

## Impact of mineral fertilization on the botanical composition and productivity of a degraded mesophytic meadow in the region of the Central Balkan Mountain

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### Abstract

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In the period 2016-2019, at the Research Institute of Mountain Stockbreeding and Agriculture, Troyan (Bulgaria), a field experiment was conducted to establish the impact of annual mineral fertilization on the bioproductive indicators of a degraded grassland with a predominant participation of grasses in mountain conditions.

The data analysis shows a significant excess in dry mass yield in the variants with annual application of the combination  $N_{12}P_{12}$  (4.03 t/ha) followed by the annual application of combined fertilization by  $N_8P_8$  (3.41 t/ha). The values of the indicator exceeded the control by 156.77% and 124.71% ( $P < 0.001$ ), respectively. The grasslands with combined application of  $N_{10}P_{10}$  (3.34 t/ha) and with alternative alternation of nitrogen and phosphorus ( $N_7/I P_7/II P_7/III/N_7/IV - 3.31$  t/ha) had a slight difference in the values of the studied trait, but with high provability compared to the control 120.10% and 118.47%, respectively ( $P < 0.001$ ). The lowest productivity (2.91 t/ha) (but with a proven excess compared to the control) were the treated grasslands from the second alternative variant ( $P_6/I N_6/II P_6/III/N_6/IV$ ). The excess over the control was 92.06% ( $P < 0.001$ ).

The applied fertilization rates contributed to the desired changes in the individual groups and species of the botanical composition of the degraded grassland. The dominant legume species were: *Trifolium hybridum* L., *Vicia sativa* L., *Lotus corniculatus* L. and *Medicago lupulina* L., and of grasses - *Festuca arundinaceae* Scherb., *Festuca rubra* L. and *Agrostis capillaris* L. The highest share of motley grasses were registered in the grasslands treated annually with  $N_8P_8$  (by 39.3% compared to the control) and the lowest in the grasslands treated annually with  $N_{12}P_{12}$  (by 18.4% compared to the control).

**Keywords:** productivity of natural grassland; mineral fertilization; botanical composition

### Introduction

In the conditions of intensive agriculture, some of the socio-economic factors in the mountainous and foothill regions of Bulgaria, as well as the free unregulated grazing of natural meadows and pastures (one of the main sources of feed for ruminants), cause negative changes in the structure of grasslands. To improve the composition of the various meadow formations (mesophytic grasslands in the process of degradation), it is necessary to conduct events

related to improving their productivity, biodiversity and feed quality (Tessema et al., 2010; Popescu & Churkova, 2015). The structure of the grass mass is directly dependent on the agro-ecological characteristics of the area and the applied management practices (Hornik et al., 2012; Mitev et al., 2011). In the mountain and foothill regions of Bulgaria, grasslands from natural meadows and pastures are used mainly for hay and grazing, and the ratio of individual plant groups in plant associations, as well as the yield obtained from them, are indicators of the effectiveness of ap-

plied agrotechnical events (Blanke et al., 2012; Tomaškin et al., 2013; Leps, 2014).

Supplying plants with mineral or organic fertilizers creates conditions for the development of valuable and high-quality cultivars with high resistance, durability and productivity (Powlson et al., 2011; Kurhak et al., 2020; Vargová et al., 2020). Nitrogen fertilizing increases the share of grasses and some high-growing species from the group of motley grasses, while phosphorus and potassium fertilizers increase the share of legumes (Yolcu et al., 2010; Bijelić et al., 2011). Changes in the botanical composition of a grassland is an indicator of the quality and biological integrity of fodder, as well as the longevity of meadow and pasture grasslands (Purschke et al., 2012; Naydenova et al., 2013).

The mineral fertilizers suppress the low-yielding and unwanted plant species, and have a positive effect on the composition of hay, grazing and yield in mesotrophic and other types of hay-making fields (Kirkham et al., 2014). The accelerated process of mineralization, associated with the release and absorption of nitrogen from the soil, favorably affects the growth and development of members of the family *Poaceae*. Inorganic fertilizing has been shown to increase the percentage share of grasses and dry mass yield (Nemera et al., 2018) and has a less pronounced effect on plant height in degraded grasslands (Ahmed & Ibrahim, 2013). The fodder mass with a predominant share of legumes is characterized by high nutritional value (Lemaire & Belanger, 2020), high crude protein content (Nilsdotter-Linde et al., 2016; Kovtun et al., 2020) and minerals (P, K, Ca, Mg).

The aim of the present study is to determine the impact of annual mineral fertilizing on the bioproductive indicators of degraded mesophytic meadow with increased participation of cereal species in the region of the Central Balkan Mountain.

## Material and Methods

The experiment was conducted in the area of the Research Institute of Mountain Stockbreeding and Agriculture, Troyan (Bulgaria) in the period 2016-2019, on a mesophytic meadow in the process of degradation. The experiment was based on the block method, in 4 replications with 5 m<sup>2</sup> plot size.

The research options are:

Control (nontreated);

- N<sub>12</sub>P<sub>12</sub> (annual combined fertilizing – from March 20 to April 10);
- N<sub>10</sub>P<sub>10</sub> (Annual combined fertilizing – from March 20 to April 10);
- N<sub>8</sub>P<sub>8</sub> (annual combined fertilizing – from March 20 to April 10);

- N<sub>7</sub>/I P<sub>7</sub>/II P<sub>7</sub>/III N<sub>7</sub>/IV (individual fertilizing with N<sub>7</sub> – first and fourth experimental years and individual fertilizing with P<sub>7</sub> – second and third experimental years);
- P<sub>6</sub>/I N<sub>6</sub>/II P<sub>6</sub>/III N<sub>6</sub>/IV (individual fertilizing with P<sub>6</sub> – first and third experimental years and individual fertilizing with N<sub>6</sub> – second and fourth experimental years).

\**Legend*: I – first experimental year; II – second experimental year; III – third experimental year and IV – fourth experimental year.

For variants 5 and 6 – fertilizing with double superphosphate was conducted once in the autumn (September-October), and with ammonium nitrate – in the spring (April).

The experimental plots were harvested in the phenophase of tasseling/ear formation (for grasses) until the beginning of the phenophase of flowering.

The following indicators were monitored:

- Dry mass yield (t/ha) – determined by weighing the mowed grass in different replications, with subsequent drying (at 105°C) to constant weight and recalculated per 1 da;
- Botanical composition of grassland (%) – determined by weight by analysis of green mass, from each variant of cutting. Weighing was performed in air dry condition. The share of grass species was determined by weight and referred to the main botanical groups (grasses, legumes and motley grasses).

Experimental data (productivity of grassland) were statistically processed by analysis of variance (ANOVA) at a *Least Significant Difference* level of probability ( $LSD = P < 0.05$ ,  $P < 0.01$ ,  $P < 0.001$ ).

## Results and Discussion

### *Dry mass yield from mesophytic meadow in the process of degradation, after applied mineral fertilizing.*

The application of nitrogen fertilizer increases the productivity and quality of the grass mass in natural meadows and pastures (Vuckovic et al., 2005; Vargová et al., 2020).

On average for the four-year test period, the productivity of grasslands in all variants with applied mineral fertilizing exceeded the proven yield of dry mass compared to the unfertilized control (Table 1). The highest yield of dry mass (4.03 t/ha) were registered in the variants with annual application by the combination N<sub>12</sub>P<sub>12</sub> followed by the annual combined fertilizing by N<sub>8</sub>P<sub>8</sub> (3.41 t/ha). The values of the indicator exceeded the control by 156.77% and 124.71% ( $P < 0.001$ ), respectively. The average yields from grasslands with combined application of N<sub>10</sub>P<sub>10</sub> (3.34 t/ha) and those

**Table 1. Dry mass yield (t/ha) of submountain mesophytic meadow, after mineral fertilizing (over the years and average for period)**

Variants	2016		2017		2018		2019		2016-2019	
	t/ha	% compared to C	t/ha	% compared to C	t/ha	% compared to C	t/ha	% compared to C	t/ha	% compared to C
Control	1.57	100.00	1.56	100.00	1.84	100.00	1.10	100.00	1.52	100.00
N <sub>12</sub> P <sub>12</sub>	3.64	232.29	3.52	226.17	4.70	255.00	4.26	387.56	4.03	256.77
N <sub>10</sub> P <sub>10</sub>	2.98	190.11	4.03	259.34	3.05	165.62	3.28	298.71	3.34	220.10
N <sub>8</sub> P <sub>8</sub>	3.53	225.20	3.44	221.16	3.52	191.17	3.14	285.30	3.41	224.71
N <sub>7</sub> /I P <sub>7</sub> /II P <sub>7</sub> /III/N <sub>7</sub> /IV	3.35	213.35	3.53	227.24	4.16	225.67	2.21	201.27	3.31	218.47
P <sub>6</sub> /I N <sub>6</sub> /II P <sub>6</sub> /III/N <sub>6</sub> /IV	2.58	164.28	3.53	227.02	3.53	191.22	2.02	183.65	2.91	192.06
LSD <sub>0.05</sub>	0.89	56.66	0.67	43.20	0.81	43.98	0.73	66.13	0.42	27.33
LSD <sub>0.01</sub>	1.24	78.48	0.93	59.84	1.12	60.91	1.01	91.59	0.58	37.86
LSD <sub>0.001</sub>	1.71	108.27	1.28	82.55	1.55	84.04	1.39	126.37	0.79	52.23

with alternative alternation of nitrogen and phosphorus (N<sub>7</sub>/I P<sub>7</sub>/II P<sub>7</sub>/III/N<sub>7</sub>/IV – 3.31 t/ha) differ slightly, but with high provability in the excess of the values of the trait compared to the control – 120.10% and 118.47% (P < 0.001). The lowest productivity was found in the treated grasslands from the second alternative variant (P<sub>6</sub>/I N<sub>6</sub>/II P<sub>6</sub>/III/N<sub>6</sub>/IV) – 2.91 t/ha (but again with a proven excess compared to the control). Dry mass yield is 1.12 t/ha lower than the maximum value of the trait registered in the treated variants. The excess over the control is 92.06% (P < 0.001), which shows that the introduction of smaller amounts of phosphorus and nitrogen alternately over the years is a good alternative to combined mineral fertilizing to increase the productivity of mesophytic meadow in the process of degradation.

During the first experimental year, the increase in the productivity of the meadow coenosis was most pronounced with the annual combined application of N<sub>12</sub>P<sub>12</sub> (3.64 t/ha). The realized yield of dry mass is 132.29% higher than the control. In the variants with combined fertilizing by N<sub>10</sub>P<sub>10</sub> (2.98 t/ha) and N<sub>8</sub>P<sub>8</sub> (3.53 t/ha) a proven higher dry mass productivity was reported by 90.11% (P < 0.01) and 125.20% (P < 0.001), respectively. In the variants with alternating fertilizing by N and P, the application of N<sub>7</sub> in the first year leads to a proven (by 113.35% at P < 0.001) higher amount of dry mass compared to the control. In the fertilizing variant with alternating phosphorus and nitrogen (6 kg/da in a year), the dry mass yields increased by 64.28% (P < 0.05) compared to the control.

The highest productivity was found in the grassland fertilized with combined application of N<sub>10</sub>P<sub>10</sub> (4.03 t/ha) in the second experimental year, with an excess of 159.34% (P < 0.001) compared to the control. The relative yield of

dry mass obtained by combined fertilizing of grassland with N<sub>12</sub>P<sub>12</sub> and N<sub>8</sub>P<sub>8</sub> exceeds the nontreated variant by 126.17% and 121.16%, respectively. The values of yields (3.44 t/ha and 3.53 t/ha) in the grasslands with alternating nitrogen and phosphorus fertilizing are almost identical. The amount of dry mass in the variants exceeds the control by 127.24% and 127.02%, respectively.

The treatment of xerophytic dry meadows (composed of the formations of bunch grasses – *Chrysopogon gryllus*) with N<sub>6</sub> (once in the first two years) and P<sub>6</sub> (in the third year) increased the productivity of the grassland by 66.49% (Iliev, 2014). In the third experimental year, the annual fertilizing with the combination of mineral nitrogen and phosphorus at a rate of 12 kg/da active substance, achieved the highest annual productivity (4.70 t/ha). The excess compared to the unfertilized variant is by 155.00%. The amount of dry mass obtained in the variants of annual combined fertilizing with N<sub>10</sub>P<sub>10</sub> and N<sub>8</sub>P<sub>8</sub>, increased from 65.62% to 91.17% compared to the unfertilized control. During the year there was a significant consumption reaction of the species in the grassland from the alternative way of alternating with mineral nitrogen and phosphorus. The effect of the applied phosphorus mineral fertilizing increased with a proof (P < 0.001) the amount of dry mass to 3.53 t/ha (after fertilizing with P<sub>6</sub>) and 4.16 t/ha (after fertilizing with P<sub>7</sub>). The excess over the control is 91.22% and 125.67%, respectively.

The yield and quality of the fodder depend mainly on the floristic composition of the lawns and the agroecological conditions of the area (Đukić et al., 2008). In the fourth experimental year, the greatest responsiveness of the plant species in the composition of the degraded grassland was ob-

**Table 2. Botanical composition (%) of submountain mesophytic meadow (over the years and groups), after mineral fertilizing**

Variants	2016			2017			2018			2019		
	grasses	le-gumes	motley grasses	grasses	le-gumes	motley grasses	grasses	le-gumes	motley grasses	grasses	le-gumes	motley grasses
Control	29.1	20.4	50.5	6.3	36.9	56.8	18.2	22.4	59.4	57.1	14.3	28.6
N <sub>12</sub> P <sub>12</sub>	5.9	14.9	79.2	22.8	45.8	31.4	30.6	40.3	29.1	75.6	5.1	19.3
N <sub>10</sub> P <sub>10</sub>	37.4	21.6	41.0	31.1	34.8	34.1	48.6	22.7	28.7	63.2	12.8	24.0
N <sub>8</sub> P <sub>8</sub>	41.8	29.2	29.0	25.0	27.2	47.8	44.5	38.6	16.9	68.3	7.1	24.6
N <sub>7</sub> /I P <sub>7</sub> /II P <sub>7</sub> /III/ N <sub>7</sub> /IV	43.5	24.9	31.6	27.1	22.9	50.0	15.9	46.2	37.9	59.5	7.4	33.1
P <sub>6</sub> /I N <sub>6</sub> /II P <sub>6</sub> /III/ N <sub>6</sub> /IV	20.3	51.1	28.6	14.9	30.0	55.1	45.4	30.1	24.5	58.5	18.2	23.3

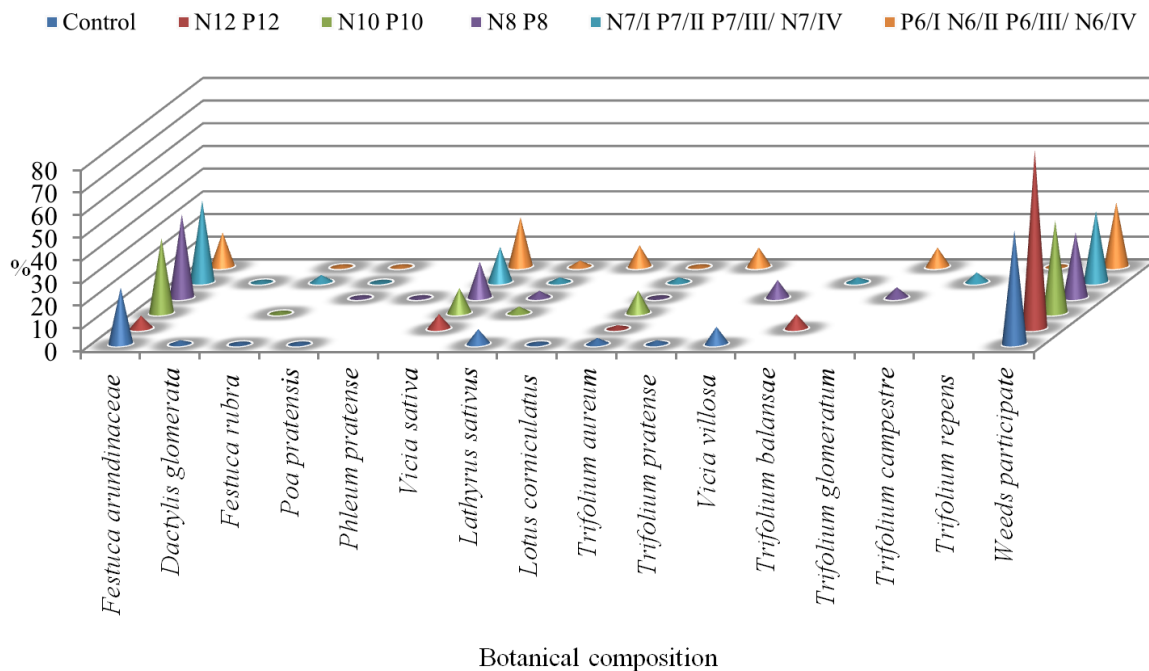
served. All fertilizing variants registered proven high productivity. The largest increase in the amount of dry biomass was realized by the annual combined application of N<sub>12</sub>P<sub>12</sub> (4.26 t/ha), where the excess compared to the unfertilized variant was 287.56% (P < 0.001). In the other variants was observed good proof of the differences in terms of the values of the attribute. Dry mass yields obtained by combined fertilizing with N<sub>10</sub>P<sub>10</sub> and N<sub>8</sub>P<sub>8</sub> increased from 198.71% to 185.30% compared to the variant without fertilizing. Supplying the grassland with N<sub>7</sub> with alternating application (every two years) and successive two-year application of P<sub>7</sub>, increased the relative dry mass yield by 101.27% compared to the control. Fertilizing with ammonium nitrate at a rate of 6 kg/da

in the variant with alternating nitrogen and phosphorus every other year, significantly affects the productivity of grassland. The registered yield is 2.02 t/ha. The amount of dry mass in the variant was significantly increased by 83.65% (P < 0.05).

#### **Botanical composition of a natural submountain mesophytic meadow in the process of degradation, after mineral fertilizing.**

The changes in the botanical composition (by groups) of the meadow grassland, after mineral fertilizing are shown in Table 2.

In the first experimental year, grasses (43.5% – N<sub>7</sub>/I P<sub>7</sub>/II P<sub>7</sub>/III/ N<sub>7</sub>/IV) and legumes (51.1% – P<sub>6</sub>/I N<sub>6</sub>/II P<sub>6</sub>/III/ N<sub>6</sub>/IV)



**Fig. 1. Botanical composition (%) of submountain mesophytic meadow after mineral fertilizing (first experimental year – 2016)**

IV) predominated with the highest percentage, respectively, in the variants with alternating intake of nitrogen and phosphorus. *Festuca arundinaceae* Scherb. (grasses) and *Trifolium aureum* Pollich. (legumes) presented in all tested variants. Their share participation in grassland varied from 5.9% ( $N_{12}P_{12}$ ) to 36.2% ( $N_7/I P_7/II P_7/III/ N_7/IV$ ) and from 0.3% ( $N_8P_8$ ) to 10.3% ( $N_{10}P_{10}$ ), respectively (Figure 1).

During the year, a higher percentage share was registered in:  
Legumes:

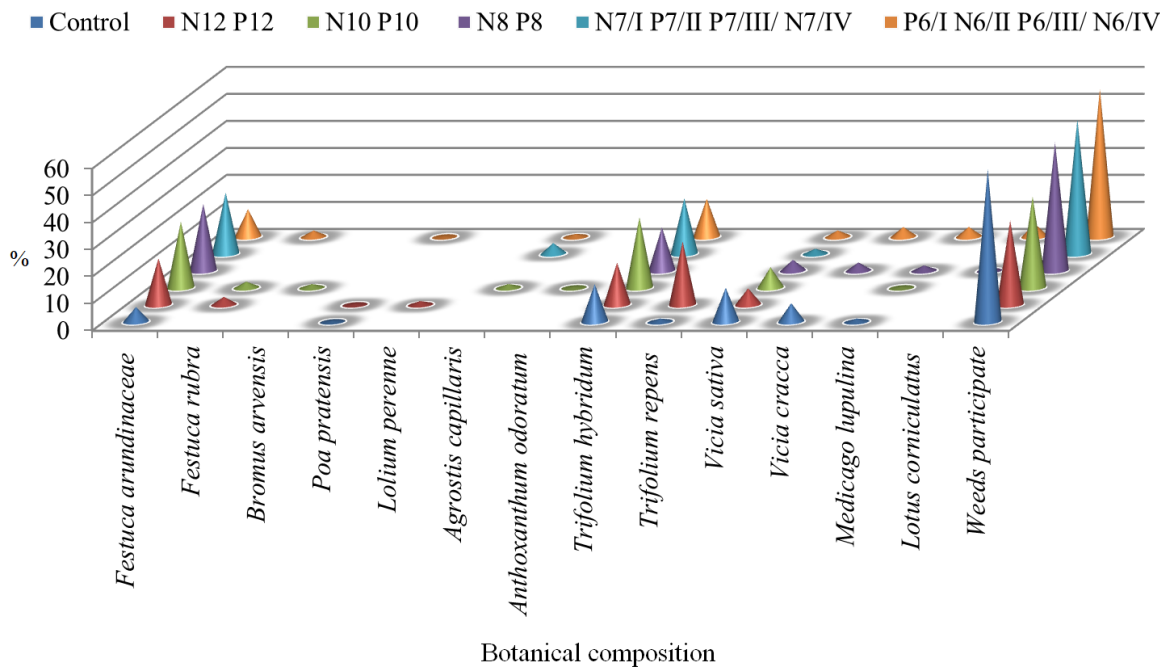
- *Vicia sativa* L. – 6.6% ( $N_{12}P_{12}$ ), 11.3% ( $N_{10}P_{10}$ ), 15.8% ( $N_7/I P_7/II P_7/III/ N_7/IV$ ) и 22.1% ( $P_6/I N_6/II P_6/III/ N_6/IV$ );
- *Lotus corniculatus* L. – 1% (Control) и 9.6% ( $P_6/I N_6/II P_6/III/ N_6/IV$ );
- *Trifolium pratense* L. – 1.9 (Control) и 8.7% ( $P_6/I N_6/II P_6/III/ N_6/IV$ );
- *Trifolium balansae* Boiss. – 4.8% ( $N_8P_8$ ) и 8.7% ( $P_6/I N_6/II P_6/III/ N_6/IV$ );
- *Vicia villosa* Roth. – 7.8% (Control) и 8.0% ( $N_8P_8$ );
- *Lathyrus pratensis* L. – 2.3% ( $N_7/I P_7/II P_7/III/ N_7/IV$ ), 2.9% ( $P_6/I N_6/II P_6/III/ N_6/IV$ ), 3.1% ( $N_{10}P_{10}$ ), 3.2% ( $N_8P_8$ ) и 6.8% (Control);
- *Trifolium glomeratum* L. – 2.3% ( $N_7/I P_7/II P_7/III/ N_7/IV$ ) и 6.6% ( $N_{12}P_{12}$ );
- *Trifolium campestre* Schreb. – 4.5% ( $N_7/I P_7/II P_7/III/ N_7/IV$ ).

Grasses:

- *Festuca rubra* L. – 1% Control,  $N_{10}P_{10}$  и  $P_6/I N_6/II P_6/III/ N_6/IV$ ) и 3.4% ( $N_7/I P_7/II P_7/III/ N_7/IV$ );
- *Dactylis glomerata* L. – 1.1% ( $N_7/I P_7/II P_7/III/ N_7/IV$ ) и 1.9% (Control);
- *Poa pratensis* L. – 0.5% ( $N_7/I P_7/II P_7/III/ N_7/IV$ ), 0.6% ( $N_{12}P_{12}$ ), 0.8% ( $N_8P_8$ ) и 1% (Control) и  $P_6/I N_6/II P_6/III/ N_6/IV$ );
- *Phleum pratense* L. – 0.8% ( $N_8P_8$ ).

In the second experimental year, the variants with annual combined fertilizing with nitrogen and phosphorus ( $N_{10}P_{10}$  and  $N_{12}P_{12}$ ) had maximum values regarding the weight percentage of grasses (31.1%) and legumes (45.8%), respectively (Table 2). Compared to the previous year (2016), the share of species in these variants marked a decreasing trend for grasses (by 6.3%) and increasing for legumes (by 30.9%). *Festuca arundinaceae* Scherb. had the strongest presence in all test variants (Figure 2).

The species predominated the most in the grasslands with combined import of  $N_8P_8$  (25.0%), followed by those with imported:  $N_{10}P_{10}$  (24.8%),  $N_7/I P_7/II P_7/III/ N_7/IV$  (22.9%),  $N_{12}P_{12}$  (17.4%),  $P_6/I N_6/II P_6/III/ N_6/IV$  (10.5%). In the studied grassland, the share of *Festuca rubra* L., *Poa pratensis* L. and *Agrostis capillaris* L. was less present. Under the impact of the combined and individual alternating mineral fertilizing, desired changes occurred in the group of legumes.



**Fig. 2. Botanical composition (%) of submountain mesophytic meadow after mineral fertilizing (second experimental year – 2017)**

*Trifolium hybridum* L. and *Vicia sativa* L. dominated in the composition of all fertilizing variants. The percentage share of both crops varied from 14.2% (Control) to 26.4% ( $N_{10}P_{10}$ ) and from 2.1% ( $N_7/I P_7/II P_7/III/ N_7/IV$ ) to 12.8% (Control), respectively. *Trifolium repens* L. (as a typical pasture legume) has the ability to fix atmospheric nitrogen and provide up to 37.0% of the total nitrogen required for plants (Lu et al., 2020). Nitrogen is one of the most important nutrients and the most common limiting factor for achieving high yields from natural lawns (LeBauer & Treseder, 2008; You et al., 2017). In the second experimental year, the species registered a share in grassland only in the variants fertilized with  $N_{12}P_{12}$  (23.7%) in the control (1.4%). Legumes in the fertilized variants include:

- *Lotus corniculatus* L. – 1.1% ( $N_8P_8$ ) и 5.2% ( $P_6/I N_6/II P_6/III/ N_6/IV$ );
- *Vicia cracca* L. – 3.3% ( $N_8P_8$ ), 3.9% ( $P_6/I N_6/II P_6/III/ N_6/IV$ ) и 7.1% (Control);
- *Medicago lupulina* L. – 0.6% ( $N_{10}P_{10}$ ), 1.4 (Control), 2.2% ( $N_8P_8$ ), 3.9% ( $P_6/I N_6/II P_6/III/ N_6/IV$ ).

The individual application of  $N_6$  improved and increased the species diversity of the members of genus *Fabaceae* as a result of the effects of phosphorus imported in the previous year.

In the third experimental year, in the volume of grasslands with annual combined fertilization with  $N_{10}P_{10}$ , grasses occupied the largest share (48.6%), followed by those

with combined alternation of  $N_6$  and  $P_6$  (45.4%), and  $N_8P_8$  (44.5%). (Table 2). The highest percentage share of legumes was observed in the variants with annual alternation of the macroelements  $N_7$  and  $P_7$  (46.2%) and annual fertilizing with  $N_{12}P_{12}$  (40.3%).

In 2017, *Festuca arundinaceae* Scherb. and *Festuca rubra* L. are available in all study variants (Figure 3).

Their percentage varied from 6.6% ( $N_8P_8$ ) to 23.4% ( $N_{10}P_{10}$ ) in the control variant – 9.6% and from 2.7% ( $N_7/I P_7/II P_7/III/ N_7/IV$ ) to 36.7% ( $N_8P_8$ ), respectively in the control variant – 6.4%. *Agrostis capillaris* L. (4.0%), *Festuca pratensis* L. (3.2%) и *Bromus arvensis* L. (2.0%) dominated in variants fertilized with  $P_6/I N_6/II P_6/III/ N_6/IV$ . During the year, *Arrhenatherum elatius* L. was present only in grassland with annual intake of  $N_{12}P_{12}$  (5.2%). Traces of *Poa pratensis* L. (0.5-1.2%) and *Cynosurus cristatus* L. (0.5%) were found in the  $P_6/I N_6/II P_6/III/ N_6/IV$  and  $N_8P_8$  variants.

From the group of legumes, the dominant species during the year was *Trifolium hybridum* L. – 17.3% ( $N_{10}P_{10}$ ), 23.3% ( $P_6/I N_6/II P_6/III/ N_6/IV$ ), 36.7% ( $N_8P_8$ ), 37.1% ( $N_{12}P_{12}$ ) и 39.3% ( $N_7/I P_7/II P_7/III/ N_7/IV$ ) in the control – 17.6%.  $P_6$  in individual application contributes to the participation of a larger range of species in legumes. The proportion of *Lotus corniculatus* L. ranged from 1.2% ( $N_8P_8$ ) to 4.1% ( $N_7/I P_7/II P_7/III/ N_7/IV$ ), for *Trifolium repens* L. – from 0.7% ( $N_8P_8$ ) to 1.6% (Control) and for *Vicia sativa* L. – from 1.4% ( $N_7/I P_7/II P_7/III/ N_7/IV$ ) to 3.2 ( $N_{12}P_{12}$ ). More significant traces

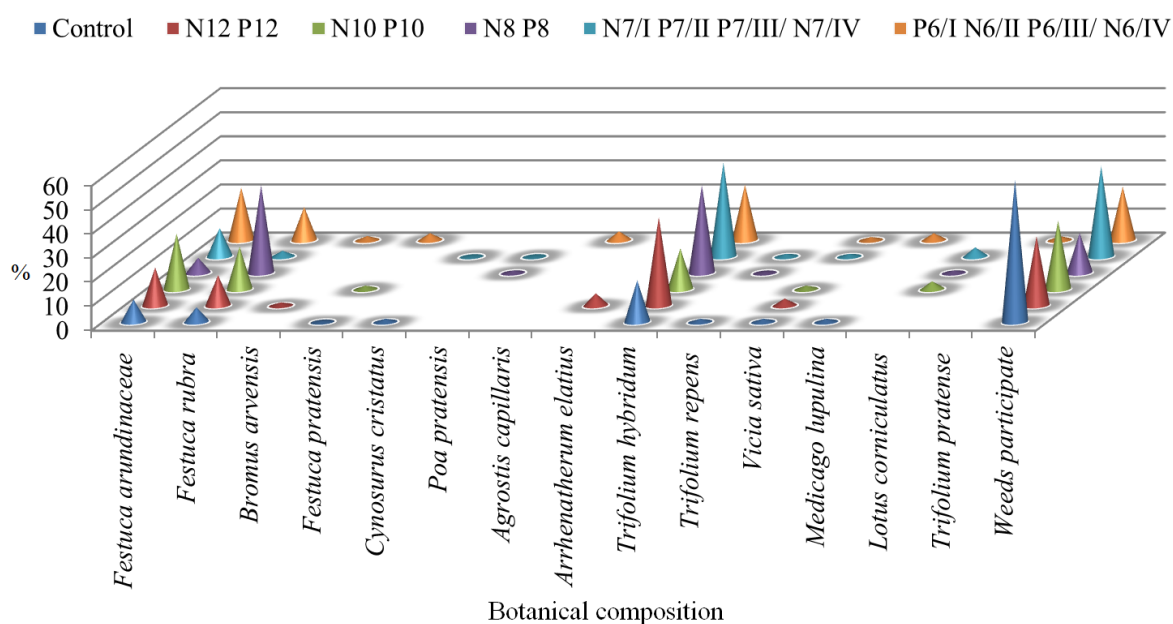


Fig. 3. Botanical composition (%) of submountain mesophytic meadow after mineral fertilizing (third experimental year – 2018)

of *Trifolium pratense* L. (2.0%) and *Medicago lupulina* L. (3.2%) were observed in the variants with combined alternation of  $N_6$  (second and fourth year) and  $P_6$  (first and third year).

The impact of mineral fertilizing in the last (fourth) experimental year significantly affected the share of grasses in all fertilizer variants (Table 2). Fertilizing regimes and management practices affect the composition of plant species (Hopkins & Wilkins, 2006). Nitrogen fertilizer has a significant effect and diversifies the composition of plant species regardless of the method (individual or in combination) of application (Hejzman et al., 2014). The data from the analysis show that due to the effect of nitrogen fertilizer (at all fertilizing rates), the groups with the highest weight percentage are the groups of grasses in the grassland with annual application of nitrogen and phosphorus ( $N_{12}P_{12}$  – 75.6%,  $N_8P_8$  – 68.3% and  $N_{10}P_{10}$  – 63.2%) followed by those with combined alternating fertilization ( $N_7/I P_7/II P_7/III/ N_7/IV$  – 59.5% and  $P_6/I N_6/II P_6/III/ N_6/IV$  – 58.5%) (Table 2).

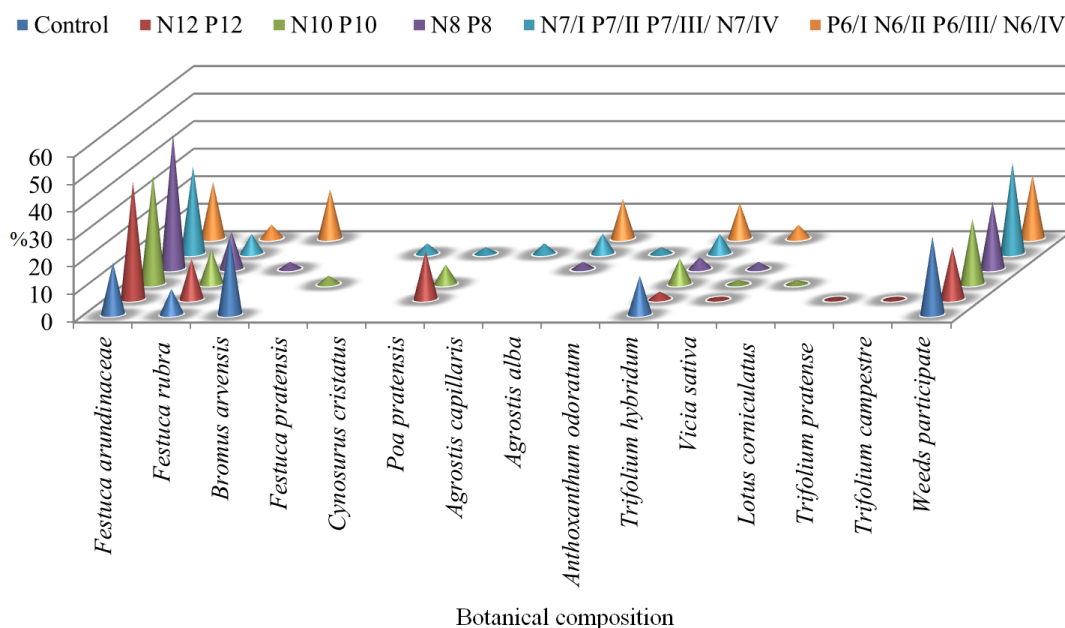
In the variants with mineral fertilizing, the participation of *Festuca arundinaceae* Scherb. (the species characterizing the studied grassland) increased in the composition of the treated grasslands from 8.9% ( $P_6/I N_6/II P_6/III/ N_6/IV$ ) to 158.9% ( $N_8P_8$ ) compared to the control (Figure 4).

*Festuca rubra* L. registered a higher presence (by 34.7 to 55.8%) in the variants with annual fertilizing with macronutrients ( $N_8P_8$ ,  $N_{10}P_{10}$  и  $N_{12}P_{12}$ ) and lower (by 22.1 to

45.3%) in the variants with alternating fertilizing ( $N_7/I P_7/II P_7/III/ N_7/IV$  и  $P_6/I N_6/II P_6/III/ N_6/IV$ ) compared with control. Only in the variants with combined fertilizing with  $N_7$  and  $P_7$  traces of *Anthoxanthum odoratum* L. (2.5%), *Cynosurus cristatus* L. (3.9%) and *Agrostis capillaris* L. (3.9%) were found, and in those with  $N_{10}P_{10}$  – of *Festuca pratensis* L. (3.2%). The percentage of *Poa pratensis* L. and *Agrostis alba* varied from 2.5% ( $N_7/I P_7/II P_7/III/ N_7/IV$ ) to 17.8% ( $N_{12}P_{12}$ ) and from 2.7% ( $N_8P_8$ ) to 14.5%, respectively ( $P_6/I N_6/II P_6/III/ N_6/IV$ ).

In the last experimental year, the share of legume component in the grassland was significantly lower. *Trifolium hybridum* L. (3.0-14.3%) and *Vicia sativa* L. (0.9-5.2%) had the highest presence in grassland in the fourth experimental year. *Lotus corniculatus* L. (1.6%), *Trifolium pratense* L. (0.6%) and *Trifolium campestre* Schreb. (0.6%) registered a share only in grasslands with annual treatment with  $N_{10}P_{10}$  and  $N_{12}P_{12}$ .

For the test period, the presence of legumes (from 8.1% –  $N_7/I P_7/II P_7/III/ N_7/IV$  to 37.9% –  $P_6/I N_6/II P_6/III/ N_6/IV$ ) and grasses (from 21.7% –  $N_{12}P_{12}$  to 62.1% –  $N_8P_8$ ) was higher in the variants with mineral fertilizing compared to the control, and the percentage of motley grasses decreased in the treated variants. For motley grasses, the maximum value of the indicator was registered in the grassland with annual application of  $N_{12}P_{12}$  (39.8%), and the minimum with annual application of  $N_8P_8$  (29.6%), in the control variant – 48.8%.



**Fig. 4. Botanical composition (%) of submountain mesophytic meadow after mineral fertilizing (fourth experimental year – 2019)**

## Conclusions

On average, during the test period, mineral fertilizing had a significant effect ( $P < 0.001$ ) on dry mass yield in all treated variants. The values of the indicator exceeded the control by 156.77% ( $N_{12}P_{12}$ ), 124.71% ( $N_8P_8$ ), 120.10% ( $N_{10}P_{10}$ ), 118.47% ( $N_7/I P_7/II P_7/III/ N_7/IV$ ) and 92.06%, respectively ( $P_6/I N_6/II P_6/III/ N_6/IV$ ) compared to the non-fertilized variant.

The applied fertilizing rates contributed to the desired changes in the individual groups and species of the botanical composition of the meadow grassland. The maximum values regarding the percentage share of legumes in the treated variants, over the years (51.1% –  $P_6/I N_6/II P_6/III/ N_6/IV$ ; 45.8% –  $N_{12}P_{12}$ ; 46.2% –  $N_7/I P_7/II P_7/III/ N_7/IV$  and 18.2% –  $P_6/I N_6/II P_6/III/ N_6/IV$ ) showed a consistent and decreasing trend. Of the legume species, *Trifolium hybridum* L. and *Vicia sativa* L. predominated, followed by *Lotus corniculatus* L. and *Medicago lupulina* L.

The lowest share of useful grasses in the mesophytic grassland was observed in the second and predominantly in the fourth experimental year. For the experimental period, the long-term mineral fertilizing with nitrogen and phosphorus positively affected mainly the quantitative share of *Festuca arundinaceae* Scherb., *Festuca rubra* L. and *Agrostis capillaris* L.

The group of motley grasses decreased in all variants with imported mineral fertilizers. The highest share of motley grasses was registered in the grasslands treated annually with  $N_8P_8$  (by 39.3% compared to the control) and the lowest in the composition of the grasslands treated annually with  $N_{12}P_{12}$  (by 18.4% compared to the control).

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