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ORIGINAL PAPER

The effect of different proportions of *Medicago media* Pers. in two-component alfalfa-grass mixtures on the macronutrient content of aboveground biomass

Marzenna Olszewska, Ewa Mackiewicz-Walec

Department of Agrotechnology and Agribusiness, University of Warmia and Mazury in Olsztyn, Poland

Abstract

The aim of this study was to evaluate the effect of different proportions of hybrid alfalfa (*Medicago media* Pers.) grown in two-component mixtures with festulolium (*Festulolium braunii* (K. Richt.) A. Camus) and orchard grass (*Dactylis glomerata* L.) on the macronutrient content of aboveground biomass. The study was conducted in Poland in 2011-2013. A small field experiment was performed at the Agricultural Experiment Station in Bałdy (53°72′N, 20°42′E) owned by the University of Warmia and Mazury in Olsztyn. The experiment had a split-plot design with four replications, and the experimental variables were: (i) mixtures of *D. glomerata* (Dg)+*M. media* (Mm), and *F. braunii* (Fb)+*M. media* (Mm), and (ii) proportion of *M. media* (Mm) seeds in the mixture: 30%, 50% and 70%. Pure-sown *D. glomerata* and pure-sown *F. braunii* were the control treatments. The proportion of hybrid alfalfa in the sward increased in successive years of the experiment. In the second and third year of the study, alfalfa was the dominant species in the mixture regardless of the proportion of sown seeds. In the analyzed grass mixtures, alfalfa was subjected to greater competitive pressure from orchard grass than from festulolium. The choice of the grass component in mixtures with alfalfa exerted a greater influence on the macronutrient content of aboveground biomass than the proportion of alfalfa seeds in the mixture. Alfalfa and orchard grass mixtures accumulated more phosphorus (P) and potassium (K) than alfalfa and festulolium mixtures. In turn, calcium (Ca) content was higher in alfalfa and festulolium mixtures. The analyzed mixtures did not differ significantly in magnesium (Mg) levels. In comparison with pure-sown grasses, the cultivation of alfalfa-grass mixtures improved the K:(Ca+Mg) ratio, which reached optimal values characteristic of high-quality fodder. The results of the study indicate that alfalfa-grass mixtures should be established based on the selection of a grass component adapted to local agroecological conditions, rather than the proportions of seeds in the mixture.

Keywords: grasses, alfalfa, potassium, phosphorus, calcium, magnesium

Marzenna Olszewska, PhD DSc, prof. UWM, Department of Agrotechnology and Agribusiness, Faculty of Agriculture and Forestry, University of Warmia and Mazury in Olsztyn, ul. Oczapowskiego 8, 10-719 Olsztyn, Poland, e-mail: marzenna.olszewska@uwm.edu.pl,
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INTRODUCTION

Nutrient concentrations and ratios in fodder play a key role in animal nutrition (Kumar and Soni 2014, Zhao and Müller 2016, Grzegorczyk et al. 2020, Rahman et al. 2022). Deficient or excessive intake of essential nutrients and low nutrient bioavailability exert a negative impact on animal health and performance (Aydin and Uzun 2008, Bijelić et al. 2013, Yoshihara et al. 2013, Jones *and* Tracy 2015, Valdez-Cepeda et al. 2017, Olszewska 2022). Nutrient concentrations in plants are influenced by numerous factors, including plant species, variety, climate, soil conditions, season, time of harvest, and fertilization (Pirhofer-Walzl et al. 2011, Kulik 2012, Wyłupek et al. 2014, Jones and Tracy 2015, Schlegel et al. 2016, Valdez-Cepeda et al. 2017, Reiné et al. 2020, Olszewska 2021, Rahman et al. 2022, Tahir et al. 2022, Olszewska 2022). Fertilizers affect the botanical composition of the sward, which directly influences the quality of green fodder because plant species differ in their ability to accumulate nutrients under the same agricultural conditions (Aydin and Uzun 2008, Finn et al. 2013, Aponte et al. 2019, Reiné et al. 2020).

Fodder quality is highly influenced by the K:(Ca+Mg) ratio, which can be imbalanced by an excess concentration of potassium (K) and a deficiency of magnesium (Mg) and calcium (Ca) (Aydin and Uzun 2008, Hejcman et al. 2016, Gao et al. 2017, Grzegorczyk and Grabowski 2019). Cations enter into antagonistic and synergistic interactions, and a macronutrient imbalance can compromise feed conversion and lead to metabolic disorders in animals (Schlegel et al. 2016, Rahman et al. 2022). A K:(Ca+Mg) ratio higher than 2.2 increases the risk of grass tetany (Jefferson et al. 2001, Aydin and Uzun, 2008, Rahman et al. 2022). The symptoms of this metabolic disease include decreased milk yields, lower weight gains, convulsions, or even death. The K:(Ca+Mg) ratio of plants is considerably affected by nitrogen (N) fertilization. Nitrogen fertilizers can increase K levels in grasses when K is highly available in soil, or decrease K content when plants are grown on K-deficient soil (Neto et al. 2009). Nitrogen also affects the concentration of Ca in plants. Staniak and Księżak (2008) reported a directly proportional decrease in the Ca content of grass biomass in response to higher N rates. Nitrogen decreases legumes' ability to compete with grasses (Aydin and Uzun 2008), which significantly affects the nutritional value of fodder.

To minimize the environmental impact of agricultural production, new solutions are being sought to reduce mineral fertilizer doses without compromising fodder quality. According to the literature, legume-grass mixtures show considerable promise in this respect (Nyfeler et al. 2009, Rasmussen et al. 2012, Lüscher et al. 2014, Aponte et al. 2019, Tahir et al. 2022). Grass and small-seeded legume mixtures decrease the demand for N and reduce the costs of roughage production (Himstedt et al. 2010, Lüscher et al. 2014, Staniak and Harasim 2018, Aponte et al. 2019). Rasmussen et al. (2012)

observed that alfalfa grown in a mixture with grasses receives around 80% of its N supply via symbiotic fixation. Alfalfa fixes more atmospheric N_2 when grown with grasses than in pure stands because grasses deplete soil N reserves and force alfalfa plants to fix larger amounts of atmospheric N (Nyfeler et al. 2009, Nyfeler et al. 2011, Bélanger et al. 2014). Alfalfa-grass mixtures are also desirable from the dietary point of view because they improve the content and proportions of nutrients in fodder (Vasileva and Naydenova 2017, Aponte et al. 2019, Yüksel and Balabanli 2021, Tahir et al. 2022). Small-seeded legumes are also less sensitive to drought than grasses (Li et al. 2015, Crème et al. 2016, Yüksel and Balabanli 2021), and both mixture components complement each other when soil moisture levels are low during the growing season (Staniak and Harasim 2018). Alfalfa plays a special role in this context. This leguminous plant has long roots, which are capable of extracting water from deep soil layers (Samuil et al. 2012, Bijelić et al. 2013, Bonfim-Silva et al. 2019, Tahir et al. 2022). It should also be noted that legume-grass mixtures promote sustainable agricultural practices in line with the European Green Deal strategy.

The aim of this study was to evaluate the effect of different proportions of hybrid alfalfa (*Medicago media* Pers.) grown in two-component mixtures with festulolium (*Festulolium braunii* (K. Richt.) A. Camus) and orchard grass (*Dactylis glomerata* L.) on the macronutrient content of aboveground biomass. The research hypothesis states that different proportions of alfalfa in the mixtures will increase the content of macronutrients and improve the K:(Ca+Mg) ratio in biomass in comparison with pure-sown grasses.

MATERIALS AND METHODS

Field experiment

The study was conducted in Poland, in 2011-2013. A small-area field experiment was performed at the Agricultural Experiment Station in Bałdy (53°72′ N, 20°42′ E) owned by the University of Warmia and Mazury in Olsztyn. The experiment was established on mineral soil of quality class IVa and very good rye complex according to the Polish soil classification system. Composite soil samples were collected from each plot to a depth of 20 cm to determine the chemical properties of soil. The arable layer was characterized by very low phosphorus (P) content (20.1 mg kg^{-1}) and moderate concentrations of K (123.8 mg kg^{-1}) and Mg (30.2 mg kg^{-1}). Soil pH was neutral (pH_{KCl} 7.2). Soil pH was measured using a digital pH meter with temperature compensation (20°C) in deionized water and 1 mol dm^{-3} KCl, at a 5:1 ratio. Plant-available P and K were measured by the Egner-Riehm method. Phosphorus was determined by the colorimetric method (UV-1201V spectrophotometer, Shimadzu Corporation Kyoto, Japan), and K was determined by atomic emission spectrometry (AES) (BWB Technologies UK Ltd. Flame Photometers).

Magnesium was determined by atomic absorption spectrophotometry (AAS) (AAS1N, Carl Zeiss Jena, Germany). Seeds were sown on 23 April 2010. The experiment had a split-plot design with four replications, and the harvested plot size was 10 m^2. The experimental variables were: (i) mixtures of *Dactylis glomerata* (Dg)+*Medicago media* (Mm), and *Festulolium braunii* (Fb)+*Medicago media* (Mm), and (ii) proportion of *Medicago media* (alfalfa) seeds in the mixture: 30%, 50% and 70%. Pure-sown *D. glomerata* (orchard grass) and pure-sown *F. braunii* (festulolium) were the control treatments. Before sowing, NPK fertilizers were applied to all plots in the following amounts: 30 kg N ha^{-1} (ammonium nitrate, 34% N), 80 kg P ha^{-1} (enriched superphosphate, 40% P_2O_5) and 60 kg K ha^{-1} (potassium salt, 60% K_2O). In pure-sown treatments, the seeding amount was 20 kg ha^{-1} for *D. glomerata* and 40 kg ha^{-1} for *F. braunii*. In the experimental treatments, the seeding amounts corresponded to the percentages of the analyzed species in the mixture. Seeds were broadcast, and cover crops were not used. In the years of full utilization, pure-sown *D. glomerata* and *F. braunii* were fertilized with N at 180 ha^{-1}, and mixtures were fertilized with N at 90 kg ha^{-1}. Nitrogen fertilizer was split into three equal doses and applied in early spring, after the first and after the second harvest. All plots were fertilized with P at 80 kg ha^{-1} (one spring application) and K at 120 kg ha^{-1} (in spring and after the first harvest).

Plant materials

The sward was mowed three times in every growing season. The first regrowth of the mixtures was mowed at the beginning of the heading stage, and subsequent regrowths were mowed in the bud development stage of alfalfa. One-kilogram green biomass samples were collected after each harvest to determine the percentage of alfalfa in the yield and analyze the chemical composition of plants. Chemical analyses were performed separately for the mixtures and individual components. Phosphorus concentration was determined by colorimetry with ammonium molybdate (UV-1201V spectrophotometer, Shimadzu Corporation Kyoto, Japan); K and Ca content was determined by flame photometry (BWB Technologies UK Ltd. Flame Photometers), and the concentration of Mg was determined by atomic absorption spectrometry (AAS; Shimadzu AA-6800 spectrophotometer – Shimadzu Corp., Kyoto Japan). The K:(Ca+Mg) ratio was calculated. The proportion of alfalfa in the mixtures was determined in botanical analyses on a green weight basis.

Statistical analysis

The results were processed statistically by analysis of variance (ANOVA). The significance of differences between treatment means was determined by the Tukey's test at $p<0.05$. All calculations were performed using Statistica v. 13.3 software (TIBCO Software Inc. 2017).

Weather conditions

Weather conditions during 2011-2013 are presented in Table 1. The growing season of 2011 was characterized by favorable conditions for plant growth and development, and average monthly temperatures approximated the long-term (1981-2010) average. With the exception of April, total precipitation in 2011 exceeded the long-term average, and rainfall levels in July were nearly three times higher than the long-term average. The second year of the experiment was characterized by moderate temperatures, high precipitation in April, June and July, and a minor water deficit in August and September. In 2013, weather conditions were less favorable for plant growth due to low temperatures in early spring and considerable rainfall deficiency in June and August.

Table 1

Mean air temperature and precipitation in 2011-2013

Years	Growing season						Mean
	Apr	May	Jun	Jul	Aug	Sep	
	Mean air temperature (^{o} C)						
2011	9.1	13.1	17.1	17.9	17.6	14.1	14.8
2012	7.8	13.4	15.0	19.0	17.7	13.5	14.4
2013	5.9	14.8	17.5	18.0	17.4	11.3	14.2
Long-term 1981-2010	7.7	13.5	16.1	18.7	17.9	12.8	14.5
	Precipitation (mm)						
2011	22.5	51.1	81.7	202.8	82.1	67.5	84.6
2012	73.1	51.1	103.2	121.0	45.1	45.7	73.3
2013	28.5	54.5	61.2	121.9	37.6	101.1	67.5
Long-term 1981-2010	33.3	58.5	80.4	74.2	59.4	56.9	60.5

RESULTS AND DISCUSSION

Proportion of hybrid alfalfa in biomass yield

In *F. braunii* (Fb)+*M. media* (Mm) mixtures, the proportion of *M. media* (alfalfa) in the total biomass yield differed significantly from the proportion of alfalfa seeds in the seed mixture. In the first year of the study, alfalfa accounted for around 40% of biomass yield in treatments sown with 30% and 50% of alfalfa seeds, and for around 48% of biomass yield in the treatment sown with 70% of alfalfa seeds (Fig. 1). In the second, fully productive year of the experiment, the share of alfalfa in the total biomass yield increased considerably, and alfalfa accounted for approximately 77%, 63% and 74% of biomass yield in treatments where the seed mixture comprised 30%, 50%

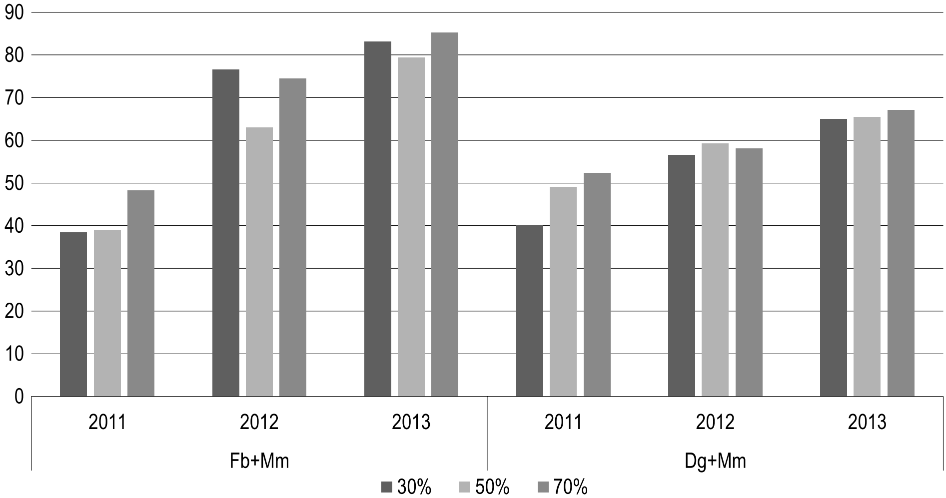

Fig. 1. The average alfalfa share from three regrowths in the green biomass yields of mixtures with various proportions of alfalfa (30%, 50%, 70%)

and 70% of alfalfa seeds, respectively. In the third year of the study, alfalfa was also the dominant component in the mixtures, and its share of biomass yield increased to 80-85%. In 2013, low temperatures in early spring and low precipitation in April, June, and August inhibited the development of festulolium, which, in contrast to alfalfa, has shallow roots and is more susceptible to periodic water deficits. In Dg + Mm mixtures, the share of alfalfa biomass in sward yields also differed from the proportion of alfalfa seeds in the seed mixture. In the first fully productive year, the proportion of alfalfa biomass in the total biomass yield was determined at around 40%, 50%, and 52% in treatments sown with 30%, 50%, and 70% of alfalfa seeds, respectively. In the second year of the experiment, alfalfa had a higher share of biomass yields, but only minor differences were noted between treatments. The proportion of alfalfa biomass was estimated at 57%, 59%, and 58% in treatments where the seed mixture contained 30%, 50%, and 70% of alfalfa seeds, respectively (Fig. 1). Considerable differences in the share of alfalfa in the total biomass yield between years could be attributed to varied weather and soil conditions, as well as changes in interspecific competition (Samuil et al. 2012, Tahir et al. 2022) The proportion of alfalfa in the analyzed mixtures probably increased because alfalfa adapted to local conditions more effectively than orchard grass and festulolium. The proportion of alfalfa biomass in sward yields continued to increase in successive years of the experiment, and the share of alfalfa biomass was higher in mixtures with festulolium than with orchard grass in the second and third year of the study. These observations suggest that alfalfa was subjected to greater competitive pressure from orchard grass than from festulolium. Bijelić et al. (2013) and Yüksel and Balabanli (2021) confirmed the high competitive advantage of orchard grass grown in mixtures with alfalfa. In turn, Tahir et al. (2022)

reported that grasses were less competitive than legumes, and the proportions of grass biomass in sward yields continued to decrease in successive years of growth.

Phosphorus (P)

In 2011, the P content of biomass was the lowest in pure-sown festulolium and in the mixture of 70% festulolium and 30% alfalfa seeds (Table 2). Phosphorus concentration was significantly higher in pure-sown orchard grass, in the mixture of 50% orchard grass and 50% alfalfa, and in festulolium mixtures containing 50% and 70% alfalfa, but significant differences were not observed between these treatments. Phosphorus content in sward biomass was associated with the proportion of alfalfa in the botanical composition of the mixture (Fig. 1). In the second year of the study, P concentration was significantly higher only in the mixture composed of 50% orchard grass and 50% alfalfa relative to the remaining treatments. In the last year of the experiment, pure-sown orchard grass and festulolium accumulated more P than their mixtures with alfalfa. Phosphorus content was highest in the biomass of pure-sown orchard grass (4.0 g kg^{-1}). Dg+Mm mixtures were also characterized by higher P content than Fb+Mm mixtures. The optimal P content of fodder has been established at 3.0 g kg^{-1} (Valdez-Cepeda et al. 2017). During the three-year study, the average P content of sward biomass ranged from 3.0 to 3.5 g kg^{-1} (Table 2).

Table 2

The average phosphorus content from three regrowths of the aboveground biomass (g kg^{-1} DM)

Treatment	Year			Mean
	2011	2012	2013	
Fb 100%	2.8[a]	3.2[a]	3.8[bc]	3.3[b]
Fb 70% + Mm 30%	2.6[a]	3.2[a]	3.1[a]	3.0[a]
Fb 50% + Mm 50%	3.3[b]	3.4[a]	3.3[ab]	3.3[b]
Fb 30% + Mm 70%	3.1[b]	3.5[ab]	3.1[a]	3.2[ab]
Dg 100%	3.2[b]	3.4[a]	4.0[c]	3.5[c]
Dg 70% + Mm 30%	2.9[ab]	3.2[a]	3.5[b]	3.2[ab]
Dg 50% + Mm 50%	3.3[b]	3.7[b]	3.4[b]	3.5[c]
Dg 30% + Mm 70%	3.0[ab]	3.3[a]	3.6[b]	3.3[b]

Means with the same letter in columns do not differ significantly at $p<0.05$ in the Tukey's HSD test.

Phosphorus concentration was highest in the aboveground biomass of pure-sown orchard grass and in mixtures composed of 50% orchard grass and 50% alfalfa. The analyzed parameter was the lowest in the mixture of 70% festulolium and 30% alfalfa. Phosphorus deficiencies were not observed during the three-year study, and the harvested biomass met the P requirements of cattle. Similar results were reported by Valdez-Cepeda et al. (2017) and Rahman et al. (2022).

An analysis of P levels in the biomass of mixture components revealed that pure-sown grasses accumulated more P than grasses grown with hybrid alfalfa. In pure-sown grasses, the P content was significantly higher in orchard grass than in festulolium (Fig. 2). In the work of Crème et al. (2016), soil P levels were lower in treatments sown with grass-alfalfa mixtures than with pure-sown grasses. These observations suggest that P uptake was higher in alfalfa than in grasses (Roscher et al. 2011, Peñuelas et al. 2012). In grasses, species-specific variations in P uptake can be attributed to differences in root length. Orchard grass has deep roots, which facilitate nutrient uptake, including in periods of water deficit. In the present study, the P content of both grass species was the lowest in mixtures with 30% alfalfa. The P content of sward biomass increased with a rise in the proportion of alfalfa in the mixture. Alfalfa has an extensive root system that transports nutrients from deep soil layers and increases their availability for shallow grass roots.

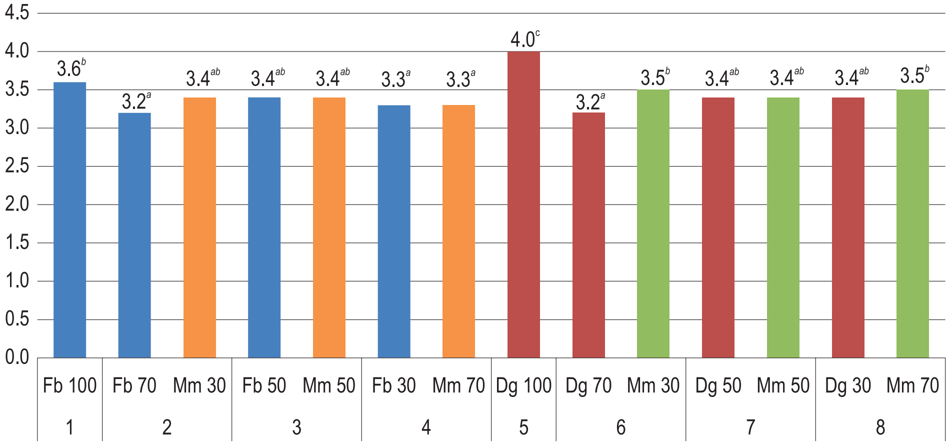

Fig. 2. The average phosphorus content from three regrowths of the aboveground biomass of the analyzed plant species (1-8- objects) during the three-year study (2011-2013) (g kg^{-1}). Means with the same letter do not differ significantly at $p < 0.05$ in the Tukey's HSD test.

Potassium (K)

In fodder crops, the demand for K and its concentration in plant tissues generally exceed the requirements of animals (Aydin and Uzun 2008, Grzegorczyk et al. 2013). The K content of fodder exceeding 30.0 g kg^{-1} contributes to hypomagnesemia in animals (Wilkinson and Mayland 1997). In the first year of the study, K levels were the highest in pure-sown orchard grass (31.0 g kg^{-1}). This parameter was significantly lower in Dg+Mm mixtures, but an increase in the proportion of alfalfa in the mixture had no significant effect on K accumulation in aboveground biomass (Table 3). Kirilov

Table 3

The average potassium content from three regrowths of the aboveground biomass (g kg^{-1} DM)

Treatment	Year			Mean
	2011	2012	2013	
Fb 100%	24.5^{a}	20.0^{b}	24.5^{c}	23.0^{b}
Fb 70% + Mm 30%	25.0^{a}	19.5^{a}	18.6^{ab}	21.0^{a}
Fb 50% + Mm 50%	24.9^{a}	19.5^{a}	18.2^{a}	20.9^{a}
Fb 30% + Mm 70%	25.1^{a}	18.6^{a}	17.5^{a}	20.4^{a}
Dg 100%	31.0^{c}	21.1^{c}	23.8^{c}	25.3^{c}
Dg 70% + Mm 30%	27.2^{b}	21.0^{c}	20.6^{b}	22.9^{b}
Dg 50% + Mm 50%	27.4^{b}	20.6^{c}	21.5^{bc}	23.2^{bc}
Dg 30% + Mm 70%	27.5^{b}	18.7^{a}	19.7^{b}	22.0^{b}

Means with the same letter in columns do not differ significantly at p<0.05 in the Tukey's HSD test.

et al. (2009) reported higher K levels in pure-sown orchard grass and its mixture with alfalfa than in pure-sown alfalfa. In the cited study, the average content of K was 58% and 30% higher in pure-sown orchard grass and its mixture with alfalfa, respectively, than in pure-sown alfalfa. The authors attributed their findings to a negative correlation between K content and crude protein (CP) content. In the present study, the K concentration was the lowest in pure-sown festulolium and its mixtures with alfalfa. Similarly to Dg+Mm mixtures, the K content of sward biomass was not significantly affected by an increase in the proportion of alfalfa in the mixture. In 2012, plants accumulated less K than in 2011. Potassium levels were the highest in pure-sown orchard grass and its mixtures with 30% and 50% alfalfa. In the third year of the experiment, the biomass of pure-sown festulolium and orchard grass was most abundant in K. The potassium content was lower in Dg+Mm mixtures, and the lowest in Fb+Mm mixtures. During the three-year study, the average K content of aboveground biomass ranged from 20.4 to 25.3 g kg^{-1} (Table 3). Pure-sown orchard grass was most abundant in K, and the K content was significantly lower in festulolium biomass. Dg+Mm mixtures accumulated more K than Fb+Mm mixtures, and the proportion of alfalfa in these mixtures had no significant effect on K levels in sward biomass. It should also be noted that the share of alfalfa in the sward differed from the proportion of sown seeds. In the second and third year of the study, alfalfa was the dominant species in all mixtures, and its share was higher in mixtures with festulolium than orchard grass.

The K content of alfalfa biomass was determined at 17.0-17.7 g kg^{-1} DM, and no significant differences in K levels were found between treatments with different proportions of alfalfa or different grass components (Fig. 3). Grasses accumulated significantly more K than alfalfa. The potassium content reached 26.2-28.5 g kg^{-1} in orchard grass and 23.7-26.2 g kg^{-1} in festulo-

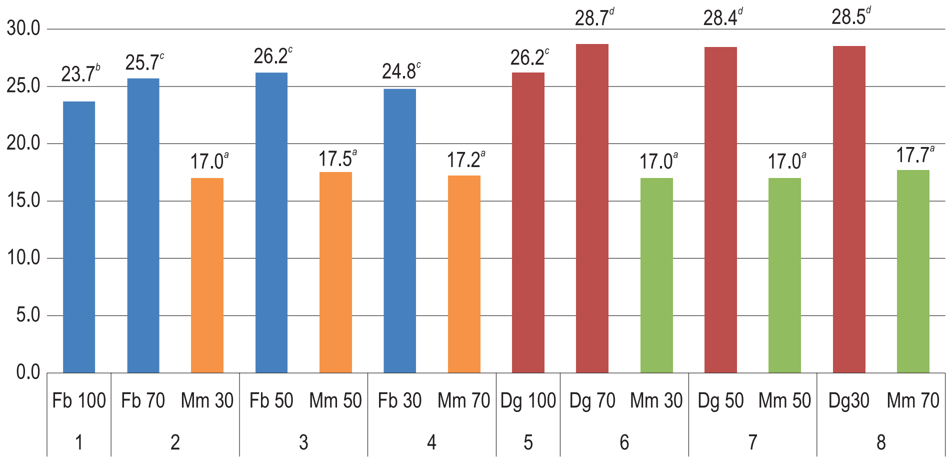

Fig. 3. The average potassium content from three regrowths of the aboveground biomass of the analyzed plant species during (1-8- objects) the three-year study (2011-2013) (g kg^{-1}). Means with the same letter do not differ significantly at $p<0.05$ in the Tukey's HSD test.

lium. It should be noted that pure-sown grasses accumulated less K than grasses grown with alfalfa, but the percentage share of alfalfa in these mixtures did not induce significant differences in K content.

Calcium (Ca)

In the first year of the experiment, the Ca content was the lowest in the biomass of pure-sown grasses, but no significant differences were observed between grass species (Table 4). The calcium content on a dry matter basis was significantly higher in Fb+Mm mixtures, and the highest in Dg+Mm mixtures. However, the proportions of sown seeds did not induce

Table 4

The average calcium content from three regrowths of the aboveground biomass (g kg^{-1} DM)

Treatment	Year			Mean
	2011	2012	2013	
Fb 100%	8.9[a]	4.7[a]	10.8[a]	8.1[a]
Fb 70% + Mm 30%	12.5[b]	8.2[c]	14.3[d]	11.7[c]
Fb 50% + Mm 50%	11.4[b]	7.6[c]	16.1[f]	11.7[c]
Fb 30% + Mm 70%	12.0[b]	8.9[d]	15.6[e]	12.2[d]
Dg 100%	9.4[a]	4.8[a]	11.1[a]	8.4[a]
Dg 70% + Mm 30%	13.2[c]	6.4[b]	12.4[c]	10.7[b]
Dg 50% + Mm 50%	13.8[c]	8.2[c]	11.5[b]	11.2[b]
Dg 30% + Mm 70%	13.6[c]	9.9[d]	11.8[b]	11.8[c]

Means with the same letter in columns do not differ significantly at $p<0.05$ in the Tukey's HSD test.

significant differences in the Ca content across treatments. The calcium concentration was determined at 11.4-12.5 g kg^{-1} DM in Fb+Mm mixtures and 13.2-13.8 g kg^{-1} DM in Dg+Mm mixtures.

The analyzed plants accumulated less Ca in the second than in the first year of the study. Similarly to the first year, the Ca content was the lowest in the biomass of pure-sown grasses. The calcium concentration was significantly higher in alfalfa-grass mixtures, and it was the highest in festulolium and orchard grass mixtures with a 70% share of alfalfa. In 2013, Ca levels were also higher in grass mixtures than in pure-sown grasses. In the tested Fb+Mm mixtures, the Ca content was the highest in that with a 50% share of alfalfa seeds.

In Dg+Mm mixtures, the Ca content was the highest in treatments with a 30% share of alfalfa seeds. During the three-year study, an average Ca content was higher in grass and alfalfa mixtures than in pure-sown grasses (Table 4). The calcium concentration was also significantly higher in Fb+Mm mixtures. It should be noted that in both Fb+Mm and Dg+Mm mixtures, the Ca content was the highest in treatments with a 70% share of hybrid alfalfa. In the work of Kirilov et al. (2009), the Ca content was also higher in orchard grass-alfalfa mixtures than in pure-sown orchard grass. The cited authors attributed their findings to a negative correlation between K and Ca concentrations in the analyzed plants. In a study by Rahman et al. (2022), the Ca content was 3-7 times higher in leguminous plants than in grasses.

In the present study, the Ca content of festulolium biomass did not differ between pure-sown Fb and Fb+Mm mixtures (Fig. 4). In turn, orchard grass accumulated significantly less Ca when sown in equal proportions with Mm. Calcium levels were also lower in the biomass of alfalfa grown in mixtures with 70% and 50% of Dg seeds than in the remaining treatments.

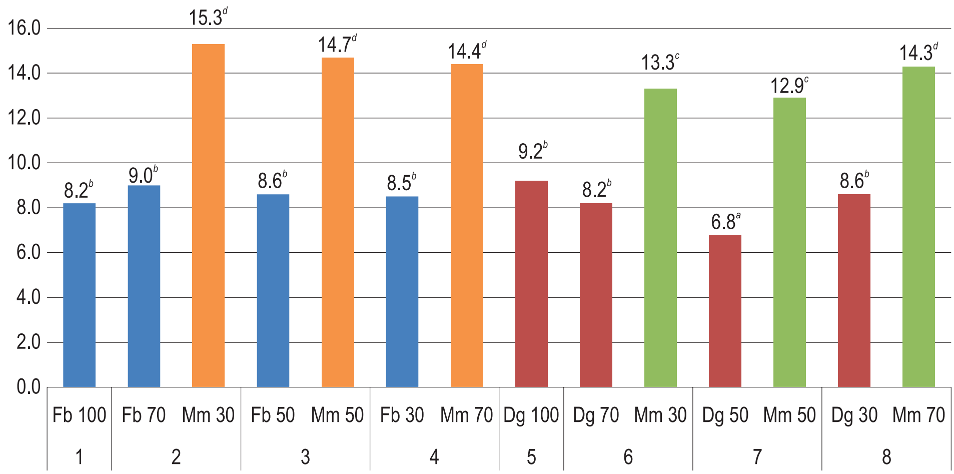

Fig. 4. The average calcium content from three regrowths of the aboveground biomass of the analyzed plant species during (1-8- objects) the three-year study (2011-2013) (g kg^{-1}). Means with the same letter do not differ significantly at $p<0.05$ in the Tukey's HSD test.

Magnesium (Mg)

The optimal supply of dietary Mg for dairy cattle has been established at 2.0 g kg^{-1} DM (Rahman et al. 2022). In the present study, the average Mg concentration in sward biomass was determined at 2.1-2.3 g kg^{-1} DM. The Mg content of aboveground biomass did not differ significantly between treatments or experimental years (Table 5). Significantly higher Mg levels

Table 5

The average magnesium content from three regrowths of the aboveground biomass (g kg^{-1} DM)

Treatment	Year			Mean
	2011	2012	2013	
Fb 100%	1.9^{a}	2.1^{a}	2.8^{b}	2.3^{a}
Fb 70% + Mm 30%	2.0^{a}	2.2^{a}	2.4^{a}	2.2^{a}
Fb 50% + Mm 50%	2.0^{a}	2.0^{a}	2.4^{a}	2.1^{a}
Fb 30% + Mm 70%	2.1^{a}	2.2^{a}	2.4^{a}	2.2^{a}
Dg 100%	2.1^{a}	2.0^{a}	2.3^{a}	2.1^{a}
Dg 70% + Mm 30%	2.4^{b}	2.0^{a}	2.3^{a}	2.2^{a}
Dg 50% + Mm 50%	2.1^{a}	2.1^{a}	2.3^{a}	2.2^{a}
Dg 30% + Mm 70%	2.1^{a}	2.2^{a}	2.4^{a}	2.2^{a}

Means with the same letter in columns do not differ significantly at $p<0.05$ in the Tukey's HSD test.

were noted only in orchard grass grown with 30% alfalfa in the first year and in pure-sown festulolium in the third year of the study. In the remaining treatments, the observed differences were not significant.

The study revealed species-specific differences in the Mg content of biomass. Orchard grass was more abundant in Mg than festulolium (Fig. 5).

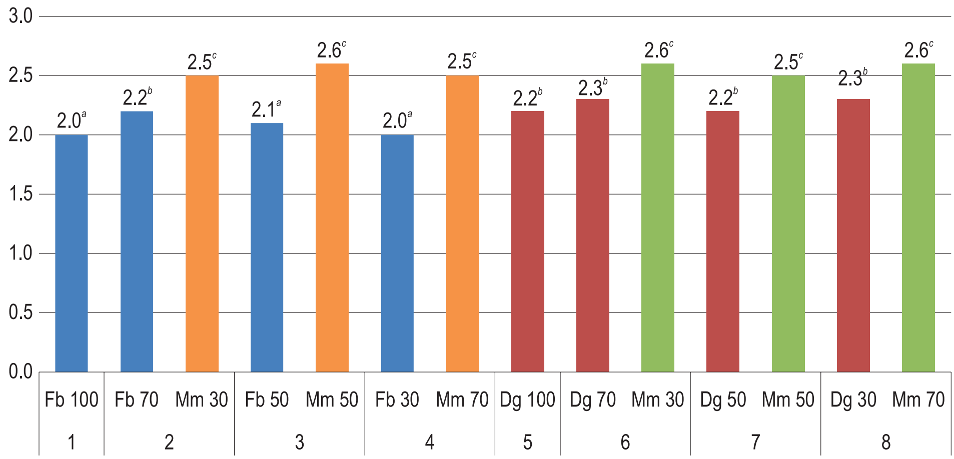

Fig. 5. The average magnesium content from three regrowths of the aboveground biomass of the analyzed plant species during (1-8- objects) the three-year study (2011-2013) (g kg^{-1}). Means with the same letter do not differ significantly at $p<0.05$ in the Tukey's HSD test.

Alfalfa accumulated significantly more Mg than pure-sown grasses, but no differences in Mg content were found between Fb+Mm and Dg+Mm mixtures. Similar results were reported by Rahman et al. (2022) who observed differences in Mg levels across plant species and varieties. In the cited study, the average Mg content was determined at 2.2 g kg^{-1} in grasses and 3.32 g kg^{-1} in leguminous plants.

K:(Ca+Mg) ratio

The K:(Ca+Mg) ratio should not exceed 2.2 in high-quality cattle feed (Aydin and Uzun 2008, Acar et al. 2009, Grzegorczyk et al. 2013, Gao et al. 2017, Rahman et al. 2022). According to Rahman et al. (2022), imbalanced supply of dietary K, Ca, and Mg can lead to metabolic disorders, such as hypomagnesemia and hypocalcemia, which can have fatal consequences for dairy cattle. High levels of dietary K decrease Mg and Ca absorption in ruminants (Mayland and Shewmaker 2001, Rahman and Saiga 2006). In the current study, the K:(Ca+Mg) ratio was determined at 1.5-2.5 on average (Table 6).

Table 6

The average K:(Ca+Mg) ratio from three regrowths of the aboveground biomass

Treatment	Year			Mean
	2011	2012	2013	
Fb 100%	2.3^{b}	2.9^{e}	1.8^{c}	2.3^{c}
Fb 70% + Mm 30%	1.7^{a}	1.9^{c}	1.1^{a}	1.6^{a}
Fb 50% + Mm 50%	1.9^{a}	2.0^{cd}	1.0^{a}	1.6^{a}
Fb 30% + Mm 70%	1.8^{a}	1.7^{b}	1.0^{a}	1.5^{a}
Dg 100%	2.7^{c}	3.1^{f}	1.8^{c}	2.5^{c}
Dg 70% + Mm 30%	1.7^{a}	2.5^{d}	1.4^{b}	1.9^{b}
Dg 50% + Mm 50%	1.7^{a}	2.0^{cd}	1.5^{b}	1.7^{ab}
Dg 30% + Mm 70%	1.7^{a}	1.5^{a}	1.4^{b}	1.5^{a}

Means with the same letter in columns do not differ significantly at $p<0.05$ in the Tukey's HSD test.

In the first year of the experiment, the K:(Ca+Mg) ratio in pure-sown grasses exceeded the optimal value, reaching 2.3 in festulolium and 2.7 in orchard grass. The value of the analyzed parameter improved when grasses were sown with alfalfa. The K:(Ca+Mg) ratio ranged from 1.7 to 1.9 in Fb+Mm mixtures, and reached 1.7 in Dg+Mm mixtures. Rahman et al. (2022) also reported a more favorable K:(Ca+Mg) ratio in grass-legume mixtures. In the present study, the proportion of alfalfa seeds did not induce significant differences in the K:(Ca+Mg) ratio. A similar trend was noted in the second year of the experiment. The K:(Ca+Mg) ratio considerably exceeded the optimal value in the aboveground biomass of pure-sown grasses, but in the analyzed mixtures, a suboptimal value of this parameter was found only in the mixture composed of 30% alfalfa and 70% orchard grass seeds. The K:(Ca+Mg) ratio was within the norm in the remaining treatments. The examined parameter was the lowest in the last year of the experiment. Optimal values of the K:(Ca+Mg) ratio were noted in all treatments, but a significantly higher value (1.8) was observed in the biomass of pure-sown grasses.

A species analysis revealed that the aboveground biomass of alfalfa was characterized by significantly lower values of the K:(Ca+Mg) ratio than the biomass of pure-sown grasses (Fig. 6). However, no significant differences

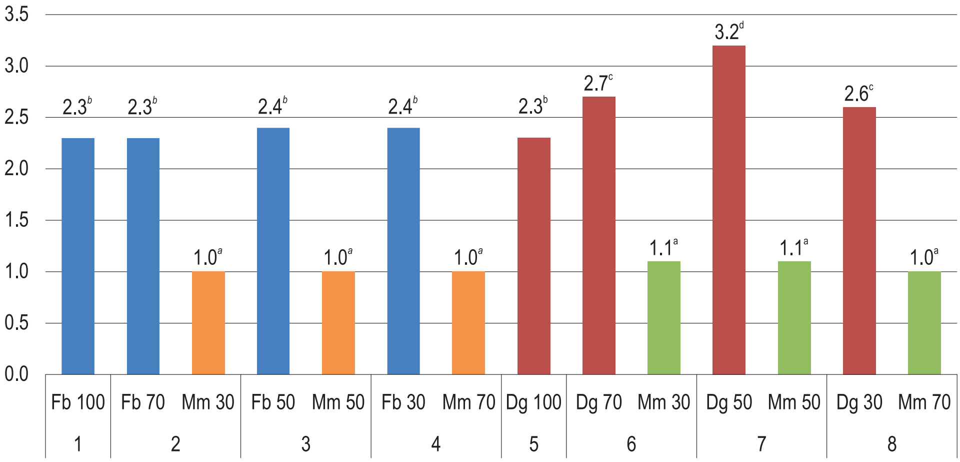

Fig. 6. The average K:(Ca+Mg) ratio from three regrowths of the aboveground biomass in the analyzed plant species during (1-8- objects) the three-year study (2011-2013) (g kg^{-1}). Means with the same letter do not differ significantly at $p<0.05$ in the Tukey's HSD test.

were found between treatments, and the K:(Ca+Mg) ratio was determined at 1.0-1.1 in both Fb+Mm and Dg+Mm mixtures. The analyzed parameter assumed significantly higher values in the biomass of pure-sown grasses. The K:(Ca+Mg) ratio of festulolium ranged from 2.3 to 2.4. Greater differences were observed in orchard grass. The K:(Ca+Mg) ratio reached 2.3 in the biomass of pure-sown grass, and it ranged from 2.6 to 3.2 in Dg+Mm mixtures. The highest value of this parameter (3.2), which considerably exceeded the norm, was noted in the mixture composed of 50% orchard grass and 50% alfalfa seeds. Such a high value of the K:(Ca+Mg) ratio is indicative of considerable Ca and Mg deficiency in plant biomass.

CONCLUSIONS

The proportion of hybrid alfalfa in the sward increased in successive years of the experiment. In the analyzed grass mixtures, alfalfa was subjected to greater competitive pressure from orchard grass than from festulolium. The choice of the grass component in mixtures with alfalfa exerted a greater influence on the macronutrient content of aboveground biomass than the proportion of alfalfa seeds in the mixture. The results of the study indicate that alfalfa-grass mixtures should be established based on the selection of a grass component adapted to local agroecological conditions, rather than the proportions of seeds in the mixture.

Author contributions

M.O. – conceptualization, investigation, methodology, visualization, writing; E.M-W. writing – review & editing and supervision. All authors helped with the interpretation of the results and provided critical feedback on the whole manuscript. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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