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Plant Diversity, Functional Group Composition and Legumes Effects versus Fertilisation on the Yield and Forage Quality

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Abstract: Elevating plant diversity and functional group composition amount in the swards may contribute to lower N fertiliser use. The excessive use of fertilisers in agriculture is one of the causes of environmental pollution issues. We investigated the effects of plant diversity, functional community composition, and fertilisation on the dry matter yield and its quality at the Lithuanian Research Centre for Agriculture and Forestry, Central Lithuania. The study aimed to determine the productivity potential of single-species and multi-species swards with three, four, six, and eight plant species in the mixtures including four grasses and four legumes. Two experimental backgrounds were used with N_0 and N_{150} kg ha^{-1} yr^{-1} for all treatments. In the two-year experiment manipulating species richness and functional group diversity had a positive effect on the dry matter yield and produced better quality of the forage when compared with single-species swards. Crude protein in the forage of grass–legume mixtures was significantly greater than for grass monocultures. Investigating fertilisation background was a concern; it had a positive effect on the single-species sward yield but decreased the yield of multi-species swards.

Keywords: environment; Fabaceae; multi-species; nitrogen; Poaceae; productivity

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1. Introduction

Grasslands are a determinant part in global food security and are economically important [1,2] in temperate regions since they comprise about 50% of the European land area covered by vegetation [3]. In the last decades many field experiments were performed to find out how functional traits of different plant species influence the productivity of swards [4–8]. Functional traits are physiological, morphological, or phenological characteristics that describe a plant's fitness by representing its ability to grow, reproduce, and survive [9]. Highly productive sward plants are characterised by dense root traits and long-lived tissue, which could inhibit nutrient deficiencies [10].

In agriculture, legumes are very important [10] and are designed to respond to nutrient deficiencies in the soil [11]. The consumption of plant-based protein is on the rise [12]. To match the global requirement for plant-based products, crop productivity must be ensured; however, this might be undermined by either environmental [13] or anthropogenic pressures [14] that lead to soil nutrient disturbance [15].

Legumes are significant for sustainable agriculture through their ability to improve soil health and fertility [16]. Legumes, having a mutually symbiotic relationship with some bacteria in soil, can improve the nitrogen (N) amount through biological N-fixation (BNF) [17]. Permanent grasslands host a high plant diversity, which sustains many ecosystem services [18]. Cultivating mixtures of different plants can be a sustainable means of increasing agricultural productivity, but it is very important to optimise functional diversity by combining different species characteristics that are well-adapted to local growing conditions [8]. The mixture composition affects total herbage in various harvests [19].

Multi-species sward field experiments can be used to investigate and understand vegetation dynamics [20]. They also demonstrate that species richness is important because it provides a pool of species with potentially relevant traits [21]. Different plant species may turn out to be valid performers or partners in new interspecific interactions [22] after environmental change.

Compared with perennial ryegrass swards, multi-species swards can sustain herbage yields [23] at reduced mineral N fertiliser inputs or increase herbage yields at high mineral N inputs, depending on the species composition of the cultivated swards [24]. The issue of fertilisation use in the high plant diversity swards has received considerable critical attention recently [24]. More research is needed on how different plant species swards and their functions are affected when using additional fertilisation treatment. The plant diversity and biomass production relationship in swards has aroused great interest among ecologists in the 21st century based on the principles of ecosystem stability, productivity, and nutrient dynamics. Therefore, the N-fixing plants that can capture biological nitrogen from the atmosphere [25] are important for swards to reduce the need for synthetic fertilisers. Reduced use of N fertiliser application may mitigate N_2O emissions [26].

The aim of the present study was to identify the influence of monocultures and different grass–legume mixtures with different species composition and functional groups on the yield productivity and quality in the first and second years of sward use.

2. Materials and Methods

2.1. Field Experiments

The field experiment was established in 2018 and will be continued until 2022 at the Lithuanian Research Centre for Agriculture and Forestry, located in Akademija (55°22′59.7″ N 23°51′42.1″ E), Central Lithuania. The sward was planted on 4 May 2018. Lithuania is in a temperate climatic zone, where the growing season lasts from 169 to 202 days. The duration of plant vegetation in the first year of sward use was 182 days (from 7 April to 1 September 2019), and in the second year—217 days (from 3 March to 10 September 2020). In the second year of sward growth, plant vegetation started earlier than in the first year, ended later, and was 35 days longer. Grasses (Poaceae) seeding rates of perennial ryegrass (*Lolium perenne* L.) 'Elena DS', Festulolium (*Festulolium*) 'Vėtra', meadow fescue (*Festuca pratensis* L.) 'Raskila', and timothy (*Phleum pratense* L.) 'Dubingiai' were 18, 18, 20, and 12 kg pure live seed ha^{-1}, respectively. Legumes (Fabaceae) seeding rates of white clover (*Trifolium repens* L.) 'Dotnuviai', red clover (*Trifolium pratense* L.) 'Sadunai', lucerne (*Medicago sativa* L.) 'Malvina', and sainfoin (*Onobrychis viciifolia* L.) 'Meduviai' were 10, 15, 15, and 80 kg pure live seed ha^{-1}, respectively. The main agrochemical characteristics of the arable (0–25 cm) layer were measured prior to undertaking the experiment in 2018 (Table 1).

Table 1. The main agrochemical soil characteristics before the experiment.

Soil Depth cm	pH_{KCl} Suspension	Phosphorus (P) Concentration mg kg^{-1}	Potassium (K) Concentration mg kg^{-1}	C_{org} %	Total Nitrogen (N) %
0–10	6.9	101	179	1.87	0.245
10–25	6.8	94	109	1.64	0.248

The soil of the experimental site was loamy Endocalcaric Epigleyic Cambisol (WRB, 2014) [27]. The characteristics of the soil's arable (0–25 cm) layer were as follows: neutral soil (pH 6.9) and relatively high soil organic carbon (1.76%) with a high content of plant available phosphorus (P) (98 mg kg^{-1}) and potassium (K) (144 mg kg^{-1}). 2018 was the year of sowing with 3 cleaning cuts; 2019 was the first and 2020 the second year of sward use. On 2 May 2018, NPK fertilisers were applied at a rate of N–P–K 5–20.5–36 kg ha^{-1}. In 2019 and 2020 two fertilisation backgrounds were used at N_{150} and N_0. The fertilised experiment received N fertiliser at 150 kg^{-1} ha^{-1} yr^{-1}: N_{60} in spring, N_{45} after the first and N_{45} after the second cut. The swards were cut 4 and 5 times in 2019 and 2020, respectively. The site was managed with no extra irrigation, and no pesticides were used.

2.2. Climatic Conditions

The weather data were collected at the stationary meteorological station located in Akademija using the temperature and rainfall sensors (Figure 1). Lithuania is in a temperate climatic zone where the mean annual air temperature is 6.9 °C and precipitation 695 mm.

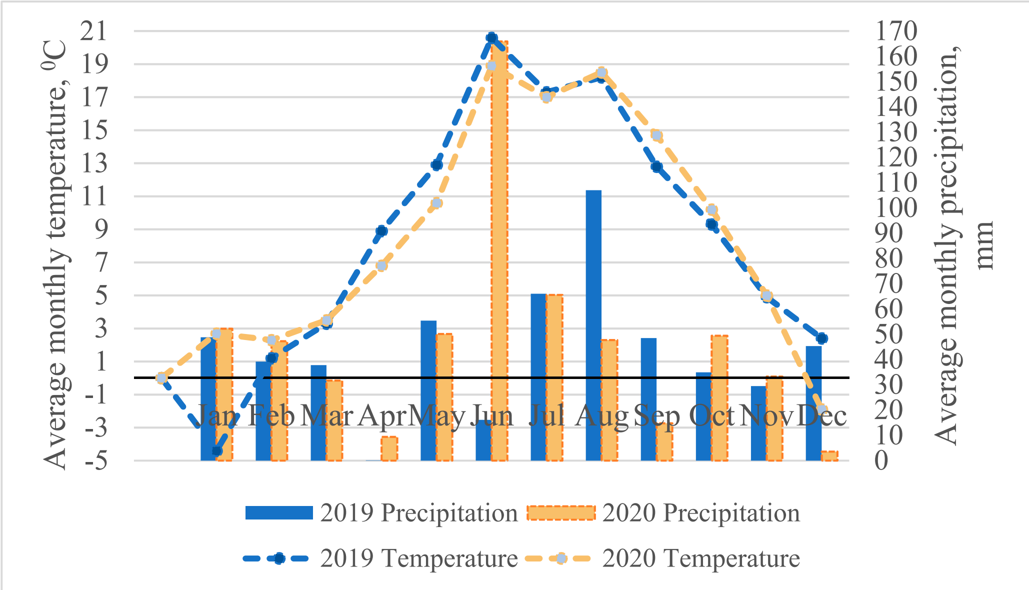


Figure 1. Meteorological conditions during the experimental years (2019 and 2020).

In the first year of sward growth, at the beginning of vegetation, which was the beginning of April, there was a very dry period—the amount of precipitation did not exceed 1 mm. In the second year of sward growth, the beginning of vegetation was more favourable for grasses. Assessing the agrometeorological conditions according to the values of the hydrothermal coefficient (HTC < 0.5), the prolonged very dry period in June of 2019 reached the indicators of a dangerous meteorological phenomenon, and in the middle of the month it reached the indicators of natural drought. Although the average annual temperature and precipitation varied slightly in both experimental years, the year 2020 was warmer and wetter. After the climatic conditions in certain months of vegetation were observed, it could be seen that there was no precipitation in April 2019.

2.3. Experimental Design, Treatments and Measurements

Two factors were investigated: A—different grass/legume mixtures (2 monoculture swards and 10 multi-species swards), and B—two levels of nitrogen (N) fertilisation (N_0 (no N fertilisation) and N_{150}). The dominant plant species were perennial ryegrass (G1), Festulolium (G2), meadow fescue (G3), timothy (G4); legumes—white clover (L1), red clover (L2), lucerne (L3), and sainfoin (L4). Each plot was 1.5 m wide and 10.0 m long. The experiment had a randomised block design with four replications. The experiment included 12 treatments consisting of perennial ryegrass and Festulolium single-cultivar swards and mixtures of grasses and legumes (Table 2). The legume and grass ratio in the sown mixtures was 40:60.

All cuts were taken depending on the predominant plant species. Grass growth stage before cut was when the flower head was enclosed in the flag leaf sheath and not showing or only partly showing. Meanwhile, legume growth stage was the start of flowering. The first yield of swards was taken 8 and 6 weeks after the start of vegetation in 2019 and 2020, respectively. All cuts were taken at the same time for all treatments. To determine the dry matter (DM) yield, samples of 1–1.5 kg fresh biomass were oven-dried at 105 °C temperature to a constant weight. Grass and legume aboveground biomass was harvested and separated by performing the sward botanical composition analysis for each species in every cut. Plant chemical analysis was performed to assess the quality of the swards. Determination of crude protein was done with an NIRS 6500 spectrometer (FOSS, Denmark) in dried grass biomass.

Table 2. Composition of single-species swards and mixtures with legumes.

Treatments		FG	Sp-r	FG of Grasses	FG of Legumes	Grasses				Legumes			
						G1	G2	G3	G4	L1	L2	L3	L4
(1)	G1	1	1	1		1							
(2)	G2	1	1	1			1						
(3)	L1 + L2/G1	2	3	0.6	0.4	0.6				0.2	0.2		
(4)	L1 + L2/G2	2	3	0.6	0.4		0.6			0.2	0.2		
(5)	L1 + L2/G1 + G2	2	4	0.6	0.4	0.3	0.3			0.2	0.2		
(6)	L1 + L2/G1 + G2 + G3 + G4	2	6	0.6	0.4	0.15	0.15	0.15	0.15	0.2	0.2		
(7)	L3 + L1/G1 + G2	2	4	0.6	0.4	0.3	0.3			0.2		0.2	
(8)	L3 + L1/G1 + G2 + G3 + G4	2	6	0.6	0.4	0.15	0.15	0.15	0.15	0.2		0.2	
(9)	L4 + L1/G1 + G2	2	4	0.6	0.4	0.3	0.3			0.2			0.2
(10)	L4 + L1/G1 + G2 + G3 + G4	2	6	0.6	0.4	0.15	0.15	0.15	0.15	0.2			0.2
(11)	L1 + L2 + L3 + L4/G1 + G2	2	6	0.6	0.4	0.3	0.3			0.1	0.1	0.1	0.1
(12)	L1 + L2 + L3 + L4/G1 + G2 + G3 + G4	2	8	0.6	0.4	0.15	0.15	0.15	0.15	0.1	0.1	0.1	0.1

FG—functional group, i.e., combination of forage species that comprises grasses and legumes; SP-r—species richness.

2.4. Statistical Analysis

Two-way factorial ANOVA was conducted for the interaction of N fertilisers and manipulation diversity of mixtures. Significant differences between the experimental treatments were determined using post hoc exploratory analysis at the 5% probability level ($p < 0.05$). To analyse the effects of the treatment on crude protein, an analysis of variance (one-way factorial ANOVA) was conducted.

3. Results

3.1. Productivity of Monoculture and Multi-Species Swards

The results obtained from the preliminary analysis of the two-year experiment are summarised in Figure 2. In the first and in the second year of sward use a two-way ANOVA revealed that statistically significant differences were obtained between monocultures and mixtures, fertilisation, and the interaction between factors A and B ($p < 0.05$). In the first year of sward use without N fertilisation significant effects ($p < 0.05$) by species richness on yield productivity were found (Figure 2A). The mixture with the highest plant species diversity (eight plants in sowing mixture) had a 48% higher dry matter yield compared to the average annual yield of perennial ryegrass and Festulolium. In the first year of sward use, 9611 kg ha^{-1} was the remarkable result of six plant species in a sown mixture including two legumes, white clover and lucerne, and it was grown without N fertilisation. However, swards with six plant species in a mixture of another composition provided lower productivity. The difference between the most productive mixture of six plant species compared with eight plant species was 7%. A comparison of the two mixtures' results, with white and red clovers, revealed that the sward with Festulolium was 10% more productive than the one with perennial ryegrass. No significant differences were found between single swards of perennial ryegrass and Festulolium. It was found that lucerne and red clover had the most positive effect on DM yields. No increase in effect on DM yield for mixtures with white clover and sainfoin was detected. The results in this section indicated that the most productive plant species in mixtures in the first year of sward use, without using mineral fertilisers, was lucerne, which indicated significant differences between DM yields. A comparison of the remaining mixture results revealed 26% lower DM yield. The DM yields were significantly increased by the diversity of multi-species, but not all species individually had a significant effect on sward productivity. The strong evidence of fertilisation in the first year of sward use was found in the single-species swards with perennial ryegrass and Festulolium. The annual yields of these monocultures were 36 and 42% higher compared with non-fertilised swards. Fertilisation also had a significant impact on two treatments with either four or six plant species in the mixture; however, lucerne and red clover did not grow in these swards.

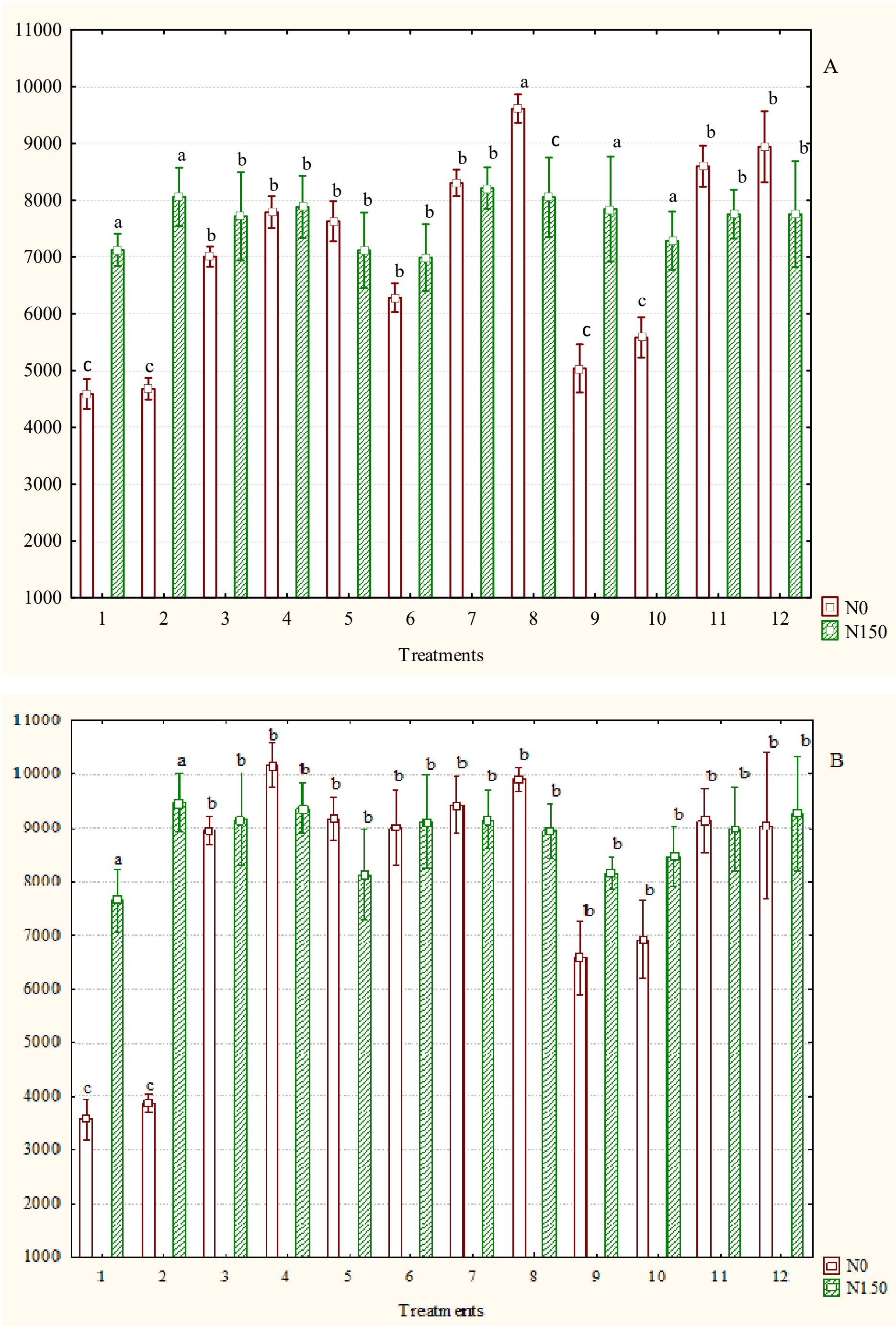


Figure 2. Annual dry matter (DM) yield kg ha^{-1} in the first (**A**) and second (**B**) years of sward use. Treatments: 1—G1, 2—G2, 3—L1 + L2/G1, 4—L1 + L2/G2, 5—L1 + L2/G1 + G2, 6—L1 + L2/G1 + G2 + G3 + G4, 7—L3 + L1/G1 + G2, 8—L3 + L1/G1 + G2 + G3 + G4, 9—L4 + L1/G1 + G2, 10—L4 + L1/G1 + G2 + G3 + G4, 11—L1 + L2 + L3 + L4/G1 + G2, 12—L1 + L2 + L3 + L4/G1 + G2 + G3 + G4; Grasses: G1—perennial ryegrass, G2—Festulolium, G3—meadow fescue, G4—timothy; Legumes: L1—white clover, L2—Red clover, L3—lucerne, L4—sainfoin.; level of N fertiliser per year: N_{150}; different letters indicate significant differences between the treatments ($p < 0.05$).

In the second year of sward use fertilisation had a significantly marked effect on monoculture swards with perennial ryegrass and Festulolium (Figure 2B) and the DM yield increased by 53 and 59%, accordingly. Indeed, this was equivalent to the yield that can be obtained by using at least two legumes suitable for growing together in a mixture with these grasses. Fertilisation did not cause a significant increase in the DM yield of swards of different species composition. Different plant species and their amounts, such as in the first year of swards use, showed significant differences between DM yields. Comparing the two years' results, it was seen that in the multi-species swards dry matter yields were higher in the second year of sward use, both using and not using N fertilisers.

To assess multi-species swards and fertilisation separately, one-way analyses of variance (ANOVAs) were used. No significant differences were found between the treatments when using additional fertilisation in both experimental years. For example, comparing monoculture and multi-species swards with no additional N, the result was significant at the $p = 0.05$ level. The obtained results showed that N fertilisers contributed to the growth of grasses in mixtures but reduced the growth potential of legumes.

Data are not shown, but in summary, the average of the two-year experiment results revealed that the most productive mixture, with 9758 kg ha^{-1} DM yield of the sward, was six plant species with white clover, lucerne, perennial ryegrass, Festulolium, meadow fescue, and timothy with N_0 rate. The monoculture sward of Festulolium showed the highest productivity with 8759 kg ha^{-1} when N_{150} was used; however, there were no significant differences between the most productive monoculture sward of Festulolium and multi-species swards that were fertilised at N_{150}.

The two-year average total sward productivity with N_0 fertilisers in mixtures with white and red clovers was 8254 kg ha^{-1}; with white clover and lucerne—9309 kg ha^{-1}; with white clover and sainfoin—6026 kg ha^{-1}; with white clover, red clover, lucerne and sainfoin—8929 kg ha^{-1}. The proportions of legumes in these swards were 51, 61, 32, and 61%, respectively. Meanwhile, total sward productivity using mineral N_{150} fertilisers in mixtures with white and red clovers was 8181 kg ha^{-1}; with white clover and lucerne—8586 kg ha^{-1}; with white clover and sainfoin—7937 kg ha^{-1}; with white clover, red clover, lucerne and sainfoin—8438 kg ha^{-1}. The proportions of legumes in these swards were 16, 26, 6, and 23%, respectively.

Meanwhile, unfertilised monoculture swards with perennial ryegrass and Festulolium showed a decreased yield in 2020 compared with 2019, by 1022 and 810 kg ha^{-1} per year, respectively. This indicated that for monoculture swards it is necessary to use additional nutrients such as mineral nitrogen fertilisers or legumes suitable for growing together with selected grasses.

3.2. Botanical Composition of the Swards

Botanical analysis was used to determine the competitive plant properties individually in the mixtures with different functional group and species richness (Figure 3). From the data of botanical composition, it was apparent that the fertilisation had a negative effect on the survival of legumes in the mixtures. Already in the first year of the sward use, after the application of the rate of N_{60} fertilisers at the beginning of vegetation, a high decrease in legumes was observed. The most productive species of legumes were lucerne and red clover; their average contents in the mixtures after N_{60} fertilisation decreased by 63 and 78%, respectively. In the second year the contents decreased by 57 and 82%. Further analysis showed that white clover yield in mixtures was the highest in the second year of sward use and only in interaction with sainfoin at N_0 fertiliser rate; however, the total yield of these mixtures was the lowest in both experimental years. Interestingly, the white clover was observed to have better growth properties in mixtures with lucerne rather than with red clover. The single most striking observation to emerge from the data comparison was that sainfoin in mixtures did not increase the total sward productivity in any conditions.

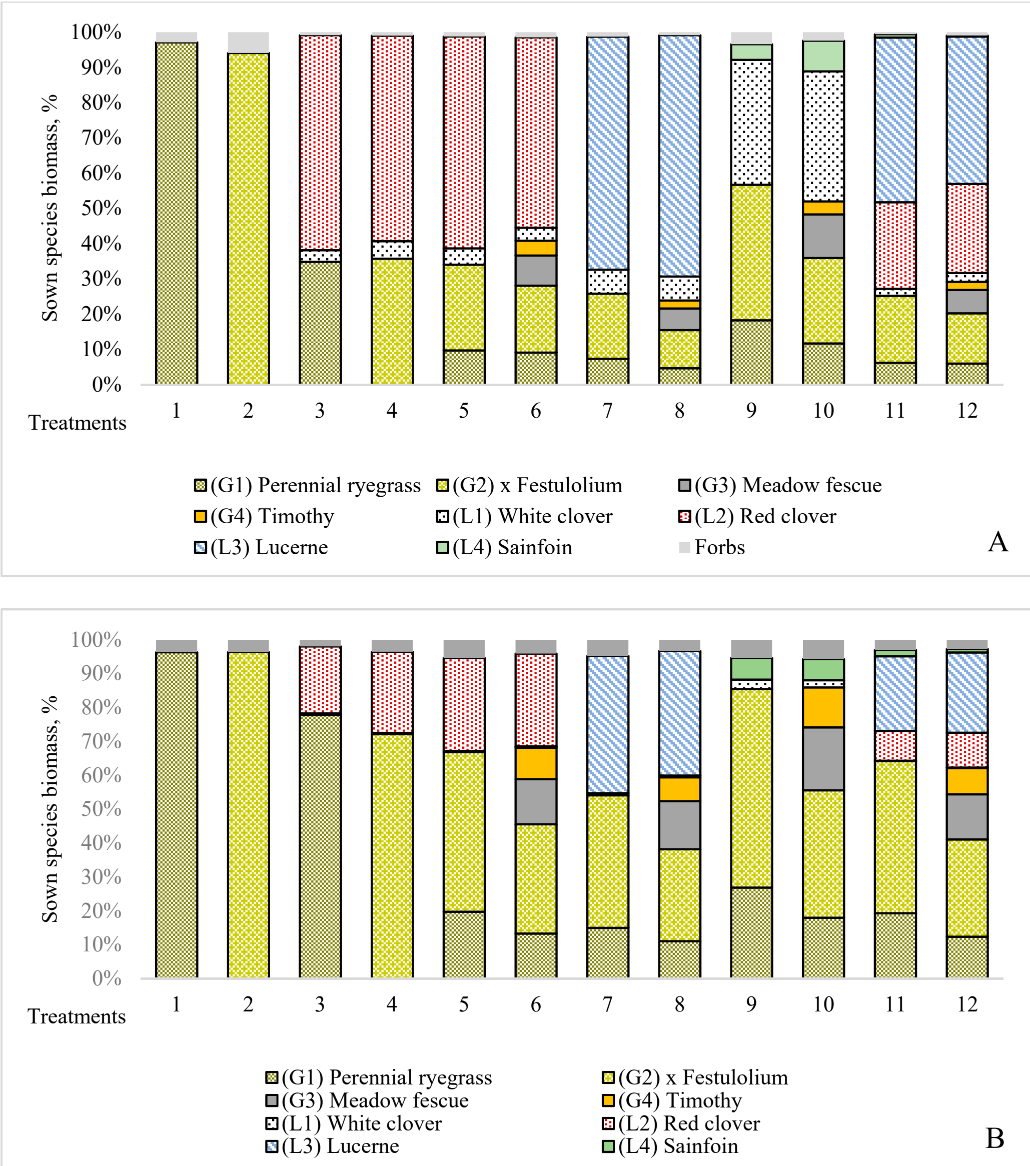


Figure 3. Two-year average botanical compositions of the swards; unfertilised N_0 (**A**), fertilised at N_{150} rate (**B**). Treatments: 1—G1, 2—G2, 3—L1 + L2/G1, 4—L1 + L2/G2, 5—L1 + L2/G1 + G2, 6—L1 + L2/G1 + G2 + G3 + G4, 7—L3 + L1/G1 + G2, 8—L3 + L1/G1 + G2 + G3 + G4, 9—L4 + L1/G1 + G2, 10—L4 + L1/G1 + G2 + G3 + G4, 11—L1 + L2 + L3 + L4/G1 + G2, 12—L1 + L2 + L3 + L4/G1 + G2 + G3 + G4.

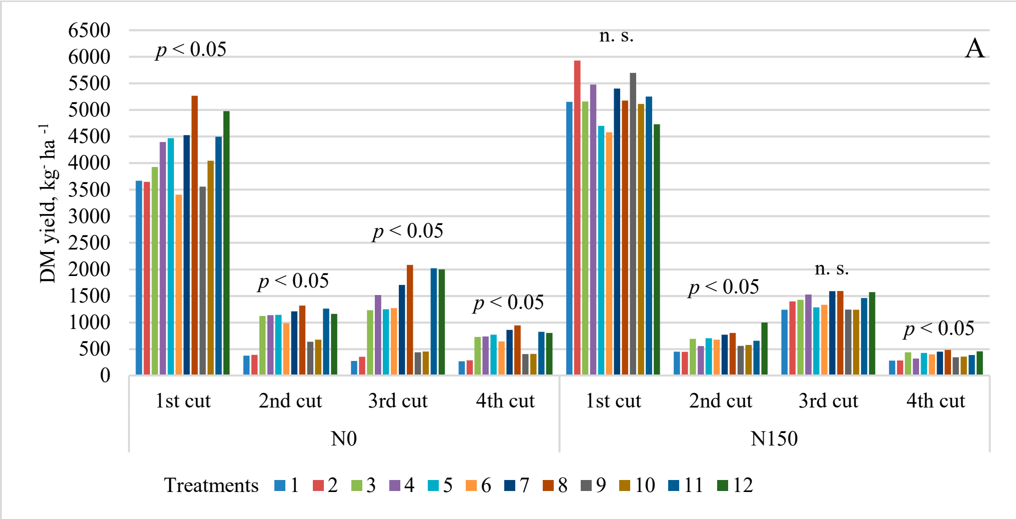
In individual growth years, no significant differences were found between monoculture swards of perennial ryegrass and Festulolium using N_0 and those with N_{150}; however, significant differences were observed between these monoculture yields when additional legume plants, white and red clover, were used in the swards. Only in the first year of sward use when using N_{150} fertilisers were no significant differences found. However, in the mixtures with white and red clovers, Festulolium grasses had insignificantly higher content in herbage: in the first year of sward use using N_0 fertilisation by 10%, in the second by 12%. When using N_{150} fertilisers Festulolium swards were more productive by 2% in the first and in the second years. Two-year average botanical composition data showed that N fertilisers strongly affected legumes in the mixtures. Decreases of 94, 68, 59, and 14% were observed for white clover, red clover, lucerne, and sainfoin, respectively. Meanwhile, the effect on Poaceae grasses was opposite; their productivity in swards was increased by 48, 42, 25 and 60% of perennial ryegrass, Festulolium, meadow fescue and timothy grass, respectively. Together these results provided important insights into fertilisation, biodiversity, and plant diversity in the multi-species swards.

3.3. The Yield of Cuts in Experimental Years

Cuttings and annual sward yield depended on meteorological conditions in the growing season. Statistically significant differences ($p < 0.05$) in cuts between treatments were found in both experimental years when no mineral N fertilisers were used. Further analysis showed that the largest portion of the annual yield was obtained in the first cuts. In the first year of sward use, the first cut accounted for 60% of the annual yield with N_0 fertiliser rate and 68% with N_{150}. In the second year of sward use, these yields were 49 and 60%, respectively. It is apparent in Figure 4 that in both cases, with N_0 and N_{150}, this accounted for more than half of the annual yield, regardless of the different number of cuts in each year of sward growth. No differences were found between the mixtures in which lucerne was grown in the first year of sward in all cuts using N_0. The mixtures of white clover and lucerne with four plant species of Poaceae were the most productive in all cuts, but there were no significant differences compared to the other three mixtures, which also included lucerne. Statistically significant differences between these treatments were found only in the third cut. However, in the second year of sward use, mixtures of white clover, red clover and Festulolium were the most productive in the three cuts. With the use of mineral N fertilisers, no trends were found between the treatments in both experimental years.

In the second year of sward use, the most productive in the first, third, and fourth cuts was a mixture of red clover, white clover, and Festulolium; however, no statistically significant differences among the mixtures of white clover and lucerne with four plant species of Poaceae were found in the first three most productive harvests compared with the mixture of red clover, white clover, and Festulolium plant. Without mineral N fertilisers in swards in both experimental years in all cuts, the lowest dry matter (DM) yields were monoculture swards with perennial ryegrass and Festulolium and two other mixtures containing white clover and sainfoin with two and four Poaceae plant species, respectively, except the first cut of the first year of sward use.

As shown in Figure 4, when evaluating annual yield, no statistically significant differences were found between the treatments in both experimental years using N mineral fertilisers. In the first year of sward use, using mineral N fertilisers on the mixture with lucerne, white clover, and two Poaceae plant species was the most productive, but no significant differences in yields were found compared to other swards. In the second year of sward use, a significantly low yield was produced by the sward with the monoculture of perennial ryegrass. N fertilisers influenced swards by reducing species composition, biodiversity, and stability, especially in adverse environmental conditions in the first year of sward use. A decrease in yield was observed compared to the unfertilised experiment; a dry period occurred before the second cut and at the end of plant vegetation in fourth cut. In the first year of sward use, significant differences were found in these two cuts between treatments with N fertilisers, so legumes improved enough to resist adverse environmental conditions, especially in mixtures with lucerne, which had good growth properties. For instance, no significant differences were found in the first and third cuts between the treatments. Meanwhile in the unfertilised experiment significant differences in yields were found in all cases.



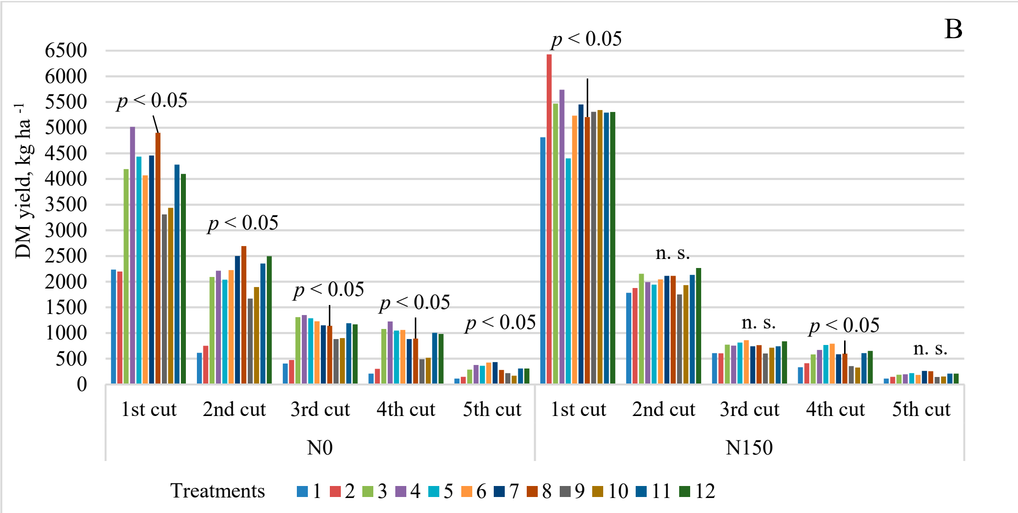


Figure 4. Dry matter (DM) yield of different treatments, by different harvest cuts in the two-year experiment: (**A**) first and (**B**) second year of sward use; *p*-value between treatments in cuts; n.s.—not significant. Treatments: 1—G1, 2—G2, 3—L1 + L2/G1, 4—L1 + L2/G2, 5—L1 + L2/G1 + G2, 6—L1 + L2/G1 + G2 + G3 + G4, 7—L3 + L1/G1 + G2, 8—L3 + L1/G1 + G2 + G3 + G4, 9—L4 + L1/G1 + G2, 10—L4 + L1/G1 + G2 + G3 + G4, 11—L1 + L2 + L3 + L4/G1 + G2, 12—L1 + L2 + L3 + L4/G1 + G2 + G3 + G4.

3.4. Crude Protein in Herbage

The results of the crude protein analysis are shown in Table 3. The data showed that the quality indicator depended on the species diversity of swards. The average crude protein of monoculture swards of perennial ryegrass and Festulolium ranged from 10 to 12% using N_0 and N_{150}, respectively. In other grass/legume mixtures, the content of crude protein ranged from 12.4 to 18.6% without the use of mineral N fertilisers and from 12.8 to 15.9% with N_{150}, depending on the species composition of the swards. Comparing the highest average dry matter yields of the two years, the mixture with legumes using N_0 was more productive than the monoculture sward with Festulolium using N_{150}, the difference in yield of these swards was 999 kg ha^{-1}. In addition, the content of crude protein in the fertilised monoculture sward was 12%, while in the grass/legume mixture using N_0 it was 17.6%. This mixture of six plant species with white clover, lucerne, and four Poaceae plant species also showed good productivity and good forage quality even when using N_{150}. It is interesting to note that in both cases of this study in mixtures with sainfoin and white clover, no significant differences between crude protein were found compared with monoculture swards. In the current study, comparing two legumes with four in mixtures showed that it was not the number of legume plants that increased yield quality or productivity but the selected species by growth properties in the mixtures with other legumes or grasses.

Table 3. Crude protein in the herbage of swards of different composition.

Crude Protein in Dry Matter, % 1st Year of Sward Use						
		N_0			N_{150}	
Treatments	1st Cut	3rd Cut	Mean	1st Cut	3rd Cut	Mean
1. G1	7.7 ab	13.9 ab	10.8 ab	9.5 abcd	16.0 ab	12.7 ab
2. G2	7.3 a	12.8 a	10.1 a	8.7 a	14.9 a	11.8 a
3. L1 + L2/G1	10.6 de	19.1 cde	14.8 def	9.5 abcd	16.1 ab	12.8 abc
4. L1 + L2/G2	9.6 bcde	18.9 cde	14.2 cd	10.4 bcd	16.4 abcd	13.4 bcde
5. L1 + L2/G1 + G2	9.8 bcde	18.9 cde	14.3 cd	9.4 ab	18.3 bcd	13.8 bcde
6. L1 + L2/G1 + G2 + G3 + G4	9.8 bcde	18.8 cde	14.3 cd	11.1 d	18.3 bcd	14.7 e
7. L3 + L1/G1 + G2	11.1 e	21.1 efg	16.1 def	8.9 ab	17.9 bcd	13.4 bcde
8. L3 + L1/G1 + G2 + G3 + G4	10.5 cde	22.7 g	16.6 f	9.6 abcd	19.6 d	14.6 cde
9. L4 + L1/G1 + G2	7.9 ab	16.2 bc	12.0 b	9.6 abcd	16.2 abcd	12.9 abc
10. L4 + L1/G1 + G2 + G3 + G4	8.1 abc	16.7 cd	12.4 bc	9.1 ab	16.6 abcd	12.9abc
11. L1 + L2 + L3 + L4/G1 + G2	10.8 de	21.1 efg	16.0 def	9.2 ab	16.9 abcd	13.1 bc
12. L1 + L2 + L3 + L4/G1 + G2 + G3 + G4	10.3 cde	19.4 de	14.9 def	9.6 abcd	16.8 abcd	13.2 bc
p-value between treatments in cuts	$p < 0.05$	$p < 0.05$	$p < 0.05$	n.s.	$p < 0.05$	$p < 0.05$
2nd year of sward use						
		N_0			N_{150}	
Treatments	1st cut	3rd cut	mean	1st cut	3rd cut	mean
1. G1	9.0 a	12.5 a	10.8 a	9.2 ab	15.4 a	12.3 a
2. G2	9.0 a	12.8 a	10.9 a	8.7 a	15.8 abc	12.2 a
3. L1 + L2/G1	13.8 defg	21.1 de	17.4 cd	10.6 cde	17.3 bcd	14.0 bc
4. L1 + L2/G2	13.9 defg	20.5 de	17.2 cd	11.1 cde	17.5 cd	14.3 bcd
5. L1 + L2/G1 + G2	13.7 de	19.7 bcde	16.7 cd	10.4 bcd	17.5 cd	13.9 bc
6. L1 + L2/G1 + G2 + G3 + G4	13.9 defg	20.8 de	17.3 cd	12.7 g	17.6 cd	15.1 cdef
7. L3 + L1/G1 + G2	14.4 efg	21.9 e	18.2 cd	11.5 defg	19.8 ef	15.7 ef
8. L3 + L1/G1 + G2 + G3 + G4	15.8 g	21.5 de	18.7 d	11.9 efg	19.8 f	15.9 f
9. L4 + L1/G1 + G2	11.9 cd	16.8 b	14.4 b	9.3 ab	15.4 ab	12.3 a
10. L4 + L1/G1 + G2 + G3 + G4	11.0 bc	17.3 bc	14.2 b	10.2 bc	16.4 abc	13.3 ab
11. L1 + L2 + L3 + L4/G1 + G2	15.0 efg	20.5 de	17.8 cd	11.0 cde	19.8 ef	15.4 def
12. L1 + L2 + L3 + L4/G1 + G2 + G3 + G4	14.3 efg	20.0 cde	17.2 cd	11.4 defg	18.8 def	15.1 cdef
p-value between treatments in cuts	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$

Dominant plant species in the swards: perennial ryegrass (G1), Festulolium (G2), meadow fescue (G3), timothy (G4); white clover (L1), red clover (L2), lucerne (L3) and sainfoin (L4). Different letters indicate significant differences between the treatments ($p < 0.05$); n.s.—not sig-nificant.

4. Discussion

DM yield and impact of legumes and mineral N fertilisers. The current study found higher forage yields in grass–legume mixtures, especially in 60–40% grass–legume mixtures, than in monocultures. This might be due to better use of the spaces by various species and the positive interaction between them. A strong relationship between species richness and yield productivity has been reported in the literature; the strength of diversity effects was improved with higher legume amounts [21,28]. The observed diversity effects may be due to the important effects of legumes even at low richness, interaction between the two grass species, or a store of N from the previous presence of legumes [28]. Strong yield reductions (e.g., −48%) were observed in single-species harvests with N_0. Similar effects were seen in monocultures and four-species and six-species mixtures containing legumes such as sainfoin and white clover, and annual yield did not depend on grass amounts in these mixtures, e.g., perennial ryegrass with Festulolium in mixture or perennial ryegrass, Festulolium, meadow fescue, and timothy grass annual yield. These two legumes in the mixtures did not have a significant effect on annual yield. Fertilisation increased yield by

24% but remained the most unproductive. Another important finding was that replacing one legume—sainfoin—in the mixture with lucerne can increase the yield of the same mixture by 48%. Therefore, we can assume that not all legumes have the same effect on sward yield and quality; prior studies have also noted the importance of this [29]. The sensitivity of sainfoin to the cutting regime might be the reason for its low productivity in this experiment, in addition to being influenced by soil properties; sainfoin shows better growth properties in gravelly soils.

Meeting the need for nutrients is important for the growth of quality swards, and nutrient richness among the diverse species can help transform food systems [5]. Previous study data have supported expectations of more rapid grass growth and augmented nutrient uptake in the presence of a legume. Legumes grew more slowly in mixtures, and the growth became more reliant on soil P [30]. In our experiment, when legumes were used the effect was equivalent to the use of N fertilisers, with additional effects in mixtures of different species composition. The value of the sward's productivity suggested that a weak link may exist between mixtures and additional fertilisation. N_{150} increased low productivity legume or other grasses' annual yield, but there was no significant effect on other grass/legume swards. The general practice of highly fertilising single-species or multi-species swards has often been criticised because of the environmental and financial costs associated with high levels of fertiliser application [12] and the inability of a monoculture to fully use system resources. Thus, there is a need for productive systems that demand less fertiliser and have positive environmental impacts [24]. These results therefore need to be interpreted with caution.

Response of legumes in multi-species swards using fertilisation. DM yield was strongly related to functional group richness and species composition. As mentioned in the literature review [26], reduced use of N fertiliser application may mitigate N_2O emissions. Comparison of our findings with those of other studies confirmed that multi-species swards have lower emissions than single-species swards. This result may be explained by the fact that emissions are lowest in combinations of species with high root biomass and diverging root morphology. The results indicated that fertilisers reduced legume plants in the multi-species swards [31]; the least resistant was white clover, followed by red clover and lucerne. Sainfoin had no effect on sward productivity at all, only traces of it were found in the mixtures. Taken together, these results suggested that there is no need to fertilise swards of different species composition, as they are more productive compared to fertilised swards. Furthermore, fertilisers could influence N_2O emissions directly and, when reduced, legumes indirectly.

Seasonal productivity and stability of multi-species cover. The present study raised the possibility that plant species diversity in swards increases and stabilises biomass yields under adverse climatic conditions. In the first year of sward use, evaluating second cut productivity when natural drought conditions were identified before the cut, it was found that the productivity of multi-species swards with legumes was about 50% higher with N_0 fertilisers compared with N_{150}. Meanwhile, the average DM yield of monoculture swards increased by only 14% under adverse climatic conditions using N_{150}. Previous studies [23] evaluating drought effects observed inconsistent results in the variability of monoculture yields, depending on the year and species, with a 1–24% reduction and a 2–14% reduction in separate years. This finding was also reported by [32], with a wide range of species-specific drought effects on annual yield (from a 6% increase to a 40% reduction). This study supported evidence from previous observations [32] that the impact of drought is mitigated by increasing diversity. This study was able to demonstrate that drought tolerance is determined by species identity and functional group diversity more than by species diversity within multi-species swards; this finding was also reported by [33,34]. These results corroborated the findings of many previous works that biodiversity promotes ecosystem functioning and stability [29,35]. In both the first and second years of sward use, the first harvest was the most productive. This is important because it can be used to make winter forage. Other harvests were also important in assessing the productivity and stability of swards. At the end of plant vegetation, yields were lowest in all cases. In our experiment, the second year of sward use was more productive with five cuts, compared to the first year with four cuts, because of drought conditions and a shorter vegetation period. However, we need more data on how physiological and ecological mechanisms drive diversity effects [36]. These results further supported the idea of studying how they may interact with soil nutrient availability.

Strong effects of functional group richness and composition on crude protein. The quality of the grasslands depended on the functional group and species composition [29]. In both experimental years no significant differences were found in the evaluation of crude proteins between monoculture swards with perennial ryegrass and Festulolium. Significantly, crude protein was increased in multi-species swards with N_0 fertiliser rate compared with monoculture swards. No higher amounts of crude protein in the swards were found with N_{150}. The highest production of crude protein in mixtures was improved only by the presence of lucerne in the swards.

Forage legumes such as lucerne, red clover, and white clover are rich in proteins, and the yield of crude proteins is known to depend on the proportion of legumes in the sward and its productivity.

5. Conclusions

The two-year research into the productivity potential of multi-species swards grown with mineral N_{150} fertilisers and without N_0 showed variability and differences between multi-species swards between and within growing seasons. The results suggested that limited enrichment of grasslands by individual species (up to 6–8 species) is valuable for the quantity and quality of sward yields in the Nemoral zone. Mixtures with legumes of different species compositions give the swards more potential for productivity and good forage quality without N fertilisers than with the use of 150 kg ha^{-1} mineral N. N fertilisers reduced legume DM yields in the multi-species swards. The use of lucerne, red clover, and white clover at different compositions in mixtures, especially in the newest swards (first two years of use), without N fertilisation avoided not only a loss of yield and quality but also, most importantly, contributed to a more moderate use of mineral nitrogen fertilisers in grasslands and thus mitigated other factors of climate change and farming systems.

The use of mineral N fertilisers had a significant impact on single-species grass sward growth, but these swards were characterised by the lowest quality of crude protein. Grasslands with productive legumes had a more stable yield of dry matter, observing separate cuts in the experimental years, but when using mineral N fertilisers, there was no trend between the productivity of monoculture grasslands or swards with different species composition.

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