Thaiszia - J. Bot., Košice, 34: 011–024, 2024
https://doi.org/10.33542/TJB2024-1-02

THAISZIA
JOURNAL OF BOTANY

Impact of fertilizing on biodiversity in pasture ecosystems

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Churkova K. & Churkova B. (2024): Impact of fertilizing on biodiversity in pasture ecosystems. – Thaiszia – J. Bot. 34: 011–024.

Abstract: Biodiversity in grazing systems determines production efficiency, system stability and resilience, and nutrient retention. In this relation during the period 2016-2018, a scientific research experiment was conducted on a natural grass stand of the *Nardus stricta* L. type at an altitude of 1400 m in the area of Chuchul (Bulgaria) of the following rates of fertilization: 1. Untreated (Control); 2. Annual fertilizing with $N_{60}P_{60}$; 3. First-year fertilizing with N_{60}, and second and third with P_{60}; 4. First-year fertilizing with P_{60}, and second and third with N_{60}; 5. First and second year fertilizing with N_{60}, and third with P_{60}; 6. First and second year fertilizing with P_{60}, and third with N_{60}; 7. First year fertilizing with N_{60}, second year with P_{60}, third year with N_{60}; 8. First-year fertilizing with P_{60}, second year with N_{60}, third year with P_{60}. The aim of the article is to analyze the impact of fertilizing with mineral fertilizers on the biodiversity of a pasture ecosystem and to propose an appropriate way of fertilizing to improve the species diversity of useful components in the grass stand. Biodiversity was determined by the indicator botanical composition of the grass stand, and the species diversity of the components in the grass stand by botanical groups was observed under eight variants of variable mineral fertilizing. It was found that the fertilization increased share of perennial grasses and motley grasses. The species *Nardus stricta* L., defining the type of pasture, was replaced from the more useful types of grasses, such as: *Nardus stricta* L., *Agrostis capillaris* L., *Phleum alpinum* L., *Festuca ovina* L., *Festuca rubra* L., *Agrostis alba* L., *Chrysopogon gryllus* L., *Dactylis glomerata* L., *Lolium multiflorum* L. The fertilizing effect is most significant in the third year, when both the representatives of grasses and legumes were significantly increased.

Keywords: pasture ecosystems, fertilizing, biodiversity, botanical composition.

Introduction

Biodiversity encompasses all forms of life on Earth with all the diversity of species, genetic variation and interactions between them. It plays a fundamental role in the functioning of terrestrial and aquatic ecosystems, soil formation and the processes taking place in soils, ensuring the circulation of nutrients and maintaining their fertility. For grazing systems, biodiversity has been shown to be positively related to primary production, system stability and resilience, as well as nutrient retention and efficiency (Tilman et al. 1996; Loreau 2000).

Biodiversity has been declining in recent years at an alarming rate. The reason for this is the way we use the lands, the pollution of the air, the ocean and the land, which leads to climate changes. The European Union is therefore committed to find solutions to the problem of biodiversity loss and the degradation of ecosystem services in the EU by 2020. The 2015 Paris Agreement on Climate Change (Paris Agreement 2015) notes that it is important to ensure the integrity of all ecosystems and the protection of biological diversity. As part of the European Green Deal, a new biodiversity strategy (Biodiversity Strategy for 2030) has been proposed, which aims to steer Europe's biodiversity towards recovery by 2030 for the benefit of people, the climate and the planet.

The Biodiversity Strategy is a key element of the European Recovery Plan (EU 2020). For biodiversity to be on the road to recovery by 2030, Europe needs to improve nature conservation and restoration. To achieve this goal, it is necessary to improve and expand the European network of protected areas and to develop an EU plan for the restoration of nature.

Biodiversity is an essential part of Europe's future and an economic and social pillar of many communities in the Union.

The Biodiversity Strategy is directly linked to the new Farm to Fork Strategy (EU 2020) and the new Common Agricultural Policy (CAP), including through the promotion of eco-schemes and result-based payment schemes. Their implementation includes compliance with climate and environmental criteria. These plans should ensure the implementation of sustainable practices (e.g. precision farming, organic agriculture, agroecology, agroforestry and low-intensity permanent grassland) and stricter animal welfare standards.

The study on biodiversity is essential for the implementation of a national policy based on knowledge in the field of nature conservation and the development of the "green strategy" of Bulgaria (Atanassova 2022). The diversity of Bulgarian flora and fauna has significant value as a biological resource important for the livelihood of the population and the economy. Their sustainable management is important for the development and increase of public welfare.

The economic value of biodiversity is becoming increasingly important to people because businesses invest in biodiversity protection and their revenues directly depend on healthy ecosystems. Grass areas represent one of the dominant biomes on Earth, accounting for up to 40% of its land area (Gibson 2023). The main components of pasture habitats are grasses and herbaceous plants, but diverse assemblages of other plant life forms and different animal communities also contribute to pasture biodiversity.

Applied agricultural practices, however, can contribute to the reduction of biological diversity to varying degrees. This in turn necessitates the provision of incentives for the implementation of sustainable practices. Improving the condition and diversity of agricultural ecosystems will increase the sector's resilience to climate change, environmental risks (Erisman et al. 2016; Çakmakçı et al. 2023). Fertilizing is such an improvement measure that preserves and improves biodiversity.

It increases the availability of nutrients to plants. The competitive ability of herbaceous species for light and water reduces species richness. Soil composition determines competition for nutrients and reduces the relative share of slow-growing species. Higher biodiversity is usually observed with lower competition (Grime 1979) and large numbers of mesotrophic species may coexist with some oligotrophic and some eutrophic species. The variety of soil, climate and type of fertilizer determine the threshold levels of herbaceous species. It is clear from various studies that, in general, there is a significant reduction in plant diversity, even at low fertilizer rates, compared to normal application rates. As the amount of applied nitrogen per 75 kg $N.ha^{-1}$ increases, the number of grass species decreases. The species diversity in the pasture increased at lower nitrogen fertilizer rates of 75 kg $N.ha^{-1}$. Research on the effect of phosphorus on the number of plant species is less studied, but Janssens et al. (1998) found a maximum of 10 dicots at values above 50 mg $P.kg^{-1}$ dry soil. Potassium has a weaker effect on biodiversity, as its high content (100 and 200 mg $K.kg^{-1}$ dry soil) preserves a large number of plant species (Peeters et al. 1994).

Manure affects biodiversity in pastures. In intensive pastures, seed import affects very few species of low ecological value. Nitrogen fertilizing increases live plant biomass and litter and reduced plant biodiversity. Litter removal led to an increase in species richness, proving the reality of this screening effect. The effects of fertilizers are not limited to plant species, as they lead to enormous changes in the chemical, biological and physical status of the soil. Soils in pastures contain an abundant and diverse community of microorganisms, micro- and macrofauna. Intensive pasture management has a negative effect on overall diversity but not on soil fauna density (Bardgett & Cook 1998). In interactions between above-ground and below-ground diversity, (Plantureux et al. 2005) decreased plant biodiversity with increasing nitrogen fertilizing treatment in pasture experiments.

Agricultural intensification is directly connected to the increase and loss of biodiversity in rural areas. Traditionally managed species-rich pastures are at risk of being abandoned with accompanying loss of species diversity (Simons & Wiesser 2017).

A potential benefit to biodiverse pastures is the maintenance of botanical composition and plant species diversity through regular use. Abandonment of pastures leads to increasing dominance of tall species and the invasion of woody plants, which reduces their productivity and nutritional value (Peeters and Janssens 1998; Tallowin & Jefferson 2008). There is a high biodiversity in these pastures and they are attractive to tourists and urban recreation seekers (Nösberger & Rodriguez 1996; Khan et al. 2009). Species diversity of plant genotypes is important for their use in breeding improvement programs (Nösberger & Rodriguez 1996). Pastures for intensive animal husbandry are usually characterized by low species diversity, with only a few valuable forage species dominating the grass areas. The species are introduced by sowing, as they possess high yield potential and nutritional value.

Pastures are dynamic ecosystems with high biodiversity and complex biogeochemical fluxes (Rumpel et al. 2015). Anthropogenic factors amplify natural changes, leading to a general decrease in ecosystem stability (Naeem et al. 2012; Vogel et al. 2012; Aubree et al. 2020) and biodiversity. Interspecific competition of biological groups and additional resources stimulate the emergence of dominance-codominance phenomena supported by interactions among species. At the same time, spatiotemporal analyzes provide qualitative and quantitative indicators of the evolution (or regression) of an ecosystem.

Pastures are a vital part of the world's ecosystems and occupy more than a quarter of the earth's land area. Maintaining the species diversity of pastures is crucial to avoid loss of productivity, which in turn affects ecosystem processes (Mogle & Mane 2010). One of the factors influencing pasture diversity is zonation, which promotes diversity (Canellas et al. 2002; Pizzeghello et al. 2013). Seed dispersal is also an important influencing the coexistence of multiple plant species (Nardi et al. 2002, 2007; Khan et al. 2009). Heterogeneticity in pastures positively affects species diversity (Benizri & Amiaud 2005; Schiavon et al. 2008). Overgrazing reduces species diversity. At the same time, the introduction of light grazing or an increase in grazing intensity has been shown to increase species diversity in some pastures. Moderate grazing promotes biodiversity (Marfà et al. 2009), and heavy grazing has an indirect effect.

The application of systems and activities for sustainable agriculture is related to the improvement of the use of pastures, the restoration of the habitats of wild species, the cultivation of rare plant cultivars, through educational programs and economic stimulation. This should also include the diversification of the seeding material used, the protection of soil-improving pasture species, the restoration of damaged agricultural lands and appropriate control over the use of artificial fertilizers and pesticides (Frison et al. 2011). The scientific research activity in this direction is not sufficient to realize the ecological effect of the measures in this direction. This necessitated a thorough study of the ways of fertilizing with mineral fertilizers on the species diversity of herbaceous plants in pasture ecosystems (Funabashi 2016).

The aim of the present research is to analyze the impact of mineral fertilizers on the biodiversity of a *Nardus stricta* L. pasture and to propose an appropriate method of fertilizing to improve the species diversity of useful components in the grass stand.

Material and Methods

During the period of three years (2016-2018), a scientific research experiment was conducted on a natural grass stand of the pasture grass association of the type *Nardus stricta* L. at an altitude of 1400 m in the area of Chuchul (Middle Stara Planina – Bulgaria) with different methods and rates of fertilizing. The experiment was conducted on a south-facing sun exposure, with a slope of 9°, near a broad-leaved forest massif. The planting method is block, with 5 m^2 plot size in four replications on mountain-meadow (humus-silicate) soil. Mineral fertilizers were introduced at the following rates and combinations presented in kg/ha: 1. Untreated (Control); 2. Annual fertilizing with $N_{60}P_{60}$; 3. First-year fertilizing with N_{60}, and second and third with P_{60}; 4. First-year fertilizing with P_{60}, and second and third with N_{60}; 5. First and second year fertilizing with N_{60}, and third with P_{60}; 6. First and second year fertilizing with P_{60}, and third with N_{60}; 7. First year fertilizing with N_{60}, second year with P_{60}, third year with N_{60}; 8. First-year fertilizing with P_{60}, second year with N_{60}, third year with P_{60}.

Fertilizing with phosphorus, introduced in the form of double superphosphate, was carried out once a year after the end of the active vegetation, and nitrogen in the form of ammonium nitrate before the start of the active vegetation of the plants, in early spring. Mineral fertilizers were applied manually, by spreading.

Harvesting of the experimental areas was carried out by mowing before ear formation of the matgrass once per year.

Biodiversity is reported by the botanical composition of the grass stand in weight %, which is determined by weight through the analysis of grass samples taken immediately before mowing, and the percentage participation of grasses and legumes species, and of the motley grasses (total) in the grassland during the years of the experimental period has been determined.

Results and Discussion

The analysis of biodiversity data by botanical groups by year and average for the period is presented in Tab. 1, indicating the relative proportion of grasses, legumes and motley grasses, which include herbaceous species outside the families Poaceae and Fabaceae. In the untreated control, in the first year, grasses prevailed with 66.7% share in the grass stand (variant 1). Their amount is almost similar when fertilizing in the first year with P_{60}, and the second and third year with N_{60} (variant 4), respectively 66.6%. Legumes occupy a small share in the grass stand, and their participation varies from 2.3 to 26.4%. The fertilizing with N_{60} (variant 5) and P_{60} (variant 6) in the first year recorded the highest relative share of legumes in the grass stand with 26.4 and 23.3%, respectively. Fertilizing with P_{60} (variant 4) contributed to the reduction of motley grasses to 25.1%.

Tab. 1 Botanical composition of a matgrass pasture in % by year and average for the period.

Fertilizing variants	Botanical groups	First experimental year	Second experimental year	Third experimental year	Average for the period
1. Untreated (Control)	Grasses	66.7	61.9	28.8	52.5
	Legumes	3.0	2.1	0.0	1.7
	Motley grasses	30.3	36.0	71.1	45.8
2. Annual fertilizing with $N_{60}P_{60}$	Grasses	42.5	52.4	12.3	35.7
	Legumes	18.2	4.8	2.9	8.6
	Motley grasses	39.3	42.8	84.8	55.6
3. First year fertilizing with N_{60}, second and third with P_{60}	Grasses	59.1	63.6	36.0	52.9
	Legumes	2.3	0.0	3.2	1.8
	Motley grasses	38.6	36.4	60.8	45.3
4. First year fertilizing with P_{60}, second and third with N_{60}	Grasses	66.6	50.0	41.5	52.7
	Legumes	8.3	5.0	0.0	4.4
	Motley grasses	25.1	45.0	58.5	42.9
5. Fertilizing in first and second year with N_{60}, and in the third with P_{60}	Grasses	29.0	60.0	11.2	33.4
	Legumes	26.4	5.0	44.9	25.4
	Motley grasses	44.6	35.0	43.9	41.2
6. First and second year fertilizing with P_{60}, and third with N_{60}	Grasses	39.9	54.2	12.7	35.6
	Legumes	23.3	0.0	41.6	21.6
	Motley grasses	36.8	45.8	45.7	42.8
7. First year fertilizing with N_{60}, second with P_{60} and third year with N_{60}	Grasses	33.3	45.8	40.7	39.9
	Legumes	4.2	0.0	1.6	1.9
	Motley grasses	62.5	41.7	57.7	54.0
8. First year fertilizing with P_{60}, second year with N_{60}, third year with P_{60}	Grasses	34.4	64.0	12.7	37.0
	Legumes	15.7	0.0	0.0	5.2
	Motley grasses	49.9	36.0	87.3	57.7
Average for variants with applied fertilization	Grasses	46.4	56.5	24.5	42.5
	Legumes	12.7	2.1	11.8	8.9
	Motley grasses	40.9	39.8	63.7	48.1

Source: Data annual reports project Zh 100 to the Bulgarian Agricultural Academy and publication of Iliev et al. (2017).

In the second experimental year, the relative share of grasses increased in all variants. Their amount varies from 45.8 to 64.0%. The legumes in variants 3, 6, 7, and 8 are completely absent from the grass stand, moreover in the other fertilizing rates they are in small quantity. Motley grasses decreased slightly compared to the first year from 40.9 to 39.8%, whereas grasses increased.

In the third experimental year, the effect of applied fertilizing was less pronounced on grasses and legumes. Grasses surpass legumes in all variants except variant 6, where the share of legumes is 41.6%. In the other variants, fertilizing stimulates the growth of motley grasses, whose share in the grass stand is from 43.9 (variant 5) to 87.3% (variant 8).

The average applied mineral fertilizing with N and P over the matgrass pasture increased the relative share of grasses, whose share was 42.5% and of motley grasses by 48.1%. The obtained data confirm the results of the studies of Stoeva & Vateva (2008) and Samuil et al. (2013) that properly regulated and balanced mineral fertilization creates conditions for favorable changes in the phytocenological and quality profile of natural grass reports. The effect of the applied fertilizing on legumes was less pronounced (8.9%). The highest share of grasses was reported in the second experimental year (56.5%) and the lowest in the third year (24.5%). Legumes have almost similar values in the first and third years, 12.7 and 11.8%, respectively, and their amount in the second year reached 2.1%. The best manifested influence of fertilizing was observed in the second experimental year, when motley grasses were the least (39.8%), as the slightest effect was noticed in the third year when they reached the highest relative share (63.7%).

Biodiversity represented by the species composition of the grass stand fertilized with mineral fertilizers in the first experimental year is presented in Fig. 1. The quantitative share of *Nardus stricta* is the highest in the untreated variant (variant 1). Its presence is relatively high in the grass stands fertilized with $N_{60}P_{60}$ (variant 2) and P_{60} (variant 4), respectively 33.3 and 27.3%. The main type of *Nardus stricta* in the grass stand was preserved under all fertilizing variants, but the fertilizing

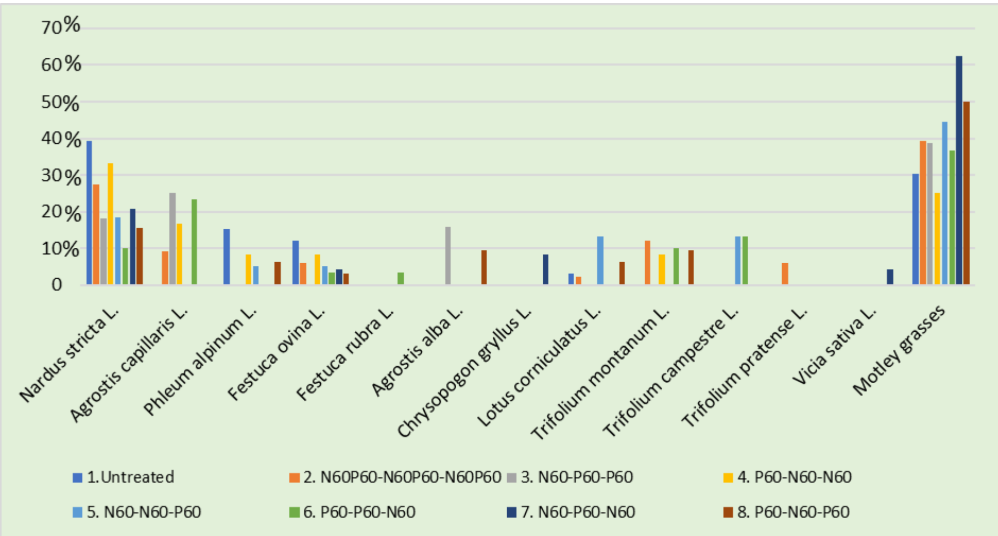

Fig. 1 Biodiversity of species from the botanical groups of grasses, legumes and motley grasses (%) in the first experimental year under the influence of variable fertilizing with nitrogen and phosphorus.
Source: Data annual reports project Zh 100 to the Bulgarian Agricultural Academy and publication of Iliev et al. (2017).

methods changed its share. Fertilizing with P_{60} (variant 6) determined the lowest share of *Nardus stricta* in the grass stand (10%), whereas the highest share of *Agrostis capillaris* L. with 25.0% was observed in N_{60} (variant 3). Close to this value is the relative share of *Agrostis capillaris* after fertilizing with P_{60} (variant 6) with 23.3%. *Phleum alpinum* L. has the highest share in the grass stand in the untreated control, whereas after fertilizing it decreased or was absent from the grass stand. A share of 8.3% was provided by fertilizing with P_{60} (variant 4). *Festuca ovina* L. again has the highest relative share in the untreated variant, and in the case of fertilizing, its quantitative presence varies slightly from 5.3 to 8.3%. *Festuca rubra* L. has a low share only when fertilized with P60 (variant 6), respectively 3.3%. Fertilizing with N_{60} (variant 3) ensures the presence of the *Agrostis alba* L. species with 15.9%. *Chrysopogon gryllus* L. was observed only when fertilized with N_{60} (variant 7) in an amount of 8.3%.

Biodiversity of legumes in the first experimental year was lower than grasses, both in terms of biodiversity and their quantitative share. They are represented by species, such as *Lotus corniculatus* L., *Trifolium montanum* L., *T. campestre* L., *T. pratense* L., *Vicia sativa* L. The relative share of *Trifolium campestre* is higher, which in fertilizing with N_{60} (variant 5) and P_{60} (variant 6) has similar values, 13.2 and 13.3%, respectively. Motley grasses are present presence in the grass stand under all fertilizing methods, as the highest percentage (62.5%) was registered in the variant with nitrogen (variant 7), whereas phosphorus at dose P_{60} (variant 4) reduced their amount to 25.1%.

Fertilizing in the second year (Figure 2) affects grasses to a greater extent than legumes. A trend of increase in grasses and decrease in the number of legumes was

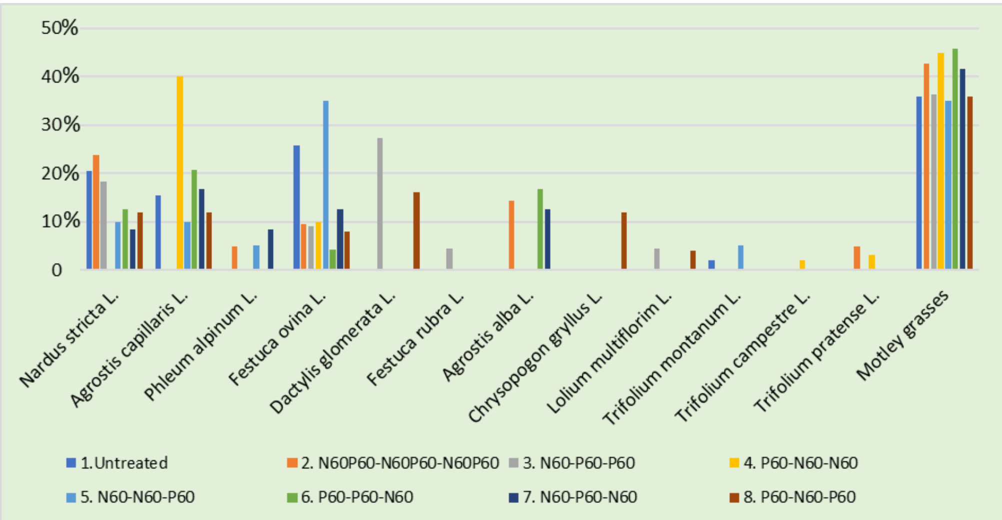

Fig. 2 Biodiversity of species from the botanical groups of grasses, legumes and motley grasses (%) in the second experimental year under the influence of variable fertilizing with nitrogen and phosphorus. Source: Data annual reports project Zh 100 to the Bulgarian Agricultural Academy and publication of Iliev et al. (2017).

observed. Compared to the first year, grasses have increased by two components. Fertilizing affected the presence in the grass stand of *Dactylis glomerata* L. and *Lolium multiflorum* L. and the decline of the legume species, such as *Lotus corniculatus* and *Vicia sativa*. The main species *Nardus stricta* was less abundant in all variants compared to the first year. It is present from 8.3% when fertilized with P_{60} (variant 7) to 23.8% when fertilized with $N_{60}P_{60}$ (variant 2). *Agrostis capillaris* took the highest share of grasses (40.0%) when nitrogen fertilizer was applied at a dose of 60 kg/ha. The effect of fertilizer rates and combinations is less pronounced on the *Phleum alpinum*, which is present with 4.8% (variant 2), 5.0% (variant 5) and 8.3% (variant 7). A significant increase of *Festuca ovina* is observed in all fertilizing variants with the maximum presence in the grass stand (35%) fertilized with N_{60} (variant 5). The presence of the grass component *Dactylis glomerata* (27.3%) proves the stimulating effect of phosphorus on the development of grasses. *Festuca rubra* is present only in the grass stand (4.5%) fertilized in the first year with N_{60}, and the second and third with P_{60} (variant 3). Fertilizing in variants 2, 6 and 7 stimulated the presence of *Agrostis alba* in the grass stand, respectively 14.3, 16.7 and 12.5%. The nitrogen fertilizer in the second year determined 12.0% share of *Chrysopogon gryllus* only in variant 8. The phosphorus fertilizing ensures the presence of *Lolium multiflorum* species in the grass stand with 4.5% (variant 3), and the self-fertilisation with nitrogen with 4.0% (variant 8). Both mineral fertilizers have an equally stimulating effect on this species. Legumes are represented only by the genus *Trifolium* with *T. montanum* (from 2.1 to 5.0%), *T. campestre* (2.0%), *T. pratense* (from 3.0 to 4.8%). The share of motley grasses maintained a relatively high presence, and the fertilizing, straight or as compound fertilizers, increased the share of motley grasses, respectively from 36.0 (variant 1) to 45.8 (variant 6). The analysis

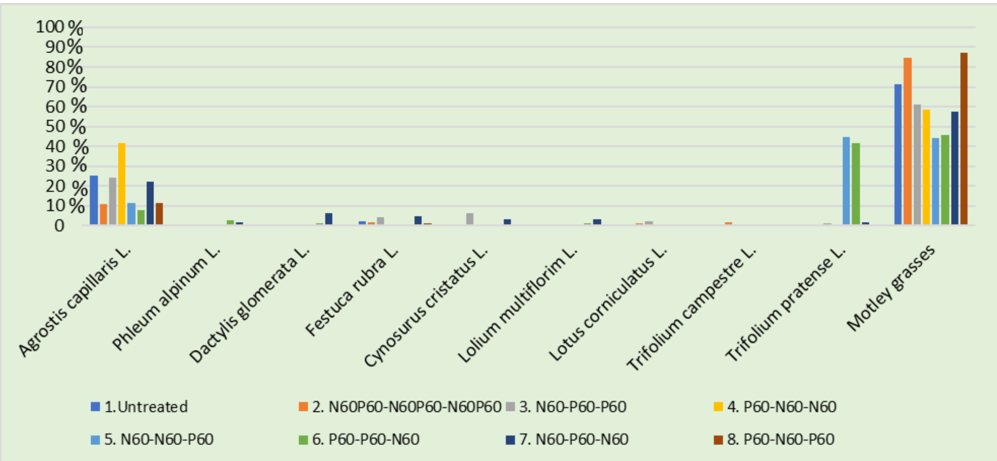

Fig. 3 Biodiversity of species from the botanical groups of grasses, legumes and motley grasses (%) in the third experimental year under the influence of variable fertilizing with nitrogen and phosphorus. Source: Data annual reports project Zh 100 to the Bulgarian Agricultural Academy and publication of Iliev et al. (2017).

of the data from the botanical composition of the grass stand shows the positive effect of the applied variable fertilizing on the biodiversity of the grasses and motley grasses and reducing the relative share of legumes. This confirms the necessity for the application of environmentally friendly fertilizer norms that sparing the environment, as well as, positively influencing the yield and quality of fodder obtained from these natural resources (Kolczarek et al. 2008). Grasses displaced *Nardus stricta* and replaced it with the beneficial grasses mentioned above. From this point of view, the applied fertilizing has a positive effect, since the grasses increased the nutritional value of the forage and their presence is desirable. The effect of fertilizing on the presence of legumes is significantly worse, which is evident from the obtained data. Fertilizers used can increase or decrease the participation of characteristic species that define meadow or grassland ecotypes, which was investigated in the research of Maruşca et al. (2014).

In the third experimental year, the effect of herbaceous species share is best expressed again in relation to grasses *Nardus stricta* completely disappears from the grass stands under all fertilization options and is mainly replaced by *Agrostis capillaris*. Fertilizing with P_{60} in the first year, and N_{60} in the second and third year (variant 4) resulted in the presence of *Agrostis capillaris* in the grassland by 41.5%. The rest of the grasses have a relatively low relative share in the grass stand. Their biodiversity is represented by *Phleum alpinum*, *Dactylis glomerata*, *Festuca rubra*, *Cynosurus cristatus* L., *Lolium multiflorum* which have a small share of the grass stand. Legumes were represented by *Lotus corniculatus* (from 1.0 to 2.0%) and *Trifolium campestre* (1.9%), which registered an extremely low presence. *Trifolium pratense* recorded a growth of 44.9% when fertilized with N_{60} in the first and second year and with P_{60} in the third year (variant 5), as a high presence (41.6%) was also realized in fertilizing in the first and second year with P_{60}, and the third with N_{60} (variant 6). *Trifolium pratense* is the dominant representative of legumes, extremely responsive to applied fertilization with good adaptability in hay and pasture grass stands in the region of Central Northern Bulgaria (Totev 1984; Naydenova & Mitev 2015). The obtained results confirm the results obtained by Maruşca et al. (2014) and Vintu et al. (2015).

The analysis of the data shows that the alternation of mineral nitrogen and phosphorus fertilizers in the indicated schemes according to variants has a positively impact on increasing the legume components in the grass stand, which suggests an increase in the content of crude protein and the nutritional value of the forage. The mineral fertilizing in the third year significantly increased the share of motley grasses. It varies from 43.9% (variant 5) to 87.3% (variant 8).

Conclusion

The applied variable mineral fertilizing, combined and by rotation, significantly affected the biodiversity of grass species in a *Nardus stricta* grass stand. The effect of fertilizing is well demonstrated on the share of perennial grasses and motley grasses. The mainly dominant species *Nardus stricta*, defining the type of pasture, was replaced from the more useful types of grasses, such as: *Nardus stricta*, *Agrostis capillaris*, *Phleum alpinum*, *Festuca ovina*, *Festuca rubra*, *Agrostis alba*, *Chrysopogon gryllus*, *Dactylis glomerata*, *Lolium multiflorum*. A weaker effect of fertilizing on legumes was registered, whose presence in the grass stands in the experimental years is different.

Under the impact of variable fertilizing, in some of the variants, the share of legumes in the grass stand increased. This interdependence is most pronounced in the third year. *Trifolium pratense* recorded the highest relative share of legumes in the grass stand when fertilized with N_{60} in the first and second year and with P_{60} in the third year (variant 5), as well as fertilizing with P_{60} in the first and second year and with N_{60} in the third year (variant 6). The applied fertilizing improved the biodiversity of legumes with representatives *Lotus corniculatus*, *Trifolium montanum*, *T. campestre*, *T. pratense*, *Vicia sativa* and their percentage share in the grass stand depended on the applied fertilizing methods and rates during the experimental years. The fertilizing effect is most significant in the third year, when both the representatives of grasses and legumes were significantly increased. The investigated species composition of the *Nardus stricta* pasture during the three-year study period is in accordance with that obtained by Iliev et al. (2017) and proves that the applied fertilization is an essential element of the surface improvement technology in order to preserve the biological diversity and balance in the ecosystem.

References

Atanassova I. (2022): Sustainable Soil Management in the European Green Deal and Biodiversity and Farm-to-Fork Strategies. – The collection of reports of the scientific conference "Ecology and agrotechnologies – fundamental science and practical implementation". p. 11–16.

Aubree F., David P., Jarne P., Loreau M., Mouquet N. & Calcagno V. (2020): How community adaptation affects biodiversity–ecosystem functioning relationships. – Ecol. Lett. 23: 1263–1275. doi.org/10.1111/ele.13530

Bardgett R. & Cook R. (1998): Functional aspects of soil animal diversity in agricultural grasslands. – Appl. Soil Ecol. 10/3: 263–276. doi.org/10.1016/S0929-1393(98)00125-5

Benizri E. & Amiaud B. (2005): Relationship between plant and soil microbial communities infertilized grassland. – Soil Biol. Biochem. 37/11: 2055–2064. doi.org/10.1016/j.soilbio.2005.03.008

Biodiversity Strategy for 2030 (2020): European Commission, Brussels. May 20, 2020, COM (2020).

Çakmakçı R., Salık M. A. & Çakmakçı S. (2023): Assessment and principles of environmentally sustainable food and agriculture systems. – Agriculture 13/5: 1073. doi.org/10.3390/agriculture13051073

Canellas L., Olivares F., Okorokova-Facanha A. & Facanha A. (2002): Humic acids isolated from earthworm compost enhance root elongation, lateral root emergence, and plasma membrane H+-ATPase activity in maize roots. – Plant Physiol. 130/4: 1951–1957. dx.doi.org/10.1104/pp.007088

Erisman J. W., Eekeren N., Wit J., Koopmans C. J., Cuijpers W. J. M., Oerlemans N. & Koks B. J. (2016): Agriculture and biodiversity: A better balance benefits both. – Agriculture and Food 1/2: 157–174. doi: 10.3934/agrfood.2016.2.157

European Commission (2020): Farm to Fork Strategy. May 20, 2020, Brussels, Belgium.

European Commission (2020): Recovery Plan for Europe. 17 December, 2020, Brussels, Belgium.

Frison E. A., Cherfas J. & Hodgkin T. (2011): Agricultural biodiversity is essential for a sustainable improvement in food and nutrition security. – Sustainability 3/1: 238–253; doi.org/10.3390/su3010238

Funabashi M. (2016): Synecological farming: Theoretical foundation on biodiversity responses of plant communities. – Plant Biotechnol. 33/4: 213–234.

Gibson D. J. (2023): Grasses and Grassland Ecology. – Oxford University Press, USA.

Grime J. P. (1979): Plant strategies and vegetation processes. – John Wiley and sons, Sheffield, United Kingdom, 186 pp.

Iliev M., Churkova B. & Mihovsky T. (2017): Influence of variable mineral fertilization with N and P over the bioproductive indicators of a natural grassland of the type *Nardus stricta*. – J. Mountain Agricult. Balkans 20/6: 189–202.

Janssens F., Peeters A., Tallowin J. R. B., Bakker J. P., Bekker R. M., Fillat F. & Oomes M. J. M. (1998): Relationship between soil chemical factors and grassland diversity. – Plant and soil 202: 69–78. https://link.springer.com/article/10.1023/A:1004389614865

Khan W., Rayirath U. P., Subramanian S., Jithesh M. N., Rayorath P., Hodges D. M., Critchley A. T., Craigie J. S., Norrie J. & Prithiviraj B. (2009): Seaweed extracts as biostimulants of plant growth and development. – J. Plant Growth Regul. 28/4: 386–399. dx.doi.org/10.1007/s00344-009-9103-x

Kolczarek R., Ciepiela G. A., Jankowska J. & Jodełka J. (2008): Influence of different forms of nitrogen fertilization on the content of macrolements (K, Na) in meadow sward - Part I. – Polish Society for Magnesium Research, Olsztyn, Poland. – J. Elementology 13/3: 341–348.

Loreau M. (2000): Biodiversity and ecosystem functioning: recent theoretical advances. – Oikos 91: 3–17.

Marfà O., Cáceres R., Polo J. & Ródenas J. (2009): Animal protein hydrolysate as a biostimulant for transplanted strawberry plants subjected to cold stress. – Acta Horticult. 842: 315–318. doi: 10.17660/ActaHortic.2009.842.57

Maruşca T., Dragomir N., Mocanu V., Blaj A., Tarjoc F., Dragomir C. & Constantinescu, S. (2014): Effect of some improvement works on the floristic composition of the vegetal cover in Nardus stricta grasslands. – Animal Sci. Biotechnol. 47/1: 144–150.

Mogle U. P. & Mane R. Y. (2010): Antagonistic effect of biofertilizers against seed born mycoflora of tomato (*Lycopersicum esculentum*). – Research J. Agricult. Sci. 1/3: 255–258.

Naeem S., Duffy J. E. & Zavaleta E. (2012): The functions of biological diversity in an age of extinction. – Science 336(6087): 1401–1406. doi: 10.1126/science.1215855

Nardi S., Pizzeghello D., Muscolo A. & Vianello A. (2002): Physiological effects of humic substances on higher plants. – Soil Biol. Biochem. 34: 1527–1536. dx.doi.org/10.1016/S0038-0717(02)00174-8

Nardi S., Muscolo A., Vaccaro S., Baiano S., Spaccini R. & Piccolo A. (2007): Relationships between molecular characteristics of soil humic fractions and glycolytic pathway and krebs cycle in maize seedlings. – Soil Biol. Biochem. 39/12: 3138–3146. dx.doi.org/10.1016/j.soilbio.2007.07.006

Naydenova G. & Mitev D. (2015): Study on selection populations of bird's foot trefoil and red clover in mixtures. – Plant Sci. LII/5: 30–36.
Nösberger J. & Rodriguez M. (1996): Increasing biodiversity through management. – Grassland Science in Europe 1: 949–956.
Paris Agreement (2015): United Nations Climate Change Conference (COP21). December 12, 2015, Paris, France.
Peeters A. & Janssens F. (1998): Species-rich grasslands: diagnostic, restoration and use in intensive livestock production systems. – Grassland Science in Europe 3: 375–393.
Peeters A., Janssens F. & Decamps C. (1994): Importance of soil phosphorus on botanical diversity. – In: Proceed of the XVth general meeting of the European Grassland Federation: 171–174.
Pizzeghello D., Francioso O., Ertani A., Muscolo A. & Nardi S. (2013): Isopentenyladenosine and cytokinin-like activity of four humic substances. – J. Geochem. Explor. 129: 70–75. dx.doi.org/10.1016/j.gexplo.2012.10.007
Plantureux S., Peeters A. & McCracken D. (2005): Biodiversity in intensive grasslands: Effect of management, improvement and challenges. – Agron. Res. 3/2: 153–164. https://www.researchgate.net/publication/234001901_Biodiversity_in_intensive_grasslands_Effect_of_management_improvement_and_challenges [Accessed 11 December 2023]
Rumpel C., Crème A., Ngo P. T., Velásquez G., Mora M. L. & Chabbi A. (2015): The impact of grassland management on biogeochemical cycles involving carbon, nitrogen and phosphorus. – J. Soil Sci. Plant Nut. 15/2: 353–371. dx.doi.org/10.4067/S0718-95162015005000034
Samuil C., Vintu V., Sirbu C. & Stavarache M. (2013): Influence of fertilizers on the biodiversity of semi-natural grassland in the Eastern Carpathians. – University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania, Notulae Botanicae, Horti Agrobotanici, Cluj-Napoca 41/1: 195–200.
Schiavon M., Ertani E. & Nardi S. (2008): Effects of an Alfalfa protein hydrolysate on the gene expression and activity of enzymes of Tricarboxylic Acid (TCA) cycle and nitrogen metabolism in *Zea mays* L. – J. Agr. Food Chem. 56/24: 11800–11808. doi: 10.1021/jf802362g
Simons N. K. & Weisser W. (2017): Agricultural intensification without biodiversity loss is possible in grassland landscapes. – Nat. Ecol. Evol. 1/8: doi: 10.1038/s41559-017-0227-2
Stoeva K. & Vateva V. (2008): Productivity and variation of the grassland of natural pastures in Strandzha mountain, fertilized with organic and mineral fertilizers. – Jubilee National Conference 1-2.10.2008, Kardzhali (Bg).
Tallowin J. R. B. & Jefferson R. G. (2008): Hay production from lowland semi-natural grasslands: A review of implications for ruminant livestock systems. – Grass and Forage Sci. 54/2: 99–115. doi:10.1046/j.1365-2494.1999.00171.x
Tilman D., Wedin D. & Knops J. (1996): Productivity and sustainability influenced by biodiversity in grassland ecosystems. – Nature 379: 718–720.
Totev T. (1984): Study on the improvement and use of natural meadows and pastures in the foothills, mountain and high mountain regions of the Central Balkan Mountain. – Dissertation, Plovdiv, Bulgaria.
Vintu V., Samuil C., Saghin G., Stavarache M., Pol-van Dasselaar A., Aarts H. F. M., Vliegher A. De., Elgersma A., Reheul D., Reijneveld J. A., Verloop J. & Hopkins A. (2015): High productivity on *Nardus stricta* L. grasslands from the Carpathian Mountains of Romania.

– In: Proceedings of the 18th Symposium of the European Grassland Federation, 15-17 June, Wageningen, The Netherlands, 340–342.
Vogel A., Scherer-Lorenzen M. & Weigelt A. (2012): Grassland resistance and resilience after drought depends on management intensity and species richness. – PLoS ONE 7/5: e36992. doi.org/10.1371/journal.pone.0036992

Received: December 15th 2023
Revised: February 19th 2024
Accepted: March 18th 2024