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Mid-term fertilisers and lime effect on grassland in the hilly-mountain region in Balkan

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Abstract: Although the effects of fertiliser addition and liming on semi-natural grassland productivity and biomass quality are well documented, less is known about how fertilisers change plant functional groups and mean ecological values. We researched the effects of liming (no lime and lime with 1 t/ha) and mineral fertilisers (control – no fertilisers, PK-P60K60, N20PK-N20P60K60, N80PK-N80P60K60, and N140PK-N140P60K60) for nine years on the *Danthonia alpina* Vest. grassland community. Based on Brown-Blanquet cover-abundance, we calculated Shannon-Wiener evenness and abundance of plant functional groups (based on height, canopy structure, storage organs presence and flowering duration). We also researched Landolt's ecological indicator values for nutrients, moisture, reaction, light, and temperature. Results revealed that fertilisers stimulated tall species with longer flowering duration. Shannon-Wiener evenness in control was 0.45, and N20PK increased to 0.71 but significantly decreased in treatment N140PK (0.25). Mean Landolt ecological value for nutrients and moisture increased while temperature dropped. The coverage of legumes and Landolt indicator value for nutrients increased because of the lime application, while the lime had no effect on Shannon-Wiener evenness and abundance of functional groups. Greater Shannon-Wiener evenness in treatments of PK and N20PK is a prerequisite for resistance to the effects of extreme climate events.

Keywords: fertilising; botanical composition; biodiversity; functional traits; ecological index

According to the current climate research, it can be assumed that by 2050, the annual average air temperature in the Alps or Highlands region will have risen about +2 °C (Gobiet et al. 2014), while rising temperatures on low altitudes could be higher. The annual average temperature, as well as drought and heat stress, have increased in recent decades. Therefore, the management of climate-resilient grassland systems is very important for stable livestock fodder production over time (Reckling et al. 2021).

Since permanent grasslands are important because they occupy enormous areas and serve as a base for developing animal husbandry, fertilising them is complex. There are difficulties in calculating the number of fertilisers. Consequently, this measure cannot be evaluated solely from the perspective of annual production since it also influences the course of long-term changes in communities. The changes tend to be long-lasting, and according to the estimate, applying nitrogen for 7 years could sustain the effects on floristic composition for several decades (Power et al. 2006). After the transformation, the key question is: What are the characteristics of these altered communities?

On the other hand, when fertilising grassland is practised, the quantity and quality of biomass are considered rather than the species' traits and long-term

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community changes. The strategy may be harmful because the biological traits of the species that live there are not considered, and species with different biological traits predominate in fertilised grasslands. The grassland's conservation value dramatically declined because mineral fertilisers led to the disappearance of endangered plant species from the landscape (Marriott et al. 2004). However, because only communities with more diversity can provide a steady yield under varying weather circumstances (Baca Cabrera et al. 2021), preserving diversity at the highest degree feasible is desirable.

Many field experiments have primarily focused on specific fertilisation effects on species productivity, with species diversity frequently used to explain changes after fertilisation. However, the relationship between biodiversity and ecosystem performance is better understood if explained by functional traits rather than species diversity (Milcu et al. 2013). Plant functional traits are explained as plant morphological and physiological characteristics that affect a plant's ability to grow, survive, etc. (Rosenfield and Müller 2020). Thus, a functional trait approach may help improve our understanding of mechanisms that drive trait changes and ecosystem function after an anthropogenic influence (Li et al. 2023). Therefore, when deciding on the amount of mineral fertilisers that should be applied to a grassland, it is important to understand species' productive properties, biological characteristics, and environmental requirements.

Danthonia alpine Vest. (syn. *Danthonia calycina*) is widespread in southern Europe but locally occurs in many other European countries, as well as in Asia Minor (Euro + Med. 2006). The species has a very broad diagnostic significance and unites the alliance *Chrysopogono-Danthonion alpinae* with all communities where *Danthonia* sp. is the dominant species. It inhabits elevations between 200 and more than 1 500 m a.s.l. Due to its widespread distribution in Serbia's mountainous region and its frequent use in high-quality meadows, communities dominated by *Danthonia alpina* Vest. are of significant economic value (Dajić-Stevanović et al. 2010).

To address how treatments have changed the habitat conditions and how the stand has adapted to the increased availability of nutrients in the soil, particularly in light of climate changes, understanding optimal niches for each species in terms of the environment is crucial. The respective values are based on the experience of field botanists, and they describe the environmental conditions and optimum ecological niche of a species on an ordinal scale. According to Bosh et al. (2019), doing research over 5.5-year periods, the mean ecological indicator value of all species in an ecosystem can be used for providing helpful site information about environmental changes caused by human activity, changes along elevation gradients, abandonment, etc. Fertilisation is a key component in changing the symmetry of grassland, as it results in changes in mean ecological values, including temperature, humidity, reaction, etc.

The study aimed to show the botanical composition changes after nine years of fertilisation on *Danthonia alpina* Vest. type grassland, how have the treatments changed the habitat's characteristics, and how has the species adjusted to the soil's enhanced nutrient availability? The goal is to explain how each specific dose of fertiliser, both with and without lime, affects the grass community and to suggest the amount of fertiliser to apply to get the desired effects.

MATERIAL AND METHODS

Locations and weather conditions. The field experiment was located in Mitrovo Polje village, in the municipality of Aleksandrovac, in the central part of Serbia (43°30'N, 20°52'E, 684 m a.s.l.). The grassland community is classified as *Danthonia alpina* Vest. type, developed in extremely acid soil (pH_{KCl} 4.09), which was poor in phosphorus (2.65 mg/100 g dry soil) and potassium (7.96 mg/100 g dry soil). The soil contained a high percentage of organic carbon (5.21%). The soil samples were taken before the experiment was set up. Before the experiment was established, the meadow had been cut annually at 4 cm stubble height for hay in the summer using an electric hay mower. Afterwards, it was grazed by grazing cattle. The grassland had an average yield of about 2.5 t/ha and was composed of 41 plant species, with *Danthonia alpina* Vest. predominating.

According to the Republic Hydro-Meteorological Institute of Serbia, the experiment location was characterised by moderate air temperature and precipitation values (Table 1).

Experiment. The field experiment was established in 2012 and conducted in a split-plot design. The plot was 10 m^2 in 3 replications of each treatment. Plots were separated with a protection path of 1 m. The fertilising treatments were: application of mineral fertilisers (control – without fertilisers addition, N20P60K60 (N20PK), N80P60K60 (N80PK),

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Table 1. Mean monthly temperature (T, °C) and monthly precipitation sum (P, mm)

Month		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
2013–2021	T	–0.1	3.7	6.0	10.3	14.0	18.0	19.9	20.1	15.8	11.1	6.9	1.3
	P	65.9	45.7	79.3	61.4	105.6	109.1	81.3	65.1	55.6	63.3	48.5	60.0
2021	T	1.2	4.1	3.2	7.8	14.3	18.1	21.6	20.2	15.2	8.3	7.2	1.6
	P	139.1	29.8	72.8	61.2	66.1	47.7	40.4	41.2	42.9	41.8	25.4	117.3

N140P60K60 (N140PK)) and liming (no lime and lime with 1 t/ha, dehydrated calcium hydroxide, repeated every five years). The liming was performed as surface treatment at the beginning of the experiment in autumn 2012 and five years later in 2017. All fertilised treatments were applied on limed and non-limed parts of the grassland.

All plots were fertilised with phosphorus and potassium at 60 kg/ha in autumn (first decade of November) every year. Phosphorus was applied as single superphosphate (8.1% P), and potassium as potassium chloride. Nitrogen was applied every year in spring as ammonium nitrate.

Analyses. Cover-abundance of individual species in each treatment was recorded right before the first cut, 2021 years, using the Braun-Blanquet scale with 6 classes (+ ≤ 1%; 1 = 1–10%; 2 = 10–25%; 3 = 25–50%; 4 = 50–75%; 5 = 75–100%). Cover abundance was estimated in 3 replications (3 different plots). The Braun-Blanquet cover-abundance values were converted to percentages (+ = 0.5%; 1 = 5%; 2 = 17.5%; 3 = 37.5%; 4 = 62.5%; 5 = 87.5%).

Calculations. Plant species were grouped according to 6 functional traits (Kahmen and Poschlod 2008) (Table 2). Cover-abundance for each group was calculated as the sum of transformed cover-abundance values of the species in the groups. Plant functional traits were analysed to show general trends in trait promotion and inhibition caused by fertilising treatments. The functional trait could be used by scientific experts and farmers or advisors with low botanical knowledge. According to the characteristics, each plant species was graded for each trait. The traits were "plant height", "canopy structure", "storage organ", and "duration of flowering". Plant species nomenclature and the species classification into functional groups were followed by Josifović (1970–1977).

The within-plot species evenness was calculated using the Shannon-Wiener evenness: $J' = H'/\ln(S)$, where H' is the Shannon-Wiener evenness index, and S is the species number. The Shannon-Wiener evenness index equals $H' = -\Sigma p_i \times \ln(p_i)$, where p_i is the i^{th} species' relative frequency (based on cover-abundance). The Shannon-Wiener evenness ranges from zero (when one species is dominant) to one (when all species are equally abundant).

The Landolt indicator value (Landolt et al. 2010) for each plot was calculated as the mean of indicator values weighted with the cover of each species present in the plot. The Landolat indicator values are based on the Elenber indicator value (according to Landolt et al. 2010) and range from 1 to 5. We calculated the following Landolt indicator values for nutrients (LIV N), humidity (LIV F), reaction (LIV R), light (LIV L) and temperature (LIV T).

Table 2. Plant functional traits, characteristics and description

Functional trait	Functional group
Plant height	maximum plant height < 0.3 m maximum plant height 0.3–0.6 m maximum plant height > 0.6 m
Canopy structure	leaves basal – the main part of phytomass near the ground leafy – the main part of phytomass along the stems
Storage organ	no storage organ storage organ
Duration of flowering	1–2 months ≥ 3 months

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Statistics. Two-way ANOVA followed by post hoc comparison using Fisher's posthoc test (at α = 0.05) was applied to identify significant differences between the treatments regarding Shannon-Wiener evenness and LIV indicator value. Redundancy analysis (RDA) for species composition change caused by NPK fertilisers and lime was applied using CANOCO for Windows 4.5 (ter Braak and Šmilauer 2002). The statistical significance of all canonical axes in RDAs was determined using Monte Carlo permutation tests, and 999 permutations were used. An ordination boxplot diagram was made in the Cano Draw program (Ithaca, USA), and it was used to represent the analyses of the results precisely. Simple linear regression was used to show the effect on plant functional groups when $R^2 > 0.6$ the coefficient of determination is considered significant.

RESULTS

After nine years, fertiliser treatment changed the *Danthonia alpina* Vest. type grassland in terms of changing the cover of specific plant species. Based on the RDA analysis (Figure 1), fertiliser treatments and lime had different effects on the cover of individual plant species. The following species were associated with N140PK treatments: *Holcus lanatus* L., *Agrostis capillaris* With., *Centaurea jacea* L., while N80PK favoured, *Festuca rubra* L., *Holcus lanatus* L. and *Agrostis capillaris* With. Fertilisation with PK and N20PK addition promoted species such as *Trifolium repens* L., *Trifolium pretense* L., *Lotus corniculatus* L., *Plantago lanceolata* L., *Festuca rubra* L. In contrast, *Danthonia alpina* Vest., *Genista tinctoria*, *Anthoxanthum odoratum* L., and *Briza media* L. did

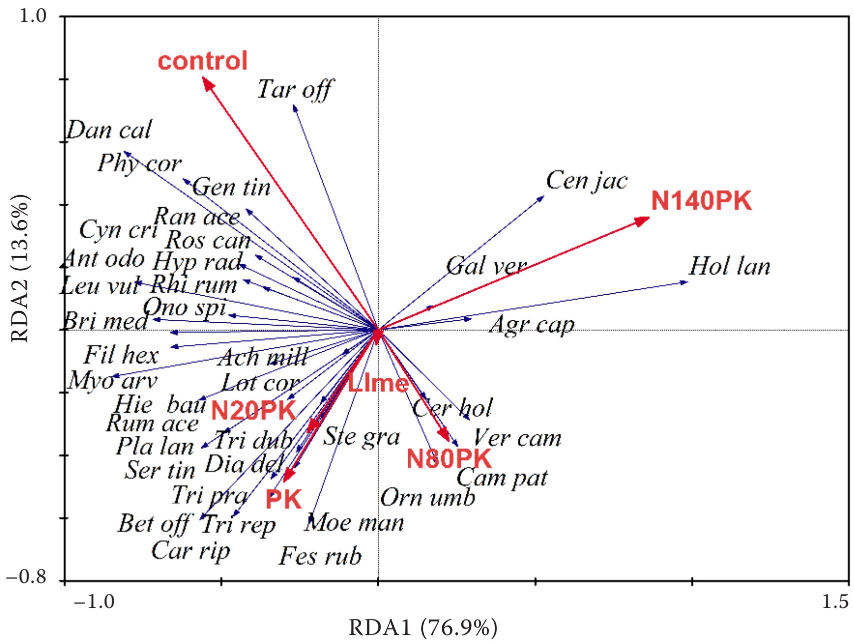

Figure 1. Redundancy analysis (RDA) results of plant species composition on *Danthonia alpina* Vest – type grassland after nine years of additional fertilisers. The sum of all canonical axes is 99.7% variability at *P*-value = 0.004. Ach mil – *Achillea millefolium* L., Agr cap – *Agrostis capillaris* With., Ant odo – *Anthoxanthum odoratum* L., Bet off – *Betonica officinalis* L., Bri med – *Briza media* L., Cam pat – *Campanula patula* L., Car rip – *Carex riparia* Curt., Cen jac – *Centaurea jacea* L., Cer hol – *Cerastium holosteoides* Fr., Cyn cri – *Cynosurus cristatus* L., Dan cal – *Danthonia alpina* Vest, Fes rub – *Festuca rubra* L., Fil hex – *Filipendula hexapetala* Gilib., Gal ver – *Galium verum* L., Gen tin – *Genista tinctoria* L., Hie bau – *Hieracium bauhinii* Schult., Hol lan – *Holcus lanatus* L., Hyp rad – *Hypochoeris radicata* L., Leu vul – *Leucanthemum vulgare* Lam., Lot cor – *Lotus corniculatus* L., Moe man – *Moenchia mantica* (L) Bartl., Myo arv – *Myosotis arvensis* (L.) Hill., Ono spi – *Ononis spinosa* L., Orn umb – *Ornithogalum umbellatum* L., Phy cor – *Physospermum cornubiense* L., Pla lan – *Plantago lanceolata* L., Ran ace – *Ranunculus acer* L., Rhi rum – *Rhinanthus rumelicus* Vel., Ros can – *Rosa canina* L., Rum ace – *Rumex acetosella* L., Ser tin – *Serratula tinctoria* L., Ste gra – *Stellaria graminea* L., Tar off – *Taraxacum officinale* L., Tri dub – *Trifolium dubium* Sibth., Tri pra – *Trifolium pretense* L., Tri rep – *Trifolium repens* L., Ver cam – *Veronica chamaedrys* L.

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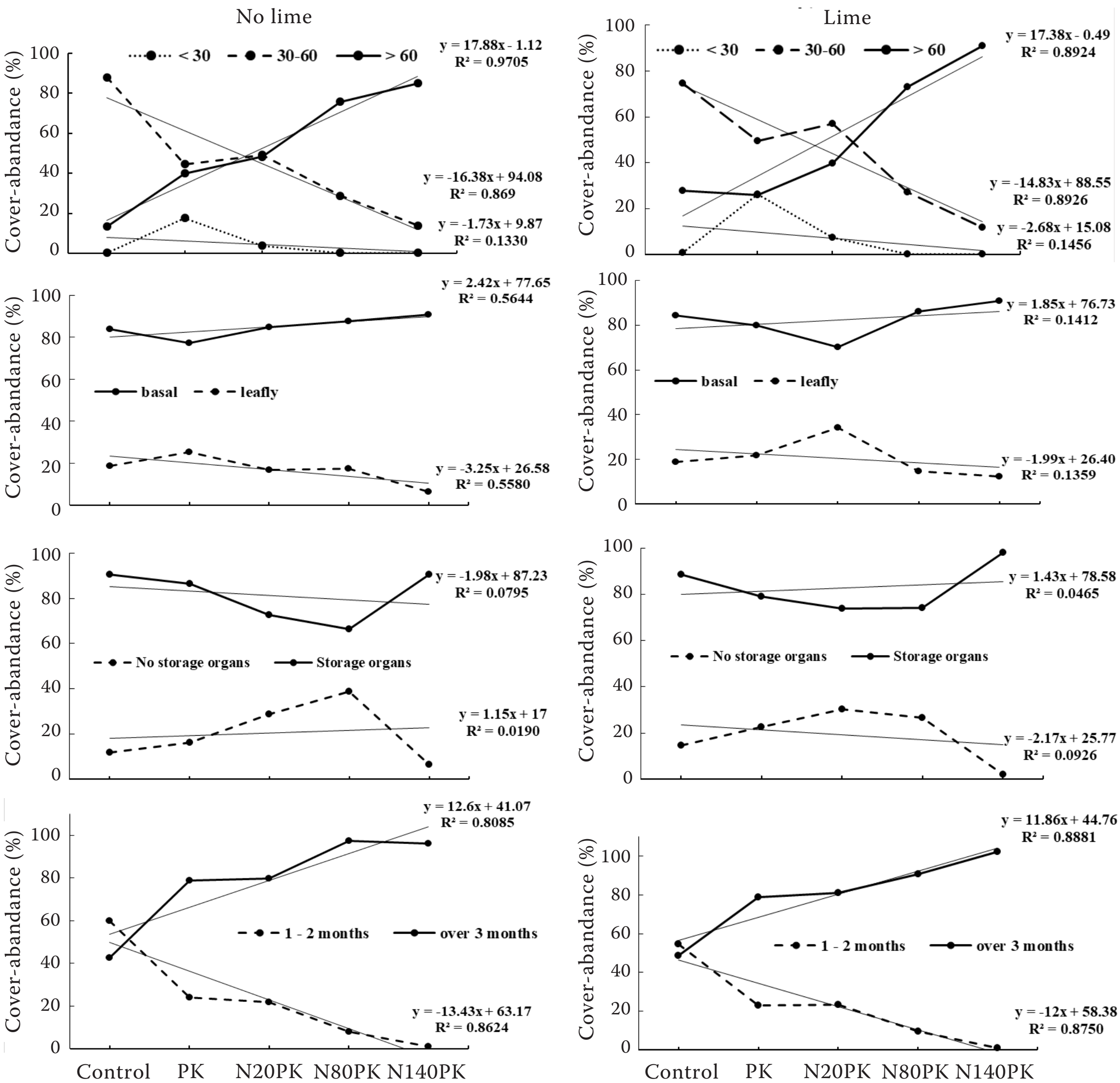

Figure 2. Relationships between fertilising, liming treatments, and plant functional traits on *Danthonia alpina* Vest. type grassland. The coefficient of determination is significant at a value of $R^2 > 0.6$

not respond to any particular fertiliser treatment. Lime had no particular effect on any plant species in the community.

As a result of the addition of mineral fertilisers, the share of several functional groups changed (Figure 2). The proportion of tall plants was higher in the fertilised treatments, followed by an increase in the proportion of plants with a longer flowering time, while the coverage of plants that generate underground organs dropped. Treatment N140PK was the exception; the percentage of plants developing underground organs remained the same as in the original community. The abundance of leafy and basal plants, as well as plants with or without storage organs, did not particularly change in fertilised treatment. Similar effects of mineral fertilisers were recorded in no limed and limed plots.

Shannon-Wiener evenness value varied widely according to treatments (Table 3, Figure 3). Treatments PK and N20PK showed a statistically significant increase in Shannon-Wiener evenness compared to the control. Conversely, treatment N140PK showed a decline in Shannon-Wiener evenness. The no-lime and limed treatments do not differ significantly.

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Table 3. Results of ANOVA and mean Shannon-Wiener evenness and Landolt ecological value depending on fertilisation addition and liming on *Danthonia aplina* Vest. type grassland (means ± standard deviations)

		Shannon-Wiener evenness	LIV N	LIV F	LIV R	LIV L	LIV T
Fertilising	*P*	0.000*	0.000*	0.000*	0.000*	0.717	0.000*
Liming	*P*	0.280	0.034*	0.824	463	0.181	0.268
Fertilising × liming	*P*	0.000*	0.511	0.178	870	0.689	0.775
Control		0.45 ± 0.05[c]	2.38 ± 0.13[c]	2.37 ± 0.11[d]	3.73 ± 0.14[a]	4.09 ± 0.13	4.51 ± 0.24[a]
PK		0.68 ± 0.04[a]	2.86 ± 0.19[ab]	2.71 ± 0.13[c]	3.37 ± 0.17[b]	3.95 ± 0.12	3.70 ± 0.25[b]
N20PK		0.71 ± 0.04[a]	2.85 ± 0.10[b]	2.82 ± 0.12[bc]	3.44 ± 0.13[b]	4.03 ± 0.13	3.76 ± 0.13[b]
N80PK		0.55 ± 0.04[b]	2.91 ± 0.12[ab]	2.91 ± 0.13[ab]	3.16 ± 0.11[bc]	3.94 ± 0.19	3.31 ± 0.11[c]
N140PK		0.25 ± 0.03[d]	3.05 ± 0.10[a]	2.98 ± 0.11[a]	3.10 ± 0.08[c]	4.03 ± 0.14	3.17 ± 0.08[c]
No liming		0.53 ± 0.18[a]	2.66 ± 0.25[b]	2.74 ± 0.26[a]	3.31 ± 0.23[a]	3.98 ± 0.17[a]	3.64 ± 0.46[a]
Liming		0.53 ± 0.17[a]	2.87 ± 0.25[a]	2.78 ± 0.23[a]	3.41 ± 0.28[a]	3.91 ± 0.10[a]	3.74 ± 0.56[a]

*Denotes that an effect is statistically significant. LIV N – Landolt indicator value for nutrients; LIV F – Landolt indicator value for moisture; LIV R – Landolt indicator value for reaction; LIV L – Landolt indicator value for light; LIV T – Landolt indicator value for temperature. Different lower-case letters indicate significant differences (Fishers *LSD* (least significant difference) test, $P < 0.05$)

The *Danthonia alpina* Vest. The grassland type was changed due to the addition of mineral fertilisers, and the new plant communities had different environmental requirements. The analysis of habitat conditions using Landolt indicator values indicates a significant impact of fertilisers on LIV N. Increasing LIV N was detected in all fertilised treatments. Applying more nitrogen led to a gradual rise in the mean ecological indicator values of nutrients and LIV N compared to the control (2.38), which was the highest in treatments N140 (3.05). The impact of fertiliser application on LIV F change was quite similar to that on LIV N. Lime addition significantly increased LIV N, and the lime impact was pronounced the most in PK treatments.

Mean LIV R and LIV T showed significant negative effects of fertilising treatment. As more nitrogen was added, the average ecological indexes for the reaction and temperature gradually fell. However, compared to N20PK and PK, plants requiring lower temperatures were more visible at N80PK and N140PK. LIV R decreased from 3.76 (N140) to 3.10, while declining LIV T varied from 4.51 (N140) to 3.17 (control). Fertiliser treatment had no impact on the LIV L.

DISCUSSION

From the perspective of plant families and other functional characteristics, the initial community of *Danthonia alpina* Vest. had been drastically changed by the application of fertilisers. This study was done after nine years of fertilising *Danthonia alpina* Vest. type grassland. PK treatments promoted legume species, while nitrogen increased *Holcus lanatus* and *Agrostis capillaris* per cent, which was confirmed by Zornić et al. (2019).

The impact of mineral fertilisers on the community can be attributed to the change in the ratio of functional plant groups. After nine years of application of mineral fertilisers, the proportion of tall plants increased, which resulted in decreasing cover of short plants in response to N application. The tall-growing neighbours probably caused this by increasing shading (Čamska and Skalova 2012). All fertilising treatments, except N140PK treatments, resulted in a decrease in the percentage of plants with storage organs. The increase in the cover of storage organ plants in plots N140PK increased the cover of rhizomatous grasses, such as *Holcus lanatus* L. (Honsova et al. 2007). In our research, the cover of basal leaf plants and cover plants with longer flowering duration was increased in treatments N80PK and N140PK, confirmed in research by Kahmen and Poschold (2008).

Fertilising generally affects Shannon-Wiener evenness, depending on the fertilisation rate. After nine years of application, PK treatments increased Shannon-Wiener evenness. According to Mrkvička et

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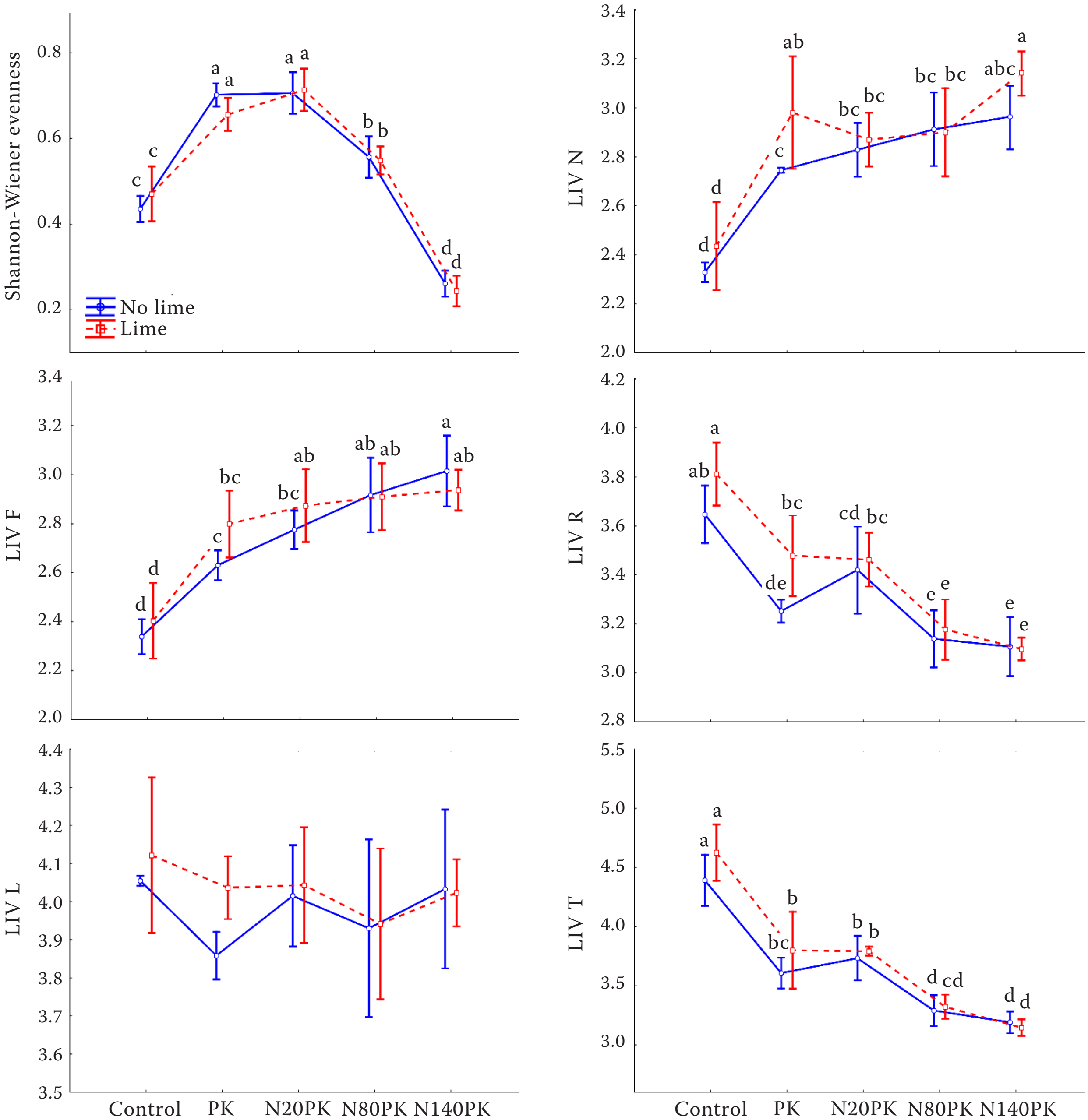

Figure 3. Effects of fertilisers and lime addition on species Shannon-Wiener evenness and Landolt indicator values for nutrients, moisture, reaction, light and temperature in *Danthonia alpina* Vest. type grassland (means ± standard deviations). Different lower-case letters indicate significant differences (Fishers *LSD* (least significant difference) test, $P < 0.05$). LIV N – Landolt indicator value for nutrients; LIV F – Landolt indicator value for moisture; LIV R – Landolt indicator value for reaction; LIV L – Landolt indicator value for light; LIV T – Landolt indicator value for temperature.

al. (2006), PK fertilisers were the most suitable combination for diverse species from a long-term point of view. In our research, Shannon Wiener's evenness was significantly decreased in treatments N80PK and N140PK. Čop and Eler (2019) recorded similar results: nitrogen amounts 120 and 220 kg/ha plus 31 kg/ha phosphorus and 174 kg/ha potassium decreased the Shannon-Wiener index in grassland. Mineral fertilisers (NPK) application has a similar effect in *Agrostis capillaris* With., *Festuca rubra* L. and *Nardus stricta* L. type grasslands (Samuil et al. 2013).

The environmental requirements of the communities developed in the fertilised treatments were completely different from those of the control. Generally speak-

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ing, the plant species that dominated the fertilised plots had higher LIV N and LIV F but lower LIV T. There was an increase in the percentage of plants that respond to a lower soil pH value in fertilised plots. No dependence was found between LIV L and fertilisation. Increasing soil fertility status caused an increase in the percentage of species with higher soil nutrient requirements that were more competitive than others (Samuil et al. 2013). Also, mineral fertiliser input favours fast-growing grass species adapted to high nutrient availability (Midolo et al. 2018). Significantly higher indicator values for nutrients were found even 15 years after the cessation of nitrogen addition in the amounts of the N50, N100, and N200 treatments. However, values in the N25 treatment were not significantly different from the control (Stevens et al. 2012). This trend is widespread in Western European countries because eutrophication causes increasing neutrophil plants in semi-natural grasslands (Boch et al. 2019). Indicator values for nutrients and moisture have a positive relationship, and according to Dostál et al. (2017), species with high nutritional requirements prefer to live in moist environments. Increasing LIV for nutrients was recorded in PK treatments, and according to Tyler et al. (2021), adding phosphorus to systems where it is the limiting factor leads to the nutrient indicator value increase. Ecological index for temperature decreases in fertiliser treatments, while Chytrý et al. (2009) found no differences between fertiliser treatment and control. These differences are probably affected by differences between communities. LIV R was significantly decreased in treatments N80PK and N140PK. Nitrogen has reduced LIV R in the grassland community, favouring grasses with low LIV R. Lime was probably added in long distances and small amounts, so LIV R was not altered significantly. Therefore, some future studies will focus on applying higher amounts and at different intervals.

Liming has significantly impacted the value of the Landolt ecological indicator for nutrients. Chytrý et al. (2009) published the same findings, stating that lime increases the availability of some nutrients as well as productivity.

For the stability of the fodder production on these lands, a deeper examination of the plant species in newly formed communities provides an assessment of their sustainability over a long period. All fertiliser treatments transformed the *Danthonia alpina* Vest. type grassland, however, lime had a much lower impact than fertilisers. Treatments with 80 and 140 nitrogen caused significantly more change than the PK and N20PK treatments. By favouring species with high water and low-temperature requirements, treatments N80 and N140 can lose production significantly during dry periods, which is becoming more frequent due to intense climate changes. It agrees with studies by Deguines et al. (2014), who reported that relative climatic adaptability decreased with increasing land use intensity.

Additionally, when fertilisers are applied, communities that demand high levels of nutrients are created, so if we cannot add them, we run the danger of either completely losing our yield or drastically reducing it over time (Piseddu et al. 2021). Although the communities created by the PK and N20PK treatments endured changes in their average ecological index and floristic composition additionally, their Shannon-Wiener evenness rose, making them more resilient in the context of rapid climate change. Evidence shows that greater plant species diversity in managed grasslands may enhance their resilience to climate change and result in more stable yields in response to disturbance (Baca Cabrera et al. 2021).

While the participation of plants with high nutritional requirements rose significantly in the liming treatments, no other significant changes in the grassland community were discovered.

The knowledge obtained can be used for grasslands where *Danthonia alpina* Vest. predominates, as well as all other grasslands that have comparable traits and grow in similar regions. The assessment requires a specific fertiliser approach for each plant community, depending on the floristic composition, intended use, climatic conditions, etc. Therefore, sustainable and high biomass production in grasslands requires a complex point of view, primarily agricultural and ecological.

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