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Article

The Effect of Fertilization on Floristic Composition and Biodiversity of Montane Grasslands (HNV) in the Eastern Carpathians

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Abstract

High Nature Value (HNV) mountain grasslands in the Eastern Carpathians are sensitive to fertilization intensification. This study evaluated the effects of organic and mineral inputs on the floristic composition, vegetation types, and diversity of an oligotrophic grassland with *Nardus stricta* in a long-term experiment established in 2002, with results presented here from three recent experimental years (2022–2024) with nine variants (unfertilized control, 10–30 t ha⁻¹ manure applied in autumn/spring, and 200–300 kg ha⁻¹ nitrocalcar). Vegetation composition was analyzed using hierarchical classification, PCoA, MRPP, Indicator Species Analysis, and α -diversity indices (species richness, Shannon, Simpson, and evenness). The results revealed six floristic types arranged along a clear trophic gradient, ranging from oligotrophic to eutrophic communities. Specifically, low-moderate manure treatments (10–20 t ha⁻¹) simultaneously maximized community diversity and balance, maintaining a rich core of oligotrophic and mesotrophic species. Conversely, high doses of manure (30 t ha⁻¹) and mineral fertilization led to a marked reduction in species richness and the dominance of a few competitive-grass species. Ultimately, this study demonstrates that moderate organic fertilization is compatible with the adaptive management of HNV grasslands, whereas intensive mineral fertilization is incompatible with conservation objectives.

Keywords: montane grasslands; High Nature Value (HNV); fertilization gradient; biodiversity; indicator species; Eastern Carpathians; sustainable grassland management

1. Introduction

High Nature Value (HNV) grasslands represent some of the most complex and valuable agropastoral ecosystems in Europe, characterized by high floristic richness, structural heterogeneity, and a long history of traditional management through mowing and extensive grazing [1–3]. These grasslands provide a wide range of essential ecosystem services, such as the production of high-quality fodder [4] for ruminants, which is rich in protein and minerals [5,6], maintaining soil structure and nutrient cycling [7,8], carbon sequestration, regulating hydro-ecological processes [9–11], providing habitat for biodiversity [12–15], and cultural services that support the identity of mountain communities [16–18].

In Romania, HNV grasslands cover extensive areas across the Eastern, Southern, and Western Carpathians, where they form core biodiversity hotspots and are included in the Natura 2000 Network. Among these, the *Nardus stricta*-dominated grasslands of the Eastern Carpathians stand out as unique oligotrophic ecosystems that are exceptionally sensitive to anthropogenic pressures [9,19,20]. These semi-natural systems are home to numerous species of conservation and economic interest, including medicinal plants such as *Arnica montana*, which is well represented in the high

areas of the northeastern Carpathians [21–23]. In addition, HNV grasslands are a central component of traditional cultural landscapes and the identity of rural communities [24,25]. Given their sensitivity to anthropogenic pressures, mountain grasslands are considered important indicators of socioecological resilience [26–29]. In recent decades, socioeconomic transformations have generated two opposing but equally problematic trends: agricultural intensification and abandonment [30,31]. Within the context of changing land use, fertilization remains one of the main factors controlling the dynamics of plant communities in mountain grasslands, contributing to increased productivity and improved nutritional value of the fodder [32–34]. However, intensification through excessive fertilization and mechanization leads to simplified vegetation cover, increased dominance of nitrophilic species, and reduced diversity ([35]. At the opposite end of the spectrum, abandonment favors secondary succession, the emergence of woody species, and a decrease in forage value [36–39].

Fertilization remains one of the main factors controlling the dynamics of plant communities in mountain grasslands, with effects depending on the dose, frequency, and nutrient source [36,40,41]. Low or moderate organic inputs can stimulate productivity while maintaining species diversity [27,42], in line with the intermediate disturbance hypothesis ([43–45]. Mulching is another method for maintaining HNV grasslands [46–48]. In contrast, repeated mineral fertilization favors competitive grasses, reduces the presence of oligotrophic species, and simplifies phytocenoses [49,50]. Recent results have shown that fertilization influences not only vegetation but also soil microbiome functions, restructures plant–microorganism relationships, and alters key nutrient cycle processes [51,52]. At the European level, recent studies have confirmed that persistent fertilization reduces diversity and favors competitive grasses in alpine and subalpine regions [53,54], and that traditional management is essential for maintaining species-rich habitats [11,28,55]. In the Carpathians, where climatic, demographic, and economic pressures are increasing, research on the interactions between fertilization and vegetation structure is fundamental for designing sustainable management strategies [56,57] for the conservation of biodiversity and the mountain cultural landscape [58–60].

Despite substantial progress in grassland ecology, long-term experimental evidence focusing on the specific effects of different organic and mineral fertilization regimes on *Nardus stricta*-dominated HNV grasslands in the Eastern Carpathians is still limited. Most previous studies either address broader geographic areas, rely on short-term observations, or do not directly compare the influence of varying fertilizer doses and application times within a single, long-term experimental framework. Filling this gap is essential for understanding how fine-scale management differences shape floristic composition and biodiversity and for developing adaptive strategies that reconcile conservation goals with agricultural needs in sensitive mountainous environments. Specifically, we hypothesized that: (1) moderate organic fertilization would maintain or increase floristic diversity compared to unfertilized controls, in line with the intermediate disturbance hypothesis; (2) high organic and mineral fertilization would lead to a significant reduction in species richness and a shift towards competitive, eutrophic species; and (3) the timing of organic fertilizer application (autumn vs. spring) would differentially influence floristic composition and diversity. Therefore, this study aimed to analyze how different fertilizer inputs (organic, mineral, moderate, and intensive) influence the floristic composition, vegetation types, and diversity of mountain grasslands in the Eastern Carpathians using an integrated analytical framework comprising vegetation classification, PCoA, MRPP, and indicator species analysis. The results have direct implications for the adaptive management of HNV grasslands, biodiversity conservation, and optimization of agricultural practices in sensitive mountain areas. This study, conducted within a long-term experimental setup initiated in 2002, focuses on the floristic composition, vegetation types, and diversity of mountain grasslands based on data collected during the 2022–2024 period.

2. Results

2.1. Hierarchical Cluster Analysis of Floristic Composition

The hierarchical classification analysis of floristic composition revealed clear differences between the experimental variants, driven by fertilization intensity and type. Using a cutoff of approximately 72% of the remaining information allowed the delimitation of six distinct floristic groups, each corresponding to a specific ecological grassland type (Figure 1). Cluster 1 (control variant T1) was characterized by the dominance of acidophilic species, particularly *Nardus stricta* associated with *Festuca rubra*, reflecting the grasslands' initial oligotrophic state. Cluster 2 included variants moderately fertilized with small doses of manure (T2 and T5). Reduced organic fertilization led to a decrease in the abundance of *Nardus stricta* and an increase in the proportion of *Festuca rubra*, marking the transition to grasslands with a moderate trophic supply of nutrients. Cluster 3 included variants at an advanced stage of the improvement process (T3 and T6). *Festuca rubra* and *Agrostis capillaris* dominated this group, but acidophilic elements were still present, indicating an intermediate stage between oligotrophic and mesotrophic grasslands. Cluster 4 corresponded to a stable type of improved grassland dominated by *Festuca rubra* and *Agrostis capillaris*. This group is associated with moderate to high doses of manure applied in both autumn and spring, resulting in a balanced floristic structure and diversification of mesotrophic species. Cluster 5 was characterized by the pronounced dominance of *Agrostis capillaris*, followed by *Festuca rubra*, especially under high doses of manure (T4 and T7). The increased competitiveness of mesotrophic species gives vegetation a more uniform and productive structure. Cluster 6 includes variants intensively fertilized with nitrocalcar (T8, T9), where *Agrostis capillaris* becomes almost exclusively dominant. High mineral fertilization significantly reduces the presence of oligotrophic species and leads to simplification of the floristic structure. Overall, the dendrogram indicated clear differences between the control, organic fertilization, and mineral fertilization, reflecting a consistent gradient of change in floristic composition. The obtained groups formed the basis for the detailed ecological interpretations presented in the Discussion section.

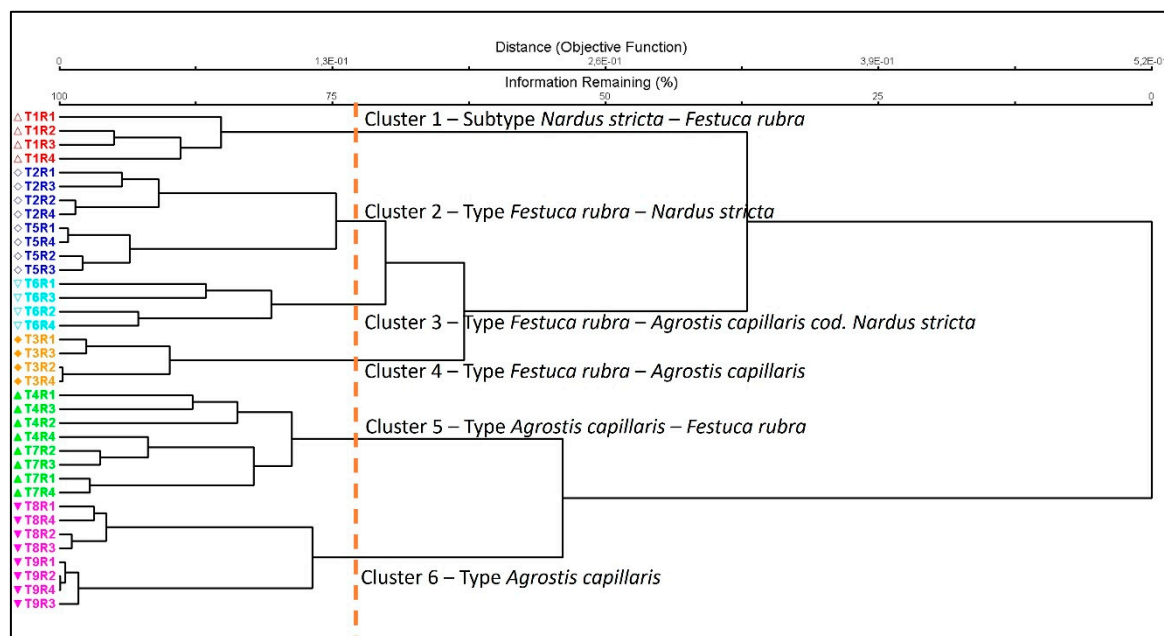


Figure 1. Floristic classification of the vegetation and changes in grassland type.

Legend: **T1** - unfertilized control; **T2** - 10 t ha⁻¹ manure applied in autumn; **T3** - 20 t ha⁻¹ manure applied in autumn; **T4** - 30 t ha⁻¹ manure applied in autumn; **T5** - 10 t ha⁻¹ manure applied in spring; **T6** - 20 t ha⁻¹ manure applied in spring; **T7** - 30 t ha⁻¹ manure applied in spring; **T8** - 200 kg ha⁻¹ nitrocalcar applied in spring; **T9** - 300 kg ha⁻¹ nitrocalcar applied in spring; **R1-R4** – replicates. The dendrogram cutoff line was set at approximately 72% of the remaining information, resulting in six clusters being identified.

2.2. Principal Coordinates Analysis (PCoA) and Floristic Differentiation

PCoA confirmed the clear separation of treatments according to the vegetation types previously identified by clustering analysis. Axis 1 explains 92.1% of the variation in species composition and represent the main fertilization gradient. Axis 2 contributed an additional 6.7%, primarily distinguishing unfertilized plots from those receiving organic fertilization applied in autumn (Table 1).

Table 1. Importance of axis.

Axis	Degree of participation (r)	Cumulative
1	0.921	0.921
2	0.067	0.988
3	0.001	0.989

Note: The correlations between the treatments and the PCoA axes (Table 2) showed that negative values on Axis 1 corresponded to unfertilized variants or variants with low organic input, and positive values on Axis 1 were associated with mineral fertilization and high doses of manure.

The strongest link was between Nitrocalc_30 and Axis 1 ($r = 0.582$, $p < 0.01$), indicating their position at the eutrophic end of the gradient. On Axis 2, only two treatments showed significant correlations: no fertilization (positive) and 20 t ha⁻¹ manure in autumn (negative), highlighting the differentiated effect of the timing of manure application on vegetation structure. Overall, Axis 1 represented the oligotrophic–eutrophic gradient, and Axis 2 differentiated the moderate treatments according to the season of manure application (Table 2).

Table 2. Correlation of experimental factors with ordination axes (PCoA).

Experimental Factors	Axis 1 (r)	Significance	Axis 2 (r)	Significance
No fertilization	-0.436	*	0.768	***
10t_man_aut	-0.340	*	-0.162	ns
20t_man_aut	-0.144	ns	-0.580	**
30t_man_aut	0.237	ns	-0.093	ns
10t_man_spri	-0.336	*	0.008	ns
20t_man_spri	-0.217	ns	-0.238	ns
30t_man_spri	0.213	ns	-0.033	ns
Nitrocalcar 200 kg ha ⁻¹	0.443	**	0.093	ns
Nitrocalcar 300 kg ha ⁻¹	0.582	**	0.238	ns
Axis importance	92.1%		6.7%	

Note: r = correlation coefficient between ordination distances and explanatory variables; significance: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns = not significant. Axis importance indicates the proportion of the total variance explained by each axis: Axis 1 = 92.1%, Axis 2 = 6.7%, cumulative = 98.8%.

The PCoA (Figure 2) showed a clear separation of areas based on floristic composition, with the first two axes explaining almost all of the variation between treatments. The areas were organized into three large groups corresponding to the types dominated by *Nardus stricta*, *Festuca rubra*, and *Agrostis capillaris*. The unfertilized areas (T1) were grouped in the area associated with *Nardus stricta*, whereas the treatments with moderate levels of manure (T2–T6) were located in the intermediate area, which was characterized by the presence of *Festuca rubra*. Mineral treatments (T8–T9) and some variants with high doses of manure (T4 and T7) were applied in areas dominated by *Agrostis capillaris*. The overlaid vectors indicate the overall direction of the fertilization gradient. *No_fertilz* was oriented toward the *Nardus stricta* group. The vectors corresponding to high fertilization levels (*Nitrocalc_20*, *Nitrocalc_30*, *Fert_high_aut*, *Fert_high_spr*) pointed towards the *Agrostis capillaris* group. The contours of vegetation types (vegetation 'hilltops', i.e., areas of maximum density in ordination space)

highlighted a clear spatial separation of the three main groups, with moderate overlap in the intermediate mesotrophic zone and complete separation at the extremes of the gradient. The treatment gradient followed the expected transition from the unfertilized control (T1) on the left side of the ordination to the high nitrocalcar doses (T9) on the right side, reflecting progressive changes in floristic composition.

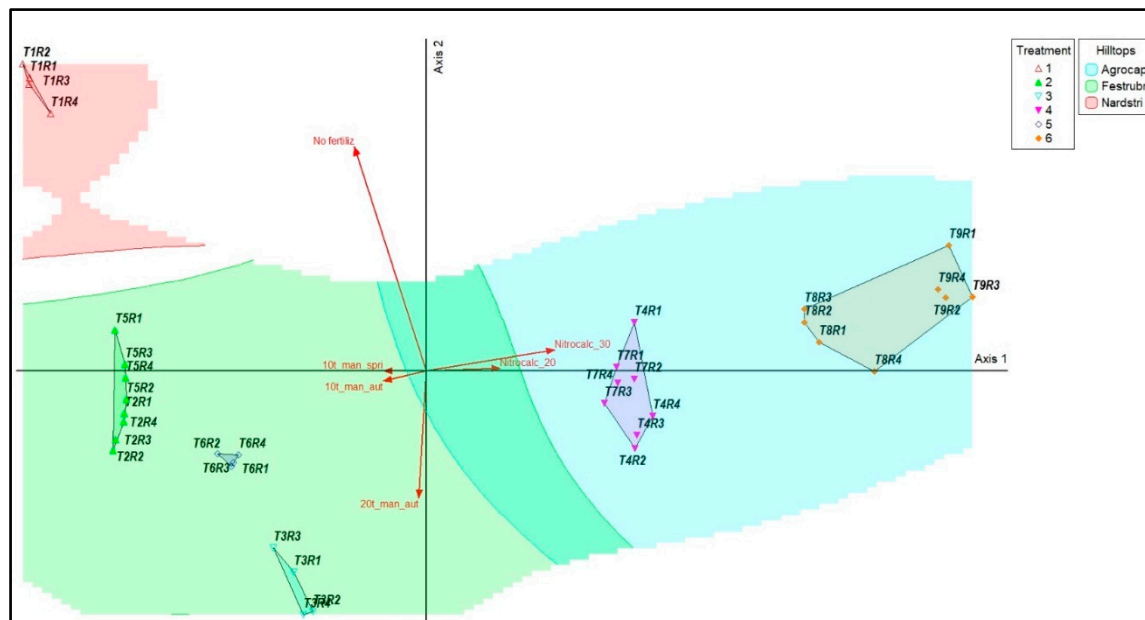


Figure 2. Principal Coordinates Analysis (PCoA) showing the floristic differentiation of mountain grasslands under manure and mineral fertilization treatments, with vegetation types delimited by the dominance of *Agrostis capillaris*, *Festuca rubra*, and *Nardus stricta*. Legend: T1 - unfertilized control; T2 - 10 t ha⁻¹ manure applied in autumn; T3 - 20 t ha⁻¹ manure applied in autumn; T4 - 30 t ha⁻¹ manure applied in autumn; T5 - 10 t ha⁻¹ manure applied in spring; T6 - 20 t ha⁻¹ manure applied in spring; T7 - 30 t ha⁻¹ manure applied in spring; T8 - 200 kg ha⁻¹ nitrocalcar applied in spring; T9 - 300 kg ha⁻¹ nitrocalcar applied in spring; R1–R4—replications. Vectors indicate the fertilization gradient: *no_fertiliz*, no fertilization (unfertilized control); *Fert_low_aut*, 10 t ha⁻¹ manure applied in autumn (T2); *Fert_med_aut*, 20 t ha⁻¹ manure applied in autumn (T3); *Fert_high_aut*, 30 t ha⁻¹ manure applied in autumn (T4); *Fert_low_spr*, 10 t ha⁻¹ manure applied in spring (T5); *Fert_med_spr*, 20 t ha⁻¹ manure applied in spring (T6); *Fert_high_spr*, 30 t ha⁻¹ manure applied in spring (T7); *Nitrocalc_20*, 200 kg ha⁻¹ nitrocalcar applied in spring (T8); *Nitrocalc_30*, 300 kg ha⁻¹ nitrocalcar applied in spring (T9).

The relationships between the three dominant species (*Agrostis capillaris*, *Festuca rubra*, and *Nardus stricta*) and the PCoA axes confirmed their essential roles in defining trophic gradients (Figure 3). *Agrostis capillaris* (panel A) appeared in the positive area of Axis 1, coinciding with the positioning of treatments T8 and T9 and variants with high doses of manure (T4 and T7). The clear positive relationship between Axis 1 scores and species abundance indicates an affinity for high trophic input levels. *Festuca rubra* (panel B). *Festuca rubra* occupied intermediate positions on Axis 1 and values close to the origin on Axis 2. Areas T2–T6 are in the vicinity of this species, reflecting its preference for moderate fertilization levels. The negative slopes of the regressions on both axes indicate that the abundance of *Festuca rubra* decreases towards both the oligotrophic and eutrophic extremes. *Nardus stricta* (panel C). *Nardus stricta* is positioned in the negative area of Axis 1 and in the positive part of Axis 2, in line with the grouping of control areas (T1). The negative relationships with Axis 1 and slightly positive relationships with Axis 2 highlight the preference of this species for oligotrophic soils with no or minimal fertilization. Overall, the overlap of treatment vectors over site scores and species abundance clearly demonstrated how the applied inputs shaped the floristic-ecological distribution of the communities.

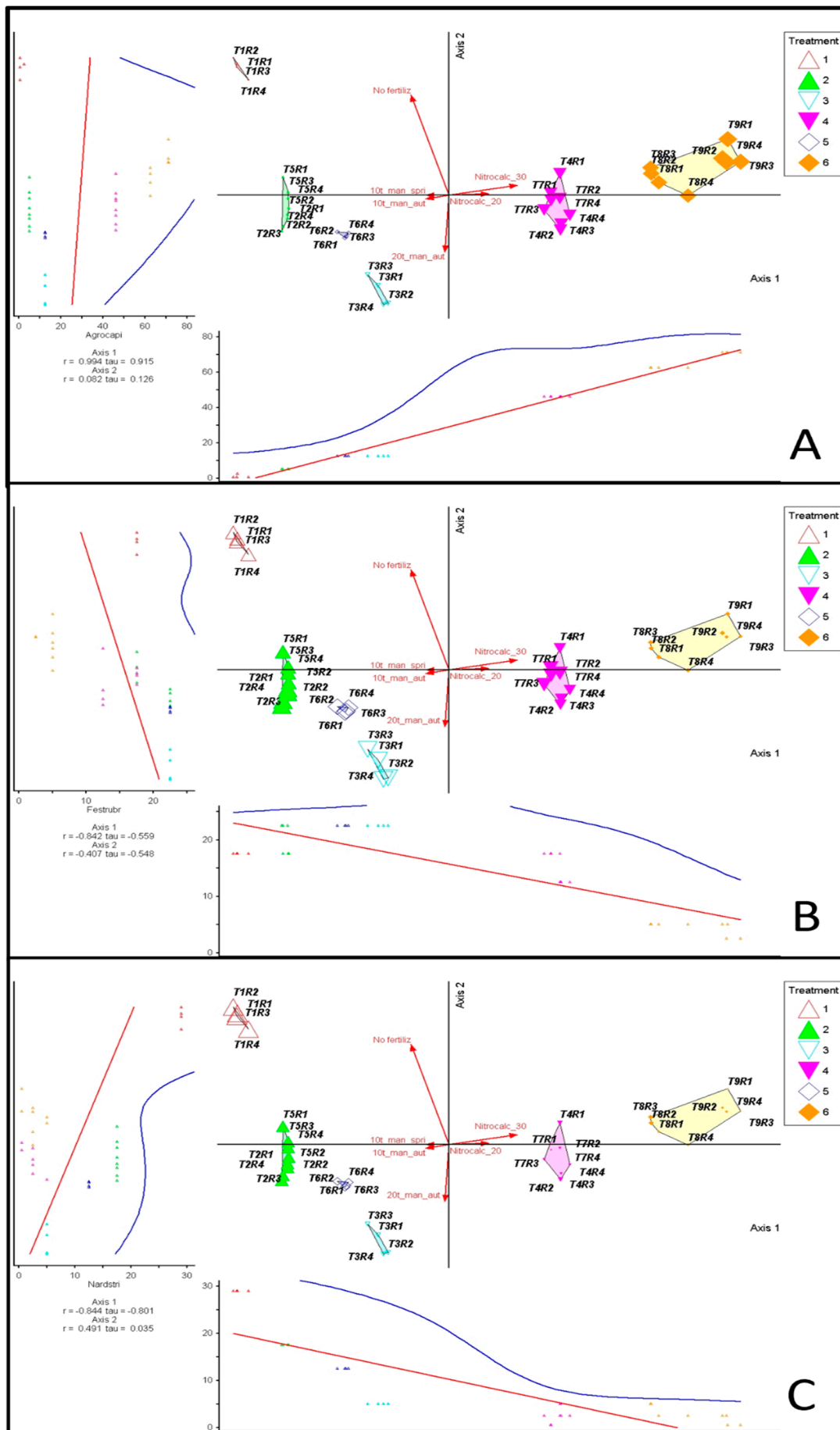


Figure 3. Species–environment relationships for the dominant grasses in the studied grasslands. Panels: (A) *Agrostis capillaris*, (B) *Festuca rubra*, (C) *Nardus stricta*. Each panel combines (i) principal coordinates analysis (PCoA) scores of vegetation plots, (ii) fitted environmental vectors (fertilization treatments), and (iii) species–environment correlations along the PCoA axes. Legend: T1 - unfertilized control; T2 - 10 t ha⁻¹ manure applied in autumn; T3 - 20 t ha⁻¹ manure applied in autumn; T4 - 30 t ha⁻¹ manure applied in autumn; T5 - 10 t ha⁻¹ manure applied in spring; T6 - 20 t ha⁻¹ manure applied in spring; T7 - 30 t ha⁻¹ manure applied in spring; T8 - 200 kg ha⁻¹ nitrocalcar applied in spring; T9 - 300 kg ha⁻¹ nitrocalcar applied in spring; R1–R4—replications. Vectors indicate the fertilization gradient: *No_fertiliz* – no fertilization (unfertilized control); *Fert_low_aut* – 10 t ha⁻¹ manure applied in autumn (T2); *Fert_med_aut* – 20 t ha⁻¹ manure applied in autumn (T3); *Fert_high_aut* – 30 t ha⁻¹ manure applied in autumn (T4); *Fert_low_spr* – 10 t ha⁻¹ manure applied in spring (T5); *Fert_med_spr* – 20 t ha⁻¹ manure applied in spring (T6);

The two main axes delimit four major floristic types (Figure 4 A+B): Subtype *Nardus stricta* – *Festuca rubra*; Type *Festuca rubra* – *Nardus stricta*; Type *Festuca rubra* – *Agrostis capillaris*; Type *Agrostis capillaris*. These were coherently arranged in the ordination space. Unfertilized areas (T1) were concentrated in the *Nardus stricta* zone, whereas low and medium manure treatments (T2–T6) overlapped in the mesotrophic zone dominated by *Festuca rubra*. Mineral (T8–T9) and high manure (T4, T7) treatments were grouped in the eutrophic zone dominated by *Agrostis capillaris*. The treatment vectors reflect the direction of the trophic gradient: *No_fertiliz* → oligotrophic zone; *Fert_high_aut* / *Fert_high_spr* / *Nitrocalc_20* / *Nitrocalc_30* → Eutrophic zone. The distribution of dominant species is consistent with this structure, confirming the concordance between classification, ordination, and ecologically defined floristic type. These floristic types, visually highlighted in the ordination space, were subsequently statistically validated using the MRPP analysis.

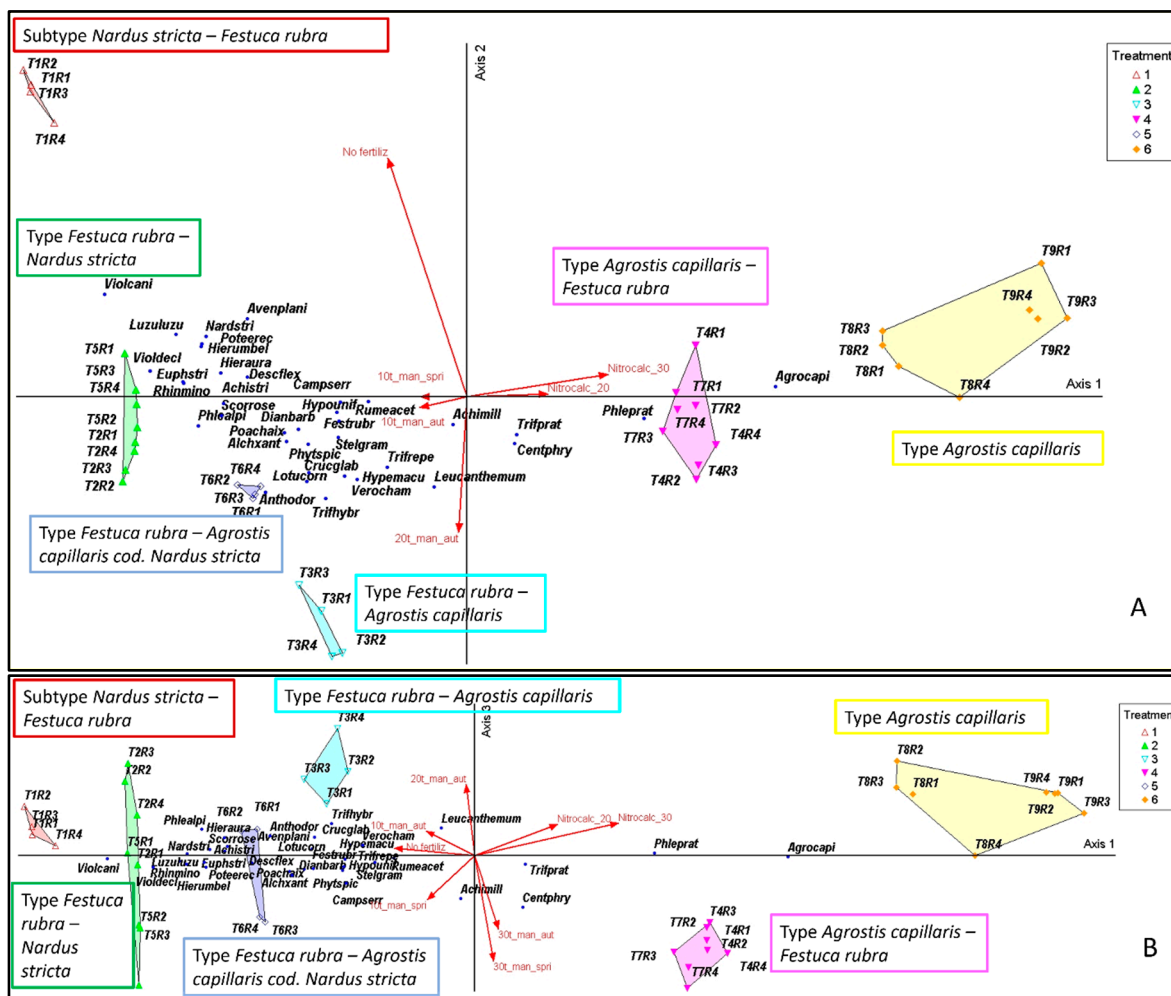


Figure 4. (A and B). Principal Coordinates Analysis (PCoA) of grassland communities under different fertilization regimes. Legend: T1 - unfertilized control; T2 - 10 t ha⁻¹ manure applied in autumn; T3 - 20 t ha⁻¹ manure applied in autumn; T4 - 30 t ha⁻¹ manure applied in autumn; T5 - 10 t ha⁻¹ manure applied in spring; T6 - 20 t ha⁻¹ manure applied in spring; T7 - 30 t ha⁻¹ manure applied in spring; T8 - 200 kg ha⁻¹ nitrocalcar applied in spring; T9 - 300 kg ha⁻¹ nitrocalcar applied in spring; R1–R4—replications. Vectors indicate the fertilization gradient: *No_fertiliz* – no fertilization (unfertilized control); *Fert_low_aut* – 10 t ha⁻¹ manure applied in autumn (T2); *Fert_med_aut* – 20 t ha⁻¹ manure applied in autumn (T3); *Fert_high_aut* – 30 t ha⁻¹ manure applied in autumn (T4); *Fert_low_spr* – 10 t ha⁻¹ manure applied in spring (T5); *Fert_med_spr* – 20 t ha⁻¹ manure applied in spring (T6); *Fert_high_spr* – 30 t ha⁻¹ manure applied in spring (T7); *Nitrocalc_20* – 200 kg ha⁻¹ nitrocalcar applied in spring (T8); and *Nitrocalc_30* – 300 kg ha⁻¹ nitrocalcar applied in spring (T9). Species abbreviations: *Agrocapi* – *Agrostis capillaris* L.; *Festrubr* – *Festuca rubra* L.; *Nardstri* – *Nardus stricta* L.; *Avenplani* – *Avenula planiculmis* (Schur) Holub; *Anthodor* – *Anthoxanthum odoratum* L.; *Phlealpi* – *Phleum alpinum* L.; *Phleprat* – *Phleum pratense* L.; *Poachaix* – *Poa chaixii* Vill.; *Descflex* – *Deschampsia flexuosa* (L.) Trin.; *Trifhybr* – *Trifolium hybridum* L.; *Trifprat* – *Trifolium pratense* L.; *Trifrepe* – *Trifolium repens* L.; *Lotucorn* – *Lotus corniculatus* L.; *Achimill* – *Achillea millefolium* L.; *Achistri* – *Achillea stricta* Schleich. ex W.D.J. Koch; *Alchxant* – *Alchemilla xanthochlora* Rothm.; *Campserr* – *Campanula serrata* (Kit.) Hendr.; *Centphry* – *Centaurea phrygia* L.; *Crucglab* – *Cruciata glabra* (L.) Ehrend.; *Dianbarb* – *Dianthus barbatus* subsp. *compactus* (Kit.) Grognot; *Euphstri* – *Euphrasia stricta* D. Wolff ex. J.F. Lehm.; *Hieraurea* – *Hieracium aurantiacum* L.; *Hierumbel* – *Hieracium umbellatum* L.; *Hypemacu* – *Hypericum maculatum* Crantz; *Hypounif* – *Hypochaeris uniflora* Vill.; *Leucvulg* – *Leucanthemum vulgare* Lam.; *Luzuluzu* – *Luzula luzuloides* (Lam.) Dandy & Wilmott; *Phytspic* – *Phyteuma spicatum* L.; *Poteerac* – *Potentilla erecta* (L.) Raeusch.; *Rhinmino* – *Rhinanthus minor* L.; *Rumeacet* – *Rumex acetosa* L.; *Scorroze* – *Scorzonera rosea* Waldst. & Kit.; *Stelgram* – *Stellaria graminea* L.; *Verocham* – *Veronica chamaedrys* L.; *Violcani* – *Viola canina* L.; *Violdecl* – *Viola declinata* Waldst. & Kit.

2.3. Multi-Response Permutation Procedure Analysis

MRPP analysis confirmed the strong statistical separation of the six floristic groups. All pairwise comparisons were significant ($p < 0.001$) with high A values (0.284–0.767), indicating internal homogeneity above the expected random level. Key findings: Group 1 (T1) was the most distinct from all other treatments ($A = 0.682$ –0.767). Group 6 (T8–T9, nitrocalcar) showed the highest internal homogeneity (A up to 0.767), confirming the uniformity of composition under intense mineral fertilization. Group 5 (high manure dose) was clearly separated from Group 6, indicating that intensive organic fertilization produced a distinct type from that obtained through mineral fertilization. Thus, the MRPP validated the stability of the floristic types delineated by the cluster and PCoA.

Table 2. MRPP pairwise comparisons of floristic composition between the four grassland groups.

Groups compared	T statistic	A (within-group agreement)	p-value
Group 1 vs. Group 2	-7.080	0.485	<0.001
Group 1 vs. Group 3	-4.450	0.721	<0.001
Group 1 vs. Group 4	-7.314	0.682	<0.001
Group 1 vs. Group 5	-4.444	0.609	<0.001
Group 1 vs. Group 6	-7.280	0.767	<0.001
Group 2 vs. Group 3	-6.736	0.458	<0.001
Group 2 vs. Group 4	-10.122	0.622	<0.001
Group 2 vs. Group 5	-6.282	0.284	<0.001
Group 2 vs. Group 6	-10.107	0.745	<0.001
Group 3 vs. Group 4	-7.253	0.562	<0.001
Group 3 vs. Group 5	-4.273	0.400	<0.001
Group 3 vs. Group 6	-7.191	0.719	<0.001

Group 4 vs. Group 5	-7.248	0.534	<0.001
Group 4 vs. Group 6	-9.513	0.462	<0.001
Group 5 vs. Group 6	-7.207	0.702	<0.001

Note: T = test statistics; A = chance-corrected within-group agreement. *Significance*: ***p < 0.001. Group 1 = T1 - unfertilized control; Group 2 = T2, T5 (T2 - 10 t ha⁻¹ manure applied in autumn; T5 - 10 t ha⁻¹ manure applied in spring); Group 3 = T6 - 20 t ha⁻¹ manure applied in spring; Group 4 = T3 - 20 t ha⁻¹ manure applied in autumn; Group 5 = T4, T7 (T4 - 30 t ha⁻¹ manure applied in autumn; T7 - 30 t ha⁻¹ manure applied in spring); Group 6 = T8, T9 (T8 - 200 kg ha⁻¹ nitrocalcar applied in spring; T9 - 300 kg ha⁻¹ nitrocalcar applied in spring).

2.4. Species–Environment Relationships Along PCoA Axes

The distribution of species in relation to the ordination axes highlighted how each species responded to the treatment gradient, particularly the differences between organic and mineral inputs (Table 4). On Axis 1, positive values were mainly associated with variants fertilized with nitrocalcar (T8–T9) and variants with high doses of manure (T4–T7). This area contained species with the highest positive correlations, such as *Agrostis capillaris* and *Phleum pratense*. These are characteristic of areas where fertilization, especially mineral fertilization, strongly influences the floristic composition. The negative values on Axis 1 were associated with unfertilized areas (T1) and treatments with low doses of manure applied in autumn and spring (T2 and T5, respectively). This area is home to *Nardus stricta*, *Festuca rubra*, *Scorzonera rosea*, *Potentilla erecta*, and other species characteristic of areas with low inputs. The association of these species with the negative area of Axis 1 corresponds to treatments in which the influence of fertilization was reduced or absent. On Axis 2, the differentiation was mainly between unfertilized areas, variants with manure applied in autumn, and treatments with medium- to high organic inputs. Thus, *Nardus stricta*, *Viola canina*, and *Luzula luzuloides* were positioned in the positive area, where repetitions from T1 and, partially, variants with low doses of manure were predominantly found. Species with negative correlations on Axis 2, such as *Trifolium repens*, *Anthoxanthum odoratum*, *Veronica chamaedrys*, *Lotus corniculatus*, and *Trifolium pratense*, were associated with variants with medium organic inputs and transitions between moderately and intensively fertilized areas. These species were found mainly in the space occupied by treatments T3, T4, and T6, where the effect of manure, regardless of the time of application, was reflected in the floristic composition. Species with low or insignificant correlations were evenly distributed in the order, with no obvious response to the inputs applied, suggesting a constant presence in most variants, regardless of the level of fertilization.

Table 4. Plant species correlation with ordination axes (PCoA).

Species	Axis 1 (r)	Axis 1 (r-sq)	Axis 1 (tau)	Signif.	Axis 2 (r)	Axis 2 (r-sq)	Axis 2 (tau)	Signif.
<i>Agrostis capillaris</i> L.	0.994	0.989	0.915	***	0.082	0.007	0.126	ns
<i>Festuca rubra</i> L.	-0.842	0.708	-0.559	***	-0.407	0.165	-0.548	*
<i>Nardus stricta</i> L.	-0.844	0.713	-0.801	***	0.491	0.241	0.035	**
<i>Avenula planiculmis</i> (Schur) Holub	-0.499	0.249	-0.534	**	0.445	0.198	-0.024	**
<i>Anthoxanthum odoratum</i> L.	-0.581	0.338	-0.403	**	-0.687	0.472	-0.592	***
<i>Phleum alpinum</i> L.	-0.532	0.283	-0.592	**	-0.146	0.021	-0.140	ns
<i>Phleum pratense</i> L.	0.842	0.709	0.773	***	-0.260	0.068	-0.026	ns
<i>Poa chaixii</i> Vill.	-0.512	0.262	-0.420	**	-0.253	0.064	-0.316	ns

<i>Deschampsia flexuosa</i> (L.) Trin.	-0.701	0.491	-0.584	***	0.154	0.024	-0.004	ns
<i>Trifolium hybridum</i> L.	-0.331	0.110	-0.310	*	-0.596	0.355	-0.537	**
<i>Trifolium pratense</i> L.	0.350	0.123	0.336	*	-0.670	0.449	-0.389	**
<i>Trifolium repens</i> L.	-0.346	0.120	-0.209	*	-0.768	0.590	-0.663	***
<i>Lotus corniculatus</i> L.	-0.488	0.238	-0.357	**	-0.647	0.419	-0.544	***
<i>Achillea millefolium</i> L.	-0.032	0.001	-0.209	ns	-0.157	0.025	-0.260	ns
<i>Achillea stricta</i> Schleich. ex W.D.J. Koch	-0.695	0.483	-0.531	***	-0.046	0.002	-0.155	ns
<i>Alchemilla xanthochlora</i> Rothm.	-0.422	0.178	-0.386	**	-0.262	0.069	-0.436	ns
<i>Campanula serrata</i> (Kit.) Hendrych	-0.312	0.097	-0.347	ns	-0.033	0.001	-0.202	ns
<i>Centaurea phrygia</i> L.	0.149	0.022	0.212	ns	-0.371	0.138	-0.379	*
<i>Cruciata glabra</i> (L.) Ehrend.	-0.426	0.182	-0.360	**	-0.512	0.262	-0.425	**
<i>Dianthus barbatus</i> subsp. <i>compactus</i> (Kit.) Grognot	-0.429	0.184	-0.468	**	-0.207	0.043	-0.362	ns
<i>Euphrasia stricta</i> D. Wolff ex J.F. Lehm.	-0.764	0.583	-0.636	***	0.093	0.009	-0.078	ns
<i>Hieracium aurantiacum</i> L.	-0.546	0.298	-0.611	**	0.133	0.018	-0.140	ns
<i>Hieracium umbellatum</i> L.	-0.579	0.336	-0.624	**	0.289	0.083	-0.083	ns
<i>Hypericum maculatum</i> Crantz	-0.365	0.133	-0.270	*	-0.591	0.349	-0.502	**
<i>Hypochaeris uniflora</i> Vill.	-0.688	0.473	-0.532	***	-0.205	0.042	-0.249	ns
<i>Leucanthemum vulgare</i> Lam.	-0.076	0.006	0.066	ns	-0.520	0.270	-0.306	**
<i>Luzula luzuloides</i> (Lam.) Dandy & Wilmott	-0.624	0.390	-0.551	***	0.336	0.113	0.159	*
<i>Phyteuma spicatum</i> L.	-0.311	0.097	-0.261	ns	-0.232	0.054	-0.270	ns
<i>Potentilla erecta</i> (L.) Raeusch.	-0.774	0.599	-0.753	***	0.366	0.134	-0.005	*
<i>Rhinanthus minor</i> L.	-0.614	0.377	-0.582	***	0.081	0.007	-0.065	ns
<i>Rumex acetosa</i> L.	-0.526	0.277	-0.399	**	-0.062	0.004	-0.160	ns
<i>Scorzonera rosea</i> Waldst. & Kit.	-0.879	0.772	-0.672	***	-0.171	0.029	-0.327	ns
<i>Stellaria graminea</i> L.	-0.554	0.306	-0.387	**	-0.436	0.190	-0.449	*

<i>Veronica chamaedrys</i> L.	-0.334	0.111	-0.257	*	-0.628	0.394	-0.619	***
<i>Viola canina</i> L.	-0.641	0.411	-0.593	***	0.453	0.205	0.242	**
<i>Viola declinata</i> Waldst. & Kit.	-0.679	0.461	-0.546	***	0.137	0.019	-0.012	ns

Note: r — Pearson's correlation coefficient; r -sq—coefficient of determination; τ —Kendall's tau correlation between ordination scores and species abundance. Significance: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns = not significant.

2.5. Indicator Species Analysis (ISA)

Indicator species analysis (ISA) revealed distinct sets of characteristic species for each of the six floristic groups identified by cluster classification (Table 5). Each group had at least one indicator species with high *IndVal* values, confirming the delimitation of vegetation types based on floristic composition.

Group 1 (oligotrophic grasslands of *Nardus stricta*-*Festuca rubra*) has the most consistent set of indicator species, including *Nardus stricta*, *Avenula planiculmis*, *Potentilla erecta*, *Viola canina*, and *Luzula luzuloides*, all with high *IndVal* values and statistical significance. These define the oligotrophic communities that are typical of nutrient-poor mountain habitats. Group 2 (communities with low manure inputs) was determined by treatments with 10 t ha⁻¹ and was mainly associated with *Phleum alpinum*, *Poa chaixii*, *Rhinanthus minor*, and *Hypochaeris uniflora*. These species have high indicator values and delimit an intermediate type of vegetation between unfertilized and moderately fertilized areas, while also indicating an early transition to mesotrophic conditions. Group 3 (communities under moderate spring manure inputs) was marked by species that prefer moderate trophic input, such as *Anthoxanthum odoratum*, *Trifolium hybridum*, *Trifolium repens*, *Lotus corniculatus*, *Hypericum maculatum*, *Leucanthemum vulgare*, *Cruciata glabra*, and *Veronica chamaedrys*. Their constant presence reflects the stability of communities with moderate nutrient input. Group 4 (communities with moderate autumn manure inputs) was defined by the presence of *Trifolium pratense* and *Centaurea phrygia*, both of which had significant indicator values. This group corresponds to moderately fertilized grasslands with organic inputs applied in the fall, which leads to a different floristic composition than that of identical fertilization applied in the spring. Group 5 (communities with high manure inputs) showed high indicator values for *Alchemilla xanthochlora* and *Dianthus barbatus* subsp. *compactus*, species characteristic of high doses of manure applied in autumn and spring, respectively. This reflects the influence of intensive organic fertilization on vegetation structure. Group 6 (communities with mineral fertilization) was associated with clear indicators of mineral fertilization, such as *Agrostis capillaris* and *Phleum pratense*. Both species showed high indicator values and almost exclusive distribution in the nitrocalcar treatments, highlighting the clear response of vegetation to mineral input, emphasizing the uniformity of communities, and the dominance of competitive grasses. Overall, ISA validated the robustness of the identified floristic types, confirming the response of plant communities to fertilization gradients.

Table 5. Indicator value of species related to the groups.

Species	Group	IndVal	Signif.
<i>Agrostis capillaris</i> L.	6	46.4	$p < 0.001$
<i>Festuca rubra</i> L.	3	22.1	ns
<i>Nardus stricta</i> L.	1	41.9	$p < 0.001$
<i>Avenula planiculmis</i> (Schur) Holub	1	50.0	$p < 0.01$
<i>Anthoxanthum odoratum</i> L.	3	39.5	$p < 0.01$
<i>Phleum alpinum</i> L.	2	51.1	$p < 0.01$
<i>Phleum pratense</i> L.	6	30.4	$p < 0.01$
<i>Poa chaixii</i> Vill.	2	40.9	$p < 0.01$
<i>Deschampsia flexuosa</i> (L.) Trin.	1	26.7	ns

<i>Trifolium hybridum</i> L.	3	48.1	p < 0.01
<i>Trifolium pratense</i> L.	4	23.3	p < 0.05
<i>Trifolium repens</i> L.	3	31.0	p < 0.01
<i>Lotus corniculatus</i> L.	3	40.3	p < 0.01
<i>Achillea millefolium</i> L.	4	34.7	ns
<i>Achillea stricta</i> Schleich. ex W.D.J. Koch	1	29.6	ns
<i>Alchemilla xanthochlora</i> Rothm.	5	47.1	p < 0.01
<i>Campanula serrata</i> (Kit.) Hendrych	5	27.0	ns
<i>Centaurea phrygia</i> L.	4	39.2	p < 0.01
<i>Cruciata glabra</i> (L.) Ehrend.	3	46.5	p < 0.01
<i>Dianthus barbatus</i> subsp. <i>compactus</i> (Kit.) Grognot	5	40.0	p < 0.05
<i>Euphrasia stricta</i> D. Wolff ex J.F. Lehm.	1	30.8	ns
<i>Hieracium aurantiacum</i> L.	1	34.8	ns
<i>Hieracium umbellatum</i> L.	1	38.2	ns
<i>Hypericum maculatum</i> Crantz	3	39.5	p < 0.01
<i>Hypochaeris uniflora</i> Vill.	2	26.1	p < 0.05
<i>Leucanthemum vulgare</i> Lam.	3	49.0	p < 0.01
<i>Luzula luzuloides</i> (Lam.) Dandy & Wilmott	1	40.0	p < 0.05
<i>Phyteuma spicatum</i> L.	5	30.0	ns
<i>Potentilla erecta</i> (L.) Raeusch.	1	36.4	p < 0.05
<i>Rhinanthus minor</i> L.	2	50.0	p < 0.01
<i>Rumex acetosa</i> L.	1	20.0	ns
<i>Scorzonera rosea</i> Waldst. & Kit.	1	23.5	ns
<i>Stellaria graminea</i> L.	2	22.9	ns
<i>Veronica chamaedrys</i> L.	3	34.8	p < 0.05
<i>Viola canina</i> L.	1	53.3	p < 0.01
<i>Viola declinata</i> Waldst. & Kit.	1	22.5	ns

Note: Groups correspond to the six phytosociological clusters identified by the cluster analysis. *Significance*: *** p < 0.001, ** p < 0.01, * p < 0.05, ns = not significant. Groups meaning: Group 1 = T1 - unfertilized control; Group 2 = T2, T5 (T2 - 10 t ha⁻¹ manure applied in autumn; T5 - 10 t ha⁻¹ manure applied in spring); Group 3 = T6 - 20 t ha⁻¹ manure applied in spring; Group 4 = T3 - 20 t ha⁻¹ manure applied in autumn; Group 5 = T4, T7 (T4 - 30 t ha⁻¹ manure applied in autumn; T7 - 30 t ha⁻¹ manure applied in spring); Group 6 = T8, T9 (T8 - 200 kg ha⁻¹ nitrocalcar applied in spring; T9 - 300 kg ha⁻¹ nitrocalcar applied in spring).

2.6. The Impact of Management Scenarios on Diversity Indices

The diversity index values showed that grasslands responded nonlinearly to the fertilization gradient. Oligotrophic communities (T1) exhibited moderate levels of diversity and uniformity, which are typical of nutrient-poor mountain grasslands ($S = 31.75$ and $H' = 2.199$). These values reflect the structure of a low-fertility mountain grassland, where the dominance of competitive species is limited and the distribution of abundance is relatively balanced. Applying a reduced amount of waste (T2, T5: low-input, maximum diversity) significantly increased diversity, especially in T5 (spring), which had the highest species richness and Shannon index ($S = 35$ and $H' = 2.857$). These results confirm the intermediate disturbance hypothesis: low-nutrient input reduces the dominance of competitive species, favoring their co-existence. Moderate fertilization applied in autumn (T3, T6: medium-input-high and stable diversity) produced high diversity values ($H' \approx 2.70-2.77$), highlighting an optimal compromise between productivity and species conservation. This level of input promotes a well-balanced community with similar proportions of species while maintaining a

high diversity. High doses of fertilizer applied in autumn (T4 and T7: high-input organic-decline in diversity) caused a reduction in diversity and uniformity (e.g., T7, $H' = 1.958$; $S = 24.25$), indicating the emergence of dominance of grasses tolerant to intensive fertilization. This indicates the emergence of imbalances in the community structure and an increasingly pronounced dominance of species tolerant to high organic inputs.

Mineral fertilization (T8 and T9 - extreme simplification) generated the lowest values for all indices, especially T9 ($H' = 0.926$ and $S = 11.25$). This confirms rapid eutrophication and the loss of sensitive oligotrophic species, as well as a strong simplification of the community, with increased dominance of a small number of species adapted to high fertility and tolerant of mineral input levels. ANOVA revealed significant differences for all variables ($p < 0.001$), indicating that the effect of fertilization on community structure was strong and consistent.

Table 6. The influence of fertilization treatments on biodiversity indices in grasslands.

Treatment	Species richness (S)	Shannon index (H')	Evenness (E)	Simpson (D)
T1	31.75 ± 0.96	2.199 ± 0.055	0.636 ± 0.016	0.789 ± 0.018
T2	32.50 ± 1.73	2.714 ± 0.043	0.780 ± 0.011	0.892 ± 0.006
T3	32.25 ± 1.50	2.770 ± 0.035	0.798 ± 0.004	0.903 ± 0.003
T4	26.25 ± 2.50	2.158 ± 0.036	0.662 ± 0.025	0.779 ± 0.007
T5	35.00 ± 1.15	2.857 ± 0.042	0.804 ± 0.016	0.912 ± 0.004
T6	32.25 ± 2.22	2.709 ± 0.037	0.780 ± 0.011	0.895 ± 0.003
T7	24.25 ± 1.71	1.958 ± 0.070	0.615 ± 0.021	0.744 ± 0.017
T8	18.25 ± 5.12	1.426 ± 0.085	0.500 ± 0.032	0.548 ± 0.009
T9	11.25 ± 2.22	0.926 ± 0.027	0.388 ± 0.037	0.387 ± 0.017
F-test	43.05	685.81 (df=8,27)	183.73 (df=8,27)	1079.41 (df=8,27)
p-value	< 0.001	< 0.001	< 0.001	< 0.001

Legend: T1 - unfertilized control; T2 - 10 t ha⁻¹ manure applied in autumn; T3 - 20 t ha⁻¹ manure applied in autumn; T4 - 30 t ha⁻¹ manure applied in autumn; T5 - 10 t ha⁻¹ manure applied in spring; T6 - 20 t ha⁻¹ manure applied in spring; T7 - 30 t ha⁻¹ manure applied in spring; T8 - 200 kg ha⁻¹ nitrocalcar applied in spring; T9 - 300 kg ha⁻¹ nitrocalcar applied in spring. Note: Values are means ± standard error (n = four replicates per treatment). One-way ANOVA was used to test for differences between the treatments. Different letters within a column indicate significant differences at $p < 0.05$, according to the LSD test

3. Discussion

3.1. Floristic Responses to Fertilization in HNV Mountain Grasslands

The results of hierarchical classification and PCoA ordination clearly show that the fertilization gradient profoundly alters the structure of plant communities, generating clear transitions between three major vegetation types: *Nardus stricta* (oligotrophic), *Festuca rubra* (mesotrophic), and *Agrostis capillaris* (eutrophic). The evolution of these floristic types is in line with the patterns reported in HNV grasslands in Romania [14,61,62] and Central Europe, where excessive fertilization leads to the simplification of vegetation cover and loss of specialized species [63,64]. In the Lucina experiment, low-moderate organic fertilization favored the *Festuca rubra* – *Nardus stricta* and *Festuca rubra* – *Agrostis capillaris* types, confirming that moderate nutrient levels can improve trophic conditions without compromising diversity. Similar results were reported in the Apuseni Mountains and

Transylvania, where moderate doses of manure maintained a stable core of mesotrophic and oligotrophic species [27,46,65].

In contrast, intensive organic and mineral fertilization led to the almost exclusive dominance of *Agrostis capillaris*, indicating that the resilience threshold of HNV habitats had been exceeded. Similar results regarding floristic simplification under intensive fertilization have been reported in mountain grasslands in Romania [66] and in long-term experiments in Central Europe [63,67]. These studies show that once fertilization exceeds moderate levels, semi-natural communities tend to be dominated by a few competitive grasses, with the loss of oligotrophic species of conservation value and HNV indicators [64,68]. In the context of HNV, and as confirmed by our results, high and repeated organic and mineral fertilization is incompatible with the maintenance of oligotrophic types of *Nardus stricta*, as defined in recent literature [56,69,70]. From this perspective, the results from the Eastern Carpathians converge with those reported in the Apuseni Mountains for *Arnica montana* grasslands, where moderate manure use has allowed the conservation of HNV habitats, whereas intensification has led to the loss of sensitive species [21,22,71].

3.2. Species–Environment Relationships and Indicator Species Patterns

The correlations between species and the PCoA axes confirmed the role of dominant grasses as effective indicators of trophic gradient. *Nardus stricta* and the associated oligotrophic species (e.g., *Potentilla erecta*, *Luzula luzuloides*, *Avenula planiculmis*, *Euphrasia stricta*, *Viola canina*) are strongly associated with unfertilized areas, characterizing the oligotrophic type typical of 6230* habitats. This group of species largely corresponds to the sets of indicators used to define oligotrophic grasslands in the Apuseni Mountains [19,28,59], and other European mountain regions [64]. Many of these species have been proposed as indicators for the management of HNV grasslands [15,116]. Mesotrophic species, especially *Festuca rubra*, *Trifolium repens*, *Anthoxanthum odoratum*, and *Lotus corniculatus*, showed intermediate correlations, being sensitive to both intensification and reduction of inputs. These marked the optimal biodiversity zones identified in treatments with moderate manure. Such responses have also been described in other grassland systems [13,80], where moderate organo-mineral inputs maintained a balance between forage value and the presence of oligotrophic species. Our results are consistent with those obtained in the Apuseni Mountains on *Festuca rubra* grasslands fertilized with manure [50,114,115].

Nitrocalcar treatments were characterized almost exclusively by *Agrostis capillaris* and *Phleum pratense*, which had the highest *IndVal* values in group 6. This pattern is typical of eutrophicated habitats, where competition for light and nutrients favors the growth of fast-growing grasses [12,68]. ISA analysis thus validated the six floristic types identified, confirming that fertilization is the determining factor of the vegetation structure. These patterns are consistent with international studies showing that in semi-natural grasslands, nitrogen fertilization rapidly reduces diversity and favors a small set of competitive grasses [72]. Recent meta-analyses have confirmed that mineral fertilization tends to homogenize communities and reduce the importance of eutrophication-sensitive species, whereas moderate organic fertilization can maintain a higher degree of functional heterogeneity [8,73–75].

3.3. Diversity Responses to Organic vs Mineral Fertilization

The analysis of diversity indices showed a typical response to the "intermediate" curve—the maximum values of specific Richness, Shannon and Simpson appeared in treatments with low or moderate doses of manure, especially T5 and T3/T6, in both autumn and spring. This structure corresponds to the intermediate disturbance hypothesis [43,44,67] and is consistent with the results obtained in other mountain experiments in Romania [50], as well as in Central Europe [63]. Moderate organic fertilization maintained a balanced distribution of species abundance, reflecting reduced competition between dominant grasses and oligotrophic species. Similar results have been reported in recent meta-analyses, which show that moderate organic amendments increase functional diversity and ecosystem stability [8,73,76,77]. In contrast, mineral fertilization with nitrocalcar

reduced diversity to extremely low levels, confirming the trend towards uniformity and loss of sensitive species. This situation has been confirmed by studies on HNV grasslands in Romania [111–113] and international research highlighting the destabilizing effect of eutrophication on the productivity and stability of grassland ecosystems [72,78]. The results of the experiment confirm that there is an "optimal window" in which moderate organic fertilization simultaneously maximizes productivity and diversity, a trade-off supported by recent studies [74,79,80].

3.4. Management Implications for *Nardus Stricta* Grasslands in the Eastern Carpathians

From the perspective of HNV grassland management in the Eastern Carpathians, the results indicate three main findings. Maintaining oligotrophic types (*Nardus stricta*). The reference grasslands (T1) and those with very low fertilization represent the core of oligotrophic habitats 6230 and HNV, comparable to those described in studies conducted in the Apuseni Mountains and Natura 2000 sites [108–110]. These ecosystems are home to numerous indicator species and plants of conservation and economic value [46–48] and are sensitive to both intensification and abandonment [39,52]. They are a priority for conservation and agricultural environmental schemes.

Moderate organic fertilization as an adaptive management tool. Doses of 10–20 t ha⁻¹ (autumn or spring) increased diversity and production without compromising community structure. This "optimal window" is applicable for both maintaining HNV habitats and sustainable fodder production in the future. Similar results have been obtained in the Apuseni Mountains and other HNV grasslands, where technologies such as mulching combined with organic fertilization have maintained high levels of diversity and a relatively stable community composition [81,104].

Limiting mineral fertilization and high doses of manure. Mineral fertilization (T8–T9) and high manure doses (T4, T7) greatly simplified the vegetation. Furthermore, the results regarding species structure and ISA values are compatible with the use of indicator species as a rapid diagnostic tool for the agrochemical status of the soil and for assessing management quality [105,106]. The integration of these indicators with modern monitoring methods, such as multivariate ordination [82–84] and remote sensing [56,85–87], can support the development of adaptive management schemes for HNV grasslands in the Carpathian region of Romania.

3.5. Study Limitations

The study was based on a single monofactorial experiment at a location representative of *Nardus stricta* grasslands in the Eastern Carpathians. Given the short duration (3 years) of the experiment, generalization of the results to the entire Carpathian range requires integration with other regional studies on fertilization, abandonment, and climate change [88–90,107].

3.6. Future Research Directions

The analysis also focused on vegetation. Further integration of data on the soil microbiome [51,91,92], edaphic fauna [12,78], and ecosystem services [93,94] would allow for a more comprehensive assessment of the effects of fertilization on the functioning of HNV ecosystems. Despite these limitations, the results clearly demonstrate that moderate organic fertilization can simultaneously support productivity and biodiversity, whereas mineral intensification leads to the loss of HNV habitats.

4. Materials and Methods

4.1. Study Area and Pedoclimatic Conditions

The experiment was conducted on a permanent *Nardus stricta* grassland in the Lucina area of the Lucina-Fundu Moldovei Depression of the major geomorphological unit Obcinele Bucovinei (Eastern Carpathians, northeastern Romania) in a mountainous region characterized by a cool and humid climate typical of the lower mountain range. The experimental field was located at an altitude of 1220

m, eastern longitude 25°10' 44" and northern latitude 47°38'44", with a 5% slope and northern exposure. The soil is of the white skeletal clinogleic luvisol type and at a depth of 0-28 cm is characterized by a low degree of base saturation, only 28-47%, strong acidity, with pH values of 4.5-4.6, low total cation exchange capacity, 6.7-13, 9 me/100 g soil, low phosphorus content of 10-15 mg/kg and very low potassium content of 4-9 mg/kg, carbon/nitrogen (C/N) ratio of 11.3, indicating a fairly slow decomposition of organic matter, the soil also being affected by excess stagnant moisture. The peat layer on the soil surface, approximately 5-7 cm thick, promotes faster infiltration of water from precipitation and prevents water loss through direct evaporation, influencing plant growth and development. The surface layer showed an accumulation of plant debris on the surface and low natural fertility, which is characteristic of oligotrophic grasslands. This study, conducted within a long-term experimental setup initiated in 2002, focuses on the floristic composition, vegetation types, and diversity of mountain grasslands based on data collected during the 2022-2024 period. The average annual temperature in the area is approximately 6–7°C, and the annual precipitation frequently exceeds 650–700 mm, with maximums recorded in the summer months (May–August) and minimums in winter. The rainfall and temperature regimes are typical of mountain depressions, with high atmospheric humidity throughout the year. Before the experiment was set up, the grassland was used extensively in a traditional system (mowing and/or grazing with low stocking rates) without systematic mineral or organic fertilization, with nutrients primarily coming from animal manure.

The multi-year average temperature is 6.4°C, and the average temperature during the growing season is 12.7°C. During the period 2022-2024, both annually and during the growing season, the average temperature was higher than the multi-year average by 0.5-1.3°C (Table 7, Table 9) and 0.1-0.9°C, respectively, and the bioactive period (with temperatures above 0°C) in the studied area was 255 days, between March 10 and November 20. The multi-annual average precipitation is 738.6 mm, with the following distribution by season: spring 175.1 mm (23.7%), summer 287.8 mm (39%), autumn 154.0 mm (20.8%), and winter 121.7 mm (16.5%). During the growing season (April-September), the multi-annual average precipitation is 493.0 mm, which represents 66.7% of the annual amount (Table 8, Table 10). During the period 2022-2024, the annual precipitation was 65 mm (9%) higher than the multi-year average, and during the growing season, it was close to the multi-year average. The highest amounts of precipitation were recorded in June and July, with positive deviations between 33.1-42.8 mm from the multi-year average.

The studied area is characterized by an average annual number of days in the cold season with snow cover between 79-100 days, an annual potential evapotranspiration of 599 mm, of which 338 (56.4%) in June -August, and a duration of sunshine of 1600-1800 hours/year, of which 1200-1300 hours (60%) in the warm season (April-September) and 400-500 hours/year (40%) in the cold season.

Table 7. Average air temperatures (°C) at Poiana Stampei Meteorological Station (2022-2023).

Specify	Oct	No	De	Jan	Feb	Ma	Apri	Ma	Jun	Jul	Au	Sep	Total	Vegetati
	.	v.	c.	.	r.	r.	l	y	e	y	g.	t.	12	on
													month	period
													s	(IV-IX)
Multi-year averages														
Decade I	7,2	4,1	-2,4	-4,2	-2,6	0,4	4,0	9,2	14,3	16,2	17,2	13,8	6,4	12,5
Decade II-a	5,9	1,7	-2,1	-5,2	-3,5	0,4	6,0	9,8	15,1	16,1	16,7	12,7	6,1	12,7
Decade III-a	6,4	-1,1	-2,2	-3,9	-1,2	2,3	7,0	11,9	16,4	17,2	15,8	9,3	6,5	12,9

Monthl y average	6,5	1,7	-2,2	-4,4	-2,4	1,0	5,7	10,3	15,3	16,5	16,6	11,9	6,4	12,7
Agricultural year 2022-2023														
Decade I	8,2	5,6	0,3	1,5	-7,7	0,6	0,6	8,0	13,4	16,7	16,1	13,2	6,4	11,3
Decade II-a	6,4	2,2	-2,7	1,3	-2,7	0,7	5,2	10,9	13,5	18,2	17,1	14,3	7,0	13,2
Decade III-a	7,4	-0,9	0,4	-2,4	0,3	2,3	5	12,6	15,8	16,2	18,1	13,3	7,3	13,5
Monthl y average	7,3	2,3	-0,7	0,1	-3,4	1,2	3,6	10,5	14,2	17,0	17,1	13,6	6,9	12,7
Multi- year average	6,5	1,7	-2,2	-4,4	-2,4	1,0	5,7	10,3	15,3	16,5	16,6	11,9	6,4	12,8
Deviatio n	+0,8	+0,6	+1,5	+4,5	-1,0	+0,2	-2,1	+0,2	-1,1	+0,5	+0,5	+1,7	+0,5	+0,1

Table 8. Atmospheric precipitation (mm) at the Poiana Stampei Meteorological Station (2022-2023).

Specify	Oc t.	No v.	De c.	Jan.	Feb r.	Ma r.	Apr il	Ma y	Jun e	July	Au g.	Sep t.	Total 12 mont hs	Vegetati on period (IV-IX)
Multi-year averages														
Decade I	13,6	11,5	14,8	12,4	19,2	11,6	18,7	24,3	31,2	29,9	19,7	19,7	226,6	143,5
Decade II-a	14,6	15,7	12,4	14,8	11,3	11,4	16,5	32,0	40,1	26,8	20,5	20,8	236,9	156,7
Decade III-a	17,2	18,3	17,4	8,1	11,3	10,0	19,2	31,4	50,6	44,6	24,4	22,6	275,1	192,8
Monthl y amount	45,4	45,5	44,6	35,3	41,8	33,0	54,4	87,7	121,9	101,3	64,6	63,1	738,6	493,0
Agricultural year 2022-2023														
Decade I	4,4	5,3	8,4	13,0	9,3	15,6	38,9	13,9	42,1	18,4	14,6	10,7	194,6	138,6
Decade II-a	15,3	40,4	28,0	27,6	24,2	4,5	10,1	7,5	89,5	39,3	2,0	10,7	299,1	159,1
Decade III-a	9,4	18,4	13,3	9,0	39,4	18,5	48,3	9,5	27,0	78,4	36,2	3,2	310,6	202,6

Monthly amount	29,1	64,1	49,7	49,6	72,9	38,6	97,3	30,9	158,6	136,1	52,8	24,6	804,3	500,3
Multi-year average	45,4	45,5	44,6	35,3	41,8	33,0	54,4	87,7	121,9	101,3	64,6	63,1	738,6	493,0
Deviation	-16,3	+18,6	+5,1	+14,3	+31,1	+5,6	+42,9	-56,8	+36,7	+34,8	-11,8	-38,5	+65,7	+7,3

Table 9. Average air temperatures (°C) at the Poiana Stampei Meteorological Station (2023-2024).

Specify	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sep.	Total 12 months	Vegetation period (IV-IX)
Agricultural year 2023-2024														
Decade I	7,9	4,5	-1,2	-2,1	2,1	3,7	9,0	10,2	12,5	17,4	16,7	15,0	8,0	13,5
Decade II-a	6,5	1,4	-1,2	-4,7	1,7	2,2	8,4	9,4	16,5	21,2	19,6	5,4	7,2	13,4
Decade III-a	9,9	-2,0	0,6	-6,1	4,2	5,1	6,8	12,5	19,2	16,7	17,7	9,9	7,9	13,8
Monthly average	8,1	1,3	-0,6	-4,3	2,7	3,7	8,1	10,7	16,1	18,4	18,0	10,1	7,7	13,6
Multi-year average	6,5	1,7	-2,2	-4,4	-2,4	1,0	5,7	10,3	15,3	16,5	16,6	11,9	6,4	12,7
Deviation	+1,6	-0,4	+1,6	+0,1	-0,3	+2,7	+2,4	+0,4	+0,8	+1,9	+1,4	-1,8	+1,3	+0,9

Table 10. Atmospheric precipitation (mm) at the Poiana Stampei Meteorological Station (2023-2024).

Specify	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sep.	Total 12 months	Vegetation period (IV-IX)
Agricultural year 2023-2024														
Decade I	11,0	28,0	23,2	16,7	15,3	4,1	10,2	16,8	21,8	79,6	45,2	4,3	276,2	177,9
Decade II-a	24,1	29,6	24,3	36,4	9,4	13,0	26,8	9,2	46,8	28,8	2,0	13,0	263,4	126,6

Decade III-a	22,7	22,4	17,5	7,0	0,1	11,8	15,8	13,8	96,1	26,0	2,2	28,3	263,7	182,2
Monthly amount	57,8	80	65	60,1	24,8	28,9	52,8	39,8	164,7	134,4	49,4	45,6	803,3	486,7
Multi-year average	45,4	45,5	44,6	35,3	41,8	33,0	54,4	87,7	121,9	101,3	64,6	63,1	738,6	493,0
Deviation	+12,4	+34,5	+20,4	+24,8	-17,0	-4,1	-1,6	-47,9	42,8	33,1	-15,2	-17,5	64,7	-6,3

4.2. Experimental Design

A single-factor experiment was organized using the randomized block method, with nine treatment variants and four repetitions, installed on a permanent grassland derived from *Nardus stricta* L (*Nardus stricta-Festuca rubra*). The experimental plots were located on gently sloping terrain under homogeneous site conditions to reduce the influence of soil and relief microvariability on the results of the study. The research covered four blocks, and the experiment had the following variants: T1 – Unfertilized control; T2 – 10 t ha⁻¹ well-decomposed horse manure applied in autumn; T3 – 20 t ha⁻¹ well-decomposed horse manure applied in autumn; T4 – 30 t ha⁻¹ well-decomposed horse manure applied in autumn; T5 – 10 t ha⁻¹ well-decomposed horse manure applied in spring; T6 – 20 t ha⁻¹ well-decomposed horse manure applied in spring; T7 – 30 t ha⁻¹ well-decomposed horse manure applied in spring; T8 – 200 kg ha⁻¹ nitrocalcar (calcium ammonium nitrate) applied in spring; T9 – 300 kg ha⁻¹ nitrocalcar (calcium ammonium nitrate) applied in spring. The horse manure used in the experiment was well decomposed (more than two years old) and originated from a traditional husbandry system, providing a complex supply of nutrients and organic matter to the soil. Its chemical composition was as follows: pH 6.7, P₂O₅ 0.334%, K₂O 0.691%, total nitrogen 0.434%, Na 0.0544%, Mg 0.1197%, Zn 5.59 mg/kg, Cu 1.358 mg/kg; Fe 65.06 mg/kg. Mineral fertilization was performed with nitrocalcar (27% total N; 8–12% CaCO₃ + MgCO₃). The balanced proportion between the nitric and ammoniacal forms ensures both rapid availability and gradual release of N. Fertilization was applied annually in spring (T5–T9) and November for the autumn treatment (T2–T4). Mechanization was minimal; mowing was performed once a year (in July), and the biomass was removed. The entire perimeter was fenced to prevent accidental grazing by animals.

4.3. Studies of Floral Composition

Floristic composition was assessed annually (in July) using the Braun–Blanquet scale modified for mountain grasslands [102]. The classic 1–5 scale was subdivided into subclasses a–c to increase the accuracy of the coverage estimates, a modification that is particularly beneficial for capturing subtle changes in species abundance in diverse and often finely structured mountain grassland communities (Figure 5 and Table 7). Floristic surveys were conducted annually in early July, when most grasses were in the flowering stage, allowing for accurate species identification and adequate comparisons between years and treatments[86]. The coverage of each species was estimated in each experimental plot (T1–T9, with four replicates per plot). Taxonomic determination of species was carried out with the help of specialized floristic works and by consulting international databases (POWO – Plants of the World Online, Euro+Med PlantBase) to ensure the use of updated and uniform nomenclature. To reduce disruptive influences, the area was protected, and mowing was performed uniformly at the same height after the surveys.

4.4. Data Analysis

Multivariate analyses were performed using PC-ORD version 7 software, which is widely applied in community ecology for classification, ordination, and testing differences among groups [83,84]. Floristic-ecological classification. To group the plots into grassland types, we used hierarchical cluster analysis based on the Sørensen (Bray–Curtis) similarity index and the unweighted pair group method with arithmetic mean (UPGMA) linkage method. This combination (Bray–Curtis + UPGMA) is considered a robust standard in plant community analysis and is frequently used to delineate phytosociological and ecological groups in semi-natural grasslands [96,97]. The dendrogram cutoff level was set at approximately 72% of the remaining information, which allowed the identification of six distinct floristic groups with clear ecological interpretations (types and subtypes of grasslands dominated by *Nardus stricta*, *Festuca rubra*, and *Agrostis capillaris*). To illustrate the trophic gradient generated by the fertilization treatments and to separate vegetation types, we performed a Principal Coordinate Analysis (PCoA) using the Bray–Curtis distance. PCoA provides a unique and reproducible solution and allows for the direct interpretation of distances among plots, which is an important advantage over iterative methods such as the NMDS [35,98]. The first two ordination axes explained over 98% of the floristic variation. The treatments were introduced into the prescription as vectors ("joint plots"), representing the input gradient: *No_fertilz* – unfertilized control (T1); *Fert_low_aut* – 10 t ha⁻¹ manure in autumn (T2); *Fert_med_aut* – 20 t ha⁻¹ manure in autumn (T3); *Fert_high_aut* – 30 t ha⁻¹ manure in autumn (T4); *Fert_low_spr* – 10 t ha⁻¹ manure in spring (T5); *Fert_med_spr* – 20 t ha⁻¹ manure in spring (T6); *Fert_high_spr* – 30 t ha⁻¹ manure in spring (T7); *Nitrocalc_20* – 200 kg ha⁻¹ nitrocalcar (T8); *Nitrocalc_30* – 300 kg ha⁻¹ of nitrocalcar (T9). The vectors were normalized, and their statistical significance was tested using permutation tests (n = 999).

Testing differences between groups. The statistical separation of floristic groups was tested using the Multi-Response Permutation Procedure (MRPP), which calculates the following metrics: T statistic (differentiation between groups), A coefficient (intragroup homogeneity), and p-value (statistical significance threshold of $\alpha = 0.05$). All pairwise comparisons between groups were tested, and A and T values were used to support the robustness of the floristic types associated with the treatments [82]. To identify the species characteristics of each type of grassland, *Indicator Species Analysis* (ISA) was used, according to the method of [99]. For each species, IndVal values (combination of frequency and relative abundance in the group) were calculated, and statistical significance was tested using permutations (n = 999). Species with high IndVal and $p < 0.05$ were considered indicator species relevant to the floristic groups corresponding to the six clusters.

4.5. Diversity Indices and Statistical Analysis

For each treatment and repetition, the classic α -diversity indices were calculated as follows: 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' 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.

These indices are widely used in grassland ecology studies and are considered robust tools for assessing plant community diversity and balance [72,76,87]. The formula and interpretation of these indices followed standard recommendations in community ecology [100] and are widely used in semi-natural grassland studies. The differences between treatments (T1–T9) for each index were tested using a one-way analysis of variance (ANOVA). The assumptions of normality and homogeneity of variance were checked before applying ANOVA [101,102], where conditions were met, and means were separated using the LSD test at $p < 0.05$. The values presented in the diversity table (Table 11) are means \pm standard error (n = 4 replicates per treatment).

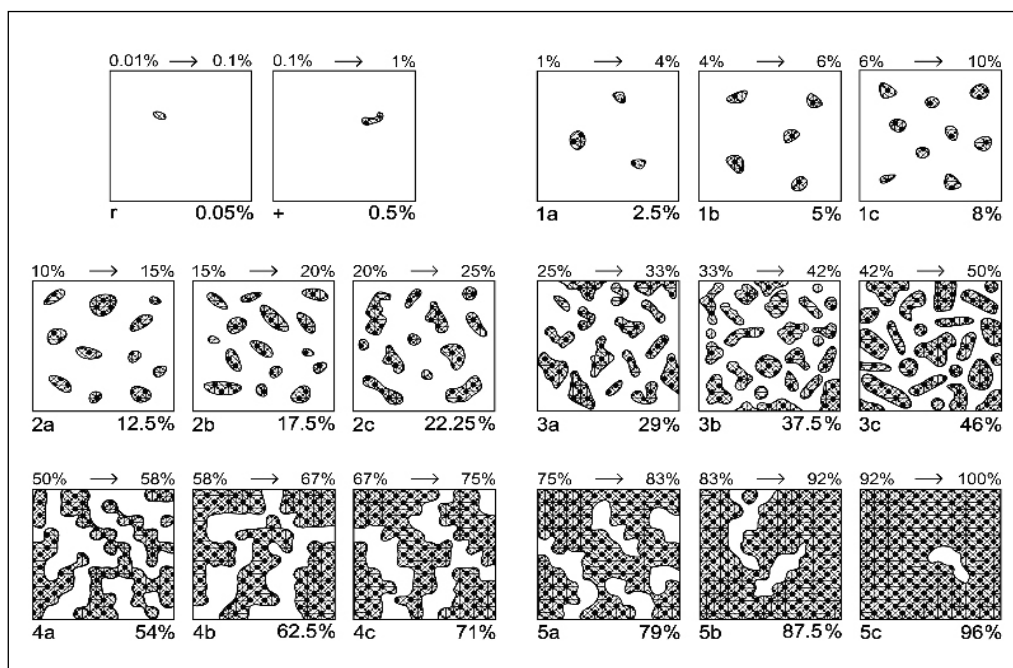


Figure 5. Modified Braun-Blanquet scale for grasslands based on species coverage [103]. Legend: 1 to 5 indicate the class of coverage; a, b, c indicate the sub-note of each class.

Table 11. Modified Braun-Blanquet scale for assessing the abundance–dominance of plant species based on classes and sub-classes [103].

Class	Coverage Interval (%)	Class Central Value (%)	Sub-Note	Sub-Interval (%)	Central-Adjusted Value of Sub-Interval (%)
5	75–100	87.5	5c	92–100	96
			5b	83–92	87.5
			5a	75–83	79
4	50–75	62.5	4c	67–75	71
			4b	58–67	62.5
			4a	50–58	54
3	25–50	37.5	3c	42–50	46
			3b	33–42	37.5
			3a	25–33	29
2	10–25	17.5	2c	20–25	22.25
			2b	15–20	17.5
			2a	10–15	12.5
1	1–10	5	1c	6–10	8
			1b	4–6	5
			1a	1–4	2.5
+	0.1–1	0.5	-	-	0.5
r	0.01–0.1	0.05	-	-	0.05

Note: a, b, and c indicate the sub-notes of each class

5. Conclusions

This study demonstrates that fertilization is a determining factor of the structure and diversity of *Nardus stricta* mountain grasslands in the Eastern Carpathians, Romania. The results showed a well-defined trophic gradient from oligotrophic communities rich in conservatively valuable species to increasingly simplified phytocenoses as the nutrient inputs increased. Moderate organic fertilization (10–20 t ha⁻¹ manure), applied in autumn or spring, supported the highest floristic diversity, confirming the existence of an optimal range in which productivity and biodiversity can be simultaneously maintained. These treatments favored the intermediate types *Festuca rubra* – *Nardus stricta* and *Festuca rubra* – *Agrostis capillaris*, which are characterized by structural heterogeneity and a stable set of indicator species.

In contrast, high doses of manure (30 t ha⁻¹) and, especially, mineral fertilization with nitrocalcar (200–300 kg ha⁻¹) led to a severe reduction in species richness, loss of oligotrophic species, and establishment of eutrophicated types dominated by *Agrostis capillaris*, which is incompatible with the objectives of the Natura 2000 network and HNV management. Overall, the results support the integration of moderate organic fertilization as an adaptive management tool for sensitive mountain grasslands, whereas the application of mineral fertilizers should be avoided in these regions. Multivariate analyses and indicator species are valuable tools for rapid diagnosis.

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