EFFECTS OF FIVE-YEAR NITROGEN AND PHOSPHORUS ADDITIONS ON PLANT BIOMASS AND NUTRIENT CONCENTRATIONS IN ALPINE GRASSLANDS

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Abstract. The Qinghai-Xizang Plateau offers a natural experimental system to evaluate vegetation responses under nutrient enrichment and climate change. We conducted nitrogen (N) and phosphorus (P) addition experiments in the alpine grasslands of the Sejila Mountain, Southeastern Xizang, to quantify their effects on plant biomass, nutrient content, and community structure. Our results revealed threshold responses with distinct optima for productivity: aboveground biomass peaked at N15, belowground biomass at P50 (+132.32%), and total productivity was maximized near P75 (+36.46%). Functional groups exhibited divergent responses, with graminae and forbs strongly stimulated under P75 (+111.54% and+58.15%, respectively), while cyperaceae declined significantly with increasing P. Combined N and P inputs produced both synergistic enhancements and localized antagonisms, reflecting a "super-mono-factor" effect. Nutrient stoichiometry highlighted shifts in limitation patterns: N addition elevated tissue total nitrogen (TN) (+8.38%) and total phosphorus (TP) (+24.20%) while lowering the N:P ratio, whereas P addition substantially increased TP (up to + 147.77%) and reduced N:P ratio without altering TN. Strong positive correlations emerged between above- and belowground biomass, and between TN and TP, whereas both TN and TP were negatively correlated with N:P ratios. The synergistic management window for balancing productivity and ecological stability was identified as N15×P50–P75, with aboveground yield optimized near P75 and belowground carbon inputs near P50. These findings demonstrate that nutrient thresholds and stoichiometric shifts govern alpine grassland responses to N and P enrichment, and providing a quantitative basis for nutrient allocation strategies. The results offer crucial guidance for policymakers and land managers in sustaining productivity while mitigating the risks of ecological homogenization under future climate change.
Keywords: *Sejila Mountain, nutrient limitation, Qinghai-Xizang Plateau, plant biomass*

Introduction

Nitrogen (N) and phosphorus (P) are the key limiting elements for plant growth and terrestrial ecosystem productivity (Elser et al., 2007). Since the Industrial Revolution, anthropogenic activities such as fossil fuel combustion and agricultural fertilization have substantially increased N and P inputs to terrestrial ecosystems (Peñuelas et al., 2013). Global reactive N inputs are projected to increase to 270 Tg N yr^{-1} by 2050, which is approximately the double of the level measured at the beginning of this century (Ackerman et al., 2019). Similarly, anthropogenic perturbation of the P cycle has elevated global P deposition from 50 Mt P yr^{-1} before the Industrial Revolution to 155 Mt P yr^{-1} by 2000 (Smil, 2000; Feng and Zhu, 2019), with further increases expected. Inputs of N and P, together with atmospheric deposition, alter nutrient availability and stoichiometric balance, with significant implications for regional and global terrestrial ecosystems, profoundly influencing their functional states (Feng and Zhu, 2019).

N and P, two essential elements sustaining ecosystem structure and function, originate from distinct sources: ecosystem N inputs are derived primarily from biological N

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fixation, whereas P inputs are predominantly supplied through rock weathering (Augusto et al., 2017; Houlton et al., 2008). On the Qinghai-Xizang Plateau, low temperatures, limited thermal accumulation, short soil development history, slow nutrient mineralization, the predominance of phosphorus in refractory forms, and rapid plant nutrient uptake during the growing season collectively contribute to the widespread N and P limitation in alpine grassland ecosystems (Fay et al., 2015; Tipping et al., 2014). To assess the impacts of N and P deposition on plant biomass and nutrient stoichiometry, N addition experiments have been widely conducted worldwide; however, most of these studies have been concentrated in temperate and northern grasslands (Chen et al., 2018; Hao et al., 2022; Xiao et al., 2024; Peng et al., 2025). Compared with N addition, studies on P addition remain limited, and those available have primarily focused on soil physicochemical properties, soil organic carbon dynamics, and microbial community responses, with relatively little attention to plant community biomass and stoichiometric ratios (Chen and Xiao, 2023; Zhou et al., 2024). Alpine grasslands are generally subject to phosphorus limitation or N–P co-limitation, exhibiting patterns distinct from temperate and boreal grasslands, which are typically more phosphorus-rich but relatively nitrogen-limited. Consequently, the effects of N and P additions on biomass and stoichiometric ratios in alpine grasslands may differ fundamentally from those in temperate or boreal systems (Wang et al., 2023; Chen et al., 2018).

Alpine grassland represents the dominant ecosystem type on the Qinghai-Xizang Plateau, covering 8.2×10^7 hm^2 and accounting for approximately 44% of China's total grassland area (Piao et al., 2012). Owing to its relatively young soils, high elevation, low temperatures, intense solar radiation, and short growing season, the Qinghai-Xizang Plateau grassland ecosystem exhibits pronounced sensitivity to environmental change (Xu et al., 2015). The Qinghai-Xizang Plateau functions as a globally critical ecological security barrier and represents a key region for investigating the impacts of climate change on grassland ecosystems (Hui et al.,2025). Ecosystem organisms are composed of multiple elements, and their ecological interactions fundamentally involve the transfer and redistribution of these chemical elements, each characterized by distinct stoichiometric properties (Wang, 2023). Biomass serves as an indicator of an ecosystem's capacity for energy acquisition (Guo and Liu, 2011). In living systems, N and P are critical elements and essential nutrients for plant growth and development (Cheever et al., 2013). The concentrations and ratios of N and P in plants, together with their transfer dynamics, directly influence plant performance and consequently shape the functioning of grassland ecosystems (Zhang et al., 2019). In China, P deposition exhibits clear spatial variation, being generally higher in northern than in southern regions, and greater in inland than in coastal areas (Wang et al., 2017). Compared with N, P has fewer input pathways and occurs in smaller quantities within ecosystems; however, P fertilizers are more stable and less volatile, thereby exerting more persistent impacts on ecosystem processes (Feng and Zhu, 2019). Consequently, N and P deposition have become a central focus in global climate change research. In this context, controlled N and P addition experiments are essential for elucidating the responses of alpine grassland plant biomass and stoichiometric ratios, which are critical for predicting future vegetation dynamics. Nevertheless, most previous studies have emphasized soil physicochemical properties, with comparatively limited attention to plant-level responses. Existing N and P addition experiments on the Qinghai-Xizang Plateau indicate that plant functional groups exhibit heterogeneous responses to nutrient enrichment (Dong et al., 2016). Since 2019, a multi-gradient N and P addition

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experiment, including both single and combined treatments, has been conducted in the alpine grasslands of Sejila Mountain to address the following objectives: (1) to quantify the response of plant biomass to nutrient enrichment; (2) to examine the dynamics of plant N and P stoichiometry under varying nutrient inputs; and (3) to evaluate the impacts of N and P additions on plant community structure. These efforts aim to provide theoretical support for understanding the mechanisms by which N and P deposition influence biomass accumulation and plant growth in alpine grasslands, to elucidate nutrient limitation processes in alpine vegetation, and to establish a scientific basis for the sustainable management and conservation of Qinghai-Xizang Plateau grassland ecosystems.

Materials and methods

Study site description

The study site is situated in alpine grassland on the western slopes of Sejila Mountain, Bayi District, Linzhi City, Xizang Autonomous Region (29°38′8″ N, 94°37′26″ E), at an elevation of 4400 m above sea level and with an average slope of 21.4° (*Fig. 1*). The area lies in the climatic transition zone between humid montane warm-temperate and semi-humid montane temperate regimes, with a mean annual temperature of –0.73°C, peaking in July at an average of 9.23°C. Mean annual precipitation is 1134.1 mm, concentrated between April and October, and the frost-free period lasts approximately 180 days. The site is characterized by broad exposure, high vegetation cover (>70%), and dominance by perennial species, including Bistorta vivipara (L.) Gray and Kobresia royleana (Nees) Bocklr. Prior to the establishment of the fertilization experiment, baseline soil physicochemical properties were determined. In May 2019, surface soil samples (0–20 cm) were collected from five randomly selected points within the experimental area and composited into three replicate samples. After removing visible roots and stones, soils were air-dried and sieved (2 mm) prior to analysis. The parameters presented in *Table 1* therefore represent the initial background soil conditions of the study site before nutrient addition treatments.

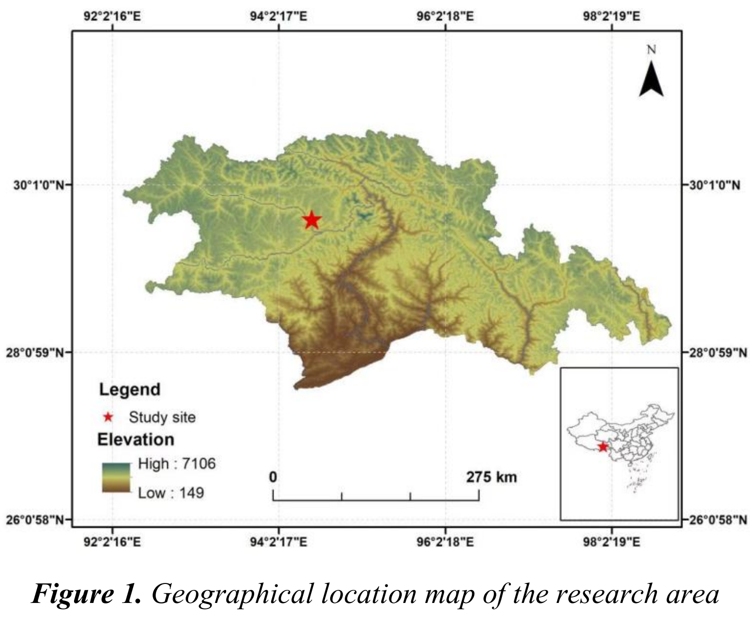

Figure 1. *Geographical location map of the research area*

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***Table 1.** Soil parameters and measurement methods of the test site*

Physical and chemical properties of soil	Test method	Unit	Value
Ph	pH meter determination		5.83
OC	Potassium dichromate oxidation-external heating method	mg/g	49.78
TN	Kjeldahl nitrogen determination method	mg/g	4.65
TP	Molybdenum anti-colorimetric method	mg/g	0.91
NH_4^+-N	Potassium chloride leaching-UV spectrophotometry	mg/g	4.53
NO_3-N	Calcium chloride leaching-UV spectrophotometry	mg/g	0.32
AP	Ammonium bicarbonate leaching-molybdenum anti-colorimetric method	mg/g	3.62

Experimental design

This experiment was conducted through controlled field applications of N and P fertilizers. The study area was established in May 2019 on the western slopes of the Sejila Mountains, with nutrient addition treatments initiated in August of the same year. The alpine grassland was treated as pastureland, and experimental plots were manually enclosed with iron fencing to exclude anthropogenic and livestock disturbance. Three replicate blocks (30 m × 30 m each) were established, within which 16 treatments were randomly assigned (*Table 2*), with each treatment plot measuring 3 m × 3 m. To minimize treatment interference, 1 m buffer strips were set between plots and 5 m buffer zones between blocks. The experimental design followed a randomized block framework, and nitrogen and phosphorus were applied sequentially to each plot. Fertilization was conducted annually at the beginning of August, with urea $CO(NH_2)_2$ dissolved in water and applied at a rate equivalent to 2 mm of natural precipitation using a manual backpack sprayer, while calcium superphosphate $Ca(H_2PO_4)_2$ was evenly broadcast. Control plots received an equal amount of water without fertilizer. The amount of water added during fertilization was carefully regulated to avoid confounding effects on ecosystem processes.

***Table 2.** Gradients of nutrient addition*

Phosphorus addition ($kg·hm^{-2}·a^{-1}$)	Nitrogen addition ($kg·hm^{-2}·a^{-1}$)			
	0	**10**	**15**	**20**
0	Control	N1	N2	N3
50	P1	N1P1	N2P1	N3P1
75	P2	N1P2	N2P2	N3P2
100	P3	N1P3	N2P3	N3P3

Sample collection and measurement

In mid-August 2024, three 20 cm × 20 cm quadrats were randomly selected within each experimental plot. Aboveground plants within each quadrat were clipped at ground level by functional group, returned to the laboratory, oven-killed at 105°C for 3 h, then dried at 65°C for 48 h to constant weight, and weighed to determine biomass by

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functional group. Root samples (0–20 cm depth) were collected using a 5 cm diameter soil auger, with three cores randomly taken per plot, composited, washed, oven-dried to constant weight, and weighed. Aboveground and belowground community biomass per unit area was calculated by conversion. The collected plant materials (above- and belowground) were ground to fine powder, digested with H_2SO_4–H_2O_2, and analyzed for TN and TP concentrations using the Kjeldahl method and the molybdenum–antimony colorimetric method, respectively.

Data analysis

Data were organized using Microsoft Excel 2019. The effects of nitrogen addition, P addition, and their interactions on above- and belowground plant organic carbon, TN, TP contents, and stoichiometric ratios were assessed with two-way ANOVA in IBM SPSS Statistics 27. Differences in plant stoichiometric traits across nitrogen and phosphorus addition gradients were further examined using one-way ANOVA with multiple comparisons. Data visualization was performed in Origin 2024, and results are reported as mean ± standard error (Mean ± SE). Relationships between nitrogen and phosphorus additions and plant biomass nutrient contents were evaluated using Spearman's correlation analysis.

Results

Community biomass

N and P additions both individually and in combination, exerted highly significant effects on community aboveground biomass ($P < 0.01$; *Table 3*). In isolated nitrogen addition, N15 significantly enhanced aboveground biomass, whereas N10 and N20 treatments showed no significant differences from the control (*Fig. 2*). In the case of separate phosphorus addition, aboveground biomass exhibited a unimodal response to P addition, peaking under P75, which was 36.46% higher than the control. In contrast, N addition alone had no significant effect on belowground biomass, while both P addition and its interaction with N were highly significant ($P < 0.001$; *Table 3*). Belowground biomass increased by 132.32% under P50, and the combined treatment N15P50 produced the greatest belowground increase 86.35%. These results demonstrate that N–P interactions exerted stronger influences on belowground biomass than single-factor additions.

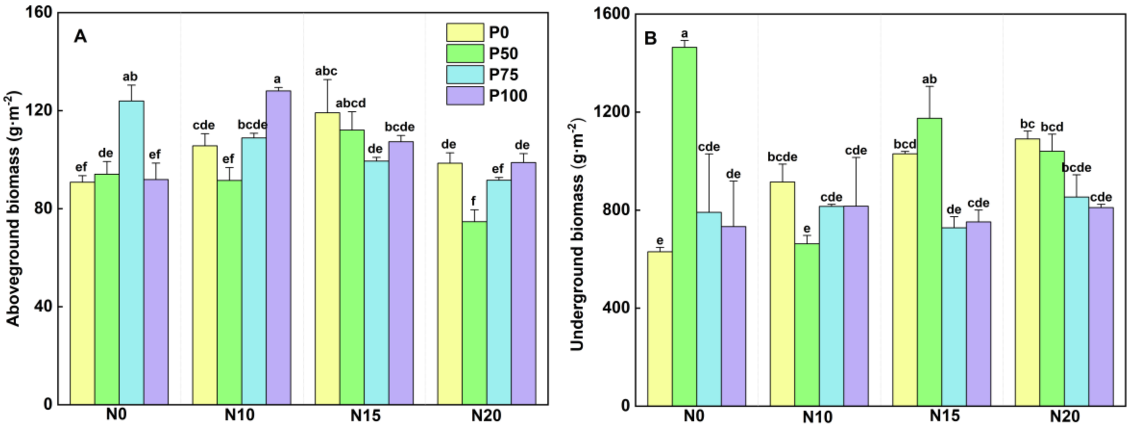

***Figure 2.** Effects of nitrogen and phosphorus addition on the biomass of plant communities*

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Table 3. *Two-way analysis of the effects of nitrogen and phosphorus addition on the biomass of alpine grassland plant communities*

Treatment	Df	Aboveground biomass	Underground biomass
N	3	9.91^{***}	1.50^{ns}
P	3	5.18^{**}	7.35^{***}
N×P	9	5.73^{***}	4.30^{***}

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, ns: not significant

Above-ground biomass of each functional group

Two-way ANOVA revealed that N and P additions, both individually and in combination, had highly significant effects on the aboveground biomass of alpine grassland functional groups (graminae, cyperaceae, and forbs), ($P < 0.001$; *Table 4*). N addition, it induced a unimodal response in the biomass of graminae and forbs (*Fig. 3*): grass biomass increased by 131.73% under N10, while for biomass peaked at N15 with a 31.20% increase. In contrast, cyperaceae biomass increased consistently with N enrichment, reaching a maximum of 65.08% above the control under N20. In the case of separate phosphorus addition, aboveground biomass of cyperaceae declined significantly with increasing P input, showing a 60.91% reduction under P50 compared with the control. In contrast, both graminae and forbs exhibited unimodal responses, with aboveground biomass increases of 111.54% and 58.15%, respectively, under P75. Under combined nitrogen and phosphorus addition, Gramineae biomass increased markedly, with N20P50 resulting in a 522.22% increase in aboveground biomass. In contrast, Cyperaceae biomass was reduced relative to nitrogen addition alone, showing a 71.07% decline under N10P50. High P input in mixed treatments promoted forb biomass, with N10P100 increasing aboveground biomass by 51.26%.

Table 4. *Two-way analysis of the effects of nitrogen and phosphorus addition on the aboveground biomass of functional groups in alpine grasslands*

Treatment	df	Poaceae	Cyperaceous	Forbs
N	3	15.79^{***}	18.21^{***}	7.72^{***}
P	3	16.14^{***}	17.46^{***}	19.30^{***}
N×P	9	17.17^{***}	4.4^{***}	9.72^{***}

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, ns: not significant

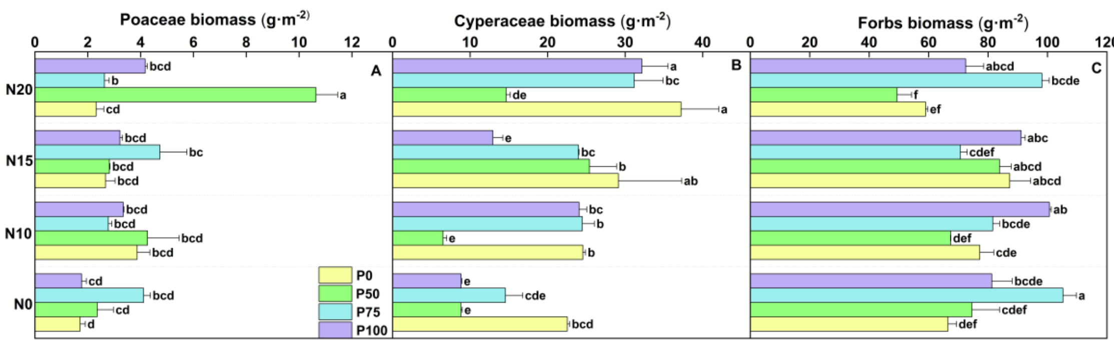

Figure 3. *Effects of nitrogen and phosphorus addition on the aboveground biomass of plants in each functional group*

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Nutrient content of plant communities

Nutrient content of above-ground parts of plant communities

Community aboveground TN, TP, and the N:P ratio responded significantly to elemental N addition (P < 0.01; *Table 5*). With increasing N input, both TN and TP contents exhibited a unimodal pattern (*Fig. 4*), reaching maximum increases of 8.38% and 24.20%, respectively, under N15 compared with the control. In contrast, the N:P ratio declined significantly, showing a 14.63% reduction under N20. “P addition had no significant effect on community aboveground TN, but exerted highly significant effects on TP and N: P. TP content increased markedly with higher phosphorus inputs, while N: P declined significantly, with TP rising by 147.77% and N: P decreasing by 57.31% under P100. Under combined N and P addition, TN, TP, and N:P all responded significantly: TN exhibited a unimodal pattern with increasing inputs, TP increased significantly, whereas N:P declined significantly.

Nutrient content of underground parts of plant communities

Elemental N addition had highly significant effects on community TP content and N:P (P < 0.01; *Table 5*). TP exhibited a unimodal response, while both TN and N:P declined initially and then increased (*Fig. 5*). At N15, TP increased by 36.59%, whereas TN and N:P decreased by 19.68% and 40.71%, respectively, compared with the control. Elemental P addition, significantly enhanced TP content but reduced N:P, with no significant effect on TN. Under P100, TP increased by 143.90% and N: P decreased by 59.58% relative to the control. Responses of TN, TP, and N:P under combined N and P additions were consistent with those observed in aboveground plant tissues.

Table 5. *Two-way analysis of the effects of nitrogen and phosphorus addition on the nitrogen and phosphorus contents of alpine grassland communities*

Treatment	df	Aboveground			Aboveground		
		TN	**TP**	**N:P**	**TN**	**TP**	**N:P**
N	3	6.62^{**}	14.73^{***}	6.09^{**}	0.36^{ns}	8.45^{***}	3.24^{**}
P	3	2.79^{ns}	189.86^{***}	205.74^{***}	2.70^{ns}	107.37^{***}	35.75^{***}
N×P	9	4.25^{**}	13.85^{***}	4.45^{***}	4.94^{***}	3.60^{**}	2.37^{**}

TN represents the total nitrogen content, TP represents the total phosphorus content, and N/P represents the nitrogen-phosphorus ratio, **P* < 0.05, ***P* < 0.01, ****P* < 0.001, ns: not significant

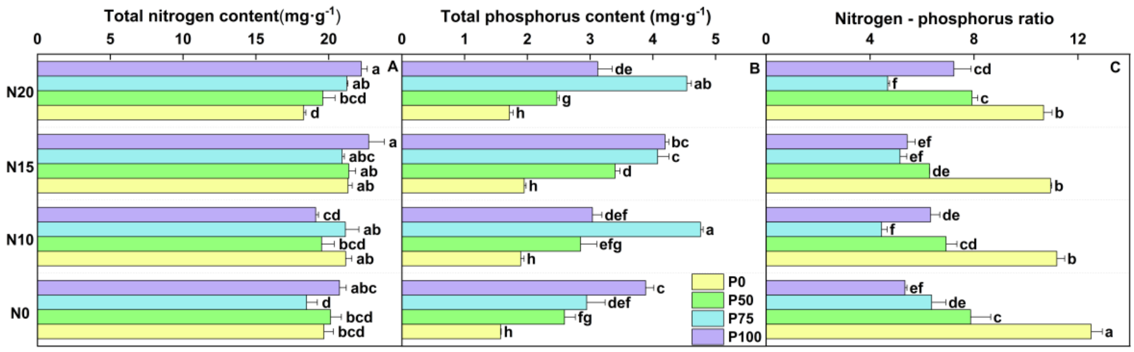

Figure 4. *Effects of nitrogen and phosphorus addition on the nutrient content of the aboveground part of plant communities*

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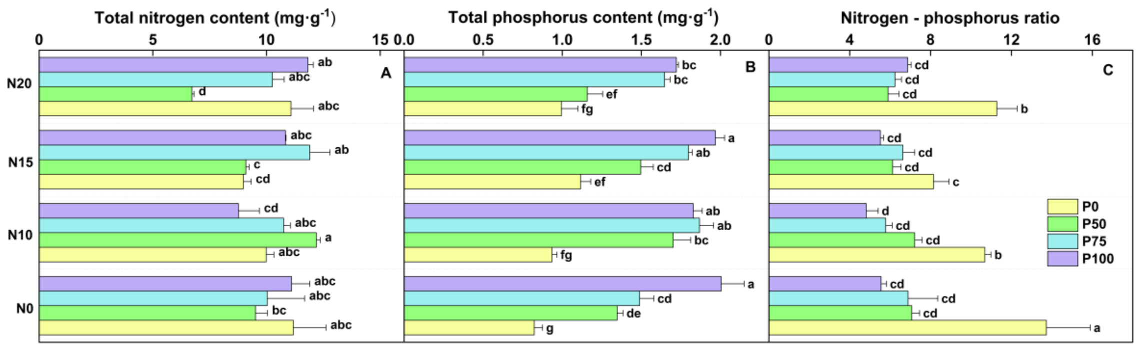

Figure 5. *Effects of nitrogen and phosphorus addition on the nutrient content of the underground part of plant communities*

Correlation analysis between plant biomass and nutrient content

The Spearman correlation heat map of plant biomass and nutrient content under N and P addition (*Fig. 6*) showed that the aboveground biomass of all functional groups was positively correlated with community aboveground biomass. In particular, the aboveground biomass of cyperaceae and forbs exhibited significant positive correlations with community aboveground biomass ($P < 0.05$). Moreover, community aboveground biomass was positively correlated with belowground biomass, indicating coordinated growth between above- and belowground components. The community root-to-shoot ratio was significantly negatively correlated ($P < 0.05$) with forb biomass and community aboveground biomass, but was highly positively correlated ($P < 0.001$) with community belowground biomass. Regarding nutrient content, community TN and TP were strongly positively correlated ($P < 0.01$), whereas TP was highly negatively correlated with N:P in both above- and belowground plant tissues ($P < 0.001$).

Discussion

Effects of nitrogen addition on plant community biomass and nutrient content

N addition had a highly significant effect on community aboveground biomass (AGB) ($F = 9.91$, $P < 0.001$; *Table 3*), exhibiting a unimodal response. The greatest increase occurred at N15, whereas N10 and N20 did not differ significantly from the control (*Fig. 2*). In contrast, nitrogen had no significant main effect on belowground biomass (BGB) (*Table 3*). Among functional groups, nitrogen significantly affected graminae, cyperaceae, and forbs (*Table 4*), but with distinct patterns: graminae and forbs increased and then declined, peaking at N10 and N15, respectively, while cyperaceae increased continuously, reaching a maximum at N20 (*Fig. 3*). In terms of stoichiometry, aboveground TN and TP increased by 8.38% and 24.20% at N15 compared with the control, whereas N:P declined significantly with increasing N (–14.63% at N20; *Fig. 4*). Belowground responses differed, with TP showing a unimodal trend, while TN and N:P decreased initially and then increased (*Fig. 5*). Correlation analysis revealed positive associations between AGB and BGB, and between aboveground TN and TP, whereas TP was negatively correlated with N:P (*Fig. 6*). Recent global and cross-climatic syntheses have demonstrated that multi-nutrient co-limitation, particularly N–P co-limitation, is the prevailing constraint on grassland productivity. Moderate N enrichment can enhance yields in the short term, but

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simultaneously reduces tissue N:P through a dilution effect, thereby shifting systems toward secondary P limitation (Fay et al., 2025). This pattern is consistent with our findings, in which aboveground biomass peaked at N15 while N:P was significantly down-regulated. Moreover, N interactions with other nutrients along drought–precipitation gradients significantly enhanced the sensitivity of grassland productivity to precipitation, providing a broader context for interpreting interannual variability in AGB. In this study, peak yield occurred at N_{15}, with no further increase at N20, accompanied by a continuous decline in N:P (*Figs. 2* and *4*). This suggests that in alpine ecosystems characterized by short growing seasons, slow phosphorus release from weathering, and depleted P substrates (Hong et al., 2025), secondary P limitation emerges earlier, constraining the conversion of high nitrogen inputs into additional aboveground productivity (Dong et al., 2023). Moreover, functional group asynchrony arising from differential thresholds—graminae and forbs responding strongly to moderate N while cyperaceae continued to increase under high N—contributed to the unimodal community-level response (*Fig. 3*). Evidence from alpine regions has similarly revealed threshold and saturation responses in co-limitation patterns and stoichiometry under elevated nitrogen inputs (Dong et al., 2023; Yang et al., 2023), consistent with our finding that moderate N addition enhances plant productivity. In this study, yield optimization was achieved at N15, whereas further N enrichment failed to sustain increases, intensified N:P imbalance, and likely reduced community stability and resilience through functional group reorganization (Bharath et al., 2020). From a management perspective, the optimal N application window should be identified and coupled with stoichiometric monitoring, particularly of N:P ratios. In years with pronounced interannual hydrothermal variability, attention should be given to the amplification of yield fluctuations caused by N interactions with water and other nutrients, to mitigate the management risks of yield gains accompanied by reduced stability (Fay et al., 2025).

Effects of phosphorus addition on plant community biomass and nutrient content

P effects were significant (*Table 3*), but above- and belowground optima differed: AGB reached a maximum at P75 (+36.46%), whereas BGB peaked at P50 (+132.32%) (*Fig. 2*). At the functional group level, graminae and forbs attained their highest biomass at P75 (+111.54% and + 58.15%, respectively), while cyperaceae declined significantly with increasing P (*Fig. 3*; *Table 4*), indicating a restructuring of taxonomic dominance. Stoichiometrically, TP increased monotonically with P addition (aboveground P100 + 147.77%, belowground P100 + 143.90%), accompanied by significant reductions in N:P (aboveground P100 –57.31%, belowground P100 –59.58%), whereas TN showed no significant response (*Figs. 4* and *5*; *Table 5*). Across treatments, TP was consistently negatively correlated with N:P (*Fig. 6*). Thus, the productivity optimum (P50–P75) occurred before the maximum accumulation of tissue P at P100. Recent global and regional studies have similarly demonstrated that P addition significantly modifies plant and ecosystem C–N–P stoichiometry, with many grasslands reaching or approaching productivity optima at intermediate P levels (Wu et al., 2025). Field experiments on the Xizang Plateau further indicate that P gains are frequently accompanied by pronounced declines in N:P ratios and reorganization of functional group structure (Zhang et al., 2025), consistent with our findings. Our results further showed that TN did not respond significantly to phosphorus addition, whereas TP increased monotonically and N:P declined continuously (*Figs. 4* and *5*). This

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indicates that in alpine systems, P enrichment was not accompanied by parallel N accumulation, potentially leading to relative N deficiency or rapid shifts in competitive dynamics. Consequently, AGB peaked earlier at P75 rather than P100, while BGB reached its maximum at P50. This root-to-shoot mismatch suggests that moderate P favored root investment, whereas higher P levels shifted allocation toward aboveground competition, consistent with the observed peaks of graminae and forbs at P75. A similar 'threshold–substitution' pattern has been reported in recent studies of phosphorus supplementation in alpine grasslands (Zhang et al., 2025). These findings indicate that maintaining moderate P inputs is more beneficial than continuous enrichment, as excessive P does not further enhance plant biomass but instead exacerbates N:P imbalance and drives taxonomic shifts. In alpine ecosystems, monitoring N:P ratios alongside functional group composition is therefore recommended to balance productivity with community structural stability (Wu et al., 2025).

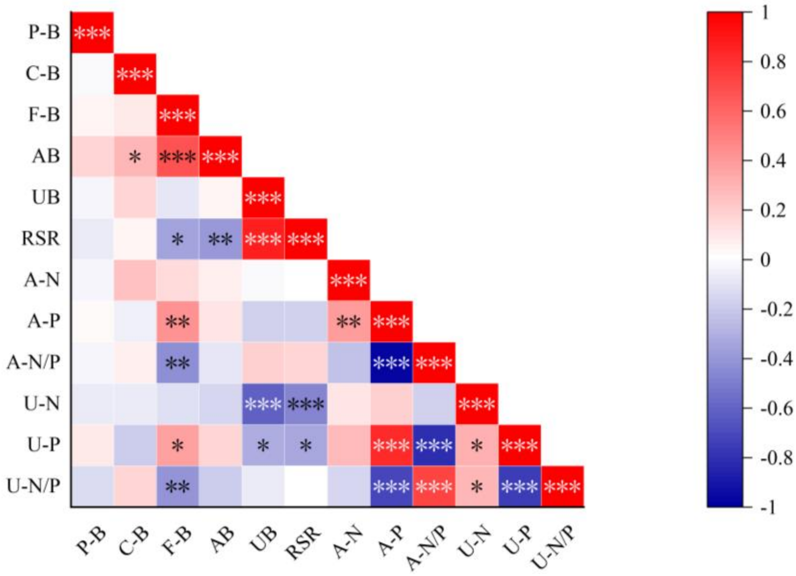

Figure 6. *Spearman correlation heat map of plant biomass and nutrient contents under nitrogen and phosphorus addition. P-B: Aboveground biomass of Poaceae; C-B: Aboveground biomass of Cyperaceae; O-B: Aboveground biomass of Forbs; AB: Aboveground biomass of the community; UB: Belowground biomass of the community; RSR: Root-shoot ratio of the community; A-N: Total nitrogen (TN) content in the aboveground part of the community; A-P: Total phosphorus (TP) content in the aboveground part of the community; A-N/P: Nitrogen-phosphorus ratio in the aboveground part of the community; U-N: Total nitrogen (TN) content in the belowground part of the community; U-P: Total phosphorus (TP) content in the belowground part of the community; U-N/P: Nitrogen-phosphorus ratio in the belowground part of the community. *, **, and *** represent significant correlations at the 0.05, 0.01, and 0.001 levels, respectively*

Effects of nitrogen and phosphorus addition on plant community biomass and nutrient content

Our results demonstrated that combined nitrogen and phosphorus additions were characterized by synergistic effects accompanied by structural substitutions. Statistically, the N × P interaction was highly significant for both AGB and BGB (AGB: $F = 5.73$, $P < 0.001$; BGB: $F = 4.30$, $P < 0.001$; *Table 3*). "In terms of magnitude,

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N15P50 increased BGB by 86.35%, and N20P50 enhanced grass AGB by 522.22% (*Figs. 2* and *3*), both exceeding the respective single-factor optima, indicating that co-limitations were alleviated synchronously to produce a super-single-factor. At the functional group level, however, phosphorus addition attenuated the positive effect of N on Salix (e.g., N10P50 reduced biomass by 71.07% relative to N10), whereas Gramineae and forbs benefited substantially (e.g., N10P100 increased forb biomass by 51.26%), resulting in overall synergistic yield gains accompanied by localized antagonism. Meta-analyses of 71 multifactorial grassland fertilization experiments worldwide have demonstrated that nutrient interactions regulate grassland productivity across climatic gradients, with synergistic effects of N and P occurring commonly in diverse climatic zones (Fay et al., 2025). Concurrently, recent process-based studies have shown that combined N and P inputs can significantly enhance microbial carbon use efficiency and modify soil enzyme activities and priming effects, thereby improving the conversion efficiency of exogenous resources (Li et al., 2024). These findings align with our observation that composite additions outperform single-factor peaks. Composite treatments elevated and advanced the 'optimum point'; for instance, N15P50 for BGB and N20P50 for grass AGB both exceeded their respective single-factor peaks (*Figs. 2* and *3*), indicating true synergism rather than simple additive effects. This response paralleled the observed increases in TP and the pronounced down-regulation of N:P (*Table 5*; *Figs. 4* and *5*). In the alpine context—characterized by basal P depletion and short growing seasons—simultaneous alleviation of N and P limitations rapidly translated into aboveground competition and enhanced dominance of graminae, while exerting local antagonistic effects on cyperaceae through competitive reorganization. This pattern of 'synergistic productivity coupled with structural substitution' is consistent with the concept of functional group dominance–productivity responses observed in recent long-term (≥8 years) N/P manipulation experiments in alpine grasslands (Chen et al., 2024). Practically, these findings suggest that total community productivity and belowground carbon inputs can be enhanced through balanced N-P ratios (e.g., N15P50, N20P50). However, two precautions are required: 1) sustained downward adjustments of N:P may induce secondary constraints or alter decomposition–sequestration equilibria; and 2) functional group monocultures or substitutions may compromise ecosystem stability and resilience. Therefore, optimizing application intensities and ratios, maintaining functional diversity, and implementing process-based monitoring (e.g., tissue N:P, functional group structure, key enzyme activities) are essential to fully exploit synergistic gains. This aligns with the governance framework of 'co-limitation–ratio optimization–stability trade-offs' proposed in recent cross-site experiments and process studies (Li et al., 2024; Fay et al., 2025).

Conclusion

In this study, we conducted a five-year multi-gradient nutrient manipulation experiment in the Sejila Mountains of Xizang, measuring soil basal nutrients along with above- and belowground plant biomass. Our results demonstrated distinct thresholds and mismatches in optimal N and P inputs for above and belowground productivity: AGB exhibited a unimodal response to N, peaking at N15, whereas P enhanced yield with optima at P75 for AGB and P50 for BGB. Functional groups responded divergently, leading to structural reorganization and significant suppression of

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cyperaceae; compound N-P additions generated ‘super–single-factor’ synergism but also exerted antagonistic effects on Saxifraga. Community stoichiometry revealed shifts in limiting factors and synergistic responses: under N addition, TN and TP increased while N:P declined, whereas under P addition, TP increased significantly and N:P decreased, while TN remained unresponsive. These findings suggest a management interval of N15 × P50-P75 to balance yield gains and ecological homeostasis, with aboveground productivity biased toward P75 and belowground carbon inputs biased toward P50. Higher peaks may be achieved under N15P50-N20P50, but continuous monitoring of tissue N:P ratios and functional group composition is required to prevent structural homogenization. Future work will incorporate key soil enzyme-microbe processes into similar experimental designs, assess sensitivities to hydrothermal variability and extreme climatic events, and integrate operational scenarios such as grazing and mowing to evaluate multi-objective trade-offs. Furthermore, threshold early-warning systems and ‘synergistic window’ identification tools will be developed through ground-remote sensing integration to support regionalized nutrient management and ecological security assessments in alpine grasslands.

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REFERENCES

[1] Ackerman, D., Millet, D. B., Chen, X. (2019): Global estimates of inorganic nitrogen deposition across four decades. – Global Biogeochemical Cycles 33: 100-107. https://doi.org/10.1029/2018GB005990.

[2] Augusto, L., Achat, D. L., Jonard, M., et al. (2017): Soil parent material—a major driver of plant nutrient limitations in terrestrial ecosystems. – Global Change Biology 23: 3808-3824. https://doi.org/10.1111/gcb.13691.

[3] Bharath, S., Borer, E. T., Biederman, L. A., et al. (2020): Nutrient addition increases grassland sensitivity to droughts. – Ecology 101: e02981. https://doi.org/10.1002/ecy.2981.

[4] Cheever, B., Webster, J. R., Bilger, E., et al. (2013): The relative importance of exogenous and substrate-derived nitrogen for microbial growth during leaf decomposition. – Ecology 94: 1614-1625. https://doi.org/10.1890/12-1339.1.

[5] Chen, C., Xiao, W. (2023): The global positive effect of phosphorus addition on soil microbial biomass. – Soil Biology and Biochemistry 176: 108882. https://doi.org/10.1016/j.soilbio.2022.108882.

[6] Chen, J.-B., Dong, C.-C., Yao, X.-D., et al. (2018): Effects of nitrogen addition on plant biomass and tissue elemental content in different degradation stages of temperate steppe in northern China. – Journal of Plant Ecology 11: 730-739. https://doi.org/10.1093/jpe/rtx035.

[7] Chen, X., Hou, G., Shi, P., et al. (2024): Functional groups dominate aboveground net primary production under long-term nutrient additions in a Tibetan alpine meadow. – Plants 13: 344. https://doi.org/10.3390/plants13030344.

[8] Dong, J., Cui, X., Wang, S., et al. (2016): Changes in biomass and quality of alpine steppe in response to N & P fertilization in the Tibetan Plateau. – Plos One 11: e0156146. https://doi.org/10.1371/journal.pone.0156146.

[9] Dong, K., Li, W., Tang, Y., et al. (2023): Co-limitation of N and P is more prevalent in the Qinghai-Tibetan Plateau grasslands. – Frontiers in Plant Science 14: 1140462. https://doi.org/10.3389/fpls.2023.1140462.

[10] Elser, J. J., Bracken, M. E., Cleland, E. E., et al. (2007): Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. – Ecology Letters 10: 1135-1142. https://doi.org/10.1111/j.1461-0248.2007.01113.x.

[11] Fan, H.-W.-Q., Li, Z.-X., Gui, J. (2025): Progress and prospects of research on sustainable development on the Tibetan Plateau. – Advances in Climate Change Research 21(3): 400-413. https://www.climatechange.cn/EN/Y2025/V21/I3/400.

[12] Fay, P. A., Prober, S. M., Harpole, W. S., et al. (2015): Grassland productivity limited by multiple nutrients. – Nature Plants 1: 1-5. https://doi.org/10.1038/nplants.2015.80.

[13] Fay, P. A., Gherardi, L. A., Yahdjian, L., et al. (2025): Interactions among nutrients govern the global grassland biomass–precipitation relationship. – Proceedings of the National Academy of Sciences 122: e2410748122. https://doi.org/10.1073/pnas.2410748122.

[14] Feng, J., Zhu, B. (2019): A global meta-analysis of soil respiration and its components in response to phosphorus addition. – Soil Biology and Biochemistry 135: 38-47. https://doi.org/10.1016/j.soilbio.2019.04.008.

[15] Guo, N., Liu, J. (2011): A review of plant biomass research. – Subtropical Plant Science 40: 83-88. DOI: 10.3969/j.issn.1009-7791.2011.02.024.

[16] Hao, J., Sun, J., Dong, K., et al. (2022): Plant C and N pools improved by N addition levels but not frequencies in a typical Grassland of Northern China. – Atmosphere 13: 851. https://doi.org/10.3390/atmos13060851.

[17] Hong, J., Pang, B., Zhao, L., et al. (2025): Soil phosphorus crisis in the Tibetan alpine permafrost region. – Nature Communications 16: 6204. https://doi.org/10.1038/s41467-025-61501-x.

[18] Houlton, B. Z., Wang, Y.-P., Vitousek, P. M., et al. (2008): A unifying framework for dinitrogen fixation in the terrestrial biosphere. – Nature 454: 327-330. https://doi.org/10.1038/nature07028.

[19] Li, W., He, Y., Shen, R., et al. (2024): Concurrent nitrogen and phosphorus enrichment increases ecosystem carbon use efficiency in an alpine grassland. – Agriculture, Ecosystems & Environment 375: 109182. https://doi.org/10.1016/j.agee.2024.109182.

[20] Peng, Y., Yang, J. X., Seabloom, E. W., et al. (2025): Multiple nutrient additions homogenize multidimensional plant stoichiometry in a meadow steppe. – Global Change Biology 31: e70123. https://doi.org/10.1111/gcb.70123.

[21] Peñuelas, J., Poulter, B., Sardans, J., et al. (2013): Human-induced nitrogen–phosphorus imbalances alter natural and managed ecosystems across the globe. – Nature Communications 4: 2934. https://doi.org/10.1038/ncomms3934.

[22] Piao, S., Tan, K., Nan, H., et al. (2012): Impacts of climate and CO2 changes on the vegetation growth and carbon balance of Qinghai–Tibetan grasslands over the past five decades. – Global and Planetary Change 98: 73-80. https://doi.org/10.1016/j.gloplacha.2012.08.009.

[23] Smil, V. (2000): Phosphorus in the environment: natural flows and human interferences. – Annual Review of Energy and the Environment 25: 53-88. https://doi.org/10.1146/annurev.energy.25.1.53.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2402_34393452

[24] Tipping, E., Benham, S., Boyle, J. F., et al. (2014): Atmospheric deposition of phosphorus to land and freshwater. – Environmental Science: Processes & Impacts 16: 1608-1617. https://doi.org/10.1039/c3em00641g.
[25] Wang, Q. (2023): Responses of community characteristics and ecological stoichiometry of typical steppe vegetation in Inner Mongolia to mowing and nitrogen and phosphorus addition. – DOI: 10.27248/d.cnki.gnjqc.2023.001020.
[26] Wang, R., Goll, D., Balkanski, Y., et al. (2017): Global forest carbon uptake due to nitrogen and phosphorus deposition from 1850 to 2100. – Global Change Biology 23: 4854-4872. https://doi.org/10.1111/gcb.13766.
[27] Wang, Y., Wang, C., Ren, F., et al. (2023): Asymmetric response of aboveground and belowground temporal stability to nitrogen and phosphorus addition in a Tibetan alpine grassland. – Global Change Biology 29: 7072-7084. https://doi.org/10.1111/gcb.16967.
[28] Wu, Y., Tie, L., Huang, C., et al. (2025): The effects, patterns and predictors of phosphorus addition on terrestrial litter decomposition. – Global Ecology and Biogeography 34: e70057. https://doi.org/10.1111/geb.70057.
[29] Xiao, H., Li, P., Monaco, T. A., et al. (2024): Nitrogen and phosphorus additions alter foliar nutrient concentrations of dominant grass species and regulate primary productivity in an Inner Mongolian meadow steppe. – Science of the Total Environment 912: 168791. https://doi.org/10.1016/j.scitotenv.2023.168791.
[30] Xu, M., Wang, G., Li, X., et al. (2015): The key factor limiting plant growth in cold and humid alpine areas also plays a dominant role in plant carbon isotope discrimination. – Frontiers in Plant Science 6: 961. https://doi.org/10.3389/fpls.2015.00961.
[31] Yang, X., Chen, Y., Zhang, T., et al. (2023): Different responses of functional groups to N addition increased synchrony and shortened community reproductive duration in an alpine meadow. – Journal of Ecology 111: 2231-2244. https://doi.org/10.1111/1365-2745.14173.
[32] Zhang, X., Ye, Y., Tao, J., et al. (2025): Responses of alpine grassland plant communities on Sejila Mountain in the Qinghai-Tibet Plateau to phosphorus addition. – Frontiers in Environmental Science 13: 1601695. https://doi.org/10.3389/fenvs.2025.1601695.
[33] Zhang, Z.-C., Hou, G., Liu, M., et al. (2019): Degradation induces changes in the soil C:N:P stoichiometry of alpine steppe on the Tibetan Plateau. – Journal of Mountain Science 16: 2348-2360. https://doi.org/10.1007/s11629-018-5346-y.
[34] Zhou, X., Hu, Y., Li, H., et al. (2024): Phosphorus addition increases stability and complexity of co-occurrence network of soil microbes in an artificial Leymus chinensis grassland. – Frontiers in Microbiology 15: 1289022. https://doi.org/10.3389/fmicb.2024.1289022.

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