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Impact of Biological Fertilizing on the Composition and Productivity of Degraded Mesophytic Meadow in Mountain Conditions

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Abstract. A field experiment was conducted at the Research Institute of Mountain Stockbreeding and Agriculture (Bulgaria) to establish the impact of annual organic fertilizing on the bioproductive characteristics of a degraded grassland with a predominant share of grasses in mountain conditions. A significant effect on the biological productivity of the at all levels of fertilizing was found. The excess in the values of the indicator compared to the control was from 49.1% to 98.8%. Desired changes in the individual groups and species of the botanical composition of the pastures were observed. *Lotus corniculatus*, *Vicia sativa*, *Trifolium hybridum* (from legumes) and *Festuca arundinaceae* Scherb., *Festuca rubra*, *Anthoxanthum odoratum*, *Agrostis capillaris* (grasses) showed the highest resistance and adaptability to mountain conditions. The elimination of *Trifolium repens*, *Trifolium aureum* Pollich., *Vicia villosa* Roth. (second year), *Vicia cracca*, *Medicago lupulina* (third year) and *Trifolium pratense* (fourth year) from the composition of the grass mass affected the fodder and nutritional value of the grasslands. The grasslands treated with 1000 and 4000 kg/da dose of manure have the highest concentration of crude protein, respectively 14.39 and 14.05%. The values of the indicator exceeded the control by 20.0% and 17.2%, respectively. With a slight difference in protein content were grasslands treated with 2000 kg/da (12.03%) and 3000 kg/da (12.52%). The excess compared to the control variants was 0.3% и 4.4%, respectively. The highest fiber (44.37%) is the biomass with an imported dose of 3000 kg/da. The fertilizing variants registered with 2.3% to 11.4% higher content of crude ash compared to the control.

Key words: productivity, organic fertilizing, botanical and chemical composition of degraded grassland.

Introduction

The quality of natural grasslands is a prerequisite for optimal consumption and efficiency in the use of fodder. The resistance, adaptation and diversity of plant species in the composition of biocenoses affect the nutritional value of grass mass and the effect on the productive and reproductive indicators of animals (Slavkova & Shindarska, 2017). Changes in climatic characteristics, specific management practices and widespread extensive animal husbandry in mountain areas lead to some changes in the structure of floristic composition and the stability of ecosystems (Allan et al., 2015; Blüthgen et al., 2016; Habel et al., 2013).

Surface fertilizing of meadows and pastures is a major agronomic measure that

increases yields, improves the botanical composition of mesophytic grasslands in the process of degradation (Popescu & Churkova, 2015; Samuil et al., 2013). Feeding the plants with organic fertilizers creates conditions for the development of valuable and high quality species (Kurhak et al., 2020; Vargová et al., 2020), which positively affects the composition of hay and pasture in mesotrophic and other types of meadows (Kirkham et al., 2014). Nonorganic and organic fertilizing has been shown to increase the percentage share of grasses and dry matter yield (Iliev, 2018; Iliev et al., 2017; Nemera et al., 2017) and has a less pronounced effect on plant height in degraded grasslands (Ahmed & Ibrahim, 2013).

In the mountaine and foot-hill regions of Bulgaria, the ratio of individual plant groups in the composition of natural meadows and pastures, as well as the yield obtained from them, are indicators of the effectiveness of applied agrotechnical events (Blanke et al., 2012; Leps, 2014; Naydenova et al., 2013; Tomashkin et al., 2013).

The objective of the study was to determine the impact of the annual organic (cattle manure) surface fertilizing on the bioproductive indicators of degraded meadow grassland (with predominant share of *Festuca rubra* L.) in the region of the Central Balkan Mountain.

Material and Methods

The experiment was conducted at the Research Institute of Mountain Stockbreeding and Agriculture, Troyan (Bulgaria) in the period 2016-2019, on degraded grassland, in the conditions of the Central Balkan Mountain. The experiment was based on the block method, in 4 replications with a plot size of 5 m^2.

Variants in the study were:

- Control (untreated)
- Fertilizing with cattle manure at a dose of 1000 kg/da;
- Fertilizing with cattle manure at a dose of 2000 kg/da;
- Fertilizing with cattle manure at a dose of 3000 kg/da;
- Fertilizing with cattle manure at a dose of 4000 kg/da.

The cattle manure treatment was applied annually and once, every year, manually, by spraying, before the onset of active vegetation in the grass cover.

The experimental areas were harvested in the phenophase of tasseling-ear formation (for grass species) until the beginning of the phenophase of flowering.

Studied indicators:

Yield of fresh and dry mass (kg/da) - the yield of fresh and dry mass was determined by mowing the area of each harvest plot (in replications) followed by drying the plant samples (0.5 kg) in a laboratory dryer at 105°C and recalculating for an area of 1 da, based on dry mass content.

Botanical analysis of grassland (%) was determined by weight by analysis of grass samples taken immediately before mowing. The percentage share of each species per year from the group of grasses and legumes, of motley grasses (total) in both modes of use and their total ratio in the main botanical groups (grasses, legumes and motley grasses) was established.

Chemical analysis was conducted on: Crude protein (CP, %) according to *Kjeldahl* (following BDS/ISO-5983); Crude fiber (CFr, %); Crude fat (CF, %) (following BDS/ISO-6492) through rxtraction in an extractor *Soxhlet*; Ash (%) (following BDS/ISO-5984) through decomposition of the organic matter by gradual combustion of the sample in a muffle furnace at 550°C; Dry matter (DM, %) was empirically calculated from the % of moisture.

The results were analyzed by the method of analyzing the variance of a single-factorial trial (ANOVA) using the SPSS 4.5 software. The significance of differences in mean values of the treatments was tested by the LSD test.

Results and Discussion

Productivity of degraded grassland after manure fertilizing

In the first experimental year, fertilizing with manure had the greatest impact on the productivity of grassland at the imported rate of 2000 kg/da (Table 1). The amount of dry matter (303.5 kg/da) of the variant significantly exceeds the untreated control by 127.7%. The degraded grassland reacted positively to the imported higher norms of manure (3000 and 4000 kg/da). Dry matter yield of the variants increased by 70.4% and 89.1%, respectively, compared to the control. An increase in grassland productivity was also reported at the lowest rate (1000 kg/da) of fertilizer application, but without statistical significance. The amount of dry matter (207.7 kg/da) exceeded the variant without fertilizing by 55.8%.

Table 1. Dry matter yield (kg/da) of foot-hill mesophytic meadow, after organic fertilizing (over the years and average for 2016-2019).

Variants	2016		2017		2018		2019		2016-2019	
	kg/da	% compared to C	kg/da	% compared to C	kg/da	% compared to C	kg/da	% compared to C	kg/da	% compared to C
Control	133.3	100.0	104.3	100.0	358.7	100.0	197.5	100.0	198.5	100.0
1000	207.7	155.8	167.6	160.8	447.7	124.8	306.1	154.9	282.3	149.1
2000	303.5	227.7	259.2	248.6	569.3	158.7	316.8	160.4	362.2	198.8
3000	227.1	170.4	313.0	300.1	551.4	153.7	318.3	161.1	352.4	196.3
4000	252.0	189.1	276.4	265.1	503.3	140.3	356.2	180.3	347.0	193.7
GD 5 %	*86.2*	*64.8*	*105.4*	*100.8*	*110.7*	*30.9*	*85.7*	*43.3*	*56.4*	*26.7*
GD 1 %	*121.1*	*91.0*	*147.9*	*141.5*	*155.4*	*43.4*	*120.3*	*60.8*	*79.1*	*37.4*
GD 0.1 %	*170.9*	*128.5*	*208.8*	*199.8*	*219.5*	*61.2*	*169.9*	*85.8*	*111.7*	*52.8*

In the second year (2017) the dynamics of dry matter productivity follows the trend of the first experimental year. The degraded grassland reacted to the highest degree when fertilizing at a rate of 3000 kg/da. Dry matter yields of the variant (313.0 kg/da) were proven to exceed by 200.0% ($p<0.001$) the untreated control. A significant positive reaction of the degraded grassland to the applied fertilizing rates of 2000 and 4000 kg/da was observed. Dry matter yields of the variants are proven to exceed the fertilized variant by 148.6% and 165.1%, respectively. The excess of dry matter yield (by 60.8%) in the variant with the lowest test dose (1000 kg/da) remained statistically unproven.

Agroecological conditions and fertilizing dose affect the productivity of natural grasslands (Iliev et al., 2020). In the third experimental year (2018), the effect of the accumulated amount of manure in the degraded grassland, as well as the favorable climatic conditions during the year allowed the formation of the highest amount of dry biomass for the entire experimental period. Yields range from 358.7 to 569.3 kg/da. The variants with doses: 2000 kg/da (by 58.7%), 3000 kg/da (by 53.7%) and 4000 kg/da (by 40.3%) are again proven to exceed the control. The effect of fertilizing at norm of 1000 kg/da was less. The registered yield of the variant was 447.74 kg/da and marked a statistically unproven increase (by 24.81%) compared to the untreated control.

Under the impact of organic fertilizing, in the fourth year (2019) of the experiment, the increase in yields had proven positive differences in all treated variants. The excess of the indicator compared to the control varied from 54.9% (at a dose of 1000 kg/da) to 80.3% (at a dose of 4000 kg/da). When monitoring the productivity results, the fertilizing rates of 2000 and 3000 kg/da registered similar values (316.8 and 318.3 kg/da), where the dry matter yields were

increased by 60.4% and 61.1%, respectively, compared to the control variant.

On average for the four-year experimental period (2016-2019), the annual manure fertilizing had the maximum impact on dry matter productivity in degraded mountain grassland. A reliable effect on the biological productivity of grassland has been found in all fertilizing variants. The excess in the productivity of the treated variants was statistically proven compared to the control and varied from 49.1% (1000 kg/da) to 98.8% (2000 kg/da).

Botanical and quality composition of degraded grassland after fertilizing with manure

The changes in the botanical composition of the grassland (by groups and years) are shown in Table 2. Fertilizing with cattle manure increases most significantly the share of legumes in the degraded grassland.

Table 2. Botanical composition (%) of foot-hill mesophytic meadow (by years and groups), after applied organic fertilizing.

Variants	2016			2017			2018			2019		
	grasses	legumes	motley grasses	grasses	legumes	motley grasses	grasses	legumes	motley grasses	grasses	legumes	motley grasses
Control	19.5	32.7	47.8	11.1	56.7	32.2	18.5	42.4	39.1	23.8	39.0	37.2
1000	9.2	53.0	37.8	1.4	79.2	19.4	9.5	70.2	20.3	44.8	29.2	26.0
2000	30.7	34.7	34.6	11.7	61.1	27.2	28.9	46.7	24.4	29.4	50.0	20.6
3000	33.3	50.8	15.9	6.7	62.0	31.2	22.1	46.1	31.8	46.0	38.0	16.0
4000	29.5	36.9	33.6	3.4	64.2	32.4	18.3	48.7	33.0	25.9	52.5	21.6

The variants with the lowest fertilizing dose (1000 kg/da) had the highest presence of the legume component. In the first, second and third experimental years, legumes accounted for 53.0%, 79.2% and 70.2% of the grassland, respectively. Similar results (with a predominance of the legume component in the grassland during the first three years of experiment) were observed in the variants with annual application of 3000 kg/da of manure. The values of the indicator were 50.8% (in 2016), 62.0% (in 2017) and 46.1% (in 2018). In the fourth experimental year, the share of legumes in the biomass of both variants was lower compared to the group of grasses by 53.4% (in the variants with manure at a dose of 1000 kg/da) and by 21.1%% (in the variants with manure at a dose of 3000 kg/da). Throughout the test period, the percentage share of legumes compared to grasses prevailed in the grassland of the variants with fertilizing rates at 2000 kg/da (34.7%, 61.1%, 46.7%, 50.0%) and 4000 kg/da (36.9%, 64.2%, 48.7%, 52.5%). Dominant representatives of legumes were mainly *Trifolium hybridum* and *Vicia sativa*, and of grasses - *Festuca rubra* and *Festuca arundinaceae* Scherb. The group of motley grasses had the highest share in the grassland of unfertilized control - from 32.2 to 47.8%.

The organic fertilizing of the degraded grassland affected the changes in the percentage share of the species in the different grass groups.

In the first experimental year grass species, such as *Festuca arundinaceae* Scherb. and *Anthoxanthum odoratum* participated in the grassland of all variants (Fig. 1). Their share varied respectively from 4.1% (variants with manure at a dose of 1000 kg/da) to 21.9% (variants with manure at a dose of 3000 kg/da) and from 1.6% (variants with manure at a dose of 2000 kg/da) to 7.7% (variants with manure at a dose of 3000 kg/da).

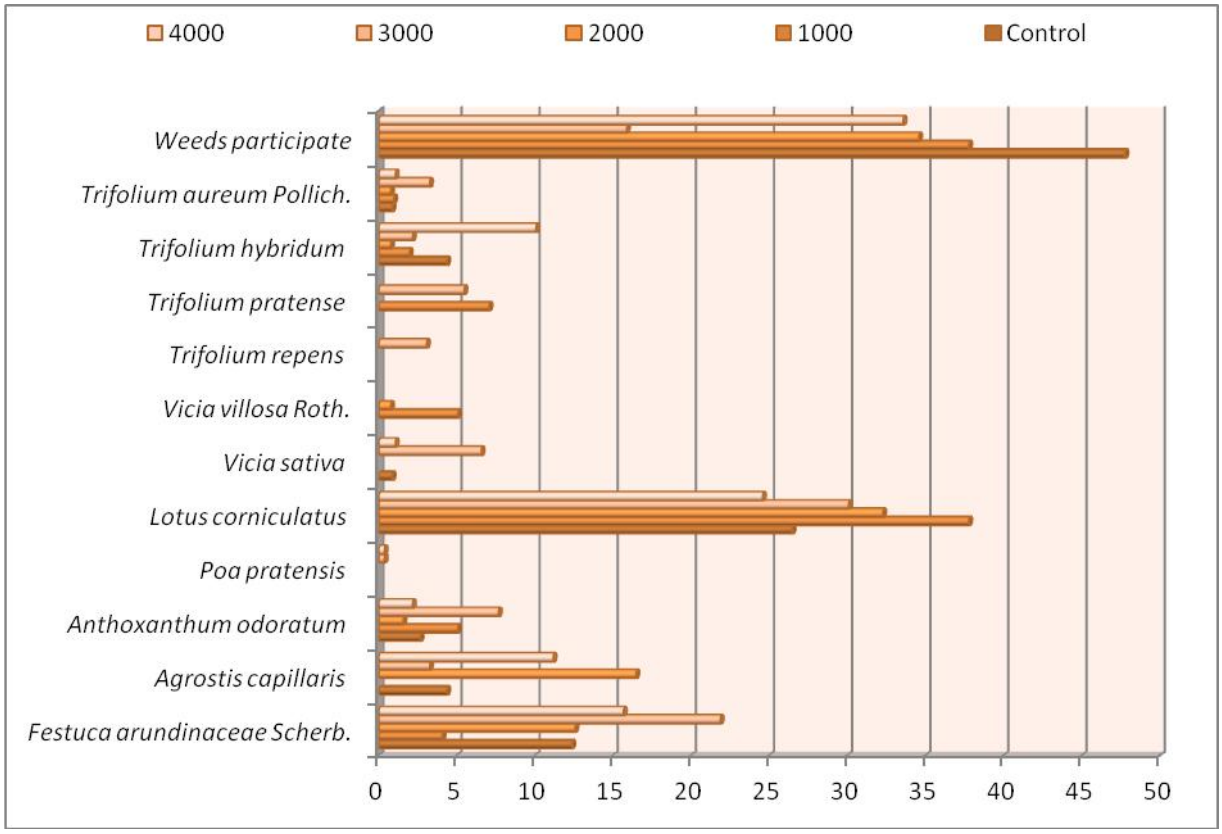

Fig. 1. Botanical composition by species (%) of foot-hill mesophytic meadow, after organic fertilizing (first experimental year - 2016).

The presence of other species was established:

- *Agrostis capillaris* - from 3.3% (variants fertilized with manure - dose - 3000 kg/da) to 16.5% (variants fertilized with manure - dose - 2000 kg/da) and
- *Poa pratensis* - 0.4% (variants fertilized with manure - dose - 3000 and 4000 kg/da).

Legumes: Lotus corniculatus - 24.6% (fertilized with manure at a dose of 4000 kg/da) to 37.8% (fertilized with manure at a dose of 1000 kg/da), *Trifolium hybridum* - 0.8% (fertilized with manure at a dose of 2000 kg/da) up to 10.1% (fertilized with manure at a dose of 4000 kg/da) and *Trifolium aureum* Pollich. - 0.8% (fertilized with manure at a dose of 2000 kg/da) to 3.3% (fertilized with manure at a dose of 3000 kg/da) participated in the grasslands of all tested variants.

The presence of other species was established:

- *Trifolium pratense* - from 5.5% (fertilized with a dose of 3000 kg/da manure) to 7.1% (fertilized with a dose of 1000 kg/da manure)
- *Vicia sativa* - from 0.9% (Control) to 6.6% (fertilized with a dose of 3000 kg/da manure)
- *Vicia villosa* Roth. - 0.8% (fertilized with a dose of 2000 kg/da manure) to 5.1% (fertilized with a dose of 1000 kg/da manure) and
- *Trifolium repens* - 3.1% (fertilized only with a dose of 3000 kg/da of manure).

In the second experimental year we observed a significant increase in useful legumes and lower participation of grasses. In the case of legumes, which had fallen out of the grassland, the species are: *Trifolium*

repens, *Trifolium aureum* Pollich. and *Vicia villosa* Roth. (Fig. 2). *Vicia cracca* (1.1-7.4%) and *Medicago lupulina* (1.2-1.5%) registered additional share in the botanical composition of the grassland. *Lotus corniculatus* had the highest share in the variant with fertilizer application - 1000 kg/da (37.0%), *Trifolium hybridum* in fertilizer application - 4000 kg/da (42.8%). The grasslands in these variants had the lowest share of grasses. The percentage share of *Vicia sativa* in the botanical composition of the grassland was from 2.2% (variants fertilized with manure at a dose of 1000 kg/da) to 22.6% (variants fertilized with manure at a dose of 3000 kg/da).

In the group of grasses additional presence of *Cynosurus cristatus* (0.5-0.8% - in the variants fertilized with a dose of 4000 and 3000 kg/da) and *Festuca rubra* (1.1% - in the variants fertilized with a dose of 4000 kg/da was found; 2.3% in the variants fertilized with a dose of 3000 kg/da and 3.7% in the unfertilized control).

In the third experimental year (2018), the applied organic fertilizing continued to have a positive effect, mainly the share of legumes in the grassland compared to that of forage grasses (Fig. 3).

Trifolium hybridum and Vicia sativa participated in the biomass of all studied variants. In grasslands that were fertilized, the percentage share of these species varied from 29.9% (at a dose of 2000 kg/da) to 50.0% (at a dose of 1000 kg/da) and from 1.7% (at a dose of 1000 kg/da) to 3.6% (at a dose of 3000 kg/da). *Lotus corniculatus* registered a presence of 4.6% (dose - 4000 kg/da) to 17.4% (dose - 1000 kg/da) only in the fertilized variants. The share of *Trifolium repens* and *Trifolium pratense* in the grassland was 0.4% (Control) to 1.1% (dose - 1000 kg/da) and from 0.9% (dose - 3000 kg/da) to 2.8% (dose - 2000 kg/da). During the year species, such as - *Vicia cracca* and *Medicago lupulina* were not present in the botanical composition of the grassland.

Subsequent fertilizing at a norm of 2000 kg/da, enriched the species diversity of grasses in the grassland, where traces of *Bromus arvensis* (0.9%) were found. *Festuca arundinaceae* Scherb., *Festuca rubra*, *Agrostis capillaris* and *Anthoxanthum odoratum*, registered the highest presence respectively in the variants fertilized annually with 2000 kg/da (11.2%), 3000 kg/da (8.2%), 4000 kg/da (7.3%).) and 2000 kg/da (8.4%). In the year, *Poa pratensis* dropped out of the grassland.

The dynamics in the change of vegetation (after the subsequent fertilizing in the fourth year - 2019) includes an increase in the degree of grass component in the composition of the grassland (Fig. 4).

Festuca rubra and *Agrostis capillaris* were dominant. Their share in the fertilized variants varied from 8.6% (for fertilizing with a dose of 1000 kg/da) to 20.5% (for fertilizing with a dose of 2000 kg/da) and from 5.5% (for fertilizing with a dose of 2000 kg/da) to 30.0%. (when fertilizing with a dose of 3000 kg/da). *Festuca arundinaceae* Scherb. predominated in the grassland of the untreated control (10.2%). *Bromus arvensis* (5.7%) and *Anthoxanthum odoratum* (2.0%) participated in the composition of the grassland only in the variants fertilized with a dose of 1000 kg/da and 3000 kg/da, respectively.

Trifolium hybridum had the largest share of legumes in the grassland - from 5.7% (at a dose of 1000 kg/da) to 38.0% (at a dose of 3000 kg/da). *Medicago lupulina* (8.6%), *Vicia sativa* (5.5%) and *Lotus corniculatus* (21.6%) registered the highest share in the variants fertilized with 1000 kg/da, 2000 kg/da and 4000 kg/da, respectively.

In the last experimental year (2019), *Trifolium pratense* and *Cynosurus cristatus* did not participate in the botanical composition of the treated grasslands, and the group of weeds showed a decreasing trend compared to the year when the experiment was set (2016). The percentage share of weeds in the variants with imported manure was reduced (except for the variant with imported dose of 3000 kg/da) by 45.4% (at a dose of 1000 kg/da), 55.6% (at a dose of 4000 kg/da) and 68.0% (at a dose of 2000 kg/da).

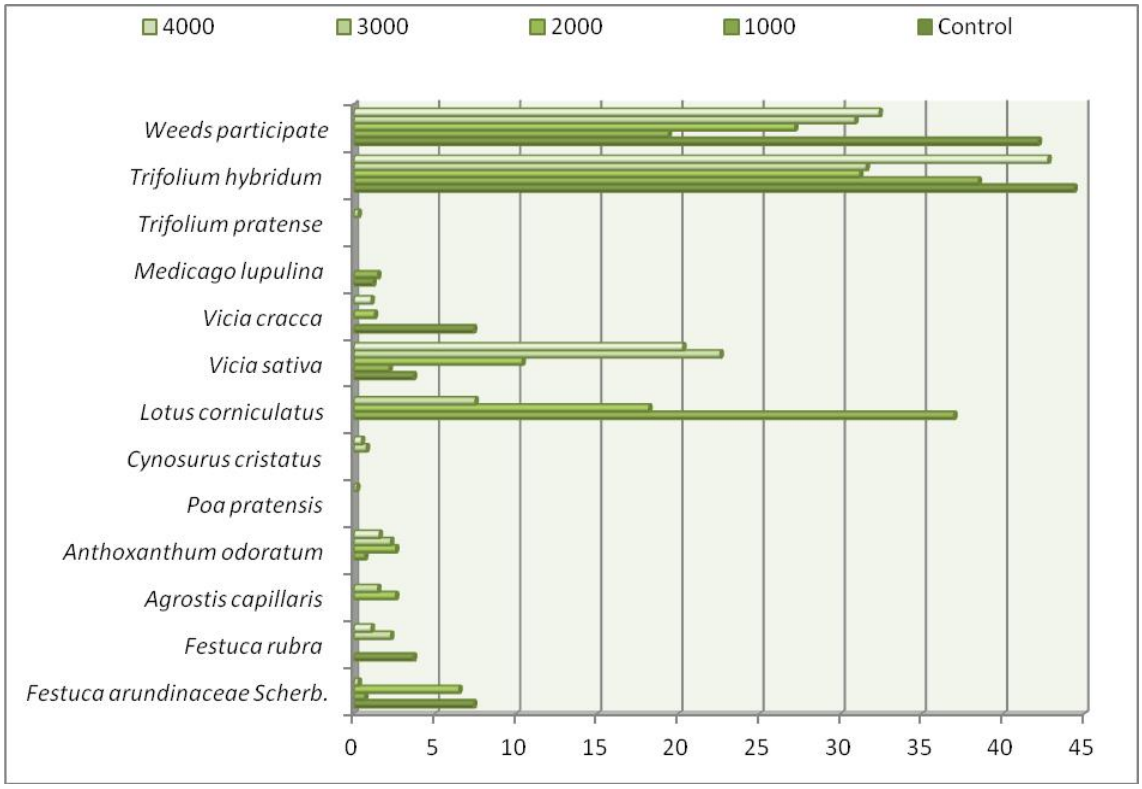

Fig. 2. Botanical composition by species (%) of foot-hill mesophytic meadow, after organic fertilizing (second experimental year - 2017).

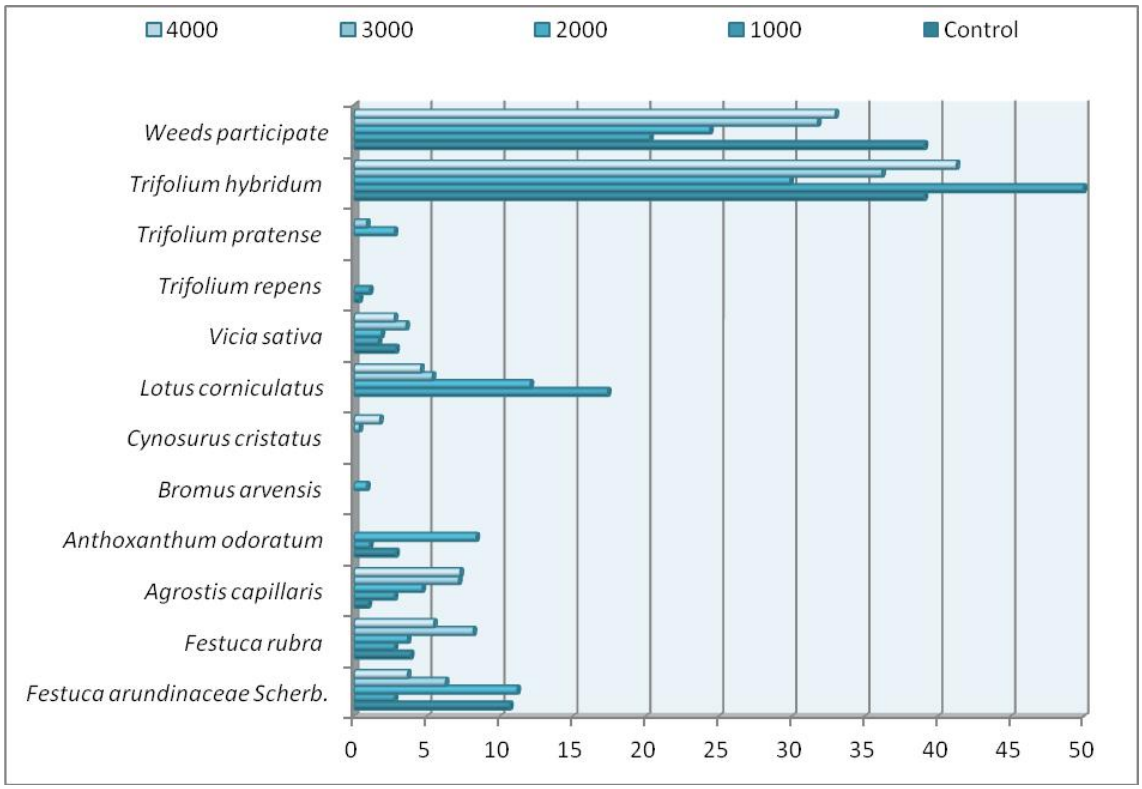

Fig. 3. Botanical composition by species (%) of foot-hill mesophytic meadow, after applied organic fertilizing (third experimental year - 2018).

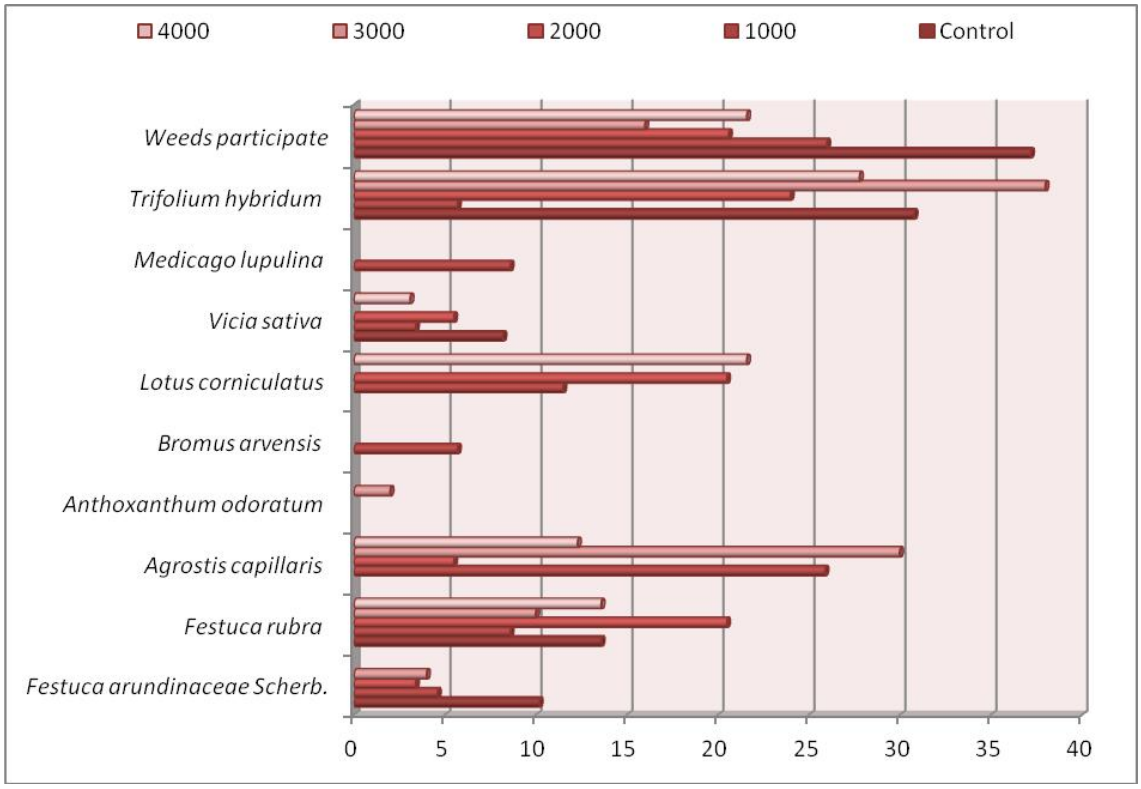

Fig. 4. Botanical composition by species (%) of foot-hill mesophytic meadow, after organic fertilizing (fourth experimental year - 2019).

Legumes were an unstable and changing component in the composition of natural grasslands. During the test period (after the application of cattle manure) the highest stability and adaptability were shown by species, such as: *Lotus corniculatus*, *Vicia sativa* and *Trifolium hybridum*. As for grass species, *Festuca arundinaceae* Scherb., *Festuca rubra*, *Anthoxanthum odoratum* and *Agrostis capillaris* showed the highest resistance to the mountain conditions of the Central Balkan Mountain. *Festuca arundinaceae* Scherb., *Festuca rubra*, *Anthoxanthum odoratum* and *Agrostis capillaris*.

Botanical composition is a factor that affects the feed and nutritional value of grass biomass (Churkova & Churkova, 2020; 2021; Naydenova et al., 2015; Naydenova & Vasileva, 2016). The dominance of legumes from the first to the third experimental year is clearly expressed in all variants of fertilizing, which implies the formation of biomass with a higher crude protein content. During the fourth (last) experimental year, organic fertilizing increased the percentage of grasses in the composition of the variants with different fertilizing rates.

All variants with organic fertilizing had higher values in terms of dry matter content, crude protein, crude fiber and crude ash compared to the untreated control. The average value of crude protein in the dry matter of the studied grasslands wass 12.995% at CV: 8.80% (Table 3). The variants with the lowest dose of manure (1000 kg/da) registered the highest content of crude protein (14.39%) followed of the grasslands (14.05%) treated with the highest dose of organic fertilizer (4000 kg/da). The values of the indicator exceeded the control by 20.0%% and 17.2%, respectively. With a slight difference in the protein content are the grasslands with annual intake of 2000 and 3000 kg/da (respectively 12.03 and 12.52%), where the excess compared to the control was 0.3% and 4.4%, respectively.

Table 3. Mean values ± SE (%) and coefficient of variation (CV %) of basic quality indicators of natural grassland cover, after applied organic fertilizing.

Variants	Dry matter	Organic matter			
		Crude protein	Crude fibre	Crude fat	Crude ash
Control	89.88	11.99	38.83	2.32	6.50
1000	90.10	14.39	42.84	2.43	6.83
2000	90.01	12.03	41.26	2.00	6.65
3000	90.18	12.52	44.37	1.37	6.98
4000	90.00	14.05	42.01	2.30	7.24
Average	*90.030±0.11*	*12.995±1.14*	*41.861±2.05*	*2.082±0.43*	*6.836±0.29*
CV	*0.13*	*8.8*	*4.9*	*20.61*	*4.21*
$LSD_{0.05}$	*0.331*	*0.763*	*4.221*	*0.723*	*0.506*

The average values for the amount of minerals (6.836%) and crude fiber (41.861%) are characterized by a low coefficient of variation (CV: 4.21-4.90%). The highest fiber (44.37%) and the lowest concentration of crude fat (1.37%) is the dry biomass from grasslands that were treated by manure with a dose of 3000 kg/da. The treated variants registered with 2.3% (2000 kg/da) to 11.4% (4000 kg/da) higher content of crude ash compared to the control. The average content of crude fat (2.082%) in the dry matter of the studied grasslands was characterized by a very high coefficient of variation (20.61%). Only the grasslands with the highest protein content exceeded by 4.7% the amount of crude fat (2.43%) in the dry matter compared to the control.

Conclusion

The applied biological fertilizing led to an increase in the productivity of the degraded grassland at all levels of fertilizing. The excess in the values of the indicator compared to the control was from 49.1% (1000 кg/da) to 98.8% (2000 кg/da). Some desired changes in the individual groups and species of the botanical composition of the grassland were also observed For the study period, with the highest resistance and adaptability in mountain conditions were species: *Lotus corniculatus*, *Vicia sativa*, *Trifolium hybridum* (**from legumes**) and *Festuca arundinaceae* Scherb., *Festuca rubra*, *Anthoxanthum odoratum*, *Agrostis capillaris* (**from grasses**). Organic fertilizing variants had a higher content of dry matter, crude protein, crude fiber and crude ash compared to untreated control. Grasslands with the lowest dose of manure (1000 kg/da) registered the highest concentration of crude protein (14.39%) followed by those (14.05%) with the highest dose (4000 kg/da) of manure. The values of the indicator exceeded the control by 20.0% and 17.2%, respectively. The difference in the protein content of the grassland treated annually with a dose of 2000 and 3000 kg/da was insignificant (12.03 and 12.52%, respectively), with an excess compared to the control - 0.3% and 4.4%. The highest fiber (44.37%) and the lowest concentration of crude fat (1.37%) were found in the biomass of grasslands with an imported dose of 3000 kg/da. In the fertilizing variants, the crude ash content was 2.3% (2000 kg da) to 11.4% (4000 kg/da) higher than the control.

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