

THE IMPACT OF FERTILIZER ON FLORISTIC COMPOSITION AND BIODIVERSITY OF FOREST-STEPPE GRASSLANDS (HNV) IN THE NORTH EASTERN OF ROMANIA

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Abstract

National natural conditions strongly influence the floristic structure of meadows, creating a wide range of meadow types and formations. This diversity of species ensures the functionality, efficiency and resilience of the ecological balance within these natural ecosystems. The objective of this study was to determine the effect of the applied management on the phytodiversity of a *Dichanthium ischaemum* permanent meadow in the Moldavian forest-steppe. The experimental factor was: fertilization with seven treatments: T_1 - unfertilized (control), T_2 - $N_{50}P_{50}$ kg/ha annually, T_3 - $N_{75}P_{75}$ kg/ha annually, T_4 - $N_{100}P_{100}$ kg/ha annually, T_5 -10 t/ha sheep manure annually, T_6 -20 t/ha annually and T_7 -30 t/ha annually sheep manure applied at two years. The applied fertilizers influenced the floristic composition, producing appreciable, quantitative and qualitative changes in the vegetal cover. The dominance and frequency of the species, as well as the variation of the Shannon diversity index, were largely influenced by the amount of mineral NP, the amount of manure, but also by the number of years of the fertilization period.

Key words: forest-steppe grasslands, *Dichanthium ischaemum*, fertilizer treatment, diversity, species frequency, sustainable management.

INTRODUCTION

Grasslands are one of the world's major ecosystems, covering nearly one-third of the Earth's land surface, and should be given greater priority in future landscape planning and management because they play important roles in agricultural landscapes and provide many benefits to society. High Nature Value (HNV) grasslands provide a wide range of services that are important for water management, livestock grazing, climate mitigation, biodiversity, and are closely linked to our agricultural history in many ways (Hopkins & Holz, 2005; Lemaire et al., 2011; Buchmann et al., 2019; Pergola et al., 2020; Petermann et al., 2021). The *Dichanthium ischaemum* (L.) Roberty (sin. *Bothriochloa ischaemum*) grasslands studied in the experiment are NATURA 2000 habitats (Sub-Pannonian Steppe grasslands – 6240*) and are specific to the steppe and forest-steppe areas, which have recently gained increasing importance in terms of climate change and biodiversity conservation. Due to their role as biodiversity hotspots, and the great threats they face, they are priority habitats of the EU Habitats Directive (Poniatowski et al., 2020).

These meadows have a xerophilic character, conferred by the characteristic and dominant species *Dichanthium ischaemum* and are established on poor, shallow, often calcareous or skeletal soils, on sunny slopes, with southern or southwestern exposition and represent a derived type of meadows. *Dichanthium ischaemum* is an oligotrophic, xerophilous species with a wide ecological amplitude (Vintu et al., 2004; Szentes et al., 2025), and due to the increased abundance of the dominant species with low potential, the vegetation has a reduced forage value, which affects sustainable use. The absence of fertilisation, abandonment and traditional land use (extensive grazing) have allowed this type of vegetation to be maintained in the long term (Doniță et al., 2005; Chifu et al., 2014; Szentes et al., 2025). Numerous long-term studies have shown that the application of fertilizers, regardless of their nature, has a significant impact on the floristic composition and biodiversity of grasslands (Vaida et al., 2021; Gaga et al., 2022; Nazare et al., 2023; Ghețe et al., 2026). In contrast, intensive management involving excessive use of mineral and organic fertilizers leads to simplification of phytocenoses and the domination of competitive

grasses and perennial legumes, while a lack of input favors oligotrophic species and reduces forage value.

In this context, the objective of the study was to evaluate how different management scenarios will affect the stability of the plant community dominated by *Dichanthium ischaemum*. For this, in 2018, a multiple treatment experiment was designed to analyze the long-term effects of organic and mineral fertilization on floristic composition and diversity, providing information on community dynamics and its responses under constant management conditions.

MATERIALS AND METHODS

Soil and climatic conditions

The experiment was conducted on a permanent *Dichanthium ischaemum* (L.) Roberty grassland in the Moldavian forest-steppe area on the territory of Andrieșeni village Iași county, between the geographical coordinates 47°02' N latitude and 27°22' - Eastern longitude, on a slightly inclined ground, with SE exposition (Photo 1).

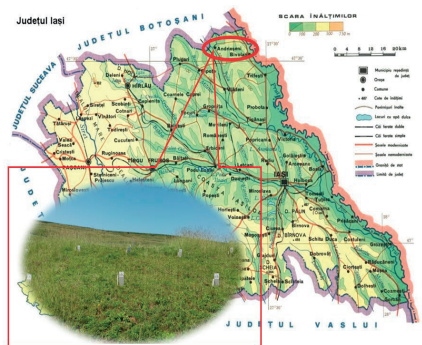


Photo 1. Location of experiences field in Iasi County

The soil type in the experimental field is kastanozem with medium fertility, having a slightly alkaline reaction, with pH values between 7.93 and 8.38 (Gafencu et al., 2023). The studied area has remarkable characteristics in terms of both precipitation and temperatures recorded during the study period and is characterized climatically by significant deviations from normal, with annual temperatures above the multi-year average and an uneven distribution of precipitation, with long periods of water stress, which negatively affects the growth and

regeneration of vegetation on the studied grasslands.

The multi-year average temperature is 9.6°C, and the average temperature during the growing season is 17.3°C. The multiannual average of precipitation is 517.8 mm, and in the area where the research was conducted, from a climatic point of view, the 2023-2024 agricultural year can be characterized as favorable for the exploitation of permanent grasslands in the forest-steppe zone. The total amount of precipitation during the study period (2023-2024) was 658.8 mm, which is higher than the multi-year average, but was unevenly distributed, with short periods of water stress in September-October 2023 and August 2024, as shown in Figure 1. The average annual temperatures were 13.7°C in 2023-2024.

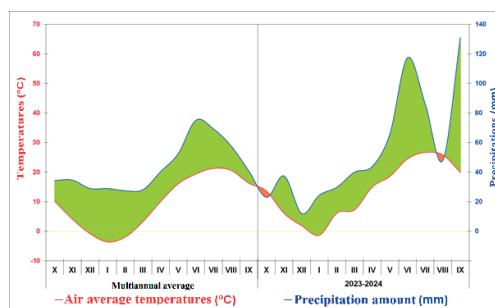


Figure 1. Climadiagram for the agricultural year 2023-2024, where green color show the precipitation that is above the evapotranspiration level, and the red color show the periods of water stress for plants

Experiment set-up

A monofactorial experiment with 7 treatments was organized according to the randomized block method in three replicates.

The research covered three blocks, and the experiment had the following treatments:

- T₁ - unfertilized (control);
- T₂ - N₅₀P₅₀ kg/ha annually (low input);
- T₃ - N₇₅P₇₅ kg/ha annually (medium input);
- T₄ - N₁₀₀P₁₀₀ kg/ha annually (high input);
- T₅ - 10 t/ha sheep manure applied annually;
- T₆ - 20 t/ha sheep manure applied annually;
- T₇ - 30 t/ha sheep manure applied at two years.

Two types of fertilizers were used: an organic one represented by well fermented sheep manure (older than two years) and a mineral one represented by a complex fertilizer with nitrogen and phosphorus (N₂₀P₂₀). The administered

manure from sheep had the following chemical composition: N-0.692%, P₂O₅-0.320% and K₂O-0.811%. Fertilizers were applied manually in early spring before the start of active vegetation growth. The area of each variant was 12 m² (3 x 4 m), and the harvested area was of 8.75 m² (2.5 x 3.5 m).

Measurements and analyses

Harvesting was done at earing-flowering stage of the dominant grasses, and the floristic composition was established using the Braun-Blanquet method (Cristea et al., 2004). For the processing of floristic data, we used the programmes Excel and PC-ORD 6 software. Within the PC-ORD multivariate analysis program (McCune & Grace, 2002), various methods were used to order the floristic relevés, namely: Summary (to determine Specific richness (S) - total number of species per plot; Shannon - Wiener index (H'), which combines species richness and relative abundance; Evenness (E), calculated as the ratio between the observed H' (Shannon-Wiener index) and the maximum possible H' for the number of species ($E = H' / \ln S$); Simpson index (D), which is sensitive to the dominance of abundant species in the community), average frequency and dominance, and multidimensional NMDS Autopilot scaling (Păcurar & Rotar, 2014).

The statistical data was analyzed by SPSS using ANOVA and the Lowest Significant Difference test (LSD 5%).

RESULTS AND DISCUSSIONS

The influence of treatments on the floristic composition

An important factor in determining fertilizer doses is the floristic composition of grasslands. Fertilizer application leads to an increase in the availability of nutrients for plants. Under these conditions, only a few fast-growing plant species can compete for light, eliminating less competitive species, which can result in a decline in species richness. On the other hand, on very poor soils, only slow-growing species are able to compete for nutrients.

The Figure 2, showing the ordering of the floral composition in 2024, that was done using NDMS Autopilot (multidimensional scaling),

confirms that the application of treatments over the years of study has produced significant changes in the floral composition of the vegetation cover. Thus, following the analysis of the ordination graph, a much clearer overlap of the experimental variants is observed, but the fact that the unfertilized phytocenosis (T₁-unfertilized) is at a great distance from the fertilized variants shows us that mineral and organic treatments have caused changes in the floristic composition of the vegetation cover, and the similarity of the phytocenoses is not very high. Depending on the management applied, phytocenoses can be grouped according to their proximity into two groups: the first group includes treatments with NP fertilizers (T₃ and T₄), and the second group includes treatments with sheep manure (T₅, T₆, and T₇). The similarity between the variants within the two groups indicates that there is a high degree of similarity between them in terms of floristic composition. In the case of treatment T₂ – low input (N₅₀P₅₀), the changes recorded in the structure of the vegetation cover were more limited. There are variants in which, following the application of organic fertilizers (T₅, T₆, and T₇), new species appeared, such as *Medicago sativa* L. in variant T₅ (10 t/ha sheep manure applied annually), *Medicago falcata* L. in variant T₆ (20 t/ha sheep manure applied annually), and *Vicia cracca* L. in the variant fertilized with 30 t/ha sheep manure applied at two years (T₇). Regarding the frequency of species in the reference phytocoenosis (type *Dichanthium ischaemum* (L.) Roberty - *Festuca valesiaca* Schleich. ex Gaudin), in addition to the dominant and co-dominant species, there are other species with higher coverage and frequency, such as: *Elymus repens* (L.) Gould., *Achillea millefolium* L., *Potentilla cinerea* Chaix ex Vill., *Thymus glabrescens* Willd and *Astragalus onobrychis* L., but also species with medium coverage and frequency: *Vicia hirsuta* (L.) Gray and *Vicia sativa* L. (Figure 3). The lowest dominance and frequency are found in the species *Poa pratensis* L. ssp. *pratensis*, *Medicago falcata* L., *M. lupulina* L., *Lotus corniculatus* L., *Achillea setacea* Waldst. et Kit., *Koeleria gracilis* Pers., *Eryngium campestre* L., *Lappula squarrosa* (Retz.) Dumort., etc. (Figure 3).

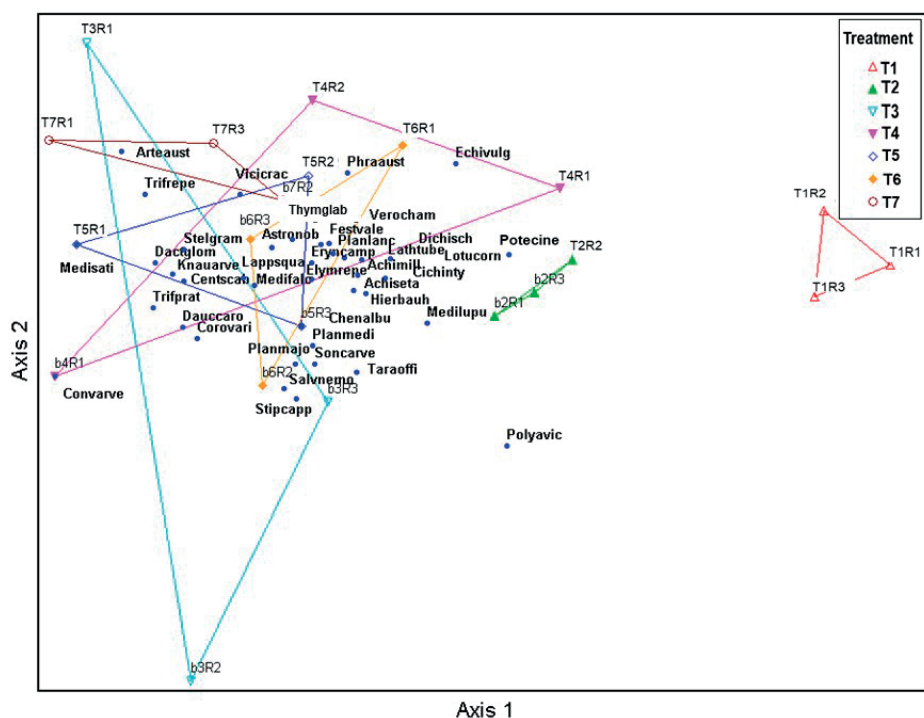


Figure 2. Ordination of floristic composition in 2024, influenced by treatments (T₁ - unfertilized (control); T₂ - N₅₀P₅₀; T₃ - N₇₅P₇₅; T₄ - N₁₀₀P₁₀₀; T₅ - 10 t/ha of sheep manure applied annually, T₆ - 20 t/ha of sheep manure applied annually and T₇ - 30 t/ha of sheep manure applied at 2 years; R₁, R₂, R₃ – replicates)

Legend: Species abbreviations: *Dactylis glomerata* L. = Dactglom, *Dichanthium ischaemum* (L.) Roberty = Dichisch, *Elymus repens* (L.) Gould. = Elymrepe, *Festuca valesiaca* Schleich. ex Gaudin = Festvale, *Poa pratensis* L. ssp. *pratensis* = Poaprat, *Phragmites australis* (Cav.) Trin. ex Steud. = Phraaust, *Stipa cappilata* L. = Stipcapp, *Astragalus onobrychis* L. = Astronob, *Coronilla varia* L. = Corovari, *Lathyrus tuberosus* L. = Lathtube, *Lotus corniculatus* L. = Lotucorn, *Medicago falcata* L. = Medifalc, *Medicago lupulina* L. = Medilupu, *Medicago sativa* L. = Medisati, *Trifolium pratense* L. = Trifprat, *Trifolium repens* L. = Trifrepe, *Vicia cracca* L. = Vicicrac, *Achillea millefolium* L. = Achimile, *Achillea setacea* Waldst. et Kit. = Achiseta, *Artemisia austriaca* Jacq. = Arteaust, *Convolvulus arvensis* L. = Convarve, *Centaurea scabiosa* L. = Centscab, *Cichorium intybus* L. = Cichinty, *Chenopodium album* L. = Chenalbu, *Daucus carota* L. ssp. *carota* = Daucaro, *Echium vulgare* L. = Echivulg, *Eryngium campestre* L. = Eryncamp, *Hieracium bauhini* Besser = Hierbauh, *Knautia arvensis* L. = Knauarve, *Koeleria gracilis* Pers. = Koelgrac, *Lappula squarrosa* (Retz.) Dumort. = Lappsqua, *Ononis arvensis* L. = Ononarve, *Plantago lanceolata* L. = Planlanc, *Plantago media* L. = Planmedi, *Plantago major* L. = Planmajo, *Potentilla cinerea* Chaix ex Vill. = Potecine, *Salvia nemorosa* L. = Salvnemo, *Sonchus arvensis* L. = Soncarve, *Stellaria graminea* L. = Stelgram, *Taraxacum officinale* Weber. = Tragoffi, *Thlaspi arvense* L. = Thlaarve, *Thymus glabrescens* Willd. = Thymglab, *Vicia cracca* L. = Vicicrac, *Vicia hirsuta* (L.) Gray = Vicihirs, *Vicia sativa* L. = Viciasati, *Veronica chamaedrys* L. = Verocham, nomenclature after Sărbu et al., 2013; Ciocărlan, 2009

Following a comparison between the floristic composition of the control and that of the fertilized variants, changes in the frequency-dominance of certain species were observed (Figure 4). Therefore, some species that had a very low frequency-dominance in the control phytocenosis (T₁-unfertilized), such as the species: *Poa pratensis* L. ssp. *pratensis*, *Achillea setacea* Waldst. et Kit., *Cichorium intybus* L., *Medicago falcata* L., *Lotus corniculatus* L., etc., are found in a higher

percentage with a medium to high frequency (Figure 4). Valuable forage species such as *Dactylis glomerata* L., *Festuca arundinacea* Schreb., and *Medicago lupulina* L., which were absent in the control phytocenosis (T₁), show an increase in frequency-dominance as a result of the management applied (Figure 4). Other species, such as *Thlaspi arvense* L., *Medicago sativa* L., and *Ononis arvensis* L., show low frequency-dominance in most of the variants studied (Figure 4).

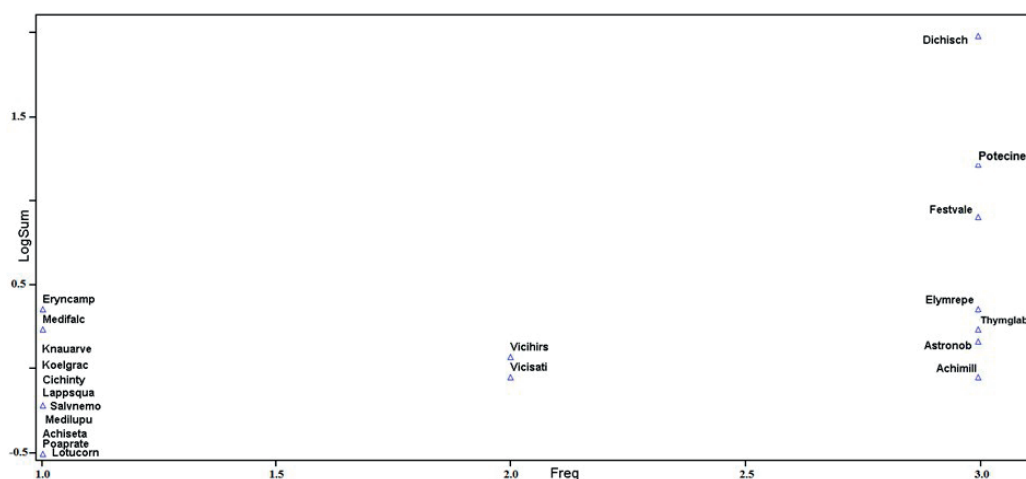


Figure 3. Dominant frequency diagram of witness phytocenosis *Dichanthium ischaemum* (L.) Roberty, in 2024 Legend: Freq= Species frequency, Logsum = Log base 10 of species dominance (for species name see the legend from Figure 2)

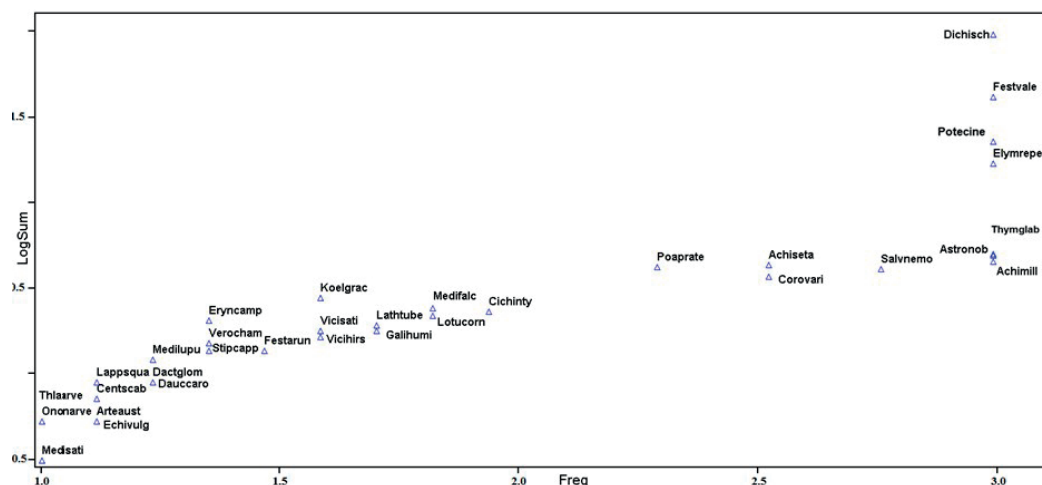


Figure 4. Dominant frequency diagram of organic and mineral fertilized variants phytocenosis *Dichanthium ischaemum* (L.) Roberty, without control variant, in 2024 (Freq= Species frequency, Logsum = Log base 10 of species dominance, for species name see the legend from Figure 2)

The Impact of fertilizer on diversity indices

To evaluate the impact of treatments on diversity indices, four biodiversity indices (S, H', E, D') were calculated, and the results are presented in Table 1. The overall assessment of biodiversity indices highlights a significant effect of mineral and organic treatments on the structure of phytocenoses. Analyzing the phytodiversity of fertilized variants, significant changes can be observed, whether we consider the number of species (S) or the Shannon (H) and Simpson (D') diversity indices. The unfertilized control plots (T₁) showed low

values of species richness and diversity (S = 19; H' = 1.314), characteristic of oligotrophic grasslands with relatively balanced species abundances. The treatments that received reduced mineral and organic inputs (T₂ - N₅₀P₅₀ and T₅; 10 t/ha of sheep manure applied annually) showed higher diversity values compared to the control treatment. Of these, treatment T₅ (10 t/ha of sheep manure applied annually) showed the highest species richness and Shannon diversity (S = 32; H' = 1.806). Moderate mineral and manure inputs (T₃ - N₇₅P₇₅ and T₆ - 20 t/ha of sheep manure applied

annually) resulted in consistently high values of diversity and evenness ($H' \approx 1.950\text{--}1.985$; $E \approx 0.641\text{--}0.625$), indicating a relatively even distribution of species abundance across these treatments (Table 1). This shows a more balanced distribution of species and greater diversity under moderate fertilization conditions. In contrast, at very high fertilizer doses ($T_4 - N_{100}P_{100}$ kg/ha annually and $T_7 - 30$ t/ha sheep manure applied at two years), although the Shannon index remains relatively high ($H' \approx 1.788\text{--}1.806$), diversity tends to be sustained by a few dominant species. Species evenness (E) followed the same trend: low values in the control (0.498) compared to the highest values in the fertilized treatments (0.571-0.625). This indicates that fertilization promotes a more uniform distribution of species abundance up to an entry threshold, after which communities become unbalanced again. The Simpson index (D) reflected the same dynamics (Table 1), with higher values in

fertilized treatments (0.634-0.715) compared to the control (0.562). Our research shows that high biodiversity can be observed in intermediate situations (T_3 and T_6) when competition is low and when a large number of mesotrophic species can coexist with a certain number of oligotrophic and eutrophic species. It also suggests that both the absence of inputs and high fertilization lead to a reduction in diversity (Table 1). These results confirm the results previously obtained by many other authors such as Mrkvička & Veselá, 2002; Rotar et al., 2003; Chytrý et al., 2009; Păcurar & Rotar, 2004; Ciobanu et al., 2013; Samuil et al., 2013; Vintu et al., 2017; Tong et al., 2019; Vaida et al., 2021; Boch ș.a. 2021; Ranta ș.a., 2021; Nazare et al., 2023; Samuil et al., 2025; Canișag et al., 2026; Ghețe et al., 2026).

Table 1. The influence of fertilization treatments on biodiversity indices in grasslands, in 2024

Treatments	Shannon Index (H')	Species Richness (S)	Evenness (E)	Simpson (D)
T_1	1.314 ^c	19 ^c	0.498 ^c	0.562 ^c
T_2	1.547*	21 *	0.571 *	0.634 *
T_3	1.950*	27 *	0.641 *	0.715 *
T_4	1.806*	25 *	0.593 *	0.667 *
T_5	1.959*	32 *	0.609 *	0.697 *
T_6	1.985*	33 *	0.625 *	0.710 *
T_7	1.788*	30 *	0.613 *	0.695 *
LSD 5%	0.31	2	0.036	0.033

Note: T_1 - unfertilized (control); $T_2 - N_{50}P_{50}$; $T_3 - N_{75}P_{75}$; $T_4 - N_{100}P_{100}$; $T_5 - 10$ t/ha of sheep manure applied annually, $T_6 - 20$ t/ha of sheep manure applied annually and $T_7 - 30$ t/ha of sheep manure applied at 2 years; * = $p < 0.05$).

CONCLUSIONS

The current results of our six-year study demonstrate that fertilization is a determining factor in the structure and diversity of oligotrophic grasslands dominated by the species *Dichanthium ischaemum* in the North-East of Romania. The results showed that the application of organic and mineral fertilizers led to a clear difference in the development of plant communities, confirming the hypothesis that management intensity is the main factor influencing the structure and diversity of grasslands in the study area. Therefore, based on the long-term study, we hypothesized that high biodiversity can be maintained in intermediate

situations, when competition is low and when a large number of mesotrophic species can coexist with a certain number of oligotrophic and eutrophic species. Our research shows that in order to preserve the floristic diversity of the studied grassland, the amount of sheep manure should not exceed 10-20 t/ha, and in the case of mineral fertilizer treatments, the dose should not exceed $N_{75}P_{75}$ kg/ha s.a.

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