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ORIGINAL PAPER

EFFECT OF THE APPLICATION OF A BIOSTIMULANT AND MINERAL FERTILIZERS ON THE CONCENTRATION OF MINERAL ELEMENTS IN THE SWARD OF FORAGE MIXTURES CULTIVATED ON LIGHT SOIL*

Karol Wolski[1], Magdalena Biernacik[1], Sebastian Świerszcz[1, 2], Marta Talar-Krasa[1], Oleksandra Leshchenko[3]

[1] Institute of Agroecology and Plant Production
Wrocław University of Environmental and Life Sciences, Poland
[2] Botanical Garden, Center for Biodiversity Conservation
Polish Academy of Sciences, Warszawa-Powsin, Poland
[3] Department of Botany
National University of Life and Environmental Sciences, Ukraine

Abstract

The content of macroelements in plant mixtures depends on their species composition and on the share of legume plants, the type and level of fertilization, especially nitrogen treatment, and finally, on the stage at which the plants are harvested. The aim of the study was to determine the effect of mineral fertilizers and a biostimulant on the mineral content of different grass and legume mixtures on sandy soil. The experiment included three factors: (A) forage mixtures (M): M_1 – *Fb* var. Lofa (15%), *Fp* var. Lipanthe (30%), *Lm* var. Mowester (10%), *Lp* (2*n*) var. Gladio (15%), *Lp* (4*n*) var. Verano (30%); M_2 – *Fp* var. Lipanther (30%), *Lm* var. Mowester (10%), *Lp* (2*n*) var. Gladio (20%), *Lp* (4*n*) var. Verano (25%), *Php* var. Liglory (10%), *Tr* var. Grasslands Huia (5%); M_3 – *Fp* var. Lipanther (30%), *Lm* var. Mowester (10%), *Lp* (2*n*) var. Gladio (15%), *Lp* (4*n*) varVerano (20%), *Php* var. Liglory (10%), *Tp* var. Nike (15%); (B) mineral fertilizers; (C) the Bio-Algeen S90 biostimulant based on marine algae. 40 kg P ha^{-1} + 100 kg K ha^{-1}, 80 kg N ha^{-1} + 40 kg P ha^{-1} + 100 kg K ha^{-1}, 160 kg N ha^{-1} + 40 kg P ha^{-1} + 100 kg K ha^{-1}, + biostimulant, 40 kg P ha^{-1} + 100 kg K ha^{-1} + biostimulant, 80 kg N ha^{-1} + 40 kg P ha^{-1} + 100 kg K ha^{-1} + biostimulant, 160 kg N ha^{-1} + 40 kg P ha^{-1} + 100 kg K ha^{-1} + biostimulant. It was found

Magdalena Biernacik, MSc, Division of Grassland and Green Areas Management, Institute of Agroecology and Plant Production, Wroclaw University of Environmental and Life Sciences, pl. Grunwaldzki 24A, 50-363 Wroclaw, Poland, tel. +48 71 320 16 45, e-mail: magdalena.biernacik @upwr.edu.pl

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that the biostimulant had varied effects, depending on the mixture and fertilizers used. The biostimulant in combination with mineral phosphorus and potassium increased the yield by up to 40%.

Keywords: algae extract, plant growth regulator, grass-legume mixtures, sandy soil, fertilizers.

INTRODUCTION

Species composition of a mixture changes in time and depends on the habitat, weather conditions, plant competitiveness (Staniak 2009), mineral fertilizers applied, and the way the crops are grown and managed (Gaweł 2009). Mixtures with an equal proportion of grasses and legumes have the highest nutritional value, with the most favourable mineral content. Likewise, Ścibior and Gaweł (2004) argued that forage for ruminant animals with a 30-50% share of legume plants and 50-70% of grasses had the best chemical composition and, consequently, a high nutritional value. An increased proportion of legume plants reduces the dose of mineral nitrogen applied to a mixture. Cultivation of legume plants, in addition to environmental benefits, is important economically (Rochon et al. 2004). Biostimulants are applied to forage crops and to other plants in order to prevent possible yield losses caused by external stress factors. Affecting plant vitality and yield potential, without changing anything in the genetic system, they neither provide nutrients nor remove the stressor (Pacholczak et al. 2012, Kurepin et al. 2014, Dudaš et al. 2016, Yakhin et al. 2017). The use of biostimulants helps plants to absorb nutrients, to grow a stronger root system, and to produce more green matter, especially leaves, thus facilitating plant survival under unfavourable ambient conditions (Ciepiela, Godlewska 2014, Zaman et al. 2016). In order to prepare plants for stress and improve their resistance to salinity, drought or lack of nutrients, the use of biostimulants is justified (Matysiak, Adamczewski 2006). Mineral fertilizers, on the other hand, have a significant impact on mixture durability, its species composition, the yield, and nutritional value (Wolski 2010). The nutritional value of grass-clover mixtures depends primarily on the macroelement content, shaped by many different factors (Cougnon et al. 2012). The above literature review suggests that there is a need for a paper dealing with the effects of mineral fertilizers and stimulators on legume-grass mixtures.

MATERIALS AND METHODS

The experiment was conducted at the Agricultural Experimental Station at Wroclaw-Swojec (PL 17°08′ E; 51°06′ N). It was set up in a split-plot design on fluvial soil of the IVb quality class made up of loamy sand, with a layer of sand below. The soil had neutral pH of 6.6, high content of phosphorus, and low content of potassium. The plots of 10 m^2 were mowed at a plant height of 5 cm; each year, the mixtures were harvested when the first ears of dominant grasses emerged and during the early budding stage of legume plants. The experiment was set up in 2010, with plant samples collected and analysed between 2011 and 2013. Seed mixtures were sown at the standard dose of 45 kg ha^{-1} and at a depth of 1.0-2.0 cm. The study included the following three factors: (A) a group of forage mixtures (M) – Table 1, (B) mineral NPK fertilizers (Table 2), and (C) the Bio-Algeen S90 biostimulant based on marine algae, containing 90 groups of chemical compounds such as amino acids, vitamins, alginic acid, and other active ingredients of marine algae. The concentration of mineral nutrients was as follows: nitrogen – 0.02%, phosphorus – 0.006%, potassium – 0.096%, calcium – 0.31%, magnesium – 0.021%, boron – 16 mg kg^{-1}, iron – 6.3 mg kg^{-1}, manganese – 0.6 mg kg^{-1}, zinc – 1.0 mg kg^{-1}. The stimulatant also contained molybdenum, selenium, and cobalt.

Table 1

Composition of the forage mixtures

Species and variety of the forage mixture	Abbreviated names	Mixture (M_1)	Mixture (M_2)	Mixture (M_3)
		participation (%)		
F. braunii (Rich.) var. Lofa	*Fb* var. Lofa	15	-	-
F. pratensis Huds. var. Lipanther	*Fp* var. Lipanther	30	30	20
L. multiflorum Lam. var. Mowester	*Lm* var. Mowester	10	10	10
L. perenne L. (2n) var. Gladio	*Lp* (2n) var. Gladio	15	20	20
L. perenne L. (4n) var. Verano	*Lp* (4n) var. Verano	30	25	25
P. pratense L. var. Liglory	*Php* var. Liglory	-	10	10
T. pratense L. var. Nike	*Tp* var. Nike	-	-	15
T. repens L. var. Grasslands Huia	*Tr* var. Grasslands Huia	-	5	-

Table 2

Fertilization of the forge mixture

Mixture	Fertilizer dose (kg ha^{-1})			(L ha^{-1})
	N	P	K	Bio-Algeen S90
M_1, M_2, M_3	-	40	100	-
	80	40	100	-
	160	40	100	-
	-	-	-	5
	-	40	100	5
	80	40	100	5
	160	40	100	5

The following mineral fertilizer treatments were used during the growing periods: phosphorus was applied once in the spring; nitrogen and potassium were applied in three doses: at the beginning of the growing period, after the first cut, and after the second cut. During the spring and after the first and second cut, the biostimulant was applied at a dose of 5 l ha^{-1} Each year between 2011 and 2013, at harvest, fresh matter samples were collected from each plot, with 0.5 kg for chemical analysis and 0.5 kg to determine botanical composition.

After wet mineralization of the plant material (concentrated H_2SO_4 + perhydrol), the dry matter content was determined with the weight method at 105°C. The content of macroelements was determined with the flow spectrophotometric method (N and P), flame emission spectroscopy (K), and with the atom absorption spectrometry method (Ca and Mg).

Statistical analysis (methods)

The research results were statistically processed using the analysis of variance for the split-plot method. Means were compared with the Tukey's test at the significance level of α = 0.05. In order to determine the effect of experimental factors (biostimulant, plant mixture, level of fertilization) on such parameters as dry matter weight, macronutrient content (N, P, K, Ca, Mg), and ratios of chemical elements (N : P, N : K, K : P, and Ca : P), three-factor ANOVA (analysis of variance) was applied with the help of Statistica 12 (www.StatSoft.en). If the data did not meet the normal distribution (P, Ca, and the ratios of N : P, N : K, K : P, Ca : P), logarithmic transformation was used. To investigate the correlation between treatments, plant mixture quality, and the ratio of chemical elements, multivariate analysis was applied. Redundancy analysis (RDA) was used (partial RDA covariate Blocks) because the gradient length was smaller than standard deviation (Lepš, Šmilauer 2003). Environmental variables included biostimulant application, the kind of plant mixture, and fertilization level, while species variables comprised plant mixture quality data, such as the content of dry matter, N, P, K, Ca, and Mg, and ratios between elements. Partial RDA Blocks were used as covariates. Before RDA analysis, all species variables were logarithmically transformed, centred, and standardized by species (species data were centered and normalized with Canoco for Windows). Applying automatic selection, simple (independent) term effect of environmental data on species data was used to determine the effect of experimental factors on the plant mixture nutritional value. After the Bonferroni correction, the statistical significance was verified with the Monte Carlo permutation test, using 999 permutations (hierarchical design); analyses were performed with the Canoco 5 package (ter Braak, Šmilauer 2012).

RESULTS AND DISCUSSION

In the years of full productive use of the mixtures, rainfall during the growing seasons was significantly higher than the average of the 1968-2009 period (404.2 mm). The course of weather conditions during the research is presented in Table 3. On the basis of meteorological data the Selyaninov's

Table 3

The weather condition in the months of the plant growing period between 2011 and 2013

Months	Sums of rainfall in the months (mm)			Average air temperature (°C)			*Hydro-meteorological index of Selyaninov		
	2011	2012	2013	2011	2012	2013	2011	2012	2013
Apr.	27.0	27.6	42.7	11.9	9.8	9.1	0.76	0.94	1.56
May	49.4	63.7	135.9	14.7	15.8	14.6	1.08	1.30	3.00
June	95.7	94.7	171.7	19.1	17.2	17.7	1.67	1.83	3.23
July	170.9	108.0	36.3	18.3	20.0	20.5	3.01	1.74	0.57
Aug.	64.8	73.2	68.2	19.3	19.3	19.0	1.08	1.22	1.16
Sept.	30.3	52.6	121.4	15.5	14.6	12.8	0.65	1.20	3.16
Oct.	42.6	35.4	7.8	9.4	8.6	10.8	1.46	1.33	0.23
Sum Apr.-Oct.	480.7	455.2	584.0						

* < 0.5 severe drought; 0.51-0.69 drought; 0.70-0.99 slight drought; > 1.0 no drought

hydrothermal coefficient (HTC) was calculated to assess rainfall and temperature ($K = 10\ P\ /\ \Sigma t$, where P is a monthly total rainfall; Σt is the sum of daily air temperature values in a given month) (Skowera, Puła 2004). In particular years of observations, coefficient values indicated a slight variation in hydrothermal conditions with the predominance of very humid months. Severe drought was only in October 2013. Unfavourable water balance was also recorded in September 2011 and July 2013, when the Selyaninov's hydrothermal coefficient indicated drought, and in April 2011 and April 2012, when the HTC values to slight drought.

During the research, species composition in mixtures changed and clearly diverged from the composition existing immediately after planting. It was found that there was a 19.2% greater share of grasses and clovers on plots with the biostimulant (94.1% DM) than on units without it (76.0% DM). The average share of grasses in the first harvest was at the level of 67.2% DM, compated to 57.6% DM on the control plot. The participation of red clover (19.5-29.1% DM) was higher than the share of white clover (17.5-24.7% DM). An opposite relationship was noted in an experiment conducted in Lithuania, where red clover initially dominated in mixtures (35.2-48.6% DM), while in the following years a twofold to threefold reduction of its participation and an increase in the proportion of white clover in the sward were found

(KLIMAS 2001). According to JODEŁKA et al. (2006), mineral nitrogen in larger doses considerably simplifies species composition and reduces or eliminates papilionaceous plants in mixtures.

Temperature and CO_2 concentration have strong influence on a plant growth increase, affecting legumes more than grasses. However, changes in seasonal precipitation can reduce those effects, especially in areas with low summer rainfall (HOPKINS, DEL PRADO 2007).

The share of white clover with the fertilization of 160 kg ha^{-1} ranged from 10.5 to 11.6% DM, and red clover from 11.7% DM on the control to 20.2% DM after the application of the biostimulant. The most balanced in terms of species composition was the mixture with meadow fescue, perennial ryegrass, and red clover, while the plots with *Festulolium* were gradually, year by year, infested with weeds. SOSNOWSKI, JANKOWSKI (2010) observed a similar relationship in the cultivation of grass mixtures with legume plants.

In the present experiment, the proportion of grasses and red clover after the application of the biostimulant remained at an equal level over three years. Weed infestation on plots with the biostimulant was nearly twofold lower than on the control. The biostimulant increased the yield by an annual average of 31.3% DM. The DM percentage was as follows: the mixture with *Festulolium* – 21.5% DM, the mixture with Tr – 25.8% DM, and the mixture with Tp 34.8% DM. On the units without NPK or the biostimulant, the dry matter concentration was 31.9%, but the PK treatment increased it to as much as 35.7%. It was somewhat lower on plots with NPK (34.2%) and the lowest as a response to the highest nitrogen fertilization (2N+PK), with 26.0% DM. According to KAYSER, ISSELSTEIN (2005), HEJCMAN et al. (2014), long-term application of mineral nitrogen limited the uptake of P and K from the soil, but an increased uptake of these elements resulted in a higher plant yield.

Results of macronutrient content analysis

Results of the ANOVA test of plant mixture quality parameters are shown in Table 4, while the means with standard deviations (SD) are presented in Table 5. The analysis of the interaction of three factors (biostimulant × mixture × fertilizers) for each variable shows no significant differences. The amount of dry matter (Table 4) differs significantly in relation to the examined factors and the interaction of these factors (biostimulant × mixture; mixture × the level of fertilization). The use of the biostimulant had an impact on the content of P and Ca (F = 10.43, p = 0.002; F = 10.53, p = 0.002, respectively). The ratio of N : P differed significantly between units (F = 8.7, p = 0.005) and mixtures (F = 4.5, p = 0016). The lowest concentration of P (Table 5) was found on plots with K (control) $+M_3$+PK (2.47 ± 0.7 g kg^{-1}), while the highest on plots with B (biostimulant)$+M_2$+PK (4.52 ± 1.73 g kg^{-1}). The Ca content ranged from 10.64 ± 1.78 g kg^{-1}

Table 4

ANOVA test results of forage quality parameters

Factors	DM (t ha^{-1})		N (g kg^{-1})		P (g kg^{-1}) (log)		K (g kg^{-1})		Ca (g kg^{-1}) (log)		Mg (g kg^{-1})	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Unit (*O*)	86.5	< 0.001	0.92	0.343	10.43	0.002	1.93	0.171	10.53	0.002	1.98	0.166
Forage mixture (*M*)	112.2	< 0.001	2.47	0.095	3.03	0.058	0.29	0.751	2.25	0.116	3.20	0.049
Fertilization level (*N*)	68.4	< 0.001	0.58	0.631	1.06	0.375	1.73	0.173	0.19	0.904	1.73	0.174
O × *M*	7	0.002	0.27	0.767	0.21	0.815	2.09	0.135	0.49	0.616	0.29	0.749
O × *N*	1.3	0.295	0.36	0.780	0.17	0.914	0.74	0.531	0.34	0.794	0.84	0.479
M × *N*	3.4	0.008	0.50	0.803	0.99	0.440	1.65	0.154	0.59	0.733	0.65	0.692
O × *M* × *N*	0.1	0.99	0.21	0.973	0.54	0.775	1.01	0.429	0.6	0.726	1.27	0.290

in K+M_2+2NPK to 15.72 ± 3.16 g kg^{-1} in B+M_3+2NPK. There were significant differences in the content of Mg between experimental units (F = 3.2, p = 0.049). The lowest content was recorded in the case of K+M_2+2NPK (1.25 ± 0.45 g kg^{-1}), while the highest one was in B+M_1+PK (2.24 ± 0.87 g kg^{-1}) and B+M_3+0 (2.24 ± 0.56 g kg^{-1}). The treatments did not significantly affect the content of N and K in the forage. The lowest amount of dry matter weight was observed in the case of B+M_2 (*Fb*+*Fp*+*Lm*+*Lp*)+PK (4.09 ± 1.32 t ha^{-1}), while the highest was in M_3 (*Fp*+*Lm*+*Lp*+*Php*+*Tp*)+PK (9.46 ± 3.82 t ha^{-1}) – Table 6. Higher values were recorded on plots without the biostimulant. The highest N:P ratio was in K + M_3 + PK (9.46 ± 3.82), while the lowest in B + M_2 + PK (4.09 ± 1.32). The N : K ratio varied in relation to the plant mixture (F = 3.19, p = 0.05) and ranged from 0.90 ± 0.11 in the case of B + M_2 + PK to 1.46 ± 0.38 B + M_1 + 2NPK. The lowest values were recorded after fertilizer application, i.e. PK and NPK, but the differences were not statistically significant. The K : P ratio differed significantly between units (F = 6.05, p = 0.018). The lowest ratio was recorded in the B + M_1 + 0 (3.63 ± 0.92) and the highest in K + M_3 + NPK (6.45 ± 2.61). The ratio of Ca : P differed between mixtures (F = 4.24, p = 0.02). The values ranged from 2.97 ± 0.32 in the case of B + M_1 + PK to 5.22 ± 1.63 in the case of the K + M_3 + PK. The highest values in the ratio were recorded in M_3, the mixture with red clover.

Multivariate analysis

The results of the RDA (Table 7) showed a strong correlation between the environmental factors and the quality of the mixture (species-environment correlation). For the first two ordination axes, the correlation was 0.897 and 0.538, respectively. These first two axes explain 22.9% of the variance

Table 5

Means and standard deviations of dry matter and chemical element (N, P, K, Ca, Mg) concentration

Unit (A)	Forage mixture (B)	Fertilizer level (C)	*DM (t ha^{-1})	N (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})
Without biostimulant	M1	0	2.55 ± 0.52	24.23 ± 3.19	4.30 ± 1.17	20.67 ± 4.51	13.13 ± 1.28	2.07 ± 0.28
		PK	3.08 ± 0.30	20.58 ± 4.71	3.41 ± 0.48	18.47 ± 0.44	11.20 ± 2.35	1.60 ± 0.51
		NPK	3.96 ± 0.46	19.93 ± 3.88	2.92 ± 0.21	16.76 ± 1.58	11.29 ± 1.66	1.53 ± 0.76
		2NPK	5.36 ± 0.79	20.80 ± 6.93	2.66 ± 0.50	15.10 ± 2.66	10.95 ± 2.33	1.60 ± 0.54
	M2	0	2.94 ± 0.35	21.26 ± 5.41	3.09 ± 0.28	15.14 ± 1.29	11.51 ± 0.63	1.71 ± 0.35
		PK	4.28 ± 0.52	18.95 ± 1.93	4.06 ± 1.51	18.51 ± 0.84	10.93 ± 0.47	1.60 ± 0.18
		NPK	5.40 ± 0.73	18.55 ± 2.42	3.54 ± 0.92	16.56 ± 3.12	11.18 ± 1.12	1.49 ± 0.05
		2NPK	6.74 ± 0.86	16.96 ± 5.29	3.23 ± 0.93	17.01 ± 2.32	10.64 ± 1.78	1.25 ± 0.45
	M3	0	5.03 ± 0.83	20.71 ± 3.17	3.27 ± 1.44	14.45 ± 1.42	11.87 ± 0.91	1.84 ± 0.10
		PK	5.40 ± 0.69	21.58 ± 4.12	2.47 ± 0.70	15.89 ± 2.97	12.14 ± 1.18	1.90 ± 0.45
		NPK	5.95 ± 0.61	20.00 ± 3.11	2.66 ± 0.94	17.36 ± 2.03	11.65 ± 0.73	1.43 ± 0.08
		2NPK	6.96 ± 0.79	21.68 ± 1.73	2.92 ± 1.31	16.64 ± 1.68	12.05 ± 1.06	1.73 ± 0.14
Biostimulant	M1	0	3.26 ± 0.29	19.77 ± 2.77	4.34 ± 1.22	15.66 ± 5.52	11.98 ± 4.35	1.30 ± 0.47
		PK	4.36 ± 0.49	18.50 ± 2.26	4.51 ± 0.96	17.93 ± 5.07	13.16 ± 1.55	2.24 ± 0.87
		NPK	5.22 ± 0.69	17.80 ± 5.22	3.65 ± 0.85	18.61 ± 0.80	12.42 ± 1.53	1.67 ± 0.41
		2NPK	5.90 ± 0.57	22.26 ± 5.46	3.80 ± 1.00	15.35 ± 0.62	13.19 ± 2.34	2.00 ± 0.59
	M2	0	3.59 ± 0.25	18.40 ± 5.62	4.29 ± 0.20	17.41 ± 2.15	13.88 ± 2.35	1.70 ± 0.14
		PK	5.25 ± 0.54	16.97 ± 1.37	4.52 ± 1.73	19.08 ± 2.02	12.90 ± 1.85	1.69 ± 0.20
		NPK	7.03 ± 0.74	19.19 ± 2.59	4.46 ± 1.53	19.33 ± 1.02	13.47 ± 0.77	1.59 ± 0.18
		2NPK	7.57 ± 0.75	17.28 ± 7.73	3.13 ± 0.78	17.27 ± 1.07	10.84 ± 1.48	1.35 ± 0.36
	M3	0	6.98 ± 0.93	21.11 ± 1.94	3.40 ± 1.25	15.47 ± 1.03	13.91 ± 3.08	2.24 ± 0.56
		PK	7.84 ± 0.43	21.84 ± 2.38	3.48 ± 0.82	18.51 ± 2.63	14.53 ± 2.93	2.05 ± 0.44
		NPK	8.29 ± 0.57	19.23 ± 4.26	3.52 ± 0.82	18.18 ± 1.90	14.07 ± 4.70	1.78 ± 0.46
		2NPK	8.85 ± 0.66	21.60 ± 4.52	3.88 ± 0.87	19.49 ± 1.54	15.72 ± 3.16	1.83 ± 0.34

Explanations: A – unit without biostimulant, B – unit with biostimulant, M_1-M_3 – forage mixtures, 0-2NPK – fertilization level;

* $LSD_{\alpha=0.05}$ for A = 0.93; $LSD_{\alpha=0.05}$ for B = 0.31; $LSD_{\alpha=0.05}$ for C = 0.17; $LSD_{\alpha=0.05}$ for A × B = 0.97; $LSD_{\alpha=0.05}$ for A × C = 0.93; $LSD_{\alpha=0.05}$ for A × B = 0.40; $LSD_{\alpha=0.05}$ for A × B × C = ns (non-significant difference)

(1 axis –16.76%, 2 axis –6.14%, respectively), while the four other axes explain 30.14%.

The graph (biplot) analysis of the RDA between environmental variables (factors) and the quality of the mixture is presented in Figure 1. The single effect of individual factors was statistically significant for M_3 (F = 7.9,

Table 6

Means and standard deviations of the relationships between elements and the examined factors

Unit (A)	Forage mixture (B)	Fertilization level (C)	N:P	N:K	K:P	Ca:P
			mean ± SD			
Without biostymulant	M1	0	5.95 ± 1.81	1.22 ± 0.38	4.88 ± 0.73	3.15 ± 0.56
		PK	6.00 ± 0.58	1.11 ± 0.24	5.48 ± 0.61	3.31 ± 0.77
		NPK	6.92 ± 1.89	1.21 ± 0.34	5.74 ± 0.37	3.90 ± 0.83
		2NPK	8.10 ± 3.13	1.44 ± 0.58	5.70 ± 0.37	4.27 ± 1.41
	M2	0	7.01 ± 2.30	1.41 ± 0.38	4.92 ± 0.63	3.75 ± 0.53
		PK	5.07 ± 1.70	1.02 ± 0.07	4.97 ± 1.65	2.94 ± 0.99
		NPK	5.57 ± 1.84	1.13 ± 0.07	5.01 ± 1.88	3.35 ± 1.07
		2NPK	5.37 ± 1.26	0.98 ± 0.17	5.54 ± 1.48	3.40 ± 0.52
	M3	0	7.34 ± 3.53	1.43 ± 0.16	5.00 ± 2.10	4.14 ± 1.73
		PK	9.46 ± 3.82	1.41 ± 0.48	6.57 ± 1.05	5.22 ± 1.63
		NPK	8.34 ± 3.46	1.17 ± 0.27	7.04 ± 2.46	4.77 ± 1.66
		2NPK	8.47 ± 3.49	1.31 ± 0.11	6.45 ± 2.61	4.72 ± 1.97
Biostymulant	M1	0	4.78 ± 1.27	1.34 ± 0.33	3.63 ± 0.92	2.98 ± 1.38
		PK	4.32 ± 1.53	1.10 ± 0.37	4.14 ± 1.64	2.97 ± 0.32
		NPK	5.20 ± 2.49	0.96 ± 0.31	5.27 ± 1.15	3.55 ± 1.08
		2NPK	6.14 ± 2.38	1.46 ± 0.38	4.20 ± 0.89	3.60 ± 0.99
	M2	0	4.30 ± 1.36	1.06 ± 0.34	4.05 ± 0.30	3.25 ± 0.67
		PK	4.09 ± 1.32	0.90 ± 0.11	4.70 ± 1.88	3.14 ± 1.30
		NPK	4.54 ± 1.16	0.99 ± 0.09	4.63 ± 1.31	3.21 ± 0.85
		2NPK	5.37 ± 1.03	0.99 ± 0.38	5.68 ± 0.95	3.54 ± 0.51
	M3	0	6.89 ± 2.78	1.37 ± 0.19	4.89 ± 1.38	4.60 ± 2.09
		PK	6.56 ± 1.92	1.20 ± 0.27	5.38 ± 0.45	4.42 ± 1.63
		NPK	5.68 ± 1.71	1.06 ± 0.23	5.28 ± 0.73	4.38 ± 2.55
		2NPK	5.80 ± 1.97	1.11 ± 0.18	5.15 ± 0.96	4.24 ± 1.50

Explanations: see Table 5.

p = 0.009), B (F = 5.5, p = 0.009), K (F = 5.5, p = 0.009), P (F = 4.2, p = 0.009), M_1 (F = 3.8, p = 0.045).

Environmental variables with their simple (independent) term effect on

Table 7

Results of RDA analyses of forage yield quality and nutrient ratios.
p Values obtained by the Monte Carlo permutation test (999 permutations)

Specification	Axis number	Sum of all canonical eigenvalues	Percentage of explained variation (cumulitive)	Pseudo-canonical correlation	Explained fitted variation (cumulative)	Pseudo-F value	p value
Forage yield quality	axis1	0.1552	16.76	0.8966	56.11	11.9	0.001
	axis2	0.0568	22.9	0.5381	76.66	4.7	0.048
	axis3	0.0505	28.35	0.4825	94.91	4.4	0.032
	axis4	0.0111	29.55	0.3673	98.92	1	0.878
Nutrient ratios	axis1	0.1656	17.62	0.4908	76.15	12.9	0.009
	axis2	0.0431	22.2	0.4607	95.96	3.5	0.459
	axis3	0.0088	23.14	0.4221	99.99	0.7	0.976
	axis4	0	23.14	0.2746	100	< 0.1	1

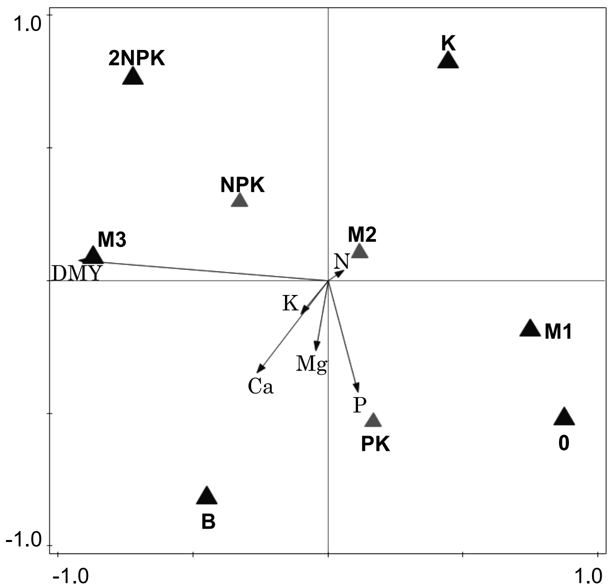

Fig. 1. RDA diagram (biplot) showing the mixture quality parameters in relation to the examined treatments. Treatments marked with black symbols and letters are statistically significant at $p < 0.05$ after Bonfferoni. Adjustment symbols in Table 6

Table 8

Environmental variables with their simple (independent) term effect on the forage quality parameters using the RDA ordination. *p* Values obtained by the Monte Carlo permutation test (999 permutations) and *p*(adj) based on Bonferroni correction

Predictor	Explains (%)	pseudo- *F*	*P*	*P*(adj)
M3	9.2	6.9	0.001	0.009
B	8.2	6.1	0.001	0.009
K	8.2	6.1	0.001	0.009
0	6.1	4.4	0.001	0.009
M1	4.9	3.5	0.011	0.099
2NPK	4.4	3.1	0.005	0.045
M2	3.7	2.6	0.025	0.225
PK	1.6	1.1	0.358	1
NPK	1.4	1	0.443	1

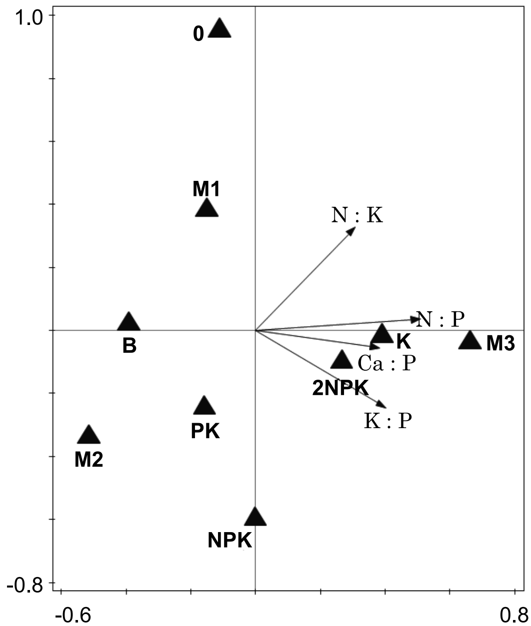

Fig. 2. RDA diagram (biplot) showing the relationship between elements relative to the examined treatments

forage quality parameters, using RDA coordination, are presented in Table 8, with the p value obtained from the Monte Carlo permutation test (permutations 999) and *p* (adj) based on the Bonferroni correction.

The ratios of N : P, N : K, K : P, Ca : P against the environmental (factors) parameters are presented in the RDA Graph (Figure 2). The ratios are positively correlated with K, M_3, and 2NPK while being negatively correlated with B. In this case, the first ordination axis is statistically significant ($F = 12.9$, $p = 0.009$). The percentage of translated volatility is 17.62, while the correlation between the ratio of the elements and environmental parameters is 0.491.

CONCLUSIONS

1. The mixture with red clover treated with NPK yielded the best production result. The application of the biostimulant together with PK fertilizer increased its yield by 40%. The highest dry matter content was in the mixture with red clover treated with the biostimulant and mineral P and K, while the lowest one was in the case of the *Festuca* and *Lolium* mixture without the use of the biostimulant and PK fertilizers.

2. The highest content of P and Ca in the mixtures was achieved after the biostimulant application. There were significant differences between the Mg content in the mixtures. Its highest content was found in the mixture with red clover, and the lowest one was in the mixture with *Phleum pratense* var. Liglory, *Trifolium repens* var. Grasslands Huia.

3. The N : P ratio differed significantly between units and forage mixtures. Higher values were found on experimental units without the biostimulant and in the mixture with red clover. The ratios of N : K and Ca : P were differentiated relative to the mixtures. The ratio of N : K reached the highest values in the mixture of *Festuca* and *Lolium* and in the one with red clover, while the highest value of the Ca : P ratio was in the mixture with *T. pratense*. The ratio of N : P differed significantly between units, assuming the highest values in the plants not treated with the biostimulant.

4. The most diverse content of microelements was observed in the mixture with Festuca and Lolium, in the one with red clover, and on plots with the biostimulant, and on those with no mineral fertilisers applied.

5. Higher ratios of elements (N : P, N : K, K : P, Ca : P) are positively correlated with the lack of the biostimulant and the highest NPK dose, while being negatively correlated with the biostimulant application.

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