



Multispecies grasslands sustain on-farm forage productivity and quality while reducing nitrogen fertilizer inputs

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Abstract

Aims Species-rich grasslands are proposed to promote biodiversity and reduce nitrogen (N) fertilization while maintaining productivity. However, the effectiveness of this strategy remains uncertain, particularly since grasslands with varying species richness are managed differently. We compared biomass yield and forage quality of three grassland management types varying in species richness and N fertilization levels in an on-farm context.

Methods We compared biomass yield and forage quality of three grassland management types: permanent grassland (*Lolium perenne* L. dominated) and multispecies grassland (sown with grasses, forbs, and legumes) fertilized with 0, 150, and 300 kg N ha⁻¹, and unfertilized semi-natural grassland (highly species-diverse) at twelve farm locations over two years. **Results** Multispecies grasslands with 150 kg N ha⁻¹ produced yields similar to permanent grasslands fertilized with 300 kg N ha⁻¹ due to higher legume cover and biological N₂ fixation. Nitrogen fertilization negatively impacted legume cover and likely reduced biological N₂ fixation. Multispecies grassland differed less in terms of yield over two years with varying weather conditions, suggesting enhanced

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drought tolerance. Semi-natural grasslands had the highest species richness but the lowest biomass yields and forage quality.

Conclusions Transitioning from permanent- to multispecies grasslands presents a promising diversification strategy for livestock farmers aiming to improve sustainability and biodiversity, while reducing N fertilization and maintaining productivity. Yet, species richness remains substantially higher in semi-natural grasslands at the cost of reduced biomass yield and forage quality. Optimization of N fertilization should be considered to improve persistence of legumes and forbs in agricultural multispecies grasslands.

Keywords Multispecies swards · Multispecies grasslands · Biological nitrogen fixation · Forage yield · Sustainability

Introduction

Management of temperate grasslands has focused on enhancing productivity through specialization and intensification over the past decades. This trend has led to the widespread use of perennial ryegrass (*Lolium perenne* L.) monocultures (Frame and Laidlaw 2011; Baker et al. 2023a). Despite its desirable agronomic qualities (Kemp et al. 2000; Lee et al. 2010; Frame and Laidlaw 2011), the production of these monocultures heavily depends on external nitrogen (N) inputs (Suter et al. 2015; Grace et al. 2019), such as manure and chemical fertilizers. The excessive use of N fertilizers challenges the sustainability of grassland systems in several ways, including demand on fossil fuels for chemical fertilizer production, nitrous oxide and ammonia emissions, nitrate leaching, biodiversity loss, and high economic costs for farmers (Frankow-Lindberg et al. 2009; Grace et al. 2019; Jaramillo et al. 2021).

One proposed solution to increase sustainability is to diversify grassland plant communities. This diversification would not only benefit biodiversity but could also reduce external N inputs while supporting productivity (Erisman et al. 2016; Schreefel et al. 2020). Grassland diversification can be implemented through different management types including extensification towards semi-natural grasslands or sowing productive multispecies grasslands. Semi-natural grasslands feature a high diversity of naturally

occurring plant species and are extensively managed with restricted external N inputs and infrequent mowing (Allen et al. 2011). However, they have lower biomass yield and reduced forage quality for livestock compared to intensified grassland systems (Tallowin and Jefferson 1999; Bruinenberg et al. 2002).

Multispecies grasslands offer an alternative strategy to promote biodiversity while also supporting high productivity levels. In this grassland management type, a combination of productive grass, forb, and legume species is sown. These species mixtures are selected to promote biological N₂ fixation to optimize resource partitioning within the sward (Sander-son et al. 2005; Høgh-Jensen et al. 2006; Lüscher et al. 2014; Hoekstra et al. 2018; De Haas et al. 2019;; Van Eekeren & Visser, 2019) often leading to transgressive overyielding (Finn et al. 2013). Researcher-managed field experiments have shown that mixtures of up to five grass, forb and legume species can maintain high productivity levels with reduced external N inputs (Moloney et al. 2020a). Furthermore, grass-clover mixtures of two or four species fertilized with 50 kg N ha⁻¹ yielded similarly to grass monocultures with 450 kg N ha⁻¹ (Nyfeler et al. 2009). In a grazing experiment, multispecies grasslands with *L. perenne*, *Phleum pratense* L., *Trifolium repens* L., *Trifolium pratense* L., *Cichorium intybus* L. and *Plantago lanceolata* L. were as productive as monocultures of perennial ryegrass while reducing N fertilization by 55% (Baker et al. 2023a). While these results are promising, more on-farm research is needed to verify these trends.

So far, the relationship between grassland diversity and productivity has been investigated under field conditions where management is mostly kept constant across treatments (Nyfeler et al. 2009; Moloney et al. 2020a; Schaub et al. 2021). Yet, in practice, increasing grassland diversity comes together with changes in management such as mowing, tillage, resowing and fertilization resulting in the comparison of distinct grassland management types rather than solely plant diversity levels (Bruinenberg et al. 2002; Van Vooren et al. 2018; Van Eekeren and Visser 2019). Moreover, evaluating grassland diversification across farms takes place under more realistic but also more heterogeneous conditions than in researcher-managed fields at an experimental station. For example, farms may vary in plant species composition, soil characteristics, grassland age, tillage, resowing and mowing

frequency (Socher et al. 2013). Hence, whether multispecies grasslands can reduce N fertilizer inputs while maintaining high biomass yields and forage quality values under these heterogeneous “real-life” farm conditions has yet to be investigated (Tonn et al. 2021). Moreover, while multispecies grasslands are also expected to promote biodiversity, this has not been compared to the plant diversity levels typical of semi-natural grasslands where biodiversity management is the prime objective.

Farmers may be reluctant to adopt species mixtures because increased plant diversity is often associated with reduced biomass yield and forage quality (Tallowin and Jefferson 1999; Bruinenberg et al. 2002; Isselstein et al. 2005). This association primarily stems from the comparison between semi-natural grasslands and species-poor intensified permanent grasslands (Tallowin and Jefferson 1999; Bruinenberg et al. 2002). While both semi-natural- and multispecies grasslands contribute to enhanced biodiversity, the latter could be managed either extensively or intensively. This offers the potential to achieve desirable yields in terms of quantity and quality with reduced N inputs while still promoting biodiversity on farms to a certain degree. Investigating the relationships between plant species composition, N fertilization and productivity of permanent-, multispecies- and semi-natural grasslands can provide better guidelines for farmers striving to enhance the sustainability of their forage operations and livestock production.

The aim of this study was therefore to compare the biomass yield, forage quality and plant species composition of three grassland management types – permanent grasslands, multispecies grasslands,

and semi-natural grasslands – under different N fertilization levels on twelve on-farm locations over a two-year period. Specifically, our study addressed the following two objectives: i) evaluate whether multispecies grasslands can reduce N fertilizer inputs while maintaining desirable biomass yields in comparison to permanent grasslands dominated by *L. perenne*, and ii) investigate plant species composition, biomass yields and forage quality of the three grassland management types across two years.

Methods

Grassland management types and site selection

This on-farm study took place at twelve locations situated in the eastern part of the Netherlands (Fig. S1) where the three grassland management types of interest to this study were already present (Table 1), amounting to a total sample size of 36 fields. We selected the three contrasting grassland management types in the following way:

- 1 High-productive permanent grasslands: fields were selected following the EU’s definition for permanent grassland: land used to grow grasses that is not included in crop rotation for 5 years or longer (Commission Regulation EU No 796/2004, 2004). These fields were intensively managed (Table 1) and featured a vegetation cover of more than 80% *L. perenne* and less than 5% legumes before the start of the experiment.

Table 1 Management parameters for permanent, multispecies and semi-natural grasslands before the start of the experiment

Parameter	Unit	Grassland management type						<i>p</i> -value
		Permanent		Multispecies		Semi-natural		<i>p</i> -value
		Mean	s.d	Mean	s.d	Mean	s.d	
Field size	ha	3.5	1.9	2.4	0.6	1.8	1.3	0.07
Grassland age at beginning of 2021	yr	8.3 ^b	5.3	1.4 ^a	0.5	21 ^b	14	<0.001
Number of cuts*	yr ⁻¹	7 ^b	1	6 ^b	1	2 ^a	3	<0.001
Mineral N from manure	kg ha ⁻¹ yr ⁻¹	123 ^c	27	66 ^b	34	1 ^a	2	<0.001
N from artificial fertilizer	kg ha ⁻¹ yr ⁻¹	155 ^c	41	29 ^b	32	0 ^a	0	<0.001

*Cuts – Cumulative mowing and grazing cuts

ANOVA *p*-values are given. Means with the same superscript letters are not significantly different ($n=12$, Tukey tests, $p<0.05$, s.d. = standard deviation).

- 2 Multispecies grasslands: These fields were intensively managed and sown with a mixture of grasses and a minimum of eight different species of legumes and forbs. Species that all farms had in common were *L. perenne*, *T. repens*, *T. pratense*, *C. intybus* and *P. lanceolata*.
- 3 Semi-natural grasslands: These fields were extensively managed and featured a plant community with a minimum of fifteen different plant species per 25 m². Most fields were used for mowing, with three out of the twelve fields also being grazed after mowing for hay before the experiment started.

Location and field selection took place in three different steps. First, we selected twelve well-established multispecies grassland fields and matched these to permanent grassland fields located closely nearby, often on the same farm. Secondly, we selected semi-natural grassland fields that were located nearby these multispecies-and permanent grassland fields, not necessarily on the same farm. All fields were situated on similar sandy soils (Soil Survey Staff 1999). The distance between the different fields within one location ranged from approximately 250 m to 6 km.

Fertilizer treatments and site management

In February 2021, a plot of 9.0×7.2 m² was established at a random location in the farmer-managed fields of permanent- and multispecies grassland enclosed with fences to exclude grazing and additional nutrient inputs from manure and urine. New plots for permanent- and multispecies grasslands were established in the same field for 2022 to prevent the impact of fertilizer residues from the previous year.

The plots of the multispecies-and permanent grasslands were further divided into subplots of 2.4×5.2 m² for fertilizer treatments of which a central area of 1.4×5.0 m² was sampled (Fig. S2). The fertilizer treatments in these subplots included 0 kg N ha⁻¹ at the center, with adjacent subplots receiving 150 and 300 kg N ha⁻¹ calcium ammonium nitrate (27%, N). Fertilizer applications were broadcasted and split into: 33% was applied before the first growing period, 27% before the second, 23% before the third, and 17% before the fourth growing period (Van Eekeren et al. 2010). The first applications occurred on the

29th of March (2021) and on the 1st of April (2022), while the other applications were applied after each cut, except for the last cut of each growing season. In addition to N fertilization, on all permanent- and multispecies grassland subplots 80 kg ha⁻¹ P and 300 kg ha⁻¹ K fertilizer was broadcasted to ensure these nutrients were not limiting factors for forage growth (Table S1). The semi-natural grassland plots did not receive any fertilization.

For both permanent- and multispecies grasslands, the sward was mowed five times per year. The mowing occurred in the first half of May, the first half of June, the second half of July, the end of August, and the second half of October. The semi-natural grassland plots were mowed two times per year. In 2021, mowing occurred in the first half of June and September; in 2022 it took place at the end of June and the first half of October. No irrigation was used in 2021; irrigation data were collected at the end of the 2022 growing season from farmers managing the fields. Plots receiving irrigation in 2022 were in locations 3, 5, 7, 9, and 10 for permanent grassland, locations 1, 3, 5, 8, 9, 10, and 12 for multispecies grasslands and none for semi-natural grasslands (Fig. S1).

Meteorological data

Weather data were obtained from weather stations near the region of the experiment, sourced from The Royal Netherlands Meteorological Institute (KNMI 2022; KNMI a, 2021; KNMI b, 2021). Precipitation data were collected and recorded at several rainfall stations close to the experimental sites (KNMI 2022). The 2021 growing season was considered normal with regards to temperature and rainfall, while 2022 was considered dry and warm (Fig. 1).

Forage quantity and quality

All subplots were harvested at a 5 cm stubble height by using a two-wheel mower (Motech M2.09 D) with a 1.4 m wide finger bar attachment (Reform-Werke, Wels, Austria). This was achieved by cutting a 1.4×5 m² strip, leaving 0.5 m from the borders of adjacent subplots, and maintaining a 0.1 m distance from the top and bottom borders. Mowed grass was recorded for dry matter (DM) yield (dried at 70 °C for 48 h) and chemical analysis. Dry material was analyzed for crude ash content (g kg⁻¹ DM) with ignition at

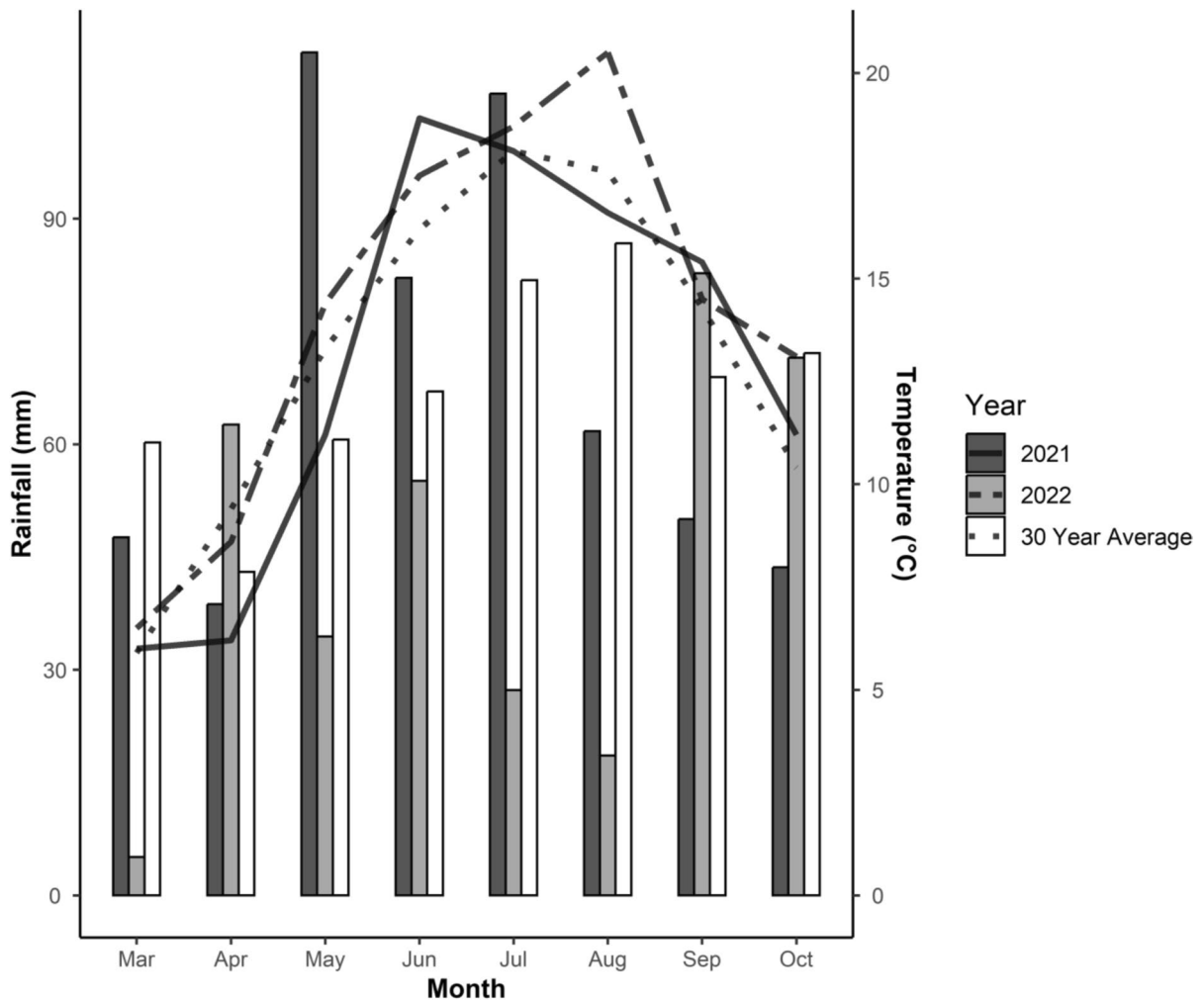


Fig. 1 Meteorological data for the growing seasons of 2021, 2022 and the 30 year average. Bars represent rainfall (mm) left y-axis and lines represent temperature (°C) right y-axis of monthly weather data

600 °C for 2 h, total N content (g kg^{-1} DM) using Dumas method, and organic matter digestibility by analyzing the in vitro digestibility coefficient of organic matter (Tilley and Terry 1963). We measured the percentage of N, calculated the N yield for each harvest and summed the N yields for all harvests. The crude protein concentration was calculated by multiplying the N yield by 6.25 (Jones 1931).

Botanical composition

Plant species cover in each field was assessed before the start of the experiment by using the plant cover

scale classification method developed by Tansley (Tansley et al. 1927). The data were transformed into species abundance values for statistical analysis (Hennekens 2020) concerning species richness and the effective number of species. The effective number of species was based on Hills number N1, i.e. the exponent of the Shannon Index (Hill 1973).

The functional group composition of the subplots was determined at the end of the growing season before the fifth cut for permanent- and multispecies grasslands in 2021 and 2022. This was done by dividing mowed grass into groups of grasses, legumes and forbs.

Soil N supply and biological N₂ fixation

To estimate N supply from the soil in permanent- and multispecies grasslands, we used a linear regression model (Fig. S3) relating soil organic matter (SOM) content to the N yield in the unfertilized subplots of permanent grasslands (Van Eekeren et al. 2010). We define N supply as non-fertilizer N coming from the soil including the atmospheric N deposition. In permanent grassland plots containing less than 5% legumes, we assumed that the N yield without fertilizer application reflects the amount of N coming from the soil. The SOM ranged between 4 to 8%, with one outlier of 14% which was discarded in the analysis. We utilized the equation from the linear model (Fig. S3) to estimate the soil N supply only in unfertilized subplots of permanent- and multispecies grasslands because of difficulties to distinguish between soil-derived and fertilizer-derived N inputs.

To calculate the biological N₂ fixation, we assumed that the biological N₂ fixation was the difference between the total measured N yield and the estimated soil N supply capacity for permanent- and multispecies grasslands. For multispecies grasslands, we further employed the calculated biological N₂ fixation as a response variable predicted by the total clover DM yield (Mg ha⁻¹) including both white and red clover (Fig. S4). This was done to determine whether the measured clover DM yield could predict the biological N₂ fixation under the zero fertilization treatment.

Statistics

All statistical analyses were performed with R version 4.2.2. (R Core Team 2022). The effects of grassland management type, fertilization level, year and their interaction on yield and quality parameters were determined using linear mixed models with location as a random effect for all models, using the ‘lmer4’ package (Bates et al. 2015), assuming that the “location” factor accounted for initial variation in, for instance, pH, soil organic matter, etc. among locations. Fertilization level, year, grassland management type and their interaction effects were included as categorical factors in all linear mixed models. Estimated marginal means were used for all models to compare treatment effects. To aid interpretation for DM yield response with fertilization as a predictor, a quadratic trend line was fitted to model for visualization

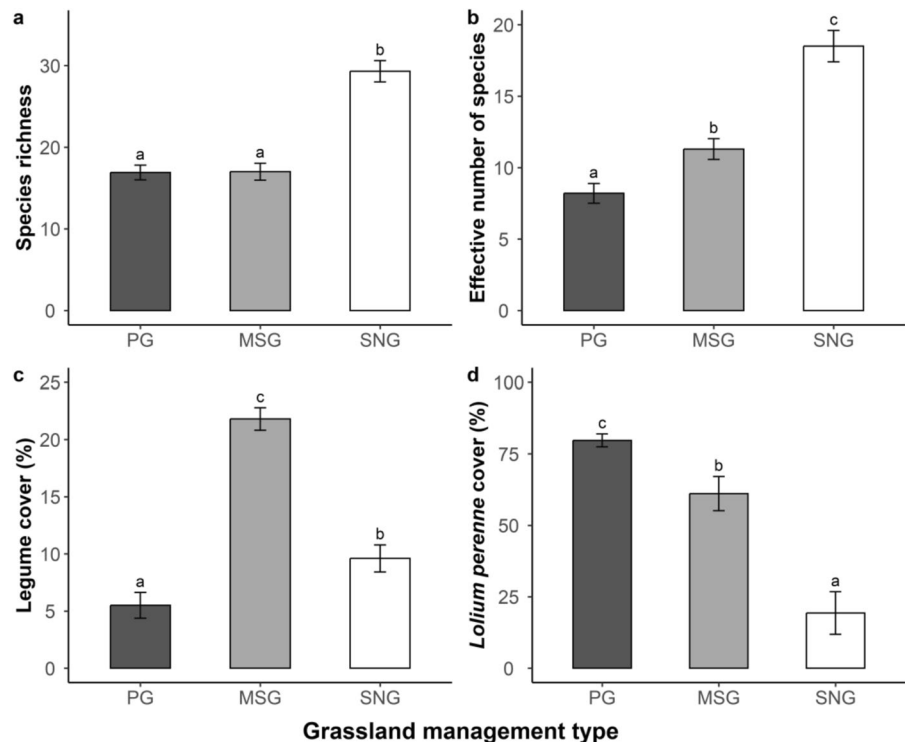
purposes. All other models used a linear trendline for visualization. For all models, backward selection was used to stepwise eliminate non-significant ($P > 0.05$) predictors. All data were balanced with equal sample sizes across treatments. Irrigation did not have any effect on any responses and was removed from all models. The regression models were fitted to predicted values from the models and subsequently used for analysis of variance (ANOVA). When significant treatment and/or interaction effects were observed, model marginal mean comparison was done utilizing Tukey’s P -value adjustment for multiple comparisons with ‘emmeans’ package (Lenth et al. 2023). For all regression models, ANOVA assumptions were tested for normal distribution and homogeneity of variances. Assumptions were not met for organic matter digestibility, which was log-transformed before analysis. Estimated means were back-transformed using the ‘emmeans’ package in R. To assess differences in yield changes between years, we calculated the Δ yield for DM and N yield as the difference between 2022 and 2021 using estimated marginal means and estimated standard errors. A t-test was conducted to compare Δ yield between the permanent- and multispecies grasslands.

Results

Botanical composition at start

Species richness, effective number of species and legume cover varied among the three grassland management types at the start of the experiment (Fig. 2). Species richness was higher in semi-natural grasslands than both other treatments, while multispecies- and permanent grasslands did not differentiate (Fig. 2a). Effective number of species was highest in semi-natural grasslands, intermediate for multispecies grasslands and lowest in permanent grasslands (Fig. 2b). Legume cover was highest in multispecies grasslands, followed by semi-natural grasslands, and lowest in permanent grasslands (Fig. 2c). Legumes with a cover higher than 1% were *T. repens* and *T. pratense*, other legumes had a cover lower than 1%. *Lolium perenne* cover was highest in permanent grasslands, intermediate for multispecies grasslands and lowest in semi-natural grasslands (Fig. 2d).

Fig. 2 Plant species richness (a), effective number of species (b), legume cover (c) and (d) *Lolium perenne* cover of the fields before the start of experiment in 2021. Bars represent means with standard errors with colors representing the different grassland management types. Means with the same superscript letters are not significantly different ($n=12$, Tukey tests, $P<0.05$). Abbreviations are PG (permanent grassland), MSG (multispecies grassland) and SNG (semi-natural grassland)



Comparison permanent vs multispecies grasslands under different N fertilization

The DM and N yields varied among grassland management types, years and N fertilization treatments (Fig. 3a-b; Table 2). In 2021, DM and N yields were larger for multispecies grasslands compared to permanent grasslands under 0 to 150 kg N ha⁻¹ fertilization, while this difference disappeared at 300 kg N ha⁻¹. Dry matter and N yields in multispecies grasslands receiving 150 kg N ha⁻¹ fertilization and permanent grasslands receiving 300 kg N ha⁻¹ were similar. In 2022, DM and N yields were larger in multispecies grasslands compared to permanent grasslands under similar fertilization levels. For both yield responses, a significant ($P<0.05$) interaction effect was observed between grassland management type and year (Table 2). In 2022, yields were reduced ($P<0.001$) compared to 2021 for both grassland management types. The yield decline (Δ yield) from 2021 to 2022 differed ($P<0.05$) between grassland management types: estimated reductions were 4.3 ± 0.3 Mg/ha in DM yield and 114 ± 11 kg N/ha in N yield for permanent grasslands, while 3.2 ± 0.3 Mg/ha and 64 ± 11 kg N/ha for multispecies grasslands (Fig. S5).

Grassland management type, year and N fertilization all significantly influenced the crude protein concentration (Fig. 3c; Table 2). In 2021, multispecies grasslands forage exhibited a larger crude protein concentration than permanent grasslands under 0 kg N ha⁻¹ fertilization and similar values under 150 to 300 kg N ha⁻¹. In 2022, multispecies grasslands forage had a larger crude protein concentration compared to permanent grasslands at 0 and 150 kg N ha⁻¹, while having similar values at 300 kg N ha⁻¹. Crude protein concentration showed an interaction effect between grassland management type and year ($P<0.05$). The crude protein concentration was larger in 2022 than in 2021 for multispecies grasslands with an increase of 9.6%, while being similar for permanent grasslands during both years. The organic matter digestibility was similar for multispecies- and permanent grasslands during both years. An interaction effect was observed between grassland management type and year ($P<0.001$). In 2022, the organic matter digestibility was reduced by 7.0% for permanent grasslands compared to 2021, while being similar for multispecies grasslands during both years ($P<0.001$; Fig. 3d; Table 2).

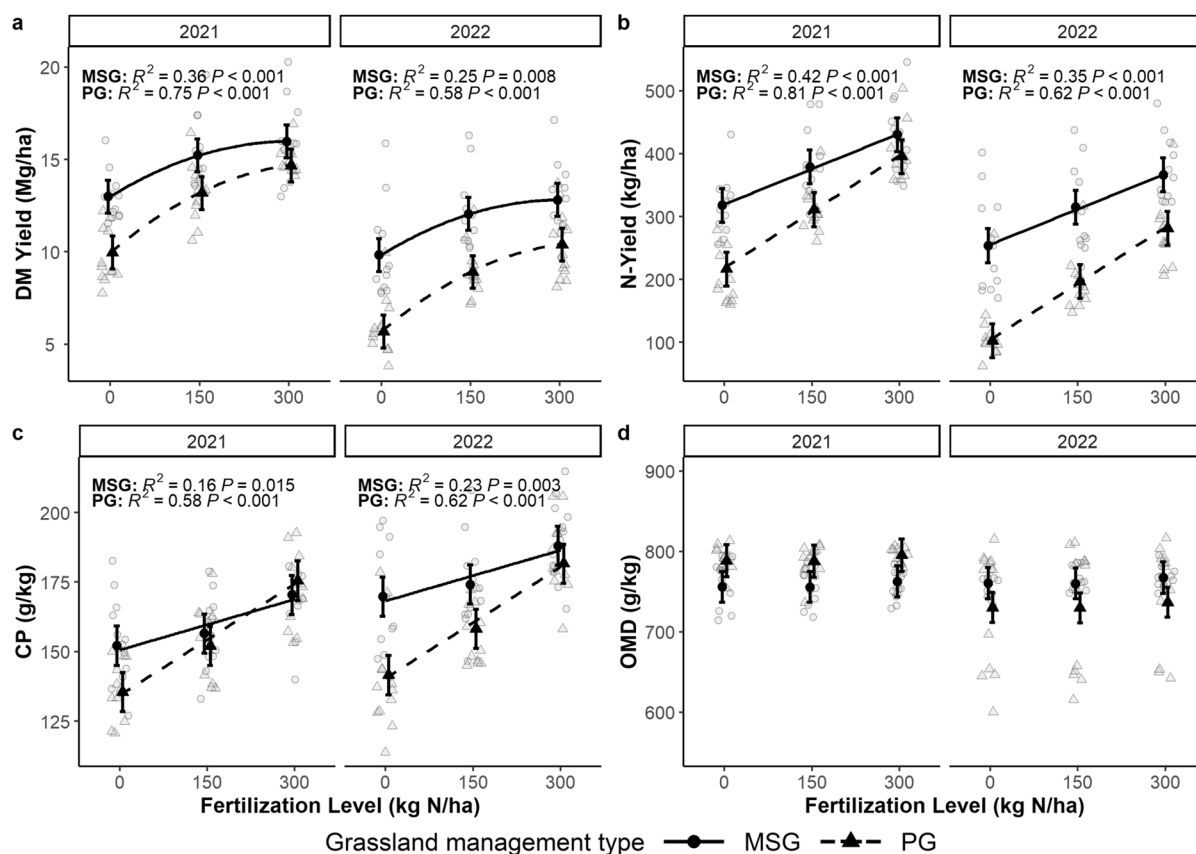


Fig. 3 Grassland management type and N fertilization effects on (a) dry matter (DM) yield, (b) nitrogen (N) yield, (c) crude protein concentration and (d) back-transformed means of organic matter digestibility in 2021 and 2022. Symbols represent model marginal means with 95% confidence interval bars.

Solid lines and circles represent the multispecies grasslands (MSG). Dashed lines and triangles represent permanent grassland (PG). Annotations represent the coefficient of determination (R^2) and the P -value for the regression coefficient

Table 2 ANOVA table for the linear mixed models for grassland management types (GMT) PG and MSG with degrees of freedom (DF), F -values with their corresponding P -values for

dry matter (DM) yield, nitrogen (N) yield, crude protein, and organic matter digestibility

Effect	DF	DM Yield	N-Yield	Crude protein	Organic matter digestibility
GMT	1	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.952$ ns
N-level	2	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.539$ ns
Year	1	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
GMT $\times$ N-level	2	$P = 0.019$	$P = 0.005$	$P < 0.001$	N/A
GMT $\times$ Year	1	$P = 0.027$	$P = 0.002$	$P = 0.012$	$P < 0.001$

This ANOVA table corresponds to Fig. 3. Interaction effects that did not meet model criteria were excluded. Cells marked as N/A were not considered in the model

Comparison among three unfertilized grassland management types

The DM and N yields under zero N fertilization varied among the three grassland management types (Fig. 4a-b). Multispecies grasslands provided the largest DM and N yields followed by permanent grasslands and lastly by semi-natural grasslands in both years. The average DM and N yields were larger in 2021 than in 2022 ($P < 0.001$).

The crude protein concentration was largest in multispecies grasslands forage, intermediate in permanent grasslands and lowest for semi-natural grasslands in both years (Fig. 4c). Both permanent- and multispecies grasslands forages had similar, but larger organic matter digestibility compared to semi-natural grasslands (Fig. 4d). The average crude protein concentration was larger in 2022 compared to 2021 ($P < 0.05$), while the average organic matter digestibility was lower in 2022 than in 2021 ($P < 0.05$).

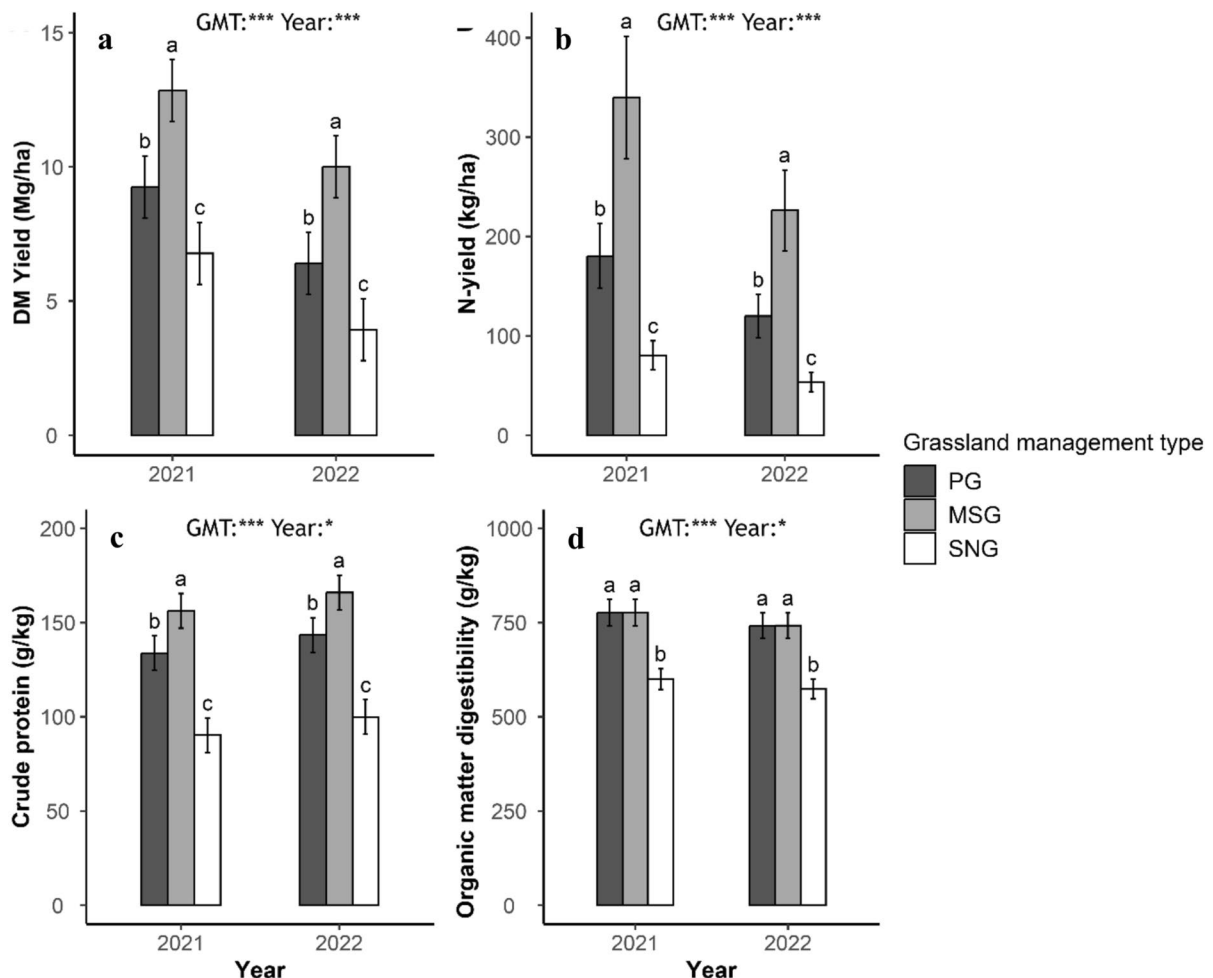


Fig. 4 The effect of grassland management type and year at zero N fertilization on (a) dry matter (DM) yield, (b) nitrogen (N) yield, (c) crude protein concentration (d) back-transformed means of organic matter digestibility. Bars represent model marginal means with 95% confidence interval error bars. Means with the same letter are not significant dif-

ferent (Tukey test, $P > 0.05$). Annotations represent ANOVA main and interaction effects (***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$). Abbreviations are GMT (grassland management type), PG (permanent grassland), MSG (multispecies grassland) and SNG (semi-natural grassland)

Functional composition

Multispecies grasslands contained larger proportions of legumes and forbs compared to permanent grasslands (Fig. 5). Nitrogen fertilization decreased the proportion of legumes and forbs while increasing the proportion of grasses in multispecies grasslands. Across both years, the legume proportion in multispecies grasslands was similar between the fertilization levels of 150 and 300 kg N ha⁻¹. However, the legume proportion was lower in 2022 than in 2021 for both grassland management types.

Soil N supply and biological N₂ fixation

The estimated N supply coming from the soil was similar in multispecies- and permanent grasslands during both years (Fig. 6). Biological N₂ fixation in multispecies grasslands was approximately seven times higher than in permanent grasslands. While the

soil N supply was lower in 2022 than in 2021, biological N₂ fixation showed the opposite trend for both grassland management types.

The estimated biological N₂ fixation per Mg of DM clover yield in multispecies grasslands was 35 and 72 kg N Mg⁻¹ clover yield in 2021 and 2022 respectively (Fig. S4). The average for both years showed that 1 Mg of DM clover yield resulted in 53 kg N fixation (Fig. S4).

Discussion

This study compared the on-farm agronomic performance of permanent, multispecies and semi-natural grasslands, over two growing seasons. We found that multispecies grasslands contained a higher effective number of species and legume cover than permanent grasslands (Fig. 2) and produced similar biomass and N yields at half the amount of N fertilizer applied (Fig. 3a-b). Moreover, multispecies grassland

Fig. 5 Plant composition based on harvested dry weight for the fifth cut (end of the growing season) for both years and three fertilization levels (0, 150, 300 kg N ha⁻¹). Stacked bars represent the mean percentage of each functional group (black: Grass, grey: Forb, white: Legume) for PG and MSG. The abbreviations are PG (permanent grassland) and MSG (multispecies grassland)

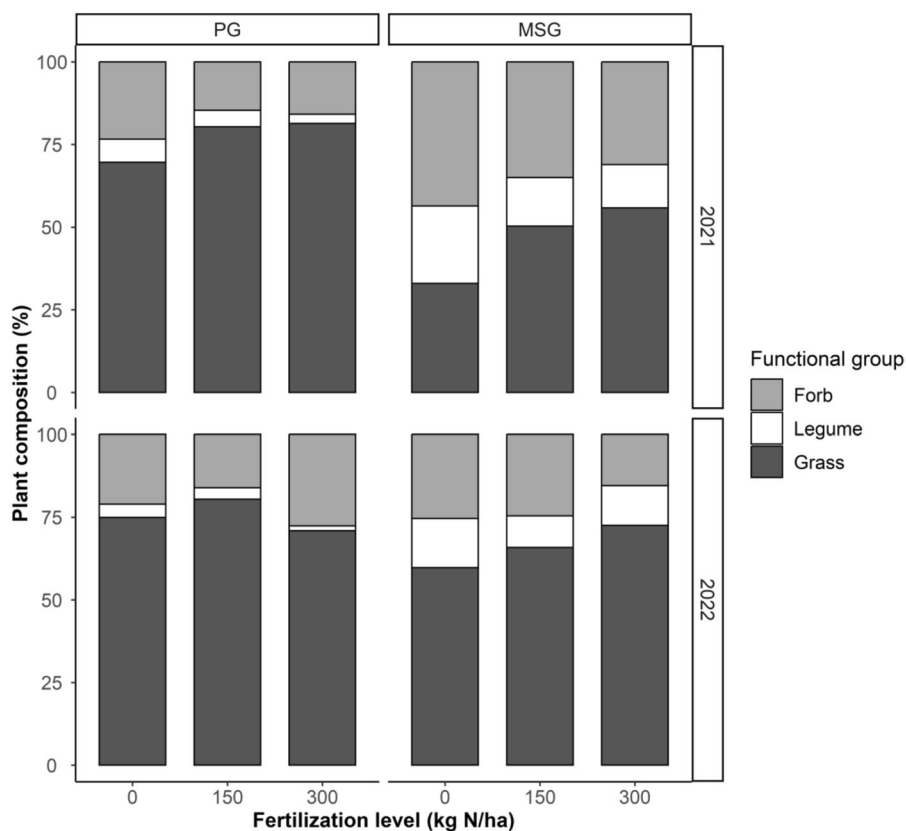
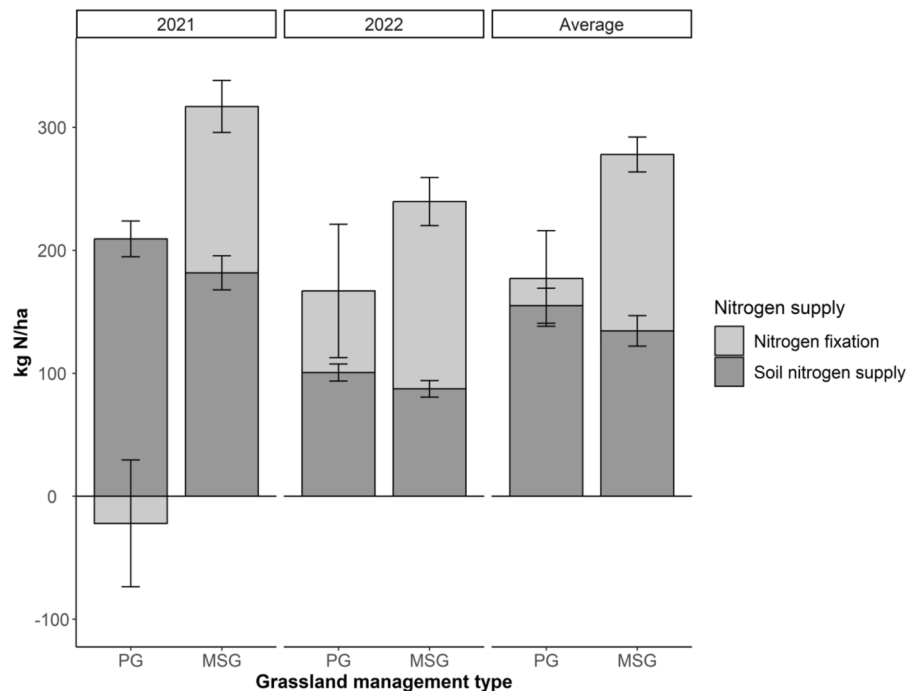


Fig. 6 The nitrogen supply from the soil and the biological nitrogen fixation in permanent grasslands (PG) and multispecies grasslands (MSG) at 0 kg N ha⁻¹ fertilization. Mean nitrogen supply is indicated by stacked bars with standard errors bars and is split up in soil nitrogen supply (dark grey) and nitrogen fixation (light grey)



exhibited smaller year to year yield declines than permanent grassland (Fig. 3a-b), indicating greater resilience under variable weather. Semi-natural grasslands hosted a substantially higher effective number of species than in multispecies grasslands (Fig. 2b), yet this came at a cost of lower biomass, N yields and forage quality (Fig. 4a-d).

Multispecies grasslands need less N fertilizer to produce yields similar to permanent grasslands

This farmer-managed research demonstrated that agricultural multispecies grasslands can reduce N fertilizer inputs by 50% while matching the biomass yield and forage quality of permanent grasslands receiving 300 kg N ha⁻¹ fertilization (Fig. 3). This confirms results of field and grazing experiments at experimental stations that demonstrated the potential of multispecies mixtures to sustain productivity with reduced N fertilization (Nyfeler et al. 2009; Moloney et al. 2020a; Baker et al. 2023a). Biomass yield in multispecies grasslands is less responsive to increasing nitrogen fertilization compared to that in permanent grasslands. Moreover, both grassland management types demonstrated similar biomass

yields when subjected to 300 kg N ha⁻¹ fertilization (Fig. 3a). This performance derives from higher legume cover and biological N₂ fixation, estimated (in unfertilized multispecies grassland plots) at 53 kg N per Mg clover DM (Figs. 5 and 6; Fig. S4). Similar findings have been reported in other grass-legume mixtures (Peoples and Baldock 2001; Carlsson and Huss-Danell 2003), with red and white clover showing values of 33 and 55 kg N Mg⁻¹ clover DM, respectively (Carlsson & Huss-Danell 2003). In line with our results, these studies conclude that permanent grasslands depend more on N fertilizer to attain desirable biomass yields, while multispecies grasslands can achieve similar yields at lower fertilization levels, primarily because of higher rates of biological N₂ fixation (Fig. 6).

The increase in N fertilizer reduced the legume cover and likely biological N fixation in multispecies grasslands. Increasing N fertilizer application from 0 to 300 kg N ha⁻¹ for multispecies grasslands decreased the legume proportion by approximately 40% and 20% and forb proportion by approximately 30% and 40% in 2021 and 2022 respectively, while the proportion of grass increased (Fig. 5). Elevated fertilizer N in grassland systems is known to

negatively impact legume cover and reduce biological N₂ fixation in swards (Nesheim et al. 1990; Nyfeler et al. 2009; Kristensen et al. 2022). These results highlight the intricate interactions and trade-offs between N fertilization, legume cover and yield. Future studies should investigate how to optimize N fertilization in multispecies grasslands to support the persistence of legume cover and the biological N₂ fixation capacity while maintaining desirable yields (Hoekstra et al. 2018; Janssen et al. 2022).

The younger grassland age of multispecies grasslands may explain its higher yield compared to the permanent grasslands (Table 1). Older grasslands like permanent grasslands may experience reduced yields due to the invasion of non-productive species (Schils et al. 2007; Reheul et al. 2008; Schils et al. 2022). On the contrary, younger grasslands such as multispecies grasslands could benefit from vegetation renewal through reseeding that enhances species composition and reduces non-productive species in the sward (Hopkins et al. 1990; Creighton et al. 2016; Schils et al. 2022). Additionally, ploughing or tilling for reseeding releases mineral N from soil organic matter decomposition, increasing N uptake and biomass yields (Seidel et al. 2007; Schils et al. 2022).

Multispecies grasslands sustain high feeding values

Some farmers are hesitant to diversify managed grasslands because it can lead to reduced forage quality, negatively impacting livestock performance (Bruinenberg et al. 2002; Sanderson 2010). However, our study shows that multispecies grasslands had a 10–20% higher crude protein concentration in the unfertilized treatment and similar values at higher fertilization treatments compared to permanent grasslands in 2021 (Fig. 3c), which is in line with results from previous studies (Grace et al. 2018; Moloney et al. 2020b; Baker et al. 2023b). Legumes have a higher crude protein concentration compared to grasses and forbs and can enhance the overall N content within grassland mixtures, resulting in higher crude protein content of the forage (Sanderson 2010; Nyfeler et al. 2011; Lüscher et al. 2014). Regardless of the fertilizer treatment, both grassland management types exhibited similar organic matter digestibility. Generally, grass has higher organic

matter digestibility compared to forbs and legumes (Sanderson 2010). However, forbs sown in multispecies grasslands such as chicory and plantain have been found to have relatively high digestibility under an intensive mowing regime and are proposed to enhance nutritional values when included in grassland mixtures (Sanderson et al. 2003). This suggests that diverse grassland mixtures with carefully selected productive species, exemplified by multispecies grasslands, can sustain high feeding values on farms compared to less diverse grassland swards like permanent grasslands.

Multispecies grasslands also provided higher forage quality than semi-natural grasslands (Fig. 4c-d). Based on findings from similar studies, forages from extensively managed grasslands usually have reduced feeding values compared to forages under intensive management (Tallowin and Jefferson 1999; Bruinenberg et al. 2002; Pavlů et al. 2006). This is attributed to the lower fertilization history, and the infrequent and delayed mowing regimes of semi-natural grasslands, resulting in a different plant community and allowing plants to reach a more advanced stage of maturity compared to intensified systems. Consequently, forage from semi-natural grasslands accumulates more stem material, resulting in reduced feeding values (Tallowin and Jefferson 1999; Bruinenberg et al. 2002). Although semi-natural grasslands have suboptimal feeding values compared to intensified grasslands (Tallowin and Jefferson 1999), it can still be of value for livestock systems. Bruinenberg et al. (2006) demonstrated that replacing 20% of silage from intensively managed grasslands with forage from semi-natural grasslands does not result in a reduction in milk yield from lactating dairy cows. Hence, semi-natural grassland produces forage that can serve as feed for livestock in addition to promoting on-farm biodiversity and supporting various ecosystem functions.

Optimizing the biodiversity-productivity trade-off

While our results on multispecies grasslands show that it is possible to diversify permanent grasslands without decreasing productivity, this is highly dependent on the functional composition of the plant community and specifically legume cover.

Considering our results from semi-natural grasslands, there seems to be a limit to maximizing plant diversity in agricultural grasslands due to a decrease in productivity. Namely, semi-natural grasslands had on average a plant species richness of 29 and effective number of species of 19, which by far exceeded the numbers in permanent- and multispecies grasslands (Fig. 2a-b). However, the biomass yield and forage quality of semi-natural grasslands were significantly lower compared to permanent- and multispecies grassland management types for the duration of this experiment (Fig. 4). Although previous grassland biodiversity experiments indicate a positive relationship between plant diversity and productivity (Marquard et al. 2009; Schaub et al. 2019, 2021; Tilman et al. 2001; Weigelt et al. 2009), our findings conducted on-farm do not support this trend.

The lower biomass yield and forage quality in semi-natural grasslands compared to multispecies- and permanent grasslands management types is likely due to long-term extensive management practices, contributing to reduced soil nutrient availability, particularly potassium (Van Eekeren et al. 2025), and the loss of functional species, such as legumes, resulting in lower productivity (Tallowin & Jefferson 1999). The productivity of semi-natural grasslands increases substantially with fertilizer application, while it causes a decrease in plant diversity (Tallowin and Jefferson 1999). As extensive management aims to maintain or enhance biodiversity, there is little room to intensify these systems. Therefore, this trade-off between plant biodiversity and agronomic productivity should be considered when applying extensive management as grassland diversification strategy. Nevertheless, future research should investigate to what extent species mixtures in multispecies grasslands could be diversified even further to promote biodiversity to a higher degree while still sustaining high agronomic productivity.

Yields fluctuate less in multispecies grasslands across years

The differences in DM and N yields across years were smaller in multispecies grasslands (3.2 ± 0.3 Mg/ha and 64 ± 11 kg N/ha) than in permanent grasslands (4.3 ± 0.3 Mg/ha in DM yield and 114 ± 11 kg N/

ha) (Fig. 3a-b; Fig. S5), likely due to enhanced tolerance to drought as shown by a significant interaction between year and grassland management type (Table 2). Permanent grasslands had larger reductions in DM yield and N yield from 2021 to the dry and hot year of 2022 compared to multispecies grasslands (Fig. S5). In 2022, multispecies grasslands had larger DM and N yields across all fertilization levels compared to permanent grasslands. Similar findings have been reported by previous studies, which indicated that multispecies swards are more stable across years (Nyfeler et al. 2009; Finn et al. 2013; Sanderson et al. 2013) and more drought tolerant compared to less species-rich swards (Haughey et al. 2018). This implies that diversified grassland communities use water resources more efficiently (Hooper et al. 2005), and/or that the individual species in multispecies grasslands are more drought and heat tolerant. Species included in multispecies grassland swards, such as red clover, plantain, and chicory, are known to be drought and heat tolerant (Stewart 1996; Li and Kemp 2005; Annicchiarico et al. 2015; Nguyen et al. 2022). In summary, these findings suggest that an additional benefit of diversifying managed grassland systems is greater tolerance to dry and hot periods during the growing season, which are expected to increase under current climate predictions (Houghton et al. 2001; Walther et al. 2002; Dai 2011).

Conclusions

This study investigated the effect of agricultural grassland diversification through three different grassland management types on biomass yields and forage quality. We found that multispecies grasslands promote the effective number of plant species, halve N fertilizer requirements and maintain target yields across variable years in comparison to permanent grasslands. While semi-natural grasslands host the greatest number of plant species, this comes at the cost of reduced biomass yield and forage quality. These findings add to the scientific literature on the positive effect of biodiversity on productivity in semi-natural experimental grasslands by showing that this effect does not hold in the context of managed grasslands.

Overall, we conclude that transitioning from permanent- to multispecies grasslands is a promising diversification strategy for livestock farmers. Such systems, however, require periodic resowing and soil disturbance due to limited legume and forb persistence. This can have economic and ecological consequences (a.o. loss of soil biodiversity) (Van Eekeren et al. 2025). Future research should investigate how to fine-tune N fertilization levels to support the persistence of legume cover without compromising yields. In addition, the choice of cultivar, soil potassium availability, mowing and grazing practices as well as overseeding are key factors that could influence the persistence of legumes and forbs and require further investigation. Yield fluctuations between years were smaller in multispecies than in permanent grasslands. Follow-up studies should further investigate this potential of multispecies grasslands as adaptation strategy to the predicted higher frequency of weather extremes.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval The research did not involve human participants or animals.

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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