar, during their respective drought seasons. All biomass (sward height ca 7 cm) in the central 2×2 m^2 of the plots was weighed fresh, and a sub sample of 1 kg was weighed fresh, and then air dried at ambient temperatures to stable weight for determination of dry matter (DM) yield. To adjust for the non-establishment of *S. guianensis*, monocultures of this species were omitted from the statistical analysis, and the proportions of other species in mixtures that included *S. guianensis* were recalibrated to sum to 1. For each harvest by site combination, the DM yield was modelled as a function of the (adjusted) sown proportions and species interactions, following the diversity-Interactions modelling approach (Kirwan *et al.*, 2009).

Results and discussion

The modelled yields differed clearly between the two sites. In the 1st harvest, the yields in Hawassa were approximately three times higher than those in Bahir Dar (Figure 1). This reflects differences in both edaphic and climatic conditions between sites. Bahir Dar has poorer, more acidic soils, and had low rainfall during establishment of the experiment, while Hawassa has richer soils and more rain.

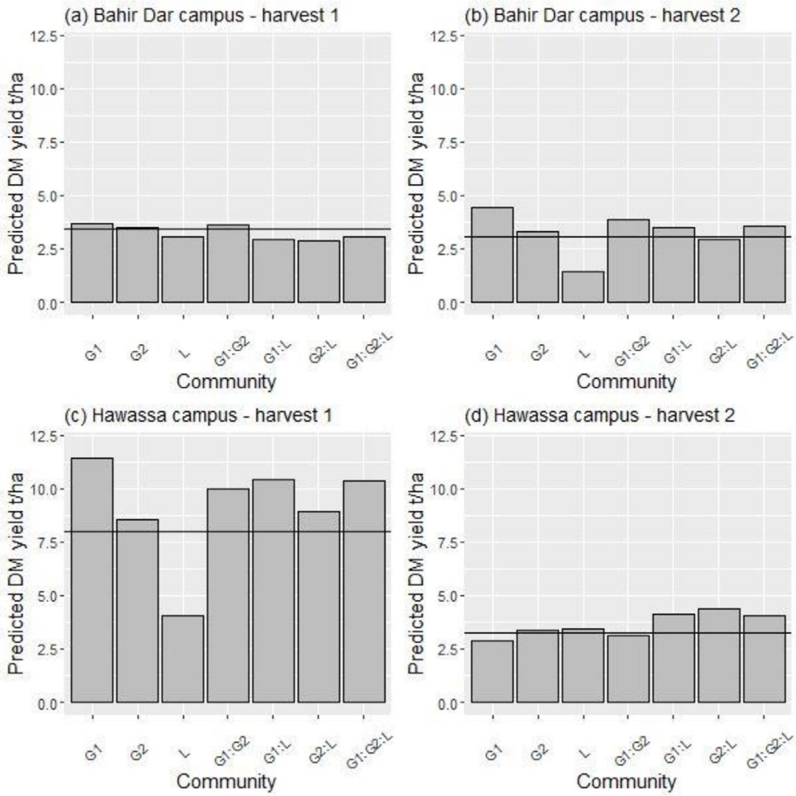

Figure 1. Model predictions of dry matter yield (t ha^{-1}) in 1st and 2nd harvest at Bahir Dar (A and B) and Hawassa (C and D) from monocultures with *P. maximum* (G1), *Brachiaria* hybrid (G2), *D. intortum* (L) and 50:50 mixtures of the two grasses (G1:G2), P. *maximum*: *D. intortum*, (G1:L) and *Brachiaria*:*D. intortum* (G2:L) and the 0.33:0.33:0.33 mixture (G1:G2:L). The horizontal line in each panel shows the average performance of the monocultures.

In the first harvest at Bahir Dar, we found no evidence of differences in modelled DM yield across the species diversity gradient (Figure 1A). In the 2nd harvest we found strong differences in species performances in monoculture (Figure 1B), with the two grasses outperforming the legume species, however, there was no evidence of interspecific species interactions or overyielding in mixtures.

In the first harvest at Hawassa, again strong differences in species performances in monoculture were identified with the similar pattern of the two grasses outperforming the legume species (Figure 1C). At both harvests in Hawassa (Figures 1C and 1D, a positive grass-legume interaction was identified, leading to improved yield of grass-legume mixtures over their respective weighted average monoculture performances.

The growth of *D. intortum* seedlings is relatively slow (Mwangi *et al.*, 2004), which may account for its lower performance in the second harvest at Bahir Dar and the first harvest at Hawassa.

Conclusions

These preliminary results show that mixing grasses with the *D. intortum* legume can potentially increase forage productivity in Ethiopian conditions. An additional benefit of including legumes in tropical productive grasslands may be improved soil health and reductions in land degradation.

Acknowledgements

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How intensification of extensive grasslands affects yield and ecosystem services

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Abstract

Forage from extensively managed grassland is often difficult to utilize. An intensification of management by increasing N input and cutting frequency could improve forage quality for use in profitable cattle production. We set up a three-year field experiment with four intensity levels (moderately managed permanent grassland, intensively managed permanent grassland, ley grass, and maize) on five sites on a climatic gradient from west to east in northern Germany. Yields of all grassland treatments were relatively low, but net energy contents and crude protein concentrations were sufficient for use in extensive to moderately intensive cattle production systems. Grass from leys had similar crude protein concentrations as that from intensively managed permanent grassland. Herbage from intensively managed permanent grassland had higher N yields than that of moderately intensive permanent grassland, while the number of plant species was not reduced. Maize yields were in a common range, but maize showed a higher risk of nitrogen leaching compared to the grassland treatments. We conclude that if the aim is to have a forage production system that delivers yields of adequate quality without a reduction in ecosystem services like biodiversity, a cautiously intensified management of permanent extensive grassland can be an option on sandy soils.

Keywords: maize, ley grass, nitrogen, biodiversity

Introduction

Extensively or moderately managed permanent grasslands deliver biomass for livestock and energy production and provide ecosystem services like biodiversity and wildlife protection, carbon sequestration, water purification, nutrient retention and also social benefits like recreation. There has been a continuing trend to intensifying forage production in cattle production systems throughout Europe (Reheul *et al.*, 2018). This process leads to an increased risk of loss of other ecosystem service functions (Allan *et al.*, 2015). In recent years, substantial areas of grassland on freely draining soils in northern Germany have been ploughed and sown with grass (leys) or maize. In this study, we wanted to find out how yield and forage quality and other ecosystem services of formerly extensive grassland are influenced by a gradual intensification of the system. Our hypothesis is that an increase in management of rather extensively managed agricultural grassland can lead to adequate yields and forage qualities while ecosystem services like biodiversity and nutrient retention are preserved.

Material and methods

We set up a three-year field experiment (2012-2014) and compared four treatments on a gradient of increasing intensity of production (represented by a combination of varying N input, cutting frequency of grassland, and land-use change from permanent grassland to arable land) on five sites along a climatic gradient from west to east in northern Germany (Table 1). There were four treatments in a randomized block designs with three replications; each plot had a size of 15 m^2 (3×5 m).

The climatic conditions in the survey area range from sub-maritime conditions (713 mm precipitation) in the west to sub-continental conditions (568 mm precipitation) with warmer and drier summers in the east. All sites are located on soils with similar soil texture (loamy sand to sandy loam). Before the start of

Table 1. Experimental design. Three replications in blocks at each site.[1]

Treatments	Cutting frequency	Amount and partitioning of N (kg N ha^{-1} yr^{-1})
1. Moderately managed permanent grassland (PGM)	2	80 (50/30)
2. Intensively managed permanent grassland (PGI)	4	240 (80/60/60/40)
3. Conversion of grassland to arable: Ley grass (Ley)	4	340 (110/90/80/60)
4. Conversion of grassland to arable: Maize (Maize)	(1)	160 (80/80)

[1] Five sites on a west-east gradient between 51°50′ to 53°05′ North, and 8°19′ to 14°38′ East in northern Germany in an area called the North German Plain. P, K, Mg as mineral fertilizer according to expected offtake.

the experiment all sites had been managed for >10 years as extensive grassland with no more than two cuts per year and N fertilization <100 kg N ha^{-1} $year^{-1}$. At the start of the experiment, vegetation on plots for conversion to arable land was killed by herbicide and the plots were ploughed in spring. Silage maize ('Ambrosini', FAO 215) was then planted with 8 seeds per m^2 for the maize treatments. Ley plots were sown with a ley grass mixture with 29% *Lolium multiflorum*, 29% *Lolium hybridum,* and 42% *Lolium perenne* and then left intact. The maize plots were ploughed each year. Before each grass harvest two randomly chosen samples were taken in an area of 1 m^2. Before harvesting the maize in mid- to end-September each year, 40 plants per plot were cut manually, the biomass weighed and chopped with a branch cutter. All plant samples were oven-dried at 60 °C for 48 h to determine dry matter (DM) and dried material was ground to 1 mm and analysed by near-infrared spectroscopy (NIRS) for forage quality parameters. Energy content (NEL) was calculated in accordance with the *German Society of Nutrition Physiology* (GfE, 2009). Nitrogen use efficiency (NUE) was calculated as the ratio between the amount of fertilizer N removed with the crop and the amount of fertilizer N applied. Plant diversity was determined in all plots on all five sites in July of the second year by visual assessment following Braun-Blanquet.

Results and discussion

Results are presented as averaged over all sites and years. Differences in DM yields and other parameters among sites did not correlate with climatic parameters along the west-east gradient (data not shown). Differences among years were also not correlated with precipitation and temperature. However, the Ley grass treatment had a slow establishment in the first year, but yields and forage quality increased substantially in the second and third year.

Usually, fertilization, with N especially, rapidly increases productivity of semi-natural grasslands (Chalcraft *et al.*, 2008). In our study, herbage from intensively managed permanent grassland had higher N yields, energy content, and crude protein concentration than that of moderately intensive permanent grassland, but a similar dry matter yield. A further step in production intensity from intensive permanent grassland to ley grass, which included ploughing of the sward and reseeding, however, showed no positive effects on forage quality parameters (Table 2). With the transition to ley grass, an established plant community that was adapted to site conditions and former management had been destroyed. Generally, the mean dry matter yields of all grassland treatments were relatively low (Table 2). Mean dry matter yields ranged from 4.8 t DM ha^{-1} in moderately intensive permanent grassland to 5.8 t DM ha^{-1} in ley grass. The mean energy content and crude protein concentrations in all grassland treatments were sufficient for most livestock feeding systems apart from intensive dairy production (Table 2).

The nitrogen use efficiency (NUE) decreased with increasing amounts of N application. The NUE of moderately intensive permanent grassland was above 100% in all years which indicates a non-balanced nitrogen supply – there was a need for N from soil resources in addition to the fertilizer N. The overall amount of soil mineral N in top soil (0-30 cm) in our experiments was generally relatively small. However,

Table 2. Dry matter yield and parameters for forage quality and ecological indicators among treatments averaged over three years and five study sites.[1,2]

Treatment[3]	DM yield (t ha^{-1})	N yield (kg ha^{-1})	NEL (MJ kg^{-1})	CP (g kg^{-1})	NUE (%)	SMN (kg ha^{-1})	Plant species richness[4] [n]
PGM	4.79^{a}	97^{a}	5.45^{a}	134^{b}	121^{c}	15^{a}	14.9^{b}
I PGI	5.53^{b}	129^{bc}	6.01^{b}	155^{c}	54^{a}	21^{ab}	14.7^{b}
Ley	5.82^{b}	143^{c}	6.15^{b}	159^{c}	42^{a}	24^{b}	10.5^{a}
Maize	15.43-	121^{b}	6.02^{b}	55^{a}	76^{b}	42^{c}	13.4-
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[1] N yield = N offtake with harvest; NEL = energy content (net energy lactation); CP = crude protein; NUE = nitrogen nutrition index; SMN = residual soil mineral N in autumn (0-30 cm).

[2] Different letters indicate significant differences among treatment means. Maize was not included in statistical analysis for dry matter yield and plant species richness. Results from ANOVA analysis.

[3] PGM, PGI = moderately and intensively managed permanent grassland, respectively.

[4] Plant species richness for second year.

42 kg ha^{-1} (0-30 cm) for maize pose a potential risk for N translocation down the profile and subsequent N leaching. The permanent grasslands in our experiment would not be categorized as 'species-rich grassland'. During the three years of the study, the larger inputs of N in the intensively managed grassland combined with a higher cutting frequency of four instead of two cuts per year did not lead to a loss of plant diversity (species number) compared to the less intensively managed grassland (Table 2).

Conclusions

There is often a need to combine the provision of ecosystem services of more extensively used grasslands with the utilization in agricultural production, especially on lighter soils.

A cautious increase of N input and a higher cutting frequency might be an option and can help to stabilize yields and increase the forage quality for agricultural use without necessarily reducing ecosystem services.

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Soil seed bank response to agrophytocenoses on hillside ecotopes

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Abstract

The objective of the study was to estimate species composition of the soil seed bank and compare it with vegetation of agrophytocenoses on hilly relief. Soil samples were taken from the permanent grassland (28 years) and cereal-grass crop rotation. The impact of the hill parts on agrophytocenoses was unequal. In spring and autumn, significantly smaller number of seeds in the seed bank (on the average 4.0 thousand seeds m^{-2}) was determined in the soil of permanent grassland compared to the soil of cereal-grass crop rotation. Thirty-eight plant species have been found in the soil seed bank: 24 species of them belonged to segetal plants, 13 meadow species and one tree species. In permanent grassland, the most similar species composition of the soil seed bank was in the summit and midslope parts of the hill (Cs=0.71-0.90), while in cereal-grass crop rotation, the closest in species composition (Cs=0.71-0.89) was the soil seed bank in the midslope and footslope of the hill. The floristic similarity of vegetation and seed bank species composition in all parts of the hill was determined to be minor (Cs=0.10-0.48).

Keywords: cereal-grass crop rotation, floristic similarity, parts of the hill, permanent grassland, soil seed bank, species of seed

Introduction

About 14% of Lithuania's agricultural land is eroded, but this proportion is higher in areas of hilly relief where it comes up to 25-53% (Lietuvos dirvožemiai, 2001). Therefore, grasslands are the most important means for soil erosion prevention (Jarašiūnas, Kinderienė, 2016). Due to uneven soil erosion in selective parts of the hill, different conditions in the hilly topography become present for the plant development, including differences in soil humidity, acidity, richness in nutrients, humus, and others (Monstvilaitė and Kinderinė, 2000). The alterations of plant communities and the contamination of soil by seeds appear because of differences in pedological aspects and crop management (Skuodienė *et al.*, 2018). The soil seed bank is essential for the stable crop system as it provides the base for biodiversity. The objective of the present study was to estimate species composition of the soil seed bank and compare it with vegetation of agrophytocenoses on areas of hilly relief.

Materials and methods

The experiment was carried out at the Vezaiciai Branch of the Lithuanian Research Centre for Agriculture and Forestry on the hilly topography of Zemaiciai Highland (55°577′ N, 22°482′ E, 185.0 m a.s.l. – WGS). The study analyses long-term monitoring data of a soil erosion experiment set up on slopes of 9-11°. The soil of the southern exposition slope was slightly eroded *Eutric Retisol (loamic) (*RT-eu.lo*).* Soil agrochemical and physical properties are presented in Table 1.

In 1983, to protect the hill from erosion, a mixture of five grass and legume species (*Phleum pratense* L. 20%, *Festuca rubra* L. 20%, *Poa pratensis* L. 20%, *Trifolium repens* L. 20%, *Lotus corniculatus* L. 20%) was sown in different parts of the hill (summit, midslope, footslope). The grassland has not been fertilised and used (abandoned).

Table 1. Agrochemical and physical properties of the soil (0-15 cm) in 2020.

Soil properties	Parts of the hill			Methods
	Summit	Midslope	Footslope	
Soil pH_{KCl}	5.58	5.65	5.05	Potentiometric method
Total N, %	0.096	0.185	0.118	Kjeldahl method
Mobile P_2O_5 mg kg^{-1}	121.4	118.8	87.3	Egner-Riehm-Domingo (A-L)
Mobile K_2O mg kg^{-1}	178.3	169.8	203.4	Egner-Riehm-Domingo (A-L)
Organic C (%)	0.94	1.20	1.23	Dumas dry combustion method
Soil moisture, %	14.8-28.8	19.4-25.8	18.8-35.2	Weight method

The six-course crop rotation consisted of *Hordeum vulgare* L. with under-sown perennial grasses: *Trifolium pratense* L. 80% and *Phleum pratense* L. 20% (2016), perennial grasses (2017), perennial grasses (2018), *Triticum aestivum* L. (winter crop) (2019), *Hordeum vulgare* (2020), and *Triticum aestivum* (spring crop) (2021).

Soil samples were taken from agrophytocenoses in 2020 and 2021. To assess the impact of the hill slope on soil contamination by seeds, the seed bank was investigated at the depths of 0-5 and 5-15 cm. The seed bank was estimated from soil samples taken in the spring (April) and autumn (September) of 2020 and 2021. In each model plot, 2 kg of soil from 20 positions was collected using a hand auger. The soil was dried. In total, five 100 g samples were removed from 2 kg of soil sample and weighed. Later, the soil samples were wet sieved through a 0.25 mm sieve until all the contents of the soil were washed out. The remaining mineral part of the soil was separated from the organic part and seeds using the saturated salt solution. The seeds were identified using binocular microscopes with 8.75× magnification. Seed species were determined by Grigas (1986). Seed viability was determined by 'destructive crushing' using forceps (Rahman, 1995). The number of viable seeds (A) was recalculated to thousands of seeds per m^2:

$$A = n \times h \times p \times 100,$$

where A is the number of viable seeds, seeds, m^2; n is the counted number of viable seeds in the soil sample; h is the depth of the plough layer (in cm); and p is the soil bulk density (g cm^{-3}).

To express the floristic similarity of phytocenoses or the similarity of the seed bank and actual vegetation, the coefficient of Sörensen (Cs) was used:

$$Cs = 2w/(A + B),$$

where w is the number of common species in both situations, A is the number of species in one of two comparable situations, and B is the number of species in another situation.

Significance of the differences between the means was determined according to the Fisher's protected least significant difference (LSD) at 0.05 probability level. The experimental data were subjected to the analysis of variance (ANOVA).

Results and discussion

Significantly smaller (6.7 times) number of seeds in the seed bank was determined in the soil of permanent grassland compared with the soil of cereal-grass crop rotation. The average data show that for depth of

0-15 cm of the pre-erosion cereal-grass crop rotation soil, the number of seeds reached 29.9, 32.5, and 18.7 thousand, respectively in the summit, the midslope, and the footslope parts of the hill (Table 2). In 1 m^2 of the unmanaged soil (permanent grassland) at depth of 0-15 cm, 4.3 thousand weed seeds were found.

In spring, irrespective of the agrophytocenoses, the greater seed number in both investigated soil depths was determined in the midslope of the hill. In autumn, in the soil of cereal-grass crop rotation the greater seed number in both soil depths was determined in the summit, while in the depth of 0-5 cm of permanent grassland – in the footslope of the hill.

During the investigation period, there were 38 plant species found in the soil seed bank: 24 species of them belonged to segetal plants, 13 species (to plants of the other growing sites and one species to trees). In most of cases, the number of seed species in the soil of permanent grassland was determined to be significantly smaller compared to cereal-grass crop rotation. Both in spring and in autumn, significantly higher number of plant species in the soil seed bank was determined in the footslope of the hill. This was influenced by better nutrition and moisture conditions (Table 1).

In permanent grassland, the most similar species composition of the soil seed bank was in the summit and midslope parts of the hill (Cs=0.71-0.90), while the least similar it was in the midslope and footslope parts of the hill (Cs=0.55-0.73). In cereal-grass crop rotation, the closest in species composition (Cs=0.71-0.89) was the soil seed bank in the midslope and footslope of the hill while the least similar (Cs=0.44-0.74) was in the summit and footslope parts. The floristic similarity of segetal plants' species in crops and seed bank species composition in all parts of the hill was determined to be minor (Cs=0.10-0.48).

Conclusions

In spring, irrespective of the agrophytocenoses, the greater seed number in both investigated soil depths was determined in the midslope of the hill. In autumn, in the soil of cereal-grass crop rotation the greater seed number in both soil depths was determined in the summit, while in the depth of 0-5 cm of permanent grassland it was in the footslope of the hill. In total, 38 seed species were identified. Seeds of dicotyledonous arable weeds comprised 63.2% of the total seed bank. There was a significantly higher number of plant species in the soil seed bank determined in the footslope of the hill.

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Table 2. The number of seeds and species.[1]

		Number of seeds				Number of species			
		Spring		Autumn		Spring		Autumn	
		0-5 cm	5-15 cm	0-5 cm	5-15 cm	0-5 cm	5-15 cm	0-5 cm	5-15 cm
PG	Summit	1,977	22,150	1,756	3,167	9.0	4.2	6.7	3.3
	Midslope	2,446	2,263	978	828**	8.0	4.8	7.7	3.2
	Footslope	2,060	2,082	1,905	2,570	9.2	4.0	7.7	5.0*
CG	Summit	9,745**	9,572**	24,966**	15,659**	6.3*	5.8*	7.0	5.2*
	Midslope	21,259**	15,192**	19,476**	9,089**	8.2	6.2*	8.2*	7.8**
	Footslope	11,106**	6,818**	12,727**	6,830**	10.7	7.7**	10.5**	6.7**

[1] PG = permanent grassland; CG = cereal-grass crop rotation; * significant at $P<0.05$, and ** significant at $P<0.01$.

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Developing locally adapted seed mixtures to reintroduce native species to degenerated medium-intensity grasslands

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Abstract

Diverse grasslands have higher ecosystem functioning, such as increased soil fertility, more carbon capture, higher-quality biomass and more-even yields. However, agricultural intensification and eutrophication are causing species losses in European grasslands, and since natural recolonization is slow, sowing species-rich mixtures might be a tool for species re-introduction. Thus, we tested four 10-species mixtures with different legume proportions and a range of rare to common forb species within a price range attractive for sowing by farmers. For a large-scale seeding experiment, 28 medium-intensity grasslands were chosen, situated in three contrasting and agriculturally disadvantaged regions of Bavaria. After seeding, agricultural management was continued to check for applicability of the seed mixtures and to maintain grassland production. Of 13 forb and legume species, nine established in the first spring after sowing. After 19 months, establishment varied among species and sites, with additional effects of biomass production. We conclude that at least some native forbs and legumes can be introduced to degenerated grasslands through reseeding, when native biomass is initially reduced.

Keywords: biodiversity, forbs, grassland, monitoring, seed mixtures

Introduction

Degraded grasslands offer opportunities for the realization of current conservation aims, due to their potential contributions to grassland biodiversity and simultaneous agricultural use. Reseeding grasslands with productive cultivars is common, while ecologically designed seed mixtures are needed for multifunctional swards. The restoration of species-rich grassland with increased forb and legume proportions creates a healthier diet for ruminants, additionally increasing the land-use elasticity for farmers (Oppermann and Gujer, 2003), and thus leading to a qualitative upgrading of unsatisfactory swards (Pirhofer-Walzl *et al.*, 2011). Seeding native grassland species creates benefits to the local fauna via specific interactions of plants and animals (Dierschke and Briemle, 2002). Unfortunately, there is a lack of practical knowledge on seed use to upgrade production grassland by sowing regional forb species.

Generally, high soil nutrients and frequent mowing, common in most production grasslands, are compromising establishment of native forbs (Socher *et al.*, 2012). However, many species respond positively to moderate fertilization (Zechmeister *et al.*, 2003) or to some disturbance (Klaus *et al.*, 2017). Furthermore, with the increasing availability of native seeds of local provenance (Bucharova *et al.*, 2019), there are new opportunities for large-scale application of species-rich mixtures of regional specialization in addition to commercially bred cultivars. Therefore, this study focussed on the response of ecologically designed forb and legume mixtures to environmental gradients, and persistence of these target species under standard grassland management.

Material and methods

Four forb-and-legume mixtures were developed composed of seven 'basic species' (*Leucanthemum vulgare, Galium album, Lotus corniculatus, Plantago lanceolata, Prunella vulgaris, Pimpinella saxifraga, Crepis biennis*) and a variable set of 'topping species'. Criteria for mixture design were the establishment of a legume gradient, a range of rare to common forb species, and an acceptable price for large-scale application by farmers. Species selection was based on availability from regional provenances, agronomic utility and ecosystem functions. In total, 28 equal field sites in three agriculturally disadvantaged regions of Bavaria were established, targeting medium-intensity grasslands with 3-4 cuts per year.

Sowing was done in 2020 and 2021 by a common drilling machine after cutting the sward close to the ground, and site management such as fertilising and mowing was continued as before. Two commercial standard grass mixtures were sown as a reference. Thereafter, the four seed mixtures were applied in the reseeding strips at two seeding densities (5 and 10 kg ha^{-1}) on 3×6 m plots. Vegetation surveys were carried out in spring and autumn in 1-m^2 plots within treatments, replicated three times per treatment; soil sampling was done in autumn 2021, and biomass sampling for determining dry matter was performed from spring 2022 onward, shortly before each cutting by the farmers was done. Using linear mixed effects models, we analysed whether the number of newly established target species correlated with time since sowing, dry matter gain or loss of each treatment through sowing (expressed as percentage change compared to unsown control), location in one of the geographical areas, the effect of gaps in vegetation cover, or soil pH.

Results and discussion

All 13 target species (except one) were found at least once; however, some disappeared again, resulting in nine target species in spring 2022. The number of established target species varied significantly among geographical areas (P<0.001), soil pH (P<0.001), time since sowing (P<0.001), vegetation gaps (P=0.001), and dry matter yield compared to the original vegetation (P<0.001) by spring 2022 (Figure 1A). This aligns with the findings of Klaus *et al.* (2017), who showed introduction of atypical species in managed grasslands to be possible, if sward disturbance was sufficient. When dry matter of the sward is increased compared to untreated control, target species establishment was inhibited; when dry matter was decreased, 4-7 target species established.

Previous studies showed that the seeding success depends on the proportion of grasses and other undesirable species that dominate after sward disturbance (Török, 2010; Warren *et al.*, 2002). Target species persistence varied among sites and sample periods; however, due to the short monitoring of <24 months, conclusions on species persistence are not yet possible.

Conclusions

We conclude that reseeding is a possible strategy to add native forbs and legumes to species-poor grassland. Establishment is species-specific, depending on pH and biomass, while persistence for up to two years is possible under conventional management.

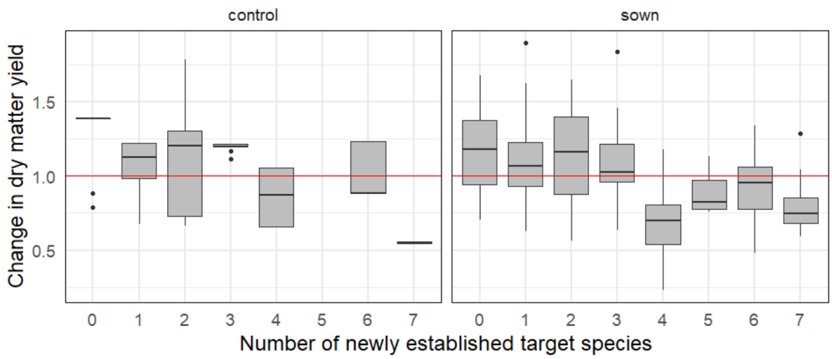

Figure 1. Number of newly established target species on field sites in correlation with the change in dry matter yield (compared to unsown plots) in grass matrix control (left) and sown areas (right) after 6-19 months of development.

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Monitoring topsoil organic carbon concentrations of two intensively managed agricultural catchments in Ireland

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Abstract

Agroecosystems are often depleted in soil organic carbon (SOC) as a consequence of historic soil cultivation. In this study, we investigated how land management (crop or grass production) influenced changes in SOC concentrations (0-10 cm depth) in two mesoscale agricultural catchments in Ireland (Arable A; well drained, and Arable B; moderate-to-poorly drained) over a 12-year period. The median SOC concentrations significantly decreased in the Arable A catchment (-2.5 g kg^{-1}; $P<0.001$; r=0.37), whilst the Arable B catchment recorded only a slight increase (0.6 g kg^{-1}; $P<0.05$; r=0.09). In Arable A, median SOC losses ranged from -2.3 to -7.2 g kg^{-1} with the most significant losses occurring in land parcels under 50%, 75% and 100% crop production. In Arable B, median SOC losses of -4.1 g kg^{-1} ($P<0.001$; r=0.57) were recorded in land parcels under 75% crop production; however, an 11.7 g kg^{-1} ($P<0.001$; r=0.65) increase in SOC was recorded in land parcels under 25% crop production. The results indicate that incorporating a temporary grass-ley into a cropped soil did not mitigate the decline of SOC in Arable A; however, full conversion of cropland to grassland was shown to increase SOC in Arable B where the baseline SOC concentration was low.

Keywords: soil organic carbon, agricultural catchment, crop rotations, grass ley, soil drainage

Introduction

Soils are a large reservoir of organic carbon (SOC) and globally contain 1500 Pg in the upper one metre of soil, approximately two-to-three times more than that stored in the vegetation and atmosphere combined (Batjes, 1996). The majority of this C is found in the topsoil (10-20 cm) where increased levels of topsoil OC are important for improving soil fertility, water holding capacity, soil structure and crop nutrition (Spink *et al.*, 2010). Intensive agricultural management is likely to have affected both the quantity and quality of SOC, and there is increasing evidence that SOC concentrations are declining in many regions of Europe, with changes attributed to land use change and climatic factors (Capriel, 2013). Estimation of SOC are typically obtained from national and regional scale soil monitoring networks, long-term experiments and modelling studies. For example, Reijneveld *et al.* (2009) highlighted uncertainties associated with detecting small mean changes in SOC changes, soil variability, heterogeneous sampling positions and land use dynamics. Moreover, assessment of SOC requires a high sampling density and time intervals ~10 years to detect large changes (Saby *et al.*, 2008). In this study, we examined archived topsoil samples (0-10 cm depth) from two mesoscale agricultural catchments (9-11 km^2) dating from winter 2009 with the most recent phase of soil sampling conducted in 2021. The combined use of measured topsoil OC with available land use data offers a unique opportunity to assess long-term changes in SOC dynamics across intensively managed agricultural catchments.

Materials and methods

Two agricultural catchments (Arable A and Arable B) with contrasting soil drainage characteristics ('Well' and 'Moderate-to-Poorly') in Ireland were selected for this study. Farming in Arable A consists of cereal cropping with predominantly spring barley (54%) with beef cattle and sheep grazing (39%), and in Arable B, winter wheat (42%), beef cattle and sheep (29%) and dairy cattle grazing (19%) (Sheriff *et al.*, 2015). Topsoil (0-10 cm depth) was taken from each catchment field at random to form a composite soil sample. Larger fields were sub-divided into smaller sampling units of ca. 2 ha to account for heterogeneity in soil type, topography and management practices. The soils were then air-dried at 40 °C, sieved to 2 mm and analysed for total organic carbon concentration (expressed in g kg^{-1}) using a CHNS analyser (LECO Tru-Spec). The soil sampling campaigns were conducted every four years between 2009 and 2022 with ca. 12 years between the initial (T_1) and final (T_2) soil samples. The Land Parcel Identification System (LPIS) was used to define the land use for each parcel at the beginning of each soil sampling campaign (n=4). Fields were classified according to when they were under cropland (C) or grassland (G), providing a snapshot for when arable land was converted to a cut/grazed grass, and vice versa if a crop was sown into a pasture field. The treatments were labelled according to the frequency of the particular land use relative to the total number of records available. For example, C3:G1 represents a sampling unit where the land use was recorded as cropland in three, and grassland in one, out of four soil sampling campaigns. Changes in SOC between the initial (T_1) and subsequent (T_2) sampling periods were examined for each catchment according to land cover and soil drainage class using Wilcoxon rank sum test and Kruskal Wallis test. Where significant differences were found (P<0.05), the effect size (r) was calculated as the ratio between the Wilcoxon test statistic (W) and the rank sum (S), where effects are considered small (r~0.1), moderate (r~0.3) and large (r~0.5), and a multiple pairwise comparisons were evaluated using Dunn's test with the Bonferroni adjustment.

Results and discussion

An overall median SOC loss of -2.5 g kg^{-1} and a gain of 0.6 g kg^{-1} were recoded for Arable A and Arable B, respectively (Table 1). The effect was moderate in Arable A and small in Arable B according to the r values suggesting a relevant loss of SOC in Arable A. There was no relationship between soil drainage class and SOC loss with the exception of well drained soils in Arable A showing a moderate decrease in SOC over time (-2.5 g kg^{-1}, P<0.001; r=0.44) (not shown).

Table 1. Median SOC concentrations (g kg^{-1}) for the initial (T_1) and subsequent (T_2) sampling periods in two agricultural catchments.

Catchment	Overall[1]	Field parcels (n)	(%) field parcels	SOC_T_1	SOC_T_2	ΔSOC	Significance (T_1 vs T_2); r value[2]
Arable A	-	662	-	35.6b	32.9b	-2.5a	***; 0.37
Arable B	-	758	-	29.2a	31.2a	0.6b	*; 0.09
	Land cover[1]	634					
Arable A	C3:G1	81	13	41.8b	32.3a	-5.2a	***; 0.67
	C2:G2	55	9	43.0bc	35.5b	-7.2ab	**; 0.55
	C1:G3	68	11	44.9c	38.2b	-6.5ab	*; 0.39
	C4 (Control)	332	52	31.4a	29.6a	-2.3b	***; 0.46
	G4 (Reference)	98	15	51.6c	54.2c	3.0c	n.s.
		704					
Arable B	C3:G1	67	10	27.8b	22.5a	-4.1a	***; 0.57
	C2:G2	35	5	29.8b	30.8b	2.3b	n.s.
	C1:G3	112	16	26.9b	51.5d	11.7c	***; 0.65
	C4 (Control)	242	34	22.3a	22.6a	-0.4b	*; 0.17
	G4 (Reference)	248	35	41.1c	42.6cd	1.1b	n.s.

[1] Kruskal Wallis test was conducted on median SOC values within groups.

[2] Wilcoxon signed rank sum test significance (P<0.05=*; P<0.01=**; P<0.001=***) and effect size (r) [z/sqrt(n)].

Differences in SOC between T_1 and T_2 showed much larger variation when distinguished by land use category. In Arable A, median SOC loss ranged from -2.3 to -7.2 g kg^{-1} (P<0.05). Large effects were observed for the C3:G1 and C2:G2 categories with considerably higher initial topsoil OC levels (41.8 and 43.0 g kg^{-1}), representing a 12.4% and 16.7% median loss in SOC, respectively. In Arable B, we observed significant decrease in median SOC in the C3:G1 category (-4.1 g kg^{-1}). A large effect was also observed in Arable A (-5.2 g kg^{-1}) suggesting a higher frequency of crop cultivation on a land parcel led to SOC loss over time. An increase in median SOC was observed in the C1:G3 category (11.7 g kg^{-1}) in Arable B, representing a 91% increase in median SOC between T_1 (26.9 g kg^{-1}) and T_2 (51.5 g kg^{-1}). Wenzel *et al.* (2022) observed ~30% increases in median SOC from (39.4 to 51.1 g kg^{-1}) for permanent grassland soils in Austria. The similar magnitude of increase between the studies suggests much of the C1:G3 land category has been converted to grassland from crop cultivation over the last decade. Interestingly, we observed a linear relationship between initial SOC (T_1) and ΔSOC where SOC loss was measured (ΔSOC = -0.2518*(Initial SOC) + 4.5787, R^2=0.83). Guillaume *et al.* (2021) reported a strong effect of cropland-grassland conversion on SOC dynamics with the rate of loss dependent on the initial SOC content.

Conclusions

Over a 12-year period, we observed a decrease in median SOC in Arable A and a small increase in Arable B. Moderate to large changes in SOC were mainly attributed to land use and initial SOC content, where crop cultivation on soils with higher OC tended to lose OC at a faster rate (Arable A) compared to soils with lowed OC (Arable B). This indicates that temporary grassland cultivation within the crop rotation had little effect on reducing the rate of SOC loss over time. The large accumulation of SOC for the C1:G3 land category (91% increase) may be due to conversion of cropland with low initial SOC to pasture-based production system during the earlier soil sampling campaigns.

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Mob grazing: impacts, benefits and trade offs

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Abstract

Mob grazing is a form of intensive managed grazing where large numbers of animals graze a small area of land for a short period of time. The overall aim of this UK Defra-funded project is to assess the practical, economic and environmental implications of transitioning from conventional set-stocked/ rotational grazing systems to mob grazing, at a farm, industry and policy level. A survey of UK farmers has provided detailed information on grazing practices including stocking intensity, frequency of livestock movements, rest periods and target pre/post grazing grass covers, enabling characterisation of regenerative 'mob' grazing systems within the context of other grazing systems. A Rapid Evidence Assessment (REA) has identified and evaluated current evidence on the impact of mob grazing systems on soil properties. Although mob grazing is frequently cited as a 'regenerative' farming practice with significant potential to increase soil carbon sequestration, the REA found limited evidence that mob grazing is any more effective than 'conventional' set stocked or rotational grazing systems in increasing soil carbon sequestration in soil.

Keywords: grazing management, mob grazing, rotational grazing, soil quality, soil organic matter

Introduction

In the UK and across Europe, continuous and rotational grazing are the most common grassland management systems for dairy, beef and sheep farms. However, there has been increasing interest in mob grazing as an alternative to conventional grazing management systems. There is no formal definition of mob grazing in terms of stocking density, the number of days a section of grass is grazed or the rest period after grazing; however, it can be characterised by short, intensive grazing events, with longer rest periods allowing the grass to recover. Mob grazing systems have been reported to have many benefits including increasing sward productivity, reducing the need for fertiliser inputs, increasing soil organic matter (SOM) and enhancing biodiversity (Briske *et al.,* 2011; Nordborg and Röös, 2016). However, there is limited European research to quantify the impact of mob grazing systems.

Materials and methods

An online survey of grazing practices was carried out to collect farm and grazing management information from UK grassland farmers, to better characterise UK grazing systems. A total of 231 responses were received between June and November 2021. This was followed by in-depth telephone interviews with a stratified sub-sample of 66 farmers who were asked questions focussing on qualitative data collection and exploring the socio-economic drivers for mob grazing. Respondents to both surveys were broadly representative of UK farming with respect to their gender and land ownership, and the land area and soil types farmed, although they were younger than the UK average. Mixed and beef-only farms were the most, and dairy-only farms were the least represented. Survey respondents were given a list of 19 grazing terms and asked which (if any) they would use to describe their grazing system. Respondents had the option of selecting multiple terms and could also provide their own if they used any other terminology.

In addition, a Rapid Evidence Assessment (REA) was carried out to assess our current knowledge on the impacts of mob grazing systems on the environment, animal health and welfare, and productivity and profitability. The REA followed standard methodology described by Collins *et al.* (2015). The primary questions were broad 'impact' questions, which aimed to assess positive and negative impacts of mob grazing on the environment, farm productivity and productivity and animal welfare: (1) What are the environmental impacts of mob grazing? (2) What are the productivity and profitability impacts of 'mob' grazing? (3) What are the impacts of rotational 'mob' grazing for animal health and welfare? Web of Science and Google were used to search published and 'grey literature' using key search terms. Searches were carried out in March 2021 and included information from all geographic regions published post 2010.

Results and discussion

Seventy-one percent of online survey respondents said they split at least some of their fields into two or more paddocks/cells which could be grazed separately. The average size of paddocks/cells was 1.24 ha. Seventy percent of respondents typically moved livestock onto new grazing at least once per week. The most common frequency of movements was every 3-4 days (24% of respondents). However, there were differences between farms depending on the main farm livestock enterprise. Dairy-only farms moved livestock most frequently (63% moved daily; 84% moved at least once per week). This was followed by beef-only farms (24% moved daily; 74% moved at least once per week); then mixed livestock farms (11% moved daily; 69% moved at least once per week), while sheep-only farms had the least frequent moves (3% moved daily; 56% moved at least once per week). The most common length of grass rest period before returning to graze again was 3-4 weeks (48% of respondents). Only 5% of respondents said their livestock were stocked and grass grazed continuously without a rest period. Thirteen percent of respondents had rest periods greater than five weeks and 5% had a rest period of more than 8 weeks. 'Rotational grazing/stocking' was the most common grazing term for all farm types, cited by 68% of survey respondents, followed by 'paddock grazing', cited by 19% of respondents. 'Mob grazing/stocking' was cited by 19% of respondents, although this varied between farm types from 5% of dairy-only farms to 27% of beef-only farms. Thirteen percent of survey respondents cited 'set stocking' and 11% 'continuous grazing'.

Around half the survey respondents had changed their grazing management system in the last 5 years, and around 40% were thinking of changing it in the future, indicating a willingness to adapt in response to the challenges facing livestock farmers. Many survey respondents had adopted some form of rotational grazing (including paddock, Techno, holistic, regenerative, precision, strip and mob grazing), with the most frequently cited reasons being to improve grass yields, utilisation and quality, increase stocking rates and improve livestock performance.

Evidence from the REA indicated no or inconsistent effects of grazing management on soil organic matter content. Despite the interconnectivity between SOM, soil structure and air and water quality, few studies have measured the impacts of mob grazing on multiple ecosystem services. There was also very limited evidence of the impact of mob grazing on insects, invertebrates, and birds in mob grazed systems either in the UK or globally. More studies have been published looking at the botanical diversity of grazed swards, which has been shown to increase or remain the same under mob grazing management regimes. However, factors such as climate and inter-year variability can have a greater impact on botanical composition than differences between grazing systems (Billman, *et al.*, 2020). In addition, interactions between site and treatment have been reported indicating that mob grazing may induce changes to botanical composition at some sites and not others (Girard-Cartier and Kleppel, 2017; Russel *et al.*, 2015).

When compared with rotational grazing practices, studies have found that mob grazing did not significantly improve biomass production or grass utilisation but did lead to a decline in sward nutritional value. When compared with continuous grazing practices, some authors have reported improvements in biomass production under mob grazing whilst others have observed no significant change.

The REA did not find clear consistent evidence of certain sward species mixes being better suited to mob grazing than others. However, species with vertical growth habits may have a competitive advantage and increased persistence compared to those with more prostrate growth habits. The long rest periods associated with mob grazing mean that swards are more mature when grazed, altering their palatability and quality, which may result in selective grazing of some plant species. Conversely, there was some evidence that mob-grazing can increase competition for resources and result in reduced grazing selection. Evidence of the impact of mob-grazing on animal grazing behaviour was extremely limited. Nevertheless, grazing the high herbage-mass swards typical of mob grazing has been shown to negatively impact on bite mass and intake rate, and significantly increase the energy expenditure required for rumination, and changes in the proportion of herbs in the mix may lead to reductions in dry matter intake.

Conclusions

There has been a clear increase in the level of interest in mob grazing in recent years. However, both the online and telephone surveys found that there was a great deal of confusion amongst the participants over what exactly constituted mob grazing, and that some terms were used interchangeably to describe similar grazing systems. For example, 'mob grazing' was often considered to be interchangeable with 'cell grazing', 'high stocking density grazing', 'holistic grazing', 'long-grass grazing', 'non-selective grazing', 'rotational grazing' or 'management intensive grazing'. Although mob grazing is frequently cited as a 'regenerative' farming practice with significant potential to increase soil carbon sequestration, the REA found limited evidence that mob grazing is any more effective than 'conventional' set stocked or rotational grazing systems in increasing soil carbon sequestration in soil. At the present time there is insufficient independent, scientific research to provide a robust evidence base to assess the impact of mob grazing systems on ecosystem services delivery, grassland productivity or animal performance and health, especially under temperate climatic conditions.

Acknowledgements

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Impact of flower plant strips on the soil parameters in an intensive farming field

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Abstract

Plants of a high environmental value, though rarely grown by farmers, are selected for flower plant strips: they are melliferous, deep-rooted plants, leaving high amounts of organic residues in soil. An experiment was installed on a clay loam soil with the aim to determine the influence of various floral sward strips on the accumulation of organic carbon and the improvement of soil physical properties. Four different plant strips were installed at the edges of an intensively cultivated arable field: perennial grass swards (PGS), perennial legume swards (PLS), annual floral plants (AFP) and seminatural grassland swards (NGS). It was found that the highest amounts of roots and plant residues in the soil were left after cultivating grass strips of PLS and NGS compared to the field where cereals had been intensively grown. The highest amount of organic residues in the subsoil were found in the PLS and NGS. In addition, the deep-rooted plants in the soil formed large pores that hold water, air, and allow soil fauna movement. During the five-year period, the plants of flowering strips improved the properties of the field edges topsoil: its bulk density decreased, moisture reserves increased, and the durability of structural aggregates improved.

Keywords: field strips, soil physical properties, roots and organic residues, organic carbon

Introduction

Compaction results from working with heavy machinery with narrow tyres, especially on the edges of the field when agricultural machinery is driven several times over the same area of land. Soil at the edge of the field is compressed, scores appear, the seed bed is poorly prepared, and the plants germination is not homogeneous. The consequence of this process is areas of bare ground and weedy edges of the agricultural fields, as well as reduced crop yields. This is particularly the case in soils with severe granulometry, where high levels of clay particles are responsible for the negative properties of this soil. Biological processes also contribute to the problem, e.g. lack of crop diversification in the crop rotation and low soil organic matter content. Reducing mechanical impact on soil creates a durable soil structure that provides optimum air and water regime, nutrient availability for plants, and soil biota (Kladivko, 2001). The goal of this research was to set flowering annual and perennial herbaceous species combinations in field edges, which might be suitable to improve soil parameters, not only to attract pollinator insects in intensive farming conditions.

Materials and methods

The experiment was carried out during five years (2013-2018) at the Lithuanian Research Centre for Agriculture and Forestry. The soil of the experimental site is *Endocalcari Endohypogleyic Cambisol* (CMg-n-w-can), topsoil texture – clay loam. In 2014, floral sward strips were established on the edges of an area of large intensive farming land (>5 ha). The strip were seeded with four plant combinations: perennial legumes (*Trifolium pratense* L., *Onobrychis viciifolia* Scop., *Medicago sativa* L., *Trifolium repens* L., *Lotus pedunculatus* Cav.) at 20 kg ha^{-1}; annual dicotyledons (*Helianthus annuus* L., *Fagopyrum esculentum* Moench, *Borago officinalis* L., *Sinapis alba* L., etc.) at 33 kg ha^{-1}; natural grassland (grasses: *Poa palustris* L., *Poa compressa* L., *Corynephorus canescens* L. etc.; perennial legumes: *Trifolium rubens* L., *Medicago lupulina* L., *Vicia cracca* L. etc.; other species: *Centaurea jacea* L., *Agrimonia eupatoria* L. etc.) 16 kg ha^{-1}; and perennial grasses (*Festuca arundinacea* Schreb., *Festuca pratensis* Huds., *Festuca rubra* L., etc.) at 20 kg ha^{-1}. The seeds of species of flowering plants from seminatural swards were collected during expeditions (in the first half of September 2013) in meadows of river valleys. The sward was cut and mulched twice in the sowing year. Soil samples for the determination of chemical characteristics were collected at the 0-25 and 20-50 cm depth layers in the last experimental year 2018. The samples were analysed for organic carbon (C) by Tyurin method modified by Nikitin, and total nitrogen (N) by Kjeldahl method. To determine the roots mass and organic residues of plants, monoliths, 25×25×24 cm in size, were extracted from the plots of each treatment (at a depth 0-25 and 25-50 cm). For the determination of soil physical properties samples were collected (at the depth of 30 cm) in the year of field experiment upon completion. The research data were processed by the two-factor analysis of variance (ANOVA version 3.1, STAT ENG version 1.5, 2000). The data were analysed when the factual Fisher criterion (F_{fact}) was higher than the theoretical one (F_{theor}).

Results and discussion

Soil analyses of flowering plant strips were carried out in the last experimental year to evaluate differences of soil parameters in flower strips and their influence on edge of soil properties relative to intensively growth wheat. In three of four strips, the mass of root and plant residues in the upper layer was higher than in the control plot (Table 1). However, the amount of underground biomass accumulated by different flowering plant strips varied significantly. The most soil enrichment with organic residues was found in the PGS and NGS strips; plants roots and residues in these strips were significantly higher than the underground mass of other flower plant strips. In the 0-25 cm soil layer depth, there were no significant differences between the amount of root and plant residues between the wheat field and strips of AFP and PLS (Table 1).

On average, the root and plant residues were 11-12 times less at the 25-50 cm soil depth than 0-25 cm depth. The studies have shown the significantly higher total N content in the plough layer of the PGS strip compared to other sown plant strips.

The lowest difference of nitrogen concentration between soil plough and deeper layers was found in the AFP strip. In the deeper soil layer, significantly higher total nitrogen concentrations were found in the soil of PGS and AFP strips compared to PLS and NGS strips. However, it was found that the highest amounts of roots and plant residues in the upper soil layer (0-25 cm) were left after cultivating sward strips of PGS and NGS compared to the field where cereals had been intensively grown. The physical and mechanical properties of the soil are very different and depend largely on the amount of soil moisture

Table 1. Mass of plant roots and residues, content of soil organic carbon and total nitrogen, Joniškėlis.[1]

Treatment	Soil layers, cm							
	0-25		25-50		0-25		25-50	
	Roots	Organic residues	Roots	Organic residues	C_{org}	N_{total}	C_{org}	N_{total}
	g from monoliths, 25×25×24 cm				mg kg^{-1} of soil			
Wheat	56.77a	16.08a	2.77a	0.93a	-	-	-	-
PGS	90.88bcd	39.33b	5.24abc	1.65ab	3.11d	17.9b	2.66d	14.5d
PLS	61.13ab	20.23a	10.00abc	2.24b	2.83ab	14.6a	1.78ab	10.9ab
AFP	46.71a	20.07a	3.84a	0.90a	2.74a	15.1a	2.27bcd	13.1bcd
NGS	114.15d	18.30a	12.66c	3.78c	2.96bcd	15.0a	1.62a	9.3a
Average	73.95	22.80	6.90	1.90	2.90	15.7	2.07	11.9

[1] PGS = perennial grass swards; PLS = perennial legume swards; AFP = annual floral plants; NGS = semi natural grassland swards. Plant residues mass <1 mm. Different combinations of letters indicate significantly different means in different years (P<0.05, Duncan's test).

Table 2. Influence of various plant strips on soil physical properties for the field edges (0-30 cm).[1]

Treatment	Soil physical properties			
	Moisture	Bulk density	Total porosity	Stability of structural aggregates %
Wheat	14.3a	1.51c	40.4ab	45.5a
PGS	16.6b	1.49abc	42.1b	75.9b
PLS	14.8a	1.48abc	41.9ab	73.0b
AFP	14.6a	1.47abc	41.8ab	79.3bcd
NGS	14.3a	1.47a	41.5ab	86.4d
Average	14.9	1.48	51.5	72.0

[1] For abbreviations see Table 1. Different combinations of letters indicate significantly different means in different years (P<0.05, Duncan's test).

in the field, plant species used, and the meteorological conditions (Mzuku *et al.*, 2005). There were significant changes of soil properties in the experiment for the different plants of strips (Table 2).

Changes in soil bulk density were less significant. Soil bulk density was the highest in the wheat field and was significantly higher than in the NGS strip. The growth of different plant species combinations in the field strips had no significant effect on soil porosity. The best soil porosity was on areas with growing strips of PGS, PLS and AFP. Assessing the agronomically important traits, the most agronomically valuable aggregates were found to be significantly different between plant strips. The amount of soil valuable structural aggregates was found two times in any growth strip compared to the in the NGS strip. The soil aggregate stability data showed a significant positive effect of grassland swards plants on the soil aggregate stability.

Conclusions

The highest amount of plant residues in the subsoil were found in the strips of PLS and NGS. Where the amounts of organic residues were determined the amount of organic carbon also increased in the soil. During the five-year period, the plants of flowering strips improved the properties of the field edge topsoil: bulk density decreased, moisture reserves increased, and the durability of structural aggregates improved.

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Modelling soil carbon measures on dairy farms

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Abstract

Soil carbon sequestration is one of the pathways for the dairy sector to mitigate climate change. Soil carbon measures have been reviewed extensively, including estimates of their impacts on regional or national scales. Eventually, these measures are to be implemented by farmers themselves, justifying an assessment at farm and field level. Here, we simulated the soil carbon changes of 96 fields on 9 dairy farms under current management and after implementation of six carbon measures. The fields were in use as permanent grassland or as a grass-arable rotation with forage maize or other crops. Under current management, the annual changes of simulated soil carbon were +0.68 and +0.39 t ha^{-1} year^{-1} for permanent grasslands and crop rotations, respectively. The simulation of carbon measures showed that converting crop rotations with temporary grassland to permanent grassland had a large effect on additional soil carbon storage (0.5 t ha^{-1} year^{-1}). Other measures like catch crops, applying more manure or compost, and increasing the sward age of permanent grassland had moderate effects (up to 0.1 t ha^{-1} year^{-1}).

Keywords: carbon sequestration, forage maize, grassland, mitigation, RothC

Introduction

Soil carbon (C) sequestration is important to agriculture to mitigate climate change by capture of atmospheric carbon dioxide (CO_2). The recent Dutch Climate Agreement includes a goal for mineral soils of 0.5 Mt CO_2 additional annual reduction by 2030 from out of a total reduction of 3.5 Mt CO_2 for the agricultural sector. Soil carbon measures have been reviewed extensively, including estimates of their impacts at regional or national scales. A recent study for mineral soils in the Netherlands (Lesschen *et al.*, 2022) modelled a potential of 0.9 Mt CO_2 annual additional soil sequestration compared to the reference year 2017. The actual effect will vary considerably between farms due to differences in farm configuration, i.e. soil type, land use and management. Hence, farm-specific estimates of the effect of carbon measures on soil carbon stocks are required to inform farmers, farm consultants and policy makers. However, farm and field specific model analyses of dairy systems are often hampered by limited data availability for model input and parameterization, complicating a correct representation of the complex interactions between animal and crop management. Here we used the RothC model on nine dairy farms across the Netherlands, all with intensive data collection plans. The objective of the present study was to quantify the effect of carbon measures on soil carbon sequestration on fields with different land use and management.

Materials and methods

We selected 96 fields from nine dairy farms on mineral soils from the Cows and Opportunities network, a group of commercial dairy farms aiming to bridge the gap in environmental performance between experimental farms and commercial farms (Oenema *et al.*, 2001). All farms are thoroughly monitored, analysed and evaluated in terms of agronomic, environmental and economic performance. The proportion of grassland varied between 80 and 100%, of which 4 to 87% was permanent grassland. Forage maize was grown on most farms, up to 20% of the area. The proportion of other arable crops was mostly lower than 10%, but a couple of farms grew considerably more arable crops arable farmers, even up to a third of the area, by renting out land to a neighbouring arable farmer once every three years. Potatoes were grown on five farms, while tulips, cereals or beans were grown occasionally on individual farms.

The historical soil data were collected for soil organic matter (SOM) and clay content and standardized to an equivalent depth of 25 cm. The bulk density was calculated with soil type specific pedotransfer functions for clay and sand. Soil organic carbon (SOC) stock to a depth of 25 cm was calculated from SOM and BD, assuming a carbon content of 54% in SOM.

Historical data on land use, manure and grazing were collected for each field. The organic matter input from crop residues was based on existing data for arable crops and a recent calibration for grassland. Annual carbon inputs from manure were based on registered field applications and analysis of farm specific manure. Monthly soil cover was based on average growing patterns for each crop. We used RothC to simulate changes in soil carbon stocks in the 0-25 cm layer. The oldest available soil sample was used to calculate the initial total soil carbon stock and its distribution among five soil carbon compartments in the RothC ll.

We simulated the effect of carbon measures on soil carbon stocks, over a period of 50 years. The measures include (1) increasing the sward age of permanent grassland through a 50% reduction of the renewal frequency, (2) increasing the proportion of grassland in crop rotations by adding one additional grass-year, (3) converting crop rotations to permanent grassland, (4) addition of 10 t ha^{-1} external organic matter from compost once every five years, (5) reduction in export of internally produced manure, and (6) introducing catch crops in rotations, if not already applied.

Results and discussion

Under current farm management, the overall average change in the modelled soil carbon stocks of 96 fields was +0.68 (sd=0.31) and +0.39 (sd=0.36) t ha^{-1} year^{-1} for permanent grasslands and crop rotations, respectively. The average carbon input from crop residues was 4.8 (sd=0.5) and 3.3 (sd=0.7) t ha^{-1} year^{-1} for permanent grasslands and crop rotations, respectively. The variation of crop inputs in rotations was mainly related to the proportion of grassland in a rotation. The average carbon input from manures was 2.2 (sd=0.5) t ha^{-1} year^{-1}, irrespective of land use.

The median value of the simulated effect of carbon measures, during the first 50 years after implementation, varied from 0.024 to 0.50 t ha^{-1} year^{-1} (Figure 1). Converting grass-arable crop rotations to permanent grasslands was by far the best measure to increase soil carbon stocks, up to nearly 1.0 t ha^{-1} year^{-1}. Other measures showed a relatively low impact, in the range of 0 to 0.2 t ha^{-1} year^{-1}. The outcomes showed a considerable variation between farms and fields that are related to soil type and current carbon stock, land use and management.

Converting rotations with temporary grasslands to permanent grasslands resulted in a median additional storage of 0.5 t ha^{-1} year^{-1}. The high variations for the measures 'convert rotation to permanent grassland' and 'increased grass in rotation' were mainly related to the current number of grass years within a rotation. The higher the current fraction of grass in a rotation, the lower the impact of these measures. Application of catch crops increased soil carbon storage by 0.1 t ha^{-1} year^{-1}. Importing compost or reducing the export of manure had a moderate effect. Although these measures might be beneficial for farm-based carbon budgets, the positive effects might be offset on a regional scale as compost or manure are limited resources.

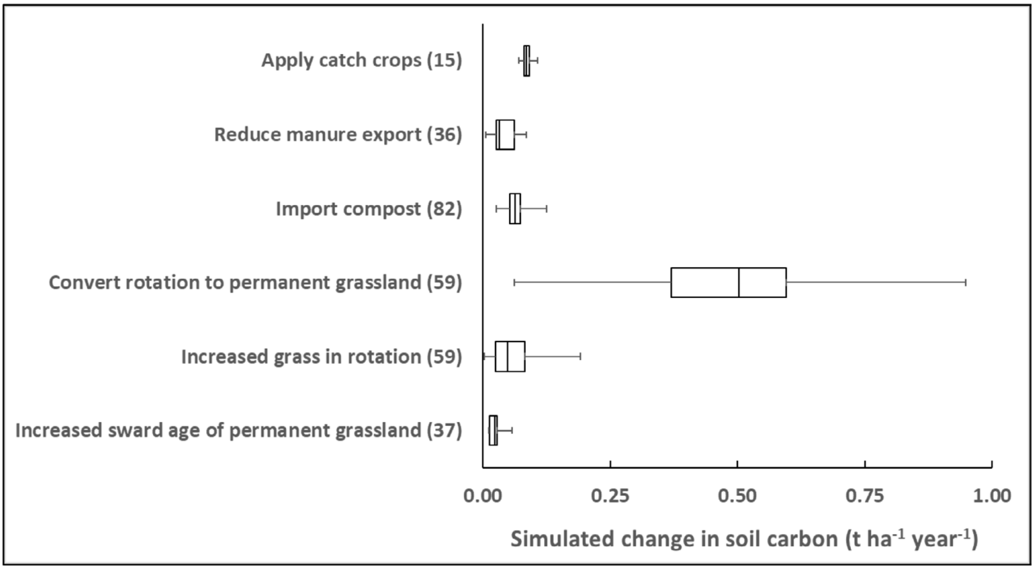

Figure 1. Average annual changes, during the first 50 years, of simulated soil carbon stocks in 0-25 cm for six measures. Boxplots indicate minimum, P25, P50, P75 and maximum values. Number of fields is given between brackets.

Increasing the sward age of permanent grasslands through a lower renovation frequency did not have a large effect on additional carbon storage. In a renovation year, our simulations show a net loss of soil carbon of approximately 0.5% of the existing carbon stock. Results of measured soil carbon losses after ploughing grassland show higher but varying loss rates (Necpálová *et al.,* 2014; Reinsch *et al.*, 2018), indicating that our simulations may underestimate the effect of grassland renovation.

Conclusions

The key to carbon sequestration on dairy farms is the proportion of grassland. All other measures showed relatively modest effects. The simulated effect of carbon measures on sequestration depends on current farm configuration.

Acknowledgements

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Mixed farming under boreal environmental conditions

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Abstract

Mixed farming in Finland relies heavily on grassland production on both mineral and drained organic soils. In northern Finland, however, organic soils are generally the most available soil types for grassland cultivation. Previous studies have shown that grasslands on mineral soils range anywhere from being a sink to a small source of carbon dioxide (CO_2) to the atmosphere, while drained organic soils are invariably a large source. The Finnish milk and beef industries are now constrained ever more than before under the changing climatic conditions to report how environmentally friendly and thus how sustainable are their business activities. Cattle methane emissions due to enteric fermentation, nitrous oxide (N_2O) emissions on mineral soils and CO_2 and N_2O (under wet soil conditions, also CH_4) emissions from organic soils are a major bottleneck in the industrial pathway to carbon neutrality in the years to come. To add to the problem, there has been a severe lack of scientific field experimental data much needed in understanding how various crop and livestock management practices influence carbon and nitrogen cycles on different soil types under the prevailing climatic conditions. While cattle emissions are not within the scope of this paper, we will present here the multi-year, continuous data comparing CO_2 exchange patterns measured using eddy covariance technique from grasslands on organic and mineral soils in eastern Finland. The presented data will highlight grassland responses to soil types, management practices and within-season and annual climatic variability with implications for milk and beef production in Finland.

Keywords: grasslands, mitigation, adaptation, sustainability, mineral soils, organic soils

Introduction

In most European countries, peat soils drained for agriculture are a considerable source of greenhouse gas emissions. Since emissions from this source have high mitigation potential, they are now a focus of the European Union's current and future climate goals (Buschmann *et al.*, 2020). In northern Finland, such soils are primarily used for mixed farming with grasslands cultivated with grass mixtures (timothy, meadow fescue, barley as a cover crop in the initial year of a new grassland establishment) on organic soils and additionally red clover on mineral soils. While the growing season in Finland is short (ranging from 180 days in the south to about 110 days in the north), climatic conditions during the season are conducive enough to allow two to three grass cuts. Winter damage to grasslands is a problem that undermines the sustainability of grassland production and thus the milk and beef production in the country. Previous field studies on grass yields under normal and elevated temperature conditions have shown that grassland productivity under future climate will increase under Finnish climatic conditions (Hannukkala, 1997). However, there is a serious shortage of information concerning Finnish grassland responses (in terms of greenhouse gas (GHG) emissions and carbon sequestration potential) to soil types, management practices, prevailing climatic conditions and grass mixture combinations. With Luke's research mandate to assess agricultural sustainability under current and future climate and a massive drive to support the development of research infrastructure to address such societal issues, we have now started continuous, year-round measurements of GHG exchange using the eddy covariance technique in grasslands on mineral and drained organic soils in the Maaninka regions in eastern Finland. We present here the preliminary results on CO_2 exchange from three grassland sites – one on a mineral soil and two on drained organic soils during 2021 and 2022 seasons.

Materials and methods

The demo farms are located near the Luke research station in Eastern Finland. The following ongoing experimental field sites will provide data from successful projects funded through many national and international sources and currently in operation and serve as demonstration farms in Finland. (A) Anttila is a 6.3 ha site on a mineral soil with a land use history of bioenergy crops and grasslands. Currently, it is a legume grassland with a grass mixture of timothy, meadow fescue and red clover. (B) Särkisuo is grassland on a drained organic soil with normal and elevated water-table levels, and (C) Pappilansuo is also another grassland on a drained organic soil under different tillage options. At each of these sites, CO_2, CH_4 and N_2O flux measurements are being carried out using the eddy covariance (EC) technique (Lind *et al.*, 2016; Shurpali *et al.*, 2010). The eddy covariance system is standardised at all of these sites and consists of a Metek 3D sonic anemometer for turbulent wind components, an IRGA (LiCor 7200 RS) for water vapour and CO_2 and a laser spectrometer (Aerodyne Inc., USA) for water vapour, N_2O and CH_4 mixing ratios. The 10 Hz EC data are stored locally on 16 GB USB disks as well as on Luke cloud servers. The data are processed, and 30 min fluxes are calculated as per the standard EC data handling procedures (see Lind *et al.*, 2020 for details). In addition, supporting meteorological variables such as air temperature, humidity, shortwave radiation, net radiation balance, photosynthetically active radiation, 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. A phenology camera captures an image of the same selected area daily for computing green index.

Results and discussion

EC flux measurements of CO_2 exchange at the Anttila site began in September 2021, in August 2020 at the Särkisuo site and in July 2021 at the Pappilansuo site, and they are continuing at all these sites (Figure 1). In the months of Oct and Nov 2020, NEE varied from -13 μmol m^{-2} s^{-1} during the daytime to about 7 μmol m^{-2} s^{-1} during the night time. Note that the negative values represent the amount of atmospheric CO_2 taken up by the grassland vegetation, while the positive values represent ecosystem respiration (comprising root, soil microbial, and above-ground vegetation respiration). With the vegetation senescence occurring towards the end of Oct 2020, NEE declined and it represented a low background emission of CO_2 throughout the 2021-22 winter until the middle of May 2022, when the vegetation began sequestering CO_2 again. There were two grass cuts during the 2022 season at Anttila. Following these grass cuts, there was a sharp decline in NEE owing to the removal of the above ground biomass capable of photosynthetic activity. A similar response can be seen in the 2021 and 2022 seasons for Särkisuo and Pappilansuo sites. The Pappilansuo site was ploughed soon after the first cut in 2022. The impact of summer tillage at this drained organic soil is quite evident as the site remained a large source of CO_2 for about a month before the grasses at the Pappilansuo site could regain their photosynthetic capacity. Based on the data presented in Figure 1, one can make a visual observation that the summertime total ecosystem respiration at the drained organic sites is higher than that from the Anttila site based on a mineral site. The continuous, multi-year EC data on CO_2 exchange in these sites highlight the grassland responses to prevailing climate, soil types and management practices (tillage, fertilization and grass harvesting) during the growing seasons.

Conclusions

The data presented in this paper are preliminary. We aim to do further data processing and gap filling and NEE flux partitioning into gross primary productivity and total ecosystem respiration and quantifying the governing climatic, soil and plant variables to CO_2 flux and finally computing the annual balances for clarification on whether the ecosystems serve as a carbon sink or source annually. This is a work in progress. Such continuous and multiyear data are needed for understanding the boreal grassland ecosystem behaviour and its responses to current climatic conditions and management practices. These data are essential for validating and improving the agroecological and regional climate models.

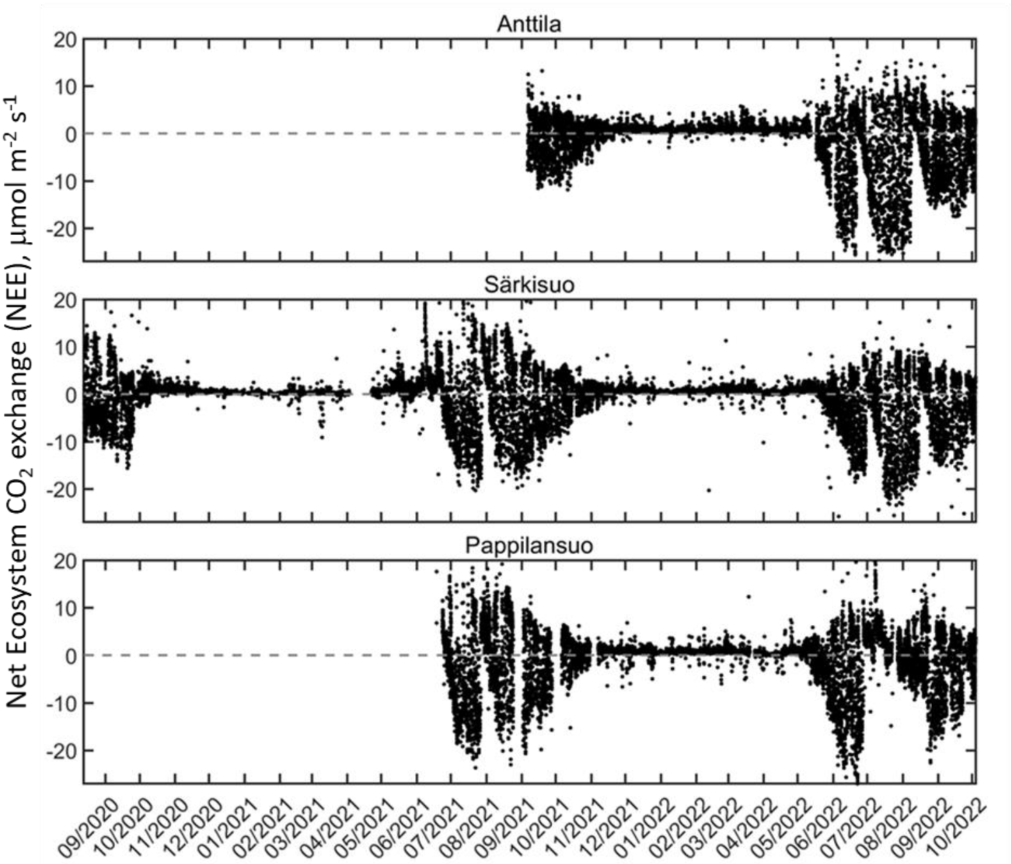

Figure 1. Seasonal patterns of NEE from grasslands on (A) mineral soil at Anttila, and on drained organic soils (B) Särkisuo and (C) Pappilansuo during 2021 and 2022.

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Plant species composition and plant species richness under long-term Brignant grassland experiment in Wales

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Abstract

To explore how long it takes for upland improved permanent pasture to revert to semi-natural grassland vegetation the Brignant grassland experiment was established in 1994 in Wales. It has seven treatments with three replications: sheep grazing with (GL+) and without (GL–) lime application; hay cutting with (HL+) and without (HL–) lime application; hay cutting followed by aftermath sheep grazing with (HGL+) and without (HGL–) lime application; control (CO) with previous grazed and fertilized plots. Percentage cover of individual plant species within randomly placed quadrats was estimated by visual observation in each plot during the growing season 2022. The mean total numbers of vascular plant species, vascular plant species with cover ≥1% and the Shannon diversity H index were significantly higher under treatments that included a cutting regime (HL+, HL−, HGL+, HGL−) than those with grazing management only (GL+, GL−, CO). Four groups of treatments were identified on the ordination diagram: CO treatment as the first group; GL+, GL− treatments as the second group; HGL+ and HGL− treatments as the third group; and HL+ and HL− treatments as the fourth group. There are still ongoing changes in the plant species composition almost after 30 years since the beginning of the experiment.

Keywords: defoliation, liming, managements, sheep grazing

Introduction

The restoration of species-rich grassland on previously agriculturally improved pasture (ploughed and reseeded, regular inputs of NPK fertilizers and lime) has several specific biotic and abiotic constraints. The potential for re-establishment of species from the seed bank, and, in the longer-term, the presence of the lost species in the locally surrounding vegetation are important for successful restoration of degraded plant communities (Van Diggelen and Marrs, 2003). Moreover, the type of applied management is also a key driver influencing floristic diversity of improved grasslands. This study addressed the related research question: What impact does type of defoliation with and without liming on plant species composition and species richness of formerly improved semi-natural grassland in the long-term?

Materials and methods

The 29-year-old Brignant grassland experiment was established in 1994. The experiment is located at 310 m a.s.l. on free-draining brown podzolic soils. The area of the experiment has a mean annual rainfall of approximately 1,850 mm and has an average annual minimum and maximum air temperatures of 5.2 and 11.9 °C, respectively. The plots are arranged in three replicated blocks, each containing seven plots of different treatments. The treatments are: sheep grazing, with (GL+) and without (GL−) lime application; hay cutting only, with (HL+) and without (HL−) lime application; and hay cutting followed by aftermath sheep grazing, with (HGL+) and without (HGL−) lime application. These six treatments are without additional fertilizer applications. Control (CO) plots continue the previous site management. These are limed and annually fertilized at a rate of 60 kg ha^{-1} N and 30 kg P ha^{-1}, with K also applied as required to maintain an index of 2+ (ADAS, 1983). The randomized block design experiment consists of

21 plots in total. The area of hay cut only plots is 0.08 ha and all grazed plots and plots with cutting and aftermath sheep grazing is 0.15 ha. The plots are stocked with sheep with numbers adjusted to maintain a sward surface height of approximately 4 to 6 cm. The HL+, HL−, HGL+, HGL− plots have a single hay harvest taken annually after 21 July, when weather conditions allow. Botanical data were collected during the growing season 2022 by one person only. Visual percentage cover of vascular plant species was estimated in five randomly located quadrats (0.4×0.4 m) in each plot. The mean of five quadrats of botanical composition was used for statistical evaluation. The scientific names of the plant species follow Kaplan *et al.* (2019). The Shannon diversity (H) index was calculated using cover data of vascular plant species from each sampling plot. A linear mixed-effects model (LMM) with fixed effects of treatment and random effect of replication and then Tukey's HSD tests were used for the univariate data. Redundancy analysis (RDA) in the CANOCO 5.0 program (Ter Braak and Šmilauer, 2012) was used to evaluate multivariate vegetation data (cover of vascular plant species).

Results and discussion

The mean total numbers of vascular plant species ($P<0.001$), vascular plant species with cover ≥1% ($P<0.001$) and the Shannon species diversity H index ($P<0.001$) were significantly higher under treatments that included a cutting (HL+, HL−, HGL+, and HGL−) than under those with grazing management only (GL+, GL−, CO) (Table 1). Based on the RDA analysis, four groups of treatments with similar plant species composition were identified on the ordination diagram: CO treatment as the first group; GL+, GL− treatments as the second group; HGL+ and HGL− treatments as the third group; and HL+ and HL− treatments as the fourth group (Figure 1). The results from this study were similar as the results obtained from the Brignant experiment after 19 years of management (Pavlů *et al.*, 2021). In contrast to previous research, we revealed significant effect of liming on the total number of vascular plant species and changes in plant species composition after application of fertilizers in the control treatment. It seems that level of dose and interval between the liming play important roles, as well as long-term application of fertilizers supporting plant species with higher nutrient demands in the control plots (CO) with long-term continuous sheep grazing (Pavlů *et al.*, 2021). Further, climate changes could also affect nutrient cycles in the soil and botanical composition under long-term lime and mineral fertilizer application.

Conclusions

The long-term impact of the different defoliation regimes or fertilizer applications resulted in different plant species composition within treatments. The treatments with grazing management in comparison to cutting has been shown to promote dominance of grasses such as *Agrostis capillaris* and *Lolium perenne* at the expense of forbs. In contrast, hay cutting and hay cutting followed by aftermath grazing was linked to increased forbs cover. Species diversity increased for all treatments which included a cutting management. Liming increased total number of species in all treatments. There are still ongoing changes in the plant species composition and species richness after almost 30 years since the beginning of the experiment.

Table 1. Numbers represent average of three replicates ± standard error of the mean (SE).[1,2]

	CO	GL+	GL−	HL+	HL−	HGL+	HGL−
Total number of vascular plant species	6.2±0.6a	8.9±0.6b	7.3±0.5ab	13.1±0.5cd	12.8±0.4c	15.0±0.6d	14.2±0.4cd
Number of vascular plant species ≥1%	5.3±0.5a	6.6±0.5ab	5.7±0.4a	8.1±0.5bc	8.6±0.4cd	10.1±0.4d	9.5±0.4cd
Shannon (H) diversity index	1.4±0.1a	1.6±0.1ab	1.4±0.1a	1.7±0.1bc	1.8±0.1bc	2.0±0.1c	1.8±0.0bc

[1] The result of post hoc comparison Tukey's HSD tests ($P=0.05$) are indicated by different lower-case letters.

[2] CO = control; G = sheep grazing; H = hay cutting; L+ = lime treatment; L– = no lime treatment.

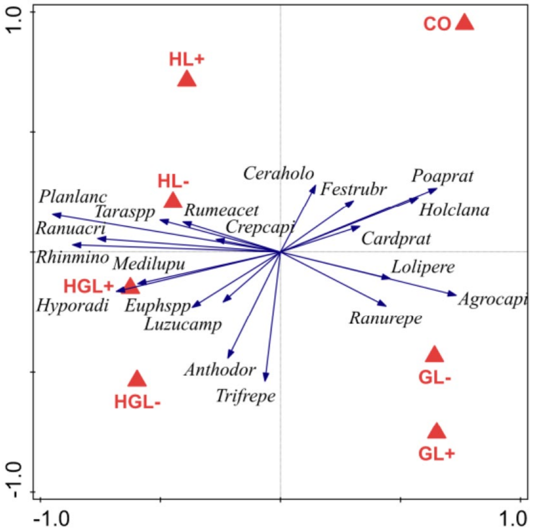

Figure 1. Ordination diagram representing the results of redundancy analysis (RDA) showing changes in plant species composition, treatments were used as predictors. Treatment abbreviations are explained in Materials and methods. Species abbreviations are based on the first four-letter of genera and the four-letter of species name: *Agrocapi* = *Agrostis capillaris*, *Anthodor* = *Anthoxanthum odoratum*, *Cardprat* = *Cardamine pratensis*, *Ceraholo* = *Cerastium holosteoides*, *Crepcapi* = *Crepis capillaris, Euphspp* = *Euphrasia* spp., *Festrubr* = *Festuca rubra* agg., *Holclana* = *Holcus lanathus*, *Hyporadi* = *Hypochaeris radicata*, *Lolipere* = *Lolium perenne*, *Luzucamp* = *Luzula campestris*, *Medilupu* = *Medicago lupulina*, *Planlanc* = *Plantago lanceolata*, *Poaprat* = *Poa pratensis*, *Ranuacri* = *Ranunculus acris*, *Ranurepe* = *Ranunculus repens, Rhinmino* = *Rhinanthus minor*, *Rumeacet* = *Rumex acetosa*, *Taraspp* = *Taraxacum* spp., *Trifrepe* = *Trifolium repens.*

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Carbon accumulation in the roots of grassland

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Abstract

The important role of SOC sequestration in grassland could be assigned to the roots of plants. However, root derived C sequestration is investigated much less than total SOC stocks in the soil. In our study, different grassland systems were investigated for biomass output and C accumulation in the roots. According to our investigations, the composition of grasslands significantly affects the biomass of roots in the soil. Results showed that the most productive mixture was red clover with festulolium, which accumulated 8,763 DM kg ha^{-1}. The carbon accumulated in the roots of grassland species mixtures differed from 3,299 to 4,388 kg C ha^{-1}. The highest C amount was found in the roots of red clover and festulolium mixture, at 4,388 kg C ha^{-1}, while for monoculture red clover it was 3,563 kg C ha^{-1} and for festulolium 3,299 kg C ha^{-1}. Meanwhile, C accumulated in the shoots varied from 2,914 to 5,134 kg C ha^{-1} and this was mostly removed from the system for livestock. All these data give important reasons to state that species diversification and the choice of the specific species can significantly elevate carbon sequestration in the roots of grasslands and thereby contribute climate change mitigation.

Keywords: alfalfa, diversification, festulolium, mixtures, red clover

Introduction

Previous studies in Lithuania show that different agro-ecosystems such as forests, grasslands, and croplands provide different soil organic carbon (SOC) stocks in the different soil types (Armolaitis *et al.*, 2022). The mean SOC concentrations in the 0-10 and 10-30 cm topsoil of most soil groups was shown to be higher for grasslands than for croplands. The total averaged SOC stock in mineral topsoil of grassland of Lithuania was 74 t ha^{-1}. The important role of this SOC sequestration in the grassland could be assigned to the roots of plants. However, root-derived C sequestration in Lithuania is investigated less than the total SOC stocks in the soil. Lithuanian and foreign researchers state that after the establishment of the plant biomass, the roots decompose more slowly than their aboveground parts.

Materials and methods

The research was carried out in the northern part of Central Lithuania lowland (56°12 N, 24°20 E). The soil of the experimental site is a limnoglacial *Endocalcaric Endogleyic Cambisol* (*Siltic, Drainic*). The soil texture is clay loam on silty clay with deeper lying sandy loam. The objective was to investigate the role of grasses in root-derived SOC. Grasses were used in crop rotation: 1. Barley (spring) + grasses (installed in autumn) → 2. Grasses for C sequestration → 3. Winter wheat → 4. Winter triticale. The experiment design included 2 factors.

Factor A – grasses species: (1) Festulolium; (2) mixture of festulolium and red clover; (3) mixture of festulolium and alfalfa; (4) red clover; (5) alfalfa.

Factor B – management type of grassland: (1) aboveground biomass removed; (2) mulched (grasses were cut 4 times and left on the soil surface); (3) mixture of both (biomass of grasses removed after first cut, and two other cuts mulched).

The shoot biomass was cut and weighed two times during the season: before cutting the first and second grass harvests, at the place from where the root samples were to be taken. Above-ground biomass was determined in 0.25 m^2 (0.5×0.5 m) plots with 3 repetitions. Roots were dug in the autumn with a

25×25×25 cm diameter cube. The dry matter (DM) content of shoot and root biomass was determined by drying samples at 105±2 °C temperature in a forced-air oven until constant weight was reached. For organic C determination, the samples of biomass (shoot and root) were oven-dried at 65±5 °C temperature to a constant weight and ground in an ultra-centrifugal mill ZM 200 (Retsch, Germany). Organic C was determined by Tyurin's method, using Nikitin's modification. Organic matter was oxidized by burning potassium bichromate in sulfuric acid solution and measured with a Cary 50 UV (VARIAN) spectrophotometer at 590 nm using glucose standards.

Results and discussion

The most productive mixture was red clover with festulolium, which accumulated 8,763 DM kg ha^{-1} (Figure 1). The carbon accumulated in the roots of grassland species mixtures differed from 3,299 to 4,388 kg C ha^{-1} (Figure 2). The highest C amount was found in the roots of the red clover and festulolium mixture, 4,388 kg C ha^{-1}, while for monoculture red clover it was 3,563 kg C ha^{-1} and for festulolium 3,299 kg C ha^{-1}. Meanwhile, C accumulated in the shoots varied from 2,914 to 5,134 kg C ha^{-1} and this was mostly removed from the system for utilization by livestock (Figure 2).

Figure 1. Root biomass (dry matter (DM) kg ha^{-1}). Differences between species and effect of mixing forage species and of management practices.

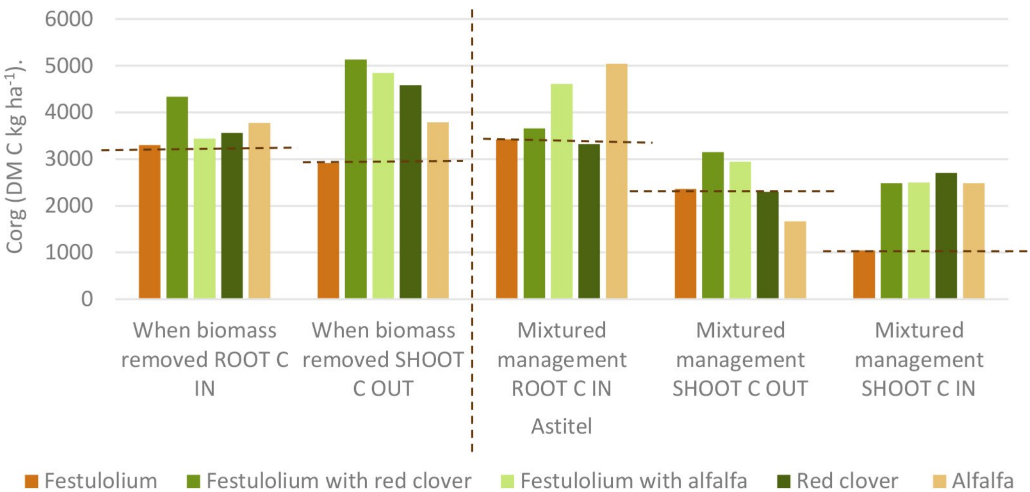

Figure 2. Organic carbon (Corg) accumulated in root and shoot biomass (dry matter (DM) C kg ha^{-1}). Differences between species and effect of species mixing and of management practices.

Conclusions

Species diversification and the choice of the specific management type can significantly elevate carbon sequestration in the roots of grasslands and therefore contribute climate change mitigation. In our case, the use of a mixed management type of cutting and mulching was the best practice for elevating C accumulation in biomass of roots and shoots during the one period of vegetation.

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Theme 3.
Multi-species swards and intercrops for crop rotations benefits

Combined adaptation to climate and mixed cropping in perennial forages

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Abstract

Both climate change and the adoption of forage species in new areas of cultivation require adaptation to new patterns of climatic seasonality and new types or levels of stress. In this context it is relevant to understand the interactions between phenology, seasonal acclimation processes, stress resistance and recovery after stress. In parallel, the benefits of mixed cropping have created a need for understanding species interactions, species compatibility and adaptation to cultivation in species mixtures versus pure stands. Plant community composition can likely affect stress prevalence, resistance and recovery, in interaction with phenology and seasonal acclimation processes. Here, I explore some of the interactions among these factors, with a focus on red clover growing in species mixtures in regions with harsh winters.

Keywords: acclimation, grassland, mixtures, phenology, season, winter

Introduction

Two current challenges for agriculture are climate change and the need for sustainable intensification. Climate change creates problems for forage production that need to be solved, such as more severe and frequent droughts, but also opportunities that can be utilized, such as increased productivity in some regions or in specific periods of the year (Ergon *et al.*, 2018; Gharahmani *et al.*, 2019). In parallel, cultivation of species mixtures which include legumes is a central element of sustainable intensification of forage production which we should take full advantage of, and which to some extent can help mitigate the problems that climate change creates (Lüscher *et al.*, 2014; Wood *et al.*, 2015).

In this paper, I will first consider adaptation of perennial forages to seasonal variation in climatic conditions, then adaptation to mixed cropping, and finally adaptation to the combination of these two, with a focus on red clover growing in species mixtures in regions with harsh winters.

Adaptation to seasonal variation in climate

In Europe, we expect, and already see, hotter and drier summers, particularly in the south, and warmer and more unstable winters, particularly in the north (Ergon *et al.*, 2018; Trnka *et al.,* 2020). These shifts exacerbate some stresses, most notably drought, while they may alleviate others. The effects on winter stresses are variable and unpredictable (Rapacz *et al.*, 2014). We expect longer thermal growing seasons, particularly in the north, and a shift of productivity towards the winter in the south. This implies shifts in seasonal patterns of temperature relative to photoperiod. Global warming also stimulates northward expansion of cultivation areas for more productive species or genotypes, which will then be exposed to seasonality patterns that are different from those they are adapted to.

Perennial plant species in regions with annual recurrent periods of winter stresses or summer droughts have evolved mechanisms that regulate and balance growth and stress resistance (in terms of survival) in accordance with the climatic seasonality. In regions with less severe or short-term stresses, some growth can be maintained throughout the unfavourable season, while in regions where severe and long-term seasonal stresses occur, plants enter an eco- or endo-dormant state enabling survival through the non-favourable season (Gillespie and Volaire, 2018). Variation in the ability to survive the severe season can be spilt into the following inter-related components: (1) variation in the ability to regulate and balance growth and stress resistance appropriately in relation to the climatic conditions; (2) variation in accumulation of organic reserves; and (3) variation in presence and efficiency of specific resistance mechanisms. I will go into each of these in the following.

Seasonal coordination of growth and stress resistance

During autumn in regions with severe winters, perennial plants stop producing leaf area, cold acclimate and enter a winter stress resistant state where meristems are dormant (Preston and Sandve, 2013). In spring, meristem dormancy is broken, growth resumes and stress resistance is largely lost (de-acclimation) (Rapacz *et al.*, 2014). Similar, but opposite processes occur in plants adapted to severe summer drought seasons (Gillespie and Volaire, 2018). The timing of these processes, both before and after the stressful season, is under genetic control and regulated by the seasonal changes in temperature and light in a coordinated manner. Both climate change and shifts in cultivation area can result in a mismatch between genetic and environmental regulatory factors (Ergon, 2017; Rapacz *et al.*, 2014; Wingler, 2015).

Negative correlations between seasonal stress resistance and potential leaf or shoot growth are also seen when comparing populations within a perennial forage species. This has been shown for cocksfoot (*Dactylis glomerata*) and perennial ryegrass (*Lolium perenne*) (Bristiel *et al.*, 2018; Cooper, 1964; Keep *et al.*, 2021) and red clover (*Trifolium pratense*) (Annicchiarico and Pagnotta, 2012; Loucks *et al.*, 2018; Zanotto *et al.*, 2021). However, at the same time these two traits are at least partly genetically uncoupled, as shown for lucerne (*Medicago sativa*) (Brummer *et al.*, 2000; Castonguay *et al.*, 2006; Claessens *et al.*, 2022), possibly due to variation in specific stress resistance mechanisms not related to growth. Moreover, stress resistance has often been found to be uncoupled from growth measured in a favourable environment or the favourable season (Bristiel *et al.*, 2018; Fletcher *et al.*, 2022; Jung *et al.*, 2020; Keep *et al.*, 2021).

Seasonal accumulation of organic reserves

Cessation of leaf expansion does not necessarily imply cessation of photosynthesis and biomass accumulation but could instead reflect a shift in allocation of photosynthates and other organic compounds from leaf blades to newly formed tillers, roots, or other storage tissues prior to the severe stress period (Hisano *et al.*, 2008; Wingler, 2015). The amounts of accumulated reserves are often found to correlate positively with stress resistance (e.g. Bertrand *et al.*, 2020; Castonguay *et al.*, 2011). Some of the accumulated compounds serve as osmolytes and cryoprotectants, and thus play a direct role in resistance to both drought and freezing (see below). They may also be needed when respiration is higher than photosynthesis over a long period, or for regrowth when the leaf and root tissue has died during the stressful season.

The perennial forage grasses and legumes that we utilize in forage production are already adapted to defoliation through co-evolution with grazing animals. Nevertheless, strong competition for light and corresponding shade avoidance responses, as well as shoot regrowth after repeated defoliation, involves allocation of resources to the aboveground parts of the shoot and may exhaust organic reserves and reduce the winter survival ability (Bélanger *et al.*, 2006; Donaghy and Fulkerson, 1998; Frankow-Lindberg *et al.*, 1997). Thus, when it comes to survival during the stressful season, interactions will exist between factors that affect competition and regrowth (e.g. sowing density, species composition (discussed below), fertilization, defoliation frequency) on the one hand, and climatic factors on the other.

Resistance mechanisms to specific seasonal stresses

Mechanisms that play a role in perennial forages tolerating extreme seasonal drought stress include accumulation of compounds that maintain cell membranes during dehydration (Norton *et al.*, 2016). Stress encountered by perennial plants during harsh winters are complex and can involve a number of different components such as freezing (which implies cellular drought), low-temperature pathogenic fungi, ice cover, water-saturated soil, soil movement and lack of light (Belanger *et al.*, 2006; Rapacz *et al.*, 2014). Mechanisms that are involved in resistance to these stresses include accumulation of osmolytes, cryoprotectants, ice-interacting proteins, increased fluidity of membranes and increased expression of pathogenesis-related proteins (Sandve *et al.*, 2011).

Adaptation to mixed cropping

There are well documented positive effects of species and genotype diversity on grassland yield and yield stability, which in sown grasslands are due to phenomena such as differentiation in the utilization of resources (niche differentiation or resource partitioning) and positive mutualistic species interactions (facilitation) (e.g. Ergon *et al.*, 2016; Finn *et al.*, 2013; Prieto *et al.*, 2015). These positive effects are stronger and sustained for longer when a certain balance is maintained among species in the mixture, both in terms of competitive ability, stress resistance and recovery (Annicchiarico *et al.*, 2019). Such species compatibility is therefore desired, and an important aspect of adaptation to mixtures.

The agronomic performance of a species or genotype is influenced by the surrounding plant community (Annicchiarico *et al.*, 2019; Litrico and Violle, 2015) due to direct and indirect plant-plant interactions, which in turn are modulated by climate and other abiotic or biotic environmental factors. For example, legumes can be outcompeted by grasses under high N fertilization or low temperatures (Brophy *et al.*, 2017) and white clover (*Trifolium repens*) growth is suppressed by shading in tall stands (Annicchiarico and Proietti, 2010) and thus compatible with perennial ryegrass and tall fescue (*Schedonorus arundinaceus*, syn. *Festuca arundinacea*) under frequent cutting, but not under less frequent cutting (Ergon *et al.*, 2016). In some cases, differential performance (competitive ability, survival) in mixed stands vs pure stands has been attributed to certain plant traits. For example, competitive ability of lucerne genotypes in mixture with tall fescue was associated with long internodes, high shoot number and a non-erect growth habit, whereas competitive ability in pure stands was associated with other traits (Maamouri *et al.*, 2017).

Stress in mixed versus pure stands

Species diversity effects on grassland resistance to drought stress and subsequent recovery has been described (reviewed by Lüscher *et al.*, 2022). Positive species diversity effects on yield and yield stability of sown grasslands have been shown to be present under experimentally applied moderate drought stress (Grange *et al.*, 2021; Haughey *et al.*, 2018; Hofer *et al.*, 2016; Prieto *et al.*, 2015), but not under experimentally applied severe drought stress (Barkaoui *et al.*, 2016; Kreyling *et al.*, 2017). Effects of species diversity on recovery of sown grasslands after severe seasonal drought stress are less consistent (Barkaoui *et al.*, 2016; Kreyling *et al.*, 2017), and have to my knowledge not yet been addressed in relation to recovery after winter.

Plant community composition can influence stress resistance or recovery of individual plants in the sward through: (1) an effect on stress levels; and (2) an effect on the individual plants' resistance levels or recovery ability. I will discuss these points in the following.

Effects of plant community composition on stress levels

An intuitive and well documented example of plant community effects on stress levels is the reduced prevalence of pathogens in mixed as compared to pure stands, both above-ground (Mitchell *et al.*, 2002) and below-ground (reviewed by Ampt *et al.*, 2018). Such effects appear to be mainly due to a certain level of pathogen-host specificity combined with a reduction in the pathogen population with a reduction in host density. Low-temperature fungal pathogens are frequently occurring stress factors for both grasses and legumes during winter, and disease pressure can be expected to be reduced in grass-clover mixtures relative to pure stands. Different grass species can be hosts for the same pathogens, and so can different clover species, but there may be some differentiation in host preference among different races within a fungal pathogen species. Effects of plant community composition on abiotic stress levels are not described, to my knowledge. It is, however, possible to speculate which mechanisms could possibly be acting. It seems likely that the presence of species with an efficient water uptake and high transpiration rates may exacerbate drought stress of species with less efficient water uptake. Stubble that is left of some species after the last harvest may trap snow which insulates well and can prevent freezing damage of species whose stubble is less rigid due to a lack of stems in the last regrowth. Stubble can also perforate ice covers, thereby allowing gas exchange and more rapid melting. Fibrous grass roots may stabilize the soil and limit soil movement and the upheaving of forage legume taproots during freeze-thaw cycles. Taproots on the other hand, promote good soil structure, and may indirectly lead to less damage to other species due to water-saturated soils and resulting anoxia, soil movement and ice cover.

Effects of plant community composition on individual plants' stress resistance and recovery

Resistance and recovery of individual plants can be related to the seasonality of growth and acclimation, organic reserves, or specific resistance mechanisms, as outlined in the section 'Adaptation to seasonal variation in climate'.

The allocation of reserves to storage organs may be affected by photosynthetic rates and thus the availability of light and N, which again is strongly influenced by the plant community. Intra- and interspecific competition for light, water and nutrients can strongly affect allocation to shoots vs storage organs, which may affect survival and spring regrowth.

Differential selection among red clover genotypes in mixtures vs pure stands has been shown to occur (Ergon *et al.*, 2019). Traits associated with this differential selection were also identified; offspring of survivor populations from mixtures harvested three times a year in Norway had more leaves with larger leaf blades and longer petioles during the vegetative stage, followed by earlier stem elongation, higher number of elongating stems, higher biomass (also when accounting for earlier stem elongation), more leaves in the regrowth after cutting and lower freezing tolerance than offspring from pure stand survivor populations (Ergon and Bakken, 2022), hinting at traits that are of importance for red clover adaptation to grass-legume mixtures. One possible interpretation of these results is that red clover individuals that are early and produce large shoots have an advantage in competition with the earlier heading grasses (in agreement with results of Hoekstra *et al.*, 2018), and that these traits are negatively correlated (genetically or physiologically) with freezing tolerance. However, in the same experiment, a higher red clover winter mortality was observed in pure stands compared to mixed stands (Ergon *et al.*, 2016), in spite of the higher freezing tolerance of their offspring. This may indicate that either the winter stresses were higher in the pure stands or, alternatively, that plants in pure stands were in a state that made them less able to survive the winter. As the same high mortality was not seen in pure stands harvested five instead of three times a year, we hypothesized that the high mortality in pure stands harvested three times a year were due to strong intraspecific competition, allocation of biomass to shoots and subsequent reduction in winter survival ability, leaving survivors with opposite growth characteristics and a higher freezing tolerance than might be needed (Ergon and Bakken, 2022).

Conclusions

Climate change, shifts in species' cultivation area, and increasing utilization of mixed cropping for sustainable intensification of crop production requires plant material with optimal performance under these new conditions. It is known that environmental and genetic regulation of annual growth cycles are important for adaptation to the seasonal variation in growth conditions and stresses a given location. It is also known that the genotypes providing the best performance in species mixtures are not the same as those providing optimal performance in pure stands. In addition, plant community composition can likely affect stress prevalence, resistance and recovery of individual plants, in interaction with phenology and seasonal acclimation processes.

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The importance of multi-species grassland leys to enhance ecosystem services in crop rotations

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Abstract

The ongoing simplification (temporal and spatial) of agricultural production systems has resulted in severe negative consequences, ranging from losses in soil organic carbon and biodiversity to a high dependency on external inputs to maintain high yields. This paper will identify how grassland leys in crop rotations are vital to mitigate these effects, by conserving soil organic carbon and enhancing nutrient efficiency. In particular, grasslands containing legumes enhance these benefits by providing nitrogen. In crop rotations, these grasslands transfer some of the acquired nitrogen to arable follow-on crops, thereby reducing the necessity for external inputs, while at the same time providing additional benefits, such as improvement of soil quality and reduction of weed pressure. However, our knowledge about the possibilities of enhancing these ecosystem services by optimising the community composition of the leys remains patchy. While the benefits of multi-species grasslands for the grassland crop have been shown repeatedly and across a large gradient of environments, further research is required to determine the benefits for follow-on crops, particularly across environments. Here, we emphasize the importance of multi-site research, such as in the research network LegacyNet. A further important question is the potential role of the functional plant diversity of the leys to achieve a range of objectives in different types of rotations, and this paper will explore the state of knowledge about this role. Finally, management techniques that are optimised for both ecosystem services and agronomic performance will be presented for cut and grazed systems. For the latter, an outlook will also be presented as to how the inclusion of bioactive plant species can additionally enhance animal health and lower methane emissions in grazing ruminants.

Keywords: crop rotations, diversification, sustainability, nutrient cycling, circularity, LegacyNet

Simplification of agricultural systems increases input dependence

Agricultural production systems have continuously become more specialized, thereby enabling an intensified production, and today less than 10% of the agricultural area in the United States and Western, Central and Northern Europe are used as Integrated Crop Livestock Systems (Garrett *et al.*, 2020). However, this led to an ongoing simplification of production systems at both temporal (i.e. monocropping) and spatial (monocultures and simplified landscapes) level. As a result, many regions developed high densities in either continuous cropping of arable crops, or livestock production with high-energy, low-fibre rations that often contain a large share of grain. Due to the arising spatial separation, manure often needs to be processed and transported to regions with lower animal density at substantial cost to avoid excessive nitrogen loads to groundwater. The processing and enhanced storage duration of manure increases ammonia volatilisation, thereby reducing the nitrogen (N) efficiency of dairy farms below 30% (Löw *et al.*, 2020). Simultaneously, 72 kg of nitrogen in the form of mineral fertilizer are applied per hectare and year on average across the EU, with large regional differences (De Vries *et al.*, 2021). A more diversified approach based on integrated crop livestock systems would render these transports and high mineral N amounts unnecessary. Thus, while providing high yield increments, the simplification of agricultural systems has resulted in severe negative consequences, ranging from losses in soil organic carbon to a high dependency on external inputs (Lemaire *et al.*, 2015). For example, global cereal yields have increased by almost 4-fold between 1961 and 2016, without expanding the production area, yet during the same time the global mineral N fertilizer production has increased by almost a factor of 10, and arrived at 123,000,000 Mg N annually in 2018 (Kopittke *et al.*, 2019). This is problematic for environmental reasons, as each kg of N fertilizer produces greenhouse gas (GHG) emissions in the range of 1.3-4 kg CO_{2eq} kg^{-1} N for urea, and 3.5-10.3 kg CO_{2eq} kg^{-1} N for ammonium nitrate (Walling and Vaneeckhaute, 2020).

Similarly, the specialisation of agriculture has resulted in substantial losses of soil organic carbon in the regions dedicated to crop production. Moreover, the increasing amount of concentrate feed in ruminant production systems is understood to have resulted in reduced grassland use throughout Europe (Van den Pol-van Dasselaar *et al.*, 2020), and thus have contributed to losses of soil organic carbon. This has direct implications for soil organic carbon stocks for three reasons: (a) grasslands have a dense rooting system, where more of the total biomass is stored belowground compared to annual crops, and even more so in species rich grasslands, (b) grasslands have a year-round soil cover with no bare ground being exposed to erosion, and (c) the amount of mechanical disturbance is reduced substantially in grasslands, thereby allowing less soil organic carbon oxidation and mobilisation. Thus, crop rotations with grassland leys are advocated to be a viable alternative that produces both arable crops and forage, while also maintaining carbon stocks. However, little is known about the general applicability of these findings across environmental conditions, leys community composition and management types. Consequently, the goal of this review paper is to address the following research questions:

- Can higher diversity in grassland leys increase ecosystem service performance and affect agronomic performance of the follow-on crop?
- How can multisite experiments help better investigate the impact of management and environmental conditions on the effects of grassland leys?
- What is the effect of more diverse plant functional traits in grassland leys on performance at the system level? and
- What is the future outlook for diverse grassland leys?

Spatial diversity: diverse grasslands are an important tool to enhance circularity expectations of leys

Historically, leys hugely contributed to improve agricultural production by replacing the grazed fallow of the medieval three-field system (Stebler, 1895). It is therefore obvious that expectations of leys include a high production of high-quality forage, as well as the maintenance of an adequate soil structure for cropping (Hoeffner *et al.*, 2021). As an important element of the crop rotation, it is moreover expected that leys suppress crop weeds and soil-borne diseases (Martin *et al.*, 2020). For instance, Dominschek *et al.* (2021) observed that weed biomass in maize (*Zea mays*) was more reduced after three years of ley than after a sunflower (*Helianthus annuus*)-maize-sunflower sequence because the relative abundance of fast-growing weeds was suppressed. Because ley cropping allows the selection of a mix of forage species and varieties with specific properties, it allows the design of forage mixtures that prioritise various functions, types of utilisation and pedo-climatic conditions (Lüscher *et al.,* 2019). For commercial seed mixtures, the range of utility nevertheless needs to remain broad enough for economic efficiency. Lately, resilience to severe weather events, resource use efficiency and multifunctionality received increased attention, as well as the potential role of plant diversity within the leys to target these multiple functions. Here, we review evidence of the effects of plant diversity on the main roles of leys.

Principles for the design of mixtures

A mixture may consist of a blend of varieties within a single species, multiple species or a combination of multiple species and multiple varieties within species. The benefits of associating contrasting complementary traits (e.g. N_2 fixing root system of legumes with capture-efficient root system of grasses) has been demonstrated in a number of studies (*e.g.* Finn *et al.*, 2013; Husse *et al.*, 2017; Nyfeler *et al.*, 2011; Suter *et al.*, 2021). Because such large contrasts in plant traits are usually not found among varieties within the same species, associating multiple species is an essential building block of forage mixtures. Emerging from experimental manipulations of plant diversity is a strong signal that (a) the identity of the plant species is crucial, and (b) more balanced mixtures tend to maximise the benefits of synergistic interspecific interactions and deliver the strongest yield responses (Lüscher *et al.,* 2019). Strong mixing effects have been achieved with four well selected species (Connolly *et al.*, 2018; Finn *et al.*, 2013) and the positive effect on biomass productivity of adding a supplementary species has been shown to decrease with the number of species already included in the mixture (Weisser *et al.*, 2017). Thus, a large number of constituent species may not be required for maximized agronomic performances. This could also be the case for multifunctionality (Gamfeldt and Roger, 2017), as observed in the experiment of Suter *et al.* (2021) in which four species (2 grasses and 2 legumes) were sufficient to induce concurrent diversity effects on yield, yield stability, weed suppression, symbiotic N_2 fixation and N efficiency, while maintaining forage quality. However, combining complementary varieties within species may further improve the agronomic performances of the mixture (Meilhac *et al.*, 2019; Prieto *et al.*, 2015).

Forage quality for livestock

Mixing grass and legume species allows for a more balanced forage in terms of energy and protein contents as compared to pure swards of either grasses or legumes (Lüscher *et al.*, 2014). Forage digestibility and protein content decrease with increasing plant maturity and the associated formation of structural tissues. Thus, for a given sward, forage digestibility and protein content usually decrease with increasing yield. Nevertheless, forage yield can be significantly increased without compromising forage digestibility and protein content by mixing grass and legume species (Sturludóttir *et al.*, 2014; Suter *et al.*, 2021). Moreover, positive mixture effects on forage voluntary intake by livestock have been observed with a binary mixture of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*), compared to perennial ryegrass and chicory (*Cichorium intybus*) mixtures (Niderkorn *et al.*, 2017, 2019). Similarly, Soder *et al.* (2006, 2007) observed that the ingestive grazing behaviour of dairy cows (grazing time, biting rate, and grazing jaw movements) were similar with a mixture containing nine species (grasses, legumes, and chicory) as with a binary mixture of cocksfoot (*Dactylis glomerata*) and white clover (Soder *et al.*, 2006, 2007). This is in accordance with a study by Loza *et al.* (2021), in which dairy cows grazing a complex 8-species mixture had higher milk yields than those cows grazing a simple binary mixture, which was likely a result of their higher herbage intake.

Benefits to yield across environmental conditions

The role of plant diversity in increasing yields has been reviewed recently e.g. (Jaramillo *et al.*, 2021; Lüscher *et al.*, 2022) and we refer briefly to it here. Climate change is already affecting both the mean and variance in climate conditions, with consequent increases in the incidence of severe weather events, including drought. Higher plant diversity within productive grassland communities is associated with higher drought resilience than less diverse mixtures or monocultures (Hofer *et al.*, 2016; Komainda *et al.*, 2020; Skinner, 2005). The strength of the relationship varies across studies and the degree of overyielding can be sufficiently strong for the more diverse mixture communities under drought to have similar or greater yields than either the average of the monoculture yields in rainfed controls (Finn *et al.*, 2018; Grange *et al.*, 2021; Hofer *et al.*, 2016) or perennial ryegrass monocultures with higher nitrogen levels (Grange *et al.*, 2021). In related drought experiments, plant diversity in mixtures also increased yield stability (Grange *et al.*, 2022; Haughey *et al.*, 2018). Finally, synergistic interspecific interactions can also occur in drought conditions, and mitigate drought effects on yield (see Lüscher *et al.,* 2022).

Nitrogen efficiency and losses

At the same level of moderate N fertilization, positive diversity effects on yield are usually produced by the interaction between grass and legumes (e.g. Nyfeler *et al.,* 2009). Correspondingly, the presence of N_2-fixing legumes grown in association with grasses did not increase N leaching as compared to pure grass swards when levels of N fertilization were adapted to crop requirements (Bracken *et al.*, 2020). Moreover, legume-based multi-species leys may have a reducing effect on N_2O emissions (Peoples *et al.*, 2019). Cummins *et al.* (2021) have observed similar N yield and DM yield from a six-species mixture compared to a perennial ryegrass monoculture while reducing N_2O emissions by 41 and 24%, respectively. Other trait contrasts that result in positive diversity impacts on yield are the combination of fast establishing and persistent species or the combination of shallow and deep rooting species (Hofer *et al.*, 2017; Husse *et al.*, 2017), however with lesser extent and less robustness than the grass-legume interaction (Finn *et al.*, 2013). A further trait that could increase N efficiency of the ley is a more thorough capture of soil available N by vertical and temporal complementarity in N capture among the species of the community, induced by contrasting rooting depth and growth pattern (Husse *et al.*, 2017). At the crop rotation level, the months following the destruction of the ley should also be included, as N leaching is generally rather low during the period of ley cultivation (Valkama *et al.*, 2016) but may be large following ley destruction (Eriksen *et al.*, 2015; Hansen *et al.*, 2019). Furthermore, residual N from ley cultivation can significantly reduce the need for fertilizer by the following crop (Fox *et al.*, 2020).

Can grassland mixtures show yield benefits within the restricted temporal duration of a ley?

Permanent grasslands are defined by not being ploughed more than every five years. In multi-year experiments using special types of species-rich grasslands, the benefit of diversity generally increases over time; for example, Van Ruijven and Berendse (2005) found no effect of diversity in the establishment year, but the effect was significant from year 2. Grassland leys, in contrast, are characterised by their short duration. A key question, therefore, is: can the known benefits of productive grassland mixtures be evident within a period of twelve to eighteen months? Here, we look at field experiments that manipulate plant diversity in productive grasslands, and present evidence on the effect of mixture diversity within that time frame.

Overall, there are strong responses of mixture yields to diversity within an eighteen-month duration. In the AgroDiversity experiment that compared monocultures and mixtures of two grasses and two legumes across 31 different international locations, there were strong diversity effects and regular occurrence of transgressive overyielding in the first full year of yield measurements after sowing (Kirwan *et al.*, 2007). An associated experiment compared the effects of three nitrogen levels (Nyfeler *et al.*, 2011), and found strong responses to diversity in the first year of measurements. An experiment across three sites that included four-species mixtures of a grass, two legumes and a herb also found responses to diversity in the first year of harvest sampling (Hofer *et al.*, 2016). A six-species experiment investigating communities assembled from two grasses, two legumes and two herbs also found strong responses to plant diversity in the first full year of yield measurements after sowing (Grange *et al.*, 2021). Using three-species mixtures of a grass, a legume and a herb, Cong *et al.* (2018b) also showed strong yield responses to diversity in year 1, when the included herb was ribwort plantain (*Plantago lanceolata*). All of these studies were conducted under a harvesting regime and were not grazed.

With respect to the temporal duration of leys and diversity benefits, the temporal evolution of the botanical composition of the grasslands should also be considered. Indeed, more balanced mixtures tend to maximise the benefits of synergistic interspecific interactions and deliver the strongest yield responses. On the other hand, mixture compositions often tend to get less balanced over time. For instance, legume relative abundance often decreases from the first to the third year of ley cultivation in favour of grass relative abundance (Brophy *et al.*, 2017; Lüscher *et al.*, 2014), with the corresponding decrease in ley performance. On the other hand, several benefits in a crop rotation are generally considered to increase with increasing ley duration (Hu and Chabbi, 2022; Lemaire *et al.*, 2015). Thus, there is a need to assess the optimal ley duration for maximal benefits from synergistic interspecific interactions.

Nitrogen legacy effects of grassland leys

A substantial amount of research has investigated the conversion from grassland to arable crops (and *vice versa*) within crop rotations, and the abiotic and biotic consequences of this (Creme *et al.*, 2018; Hoeffner *et al.*, 2021; Martin *et al.*, 2020). A variety of legacy effects are possible that include the stocks and flows of specific nutrients (especially carbon and nitrogen); attributes of soil structure and hydrology; incidence of weeds, pests and pathogens; soil biodiversity, and crop or forage production. Yet there has been much less research on the legacy effects associated with the diversity of grassland species in a grassland ley.

Fox *et al.* (2020) used a systematically varying proportion of legumes across plant communities assembled from a grass, two legumes and a herb, to find that the yield of a follow-on monoculture of Italian ryegrass (*Lolium multiflorum*) (the legacy effect) was strongly related to the legume proportion. Mixture communities with >20% legume proportion had a significantly higher legacy effect than that from grass monocultures. Communities with 50% legume proportion in the preceding grassland exerted the same legacy effect as a 100% legume proportion (legume monoculture), and the legacy effect was evident for at least 12 months. Similarly, in a six-species experiment by Grange *et al.* (2022) an experimental drought was imposed across all communities, and compared with the rainfed control. Furthermore, all plots received 150 kg N ha^{-1} yr^{-1} of nitrogen fertiliser (150N) in the grassland phase, but were compared to a high N perennial ryegrass monoculture receiving 300 kg N ha^{-1} yr^{-1} of N (300N). They found a strong legacy effect that was related to the legume proportion in the grassland ley, and persisted across four successive yields over four months. The legacy effect was negatively affected by the drought, but to a modest degree in comparison to the effect of legume proportion. In this experiment, the legume proportion in the pre-crop was positively correlated with the benefits in the follow-on crop. Accordingly, at the crop rotation level, the legume monoculture resulted in the highest N transfer to the follow-on crop. This is different to the legume derived N benefits in the grassland ley itself, where a legume share of 25-33% has generally been sufficient to provide the maximum Nitrogen yield across a large gradient of environments (Suter *et al.*, 2015). Finally, Komainda *et al.* (2022) also observed that the yield of a follow-on crop of Italian ryegrass with a simple mixture as pre-crop was only 45% of the yield that was obtained when the pre-crop was a more complex lucerne-dominated multispecies grassland mixture.

Additional benefits of grassland leys in crop rotations

In the AgroDiversity experiment mentioned above, combining two grass and two legume species also strongly improved weed suppression during the period of ley cultivation, especially compared to the legume pure swards, but also compared to the grass pure swards (Connolly *et al.*, 2018). For the function of weed suppression, the interaction between grass and legume species had the strongest positive effect, like for yield, but the interaction between the fast and the persistent species played a major role as well. Thus, multi-species leys are more stable against weed invasion and might therefore help reducing the use of herbicide. Additional benefits of grasslands in crop rotations have for example been illustrated by Colombi *et al.* (2017), who showed that in compacted soils, soil macropores from tap-roots are a tool to provide a path of least resistance, as well as oxygen, thereby enhancing yields of wheat (*Triticum aestivum*), maize and soybean (*Glycine max*) as follow-on crops. However, Kautz *et al.* (2014) showed that even in non-compacted soils, taproots from chicory and lucerne (*Medicago sativa*) resulted in an increased density of biopores compared to tall fescue (*Festuca arundinacea*) when grown for up to 3 years as a ley. In the same time frame, anecic earthworms (*Lumbricus terrestris*) were unable to markedly increase the sub-soil's biopore density, indicating that in these ley durations, deep-rooting species with tap-roots have a stronger importance on the creation of biopores than earthworms (Kautz *et al.*, 2014). In grass-clover swards with added manure, however, it was identified that 3 years are sufficient to create biopores that aid the increased impact of the epi-anecic earthworm species *Aporrectodea longa* and *Lumbricus herculeus* (Krogh *et al.*, 2021). These benefits have been shown before and are especially prominent in no till systems, where the soil structure is not destroyed during the conversion of a follow-on crop (Alhameid *et al.*, 2020). Yet, while little research has been conducted on the potential of multispecies leys directly to increase macropores via the inclusion of forages with deep tap-roots, similar studies on multispecies cover crops indicated their suitability to establish macropores in even a shorter time frame, especially if these cover crops were grazed, thereby allocating more resources to form root biomass (Singh *et al.*, 2021). Thus, further research on the potential of multispecies grassland leys and their impact on macropore creation for follow-on crops would be promising.

Flower resources for pollinators

Pollination is important for maintenance of wildflower communities and crop production as many crops are partially or fully dependent on animal pollination for seed or fruit set (Klein *et al.*, 2006), but a historical decline is documented for wild pollinators in Europe (Biesmeijer *et al.*, 2006). However, multispecies grasslands can be designed to support a higher diversity of pollinators if plants with different pollinator profiles are included in flower mixtures to enhance flower-visiting insects (Cong *et al.*, 2020). In a comparison of grassland leys with increasing levels of diversity compared to a conventional monoculture of perennial ryegrass as permanent grassland, pollinator abundance increased drastically, with 541 wild bees of 10 species in the diverse grassland leys, compared to no wild bees in the permanent grassland due to the absence of flowers. While pollinator abundance was not affected by the grassland ley diversity, the simple mixtures consisting of perennial ryegrass and white clover were only visited by common generalist species, whereas the multispecies grassland ley increased the abundance of rare long-tongued bumblebee to 10% in grazed areas, and even to 20% in ungrazed exclosures (Beye *et al.*, 2022). Typically, legumes were visited mainly by large bees (honeybees and bumblebees) while some of the forbs attracted syrphids and other flies. Attracting pollinators with nectar delivering flowers must imperatively be combined with an adapted management, as mowing at an unsuitable time can amount to a fatal trap for the insects (Kenyeres and Varga, 2023). Spatially heterogeneous management can better support bees and hoverflies through delayed mowing and leaving uncut refuges in extensively managed grasslands (Meyer *et al.*, 2017). An alternative to wildflower strips to achieve this is to systematically leave strips of uncut multispecies grassland at each mowing date, to be included in the following harvest (Cong *et al.*, 2020). This may improve resource availability for pollinators considerably, with only marginal yield and quality loss.

Tailoring management for different ecosystem service priorities

Management is an important tool to steer the performance of swards and crop rotations between maximised yields and maximised ecosystem services. From an agronomic point of view, ploughing-in of grassland provided the highest benefits for the follow-on crop in a crop rotation, combined with a ley duration that spans several years. Accordingly, the incorporation of a three-year-old grassland provided higher gains compared to an annual grass-clover sward, arable legume (field bean) or the manure fertilization of previous crop (Kayser *et al.*, 2010). With regards to the reduction of environmental disservices, nitrate leaching was lowest if the arable crop in the crop rotation was undersown with a catch crop to ensure an additional uptake of nitrogen in autumn (Biernat *et al.*, 2020; Eriksen *et al.*, 2015; Hansen *et al.*, 2007). Accordingly, barley (*Hordeum vulgare*) with an undersown Italian ryegrass (the barley was harvested as whole crop silage to permit enough time for the Italian ryegrass to establish) was the only crop in a study by Eriksen *et al.* (2015) to result in low nitrate leaching of less than 10 kg N ha^{-1} yr^{-1}, whereas maize as a follow-on crop resulted in high nitrate leaching amounts of more than 50 kg N ha^{-1} yr^{-1} for five consecutive years.

Regarding the C inputs, grasslands leys and permanent grassland can actually exhibit similar carbon inputs, but permanent grasslands retain the carbon longer in the soil, thus only permanent grasslands result in substantially enhanced C stocks (Hu and Chabbi, 2022).

As a result, the modelling of changes in soil organic carbon (SOC) stocks, based on a 7-year experiment in Northern Germany and initial SOC stocks of around 50 Mg ha^{-1}, estimated annual losses across a 100 year time horizon to be on average 76 kg C ha^{-1} yr^{-1} for the monocropping of silage maize, fertilized with 240 kg N ha^{-1} $yr^{-1}$from cattle slurry, compared to gains in SOC of 413 kg C ha^{-1} yr^{-1} in an equally fertilized permanent grassland. In that same experiment, a crop rotation consisting of two years of grassland, followed by maize and winter wheat resulted in slight annual gains of 15 kg C ha^{-1} (Loges *et al.*, 2018). This is in accordance with a study by Jensen *et al.*, 2022, who identified that introducing a 2-year-old grass-clover ley into a six-year arable crop rotation increased carbon stocks by 5 Mg ha^{-1}, before a new steady-state condition was achieved after 20 years. Subsequent conversion to a 4-year grass-clover ley resulted in additional C stock increments of 4.2 Mg ha^{-1}, with no new steady state being achieved after 13 years. Similarly, Guillaume *et al.* (2022) have shown a clear benefit of leys for improved SOC, with a positive correlation between the proportion of leys in the crop rotation and SOC stocks (Figure 1). This is due to a combination of the negative impact of tillage on SOC stocks (Haddaway *et al.*, 2017) and the fact that the root biomass only reaches its maximum in the second year (Weisser *et al.*, 2017). After that peak, only grassland mixtures with a higher species richness were able to maintain high levels of root biomass, whereas grass monocultures had decreased their root biomass in year five by more than 50% compared to the first experimental year, and by approximately 75% compared to the maximum root biomass.

To balance soil C stocks and food production, Hu and Chabbi (2022) proposed the incorporation of grassland leys with high N application for three years embedded into a crop rotation. However, with regards to the high N fertilisation, this should not be combined with grazing, as the combination of grazing and slurry application has generally resulted in the highest leaching rates of grasslands (Eriksen *et al.*, 2015), whereas cutting generally produced the lowest leaching rates (Wachendorf *et al.*, 2004). However, reducing fertilizer to reduce nitrate leaching or gaseous emissions also reduces C sequestration, thereby resulting in a trade-off between C accumulation and N losses, as well as yields (Allard *et al.*, 2007)

Performance of diverse crop rotations is dependent on environmental and soil conditions

A recent meta-analysis from China has shown that the potential yield benefits of crop rotations are largest if: initial soil nitrogen concentrations are low; soil organic carbon stocks are intermediate; and soil textures are coarse to intermediate (Zhao *et al.*, 2020). This is to be expected, that yield gains are largest under conditions where nutrient statuses are most limiting. However, environmental conditions also affect the soil microbial conditions and with that the turnover of residual biomass and the transfer of nutrients in a crop rotation. For example, using laboratory conditions, a study by Taghizadeh-Toosi *et al.* (2021) found that soil moisture levels of 60% water-filled pore space (WFPS) resulted in higher N mineralisation rates of red clover residues compared to 40% WFPS. Furthermore, a previous cropping history of diverse crop rotations instead of cereal monocropping has been shown to result in up to 80% higher recovery of nitrogen in follow-on crops, both under drought and controlled conditions (Bowles *et al.*, 2022). As this is likely a result of changes in the soil microbial composition, soil-plant-microbial interactions will need to be analysed in detail to understand their impact on the N transfers. Repeated drying-rewetting cycles might have particularly long-lasting impacts on C and N dynamics and increase N losses from the system via enhanced nitrifier activity (Fierer and Schimel, 2002).

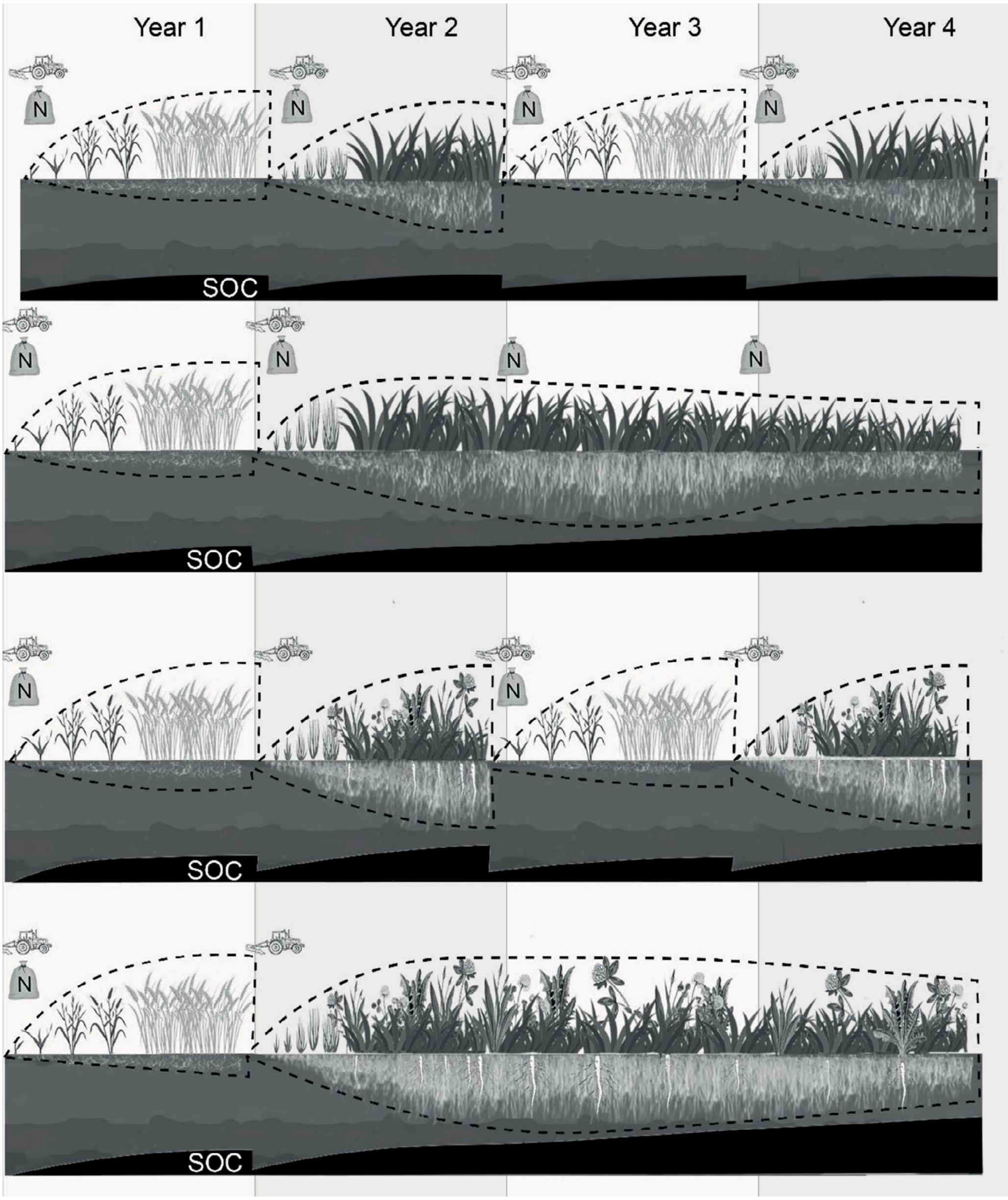

Figure 1. Conceptual representation of a crop rotation comprising a cereal crop followed by grassland leys of different duration (short ley: a,c; long ley: b,d) and species diversity (grass monocultures in the leys in panels a and b; multispecies grassland mixtures in panels c and d) on above- and belowground biomass production over time (dashed lines), as well as soil organic carbon (SOC) stock changes over time (black area at the bottom of each panel). Pictograms of tractors indicate ploughing events, while images of fertiliser bags of N indicate requirement of large amounts of external fertilizer applications. Overall, ley duration affects soil carbon stocks; legume-based mixtures reduce the requirement for nitrogen fertiliser inputs; mixtures maintain above- and below-ground biomass over time, and; grasslands have more root biomass than cereals.

New experimental approaches to account for multitude of factors affecting ecosystem services

The impact of environmental conditions, soil types and the resulting plant-soil-microbiome interactions require our experimental approaches to account for these levels of complexity. If an experiment is repeated at multiple sites, the inference and generalisability of the results from the experiment can be enhanced compared to a single-site study. If multiple sites are selected at random across a country (or alternative spatial scale) and a common experiment implemented at each site, the results can generalise to the scale of the country, whereas the results from a single-site study may be confounded with the conditions of the selected site. For example, in a grassland biodiversity experiment, the species diversity and other treatment outcomes may be affected by factors such as the soil type, previous land use history and climatic conditions at the site (Finn *et al.*, 2013). However, the impact of these differences across sites requires a revised understanding of experimental, as well as analytical approaches.

There are several ways to analyse data from multi-site experiments; for example, including site as a random effect in a linear mixed modelling framework would account for the hierarchical nature of the data. However, the effects from experimentally manipulated treatments could vary from site to site and in this case, random coefficient models, where individual model parameters are assumed to vary from site to site, may be more suitable. Such approaches account for site-to-site variation and appropriately model the correlations between observations from the same site.

One example of a multi-site international experiment that attempts to prioritize the potential generalization of the impact of grassland leys in crop rotations across environments and soil types is the LegacyNet experiment (LegacyNet 2023). In this study, a common experiment is implemented at 32 sites spread across Asia, Europe, New Zealand and North America. The experiment aims at understanding how increased diversity in grassland leys can enhance not only the performance of the ley itself, but also in a follow-on crop. Therefore, the experiment consists of a grassland ley phase of at least 18 months, followed by a follow-on crop phase. In the grassland stage, systematically varying combinations of six forage species (two grasses, two legumes, two herbs), are established and dry matter and botanic composition and nitrogen concentration will be measured. The follow-on crop is then a monoculture of either a grass 'model' crop, or a cereal. Yield, nitrogen yield and other quality variables are recorded on each plot in this phase, as the project assumes that even the recording of a small number of responses can lead to deep knowledge increase, via the value from the distributed and coordinated data collection effort across multiple sites.

Thus, this approach enabled considerable variation of sites across geographical, environmental and soil conditions, and the results of this study will be powerful in testing the robustness of species diversity.

Re-evaluating traditions and preparing them for the future

Bioactive herbs and legumes that contain tannins or other plant specialized metabolites (PSM) have been pursued as a promising route to be included in future grasslands. The PSM in these plants have been identified to act anthelmintic, reduce methane emissions from ruminants and potentially enhance carbon stabilization rates in the soil (Adamczyk *et al.*, 2017; Mueller-Harvey *et al.*, 2018). Consequently, their inclusion in grasslands could reduce the dependence on external inputs further, while generating healthy and nutritious diverse forages. However, to date, the agronomic potential of these bioactive species is often low and the bioactivity has been too variable as a result of environmental impacts and a large variability within species or even cultivars, due to lack of breeding efforts (Verma *et al.*, 2021). Therefore, further efforts will be required to achieve a large share of continuously high bioactivity.

In the future, grasslands may also play a more important role in stockless plant production systems. Not only for maintaining soil fertility and combat weed problems, but also for delivering bioenergy. There is an increasing interest in using grassland biomass for bioenergy production in Europe and North America (Prochnow *et al.*, 2009; Wahid *et al.*, 2018), which is further intensified with the current energy crisis. Biogas production can be enhanced through mixing of two or more substrates with complementary characteristics leading to improved efficiency of microorganisms involved in anaerobic digestion. It has been demonstrated that co-digestion of grass and forbs (chicory and plantain) synergistically enhanced methane yields (Cong *et al.*, 2018a). The synergistic effects were attributed to a more balanced nutrient composition (C/N ratio and micronutrients) in the grass-forb mixture promoting the utilization of multi-species grasslands for bioenergy production.

Conclusions

Increasing species richness has been shown to provide substantial benefits for grassland leys by reducing external inputs and enhancing ecosystem services. Similar benefits have been indicated at the systems level in crop rotations, but complex plant-soil-microbe interactions make it difficult to generalize and quantify these benefits. Future developments will, however, increase the importance of grasslands both in mixed and stockless plant production systems, making the research at the system level even more pressing. Therefore, more multisite experiments will be required to gain an understanding of the extent of these benefits across a gradient of soil and environmental conditions.

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Mechanical and thermal suppression of Kura clover living mulch for maize production

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Abstract

A maize (*Zea mays* L.) Kura clover (*Trifolium ambiguum* M. Bieb.) living mulch system that reduces fertilizer inputs and negative environmental impacts of maize production has been developed. This study tested mowing and/or propane flaming as alternatives to herbicides in maize-Kura clover living mulch production. In 2 years at two Wisconsin USA locations, maize was planted into zone-tilled strips, and inter-row Kura clover suppression was accomplished with: (1) herbicide-killed Kura clover for monocrop maize production; (2) one mowing; (3) two mowings; (4) three mowings; (5) one flaming; (6) two flamings; (7) three flamings; (8) one flaming and one mowing; (9) one flaming, one mowing, and one flaming. After planting, mowing or flaming events were imposed biweekly or weekly, respectively. Maize whole-plant DM yield ranged from 10.9 to 21.8 Mg ha^{-1}, and grain yield from 8.0 to 14.6 Mg ha^{-1}. Whole-plant yield of maize in living mulch was 37 to 50% less, and grain yield was 32 to 46% less than monocrop maize. By increasing mechanical suppression of the living mulch, maize grain yield was increased by 0.6 Mg ha^{-1} per additional mowing or 0.7 Mg ha^{-1} per additional flaming. Whole plant yield was increased 0.8 Mg ha^{-1} per additional mowing and 1.2 Mg ha^{-1} per additional flaming. Although many challenges to controlling competition in this system without herbicides remain, the yields were still high for organic maize.

Keywords: intercropping, legume, organic agriculture, cover crop

Introduction

We have developed a maize-Kura clover living mulch system (Affeldt *et al.*, 2004; Zemenchik *et al.*, 2000) that, because this clover is a long-lived, rhizomatous, perennial, provides permanent soil cover for maize grain and silage production. Previous results indicate that this system eliminates the need for N fertilisation (Berkevich and Albrecht, 2007), reduces nitrate leaching to the groundwater (Ochsner *et al.*, 2010), increases water infiltration and decreases soil erosion and nutrient runoff by 80% compared to conventional methods (Siller *et al.*, 2016). In this system, herbicide-resistant maize is no-till planted into an established Kura clover field in band-killed strips, and inter-row clover growth is suppressed with herbicides. Maize grain and whole plant (as for silage) yields in this system are equal to conventional production methods, given adequate clover suppression (Affeldt *et al.*, 2004; Zemenchik *et al.*, 2000). Currently the system relies on herbicide inputs similar to conventional maize production, however we now have the goal of developing alternatives acceptable for organic production. The objective of this research was to test whether mechanical suppression of Kura clover by tillage, plus mowing or flaming, can replace herbicide suppression of the clover.

Materials and methods

This experiment was conducted at University of Wisconsin Agricultural Research Stations near Arlington, WI (43°18'N, 89°21'W) on a Plano silt loam, and Lancaster, WI (42°18'N, 90°47'W) on a Fayette silt loam, over 2 years. 'KTA202' Kura clover was established 2 years before the experiment was initiated. A rototiller was used to till 30-cm strips on 76-cm centres in the Kura clover fields in late April. One month later, the entire plot area was mowed to a height of 5-cm and the tilled strips were propane flamed at a rate of 166 kg ha^{-1}, creating a stale seedbed. Maize was then planted into the strips. Inter-row Kura clover suppression was accomplished with different frequencies of mowing and flaming (1, 2, or 3 mowing or flaming events imposed biweekly or weekly respectively, after maize planting) or a combination of these (Table 1), and were compared to a control treatment of killed Kura clover. Maize plots were 9.1 m long by four rows, spaced at 76 cm, wide and arranged in a randomized complete block with nine treatments and four replications in all site-years. One of the centre rows was harvested when control plots reached 50% kernel milkline, as for silage, and yield expressed on a dry matter basis. The other centre row was harvested for grain at maturity, and grain yield adjusted to 155 g kg^{-1} moisture. Analysis of variance procedures were run using the PROC MIXED statement of SAS, and separation of treatment means was conducted using Fisher's protected LSD (P=0.05). Single degree-of-freedom contrasts were used to compare suppression type. Linear regression was conducted in SAS, by making the suppression frequency within mowing or flaming treatments a continuous variable.

Results and discussion

Treatment by location or year interactions were not significant (P<0.05), therefore maize whole-plant and grain yields were pooled across all environments. Single degree-of-freedom contrasts showed whole-plant yield in the control treatment was greater than all other treatments (Table 1). Whole-plant yield in the living mulch ranged from 50 to 63% of the control. Increased clover suppression frequency increased whole-plant maize yield, however this increase was not consistent. A second mowing was not different than one mowing (P=0.14) or three mowings (P=0.097), however a third mowing increased yields by 13% compared with one mowing (P<0.01). Two flamings increased whole-plant yields by 12% compared to one flaming (P<0.01), and third flaming increased whole-plant yields by 8.6% compared to two flamings (P<0.05).

Table 1. Mean maize whole-plant and grain yields across all environments.

Treatment	Whole-plant		Grain	
	Mg ha^{-1}			
Control	21.8	a[1]	14.6	a
One mowing	12.2	d	8.8	c
Two mowings	12.9	bcd	9.2	c
Three mowings	13.8	b	10.0	b
One flaming	10.9	e	8.0	d
Two flamings	12.2	d	8.7	cd
Three flamings	13.2	bc	9.4	bc
Combo 1[2]	12.1	d	8.7	cd
Combo 2[2]	12.7	cd	9.1	c
LSD 0.05	1.0		0.7	
Contrasts	Significance[3]			
Control vs living mulch	***			***
Control vs mowing	***			***
Control vs flaming	***			***
Mowing vs flaming	‡			*
Mow/flame vs combo	NS			NS

[1] Within columns, means followed by the same letter are not significantly different at P=0.05 according to Fisher's protected LSD.

[2] Combo 1 is flame, mow and Combo 2 is flame, mow, flame.

[3] ‡, *, *** Significant at the 0.1, 0.05, and 0.001 probability levels, respectively.

Control maize grain yields were also greater than all living mulch treatments by single degree-of-freedom contrast (P<0.001). Grain yields in living mulch treatments ranged from 54 to 68% of the control, and displayed similar yield trends as whole-plant (Table 1). Maize grain yield did not differ between one and two mowings (P=0.303), however a third mowing increased grain yield compared with one mowing by 14% (P<0.01) and two mowings by 9% (P<0.05). Corn grain yields were not different between one and two flamings (P=0.053) or between two and three flamings (P=0.064), however a third flaming increased grain yields by 18% compared to flaming (P<0.001). Zemenchik *et al.* (2000) and Affeldt *et al.* (2004) also observed greater maize silage and grain yields with increased suppression of Kura clover living mulch with herbicides.

Combinations of mowing and flaming (Combo 1 and Combo 2) did not affect grain or whole plant yield compared with mowing or flaming alone by single degree-of-freedom contrast (P<0.05), and neither combination treatment was different from just one mowing (Table 1). Greatest grain and whole plant yield in the living mulch was accomplished with three mowings and three flamings. Two mowings were not different from three flamings or from Combo2, which both had one more suppression event. Single degree-of-freedom contrasts showed that mowing yielded more whole-plant (P<0.10) and grain (P<0.05) than flaming.

Regression analyses revealed that increasing the number of suppression events linearly increases grain and whole-plant yield (data not shown). The slope was significant for all regression lines (P<0.01) and suggests an increase of 0.8 Mg ha^{-1} of whole-plant and 0.6 Mg ha^{-1} of grain per additional mowing, and an increase of 1.2 Mg ha^{-1} of whole-plant and 0.7 Mg ha^{-1} of grain per additional flaming. It is unclear how many mowing or flaming events would continue to sustain yield increases, but the data suggest that it is likely more than three.

Low maize whole-plant and grain yields relative to the control indicate competition from the Kura clover living mulch. Oschner *et al.* (2010) reported that Kura clover living mulch with herbicide suppression used about 8 cm more water than conventional corn. Both mowing and flaming reduce clover transpiration via leaf removal, but this suppression is fleeting with rapid regrowth of clover leaves, whereas herbicides provide longer-term suppression.

Conclusions

Whole-plant yield of maize in mechanically or flame suppressed living mulch was 37 to 50% less, and grain yield was 32 to 46% less than monocrop maize. Yields were still high for organic maize, and yield loss may be more than compensated for by the near double price per unit of organic maize grain and silage compared to conventionally grown maize.

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Best *Festuca apennina* accessions from alpine grassland show potential as productive forage grasses at higher elevation

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Abstract

The allotetraploid grass species *Festuca apennina* De Not. is a close relative of *Festuca arundincea* and *Festuca pratensis*. In nature, it hybridizes frequently with diploid *Festuca pratensis* to form a sterile triploid which was shown to outyield both parents at mid-altitudes in the Swiss Alps. We conducted row trials to compare 20 accessions of *Festuca apennina* collected at Swiss grassland sites at Malchow (D), near sea level, and Früehbüehl (CH), at 1000 m a.s.l. Productivity was assessed visually and compared with two *Festuca pratensis* cultivars (one diploid and one tetraploid) as well as two diploid *Festuca pratensis* ecotypes. The accessions responded differently to conditions at experimental sites. Near sea level, all *Festuca apennina* accessions performed clearly poorer than *Festuca pratensis*, with inferiority increasing with time. Their inferiority became more on more visible from the sowing year to the year after the second winter. At 1000 m a.s.l., *Festuca apennina* accessions differed much more in productivity, and their performance relative to *Festuca pratensis* improved over the years. We conclude that *Festuca apennina* is a promising forage grass for use at higher elevation, and that selection should be carried out at higher elevation as well.

Keywords: fescue, productivity, genetic resources

Introduction

Festuca apennina De Not. (4× Fape) is an allotetraploid species of the *Festuca-Lolium* complex, combining a *Festuca pratensis*-like and a *Festuca glaucescens*-like genome (Kopecký *et al.* 2016). Thus, it is a close relative of *Festuca pratensis* Lam. (2× Fp) and *Festuca arundinacea* Schreb. It occurs quite frequently in alpine grassland above 1,500 m a.s.l., and triploid hybrids resulting from spontaneous 4× Fape × 2× Fp crosses can even dominate pastures around 1,400 m a.s.l. (Boller *et al.* 2022). More than 100 years ago, Stebler (1904) postulated a potential of 4x Fape – which he named *Festuca pratensis* var. *megalostachys*, ignoring the pre-existing species description *Festuca apennina* by De Notaris (1844) – as a forage grass in seed mixtures for high elevation. However, no efforts to select cultivars of the species were since undertaken, and virtually nothing is known about genetic variability of agronomically important traits. The present investigation aimed at first insights into the agronomic potential of the species compared to *Festuca pratensis* and its variability among 4× Fape ecotype accessions.

Materials and methods

In September 2017, seeds or ramets of at least 50 randomly selected individuals of 4× Fape were collected at 20 sites in the Swiss Alps. Sites ranged from 1,480 to 2,000 m a.s.l. in elevation; they lay in 9 different cantons and were predominantly cattle-grazed pastures covered by Cynosurion or Rumicion alliances (7 each). Remaining alliances observed were Adenostylion, Poion alpinae, Poion supinae or Calthion (1 or 2 each). Plant species diversity at these sites ranged from 13 to 35 species. One generation of panmictic propagation in rye isolation was carried out for each accession at Malchow and obtained seeds were used to establish trials in 2019. Row trials with 4 replications were sown at two sites: Malchow DE (near sea level) and Früehbüehl CH (1000 m a.s.l.). One 2× Fp (Preval) and one 4× Fp (Tetrax) cultivar, and two 2× Fp Swiss ecotype accessions were included for comparison. Productivity of each accession was assessed visually at 3 to 5 occasions each in 2019, 2020 and 2021, using a 1 (no biomass) to 9 (very high biomass) scale. Mixed models in R including accession type (Fp cultivar, Fp ecotype, Fape) and accessions nested within type were used to assess significance of the results.

Results and discussion

Productivity of the different accessions varied in a similar way at the two experimental sites (Figure 1), but Pearson correlation coefficients between response at Malchow and Früehbüehl for annual means of biomass scores decreased from 0.75 in the seeding year to 0.69 and 0.52 in the two consecutive years, respectively. This was mainly due to the fact that the two cultivars (one 2× one 4×) were clearly the most productive at Malchow (near sea level) throughout the years, with a tendency for even greater differences as time progressed. Both Fp cultivars were significantly more productive than all but two, all but one and all Fape accessions in the year of sowing, the year after the first winter, and the year after the second winter, respectively. At this site, both 2× Fp ecotypes were also more productive than all 4× Fape accessions in all three years, significantly so with few exceptions. On the other hand, at Früehbüehl (1000 m a.s.l.), three Fape accessions performed similarly as the Fp cultivars and slightly, though not significantly exceeded Fp ecotypes in productivity in the years after the first and second winter. In the year after the second winter, the best 4× Fape accession performed even slightly better than both Fp cultivars and significantly better than one of the Fp ecotypes.

There was no significant correlation between elevation at collecting sites, their slope or aspect, with any of the performance traits shown in Figure 1. However, performance of 4× Fape accessions was different depending on the plant alliances prevailing at the sites where they were collected (Table 1). Best performing accessions originated from alliances which are typical of nutrient rich sites where cattle like to rest (Lägerfluren, such as Poion supinae and Rumicion). At both sites and in all years, these accessions performed significantly better than the group originating from sites of relatively low intensity of utilization (Adenostylion, Calthion, Poion alpinae). Accessions from Cynosurion, typical of moderately intensive grazing and nutrient availability, performed intermediately. It appears that strong competition by nitrophilous plants such as *Rumex alpinus* provides a suitable environment for natural selection of highly productive 4× Fape genotypes.

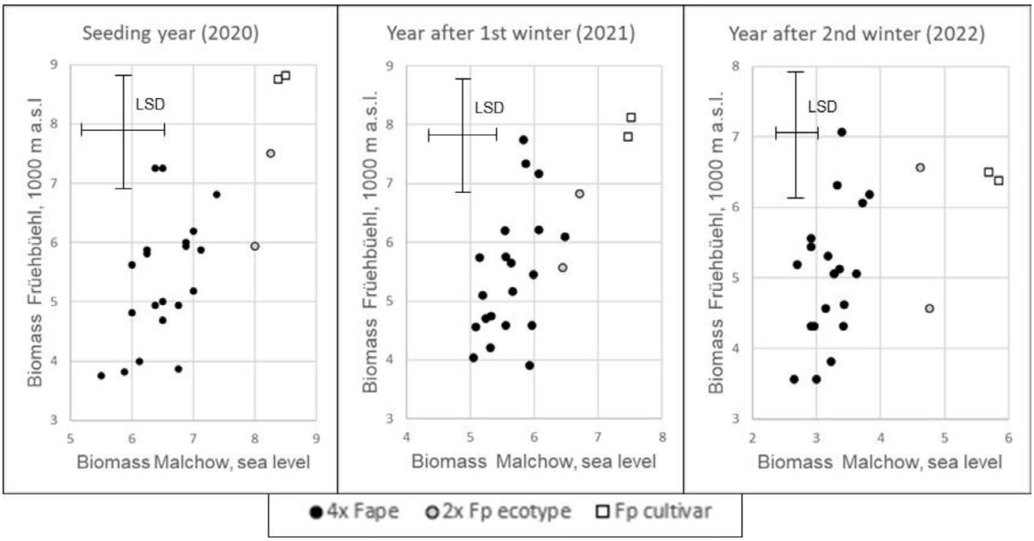

Figure 1. Mean biomass score per year (3 to 5 visual assessments) of 20 *Festuca apennina* (4× Fape) accessions, 2 diploid *Festuca pratensis* (2× Fp) ecotypes and 2 *Festuca pratensis* (Fp) cultivars grown in row trials at two locations of different elevation. Bars indicate least significant difference (P=0.05).

Table 1. Performance (annual means of biomass scores in row trials) of 4× Fape accessions originating from different plant alliances in the seeding year (2020) and two consecutive years (2021, 2022) at two sites.[1]

Plant alliance	n	Malchow			Früehbüehl			Both sites
		2020	2021	2022	2020	2021	2022	all years
Poion supinae, Rumicion	7	6.77 a	5.77 a	3.40 a	6.25 a	6.19 a	5.69 a	5.60 a
Cynosurion	7	6.48 ab	5.76 a	3.29 a	5.24 ab	5.51 ab	5.03 ab	5.17 a
Adenostylion, Calthion, Poion alpinae	6	6.21 b	5.31 b	2.89 b	4.53 b	4.51 b	4.44 b	4.55 b

[1] Plant alliances were grouped according to intensity of utilization (see text). Within columns, values not followed by the same letter were separated by non-overlapping confidence intervals (P=0.05).

As a consequence of global warming, the altitudinal limits of crop farming in alpine regions tend to rise in elevation (Holzkämper *et al.* 2015). Therefore, ley farming may become of interest at higher elevations than is common nowadays. This calls for forage species and varieties that are well adapted for temporary grassland sown at high elevation, and 4× Fape could be such a species. Here we provide evidence of considerable genetic variation in productivity of 4× Fape accessions collected in the Swiss Alps. The best accessions may constitute a basis for breeding of competitive cultivars. Such breeding and selection should be carried out at higher elevation to allow for expression of genetic differences.

Conclusions

When tested at an appropriate site – elevation of 1000 m – productivity of the best accessions of 4× Fape is comparable to elite cultivars of *Festuca pratensis*, especially when looking at their persistence over the years. Collection of 4× Fape accessions appears to be most promising at nutrient-rich sites with strong competition of nitrophilous plants.

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Forage production and nitrous oxide emissions from grass-legume swards

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Abstract

Improving the efficiency of N use to reduce environmental impact without reductions in herbage production is one of the main challenges for livestock systems. The aim of this study was to evaluate the effects of including legumes or using different sources of N fertilizers on herbage production and nitrous oxide (N_2O) emissions. The treatments were a binary mixture of the same grasses (*Lolium multiflorum* L. and *Secale cereale*) without N fertilization (control) or receiving 100 kg of N ha^{-1}, from one of three synthetic N sources: common urea, urea with urease inhibitor (NBPT urea) and ammonium nitrate. A fifth treatment was a mixture of the same two grasses + three legumes (*Vicia sativa*, *Trifolium repens* L. and *Trifolium pratense* L.) without N fertilization. The experimental design was randomized blocks with four replications. Swards were mechanically harvested whenever the pre-defoliation sward height averaged 20 cm. The herbage mass was measured by cutting three samples per experimental unit, at ground level, using scissors. The sampling area was 0.240 m^2 (0.8×0.3 m). The N_2O flow, was assessed using the methodology of static chambers. The lowest herbage production was observed in the control treatment; but there were no differences between treatments with different fertilizer sources and non-fertilized multi-species swards including legumes. The highest N_2O emission was observed in areas receiving ammonium nitrate or urea fertilization, while the control, urea NBPT and multi-species swards had similar N_2O emissions. In conclusion, multi-species swards that include legumes may be a tool to mitigate greenhouse gas emissions from livestock systems, without decrease in the primary productivity of pastures.

Keywords: legumes, nitrogen, greenhouse gases, multi-species, fertilizers

Introduction

Herbage ecosystems are dependent of N fertilization to ensure high DM yield. However, large amounts of nitrous oxide (N_2O) may be released following N fertilizer applications (Cummins *et al.*, 2021). Nitrous oxide is a greenhouse gas (GHG) with a global warming potential 265 times greater than carbon dioxide (CO_2) (IPCC, 2014), and its emission is one of the main sources of GHG from dairy and beef cattle production. Thus, improving N-use efficiency to reduce the environmental impact without affecting herbage productivity is one of the main challenges of livestock systems. In this context, the effect of different sources of N fertilizers as well as the effect of legume inclusion on N_2O emissions and herbage production of multi-species swards deserves to be better studied. This research aimed to assess the effect of different sources of N fertilizers or the inclusion of legumes as a tool to mitigate GHG emissions without adversely affecting herbage productivity.

Materials and methods

The experiment was conducted in Lages, SC, Brazil (50.18°W, 27.47°S; 920 m above sea level), from April 2022 to November 2022. The treatments were a binary mixture of grasses (*Lolium multiflorum* L. and *Secale cereale*) without N fertilization (control), or receiving 100 kg N ha^{-1} from one of three synthetic sources: common urea, urea with urease inhibitor (NBPT urea), and ammonium nitrate. A fifth treatment was a mixture of the same two grasses + three legumes (*Vicia sativa*, *Trifolium repens* L. and *Trifolium pratense* L.) without N fertilization. The experimental design was randomized blocks, with four replications having 37.5 m^2 each (6.0×6.25 m). The N_2O flux was determined using the closed static chamber technique (Mosier, 1989). For this purpose, each experimental unit received a rectangular metal base fixed in the ground at a depth of 5 cm, which remained in place throughout the experimental period. The chambers were made of galvanized steel with a volume of 108 L, equipped with internal fans, an outlet valve for collecting air samples and a thermometer for checking the temperature of the internal air. Collections of gas samples were performed on days 0 (zero, before N fertilizer application), 1, 3, 5, 7, 9, 13, 17, 21, 27, 31, 36, 41, 48, 55, 62, 69, 76, 83 and 90 after applying N fertilizer. Each day, air samples were collected at 0, 15, 30 and 45 min after closing the chamber at the base, using 10 ml polypropylene syringes. Samples were transferred to pre-evacuated flasks, and N_2O concentrations were measure by gas chromatography. The N_2O fluxes (μg $(m^2)^{-1}$ h) were calculated according to the increase in gas concentration inside the chamber over time. Herbage DM yield was determined by mechanical cutting whenever the average pre-defoliated pasture height reached 20 cm. Total herbage DM production was measured by adding the first pre-cutting herbage mass plus DM accumulation rate throughout the growing season. Herbage DM accumulation rate was calculated by the difference between pre-defoliation herbage mass and previously post-grazing defoliation herbage mass divided by the number of days between each defoliation event. The dependent variables were analysed using PROC MIXED of SAS (version 9.4). Significant differences between experimental treatments were determined using Tukey's test at the 5% probability level ($P<0.05$).

Results and discussion

Total DM yield was similar in the mixed grass-legume swards compared with those receiving urea and NBPT urea (Table 1). Sward treatments that received ammonium nitrate had the highest DM yield compared with other treatments. The lowest N_2O-N emissions (between 2 and 12 mg N-N_2O $(m^2)^{-1}$ h; data not shown) was observed in the two treatments without N fertilization (control and grasses+legumes), whereas those receiving ammonium nitrate showed lower N_2O-N emission compared to the urea and NBPT urea treatments (Figure 1). This result was relatively unexpected because NBPT urea has been shown to be an option for mitigating N_2O emissions (Krol *et al.*, 2020). The higher N_2O-N emissions from treatments receiving synthetic-N fertilizers when compared with legumes may be explained due to the greater availability of soluble N in the soil. In this context, ammonium nitrate seems to have better N-use efficiency (lower N_2O-N emission and better productivity) than the other tested mineral-N sources. However, it is worth highlighting that non-fertilized grass-legume swards had a DM yield greater than 80% of that observed for the ammonium nitrate treatment. This result may be partially explained by the supply of the N requirements through biological N fixation from legumes (Lüscher *et al.*, 2014). Finally, regardless of the treatment, maximal N_2O emissions were observed at day 36 after fertilization, and a subsequent increase of N_2O emissions at day 40 may be explained by precipitation events.

Table 1. Herbage dry matter yield of sward treatments receiving different sources of N fertilization or mixed with legumes.

N source	Dry matter yield (kg ha^{-1})
Without N	1,558^{C}
Common urea	4,038^{B}
NBPT urea	3,766^{B}
Ammonium nitrate	4,952^{A}
Legumes	4,020^{B}
P<	0.001
Standard error	0.539

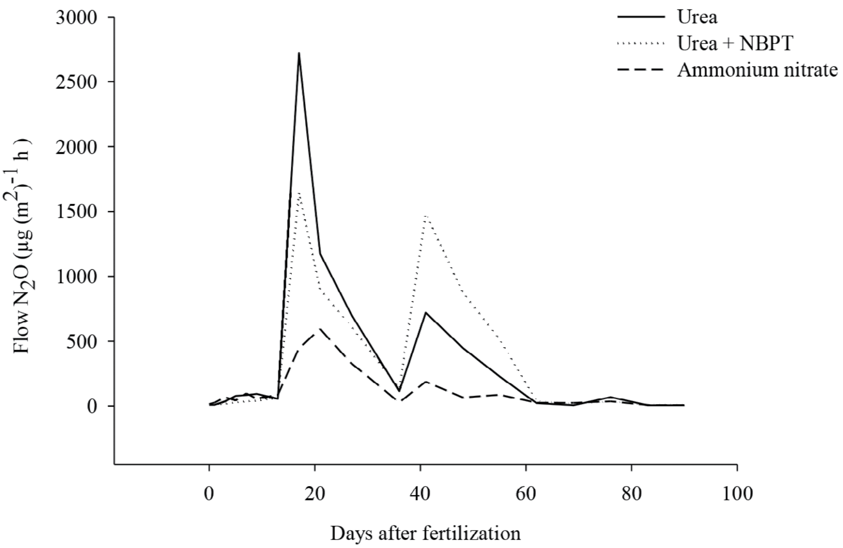

Figure 1. Nitrous oxide (N_2O-N) flow from sward treatments receiving different sources of N fertilization.

Conclusions

Mixed-species pasture swards that incorporate legumes should be better explored as an option to reduce the environmental impact of livestock systems, and ammonium nitrate seems to be a better alternative than urea fertilization in more intensive scenarios.

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Effects of regrowth periods on yield and quality traits in different grass-clover mixtures

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Abstract

To evaluate the effect of different cutting schedules in different grass mixtures, two trials were established in 2017 in Denmark with 11 commercial grass mixtures with different proportions of the grass species: (1) perennial ryegrass (*Lolium perenne*); (2) hybrid ryegrass (*Lolium hybridum)*; (3) Festulolium and (4) tall fescue (*Festuca arundinacea*) and legumes; (5) white clover (*Trifolium repens*); and (6) red clover *(Trifolium pratense*). In 2018-2020 the plots were harvested four times with a weekly interval from 1st to 4th cut with a Haldrup harvester. The yield results show that all mixtures had similar daily growth rates for 1st and 2nd cut, while the daily growth rate of mixtures including red clover or tall fescue were higher, compared to mixtures with white clover or perennial ryegrass in 3rd or 4th cut. The decline of digestibility of organic matter was rather similar across mixtures for 1st cut, while the decline was much lower in 3rd cut in mixtures containing only white clover compared to mixtures including red clover. The crude protein concentration increased by order of cut and declined within the growing period of each cut. This was related to yield level and the daily growth rate, indicating a dilution of protein depending on yield level. The initial protein concentration was higher in mixtures including red clover. Our results indicate that the cutting interval needs to be shorter for mixtures containing red clover or tall fescue to achieve a digestibility of organic matter at approx. 80%.

Keywords: mixtures, regrowth period, digestibility

Introduction

Grass silage is a major source of protein and fibre in the diet for dairy cattle. However, the digestibility of organic matter and fibre is limiting the amount of grass silage in the diet for high yielding dairy cattle. Previous studies by Johansen *et al.* (2017) and Egan *et al.* (2018) demonstrated a positive correlation between digestibility of organic matter in grass silage, the legume proportion, and the ECM (energy corrected milk) yield of lactating cows. The milk yield peaked when organic matter digestibility (DOM) reached 82%, but the marginal effect from 80 to 82% was rather small. Typically, we find a negative correlation between grass yield and digestibility of organic matter, so achieving the best combination of a high yield and digestibility requires the correct timing of cutting during the season.

Materials and methods

Two trials were established in 2017 in Denmark with 11 different commercial grass mixtures with different proportions of the grass and legume species based on weight proportion in seed mixtures as shown in Table 1. The seed rate of mixtures was adjusted according to seed weight to obtain the same plant density. The soils of the trial sites were predominantly sandy soils with 6 and 12% clay, and the trials were not irrigated. All mixtures were fertilised each year with 270 kg N, 32 kg P, 268 kg K and 68 kg S ha^{-1} applied in descending quantities from the 1st to the 4th cut.

Five plots per replicate with each mixture were subdivided into 3 subplots in a randomised split-plot design, so each plot was used for only one cut to avoid carry-over effects of previous cuts. In 2018-2020 the plots (1.5×12 m) were harvested with a Haldrup harvester four times, with a weekly interval from 1st to 4th cut. A subsample of each plot was mixed from the 4 replicates and was dried at 60 °C for 48 hours and subsequently milled in a Cyclotec mill with 1 mm sieve. Forage quality was determined by content of crude protein, sugars, neutral detergent fibres (NDF), digestibility of organic matter (Tilley and Terry, 1963) and the legume proportion using dry NIRS.

Table 1. Seed composition (%) of 11 mixtures based on weight basis.

Mixture no.	Perennial ryegrass (*Lolium perenne*)	Hybrid ryegrass (*Lolium hybridum*)	Festulolium	Tall fescue (*Festuca arundinacea*)	Meadow fescue (*Festuca pratensis*)	White clover (*Trifolium repens*)	Red clover (*Trifolium pratense*)
1	87					13	
2	52			30		9	9
3	57			30		13	
4	60	23				9	8
5	47	40				13	
6	42		25		15	9	9
7	37		45			7	11
8	37		50			13	
9	32		33			5	30
10	15			70		6	9
11	15			75		10	

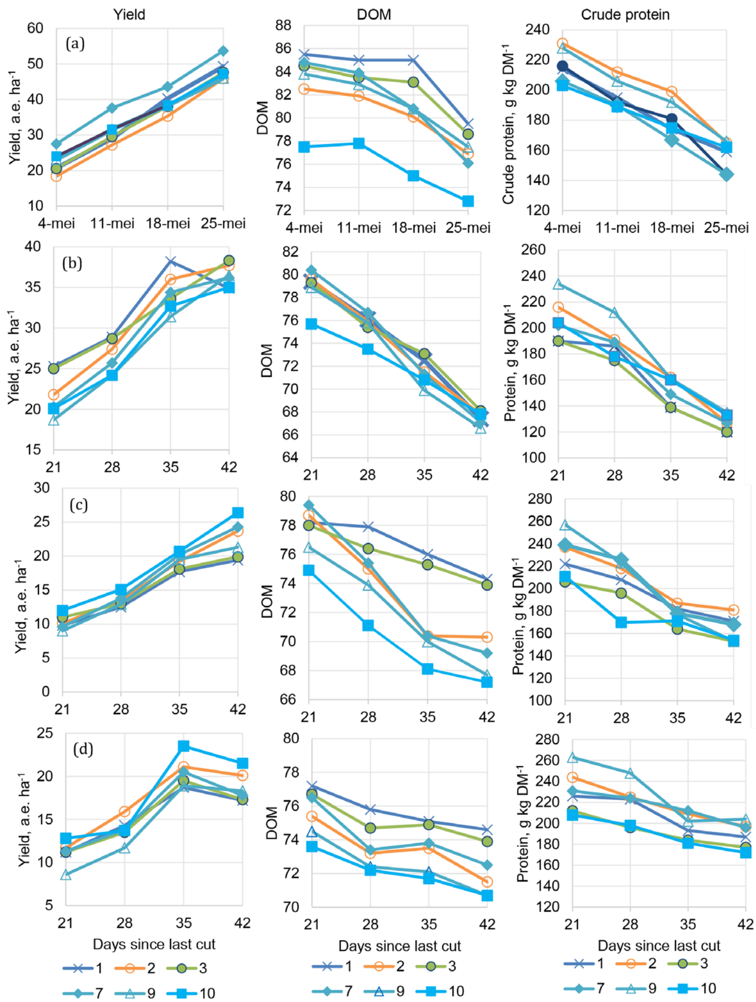

Figure 1. Yield of metabolisable energy pr ha, digestibility of organic matter and content of crude protein for 6 selected mixtures for 1st (A), 2nd (B), 3rd (C) and 4th (D) cut. Values are means of 2019-2020 across both sites.

Results and discussion

The daily growth rate for 1st and 2nd cut was quite linear and similar for all mixtures, with an average daily growth rate of 150 kg dry matter ha^{-1}. The mixtures containing a high proportion of Festulolium had the highest yield, but also the lowest content of crude protein, which was probably due to the higher yield causing a dilution effect of the crude protein. DOM was highest for the mixture containing only perennial ryegrass and white clover, and lowest for the mixtures with a high proportion of tall fescue. The decrease in DOM was highly correlated with the decrease in digestibility of NDF and content of sugar and protein. For 3rd and 4th cut, the mixtures containing only white clover as legume maintained a higher DOM than mixtures including red clover, but also a lower yield and daily growth rate.

Conclusions

Our results indicate that the cutting interval needs to be shorter for mixtures containing red clover or tall fescue to achieve a digestibility of organic matter at approx. 80% compared to mixtures containing white clover and/or perennial ryegrass.

Acknowledgements

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Yield and forage quality of 11 different grass-clover mixtures during five years

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Abstract

Grass silage in Denmark is predominantly produced from temporary grasslands which typically persist for only 2-3 years as the yield typically decreases by10% annually. To evaluate different grass mixtures, two trials were established in 2017 in Denmark with 11 commercial grass mixtures with different proportions of the grass species: (1) perennial ryegrass; (2) hybrid ryegrass; (3) Festulolium; and (4) tall fescue and legumes; (5) white clover; and (6) red clover. In 2018-2022 the plots were harvested 5 times annually with a Haldrup harvester. The results show that the highest yielding mixtures, in their metabolisable energy and crude protein, as average of 1st and 2nd years, contained higher proportions of Festulolium and red clover, while mixtures containing tall fescue showed the highest yield in 4th and 5th year. Digestibility of organic matter and legume proportion were lowest in mixtures with an initial high proportion of tall fescue, while mixtures containing only white clover as legume showed the highest digestibility of organic matter during all trial years. Mixtures with a moderate proportion of tall fescue in combination with perennial ryegrass and legumes can provide a more persistent mixture than a perennial ryegrass-white clover binary mixture.

Keywords: multi-species, yield-stability, persistence

Introduction

Temporary grasslands in Denmark are mostly used for cutting and typically consist of mixtures of perennial ryegrass, festulolium, white clover and, to some extent, red clover as the yield and forage quality are more stable compared to monocultures of species (Lüscher *et al.*, 2008; Suter *et al.*, 2021). However, temporary grasslands in Denmark persist for only 2-3 years as the yield typically decreases 10% annually. Hence, it is interesting to evaluate combinations of different grassland species in terms of yield and forage quality over a five-year period.

Materials and methods

Two trials were established in 2017 in Denmark, with 11 commercial grass mixtures with different proportions of grass and legume species based on weight proportion in seed mixtures (Table 1). The seed rate of mixtures was adjusted according to seed weight to obtain the same plant density. The soil type of the trial sites was predominantly sandy soils, with 6 and 12% clay, and the trials were not irrigated. All mixtures were fertilised yearly with 270 kg N, 32 kg P, 268 kg K and 68 kg S per ha applied descending from 1st to 4th cut.

In 2018-2022 the plots (1.5×12 m) were harvested 5 times annually with a Haldrup harvester. A sample of each plot was dried at 60 degrees for 48 hours and subsequently milled at a Cyclotec mill with 1 mm sieve. Forage quality was determined by content of crude protein, sugars, neutral detergent fibres (NDF), digestibility of organic matter (Tilley and Terry, 1963) and the legume proportion using dry NIRS. The results were evaluated using a mixed model for a randomised split-plot design in R.

Results and discussion

During the 1st and 2nd year of growth the yield of metabolisable energy of mixtures containing Festulolium, red clover and/or tall fescue was slightly higher than of mixtures that contained only white clover as the

Table 1. Seed composition (%) of 11 mixtures based on weight basis.

Mixture no.	Perennial ryegrass (*Lolium perenne*)	Hybrid ryegrass (*Lolium hybridum*)	Festulolium	Tall fescue (*Festuca arundinacea*)	Meadow fescue (*Festuca pratensis*)	White clover (*Trifolium repens*)	Red clover (*Trifolium pratense*)
1	87					13	
2	52			30		9	9
3	57			30		13	
4	60	23				9	8
5	47	40				13	
6	42		25		15	9	9
7	37		45			7	11
8	37		50			13	
9	32		33			5	30
10	15			70		6	9
11	15			75		10	

legume. The legume proportion was highest in mixtures containing both white and red clover, while the digestibility of organic matter was higher in mixtures without red clover or tall fescue.

The legume proportion, and especially the proportion of red clover, decreased markedly in the 3rd year. During 4th year the legume proportion decreased further for nearly all mixtures. During the 5th year the legume proportion stabilised or even increased as the grass sward had become patched for most mixtures and this favoured an increasing white clover proportion. Hence the difference in digestibility of organic matter between mixtures with/without red clover diminished. The yield throughout the experiment tended to be higher in mixtures containing a high proportion of tall fescue; mixtures with this species also had the lowest digestibility of organic matter and legume proportion, as found by Cougnon *et al.* (2013).

Table 2 includes the yield of metabolisable energy relative to that of mixture 1, as reference. The absolute yield of mixture 1 was 95.2 crop units ha^{-1} in the 1st year in 2018. The yield was rather low due to severe drought in 2018. The yield increased by 39% for the 2nd year where growing conditions were nearly optimal. In the following years, the absolute yield decreased by 11, 18 and 15%, respectively for 3rd, 4^{th,} and 5th year. As the trials have not been replicated yearly, the effect of harvest year and year of ley is confounded and hence it is difficult to generalise these results to any growing period.

Conclusions

Inclusion of a moderate proportion of tall fescue in combination with perennial ryegrass and white and red clover could be a promising mixture providing a good combination of a persistence, digestibility of organic matter and a fair legume proportion even though it decreases over time.

Table 2. Legume proportion, digestibility of organic matter and the relative yield of metabolizable energy of 11 mixtures for 1st to 5th year of ley.

Mixture	Legume proportion % dry matter					Digestibility organic matter					Yield of metabolizable energy (relative)				
	1. year 2018	2. year 2019	3. year 2020	4. year 2021	5. year 2022	1. year 2018	2. year 2019	3. year 2020	4. year 2021	5. year 2022	1. year 2018	2. year 2019	3. year 2020	4. year 2021	5. year 2022
ref: mixture 1 (absolute values)											*95.2*	*132.7*	*117.1*	*96.3*	*82.2*
1	18	16	12	8	18	76.8	78.8	76.4	78.3	79.4	100[ab]	100[a]	100	100[abc]	100[ab]
2	44	50	28	11	6	75.0	75.2	73.1	75.0	76.4	106[a]	97[ab]	97	103[abc]	105[a]
3	21	17	11	6	8	76.8	78.2	76.0	75.8	76.5	99[ab]	93[b]	97	108[ab]	96[bc]
4	31	45	29	19	20	73.9	75.4	74.4	76.6	78.2	100[ab]	97[ab]	95	94[bc]	94[bc]
5	16	24	19	18	33	75.4	77.1	75.9	76.4	75.4	97[b]	91[b]	94	96[bc]	93[bc]
6	35	48	32	21	34	74.0	75.7	74.9	76.4	76.9	95[b]	97[ab]	95	92[c]	89[c]
7	34	42	24	21	22	73.3	75.1	74.6	76.6	77.1	103[ab]	102[a]	101	98[bc]	95[bc]
8	21	30	23	19	28	73.7	76.0	76.0	76.5	77.0	97[b]	97[ab]	96	89[c]	87[c]
9	62	68	38	35	44	73.6	73.7	72.9	75.4	75.5	105[a]	94[b]	95	86[c]	81[c]
10	34	37	9	3	3	73.9	73.0	71.0	72.4	72.7	102[ab]	101[a]	99	108[ab]	107[a]
11	5	5	2	1	2	74.9	74.7	73.3	72.9	73.8	104[ab]	100[a]	103	115[a]	107[a]

[1] Different combinations of letters indicate significantly different means in different years ($P<0.05$; Tukeys test).

Acknowledgements

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Crop yield following barley, ryegrass ley, or a ryegrass-clover mixture in crop rotations

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Abstract

How type and duration of leys affect carryover nitrogen to following crops is still poorly quantified. We established ten crop rotations to compare effects of: (1) leys during 18 or 30 months; (2) legumes, either as clover in leys or as grain legume; and (3) improved soil cover by catch crops. We assess effects on the yield and N nutrition of the crops, as well as on the N efficiency of the entire crop rotation. At this mid-stage of the experiment, we compare the effects of a perennial ryegrass-red clover mixture, a pure ryegrass ley, a barley-catch crop and a barley-fallow sequence on the yield of following spring wheat and pea crops. The leys and the barley were established in autumn 2020. In early spring 2022, stands were terminated to plant either wheat or peas. Wheat yield following grass-clover ley was on average 46% larger than following barley or pure grass. Pure grass did not benefit wheat yield compared to barley. After a single catch crop period, the effect of improved soil cover was trending beneficial for both peas and wheat, but differences were not significant. We conclude from these preliminary results that grass-clover is a clearly superior preceding crop for spring wheat compared to pure grass or barley.

Keywords: legacy effect, carryover nitrogen, legumes, yield benefits

Introduction

Improving nitrogen (N) efficiency of crop rotations is key to reducing agricultural inputs. Recent research demonstrated that inclusion of pure grass leys into crop rotations provides several benefits (Hoeffner *et al.*, 2021), but to be productive such leys require substantial N fertilizer applications. Grass-legume mixtures incorporate large amounts of N into the system through symbiotic N_2 fixation and may be superior to pure grass leys in terms of carryover nitrogen (N) to the following crop (Fox *et al.*, 2020). In addition, benefits of leys might be derived from the absence of tillage for often two or three years, improved soil cover and positive effects on soil humus content (Guillaume *et al.*, 2022). Nevertheless, the amount of residual N delivered to the following crop remains difficult to predict, and the effect of ley duration is largely unexplored. Moreover, direct comparison with rotations lacking leys but optimising soil cover with catch crops and using grain legume is lacking. The objective of this study is to compare the effects of incorporating: (1) leys during 18 or 30 months; (2) legumes, either as clover in leys or as grain legume; and (3) catch crops to improve soil cover, on the yield and N nutrition of the following crops, as well as on the N efficiency of the entire crop rotations. At the current stage of this ongoing experiment, we compare effects of the preceding crops (pure grass leys, grass-clover leys, and winter barley) with subsequent fallow or a catch crop (phacelia) on grain yields of the following crops spring wheat or peas (treatments 1 to 6, Crop 22, Table 1).

Materials and methods

A field experiment was set up with 10 rotations differing in three factors: (1) duration of ley; (2) presence of legumes, either as clover in the ley or as a pea crop; and (3) continuity of soil cover (Table 1). Plots (4.5×6.5 m) were established near Zürich-Reckenholz (490 m a.s.l) in autumn 2020 on a eutric cambisol, arranged according to a randomized complete block design with five replicates. The field was uniformly used for a wheat crop prior to establishment of the experiment. Pure grass was *Lolium perenne* and grass-clover leys consisted of *L. perenne* and *Trifolium pratense*. Clover proportion in the grass-clover leys was 65% on average across 2021, assessed by sorting three of the five harvests. The leys (treatments 5 & 6,

Table 1) were destroyed chemically in February 2022 and spring wheat and pea crops sown in early March after minimal tilling. Nitrogen fertilization was as follows: barley and wheat: 100 kg N ha^{-1}; pure grass: 250 kg N ha^{-1} $year^{-1}$; grass-clover: 100 kg N ha^{-1} $year^{-1}$. Peas and catch crops were not N fertilized. All plots received P and K in non-limiting amounts for crop growth. Yields were measured on the central 1.5×6.5 m of each plot. Data were analysed by analysis of variance, and differences between treatments were tested post-hoc using Tukey's multiple comparison test.

Results and discussion

Grain yields in 2022 were significantly affected by the identity of the previous crop (ANOVA: $F_{5,24}$=10.1, P<0.001). On average, pea crops had 1.2 t ha^{-1} higher grain yields than wheat (Figure 1). While the yield of the N_2-fixing crop (pea) was as expected under Swiss conditions, wheat yield following barley and pure grass was clearly below average (Richner and Sinaj, 2017). This indicates that N availability to wheat was limiting in these treatments. In contrast, wheat following grass-clover ley had yields as high as pea and significantly higher than following barley or pure grass (+46%, Figure 1), demonstrating a distinct yield advantage through a legacy effect of the presence of legumes in the previous crop. This strong legacy effect was most probably due to residual N from the N_2-fixing activity of the grass-clover ley (Fox *et al.*, 2020). Plant analyses and the use of ^{15}N labelled fertilizer will allow us to assess how much N from non-fertilizer sources was taken up by the crops. Wheat yield following the 18-month old pure grass ley did not significantly differ from wheat yield following barley (Figure 1). Pure grass leys may increase soil humus content and therefore soil N content as compared to crops (Crème *et al.*, 2018; Hoeffner *et al.*, 2021), but at this intermediate stage of the experiment, the duration of the ley was probably too short for such an effect to occur. Moreover, we observed a poorer root and stubble decomposition of the pure grass ley compared to barley and the grass-clover ley, which might have affected N dynamics and impaired wheat establishment. Regarding the effect of soil cover, pea and wheat following barley with catch crop had 0.4 t ha^{-1} higher yields (on average) than when following barley with fallow; the effect, however, was not significant (Figure 1). Further results from this experiment will provide insights into the processes involved in N legacy effects within crop rotations: plant N content is being analysed to determine the nutritional status of the crops and ^{15}N labelled fertilizer is applied on a subplot to determine fertilizer N capture. In addition, the effects of ley duration will be tested.

Table 1. Factor levels and crop rotation in the 10 treatments.[1]

	Over whole experiment			Crop sequence to date				Forthcoming	
Treat. #	Duration of ley[2]	Legume presence	Soil cover	Winter 2020-2021	Crop 2021	Winter 2021-2022	Crop 2022	Winter 2022-2023	Crop 2023
1	0	–	–	Barley		Fallow	Wheat	Fallow	Barley
2	0	+	–	Barley		Fallow	Pea	Fallow	Barley
3	0	–	+	Barley		Catch crop	Wheat	Catch crop	Barley
4	0	+	+	Barley		Catch crop	Pea	Catch crop	Barley
5	18 m.	–	+	Pure grass			Wheat	Catch crop	Barley
6	18 m.	+	+	Grass-clover			Wheat	Catch crop	Barley
7	18 m.	–	+	Barley		Pure grass			Barley
8	18 m.	+	+	Barley		Grass-clover			Barley
9	30 m.	–	+	Pure grass					Barley
10	30 m.	+	+	Grass-clover					Barley

[1] Grey shaded cells indicate the crops for which results are shown in this article.

[2] m. = months.

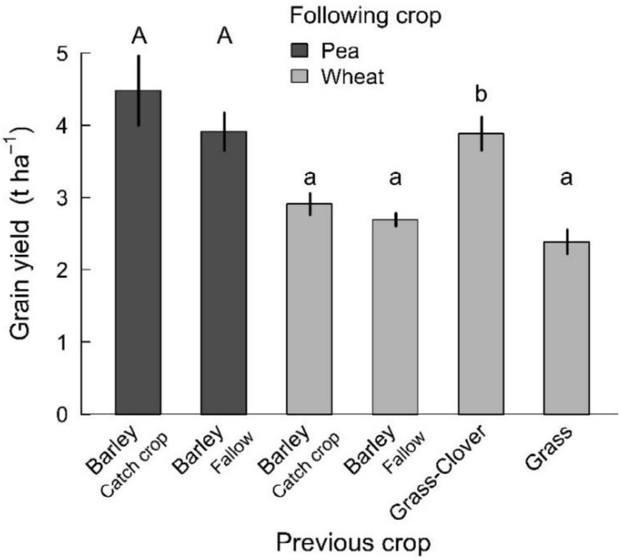

Figure 1. Grain yield of pea and wheat as affected by six previous crops differing in the presence of legumes and soil cover. Different letters indicate a difference at $P \leq 0.05$ (Tukey's multiple comparison test within each following crop; Pea: upper case, Wheat: lower case). Shown are means of 5 replicates ± standard error of the mean.

Conclusions

This ongoing experiment highlights the benefit of an 18-month period of grass-clover ley with a clover proportion of about two-thirds on the grain yield of the following crop in sparingly fertilized rotations. This short period of time was not sufficient for pure grass leys or enhanced soil cover by catch crop to have significant effects on the grain yield of the following crop. The next experimental year will allow us to test for a longer period and broaden the options for treatment comparisons.

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White clover (*Trifolium repens*) population dynamics are partly dependent on timing of seminal taproot death

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Abstract

The expanded usage of white clover has increased the importance of understanding white clover dynamics. It is assumed that clover plants have a higher tolerance to moisture and nutrient deficiencies when the taproot is still present. Therefore, the survival of the seminal taproot can influence the dynamics of clover. There is no direct evidence whether increased survival of the taproot results in increased performance of white clover. In this study, we aimed to: (1) assess the relationship between taproot volume and taproot survival; and (2) whether the timing of death of the seminal taproot influences the population dynamics of white clover varieties. In a two-year field experiment with 18 white clover varieties grown in monoculture and in mixture with *Lolium perenne* L, the taproot characteristics and population dynamics were studied. It was shown that taproot volume was positively correlated to both leaf size and taproot presence during autumn 2017. The combination of the timing of death of the seminal tap root and the development of stolons seems to play a more important role in increasing the persistence of white clover than the survival of the seminal taproot. Future research should focus on understanding the transition from a taprooted white clover to a stolonous white clover plant.

Keywords: nodal roots, breeding, morphology, stolons, regenerative agriculture, organic agriculture

Introduction

An issue concerning the use of white clover is the persistence of white clover over years in grass-clover pastures, which influences the stability of dry matter production and feeding value of grass-clover. Reasons for reduced persistence of white clover include diseases, pests and drought stress, possibly related to the survival of the taproot (Nichols *et al.*, 2015). A possible change in the root morphology of white clover could be the increase in lifetime of the seminal taproot. A major decline in clover content has been observed after the death of the seminal taproot (Nichols *et al.*, 2015; Westbrooks and Tesar, 1955). Death of the taproot usually occurs between twelve and eighteen months after plant formation, but the variation is large, ranging from six months to over 24 months (Brock and Tilbrook, 2000; Nichols *et al.*, 2015). Therefore, in this study we investigated the relationship between taproot characteristics of white clover and the persistence of the taproot in 18 white clover varieties and related this to the population dynamics of white clover in the period during which the taproot disappears. We hypothesized that: (1) a higher taproot volume will increase the lifespan of the seminal taproot; and (2) the timing of death of the seminal taproot has an effect on the short-term population dynamics and performance of white clover varieties.

Materials and methods

In an experiment, which was part of the Dutch VCU (Value for Cultivation and Use) testing programme, 18 white clover varieties (*Trifolium repens* L.) were tested in a randomized block design with four replicates. The different clover varieties were sown both in monoculture and in mixture with perennial ryegrass (*Lolium perenne*, varieties Sputnik and Dromara (50/50)). In these plots, clover cover was determined according to VCU protocol, leaf size of white clover was measured, presence of taproot was determined at four different times and size of taproot was determined during first taproot presence measurement. On each monoculture plot, a clod of 20×20 cm square and 25 cm depth was harvested in a place where white clover was abundantly present. Taproots were measured at base, 1 cm below base and 10 cm below base. Taproot volume was determined through these three measurements. Correlation coefficients between variables were based on Pearson's R test. Data were checked for normality and log-transformed if required. The effect of white clover variety and leaf size group was tested in separate Anovas. Post hoc analysis was done using Fishers test of least significant difference for varieties and Tukey's test for honest significant difference for differences between leaf size groups.

Results and discussion

Taproot survival showed a gradual decline from an average of 85% in spring 2017 to 31% in autumn 2018 (Figure 1). The survival of the taproot measured during autumn 2017 significantly positively correlated with taproot volume ($P<0.05$), average clover cover over all years ($P<0.01$, Table 1), leaf size ($P<0.001$), average clover cover in 2017 ($P<0.01$) and average clover cover in 2018 ($P<0.05$). A significant correlation was observed between the taproot volume and the survival of the taproot in autumn 2017 ($P<0.05$, Table 1) but not with the survival of the taproot in spring 2017, spring 2018 and autumn 2018. Previous research and breeding efforts have focussed on a longer lifespan of the seminal taproot (Caradus and Woodfield, 1998). It has, however, remained unclear to what extent the longer lifespan and timing of death of the seminal taproot has in terms of effect on white clover performance in pastures. The first hypothesis of this research was that an increase in taproot volume would increase the survival of the taproot in white clover. However, despite the large range of taproot sizes in our experiment, we only measured a significant positive correlation of taproot volume with taproot survival in the autumn of 2017, but not in the other periods. Therefore we cannot conclude that an increase in taproot volume increased the survival of the taproot over the measured years. The second hypothesis of this research was that the timing of death of the taproot would have an effect on the persistence of white clover. At the end of the experiment in September 2018 we did not find a relation between taproot survival and the persistence of white clover in the monoculture and mixture. However, we did find a positive correlation between the survival of the seminal taproot in autumn 2017 and the white clover cover in 2018. A cause that has been mentioned for the decline of white clover is its winter survival.

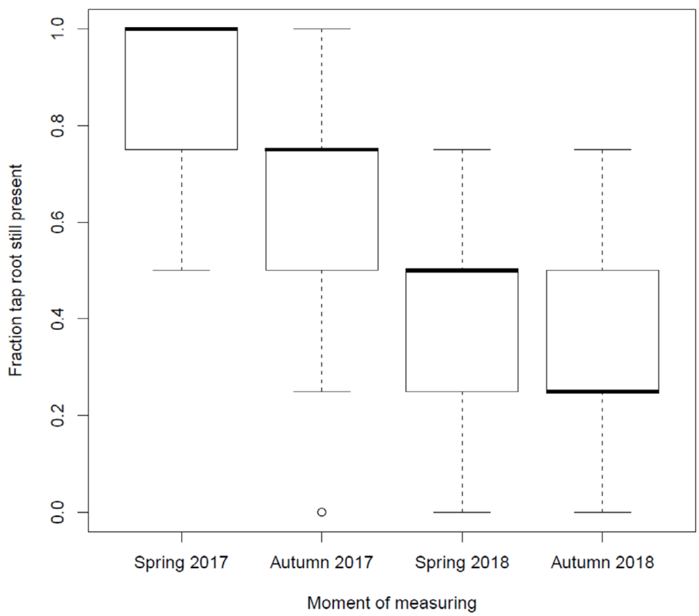

Figure 1. Fraction of taproot still present at four measuring dates after sowing (n=18).

Table 1. Correlation table of taproot volume, leaf size and taproot survival with taproot volume, leaf size and clover cover.[1]

Correlation matrix	Taproot volume (cm^3)	Leaf size (cm^2)	Plant density	Average clover cover mono (%)	Average clover cover mono 2017 (%)	Average clover cover mono 2018 (%)
Taproot volume (cm^3)		0.27*	-0.35**	0.22	0.19	0.17
Leaf size (cm^2)	0.27*		-0.04	0.28*	0.35	0.08
Plant density (# plants m^2)	-0.35**	-0.04		-0.13	-0.14	-0.06
TR survival spring 17	-0.02	0.11	0.04	0.10	0.11	0.05
TR survival autumn 17	0.29*	0.43***	-0.25*	0.38**	0.36**	0.26*
TR survival spring 18	-0.21	-0.21	-0.10	-0.17	-0.13	-0.16
TR survival autumn 18	-0.06	0.16	0.07	0.00	-0.08	0.09

[1] *P*-values: *** <0.001, ** <0.01, * <0.05. Taproot volume and plant density were measured in spring 2018, leaf size was measured autumn 2018. TR = Taproot, TR survival is fraction.

Conclusions

We found a positive correlation between leaf size and taproot volume. However, no correlations were found between taproot characteristics and taproot survival. We found indications that the timing of death of the seminal taproot can contribute to the winter hardiness of these varieties, possibly resulting in a higher persistence over the years. The combination of the timing of death of the seminal tap root and the development of stolons seems to play a more important role in increasing the persistence of white clover than the absolute survival of the seminal taproot. Future research should focus on understanding the transition from a taprooted white clover to a stoloniferous white clover plant in relation to specific weather events such as winter frost conditions.

Acknowledgements

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Green rye fermentation pattern and aerobic spoilage could be modified with silage additives

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Abstract

The aim of the present study was to evaluate the potential of the blends of homo- and heterofermentative lactic acid bacteria (LAB) as an additive for rye silage. Rye (*Secale cereale* L.) at soft dough stage of grain was ensiled in mini-silos without additives or with three different blends of homofermentative and heterofermentative LAB strains at the application rate 1.5×10^5 cfu g^{-1} fresh forage. There were 60 days of fermentation followed by 15 days of aerobic exposure. The data show that the homofermentative LAB strains inoculant increased the concentration of lactic acid, decreased the concentration of acetic acid and reduced the fermentation losses. However, homofermentative LAB impaired the aerobic stability. In contrast, applying the hetero- and homofermentative LAB strains inoculant in combination increased the concentration of acetic acid, decreased the numbers of yeasts and moulds and improved aerobic stability. Under aerobic conditions, this silage had the lowest pH, lowest heat production and the lowest numbers of yeasts and moulds among other silages. While the addition of homofermentative LAB strains was effective in reducing fermentation losses they did not improve the aerobic stability of the rye silage. In contrast, the combination of hetero- and homofermentative LAB strains was effective in reducing aerobic deterioration of the rye silages by supporting low pH values and inhibiting the proliferation of yeast and moulds.

Keywords: aerobic deterioration, bacteria strains, *Secale cereale*, mould, yeast

Introduction

Cover crops add organic matter to the soil, reduce the nitrate loss to groundwater and surface water, reduce soil erosion, suppress weeds, and keep valuable nutrients in the soil, thereby increasing the sustainability and reducing the environmental impact of the production system (Everett *et al.*, 2019). Like other small grain winter annuals, whole-crop rye (*Secale cereale* L.) can be a valuable forage for silage production for many ruminants including lactating dairy cows (Harper *et al.*, 2017). However, Auerbach and Theobald (2020) have shown poor fermentation quality in whole-plant rye despite the high content of water-soluble carbohydrates at harvest. Moreover, due to the hollow structure of the stem and high porosity at later phenological stages of rye, rye silages can be prone to aerobic deterioration. Application of the right silage inoculant can facilitate a successful fermentation process and inhibit aerobic deterioration during feed out (Wilkinson and Muck, 2019). The current research analyses the effects of combined lactic acid bacteria (LAB) strains to affect the fermentation characteristics, dry matter loss, microbial population, and the aerobic stability of whole-crop rye at soft dough stage of grain ensiled in a mini-silo setup.

Materials and methods

Rye (*Secale cereale* L.) was harvested and ensiled at the soft dough stage of grain, when forage contained 457 g kg^{-1} DM after harvest at a residual stubble height of 20 cm. Prior to ensiling, the rye fresh matter was separated into four piles and four additive treatments were applied: control (T0) with no additive (only tap water added) and three commercial (Chr. Hansen A/S, Denmark) LAB inoculants: (T1) a homofermentative SiloSolve® MC containing *Lactobacillus plantarum* (DSM26571), *Enterococcus faecium* (DSM22502) and *Lactococcus lactis* (NCIMB30117 (T2) a combination of hetero- and homofermentative SiloSolve® AS200 containing *Lactobacillus plantarum* (DSM26571), *Enterococcus* *faecium* (DSM22502), and *Lactobacillus buchneri* (DSM22501) and (T3) a combined hetero- and homofermentative SiloSolve® FC containing *Lactobacillus buchneri* (DSM22501) and *Lactococcus lactis* (DSM11037). The products were applied at the dose of 150,000 cfu g^{-1} fresh forage. Silages were packed tightly into 3-l glass jars at a storage bulk density 239±9 kg/m^3 and stored for 60 days in a dark room at ambient temperature (20-22 °C). After 60 days of fermentation a subsample was obtained and subjected to analysis of nutrients, fermentation and microbial characteristics. For the aerobic stability test, 1200 g of representative fermented silage samples were aerated in open polystyrene boxes under constant ambient temperature of 20 °C. Silage temperature was measured using data loggers (MS3+ (Comet System s.r.o., Czech Republic) that recorded temperature readings every six hours. Aerobic deterioration was evaluated by observing temperature dynamics inside the silages, the pH and the number of yeasts and moulds at the end of the aerobic stability test. Data were statistically analysed as a randomized complete block by using the GLM procedure of SAS.

Results and discussion

Inoculant application had an effect on the silage fermentation profile (Table 1). The use of homofermentative LAB (T1) and hetero- and homofermentative LAB (T2) resulted in the largest concentration of lactic acid ($P<0.05$). The T1 silage showed the lowest DM loss ($P<0.05$). According to Muck *et al.* (2018), homofermentative LAB usually promote lactic acid fermentation by shifting fermentation products towards lactic acid and away from ethanol and acetic acid fermentation products with a decreased DM loss as the main benefit. The lowest concentration of acetic acid ($P<0.05$) was detected in control (T0) and homofermentative LAB (T1) silages. Hetero- and homofermentative LAB silage (T3) caused the largest concentration of acetic acid ($P<0.05$) between all treatments. All three inoculant treatments suppressed the formation of ammonia-N, alcohols, and butyric acid.

Homofermentative LAB-treated silage (T1) began to deteriorate 65 h after aerobic exposure, and it took the control (T0) and hetero- and homofermentative LAB (T2) silages 176 h and 116 h, respectively, to reach a temperature of more than 3 °C above ambient (Figure 1). At the end of the aerobic stability test, T1 and T2 silages reached the largest pH, fresh weight loss, and the largest number of yeasts and mould (Table 2). Application of hetero- and homofermentative LAB (T3) supported aerobic stability for almost 340 h and showed the lowest pH value, fresh weight loss, and the lowest number of yeasts and moulds among all inoculant treatments. Tabacco *et al.* (2011) indicated the power of *L. buchneri* to inhibit the aerobic deterioration of silages through the fermentation of lactic acid to acetic acid and the inhibition of yeast and clostridial growth.

Table 1. Fermentation characteristics and dry matter losses of whole-crop rye silage.

	pH	Ammonia-N, $g\ kg^{-1}$ total N	$g\ kg^{-1}$ DMc LA	AA	Alcohols	BA	DM loss
T0	4.41^a	51.32^a	12.58^a	5.89^a	12.52^a	6.93^a	75.2^a
T1	3.70^b	32.73^b	34.57^b	5.65^a	8.32^b	0.65^b	29.7^b
T2	3.77^c	34.97^b	32.74^b	10.69^b	5.25^c	0.71^b	36.1^c
T3	3.96^d	39.06^c	7.78^c	29.26^c	6.78^d	0.73^b	36.8^c
SE	0.014	1.106	1.030	0.997	0.482	0.265	1.555

[1] DMc = dry matter corrected for volatiles; T0, = control, SE = standard error; LA = lactic acid; AA = acetic acid; BC = butyric acid. Within a column mean values followed by different letter differ significantly ($P<0.05$).

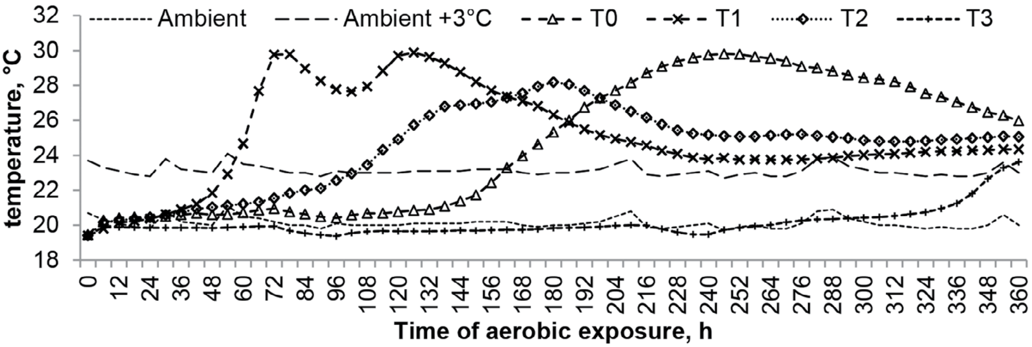

Figure 1. Temperature changes of whole-crop rye silage during aerobic exposure period.

Table 2. Characteristics of whole-crop rye silage at the end of the aerobic stability test.[1]

	pH	DM, g kg^{-1}	Weight loss, %	AS, h	Highest temp., °C	Log_{10} cfu g^{-1} of FF	
						Yeast	Mould
T0	6.23[a]	396.5[a]	5.29[a]	176.4[a]	29.8	7.60[a]	8.06[a]
T1	7.17[b]	407.4[b]	6.44[b]	64.8[b]	29.9	8.34[a]	7.26[b]
T2	7.63[c]	409.7[b]	5.96[ab]	116.4[c]	28.2	7.71[a]	7.05[b]
T3	4.32[d]	411.5[b]	2.51[c]	339.6[d]	23.3	4.78[b]	2.24[c]
SE	0.149	3.303	0.289	11.392	-	0.255	0.214

[1] DM = dry matter; AS = aerobic stability; CFU = colony forming units; FF = fresh forage. Within a column mean values followed by different letter differ significantly ($P<0.05$).

Conclusions

The homofermentative inoculant treatment improved the fermentation profile and reduced DM losses but impaired aerobic stability and increased fresh weight loss during the period of exposure to air. The product containing homofermentative *E. faecium*, *L. plantarum*, and heterofermentative *L. buchneri* improved fermentative profile, reduced DM losses at all stages of maturity, but aerobic stability was not improved. The dual-purpose inoculant containing *L. buchneri* and *L. lactis* supported good fermentation with reduced DM losses and ensured long-lasting protection against aerobic deterioration caused by yeasts and moulds of whole-crop rye silage at soft dough stage maturity of grain. The results of this experiment should support and promote the use of the combination of these LAB strains in the ensiling whole-plant of rye at the soft dough stage of maturity as forage for ruminants.

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Evaluation of alfalfa populations selected for acid soil tolerance in Lithuania

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Abstract

As climatic conditions change, there is an increasing need for fertile and high-quality alfalfa cultivars adapted to regional conditions. Field experiments were carried out in the western and central locations of Lithuania. In 2020, the average fresh matter yield of alfalfa populations was 20.1 t ha^{-1} higher in the alkaline soil (pH 7.0) compared with the fresh matter yield in the acid soil (pH 4.4). In 2021, the fresh matter yield was 9.5 t ha^{-1} higher in the acid soil compared to the alkaline soil. Warm and dry weather in 2019 determined high seed yield: 324.2 kg ha^{-1} in acid and 670.6 kg ha^{-1} in alkaline soil. In 2020, due to the rainy and warm weather conditions, the seed yield was lower by 180.5 kg ha^{-1} and 223.6 kg ha^{-1}, respectively in the acid and alkaline soils, compared with 2019. In the acid soil, the populations 3056, 3132, 3130 and 3058 differed by the fresh and dry matter yields in the period of 2019-2020. The populations 3131 and 3057 were distinguished by seed yield in 2019 and 2020, respectively. In the alkaline soil, the population 3057 was the most yielding by the seed yield in 2019 and 2020.

Keywords: *Medicago sativa*, fresh and dry matter yield, region, soil reaction, seed yield

Introduction

Alfalfa (*Medicago sativa* L.) is an important legume crop and cultivated in southern Europe, and also under temperate and Nordic or Baltic areas (Annicchiarico *et al.,* 2015). As climatic conditions change, there is an increasing need for more yielding and high-quality alfalfa cultivars that are adapted to regional conditions. Research should be expanded selecting genotypes of alfalfa with high adaptive capacity and good productivity (Cacan *et al.,* 2018). Alfalfa is usually grown on neutral soil, with a pH range of 6.2 to 7.8 (Lakić *et al.,* 2019). On low yielding soils, alfalfa growth is very slow, and productivity of genotypes is very low. As a result, new genotypes of alfalfa are being developed that are tolerant of low pH of soils. However, alfalfa cultivars and populations respond differently to low pH of soil and mobile Al concentrations (Liatukienė and Skuodienė, 2021). The resistance of alfalfa to mobile Al can be assessed based on laboratory methods and in field conditions when genotypes of alfalfa are grown in soil of low pH and with high concentrations of mobile Al (Stevović *et al.,* 2010). The aim of this study was to investigate the seed, fresh and dry matter yields of populations of alfalfa under different environmental conditions and select the promising populations of alfalfa under different pH of soil with mobile concentrations of Al.

Materials and methods

Experimental material consisted of 11 populations, which were selected after tests under laboratory and greenhouse conditions during 2008-2014. The populations of alfalfa were established in 2018 on two experimental sites of the Lithuanian Research Centre for Agriculture and Forestry. Experimental site I was in Vėžaičiai Branch in western Lithuania (55°70 N., 21°49 E.), and the experimental site II was in the Institute of Agriculture in central Lithuania (55°23′ N., 23°51′ E.). The soil of the experiment site I was *Retisol* with pH_{KCl} 4.4 and mobile Al mg kg^{-1} ranged 1.78-23.41. The soil of experiment site II was *Cambisol* with pH 7.0. The populations of alfalfa were sown using a randomised block design with four replications in the two experimental plots to evaluate the fresh and dry matter yields and the seed yield. The plot size was 1.5 m^2. The populations of alfalfa were assessed in 2019-2021. The fresh matter, dry matter (FM, DM in t ha^{-1}) and seed yields (SY in kg ha^{-1}) were evaluated. The one-factor analysis of variance (ANOVA) was used followed by Tukey's least significant difference at the P<0.05 significant levels.

Results and discussion

The significant (P<0.05) influence of the year was determined for fresh and dry matter yields in the acid and alkaline soils. Fresh matter yield was the highest in 2020, due to rainier and warm weather conditions at both experimental sites. The average fresh matter yield of alfalfa populations was 20.1 t ha^{-1} higher at the site with alkaline soil (pH 7.0) compared with fresh matter yield at the site with acid soil (pH 4.4) (Table 1). The fresh and dry matter yields depend on regional climate, soil conditions, plant genetics and sowing time (Luo *et al.* 2016).

Table 1. The fresh, dry matter yields (t ha^{-1}) and seed yield (kg ha^{-1}) of populations under different experimental sites.[1]

Populations	Acid soil (pH 4.4)						Alkaline soil (pH 7.0)					
	2019		2020		2021		2019		2020		2021	
	FM	DM	FM	DM	FM	DM	FM	DM	FM	DM	FM	DM
3056	95.0^{h}	23.3^{h}	94.7^{d}	23.2^{b}	80.3^{b}	20.7^{a}	118.2^{a}	25.6^{a}	128.9^{a}	26.1^{a}	99.8^{b}	24.0^{a}
3057	70.3^{d}	18.7^{e}	93.0^{d}	27.6^{f}	85.7^{c}	24.7^{e}	107.8^{a}	23.3^{a}	106.3^{a}	20.8^{a}	73.3^{ab}	18.3^{a}
3058	43.3^{a}	11.4^{a}	89.7^{c}	26.0^{d}	95.0^{e}	28.3^{g}	110.2^{a}	24.4^{a}	117.4^{a}	23.8^{a}	74.9^{ab}	19.3^{a}
3059	56.7^{b}	15.5^{b}	93.0^{d}	28.4^{g}	79.7^{b}	22.6^{d}	117.6^{a}	26.5^{a}	124.9^{a}	25.8^{a}	88.4^{ab}	22.0^{a}
3060	75.7^{e}	17.2^{c}	93.0^{d}	25.9^{d}	73.7^{a}	20.7^{a}	110.2^{a}	24.5^{a}	117.6^{a}	23.3^{a}	92.2^{ab}	23.5^{a}
3061	59.3^{c}	15.4^{b}	74.7^{a}	20.8^{a}	93.7^{d}	26.1^{f}	122.5^{a}	27.0^{a}	114.4^{a}	23.2^{a}	86.7^{ab}	21.0^{a}
3131	70.7^{d}	18.6^{e}	94.7^{d}	26.4^{de}	99.7^{f}	21.3^{b}	118.0^{a}	25.8^{a}	111.9^{a}	23.6^{a}	66.7^{a}	16.1^{a}
3132	78.3^{f}	20.6^{f}	106.3^{f}	32.2^{i}	101.0^{g}	24.5^{e}	118.2^{a}	25.6^{a}	100.2^{a}	20.2^{a}	71.8^{ab}	17.7^{a}
3130	71.3^{d}	17.8^{d}	106.3^{f}	29.7^{h}	106.3^{h}	24.7^{e}	99.1^{a}	21.8^{a}	101.5^{a}	18.6^{a}	66.9^{a}	15.7^{a}
3129	80.0^{g}	20.8^{f}	99.7^{e}	26.9^{ef}	86.3^{c}	22.4^{d}	106.9^{a}	24.0^{a}	129.8^{a}	26.1^{a}	84.7^{ab}	22.0^{a}
3086	77.7^{f}	22.8^{g}	86.3^{b}	24.2^{c}	86.3^{c}	22.1^{c}	108.4^{a}	23.0^{a}	100.2^{a}	19.1^{a}	77.3^{ab}	19.3^{a}
Mean	70.8	18.4	93.8	26.5	89.8	23.5	112.5	24.7	113.9	22.8	80.3	19.9

Populations	Seed yield					
	2019	2020	2021	2019	2020	2021
3056	432.3^{bc}	58.1^{a}	15.3^{a}	746.0^{a}	497.3^{a}	406.9^{a}
3057	271.5^{abc}	309.2^{c}	15.0^{a}	1,115.2^{b}	743.5^{b}	311.1^{a}
3058	324.1^{abc}	68.4^{a}	15.0^{a}	653.6^{a}	435.7^{a}	304.2^{a}
3059	243.8^{ab}	269.5^{bc}	14.3^{a}	534.0^{a}	356.0^{a}	244.9^{a}
3060	375.4^{abc}	167.5^{abc}	14.0^{a}	625.0^{a}	416.6^{a}	384.7^{a}
3061	181.9^{a}	102.4^{ab}	13.7^{a}	543.6^{a}	362.4^{a}	296.7^{a}
3131	498.8^{c}	164.7^{abc}	14.3^{a}	608.3^{a}	405.5^{a}	469.3^{a}
3132	314.0^{abc}	139.0^{abc}	14.7^{a}	727.3^{a}	484.9^{a}	281.8^{a}
3130	318.2^{abc}	79.6^{a}	13.2^{a}	465.4^{a}	310.3^{a}	348.2^{a}
3129	387.2^{abc}	93.4^{ab}	14.0^{a}	608.8^{a}	405.9^{a}	311.1^{a}
3086	219.2^{ab}	129.3^{ab}	13.7^{a}	749.1^{a}	499.4^{a}	504.2^{a}
Mean	324.2	143.7	14.3	670.6	447.0	351.2

[1] FM = fresh matter; DM = dry matter. Averages followed by the same letter in column do not differ from each other (P<0.05, Tukey's test).

In 2021, the fresh matter yield was lower, due to the dry and warm weather conditions compared to 2020, respectively by 4.0 t ha^{-1} for the acid soil site and by 33.6 t ha^{-1} for the alkaline soil. Also, in 2021, the fresh matter yield was 9.5 t ha^{-1} higher at the acid soil compared to the alkaline soil site. In terms of fresh matter yield, at the acid soil site populations 3056, 3132 and 3130 were the highest yielding, compared to other populations, in 2019, 2020 and 2021 respectively. In 2020, the populations 3132 and 3130 were more yielding by the fresh matter yield compared to populations 3061 (by 31.6 t ha^{-1}), 3086 (by 20.0 t ha^{-1}). In 2021, the populations differed more in their fresh matter yield at the site with alkaline soil. At the alkaline soil site the fresh matter yield of population 3056 was more yielding compared to populations 3131 and 3130 (by 33.0 t ha^{-1}). At the acid soil site, the dry matter yield of the populations was the highest, respectively 3056 – 23.3 t ha^{-1} in 2019, 3132 – 32.2 t ha^{-1} in 2020 and 3058 – 28.3 t ha^{-1} in 2021. However, the dry matter yield of populations was similar in the alkaline soil site in all experimental years. Also, in our study, there was a significant influence of the year for seed yield in acid and alkaline soils. The growing season in 2019 was very warm and dry at both experimental sites. The seed yield was higher than in the other years, in both acid and alkaline soils, with 324.2 kg ha^{-1} and 670.6 kg ha^{-1}, respectively (Table 1). The seed yield of alfalfa depends on upon the activity of pollinators and the weather conditions during seasons, especially during the flowering and ripening stage (Karar *et al.,* 2021). In 2020, due to the rainy and warm weather conditions, the seed yield was lower in the acid and alkaline soils, respectively by 180.5 kg ha^{-1} and 223.6 kg ha^{-1} compared with 2019. At the site with acid soil, the seed yield was highest for population 3131, at 498.8 kg ha^{-1} in 2019, and the population 3057, at 309.2 kg ha^{-1} in 2020. In 2021, the seed yield of all populations was very similar at the acid soil site. The seed yield of population 3057 was significantly the most yielding by the seed yield at the alkaline soil site in 2019 and 2020.

Conclusions

In the acid soil, the populations 3056, 3132, 3130 and 3058 were distinguished in terms of their fresh and dry matter yields in the period of 2019-2021. In the acid soil, population 3131 differed in the seed yield in 2019 and the population 3057 differed by the seed yield in 2020. However, at the site with alkaline soil the differences between populations were less pronounced for all yield measures: fresh matter, dry matter and seed yields.

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Factors related to grassland management affecting clostridia contamination in farm tank milk

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Abstract

Since January 2021, South Tyrolean dairies have banned the food preservative lysozyme in cheese production. In order to prevent late-blowing, it is therefore important that the farm tank milk (FTM) has clostridia values as low as possible. A survey addressing several aspects related to forage production was conducted in spring 2021. The answers of 535 valid questionnaires were linked to the respective clostridia levels in the FTM from September 2020 to April 2021. The results show that farms producing and feeding silages had higher clostridia levels in the FTM than farms feeding hay. Complying with the good practice rules of silage-making effectively reduced clostridia contamination in FTM.

Keywords: *Clostridium tyrobutyricum*, contamination, leys, permanent grassland, prevention

Introduction

Soil is considered to be the initial source of clostridia contamination. Forage plants are already contaminated during their growth or during the harvesting process by soil with the contamination level depending on different factors, e.g. harvesting technique and weather conditions (Brändle *et al.*, 2016). The endospores of clostridia producing butyric acid (especially *Clostridium tyrobutyricum*) can cause the so-called late-blowing defect in hard and semi-hard cheeses, which is not a health hazard but causes high economic losses (Brändle *et al.*, 2016). The most relevant action to prevent the late-blowing defect is to ensure that the contamination level of farm tank milk (FTM) is as low as possible. Since January 2021, the South Tyrolean dairies have banned the food preservative lysozyme, an enzyme attacking the cell walls of clostridia endospores, in order to match consumers expectations, to avoid allergic reactions because of its origin from hens' eggs, and to improve the usability of the whey left over from cheese making. In order to identify grassland management practices affecting farm tank milk (FTM) contamination with clostridia, a survey addressing several aspects related to grassland management and forage production was conducted.

Materials and methods

In spring 2021, a questionnaire was developed and distributed by three South Tyrolean dairies producing hard cheese, by addressing their 1,127 cooperative members. The questionnaire was set up in two different versions for milk farms feeding hay and farms producing and feeding silage. Before data analysis, a data validation of the 582 filled out questionnaires was conducted, and questionnaires with inconsistent answers were removed, resulting in 535 valid questionnaires, 325 of which referred to silage farms. The ten explanatory variables related to grassland management considered in this study, common to all investigated farms, were: inclusion of silage in the diet; exposure to rain damage during field drying; mowing at the right time (in the morning); keeping a minimum stubble height of 5-7 cm; occurrence of subterranean rodents; periodic overseeding and reseeding; application of biogas slurry; application of separated slurry; farmyard manure application in spring; application of liquid manure early in spring or just after mowing; splash plate application of slurry. The eleven variables available for the silage farms only were: silage proportion in the ration >50%; feeding baled silage; feeding clamp silage; feeding silage from tower silos; chopping of herbage prior to ensiling; use of silage additives; typical silage odour; favourable (sunny) exposition of the silage meadows; botanical composition rich in grass; altitude of the silage meadows (<1000/1000-1,250/1,250-1,500/>1,500 m a.s.l.); mowing frequency (cuts year^{-1}). All but the last two variables were binary scaled with 'yes' or 'no' as possible answers. The questionnaires were linked to the respective amount of clostridia endospores per litre milk (AC) measured in FTM by the Affect Misattribution Procedure (AMP)-method from September 2020 to April 2021 (15±1.9 standard deviation measurements per farm, performed every 1-2 weeks). Data analysis was performed by means of generalized linear mixed models using a Poisson distribution coupled to a log link function. The degrees of freedom were computed by Satterthwaite method. The statistical model accounted for the repeated measurements over time with the farm as a subject with a first-order autoregressive covariance type. We analysed two subsets of the data: (1) that including all explanatory variables common to all investigated farms; and (2) that including the silage farms only and, in addition to the explanatory variables common to all farms (except the inclusion of silage in the diet). In (1) we started from a full model including all selected explanatory variables as well as their interactions with the use of silage. In a further step, non-significant interactions (P>0.1) were dropped by the model and then the final model was computed by deleting stepwise backward the non-significant main terms. In (2) we tested for all main effects and then proceeded as described for (1). Estimated marginal means on the original scale were computed and, for significant interactions, multiple comparisons were performed by LSD test.

Results and discussion

Concerning the factors common to all farms, the inclusion of silage in the diet was the factor that mostly affected AC in FTM (Table 1) as shown by the interactions with the occurrence of subterranean rodents, no periodic overseeding and reseeding, and farmyard manure application in spring, leading to an increase of AC only for the farms feeding silage. This is consistent with Vissers *et al.* (2007) who consider silage as the most relevant factor regarding AC in FTM. Both the exposure to rain damage during field drying and an insufficient stubble height led to increased AC in all farms, whilst no periodic overseeding and reseeding, problems with subterranean rodents and the contamination potential represented by the application of farmyard manure in spring proved to be detrimental factors for silage farms only. All these variables are also described by Brändle *et al.* (2016) and Pahlow *et al.* (2003) as factors influencing the extent of contamination during forage production. The analysis concerning silage farms only (Table 2) showed that besides the factors already described above, AC were higher if forage was conserved in tower silos, which may be due to slow acidification because of delayed sealing, oxygen ingress because of improper sealing, or aerobic spoilage because of slow unloading (Pahlow *et al.*, 2003). AC were lower if silage additives were used. Especially under sub-optimal conditions, additives shall ensure reaching the critical pH to stop clostridial growth (Pahlow *et al.*, 2003). Furthermore, increased AC were found if the herbage was chopped before ensiling. This may be the consequence of higher soil contamination due to field operations such as chopping, even if positive effects of chopping on limiting clostridia proliferation are reviewed by Pahlow *et al.* (2003).

Conclusions

It can be concluded that including silage in the diet highly increases the risk of clostridia contamination of FTM, but complying with the good practice rules of silage making aiming at reducing forage contamination with soil effectively reduces clostridia contamination. Moreover, the results suggest that poorly maintained permanent or temporary grasslands without periodic overseeding or reseeding lead to higher clostridia levels.

Table 1. Effect of factors common to all farms on the clostridia contamination level in farm tank milk.[1]

Source	F	P-value	Main effect		Use of silage			
			yes	no	yes		no	
					other factor		other factor	
					yes	no	yes	no
Inclusion of silage in the diet (S)	90.220	<0.001	-	-	-	-	-	-
Exposure to rain damage	17.329	<0.001	1,175.2	732.3	-	-	-	-
Minimum stubble height 5-7 cm	5.165	0.023	766.2	1,123.2	-	-	-	-
Occurrence of subterranean rodents (R)	0.002	0.963	-	-	-	-	-	-
Periodic overseeding and reseeding (OR)	0.146	0.703	-	-	-	-	-	-
Farmyard manure application in spring (F)	0.259	0.611	-	-	-	-	-	-
S × R	4.718	0.030	-	-	2,426.1[a]	1,728.9[b]	357.2[a]	494.2[a]
S × OR	8.771	0.003	-	-	1,684.2[b]	2,490.4[a]	542.0[a]	325.8[a]
S × F	3.860	0.050	-	-	2,307.6[a]	1,817.6[b]	343.0[a]	514.8[a]

[1] Estimated marginal means are provided at the interaction level where appropriate. For multiple comparisons, means sharing superscript letters within the same silage use category do not significantly differ from each other.

Table 2. Effect of factors common to all silage farms on the clostridia contamination level in farm tank milk. Estimated marginal means are provided.

Source	F	P-value	Factor level	
			yes	no
Exposure to rain damage	6.7	0.010	2,778.8	1,910.0
Minimum stubble height 5-7 cm	6.8	0.009	1,759.8	3,015.9
Occurrence of subterranean rodents	7.0	0.008	2,747.7	1,931.6
Periodic overseeding and reseeding	4.4	0.036	2,012.4	2,637.3
Feeding silage from tower silos	27.3	<0.001	3,209.0	1,653.9
Chopping of herbage prior to ensiling	13.5	<0.001	2,949.1	1,799.7
Use of silage additives	10.8	0.002	1,879.4	2,824.0

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Effects of perennial ryegrass on the yield and nutritive value of grass mixture with legumes

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Abstract

Alaska brome cv. Hakari and smooth brome cv. Lehis were grown in pure stand and in combination with perennial ryegrass cv. Raite, lucerne cv. Karlu, and red clover cv. Varte in a field trial during 2017-2020 with or without fertilisation. With fertilisation, Alaska brome and smooth brome plots were cut three times and received a total of 200 kg ha^{-1} N (80-60-60 kg ha^{-1} after each cut) plus autumn fertiliser (7-20-28) of 300 kg ha^{-1} ($N_{21}P_{60}K_{84}$). The average dry matter (DM) yield in perennial ryegrass mixture with red clover was 3.6 Mg ha^{-1} for smooth brome, and 4.4 Mg ha^{-1} for Alaska brome. In the mixture with lucerne, the corresponding DM yields were 9.2 and 7.9 Mg ha^{-1}. The forage with the highest nutritive value was obtained when growing grasses of perennial ryegrass with red clover: digestible dry matter (DDM) 645-706 g kg^{-1} DM, metabolisable energy (ME) 9.6-10.5 MJ kg^{-1} DM. Similar results were found in the mixture with lucerne. The nitrogen used in the fertilised plots increased the DMY and protein content of the forage. All mixtures with perennial ryegrass improved nutritional value, with Alaska brome having a better nutritional value than smooth brome.

Keywords: Alaska brome, smooth brome, perennial ryegrass, lucerne, red clover, nutritive value

Introduction

Perennial ryegrass (*Lolium perenne* L.) is considered to be the most important grass species in temperate climates due to its high digestibility and good grazing tolerance (Wilkins, 1991). An optimal combination of suitable grass and legume companion species is needed to obtain high N-use efficiency, high herbage yield and high contents of nutritive compounds in grass-legume mixtures (Adamovics and Gutmane, 2018; Elgersma and Søegaard, 2015; Moloney *et al.*, 2016). Alaska bromegrass (*Bromus sitchensis* Trin. in Bong.) is a relatively new species in Estonia, and it is frost-resistant and has rapid regrowth. It has been investigated and cultivated here only very recently (Tamm *et al.,* 2018). When choosing legumes for grass-legume mixtures, the rate of phenological development of the species, persistency and nutritive value should be considered. The nutritive value is highest when the 1st cut is taken at early heading for grasses, and budding or the beginning of flowering for legumes. The aim of this study is to compare production abilities and forage quality of two *Bromus* species in pure stand and in mixture with lucerne or red clover cultivars with added perennial ryegrass in Estonia.

Materials and methods

The experimental field was established in 2017 in Saku, Estonia (latitude 57° 25'). The study included data from three years (2017-2020). The trial plots were on a typical soddy-calcareous soil where the agrochemical indicators were as follows: pH_{KCl} 7.4 (ISO 10390); soil carbon content C_{org} 3.0% (Tyurin method) and concentration of lactate soluble phosphorus (P) and potassium (K) 53 and 97 mg kg^{-1} (Mehlich III method) respectively. The trial was established without fertilisation. The sowing rate of Alaska brome (*Bromus sitchensis* Trin. in Bong) cv. Hakari *(Bs)* 20 kg ha^{-1} and smooth brome (*Bromus inermis* Leysser) cv. Lehis *(Bi)* was 20 kg ha^{-1}; perennial ryegrass (*Lolium perenne)* cv. Raite (*Lp*) 6 kg ha^{-1}; lucerne (*Medicago sativa* Lam) cv. Karlu (*Ms*) 12 kg ha^{-1} and red clover cv. Varte (*Tp*) 10 kg ha^{-1}. These trials had four replicates with a split-plot design. Ten different mixtures plus two pure stands were compared: mixture of Alaska brome or smooth brome with either red clover or lucerne and with addition of perennial ryegrass (Table 1). Pure stands were fertilised with 200 kg N ha^{-1} in three applications

(80+60+60 kg ha^{-1}). Autumn fertiliser (7-20-28) was also given at 300 kg ha^{-1} ($N_{21}P_{60}K_{84}$) in all treatments. A three-cut system was used during harvest. First cut was taken during May 28 to June 2, second during July 3 to July 18 and third cut during September 2 to September 18. The crop was cut, weighed and samples were taken for laboratory analyses and for estimation of botanical composition. The following data were collected: dry matter (DM) yield, crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), metabolisable energy (ME) content and digestible dry matter (DDM). The sum of effective temperatures (over 5 °C) for the growing season (May-September) were 1,700 °C in 2018, 1,397 °C in 2019, and 1,384 °C in 2020. Rainfall occurred in the growing season were 229 mm in 2018, 343 mm in 2019, and 419 mm in 2020 (long term average 353 mm). Statistical analyses (ANOVA and Fisher´s LSD) were carried out by Agrobase 20™.

Results and discussion

The growth and development of legumes and Lp are slower in spring as they require warmer temperatures, compared to the cooler climate-adapted *Bromus* species. The high air temperatures and drought in the summers of 2018 and 2019 reduced the late yields of mixtures. The addition of *Lp* reliably reduced the dry matter yield of mixtures. The 3-year average DM yield for *Bi* and *Tp* mixture with *Lp* was 3.6 Mg ha^{-1} and 4.4 Mg ha^{-1} for *Bs* with *Tp* and *Lp* mixture. The highest DM yields of *Bs* and *Bi* were obtained with pure stand supplied with N fertiliser, with the 3-year average yields of 10.1 and 11.0 Mg ha^{-1}, respectively (Table 1).

The three-year average DM yields were 9.2 and 7.9 Mg ha^{-1} for *Bi/Ms* and *Bs/Ms* mixtures. The botanical composition of the mixtures as an average of the experimental years was relatively similar: in the mixtures of *Lp*/*Bs/Ms* and with *Lp/Bs/Tp* were: grasses 72%, (*Lp* 42%, *Ms* 21% and *Tp* 24%). In the mixtures with *Lp/Bi/Ms* and *Lp/Bi/Tp*: grasses averaged 75%, (*Lp* 43%), *Ms* 27% and *Tp* 27%). Averaged over the three years, the addition of *Lp* reliably increased crude protein content in the first cut only in the *Bi* yield in the N 200 variant. In *Bs/Lp* mixtures, the increase was within the limits of the test error. In mixtures of *Bs* and *Bi* with lucerne, TP was higher (158-188 g kg^{-1} DM) in both cuts than in mixtures of the same grass species with red clover (Table 2). The mixtures with *Bi* (*Bi* as botanical composition 26%) was less competitive than *Bs* (*Bs* as botanical composition 32%). The CP concentration of the *Bs/Tp* mixtures was

Table 1. The dry matter yield (Mg ha^{-1}) of perennial ryegrass and grass-legumes mixtures 2017-2020.[1,2]

Species	Treatment	2018	2019	2020	Average
Bs	N200	$6.1^{b/C}$	$7.2^{a/B}$	$17.1^{a/A}$	10.1^{a}
Bs/Lp	N200	$6.7^{a/B}$	$5.8^{b/C}$	$13.7^{b/A}$	8.7^{b}
Bs/Ms	N0	$4.3^{a/C}$	$6.8^{a/B}$	$12.8^{a/A}$	7.9^{a}
Bs/Ms/Lp	N0	$3.9^{b/B}$	$2.5^{b/C}$	$8^{b/A}$	4.8^{b}
Bs/Tp	N0	$4.9^{a/C}$	$6.3^{a/B}$	$7.7^{a/A}$	6.3^{a}
Bs/Tp/Lp	N0	$4.2^{b/B}$	$2.7^{b/C}$	$6.3^{b/A}$	4.4^{b}
Bi	N200	$6.5^{a/C}$	$9.5^{a/B}$	$17^{a/A}$	11^{a}
Bi/Lp	N200	$6.3^{a/B}$	$6.6^{b/B}$	$15.6^{b/A}$	9.5^{b}
Bi/Ms	N0	$3.9^{a/C}$	$7.7^{a/B}$	$16.1^{a/A}$	9.2^{a}
Bi/Ms/Lp	N0	$3.3^{a/B}$	$2.6^{b/B}$	$8.5^{b/A}$	4.8^{b}
Bi/Tp	N0	$3.9^{a/C}$	$5.9^{a/B}$	$8.1^{a/A}$	5.9^{a}
Bi/Tp/Lp	N0	$3.3^{b/B}$	$2.3^{b/C}$	$5.2^{b/A}$	3.6^{b}

[1] Mean values with different lowercase letters (a,b,c,d) within years (in columns between lines) and mean values with different uppercase letters (A,B,C,D) in rows between lines are statistically different (*P*<0.05, Fisher LSD test).

[2] *Bs* = Alaska brome, *Bi* = smooth brome; *Lp* = perennial ryegrass; *Ms* = lucerne; *Tp* = red clover.

lower than that in the *Bs/Ms* mixture (Meripõld *et al.*, 2022). In all cuts the NDF values of the mixtures were lower than those of the pure grass variants because fibre content was higher in the grasses than in the lucerne and red clover. *Lp* increased the feed digestibility and metabolizing energy value in first cut grass-legumes mixtures (DDM 689-706 g kg^{-1} DM, 10.3-1.5 ME MJ kg^{-1}).

Table 2. The average nutritive value of perennial ryegrass and grass-legumes mixtures 2017-2020.[1,2]

Species	Treatment	First cut				Second cut			
		CP (g kg^{-1})	NDF (g kg^{-1})	DDM (g kg^{-1})	ME (MJ kg^{-1})	CP (g kg^{-1})	NDF (g kg^{-1})	DDM (g kg^{-1})	ME (MJ kg^{-1})
Bs	N200	142^a	620^a	635^a	9.3^b	144^a	673^a	599^a	9.0^b
Bs/Lp	N200	146^a	481^b	680^b	10.0^a	146^a	546^b	658^a	9.8^a
Bs/Ms	N0	158^a	477^a	671^b	10.0^a	178^a	489^a	643^b	9.5^a
Bs/Ms/Lp	N0	154^a	408^b	703^a	10.5^a	138^b	508^a	648^a	9.7^a
Bs/Tp	N0	140^a	505^a	666^b	10.0^b	142^a	513^a	637^a	9.4^a
Bs/Tp/Lp	N0	155^a	420^a	706^a	10.5^a	118^b	514^a	639^a	9.6^a
Bi	N200	145^b	641^a	618^b	9.1^b	163^a	662^a	609^b	9.2^a
Bi/Lp	N200	162^a	472^b	687^a	10.0^a	146^b	568^b	649^a	9.6^a
Bi/Ms	N0	164^a	475^a	666^a	10.0^a	188^a	477^a	649^a	9.5^a
Bi/Ms/Lp	N0	138^b	449^a	689^a	10.3^a	175^a	483^a	656^a	9.7^a
Bi/Tp	N0	135^a	506^a	659^b	9.8^b	144^a	499^a	645^a	9.5^a
Bi/Tp/Lp	N0	136^a	415^b	706^a	10.5^a	123^a	499^a	645^a	9.6^a

[1] Different lowercase letters within column (between lines) are significantly different (P<0.05; *ANOVA, Fisher LSD* test).

[2] *Bs* = Alaska brome, *Bi* = smooth brome; *Lp* = perennial ryegrass; *Ms* = lucerne; *Tp* = red clover; CP = crude protein; NDF = neutral detergent fibre; DDM = digestible dry matter; ME = metabolizable energy.

Conclusions

The addition of *Lp* reduced dry matter yield of mixtures. High summer temperatures and drought reduced the late harvest of legume-grass mixtures. The lucerne mixtures with brome grass species had high DM yields. The legumes and perennial ryegrass increased metabolizable energy and digestible dry matter content in a forage. *Lp* increased feed digestibility and metabolizing energy value in first cut grass-legumes mixtures. Altogether, these results suggest that addition of *Lp* increased forage quality whereas the yield remained unchanged.

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Resistance of diploid and tetraploid clover (*Trifolium* spp.) to fungal leaf diseases

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Abstract

A clover collection was investigated at the Lithuanian Research Centre for Agriculture and Forestry, Institute of Agriculture Grass Breeding Department in 2018-2020. The present study aimed at determining diploid and tetraploid clover (*Trifolium pratense* L., *T. hybridum* L., *T. repens* L.) resistance against the foliar diseases common leaf spot (*Pseudopeziza trifolii*) and powdery mildew (*Erysiphe trifolii*) recorded under the field conditions. Common leaf spot occurred in all the studied years. In 2018-2019, the average disease severity of diploid and tetraploid red clover was almost the same, 5.8 scores for diploid and 6.0 scores for tetraploid, 4.7 and 4.8, respectively, in 2019-2020. Leaf spot severity of white clover was similar to red clover. The analysis of incidence and severity of powdery mildew indicated that tetraploid red clover was more susceptible (6.3 scores in 2018 and 4.6 scores in 2019), whereas powdery mildew was not noticed in white clover. Obtained results demonstrate that ploidy level is not a determining factor affecting resistance to red clover diseases. White clover was outstandingly resistant to powdery mildew.

Keywords: clover, *Erysiphe trifolii*, *Pseudopeziza trifolii*, resistance

Introduction

Clover is cultivated as a perennial leguminous forage crop in various regions of the world in temperate climatic conditions. It is short-lived, perennial, best suited for hay, silage or pasture and its stand life is generally limited to 2 or 3 years (Bhardwaj *et al.*, 2022). However, clover is infected by several pathogens affecting its growth, resistance and overwintering capacity (Wallenhammar *et al.*, 2005). Common leaf spot (*Pseudopeziza trifolii*) primarily attacks red clover but also white and crimson species. Leaf spot occurs throughout the growing season but is most severe in autumn. Long-term infections may reduce plant vigour and contribute to winter injury. The fungus survives in plant residue. No resistant varieties are known, tetraploid varieties are more susceptible than diploids. Powdery mildew (*Erysiphe trifoliorum*) overwinters on the plant and on the diseased crop residue. It seldom seriously reduces seed production but it can reduce forage yield and quality. The disease is common on red clover and occasionally on white and alsike clovers (Pscheidt and Ocamb, 2022). The development of resistant varieties is the most efficient and eco-friendly way of controlling the diseases in legume forage crops (Taylor, 2008). Hence, there is a need to find alternative strategies for efficient management of these diseases to minimize the seed yield loss in clover (Bhardwaj *et al.*, 2022).

The aim of this study was to determine diploid and tetraploid clover (*Trifolium pratense* L., *T. hybridum* L., *T. repens* L.) resistance against foliar diseases powdery mildew (*Erysiphe trifolii*) and common leaf spot (*Pseudopeziza trifolii*) in the field conditions.

Materials and methods

Investigations were carried out at the Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry. Resistance of clover varieties to powdery mildew and leaf spot was investigated in 2018-2019 and 2019-2020 in natural field conditions. The soil of the experimental site is Endocalcari-Epihypogleyic Cambisol (CMg-p-w-can). Seeds were sown in June in 2017 and 2018. Breeding nurseries and cultivar testing trials of perennial legumes were used for two years. All genotypes were sown in 8.25 m^2 (5.5×1.5 m) plots in three replications using a specialist seed drill (Hege). The collection consisted of 32 varieties of different origin (20 red clover, 5 alsike clover and 7 white clover). The severity of leaf diseases (powdery mildew and common leaf spot) was assessed on a 1-9 score scale: (1) – 0%, (2) – 0.1%, (3) – 1%, (4) – 5%, (5) – 10%, (6) – 20%, (7) – 40%, (8) – 60%, and (9) ≥80% of the disease-affected leaf area (Hartung *et al.*, 2007). Disease assessments in the collection were started when the first symptoms of the disease occurred (51 BBCH) and were continued until the end of the growing season. Leaf diseases were assessed every 2-3 weeks. A total of 10 plants per plot were assessed (scores), and the overall average was calculated. Analysis one-way of variance (ANOVA) was used followed by Fisher's the least significant difference at the $P<0.05$ significance levels.

Results and discussion

Disease resistance is one of the most important traits of a good clover variety. In our investigation, 32 genotypes of various origin were investigated for resistance to common leaf spots and powdery mildew.

Common leaf spot occurred in all the studied years. The average damage score for clover in the 2017 sowing was 5.7 and 4.7 in 2018 (Figure 1). In 2018-2019, the average disease severity of diploid and tetraploid red clover was almost the same, scores 5.8 and 6.0 for diploid and tetraploid, respectively, and scores 4.7 and 4.8 in 2019-2020. We were unable to assess the resistance of alsike clover in relation to ploidy because only a few varieties were tested. Leaf spot severity of white clover was similar to red clover. The analysis of the 2018-2019 data showed that the most resistant red clover variety was Arimaičiai (score 5.5), alsike clover – Poliai (score 4.8) and white clover – Sūduviai (score 5.6). In 2019-2020 red clover variety Kaive (score 4.0), alsike clover – Menta (score 4.8) and white clover – Rivendel (score 4.7) were most resistant to common leaf spot. The second widespread leaf disease is powdery mildew. Powdery mildew occurred only under favourable conditions in 2018 and 2019. The red clover damage score ranged from 5.0 to 7.3 in 2018 and 4.7 to 4.8 in 2019 (Figure A, B). The analysis of incidence and severity of powdery mildew indicated that tetraploid red clover was more susceptible (score 6.3 in 2018 and score 4.6 in 2019). In 2018 the variety Sandis was the most resistant to powdery mildew (score 5.0), while Vyčiai (score 7.1), Marita (score 7.1) and Skriveru tetra (score 7.3) were the most susceptible. The alsike clover varieties Menta (score 3.8) and Lomiai (score 5.6) were more susceptible than the standard Poliai (score 3.5). In 2019 red clover Vesna (score 5.0) was more sensitive than the standard variety Arimaičiai (score 4.0). The susceptibility of alsike clover to powdery mildew was similar. Powdery mildew was not noticed in white clover. The slow spread of the disease was influenced by meteorological conditions, with hot weather and low rainfall. The most reliable way to control diseases is to breed resistant varieties.

Conclusions

The obtained results demonstrated that ploidy level is not a determining factor affecting resistance to red clover diseases. White clover was outstandingly resistant to powdery mildew.

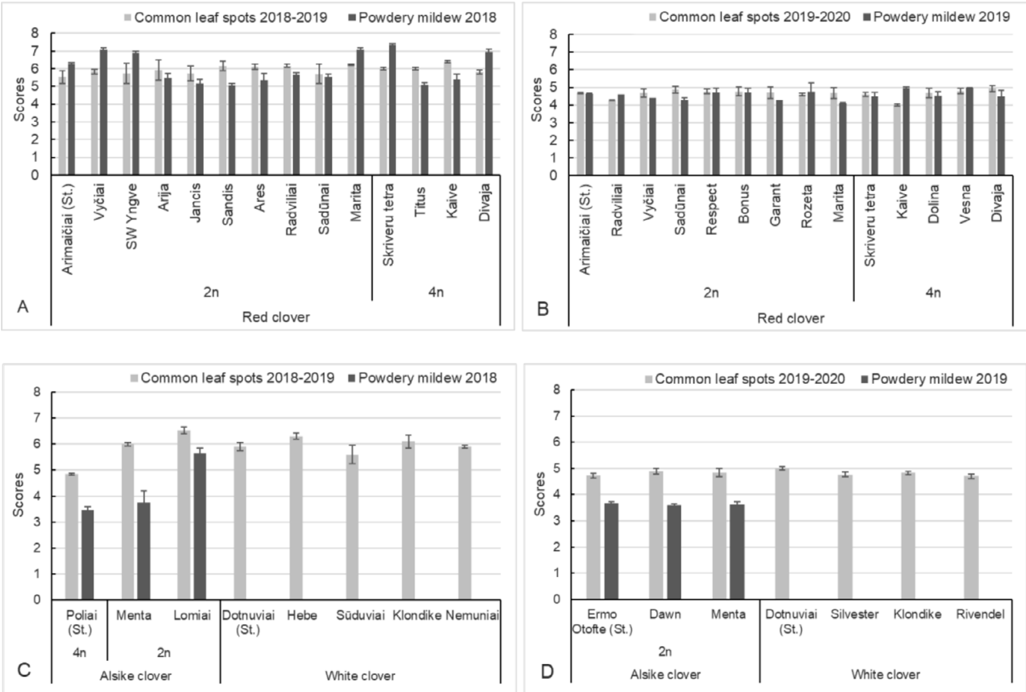

Figure 1. Resistance of clover to common leaf spot and powdery mildew (scores), 2018-2020.

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Effect of different chemical additives on the fermentation of *Cichorium intybus* and *Plantago lanceolata*

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Abstract

Future cropping conditions might be affected by more warm and dry periods. Suited fodder crops are needed for adaptation to these conditions. As well as the use of better adapted grasses and legumes, herbs like *Cichorium intybus* (CI) and *Plantago lanceolata* (PL) may become more important in a drier climate. In the southern hemisphere both these herbs are used quite often in pastures as well as in silages. Data from central Europe are still lacking. We ensiled pure crop stands of CI and PL in 1.5 L jars treated with additives based on nitrite, HMTA, formate and propionate. The herbs had been wilted slightly. The DM for CI and PL was 142 and 197 g kg^{-1}, respectively. The control silages were characterized by a butyric acid content of 8.6 and 4.0% of DM, for CI and PL respectively. The fermentation losses were 14.1 and 8.7 for CI and PL, respectively. The additives used could improve the silage a lot by controlling mal-fermentation. In the additive treatments of both herbs butyric acid was 0.3% of DM compared to >4% in control and the fermentation losses dropped from >9% in control to 5…7% of DM in the treatments. The protein fraction A was low (<45% of CP) for both herbs. For most of the fermentation traits PL compared to CI showed better data. That might come from the secondary plant ingredients of PL like aucubin or acteoside. Both herbs are difficult to ensile and for preventing mal-fermentation additives are needed.

Keywords: herbs, *Plantago lanceolata*, *Cichorium intybus*, fermentation, additives

Introduction

Increasing periods of dry, hot weather as a result of climate change in Europe may require the use of more drought-resistant crops in leys or grasslands. Drought-tolerant species are tall fescue or alfalfa or crops such as *Cichorium intybus* (CI) and *Plantago lanceolata* (PL) which are not widely used in European grassland. Both crops, CI and PL are winter dormant, winter hard and drought resistant herbs characterized by a deep root system, bearing a mycorrhiza or antioxidative system to repair drought damage (Ghanaatiyan and Sadeghi, 2017; Li and Kemp, 2005; Pol *et al.*, 2021). For both herbs the main use is grazing. The feed value is often better than ryegrass-dominated swards (Somasiri *et al.*, 2015). But there are limited data available regarding the ensilability of CI and PL.

Material and methods

In the first week of October pure crop stands of *Cichorium intybus* and *Plantago lanceolata* were harvested and ensiled after 5 hours wilting. The crop traits for CI and PL were (g kg^{-1} DM): DM 142/197, crude protein (CP) 151/122, crude ash (CA) 286/196, water soluble carbohydrates (WSC) 68.5/85.6, buffer capacity (BC) 11.0/9.2 g lactic acid 100 g^{-1} DM, WSC/BC 0.6/0.9 and the fermentation coefficient (FC) were 19/27, respectively. Thus, the fermentation conditions were very poor. The crop material was ensiled in 1.5 litre jars. Each treatment was prepared from 5 kg slightly wilted crop (FM) and treated with: (1) sodium nitrite + HMTA (KL, 3 litres t^{-1} FM, KOFASIL liquid); (2) sodium nitrite + sodium formate (EP3, 4 litres t^{-1} FM); and (3) sodium formate + sodium propionate (BU, 4 litres t^{-1} FM, Kofasil BU). The additives are all from ADDCON Europe GmbH. Per treatment we applied 15 and 20 ml 5 kg^{-1} FM for the application amount of 3 and 4 litres t^{-1} FM, respectively. For a better distribution, the additives were mixed with 35/30 ml water. Fifty ml water were sprayed on the control plant material. The silages were opened after 110 days. Chemical composition from fresh crop as well from the silages was evaluated based on the VDLUFA protocol and the following fermentation traits were analysed: lactic-, acetic- and butyric acid using high pressure liquid chromatography (HPLC). The protein fractions were analysed according to Licitra *et al.* (1996). The DM-fermentation losses (DMFL) were calculated after Weißbach (2005). The setup of the experiment was a random block design with 3 repetitions. Statistical analysis was undertaken using PROG ANOVA followed by post-hoc t-test in SAS.

Results and discussion

The fermentation coefficient (FC) for the crops CI and PL was 19 and 27, respectively, and thus was not optimal for fermentation. The low FC was the result of a low content of WSC, DM, and a high content of crude ash. High contents of minerals as well as low WSC are described for both crops (Kälber *et al.*, 2012; Kara, 2022). But the high figures of CA (Table 1) might be caused by soil contamination as well due to an open sward. These conditions resulted in a mal-fermentation in the untreated silages, characterized by high amounts of butyric acid and DMFL (Table 1). The DM fermentation loss was reduced by half by the additives. In addition, butyric acid fermentation was suppressed and the amount of lactic acid was significant higher in the treated silages. The acetic acid was controlled in the CI crop by the treatments but not for PL. For PL the acetic acid was low in the control as well. Although a clear suppression of a butyric acid fermentation was given by the additives the effect was relatively weak in the *Plantago lanceolata* silages. All fermentation induced effects in addition to the acids like the increase of the less metabolizable substances like CA and ADFom or the decrease of DM content, all as a result of the catabolism of organic material, were less visible for PL. Concerning the protein values, we found a low protein solubility for both herbs in all treatments, but in the case of PL the soluble protein was much lower (CI=43, PL=15) and the fraction C was, with 48%, very high (data not shown). These results for PL might come from the secondary plant ingredients like aucubin or acteoside. These substances obviously have effects on microbes and can affect soil-N-mineralization or rumen fermentation (Dietz, 2013; Navarrete, 2016). Substances such as sesquiterpene lactones which carry antibacterial traits and formed by CI as well (Chaturvedi, 2019) did not positive affect the fermentation of CI in this experiment.

Conclusions

Both crops, CI and PL, are difficult to ensile and need to be wilted, which seems to be difficult due to the leaf proportion and structure. Pure stands of *Cichorium intybus* and *Plantago lanceolata*, not or slight wilted, need to be supported in fermentation by additives. Otherwise, mal-fermentation (butyric acid) and high DMFL may occur. Mixed swards with grasses may improve the fermentation.

Table 1. Effect of the additive treatments onto fermentation parameters of *Cichorium intybus* (CI) and *Plantago lanceolata* (PL).[1]

	DMFL	DM	CA	ADFom	CP	A	B2	LA	AA	BA
	(%)	(g kg^{-1} DM)				(% CP)		(% DM)		
CI										
Control	14.1^a	128.3^a	318.5^a	201.9^a	110.9^a	40.8^a	41.0^a	0.4^a	8.2^a	8.6^a
KL	6.0^b	156,2^b	283.6^b	190.5^{ab}	143.2^b	41.3^{ab}	43.0^a	10.2^b	2.9^b	0.3^b
EP3	6.7^b	151.1^b						9.5^b	4.8^c	0.3^b
BU	7.2^b	150.6^b	286.9^b	167.6^c	136.0^c	43.3^c	40.2^a	10.5^b	4.5^c	0.3^b
PL										
Control	8.7^a	183.8^a	253.1^a	305.3^a	124.3^a	11.4^a	14.4^a	9.5^a	2.2^a	4.0^a
KL	5.3^b	199.6^b	235.4^a	287.5^a	127.7^a	19.4^b	14.2^a	12.4^a	2.1^a	0.2^b
EP3	5.0^b	192.1^c						13.5^a	2.9^b	0.3^b
BU	5.0^b	194.5^c	222.6^a	299.6^a	118.3^b	13.7^b	12.3^a	13.8^a	2.2^a	0.3^b

[1] ADFom = acid detergent fibre of organic matter; DM = dry matter; DMFL = DM fermentation loss; CA = crude ash; CP = crude protein; A, B2 = protein fractions; LA = lactic acid; AA = acetic acid; BA = butyric acid. Different letters indicate significant differences between treatments within a crop ($P<0.05$, t-test).

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Establishing multi-species leys – challenges and benefits

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Abstract

There is increasing interest in establishing multi-species (MS) leys within arable rotations for multiple benefits. The diversity of species can provide climatic and economic resilience within mixed farming systems due to their ability to continue growing during drought conditions and fix nitrogen from the atmosphere. In addition, there is increasing evidence that the varied mineral composition and tannin content of MS leys can help improve livestock performance. The ley phase also provides an opportunity to control weeds and can result in yield benefits for following arable crops. However, establishing MS leys at minimal cost and achieving persistence of desired species can be significant challenges. This paper presents findings from work with farmers in England and Northern Ireland.

Keywords: resilience, sustainable systems, herbs, legumes, grazing

Introduction

Across Europe, the farming community and society in general are facing a number of significant challenges. In 2021-22, agricultural input costs increased by around 20-30% with feed prices increasing by 40% and fertiliser by 150-250% (e.g. Defra, 2022). At the same time, in many regions the control of arable weeds is becoming increasingly challenging (e.g. Délye *et al.*, 2013). There is an urgent need to reduce the cost base of agricultural production and face the twin challenges of climate change and biodiversity loss. Agricultural systems that preserve or sequester carbon, fix atmospheric nitrogen, and support nature by providing habitats for pollinators and natural predators will help to meet these challenges (Poux and Aubert, 2018). The inclusion of multi-species (MS) leys in cropping systems can help address multiple agronomic and environmental challenges. MS leys are areas of temporary grassland that include several plant species, including grasses, herbs and legumes. There is a growing body of evidence that MS leys and the livestock they support can form an important part of sustainable agricultural systems. This paper discusses some of the issues and opportunities associated with the integration of MS leys into cropping systems through the findings and experiences gained from working with farmers in the UK.

Materials and methods

A series of surveys, field trials and demonstrations have been carried out since 2018 with farmers in the SUPER-*G* and EcoSward projects; the ADAS, AHDB (Agriculture and Horticulture Development Board) and Defra (Department for the Environment and Rural Affairs) 'Grass and Herbal Leys Network'; and a European Innovation Partnership (EIP) Operational Group. An online ley management survey was used to collect information on ley establishment, duration and management methods. Field investigations included replicated plot field experiments in northeast (NE) and southwest (SW) England using contrasting MS mixes; a two-year demonstration in Northumberland (England) using separate groups of ewes and lambs on separate grazing cells, each with a different MS mix to monitor the effect on livestock performance up to weaning; and a replicated livestock performance experiment comparing grass-clover (GC) and MS swards in Northern Ireland. In the latter experiment, each of two replicated blocks of GC and MS swards were sub-divided into 4 equal paddocks for grazing. A group of autumn born dairy-origin beef calves were turned out onto each block in spring 2020 and 2021, and were rotationally grazed around the 4 paddocks during the grazing season. Stocking rate was similar for both treatments. Across all the field trials, swards were established in autumn 2019 or spring 2020. The farmers provided sward management information, including manufactured-fertiliser application rates. Other measurements included pre-and post-grazing sward covers, dry matter (DM) yield, botanical assessments, herbage quality and daily liveweight gain (DLWG). Where relevant, conventional analysis of variance (ANOVA) was used to test for statistical differences between replicated treatments.

Results and discussion

In the online ley management survey, around 75% of farmers had leys as part of their rotation, either as part of a mixed farming system or for beef/lamb production, with the leys both cut for silage and grazed. About 80% of farmers included legumes/herb species in the ley seed mix (8% had pure grass swards), with leys typically in place for 3-5 years (73%). Just under half of farmers with leys used plough-based cultivation to establish arable crops after the ley, 28% used minimum tillage and 26% direct drill. More than 90% of farmers with leys said improvements in soil quality were an important consideration in their use of leys and 53% said the leys helped them control black-grass (*Alopecurus myosuroides*).

In the field trials, a variety of establishment methods were used including plough-based cultivation, reduced cultivation using tines and discs, and direct drilling. Where cultivation was used in combination with stale seed-bed techniques, there tended to be reduced weed burden in the newly established MS ley. Findings from the 2021 grazing season in Northern Ireland showed that the MS swards had a 7% higher DM yield than the ryegrass-clover control, while using 11% less nitrogen. Herbage mineral analysis also showed that MS leys had considerably higher mineral content (44% higher for boron, 11% higher for copper, 32% higher for calcium and 15% higher for phosphorus). On the field experiments in NE England, in 2022, when no manufactured N fertiliser was applied, dry matter yields on MS sward plots were ca. 40% higher than on the ryegrass-white clover control plots (n=3, $P<0.05$). However, on the organic site in SW England, where MS mixes were compared with the original grass-clover sward, there were no significant yield differences between treatments. On the grazing plots in Northumberland, there were indications that grass-herb mixes with Bird's foot trefoil (BFT – *Lotus corniculatus*) resulted in the best livestock performance (lamb DLWG) in 2020 and 2021. The farmer also observed a reduction in intestinal torsions (and mortality) in lambs where Sainfoin (*Onobrychis viciifolia*) or BFT were included in the mix. On the replicated livestock performance experiment in Northumberland, dairy-origin beef calves grazing MSS had a higher daily live weight gain during both 2020 and 2021 ($P<0.001$) than those grazing grass-clover (Figure 1).

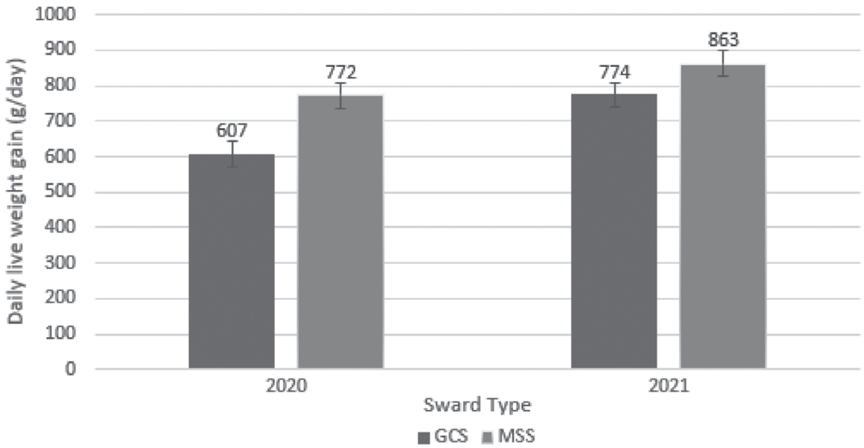

Figure 1. Live weight gain of dairy-origin calves grazing either grass-clover swards (GCS) or multi-species swards (MSS) during 2020 and 2021.

All the host farmers observed that MS leys tend to perform considerably better than grass-clover swards during drought periods. Other observations from the trials related to seed mix choice and grazing management. Different grass, herb and legume species are sensitive to different soil pH and drainage conditions. It can therefore take a few years to find the right mix for a particular farm. The trials also emphasised the importance of rotational grazing to maximise herb persistency in MS leys. Using higher entry and residual sward heights and increasing the grazing interval to at least 3-4 weeks improves herb persistency and reduces the animal's ability to selectively graze certain species (e.g. plantain). Furthermore, selecting a base mix such as ryegrass, white clover and plantain will provide a sward that performs well after 3-4 years when the presence of many herbs can start to decline.

Concluding remarks

Evidence from a survey and field trials in England and Northern Ireland has shown good potential for the use of MS leys within sustainable cropping systems. The number of MS sward establishment techniques is increasing and their optimal management (e.g. rotational grazing) and benefits are better understood. The major challenges include minimising the weed burden and maximising persistence of beneficial herbs beyond 3-4 years.

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LegacyNet: introducing an international multi-site experiment investigating potential benefits of increasing the species diversity of grassland leys within crop rotations

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Abstract

LegacyNet is a voluntary network of 32 international sites, established to investigate the yield benefits of multispecies grassland leys and their legacy effects on a follow-on crop. Relatively few experiments have investigated the impact of manipulating species diversity in grassland leys within crop rotations, and fewer still have accounted for variability across environments and soil types. A common experiment is being conducted at all 32 LegacyNet sites, with 52 grassland plots of systematically varied combinations of six forage species from three functional groups (two grasses, two legumes and two herbs) being sown at each site. The plots are measured and harvested for a period of at least 18 months. After this time, grassland plots are terminated, and a follow-on crop established on each plot (retaining the same plot structure). Measurements taken during the grassland and follow-on crop stages include dry matter yield, forage quality, botanical composition, and legacy effects. In this paper, we introduce the LegacyNet international experiment, its design, and overall aims and objectives.

Keywords: crop rotation, grasses, grassland leys, herbs, legumes, multi-site experiment

Introduction

Agricultural systems have been subjected to ongoing simplification, leading to the widespread use of monocropping (single crop being used continuously) and monocultures (single species) that are only high yielding with high inputs of inorganic nitrogen fertiliser, herbicides and pesticides. This lack of diversity lowers the resilience of the agri-ecosystems and reduces environmental quality and the supply of ecosystem services. For grassland leys, a previous pan-European multi-site experiment (the 'Agrodiversity' study across 31 international sites) showed that increases from one to four species increased forage yield and reduced weeds in grassland communities, compared to monocultures (Connolly *et al.,* 2018; Finn *et al.,* 2013). Through grass-legume mixture effects, four-species grassland mixtures greatly enhanced nitrogen use efficiency (Nyfeler *et al.,* 2009; Suter *et al.,* 2015). However, the benefits of diverse leys in crop rotations remain under-investigated. Legume-based mixtures have great potential to contribute legacy effects to subsequent follow-on crops within rotations; recent studies showed a significant legume-legacy induced increase in biomass yield of a follow-on model crop (Fox, 2020; Grange *et al.,* 2022). There is also evidence that including herb species (or forage forbs) in multi-species grasslands will provide additional benefits to those provided by legumes (Grange *et al.,* 2021; Jaramillo *et al.,* 2021).

LegacyNet, an international research network, tests whether this increase in diversity will also enhance the ley benefits in crop rotations, and whether these benefits are generally applicable across a gradient of soil and environmental conditions. The LegacyNet experiment is still ongoing at many sites; thus, in this paper, we introduce the experiment and discuss its research objectives and potential impact.

Methods

The LegacyNet experiment was established at 32 sites across 17 countries between 2018 and 2022 (LegacyNet 2022). The common experiment consists of two stages implemented over a three-year crop rotation, the grassland ley phase (~ two years) and the follow-on crop phase (one growing season). In the grassland stage, an innovative experimental design is implemented, consisting of forty systematically varying combinations of six forage species, including two species from each of the three functional groups: grasses, legumes, and herbs. The core design consists of 47 plots: 18 monoculture plots and 29 mixture plots consisting of either two, three, four, or six species. All 47 plots are managed at the same level of nitrogen (N) fertiliser (dictated by local practice). An additional five monoculture plots of one of the grasses are managed at a higher level of N (at least 100 kg N ha^{-1} higher). The total of 52 plots are arranged in a completely randomised design at each site.

In the grassland stage, plots are sown, fertiliser applied, and harvested by mowing for at least 18 months. At each harvest during this period, dry matter yield is recorded per plot along with a measurement of the plot's botanical composition and forage quality. After a minimum of 18 months, the grassland ley is terminated, but the 52-plot structure is retained, and a follow-on crop is established on each plot, with no or low nitrogen application. The follow-on crop varies across the sites, either a grass monoculture 'model' crop, wheat, barley or maize is used. Yield, nitrogen yield and other quality variables are recorded on each plot in this phase.

The data from all sites in the experiment will be collated and analysed using the Diversity-Interactions modelling approach (Kirwan *et al.* 2009), embedded in a multi-site framework. The main research aims of LegacyNet are to assess and quantify: (1) the role of up to six species across three functional groups of grasses legumes and herbs, in grassland mixtures on yield, weed invasion and forage quality, (2) the effect of varying the species diversity of a grassland ley on the transfer of legacy benefits to a follow-on crop, (3) the resource use efficiency of the full crop rotation under varying ley manipulations. A major strength of LegacyNet will be the ability to test the robustness of the effects across wide geographic and climatic gradients; this is an important benefit of multi-site experiments.

Results and discussion

A small number of the 32 LegacyNet sites have already completed the experiment, however, the experiment is still ongoing at most sites; all experiments will be completed by the end of 2024. It is anticipated that the results from LegacyNet will help identify optimal designs of grassland leys within crop rotations (e.g. what is the recommended proportion of each functional group) and how (or if) the optimal design varies with climatic and other local conditions, and the extent to which the optimal grassland ley designs are an improvement over conventional 'low-diversity high-input' grassland leys in crop rotations.

Conclusions

The LegacyNet experiment is expected to provide important contributions to knowledge on the effects of species diversity in grassland leys and subsequent effects on follow-on crops, across a climatic gradient. Through a common 32-site experiment implemented across 17 countries, the results are expected to be internationally relevant to farm management practices and to contribute to reducing the environmental impact of crop rotation systems.

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Breeding *Trifolium medium* L. for enhanced isoflavanoid content

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Abstract

Species of genus *Trifolium* produce secondary metabolites and other physiologically active compounds in different proportions. The highest content of active compounds was found in *Trifolium pratense* L. and *Trifolium medium* L. Isoflavones (biochanin A, biochanin B, daidzein, genistein) which were found in clovers, are already used as food supplements to alleviate the negative symptoms of menopause. Moreover, there are reports that seeds and young sprouts of red clover can be used to enrich the nutritional properties of baked foods and beverages. However, varieties of red clover have been bred for animal feed, and their biochemical composition has not been well studied. For instance, autochthonous red clovers are less suitable as fodder crops because of their low biomass yield, but they do accumulate a higher concentration of secondary metabolites than other varieties. Meanwhile, there are only a few varieties of zigzag clover because this species is not particularly suitable for animal feed. Nevertheless, zigzag clover tends to produce higher concentrations of isoflavones than red clover. In this study, wild population 2,148 of *T. medium* L. was found to produce 20.41 mg g^{-1} isoflavanoids, while *T. pratense* L. produced only 9.69 mg g^{-1}. Therefore, zigzag clover could be used for a new breeding direction.

Keywords: legumes breeding, functional food, crop wild relatives

Introduction

Red clover is one of the most popular fodder crops for animal feed. Meanwhile, only a few varieties of zigzag clover have been bred and officially registered worldwide. *Trifolium pratense* L. and *Trifolium medium* L. could be used for other purposes, as it was found that these species are a rich source of isoflavonoids, coumarin derivatives, cyanogenic glycosides, volatile oil, saponins, etc. (Butkutė *et al.*, 2014; Maciejewska-Turska and Zgórka, 2022). Epidemiological studies have shown that foods enriched in isoflavonoids have a positive effect on hormone functions related to cancer, vascular diseases, osteoporosis, menopausal symptoms, etc. (Maciejewska-Turska and Zgórka, 2022; Zgórka *et al.*, 2022). *T. pratense* and *T. medium* could be used to supplement foods with the bioactive compounds required in human diet. However, the nutritional, mineral, and phytochemical values depend on the vegetation period and structural parts of the plant within and among species (Butkutė *et al.*, 2018). The aim of this research is to determine the isofalanoids content in wild zigzag and red clovers. Furthermore, morphological and agrobiological features were analysed to identify the most promising genotypes for new varieties addressed for beekeeping or landscaping. This could be the basis for new breeding directions for *Trifolium* species.

Materials and methods

This research included *T. pratense* L. and *T. medium* L. species. Isoflavonoids (daidzein, formononetin and genistein) from different plant parts (leaves, flowers, stems) were analysed. The isoflavonoid content was determined via high-performance liquid chromatography. The Waters 2695 chromatographic system with UV/Vis detectors was used for phytochemical analysis via ESC method (Waters, Milford, USA). Daidzein and formononetin were determined at 301 nm, genistein at 258 nm wavelength. XTerra RP18 150×3.9 mm column with a sorbent particle size of 3.5 μm was used in this research (Waters, Milford, USA). The Waters Empower 2 chromatographic manager system was used for chromatogram management (Waters, Milford, USA).

Elution was performed using reversed-phase, high-performance liquid chromatography (RH-HPLC). A system of two eluent was used. Mobile phase A was a 0.1% TFA solution in water, and mobile phase B was a 0.1% TFA solution in acetonitrile. Purified deionized water and gradient purity acetonitrile were used for analysis.

The morphological and agrobiological traits of red clover and zigzag clover were assessed based on The International Union for the Protection of New Varieties of Plants recommendations.

Results and discussion

Based on research data, one red clover and two zigzag clover populations were distinguished by their high content of isoflavonoids. The highest content of isoflavanoids was identified in *T. medium* population 2148 (20.41 mg g^{-1}), which was twice as high as that in *T. pratense* population 2426 (9.69 mg g^{-1}) (Table 1). However, population 2148 produced a lower number of stems and flower heads per plant. Nevertheless, most isoflavones accumulate in leaves. Population 2148 was characterized by a 47.7 leaf ratio, which was slightly higher than that in the 'Ruža' and 'Melot' varieties but significantly higher than that in 'Vytis'. Despite a low seed set, population 2148 of *T. medium* is a promising gene donor for breeding a new variety directed to a high content of isoflavones.

The concentrations of isoflavones were different in *Trifolium* species and structural parts of their plants. The concentration of genistein was higher than that of daidzein or formononetin in zigzag clover (Figure 1). *T. medium* plants accumulated genistein mostly in their leaves, and the highest content was found in population 2148 (6.75 mg g^{-1}). Meanwhile, *T. pratense* population 2426 had a low genistein content in all structural parts and did not exceed 1.0 mg g^{-1} (0.61 mg g^{-1}) in the whole plant. Red clover accumulated formononetin (5.65 mg g^{-1}) the most among the analysed isoflavones. Its concentration was slightly higher in zigzag clover populations 2148 (6.96 mg g^{-1}) and 41 (5.75 mg g^{-1}). Finally, zigzag clover tended to accumulate lower concentrations of daidzein than genistein or formononetin.

Different results from ours were obtained by Maciejewska-Turska and Zgórka (2022). Formononetin and its glucosides were identified as major isoflavones, while daidzein and genistein were present in lower concentrations. However, the explanation for this phenomenon could be various biotic and abiotic factors that may have contributed to characteristic changes in polyphenol metabolism (Zgórka *et al.*, 2022). Moreover, isoflavone content is strongly correlated with plant-growth stages (Akitha Devi *et al.*, 2018).

Table 1. Population richness by isoflavonoids and the most important quantitative and qualitative traits.

Population/cultivar	Overwintering, %	Number of stems, no.	Flower heads per plant, no.	Leaves ratio, %	Seed set, %	Isoflavonoids content, mg g^{-1}
T. medium						
41	90.0	36 ± 6.7^{a}	67 ± 7.17^{a}	42.8	41.8	17.68
2148	75.0	29 ± 4.0^{ab}	42 ± 6.19^{b}	47.7	52.4	20.41
RŮŽA	85.0	21 ± 4.8^{b}	51 ± 5.59^{b}	45.3	84.2	-
T. pratense						
2426	91.2	38 ± 5.6^{b}	130 ± 16.99^{a}	44.0	64.2	9.69
VYTIS	83.3	54 ± 9.1^{a}	72 ± 15.18^{b}	27.3	70.2	9.85

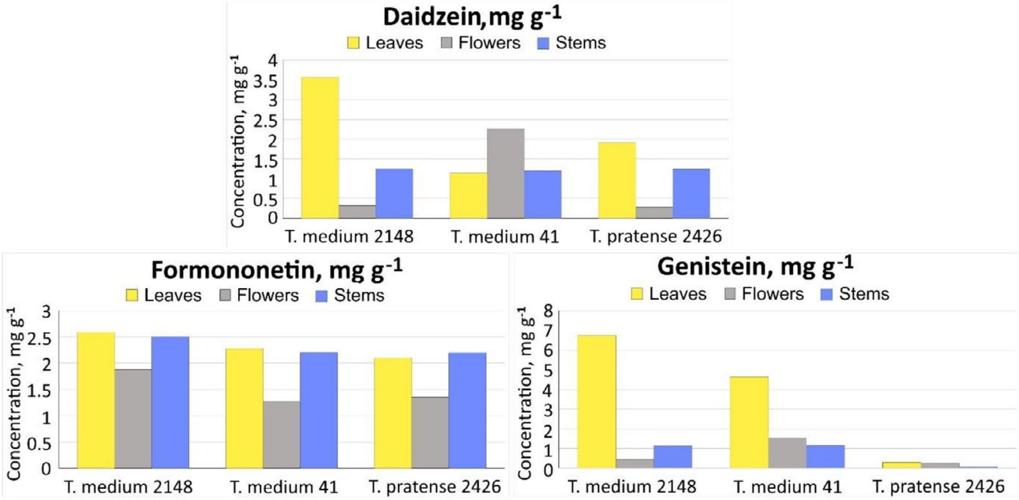

Figure 1. Concentration of isoflavones in different parts of *Trifolium pratense* and *Trifolium medium* plants.

Considering our research data, gene donor 2148 of *T. medium* was used for further breeding. Thus, selection line 260 was created by mass crossbreeding and individual selection methods. Recently, breeding line 260 has been referred for distinctiveness, uniformity, and stability (DUS) testing in the Czech Republic.

Conclusions

Research has revealed that *T. medium* populations have accumulated twice as high concentrations of isoflavonoids as *T. pratense* populations. Moreover, it was found that the highest concentrations of isoflavones (daidzein, genistein, and formononetin) accumulated in leaves. Finally, population 2148 was not inferior to the varieties by isoflavone content and morphological features. As a result, breeding line 260 was bred by using the 2148 population as a gene donor.

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Which grass species are the most resilient under stress conditions in Baltic areas?

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Abstract

Periods of prolonged heat and drought are becoming more frequent even in areas where these were not pronounced before, including the Nordic-Baltic region. It is increasingly relevant to identify species and genotypes that would be sufficiently resilient under different stress conditions. It is important to select species that can withstand prolonged summer drought and at the same time be resistant to long-term humidity, followed by black frost in winter. In field trials in Latvia, various grass species and genotypes were evaluated in periods of several years and growing cycles. The dry matter yield, winter-hardiness, drought resistance (DR) and persistence were evaluated. Persistence and productivity were strongly affected by the grass: (1) species; (2) genotype; (3) year of use. In the conditions of increased drought, the year of use was particularly important, and swards of 1st and 2nd year of use showed a much better regrowth ability. Critically low DMY were found in the extremely dry vegetation period of 2018, critically low DMY were found especially for swards of the 3rd year of use, and among species it ranged from an average of 1.26 Mg ha^{-1} for *Lolium perenne* to 4.9 Mg ha^{-1} for *Dactylis glomerata*. Based on the results, the grass species ranked in the following descending drought-resistance sequence: *Festuca arundinacea* > *Dactylis glomerata* > *Festuca pratensis* > *Phleum pratense* > *Festulolium* > *Lolium perenne*.

Keywords: grasses, dry matter yield, persistence, regrowth intensity, drought resistance

Introduction

Climate change is expected to lead to a higher probability of drought periods in summer, more rainfall in winter and a generally prolonged vegetation period in North-West Europe (Fuhrer, 2007). This is also relevant in the Nordic-Baltic region, where earlier resumption of vegetation in the spring, warmer and longer autumn periods and increasingly unstable winter conditions with sharp temperature fluctuations, prolonged rains followed by periods of black frost have been observed. Increased drought and heat periods as well as long-lasting rains causing flooded areas reduce yield and persistence of perennial grasses, influence species abundance and distribution (Tilmann and Haddi, 1992). Species drought resistance and ability to withstand periods of low water availability varies widely. It is necessary to look for species and genotypes that are resilient, and able to tolerate both drought and flooding.

Materials and methods

Field trials were conducted at the Research Institute of Agronomy of Latvia University of Life Sciences and Technologies in Skriveri (56°37 N, 25°07 E) in the sod-podzolic sandy loam soil (Eutric Retisol – WRB 2015) with pH $_{KCl}$ 5.8-6.4 and medium plant available phosphorus and potassium content. Data were collected for 1st, 2nd and 3rd year of sward use on dry matter yield (DMY), regrowth intensity, drought resistance (DR) and winter hardiness (WH) for the following grass species: *Lolium perenne* L. (*Lp*), *Festulolium* (*FL*), *Phleum pratense* L. (*Php*), *Festuca pratensis* Hudds (*Fp*), *Festuca arundinacea* Schreb. (*Fa*), *Dactylis glomerata* L. (*Dg*). Trials were established in 2017, 2016 and 2015, respectively. DR was evaluated by combining a set of parameters: green leaf colour intensity, regrowth intensity after mowing and sward height. Score of each parameter was carried out independently in a 9-point scale: (1) – the lowest expression of the characteristic; (9) – the highest. The vegetation period of 2017 was very wet, June to October precipitation exceeded the long-term average by 10-30% on average, but in

September it rained almost twice as much (139 mm). In contrast, 2018 was the driest in the history of weather observations in Latvia. Annual precipitation was 30% below average, but in the months of active vegetation it was 2-3 times less than the long-term average. The effect of drought was reinforced by the heat, from April to September, air temperatures was on average 2-4 °C higher than the long-term average. Experimental data were statistically analysed using an analysis of variance (ANOVA). The tests of the statistically significant differences (LSD $_{0.05}$) and Fisher criterion (F-test) were used.

Results and discussion

Atypically long periods of drought during the growing seasons, including dry summer of 2018 with 2-3 times less rainfall than the long-term average, made it possible to assess the persistence and drought resistance (DR) of different grass species and genotypes (Table 1).

As criteria for the assessment of drought resistance (DR), the rating of regrowth intensity and DMY proportion obtained from aftermath (2^{nd} and next cuts, if applicable) in two drastically different years – wet 2017 and dry 2018 – were evaluated. In conditions of increased drought and heat during summer 2018, many genotypes of *FL* and *Lp* after the 1^{st} mowing showed almost no signs of regrowth, while *Fa* and *Dg* produced considerable aftermath yield. Next to the species, the genotype was of great importance.

Winter conditions in 2017/2018 were satisfactory, however the WH of *Lp* and *FL* in the 3^{rd} year of use was relatively weak; on average it was evaluated with 2.4 and 3.2 points, respectively, while in the 2^{nd} year of use WH was 7.3-7.4, on average. Usually, in the 1^{st} year of use, there is no problem with wintering of mentioned species. Later, in the 2^{nd} and subsequent years, WH for *Lp* and *FL* could be strongly influenced by weather conditions and the age of the sward (Berzins *et al.*, 2018). In unfavourable climatic conditions the noticeable thinning of *Lp* swards begins already in the 2^{nd} year of use, but mostly it happens in the 3^{rd} year of use. In favourable conditions, a significant thinning of the sward can happen even later (Bothe *et al.*, 2018). Winter hardiness of *Fp* in the 3^{rd} year of use was much better (6 points, on average) than that of *Lp* and *FL*. Mostly WH is strongly influenced by the genotype and growing conditions (Berzins *et al.*, 2019; Brink *et al.*, 2010).

The smallest yield drop under increased drought and heat conditions of 2018 was for *Fa* and *Dg*: amount of DMY in the 3^{rd} year of use compared to the previous year was around 60% for *Fa* and *Dg*; 50% for *Fp*; 20% for *Lp* and *FL*. A much smaller yield reduction in the same conditions was observed for younger swards: in the 2^{nd} year of use DMY for *Lp* anf *FL* were 54% and 56%, respectively. The overall analysis of the obtained data showed that the grass species ranked in the following descending drought-resistance sequence: *Festuca arundinacea* > *Dactylis glomerata* > *Festuca pratensis* > *Phleum pratense* > *Festulolium* > *Lolium perenne*.

Conclusions

Increased drought stress negatively affected the regrowth intensity and productivity of all grass species; however, *Festuca arundinacea* and *Dactylis glomerata* proved more drought-resistant. Based on field trials grasses could be ranked in terms of drought resistance in descending order: *Festuca arundinacea* > *Dactylis glomerata* > *Festuca pratense* > *Phleum pratensis* > *Festulolium* > *Lolium perenne*. For all species, age of the sward had a decisive role in the intensity of regrowth and formation of dry matter yield in conditions of increased drought, and the more resilient and productive were swards of the 1^{st} and 2^{nd} year of use.

Table 1. Evaluation of different grass species: winter hardiness (WH), drought resistance (DR), dry matter yield (DMY) and distribution of the total DMY (proportion of aftermath from total DMY) depending of year of use.

Species and sowing year	Parameter	Total DMY, Mg ha^{-1}		The proportion of aftermath yield, %		WH (1-9)	DR (1-9)
		2017	2018	2017	2018		
Festulolium 2015 (6)[1]	average	6.83	1.38	43.9	-	3.2	4.4
	max/min[2]	7.5/5.9	1.7/0.7	58.5/37.5	-	4.5/1.0	7.0/2.3
	$LSD_{0.05}$	0.52	0.23	26.92	-	0.8	1.35
Festulolium 2016 (17)	average	8.79	4.63	63.22	18.07	7.3	5.80
	max/min	11.1/7.2	5.4/4.0	68.6/58.8	24.4/13.1	8.5/6.0	7.5/3.0
	$LSD_{0.05}$	0.54	0.36	40.7	5.5	0.62	1.4
Festulolium 2017 (24)	average	-	7.61	-	21.81	-	5.72
	max/min	-	11.9/5.1	-	28.4/14.2	-	8.0/3.0
	$LSD_{0.05}$	-	0.36	-	-	-	2.27
L. perenne 2015 (10)	average	6.21	1.26	52.52	-	2.4	3.38
	max/min	7.5/4.9	1.7/1.0	77.6/45.2	-	3.0/1.5	5.0/2.0
	$LSD_{0.05}$	0.58	0.18	41.4	-	0.83	1.35
L. perenne 2016 (11)	average	8.74	4.41	61.33	18.05	7.4	7.01
	max/min	10.3/6.2	5.4/2.6	66.8/52.0	22.1/15.4	9.0/6.0	8.3/3.0
	$LSD_{0.05}$	0.60	0.58	18.33	1.72	1.16	1.01
L. perenne 2017 (12)	average	-	5.63	-	12.62	-	6.60
	max/min	-	7.6/3.4	-	23.2/6.1	-	7.5/4.7
	$LSD_{0.05}$	-	0.76	-	-	-	1.75
F. pratensis 2015 (8)	average	7.51	3.78	43.46	9.83	6.0	3.99
	max/min	8.1/6.9	4.2/3.5	49.9/34.0	13.6/5.9	7.0/2.0	7.0/2.3
	$LSD_{0.05}$	0.63	0.67	9.5	-	0.46	1.25
F. pratensis 2017 (9)	average	-	6.44	-	35.58	9.0	6.83
	max/min	-	7.7/5.7	-	38.8/30.9	9.0/9.0	7.3/6.3
	$LSD_{0.05}$	-	0.5	-	-	0	0.6
F. arundinacea 2015 (6)	average	7.53	4.42	57.63	33.10	8.0	4.38
	max/min	8.4/6.2	5.5/3.6	66.0/52.0	43.1/22.7	8.0/8.0	6/1.35
	$LSD_{0.05}$	0.57	0.98	29.8	8.16	0.00	1.35
Dactylis glomerata 2015 (19)	average	7.95	4.90	57.78	34.14	6.2	6.54
	max/min	9.4/6.7	6.0/3.8	69.4/48.1	42.5/27.0	7.0/4.5	9.0/5.0
	$LSD_{0.05}$	0.60	0.58	28.3	8.62	0.53	3.7
Ph. pratense 2017 (10)	average	-	4.45	-	17.28	9.0	7.03
	max/min	-	4.9/3.8	-	22.0/13.4	9.0/9.0	8.0/5.7
	$LSD_{0.05}$	-	0.68	-	53.2	0	2.28

[1] Number of tested cultivars or candivars.

[2] Maximum and minimum value that varied among different genotypes within a species.

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The effects of legume-rich mixtures on the soil organic carbon after three years of sward use

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Abstract

Decreasing soil fertility and declining soil health are among the main challenges for ensuring food production. Perennial plants can improve water and nutrient use efficiency, thereby increasing soil C storage and reducing the rate of organic matter loss. The aim of this study was to determine, in a field experiment, the effect of sward fertilization and different species composition of grasses on soil organic carbon content. The experiment was carried out with single-species and multi-species swards with three, four, six, and eight plant species in the mixtures, including four grasses and four legumes, with annual fertilization rates of N_{150} and N_0 kg ha^{-1}. At the soil depth of 0-20 cm, no significant differences were found in soil organic carbon between the use of swards of different species composition and mineral nitrogen fertilizers, after three years of sward use. The soil organic carbon amount was lower in the 10-20 cm depth than in the top layer, but not significantly, on average by 4.1 and 4.3% for N_0 and N_{150} sward fertilization rates, respectively.

Keywords: diversity, food production, legumes, organic carbon, soil health

Introduction

Perennial grasses are considered to have an abundant and deep root system and therefore high potential to sequester carbon (C) into the soil. In addition, grassland forms a permanent soil cover that helps reduce soil erosion and the leaching of nutrients (Marshall *et al.*, 2016). Many of the environmental and ecological benefits provided by grasslands arise from the interactions between the roots of plant species and the soil in which they grow (Lüscher *et al.*, 2022). Soil carbon sequestration by plant roots is an important means for net removal of CO_2 from the atmosphere (Panchal *et al.*, 2022) and to the accumulation of organic matter in the soil. Increasing the content of organic matter in the soil maintains the production of aboveground and underground phytomass (Voltr *et al.*, 2021) and enhances the stability of soil fertility (Gautam *et al.*, 2022) and also improves ecosystem resilience to extreme climatic conditions.

Materials and methods

The field experiment was established in 2018 at the Lithuanian Research Centre for Agriculture and Forestry. The experiment was sown on an agricultural field with loamy Endocalcaric Epigleyic Cambisol. The experiment had a randomized complete block design with four replicates; each plot size was 15 m^2. Soil chemical analyses were carried out twice – before the experiment was set up and after four years of sward growth from each plot. Before the experiment commenced, agrochemical characteristics in the 0-20 cm soil layer were determined: soil organic matter (SOM) was 3.06%, mobile phosphorus was 220 mg kg^{-1} and potassium was 173 mg kg^{-1}, average soil pH was 6.9 (neutral soil acidity). Soil organic carbon content (SOC) was calculated using the formula of Sanderman *et al.* (2011). The treatments comprised different combinations of plant species, with two sown monoculture swards and swards with 40% of legumes and 60% of grasses in the mixtures. The seed rate was 18 kg ha^{-1} of perennial ryegrass cv. Elena DS and × *Festulolium* cv. Vėtra, 20 kg ha^{-1} of meadow fescue cv. Raskila, 12 kg ha^{-1} of timothy cv. Dubingiai, 10 kg ha^{-1}of white clover cv. Dotnuviai, 15 kg ha^{-1} of red clover cv. Sadūnai, 15 kg ha^{-1} of lucerne cv. Malvina and 80 kg ha^{-1} of sainfoin cv. Meduviai. Swards were grown without mineral N fertilizers and also with 150 kg N ha^{-1} yr^{-1} except in the sowing year (fertilized 60 kg N ha^{-1} in the start of vegetation, each 45 kg N ha^{-1} after first and second cut). To analyse the effects of the treatment, an analysis of variance two-way factorial ANOVA was conducted, probability level (P<0.05).

Results and discussion

A two-factor analysis of variance (ANOVA) showed that neither the different sward compositions (grass-legume mixtures), fertilization, or their interaction had significant effects on soil organic carbon at the end of the experiment (Table 1).

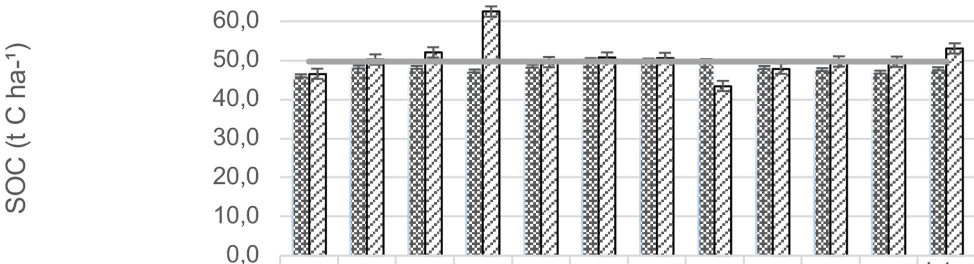
Fertilization did not have significant effect for SOC content in swards of different species composition, but SOC content varied from 43.4 to 62.5 t C ha^{-1} in fertilized swards (Figure 1). As observed by Poeplau *et al.* (2018) fertilization with nitrogen (N), phosphorus (P) and potassium (K) or only (P) and (K) increased grassland soil carbon stocks. In the unfertilized swards of our experiment the SOC content differences between treatments were lower than in the fertilized swards; SOC content was not significantly different between treatments but variation was from 46.0 to 50.2 t C ha^{-1}. The experiments of Poeplau *et al.* (2018) also showed that root carbon stocks tended to be higher in unfertilized treatments in comparison with fertilized treatments. Rates of soil organic carbon accumulation are quite variable, and therefore rates of carbon sequestration are also highly uncertain, especially for deeper soil carbon stocks and in the early years after sward establishment. On average, 48.3 t C ha^{-1} is accumulated in swards of different species composition at a soil depth of 0-20 cm without N fertilizers, and 50.5 t C ha^{-1} when using mineral nitrogen fertilizers. No significant differences of SOC in 0-20 cm soil depth were found in the assessment of monoculture swards and swards with different species composition; however, an increase in soil organic carbon using legumes by 0.6 t (N_0) and 1.3 t C ha^{-1} (N_{150}) is possible. Values of soil organic carbon content appeared lower, but not significantly, in the 10-20 cm soil depth compared with the topsoil layer; on average SOC content was lower by 4.1% without mineral nitrogen fertilizers in the swards and by 4.3% when using mineral N fertilizers. An increase in the concentration of organic carbon in the soil in cultivated fields is observed only after 7-10 years (Laganière *et al.*, 2010). It was reported (Ghimire *et al.* 2019) that grassland systems sequester more SOC than other agricultural soils, but as the SOC is sensitive to disturbance the accrued SOC is easily lost when the grasslands are cultivated, and therefore short-term studies such as these do not necessarily show subtle changes over time.

Therefore, the main advantage of using legumes is to ensure that the sward benefits from biological nitrogen fixation, as this in turn allows reduction of the amount of nitrogen (N) fertilizer required to support productivity and stability.

Table 1. Results of two-factor analysis of variance of the soil organic carbon after three years of sward use in 0-20 cm soil depth.[1]

Year	**2021**		
Source of variation	**df**	**F**	***P*-value**
Factor A (grass-legume mixtures)	11	2.00	0.692
Factor B (fertilization)	1	4.05	0.142
Interaction A × B	11	2.00	0.518

[1] df = degrees of freedom, F = the test statistic from the F-test, *P*-value = the critical value of the Fisher test at a significance level 0.05, effect is significant under *P*>95%.



	G1	G2	L1+ L2/ G1	L1+ L2/ G2	L1+ L2/ G1+ G2	L1+ L2/ G1+ G2+ G3+ G4	L3+ L1/ G1+ G2	L3+ L1/ G1+ G2+ G3+ G4	L4+ L1/ G1+ G2	L4+ L1/ G1+ G2+ G3+ G4	L1+ L2+ L3+ L4/ G1+ G2	L1+ L2+ L3+ L4/ G1+ G2+ G3+ G4
0-20 cm (N0)	46,0	48,3	48,2	47,3	48,5	50,2	50,1	50,0	48,2	47,6	47,0	47,9
0-20 cm (N150)	46,6	50,2	52,0	62,5	49,6	50,7	50,6	43,4	47,9	49,7	49,6	53,1
Before experiment	49,7	49,7	49,7	49,7	49,7	49,7	49,7	49,7	49,7	49,7	49,7	49,7

Figure 1. Soil organic carbon content in 2018 and after three years of swards use in 2021. Grasses: G1 = perennial ryegrass, G2 = × *Festulolium*, G3 = meadow fescue, G4 = timothy; Legumes: L1 = white clover, L2 = red clover, L3 = lucerne, L4 = sainfoin.

Conclusions

This study showed that after three years of using perennial grasses in the swards, the content of organic carbon in the soil did not decrease significantly compared to the beginning of the experiment. There is a need for further investigation of SOC in different soil management systems with different plant species, especially perennials, that could lead to increasing nutrient supply.

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Deficiencies of selected micronutrients as factors of red clover and lucerne decline

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Abstract

The functions of red clover (*Trifolium pratense* L.) and lucerne (*Medicago sativa* L.) as improvers of soil fertility and as protein source for livestock can be severely impaired by the phenomenon of legume decline, in which legumes show reduced vitality after repeated cultivation. The project TriSick investigates various abiotic and biotic factors that possibly influence the occurrence of decline in stands of red clover and lucerne. After comprehensive nutrient analysis in soil and biomass of 24 German locations, various micronutrients were suspected to be deficient at several locations. Those micronutrients (B, Cu, Fe, Mn and Zn) were investigated further, aiming for the detection of content differences in soil and biomass between areas with and without symptoms of decline. Significant differences were found only for B and Cu in relation to soil contents. These contents were lower in decline areas, implying a possible influence of B and Cu deficiency on the occurrence of legume decline at the corresponding locations. Potential interactions between different nutrients, as well as with other influencing factors, will be subject of further analyses.

Keywords: legume decline, red clover, lucerne, micronutrients

Introduction

Legume decline or clover sickness is the phenomenon of reduced growth and vitality of legumes after repeated cultivation, known and studied at least since the nineteenth century (Haywood, 1842). While interest in this topic declined with reduced red clover and lucerne cultivation in the second half of the twentieth century, the current advance of organic farming restores its relevance. Legume decline is mostly associated with legume-specific diseases and pests but additional factors like nutrient deficiencies may play an important role in the occurrence of decline (Pommer, 2004). In the project TriSick, a multitude of German locations with red clover or lucerne cultivation were examined for various potential biotic and abiotic factors (diseases, pests, soil nutrients, soil compaction, nodulation and mycorrhizal colonisation), comparing areas with and without symptoms of legume decline. Several micronutrients (B, Cu, Fe, Mn and Zn) proved to be deficient at multiple locations, leading to the hypothesis that said deficiencies are a cause of the decline symptoms.

Materials and methods

In autumn of 2021, and in spring to early summer of 2022, a total of twenty-four locations throughout Germany (reaching from Lake Constance to the Baltic Sea) were examined. At each location, an area with symptoms of decline, as well as a nearby area (the same field or a nearby field of the same farm, max. distance: 2.5 km) with normal growth were sampled. The predominant criterion for assignment was biomass production. Sampled areas were usually part of diversified crop rotations. Nutrient analyses were carried out on air-dried soil samples (0-10 cm depth) and oven-dried (55 °C) biomass samples (in each case bulk samples from throughout the area). Soil samples were tested by certified laboratories for P (Double-lactate (DL)), K (DL), Mg (DL), B, Cu, Fe, Mn, Zn, soil type and pH, biomass samples for N, P, K, S, Mg, Ca, B, Cu, Fe, Mn, Mo and Zn. For the micronutrients B, Cu, Fe, Mn and Zn, several locations showed signs of deficiency, calling for further investigation. For each of these nutrients, locations with signs of deficiency were considered, independent of their occurrence on areas with or without decline. For a location to be considered, at least one of the areas either had to show soil contents classified as deficient

(Kape *et al.,* 2019) or substandard biomass contents (Kape *et al.,* 2019; LfL, 2021) combined with soil contents classified as below excessive (classes deficient or sufficient; Kape *et al.*, 2019), to make sure that low biomass contents are connected to availability in soil. Both areas had to show the same soil type. This resulted in fifteen locations with possible B deficiency, ten for Cu, five for Fe and three for Mn and Zn. Soil and biomass contents of areas with and without decline were compared using the paired samples Wilcoxon test. Analyses and figure creation were conducted in R (Version 4.1.1; R Core Team, 2021).

Results and discussion

Average contents of B, Cu and Zn in soil and biomass of from the considered locations were lower in decline areas than in areas with normal growth; however, the opposite was the case for Fe and Mn (Table 1). These differences were significant for B and Cu in soil (Figure 1), implying deficiencies in considered decline areas to be a possible cause of reduced growth. Deficiencies in both B and Cu have previously been shown to reduce biomass production in red clover and/or lucerne (Jarvis and Robson, 1982; Sherrell, 1983). However, additional influencing factors correlating with B and Cu contents may contribute to the observed effects and therefore prohibit proof of a cause and effect relationship. While at the considered locations differences were not significant for Zn, Fe and Mn, previous studies have implied there are

Table 1. Mean values of soil and biomass contents of B, Cu, Fe, Mn and Zn of normal growth and decline areas of considered locations alongside *P*-values of the paired samples Wilcoxon test.[1]

	Soil				**Biomass**			
	n	**Mean value of normal areas (mg kg^{-1})**	**Mean value of decline areas (mg kg^{-1})**	***P*-value (paired Wilcoxon)**	**n**	**Mean value of normal areas (mg kg^{-1})**	**Mean value of decline areas (mg kg^{-1})**	***P*-value (paired Wilcoxon)**
B	15	0.41	0.34	0.026*	16	22.35	20.47	0.478
Cu	10	2.39	1.41	0.014*	10	7.04	6.75	0.610
Fe	5	98.20	107.00	0.813	6	48.88	53.08	0.281
Mn	3	29.67	56.00	1.000	3	23.63	34.26	0.500
Zn	3	2.67	1.17	0.586	3	25.33	21.33	0.250

[1] Significance level * $P<0.05$.

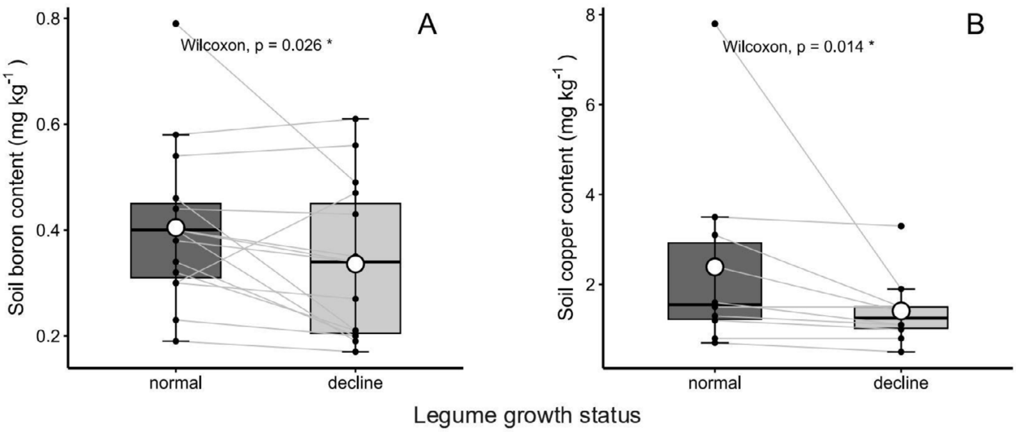

Figure 1. Soil boron (A) and copper (B) contents of normal growth and decline areas. Samples of the same location are connected by a grey line, the mean (see also Table 1) is indicated by a white circle. Significance level of differences between normal growth and decline area * $P<0.05$.

effects of deficiencies, or influence, on the occurrence of diseases for all of the considered micronutrients (e.g. Jin *et al.,* 2007; Stoltz and Wallenheimer, 2012). Nonetheless, they are rather rare, the focus instead often lying on toxicity or stress induced by oversupply (e.g. Chen *et al.* 2022). Therefore, further research is necessary to fully clarify their potential influence on the occurrence of legume decline, especially in combination with other biotic or abiotic factors.

Conclusions

At the considered locations, contents of B and Cu were significantly lower in the soil of decline areas compared to areas with normal growth, implying deficiencies of these micronutrients to be a probable reason for the decline. Further research is needed to fully understand micronutrients as factors for the occurrence of legume decline and to proof a cause and effect relationship.

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Value of grasses and legumes as forecrops for winter wheat cultivation

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Abstract

We present the results of a study on the impact of different forecrops on productivity of winter wheat (*Triticum aestivum*). Winter wheat was cultivated in monoculture and undersown with the fodder plants, which became a forecrop for wheat. They were: red clover (*Trifolium pratense*), Italian ryegrass (*Lolium multiflorum*) and a mixture of these two plant species. Winter wheat was cultivated in crop rotation after the following crops: red clover, Italian ryegrass and mixtures of these two species, combined with the nitrogen at 60 kg N kg^{-1}, provided similar wheat grain yields as nitrogen at the rate of 90 kg N kg^{-1} applied to other types of forecrop. It was found that companion crops and their subsequent forecrops for wheat compared to cultivated in monoculture slightly increase protein crude protein content. Winter wheat cultivated as monoculture influenced the soil chemical properties. This is evident in the tendency to reduce soil pH and humus content.

Keywords: arable leys, wheat, clover, grass, yield

Introduction

Winter wheat (*Triticum aestivum* L., syn. *Triticum vulgare* Vill.) is the most important cereal cultivated in Poland. Wheat grain is the most valuable bread and fodder raw material. However, this plant requires quite fertile soils and good forecrops in a rotation, but it is often grown after other cereals and in monoculture. Abandonment of the classical principles of crop rotation leads to so-called 'soil fatigue', i.e. destruction of biological and chemical balance. The result is changes in wheat yield components and a decrease in grain yield of up to 20-30%. We investigate how to reduce the negative effects resulting from simplifications in cereal cultivation by using the intercrops in the form of relay intercrop or catch crops. Opinions on the usefulness of legumes and grasses as a forecrop of cereals, including winter wheat, are divided. Some (Woźniak, 2006) confirm their beneficial effect on the yield and quality of wheat grain. Thomsen *et al.* (2010) found that use of a ryegrass catch crop with clover increased winter wheat yield. According to Wanic *et al.* (2013), the use of red clover and Italian ryegrass as a forecrop in spring barley negatively affects the development and yield of barley. The main objective of the study was to evaluate Italian ryegrass, red clover and their mixture as a forecrop on the productivity of winter wheat and the chemical properties of the soil in order to confirm or deny their usefulness in this way.

Materials and methods

The experiment was conducted at the experimental station in Prusy, Krakow, Poland (47°24′ N, 7°19′ E; 300 m a.s.l.). The soil was characterized by a slightly acidic reaction. Its pH in water was on average 6.53, and 5.60 in KCl units. Although it is classified as a degraded chernozem, it was characterized by a relatively low content of soil humus (2.07%) and total nitrogen (1.323%). It was also poor in absorbable forms of phosphorus and potassium, and relatively rich in magnesium. The content of these components on average was: 21.3 mg, 74.0 mg, and 63.1 mg kg^{-1} soil, respectively.

The experiment was set up in a randomized block design in triplicate. Each plot had an area of 10 m^2. Winter wheat (cultivar Ludwig) was cultivated in monoculture and as a nurse crop. After harvesting of wheat, fodder plants were grown as intercropping relay – undersown in the spring – which became a forecrop for wheat. Thus, the following experimental objects were obtained:

1. Winter wheat monoculture (WW);
2. Red clover (RC);
3. Italian ryegrass (IR);
4. Red clover and Italian ryegrass mixture 50:50 (M).

Mineral fertilizers were applied before sowing in the amount of 35 kg ha^{-1} of P, and 50 kg ha^{-1} of K. Basic nitrogen fertilization (90 kg ha^{-1} of N) was reduced to 60 kg at red clover component and applied in two equal amounts before sowing and in spring.

The yield of the winter wheat grain and the weight of a thousand seeds were measured. In the grain of wheat the crude protein content (Kjeldahl method) and macronutrient content (by Perkin-Elmer Model Optima 7300 DV Inductively Excited Plasma Atomic Emission Spectrophotometer (ICP-AES)) were measured. Soil pH was determined potentiometrically using a glass and calomel electrode, the humus content was determined by the Tiurin method, the absorbable forms of phosphorus and potassium by the Egner-Riehm method and the magnesium content by the Schachtschabel method. The results of winter wheat productivity were developed using a one-factor analysis of variance. To determine the significance of differences in yield, Student's t-test was used at α=0.05.

Results and discussion

The yield of winter wheat grown in monoculture and after a forecrop with Italian ryegrass was statistically similar (Table 1). On the other hand, in the treatment with red clover as a forecrop, the harvested grain yield was significantly higher. Grain of wheat grown in monoculture was less abundant (filled). The differences in the mass of 1000 seeds between the other treatments were non-significant. The lowest content of total protein was characterized by the wheat grain from the treatment where Italian ryegrass was a forecrop. The grain yield harvested after the red clover crop was almost 10% higher than the yield of fertilized wheat monoculture. A similar result was also obtained by Soon and Clayton (2002). Grain yield was 20% higher when red clover was the forecrop compared to monoculture cultivation. However, it should be emphasized that the increased yield was obtained with a reduced application of nitrogen. This represents a measurable benefit for the farmer. In these studies, a slight variation in the total protein content was obtained. Thomsen *et al.* (2010) suggest that the potential for improving grain quality by adjusting the cropping system is rather limited when compared with differences between varieties.

The lowest $pH_{(KCl)}$ value and the lowest humus content were characterized by the soil of treatment WW (Table 2). With regard to the initial state, the values of these parameters were slightly lower. In the soil of the other treatments, the values of the tested parameters were similar to the beginning of the study. The content of other macronutrients was at a similar level and, regardless of the forecrop used, was slightly

Table 1. Winter wheat yield, seeds filling and crude protein content.[1]

Forecrop[2]	Yield (Mg ha^{-1})	Weight of 1000 seeds (g)	Crude protein (g kg^{-1})
WW	9.00 ^{a}	45.0 ^{a}	132 ^{b}
RC	9.78 ^{b}	48.1 ^{b}	128 ^{b}
IR	8.81 ^{a}	49.3 ^{b}	116 ^{a}
M	9.11 ^{a}	47.1 ^{b}	128 ^{b}

[1] The same letter indicates no significant differences.

[2] WW = winter wheat; RC = red clover; IR = Italian ryegrass; M = 50:50 mixture RC and IR.

Table 2. Properties of soil after harvesting of winter wheat.

Forecrop[1]	pH_{water}	pH_{KCl}	C-total (%)	N-total (%)	Available (mg kg^{-1})			
					P	K	Mg	Na
WW	6.65	5.48	1.96	0.14	22.9	72.4	61.9	33.0
RC	6.72	5.58	2.01	0.15	19.9	69.2	61.7	36.7
IR	6.72	5.55	2.01	0.14	18.7	66.4	63.0	36.6
M	6.69	5.58	2.00	0.14	23.7	71.5	61.7	35.7

[1] WW = winter wheat; RC = red clover; IR = Italian ryegrass; M = 50:50 mixture RC and IR.

higher in relation to the initial level. The chemical properties of the soil can be varied not only according to the cultivated plants and fertilization, but also to the duration of the experiment. In the research of Bałuch and Benedycki (2004), the increase in the content of phosphorus and potassium in the soil occurred only after 5 years of cultivation of grass-legume mixtures.

Conclusions

Wheat grown in monoculture provided a similar amount and quality of grain as that grown in crop rotation after fodder crops. This indicates that on relatively fertile soils, short-term cultivation of winter wheat in monoculture is possible and ensures high yield. The use of red clover as a component of the catch crop can compensate for reduced nitrogen use in winter wheat cultivation without a significant decrease in the quantity and quality of the crop.

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Effect of different condensed and hydrolysable tannin-rich extracts on methane production *in vitro*

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Abstract

Tannins have been found to reduce methane (CH_4) production in ruminants; however, making their widespread inclusion in an animal diet is difficult due to their varied effects. This study aimed to examine the effect of tannin composition on their antimethanogenic activity. Plant extracts from four forage species – birdsfoot trefoil (*Lotus corniculatus)*, big trefoil (*Lotus pedunculatus)*, sulla *(Hedysarum coronarium),* and salad burnet (*Sanguisorba minor*) – were purified and their tannin composition was analysed using UPLC-MS/MS. Sulla and big trefoil extracts contained condensed tannins (CTs) rich in prodelphinidins (PD) with high mean degrees of polymerisation (mDP). Birdsfoot trefoil was rich in procyanidins with low mDP, and salad burnet contained mainly hydrolysable tannins (HTs). A Hohenheim gas test was performed using pure perennial ryegrass (*Lolium perenne*, control) as substrate with different extracts being included at the rate of 10, 20, and 30 g kg^{-1} DM. The largest CH_4 reduction (15%, at 30 g kg^{-1} dry matter (DM)) was observed from sulla and big trefoil extracts compared to control. However, their concomitant reduction in gas production (GP, 11%) indicated a reduction in feed digestibility. On the contrary, birdsfoot trefoil and salad burnet extracts reduced CH_4 up to 12% without significantly reducing GP (4%), indicating a strong effect of tannin composition on ruminal fermentation.

Keywords: legumes, condensed tannins, hydrolysable tannins, methane emissions, ruminants

Introduction

Methane (CH_4) emissions from enteric fermentation contribute to around 17-30% of the anthropogenic CH_4 produced globally. Inclusion of tannin-rich forages in animal diets is one of the promising dietary strategies to mitigate CH_4 emissions in ruminants (Beauchemin *et al.,* 2009). However, the effects of these forages on ruminants have been contradictory until now, ranging from beneficial to anti-nutritional. These diverse effects of tannins on ruminants can be at least partially attributed to the large inter- and intraspecies variability in tannin concentration as well as tannin structure in the plants. Furthermore, the bioactive effects of the tannins are often confounded due to concomitant changes in forage quality that also affect CH_4 emissions and digestibility (Mueller-Harvey, 2006). Thus, the current study aimed to assess the effect of tannin extracts from four tannin-containing forages on the digestibility and CH_4 emissions of a homogenised perennial ryegrass sample. It furthermore attempted to examine the impact of differences in the tannin composition on their bioactivity.

Materials and methods

The tannin extracts were isolated from the leaves of four forage species, viz birdsfoot trefoil (*Lotus corniculatus*), big trefoil (*L. pedunculatus*), salad burnet (*Sanguisorba minor*) and sulla (*Hedysarum coronarium*). These were harvested at vegetative stage under a greenhouse experiment. The extracts were then purified by gel chromatography with Sephadex LH-20 as described in the study by Verma *et al.* (2021). The purified tannin fractions were then analysed using UPLC-MS/MS for detailed tannin composition including subunits of hydrolysable tannins (HTs) and condensed tannins (CTs) as well as

CT structural features, such as prodelphinidin percentage (PD%) and mean degree of polymerisation (mDP). The antimethanogenic potential of these forages was determined using the Hohenheim gas test (Menke, 1998). A homogenised perennial ryegrass (*Lolium perenne*) defoliated at the vegetative stage was used as a base substrate. Purified extracts were tested at different concentrations of 0 (control), 10, 20, and 30 g kg^{-1} dry matter (DM) into the base substrate. Each sample was run in triplicate on two different dates making a total of 6 replicates. Gas (GP) and CH_4 production from the samples were measured at 8 and 24 hours of incubation. The CH_4 percentage in total gas (MP) was determined using an infrared spectrometer. Statistical analysis of the data was performed using the software R (R Core Team 2021). The influence of factors on GP, CH_4 production and MP were determined using a linear mixed model, with species, concentration, and their interaction effects as fixed factors, and replicates nested in the date of experiment was used a random factor.

Results and discussion

The extracts from sulla, birdsfoot trefoil and big trefoil were found to be rich in CTs, and salad burnet extracts comprised mainly HTs (Table 1). Among the CT-containing forages, birdsfoot trefoil was rich in procyanidins (PC, 51%), and sulla and big trefoil had PD-rich CTs (88-93%) with larger polymer sizes (mDP, 21-25). Salad burnet was found to be rich in ellagitannins (96%). Tannin concentration and composition quantified in the current study were within the range described in previous studies conducted on these species (e.g. Jonker and Yu, 2017). Both tannin concentration and species significantly influenced GP and CH_4 production from these extracts. With the increase in concentration, a simultaneous reduction in CH_4 production was observed from all the extracts (Table 2). Furthermore, a concomitant decrease in GP was observed with increasing tannin concentration as seen in previous studies where increase in concentration led to simultaneous reductions in both GP and CH_4 production with significant reductions observed at tannin concentrations exceeding 50 g kg^{-1} DM in the diet (Naumann *et al.,* 2013). Tannins have the propensity to bind with protein in the rumen and increase the amount of protein available for digestion in the small intestine for ruminants. The lower GP in the presence of tannins could result in decrease in ruminal protein digestion. However, this mechanism and its effect on nutrient utilisation are not yet clearly understood (Mueller-Harvey, 2006). Largest variation in CH_4 reduction potential across the species was observed at the tannin concentration of 30 g kg^{-1} DM where it ranged from 10% in birdsfoot trefoil to 15% in big trefoil, compared to the control. Tannin structural characteristics can play an important role in determining their antimethanogenic activity. Higher PD% and mDP values of CTs are able to reduce protein degradation more effectively than PC-dominant forages (Huyen *et al.,* 2016). Tannin extracts from all the species had lower MP compared to control and the lowest MP was attained with salad burnet inclusion (MP: 20%). This could be attributed to the lower negative effect of salad burnet extracts on GP (up to 3%) compared to CT-rich big trefoil and sulla extracts (up to 12%). This is in line with the study by Jayanegara *et al.* (2015) where CT-rich extracts had stronger detrimental effect on *in vitro* digestibility of the feed compared to HT-rich extracts.

Table 1. Chemical composition of the pooled sample of tannin extracts

Species	Total tannins (mg g^{-1})	Hydrolysable tannins (mg g^{-1})	Condensed tannins (mg g^{-1})	Mean degree of polymerisation	Prodelphinidin share (%)
Birdsfoot trefoil	208.5	10.0	162.3	13.9	49
Sulla	341.4	0.1	340.9	24.7	93
Big trefoil	292.2	0.2	291.3	21.5	88
Salad burnet	178.4	171.1	7.3	4.3	37

Table 2. *In vitro* fermentation end-products after 24 hours incubation from different plant species under different concentrations (10, 20 and 30 g kg^{-1} dry matter) with grass as a base substrate.[1]

Species	Concentration	Total gas (ml 200 mg^{-1})	Methane (ml 200 mg^{-1})	Methane percentage in total gas
Grass	Control	62.9±0.9a	13.6±0.3a	21.7±0.5a
Sulla	1	64.8±1.47a	12.8±0.4b	19.7±0.5b
Sulla	2	60.4±1.2ab	12.4±0.2ab	20.6±0.5ab
Sulla	3	56.0±1.3b	11.7±0.5b	21.0±0.7b
Birdsfoot trefoil	1	61.9±1.9a	12.8±0.2b	20.8±0.4b
Birdsfoot trefoil	2	61.2±2.1ab	12.4±0.2b	20.2± 0.4ab
Birdsfoot trefoil	3	60.5±1.7b	12.2±0.1ab	20.2±0.4b
Big Trefoil	1	60.7±1.3a	12.3±0.2b	20.3± 0.2ab
Big Trefoil	2	55.1±2.1b	11.6±0.2b	21.1±0.6b
Big Trefoil	3	56.9±1.4b	11.6±0.2ab	20.4±0.4b
Salad Burnet	1	64.5±1.3a	12.9±0.3ab	20.0±0.4b
Salad Burnet	2	61.4±1.4ab	12.1±0.5ab	19.7±0.5b
Salad Burnet	3	60.5±2b	11.9±0.3ab	19.7± 0.6ab

[1] Lowercase letters indicate significant differences (P<0.05) within a column across different extracts.

Conclusions

This study highlights the influence of tannin chemical composition on their antimethanogenic potential. At the inclusion level of 1 and 2%, the CT-rich extracts reduced CH_4 production without significant reduction in GP except the extracts from big trefoil compared to control. Hydrolysable tannins were more efficient in reducing CH_4 concentration in the total GP with lower effect on digestibility compared to CTs.

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Keyword index

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