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ORIGINAL PAPER

Long-term effects of N, P and K fertilization on mountain grassland vegetation: productivity, species composition and plant N:P stoichiometry*

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Abstract

Increased nutrient availability, especially nitrogen and phosphorus from fertilization and pollution, affects plant communities. Nutrient concentrations in plant tissues and their stoichiometric ratios can indicate nutrient availability. This study aimed to evaluate the use of nutrient concentrations and stoichiometric ratios as indicators of nutrient addition effects on productivity, species composition, and functional group proportions in a mountain meadow. In a long-term grassland experiment in the Polish Carpathians, various fertilizer combinations (90NPK, 180NPK, N, P, PK, control) and liming were applied. Over 39 years, species composition was assessed, and nitrogen, phosphorus, and potassium concentrations in plant biomass were measured. Liming had no effect on vegetation parameters or nutrient concentrations. The N:P ratio remained low across all treatments, ranging from 4.9 in 90NPK to 11.7 in 90N, suggesting nitrogen limitation. Despite significant variations in species composition and productivity between the NPK-fertilized treatments and the remaining treatments, the N:P ratio varied only slightly. The N:P ratio was influenced not only by nutrient availability but also by other factors, such as the capacity of some species characteristic of oligotrophic habitats to accumulate excess nitrogen in the 90N treatment, and the increase in the proportion of nitrogen-fixing legumes following potassium application in the PK treatment. Conclusion: Stoichiometric ratios may serve as useful indicators of nutrient effects on vegetation; however, they should be interpreted with caution because they are also affected by various environmental factors.

Keywords: nitrogen, phosphorus, vegetation stoichiometry, mountain grassland, plant functional groups

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INTRODUCTION

Different types of grasslands provide a wide range of crucial ecosystem services, including climate regulation, soil carbon storage, maintenance of plant diversity, and support for pollinators, while also contributing to human nutrition (Bengtsson et al. 2019). However, grasslands remain among the ecosystems most vulnerable to environmental and land use change (Dengler et al. 2014, Bengtsson et al. 2019). One of the most important factors affecting species diversity in these ecosystems is biomass production. According to the well-known theory linking productivity to species richness, the relationship becomes negative beyond a certain biomass threshold (Grime 1973). Nutrient availability is a key driver of both productivity and diversity in plant communities (Elser et al. 2007, Fay et al. 2015). In terrestrial ecosystems, nitrogen (N) and phosphorus (P) are generally the primary limiting nutrients (Elser et al. 2007, Soons et al. 2017, DeMalach 2018), while potassium (K) often acts as a secondary limiting factor (Dey et al. 2024). Numerous studies confirm that nitrogen addition increases aerial biomass production in grasslands, underscoring its role as a primary limiting nutrient (Song et al. 2014, Dey et al. 2024). However, nitrogen addition can also negatively impact biodiversity, reducing plant species richness in diverse grassland ecosystems (Francksen et al. 2022). An increased nitrogen supply often shifts the primary nutrient limiting factor to phosphorus (Harpole, Tilman 2007, Peñuelas et al. 2012). Consequently, phosphorus enrichment may be equally, or even more, influential than nitrogen in driving species loss, as many plant species can persist under nitrogen-rich but phosphorus-limited conditions (Wassen et al. 2005, Ceulemans et al. 2013). Liming, a common practice used to increase soil pH, can improve productivity, soil structure and benefit biodiversity, particularly when integrated with other appropriate management practices (Macholdt et al. 2023). However, its ecological effects are context-dependent, and liming in some ecosystems may contribute to declines in rare or threatened species (Spiegelberger et al. 2006, Macholdt et al. 2023).

By inducing convergence in the multidimensional stoichiometry of plants, combined applications of N, P, and K (NPK) often produce greater negative effects on plant diversity compared to single-nutrient additions (Peng et al. 2025). As a result, competitive grasses outcompete less competitive forbs and legumes under high nutrient availability (Harpole et al. 2016). This shift leads to characteristic increases in grass biomass and declines in legumes and forbs (You et al. 2017). Understanding the primary nutrients limiting productivity in grasslands is essential for predicting ecological changes and guiding management aimed at balancing agricultural production with biodiversity conservation (Roeling et al. 2018). A useful approach to identifying nutrient limitation is analyzing nutrient concentrations and their stoichiometric ratios in plant biomass (Olde Venterink et al. 2003, Güsewell

et al. 2005, Elser et al. 2010). Shifts in nutrient limitation often translate into changes in species composition and the proportions of functional groups (Güsewell et al. 2005, Fujita et al. 2014, You et al. 2017, Roeling et al. 2018). Numerous studies have explored stoichiometric ratios in the context of different grassland types, such as wetlands (Olde Venterink et al. 2003, Roeling et al. 2018), dry grasslands (Palpurina et al. 2018) or management practices, including grazing (Yu, Li 2021), mowing (Olde Venterink et al. 2009, Pavlů et al. 2013, Yang et al. 2021) and liming (Heyburn et al. 2017).

Although stoichiometric indicators hold significant promise for assessing the impacts of nutrient availability on grassland ecosystems, the complexity of these interactions remains incompletely understood. The purpose of this study was to assess the potential of using the aerial biomass N:P ratio as an indicator of vegetation parameters under different fertilizer combinations and liming. We tested the hypothesis:

Long-term application of different combinations of nitrogen, phosphorus, and potassium fertilization significantly alters the species composition of mountain grassland communities.

The N:P stoichiometric ratio in plant biomass reflects the type of nutrient limitation and can serve as an indicator of nutrient-driven changes in productivity, plant species composition and share of plant functional groups.

Liming can affect species composition, nutrient concentrations and N:P ratios in semi-natural mountain grasslands under long-term fertilization.

MATERIALS AND METHODS

The experiment was established in Czarny Potok (20°54′53″ E, 49°24′35″ N) in the Beskid Sądecki region (the Polish Carpathians), at an altitude of 720 m a.s.l. and in a complex of semi-natural mountain grasslands. The soil was classified as an Endoskeleton District Cambisol with the granulometric composition of light silty loam (% of 1-0.1 mm fractions: 40; 0.1-0.02 – 37 and <0.02 mm – 23) and three characteristic genetic horizons: turf – AhA (0-20 cm), browning – ABbr (21-46 cm) and matrix BbrC (47-75 cm). The soil properties before the experiment are presented in Table 1. The average annual precipitation in the years 1968-2007 was about 880 mm, and the average annual air temp. was 5.9°C. At the beginning of the experiment, the vegetation cover was classified as poor mountain meadow.

Table 1

Selected properties of soil in the 0-10 cm layer before setting up the experiment in Czarny Potok

pH H_2O	pH KCl	Hh	Hw	P	K	Ca	Mg	Na	K
		cmol(+) kg^{-1} soil		mg g^{-1} soil					
5.20	4.38	4.42	0.46	4.80	112.00	680.00	38.00	20.00	69.00

The experiment was established in 1968 with five replications. Fertilized plots measured 6 × 7 m (42 m²), with 20 m² used for harvest and species assessment. Treatments included full NPK fertilization at two N levels, PK fertilization, single N or P fertilization, and an unfertilized control (excluded from statistical analyses due to the lack of replication) (Table 2). Urea and ammonium nitrate were applied from 1968-2004.

Table 2

List of treatments and number of replications used in analysis

Treatment	Number of replications (1967-1985)	Number of replications in 2007 for liming effect	Number of replications in 2007 for analysis of vegetation parameters
PK	5	5	10
90NPK	10	10	20
180NPK	10	10	20
90N	5	5	10
P	5	5	10
PK + Ca		5	
90NPK + Ca		10	
180NPK + Ca		10	
90N + Ca		5	
P + Ca		5	

N – 90 or 180 kg ha^{-1}, P – 39.24 kg ha^{-1}, K – 124.5 kg ha^{-1}

Since no differences in response to the different forms of nitrogen were observed, data for the same N dosage were analyzed together. In the statistical analyses for the period 1971-1981, three fertilization treatments (PK, 90N, and P) were represented by five replicates each, whereas two treatments (90NPK and 180NPK) had ten replicates each. In 1985, each experimental plot was divided into limed and non-limed subplots. Consequently, from 2007 onwards, the number of experimental plots included in the study doubled (Table 2), although the area of each plot was reduced by half. Data from the unfertilized control treatment were excluded from the inferential statistical analyses because this treatment was not replicated. However, the control data are presented in the tables and figures as descriptive reference values to facilitate interpretation of the long-term effects of fertilization. No statistical comparisons involving the control treatment were performed. Fertilization was suspended from 1974 to 1975, replaced by sheep grazing in 1993-1994, and supplemented with Cu and Mg in 1994. In 1985, plots were split into limed and non-limed series. The lime doses in 1985 and 2005 were calculated based on 0.5 of the hydrolytic acidity (Hh) value, while in 1995, they were based on the total hydrolytic acidity of the soil determined in the previous year. Foliar micronutrients were applied in 2000-2004. Further details are given in (Kopeć et al. 2021).

Data from 1971, 1975, 1978, 1981 and 2007 were analyzed. Species composition was assessed annually before the first cut (June) using the Braun-Blanquet scale (Braun-Blanquet 1964). Functional groups share (grasses, herbs, legumes) were quantified by percentage cover. Biomass was harvested twice yearly and the total biomass from both cuts was used for analysis. Samples of vegetation were analyzed for their content of basic macronutrients (nitrogen, phosphorus and potassium). The nitrogen content was determined using the Kjeldahl method (Kirk 1950), following prior mineralization of the plant material in sulfuric acid, with an automatic analyzer model K9840 by Hanon Instruments. The concentrations of potassium and phosphorus were determined by inductively coupled plasma optical emission spectrometry (ICP-OES), using an Optima 7300 DV spectrometer from PerkinElmer Inc., after dry ashing (Matusiewicz 1998) and dissolution in HNO_3 and HCl. The N:P ratio was calculated to identify nutrient limitation. In 1971-1981, samples were pooled by treatment (no statistical analysis), whereas the analyses in 2007 were plot-based. Habitat changes were evaluated using the mean Ellenberg N indicator values (Ellenberg 1992).

Preliminary analysis showed relatively low variation in species composition in the years 1967-1981 (gradient length in detrended correspondence analysis (DCA) was 2.3 SD), and environmental variables (treatments) were nominal. For these reasons, plant species composition (1971-2007) and their relationships with N, P, N:P and Ellenberg N were analyzed using principal component analysis (PCA), as a method recommended in such cases (Lepš and Smilauer, 2003). In 2007, treatment and liming effects on species composition were tested with redundancy analysis (RDA) as gradient length in detrended correspondence analysis (DCA) was 2.96 SD).

The effects of liming and fertilization on soil pH, nutrient concentrations, and the N:P ratio were assessed using two-way ANOVA. As liming had no significant effect on any of the analyzed variables, data from the limed and unlimed plots were pooled for subsequent analyses. The effects of fertilization treatments were then evaluated using one-way ANOVA followed by the Tukey's *post hoc* test. Phosphorus concentration, the N:P ratio, and the Ellenberg N indicator values did not meet the assumption of normality (Shapiro-Wilk test) and were therefore log-transformed prior to analysis. Percentage variables (functional groups) were subjected to the Bliss transformation ($y = \arcsin\sqrt{x}$). Relationships between nutrient concentrations and vegetation parameters were assessed using Pearson's correlation analysis. Analyses were performed in CANOCO 5.1 (ter Braak, Smilauer 2018) and Statistica 13.1.

RESULTS AND DISCUSSION

The applied fertilization led to the formation of three groups of communities with clearly different species composition already in the third year of the experiment (Figure 1). The first group consists of the communities fertilized with three basic nutrients (N, P, K) 90NPK and 180NPK. The second group includes communities under no fertilization (0) or fertilized with only one nutrient: nitrogen (90N) or phosphorus (P). The third group is the PK treatment, which shows intermediate parameters between the two groups (Figure 1).

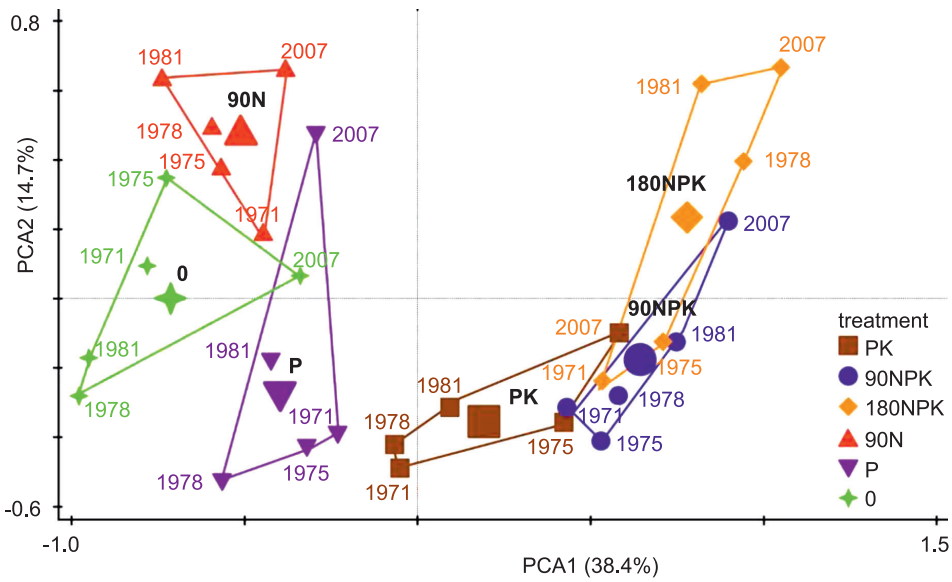

Fig. 1. Ordination diagram of the Principal Component Analysis (PCA) showing the plant species composition changes from 1971 to 2007 in relation to fertilization treatments

Species composition (Figure 1), consistent with other long-term experiments (Chytrý et al. 2009), showed persistent differences among fertilization treatments (PCA analysis: adjusted R^2=0.28, variance explained by ax 1 = 38.4%, ax 2 = 14.7%). The relationship between nutrient concentrations and the N:P ratio also remained stable over nearly four decades despite pronounced interannual variability (Figure 2). Treatments including phosphorus and potassium maintained elevated tissue concentrations, confirming earlier findings (Hejcman et al. 2010, Hrevušová et al. 2015). In contrast, nitrogen concentration and the N:P ratio in nitrogen-only treatments remained similar to those in unfertilized plots.

Nitrogen concentration in years 1971-2007 varied significantly between years and treatments (Figure 2), with generally higher values in 180NPK

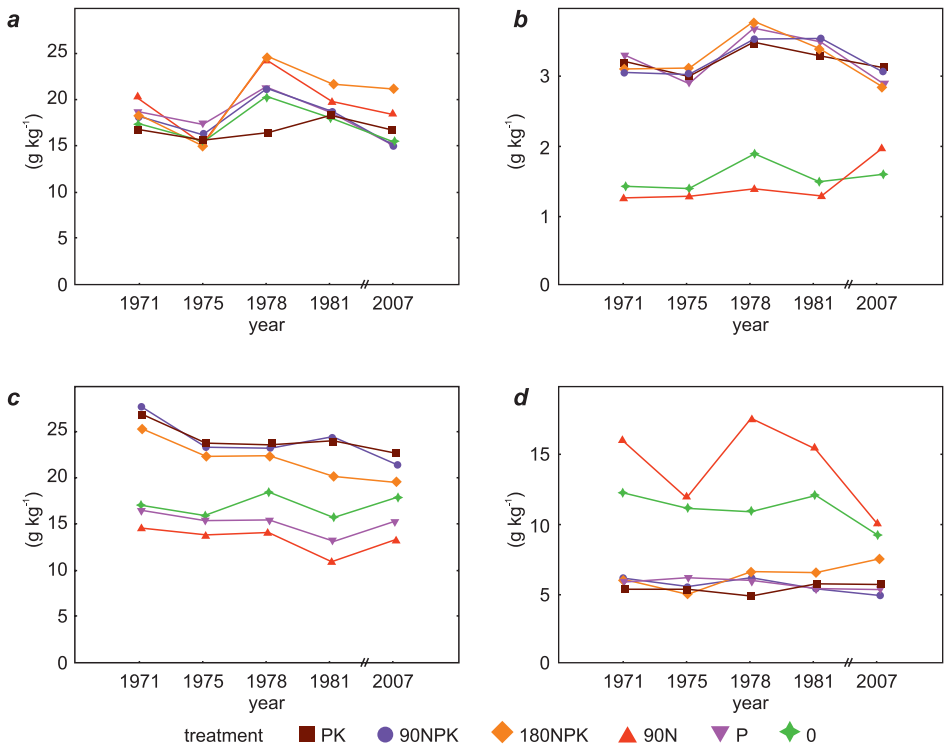

Fig. 2. Nutrient concentrations in biomass under different fertilization treatments from 1971 to 2007: *a* – nitrogen, *b* – phosphorus, *c* – potassium, and *d* – N:P ratio. No statistical analysis was performed for the years 1971-1981 due to the use of composite samples. Statistical significance for the year 2007 is presented in Table 3

Table 3

Mean values of biomass nutrient concentrations (g kg^{-1}) and N:P ratio under different fertilization treatment in 2007

Nutrient	Treatment					
	PK	90NPK	180NPK	90N	P	0
Nitrogen	17.00 ± 2.6*abc*	14.60 ± 2.7*a*	20.20 ± 3.7*c*	18.70 ± 3.3*bc*	15.00 ± 1.0*ab*	14.80
Phosphorus	3.10 ± 0.2*b*	3.00 ± 0.3*b*	2.80 ± 0.6*b*	1.80 ± 0.9*a*	2.80 ± 1.0*b*	1.600
Potassium	21.70 ± 2.8*c*	19.70 ± 3.4*bc*	17.50 ± 5.1*b*	13.00 ± 3.0*a*	16.20 ± 1.1*ab*	16.90
N:P	5.40 ± 0.6*ab*	4.90 ± 0.8*a*	7.60 ± 2.3*b*	11.70 ± 3.9*c*	6.20 ± 2.7*ab*	9.40

Different lowercase letters in the same row indicate significant differences in biomass parameters under different treatments (one-way ANOVA followed by the Tukey HSD test, $p<0.05$). The unfertilized control treatment (0) is shown for descriptive purposes only. Because the treatment was not replicated, it was excluded from all inferential statistical analyses and no SD was presented

and 90N and lower in PK and 0; no treatment showed stable levels over time. Phosphorus clearly differentiated fertilized and non-fertilized treatments: 90N and 0 had lower concentrations (1.2-2.0 g kg^{-1}), while the others ranged from 2.8 to 3.8 g kg^{-1}. Potassium content depended on K fertilization, being lower in 90N, P and 0 (11.0-19.0 g kg^{-1}) and higher in PK, 90NPK and 180NPK (19.0–28.0 g kg^{-1}), with minor annual fluctuations. The N:P ratio remained relatively stable (4.7-7.4) in most treatments, but was higher in 0 and highest in 90N, where N concentration fluctuated most (9.4-17.5 g kg^{-1}).

In 2007 fertilizer treatments significantly affected nutrient concentrations and the N:P ratio (Table 2). Nitrogen ranged from 14.6 g kg^{-1} (90NPK) to 20.2 g kg^{-1} (180NPK). Phosphorus was highest in PK (3.1 g kg^{-1}) and lowest in 90N (1.8 g kg^{-1}). Potassium was higher in K-fertilized treatments (PK, 90NPK, 180NPK) and lowest in 90N (13.0 g kg^{-1}). The N:P ratio varied from 4.9 (90NPK) to 11.7 (90N).

The relatively narrow range of N:P ratios in our experiment (4.9-11.7) was similar to those determined in other fertilized meadow experiments. For example, N:P values ranged from 6.22 to 9.29 in a multi-year meadow experiment (Hrevušová et al. 2015) and from 4.7 to 17.7 in the Rengen Grassland Experiment (Hejcman et al. 2010). The N:P ratio varies widely among grassland types. In Eurasian dry grasslands, N:P ranges from 4.0 to 26.5 (Palpurina et al. 2018), while European wet grasslands show a broader range (3.1-52.9, Roeling et al. (2018). Values between 6.4 and 23.1 have been reported for mire vegetation (Wassen et al. 1995).

Interpretation of nutrient limitation based on N:P ratios depends on habitat-specific thresholds. Commonly, N:P <10 indicates nitrogen limitation and >16 phosphorus limitation (Palpurina et al. 2018), with lower values reflecting stronger nitrogen limitation (Güsewell 2004). The N:P range in our study therefore indicates overall nitrogen limitation, with only the nitrogen-only (90N) treatment approaching potential co-limitation. This interpretation is consistent with long-term nutrient balances in this experiment (Zarzycki, Kopeć 2020).

The highest N:P ratio occurred in the nitrogen-only treatment, where nitrogen balance was positive but phosphorus balance negative. The lowest N:P ratio, indicating strongest nitrogen limitation, was found in the 90NPK treatment. Similar patterns were reported from the Rengen experiment, where phosphorus-fertilized treatments showed lower N:P ratios than non-phosphorus treatments, and the highest N:P occurred under nitrogen--only fertilization (Hejcman et al. 2010).

In our experiment, liming applied every five years since 1985 increased soil pH in all treatments (Table 4). However, no significant differences were found between the treatments, nor was there a differential response of the treatments to liming (treatment × Ca). The application of lime did not significantly affect vegetation species composition (RDA analysis: adjusted R^2=0.0073, total variance explained 2.13%, unrestricted permutation, n=999,

Table 4

Treatment and liming (Ca) effect on soil pH, nitrogen, phosphorus, potassium concentrations, and N:P ratio in biomass in 2007

Specification	Soil pH_{KCL}			Nitrogen			Phosphorus			Potassium			N:P		
	F	*p*	η^2	*F*	*p*	η^2	*F*	*p*	η^2	*F*	*p*	η^2	*F*	*p*	η^2
Treatment	3.43	0.14	0.08	10.15	**0.00**	0.41	4.34	**0.01**	0.32	9.64	**0.00**	0.40	11.11	**0.00**	0.55
Ca	110.14	**0.00**	0.61	0.01	0.91	0.00	2.90	0.10	0.02	4.78	0.06	0.07	2.12	0.16	0.00
Treatment Ca	2.31	0.67	0.18	0.39	0.82	0.02	0.27	0.90	0.01	1.30	0.29	0.08	1.43	0.25	0.03

Two-way ANOVA test, $p \leq 0.05$ in bold

pseudo-F = 1.5, p=0.06)) and had no significant impact on nutrient concentrations, or biomass N:P ratio in 2007. The treatments had a significant effect on nutrient concentrations and the N:P ratio, whereas liming had no significant effect (Table 3). This supports the view that changes in soil chemistry do not necessarily translate into immediate changes in vegetation structure or stoichiometry (Holland et al. 2018).

The main gradient in species composition in 2007 (PCA analysis: adjusted R^2=0.54, variance explained by ax 1 = 33.76%, ax 2 = 13.08%) reflected fertility, as confirmed by its correlation with the Ellenberg N indicator (Figure 3). Unfertilized and single-nutrient treatments (0, 90N, P) were associated with oligotrophic species (e.g., *Nardus stricta, Potentilla erecta, Briza media*), with little difference between 90N and P (RDA analysis: adjusted R^2=0.0035, total variance explained 8.81%, unrestricted permutation, n=999, pseudo-F = 1.6, p=0.07). Fully fertilized treatments (90NPK, 180NPK) were dominated by grasses of fertile habitats (e.g., *Dactylis glomerata, Festuca pratensis*), with 180NPK also showing ruderal, nitrophilous species (*Urtica dioica, Rumex crispus*). The PK treatment had intermediate composition and a high share of legumes (*Vicia cracca, Trifolium repens*).

Nitrogen-only or phosphorus-only fertilization, leading to divergent N:P ratios, often results in different species composition (Hejcman et al. 2007, Olde Venterink 2011). Our experiment revealed a lack of strong relationship between N:P ratio and species composition. This may reflect relatively small differences in species composition between phosphorus-only and nitrogen--only treatments despite their contrasting N:P ratios. In nitrogen-only plots, species from nutrient-poor habitats were capable of luxury nitrogen uptake, resulting in high tissue nitrogen concentrations despite low productivity (Güsewell 2004).

In 2007 species richness was lowest in 180NPK (16.9 species/plot) and highest in partially fertilized treatments (PK, 90N, P) – Table 5. Grasses dominated in 180NPK, while legumes varied most, from 0.5% (180NPK) to 27.9% (PK). Herbaceous species were most abundant in P (30.3%) and least in PK (13.4%). Biomass was highest under full fertilization (180NPK) and lowest in P. The Ellenberg N indicator was highest in 180NPK and lowest in 90N.

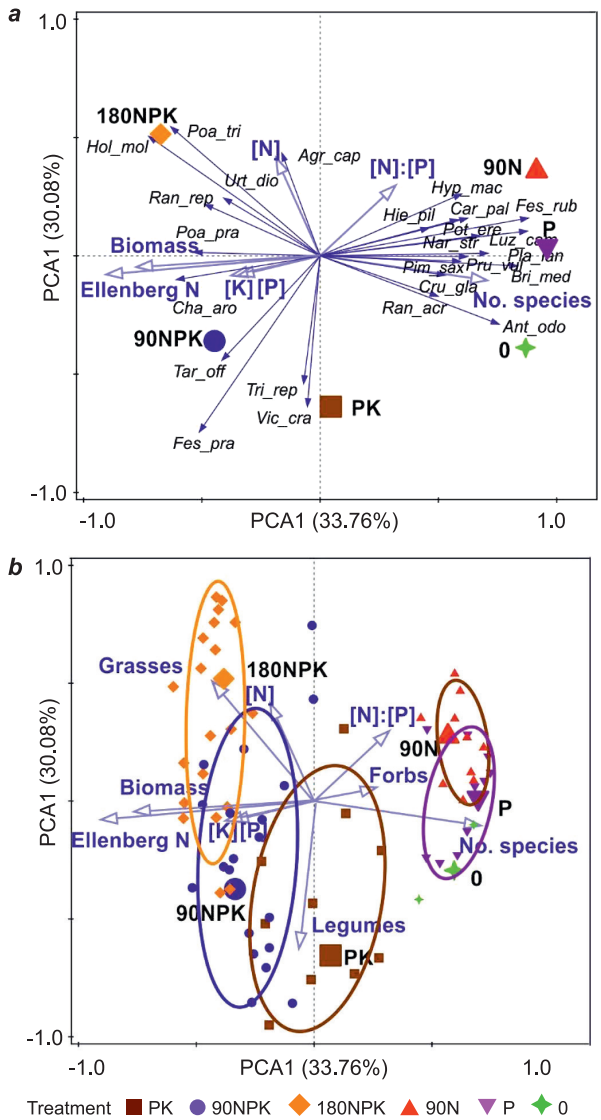

Fig. 3. Ordination diagram of the Principal Component Analysis (PCA) of plant species composition data in 2007: *a* – species, *b* – plots. Concentration of nitrogen, phosphorus, potassium, N:P ratio, biomass, no. of species and Ellenberg N are shown as passive varieties. Only the best fitted species are presented

Species richness is frequently linked to N:P ratios, although the relationship varies with nutrient limitation and region (Palpurina et al. 2018). Resource competition theory predicts maximum species coexistence under nitrogen and phosphorus co-limitation (Fargione, Tilman 2006). Empirical evidence supports this pattern: species richness increased with rising N:P in nitrogen-limited German grasslands (Klaus et al. 2011) but declined with

Table 5

Mean values and standard deviations of number of species per plot, functional group share (%), biomass (t ha^{-1}) and Ellenberg indicator values for fertility (N) under different fertilization treatment in 2007

Parameter	Treatment					
	PK	90NPK	180NPK	90N	P	0
No. of species	19.7 ± 2.2*ab*	17.6 ± 2.7*a*	16.9 ± 2.9*a*	23.3 ± 2.7*b*	22.3 ± 1.9*b*	24.0
Grasses	57.2 ± 8.2*a*	64.5 ± 10.8*a*	74.1 ± 8.4*b*	60.3 ± 7.1*a*	60.9 ± 6.9*a*	48.1
Legumes	27.9 ± 5.1*c*	9.4 ± 10.0*b*	0.5 ± 1.2*a*	0.7 ± 0.3*a*	0.9 ± 1.1*a*	9.9
Herbs	13.4 ± 5.9*a*	23.7 ± 10.8*ab*	25.6 ± 8.4*b*	27.0 ± 7.2*b*	30.3 ± 6.9*b*	38.3
Biomass	5.4 ± 0.7*bc*	4.7 ± 1.1*b*	6.4 ± 1.3*c*	3.0 ± 0.6*a*	2.4 ± 0.6*a*	2.5
Ellenberg N	4.9 ± 0.3*a*	5.4 ± 0.3*b*	5.6 ± 0.4*b*	3.8 ± 0.3*d*	4.2 ± 0.3*c*	4.5

Different lowercase letters in the same row indicate significant differences in vegetation parameters under different treatments (one way ANOVA followed by the Tukey HSD test, $p<0.05$). The unfertilized control treatment (0) is shown for descriptive purposes only. Because the treatment was not replicated, it was excluded from all inferential statistical analyses and no SD was presented

increasing N:P in phosphorus-limited Chinese grasslands (Yan, Lu 2020). In our experiment, species richness was weakly positively correlated with N:P ratio but strongly negatively correlated with biomass. This supports Grime's (1973) hypothesis that species richness peaks at intermediate productivity. Nutrient effects on richness were therefore mediated mainly by biomass rather than stoichiometry (DeMalach 2018).

Nitrogen concentration showed a positive correlation only with grass share and biomass. Phosphorus concentration was uncorrelated only with grass share, while potassium concentration showed no correlation with biomass and grass share. The N:P ratio was negatively correlated with Ellenberg N indicator value and the share of legumes and positively correlated with the number of species and the share of herbs but not correlated with biomass (Table 6).

Table 6

Pearson correlation coefficients (N=72) of nutrient concentration and N:P ratio in biomass to vegetation parameters in 2007

Nutrient	Ellenberg N		Grasses		Legumes		Herbs		Biomass		No. of species	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Nitrogen	0.10	0,38	**0.26**	0,03	-0.21	0,08	-0.06	0,63	**0.31**	0,01	-0.21	0,08
Phosphorus	**0.35**	0,00	0.22	0,07	**0.23**	0,05	**-0.41**	0,00	**0.26**	0,03	**-0.37**	0,00
Potassium	**0.33**	0.00	0.11	0,37	**0.46**	0,00	**-0.44**	0,00	0.16	0,18	**-0.32**	0,01
N:P ratio	**-0.36**	0.00	-0.09	0,43	**-0.32**	0,01	**0.37**	0,00	-0.16	0,17	**0.31**	0,01

Statistically significant values ($p\leq0.05$) in bold

Numerous studies indicate that the highest biomass production occurs under relatively balanced N and P availability. Increasing vegetation N:P often coincides with reduced biomass because elevated values may reflect phosphorus limitation of growth. For example, biomass was negatively related to shoot N:P ratio and positively associated with plant P concentration (Yan, Lu 2020). Fertilization experiments showed that the highest-yield treatments reduced plant N:P values while simultaneously increasing biomass production (Vargova et al. 2020). Therefore, vegetation N:P should be interpreted primarily as an indicator of nutrient conditions underlying biomass production rather than as a direct predictor of yield. However, this relationship is strongly modified by management practices and changes in community composition, which can simultaneously influence productivity and vegetation stoichiometry (Jin et al. 2022).

Nitrogen typically increases grass dominance while reducing legumes and forbs (Chen et al. 2024), whereas legumes respond positively to phosphorus addition, increasing their biomass and tissue nitrogen via N-fixation (Ren et al. 2017, Herion et al. 2025). In our experiment, functional group proportions were largely similar among treatments, except for a significantly higher legume share in the PK treatment, suggesting potassium limitation in the other treatments. The increase in N-fixing legumes under PK fertilization may stimulate biomass production more strongly than N accumulation in plant tissues, resulting in lower tissue N concentrations (dilution of tissue nitrogen) and reduced vegetation N:P ratios (Güsewell 2004). Consequently, both PK and NPK fertilization lowered N:P ratios. This biomass dilution effect has also been reported in other long-term studies (Loeb et al. 2009, Hejcman et al. 2010).

CONCLUSIONS

Fertilization treatments produced distinct species compositions linked to biomass nutrient concentrations and N:P ratios. Despite strong year-to-year climatic variability, these patterns remained consistent across nearly four decades, confirming the robustness of stoichiometric indicators in long-term grassland research. The persistently narrow N:P range (4.9-11.7) points to chronic nitrogen limitation, with potential co-limitation occurring mainly under nitrogen-only fertilization. Although liming raised soil pH, it did not significantly alter nutrient concentrations, species richness, or N:P ratios, indicating that shifts in soil reaction alone do not necessarily trigger rapid vegetation changes. Biomass production showed no clear relationship with N:P ratios, underscoring the complex and non-linear links between nutrient limitation and productivity. Species richness exhibited only a weak positive association with N:P but a stronger negative relationship with biomass, suggesting that overall productivity plays a greater role in shaping diversity

than stoichiometry alone. Potassium addition increased legume abundance, revealing potassium limitation within this functional group. Overall, stoichiometric ratios remain useful indicators of nutrient effects, but their interpretation requires caution due to interacting environmental drivers.

Author contributions

J.Z. – Conceptualization, material, methodology, investigation, original draft preparation, M.K. – material, methodology, investigation, writing – review. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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