



# Importance of plant community composition and aboveground biomass in shaping microbial communities following long-term nitrogen and phosphorus addition in a temperate steppe ecosystem

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Received: 27 February 2024 / Accepted: 25 July 2024  
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## Abstract

**Background and aims** Human activities have increased the input of nitrogen (N) and phosphorus (P) into terrestrial ecosystems since the industrial revolution. These activities are expected to increase aboveground biomass (AGB) and further affect plants and soil microbial communities. Plant–microbe interactions play a significant role in shaping microbial communities. However, how soil microbial community respond to change in plant communities after N and P addition remains unclear, particularly in temperate steppe ecosystems.

**Methods** A 12-year factorial combination experiment of N and P addition was conducted in a

temperate steppe ecosystem to evaluate soil microbiomes in relation to plant communities and soil characteristics.

**Results** Long-term N addition shifted the dominance of plant community from multiple species to sole dominance by *Leymus chinensis*. N addition did not significantly affect microbial  $\alpha$ -diversity. However, P addition led to significantly increased bacterial richness, while NP addition led to significantly decreased arbuscular mycorrhizal fungal richness. Structural equation modeling indicated that available phosphorous (AP) significantly affected bacterial richness, while AP, dissolved inorganic nitrogen (DIN), and AGB significantly influenced arbuscular mycorrhizal fungal richness. Nutrient addition also significantly altered soil microbial community structures that can largely be explained by AGB and plant community compositions. Finally, network analysis revealed strong correlations between plant functional groups and dominant microbial taxa.

**Conclusions** Microbial communities can be influenced by both N and P addition-induced changes in soil properties and plant communities. The significant associations between plant functional groups and dominant microbial taxa emphasize the important roles of plant-mediated effects on microbial communities after N and P addition.

Responsible Editor: Raúl Ochoa-Hueso.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s11104-024-06881-7>.

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**Keywords** Long-term nutrient addition · Plant functional group · Soil microbial diversity · Community structure · Plant–microbial interactions · Temperate steppe

## Introduction

The longstanding maxim in environmental microbiology that “everything is everywhere but the environment selects” (Baas Becking 1934) has been used to describe the global distributions of microorganisms. The concept suggests that although microorganisms are widely distributed globally (including in extreme environments) and possess exceptional diversity, substantial variation is observed in their spatial microbial diversity patterns that is primarily driven by environment heterogeneity (Fuhrman 2009; Green and Bohannan 2006; Pan et al. 2020; Shu and Huang 2022). Variation in climatic conditions (e.g., temperature and precipitation) among different regions occurs due to geographically variable parameters like latitude and altitude, resulting in biogeographical variation of microorganisms (Peay et al. 2017). Furthermore, the continuous impact of land use changes and global climate change caused by human activities on regional environments have been identified as key drivers underlying changes in soil microbial diversity. Intensification of human activities has not only caused global climate warming but also significantly impacted nitrogen (N) cycling within ecosystems (Peñuelas et al. 2013; Wen et al. 2022). Unprecedented input of N to ecosystems has occurred due to human activities, although minimal changes in phosphorus (P) deposition have been observed (Wen et al. 2022). This disproportional anthropogenic input of N relative to P can cause substantial changes in N and P balances, such that continual anthropogenic P input is needed to sustain stable ecosystem productivity (Fleischer et al. 2019; Peñuelas et al. 2013; Vitousek et al. 2010). These changes have significantly impacted grassland ecosystems that cover 40% of the global land area and particularly biodiversity.

The importance of bacteria and fungi in terrestrial soil habitats is evident in their control of biodiversity and their regulation of diverse critical soil biogeochemical processes (Bahram et al. 2018). Much research attention has been given toward understanding the impacts of N and P additions on soil microbial

communities, but these studies have yielded mixed results. In addition to climatic factors, soil pH is a significant determinant of soil microbial community composition and functioning at global and regional scales (Fierer and Jackson 2006; Liu et al. 2020; Peay et al. 2017). Most bacteria generally exhibit optimal growth at neutral pH (Fierer and Jackson 2006; Zhou et al. 2020), and N-induced soil acidification often leads to reduced microbial diversity (Dai et al. 2018; Yang et al. 2021). However, some studies have suggested that bacterial diversity may not significantly decline until N additions reach a certain critical threshold (Liu et al. 2020). Soil fungi exhibit lower sensitivity to pH changes induced by N enrichment, and fungal diversity exhibits lesser responses to N addition (Wang et al. 2023). Moreover, increased soil nutrient effectiveness resulting from the addition of N and P may influence microbial community composition at the local scale (Kaspari et al. 2017; Philippot et al. 2013b; Yang et al. 2021; Zhu et al. 2023). These processes occur because changes in nutrient effectiveness affect the biodiversity of microorganisms with different life history strategies (Li et al. 2023b; Philippot et al. 2013a), thereby further impacting potential multi-trophic interactions among soil microbial communities (Zhu et al. 2023).

N and P are critical for increasing aboveground biomass in temperate grasslands. Their application consequently typically exerts diverse influences on biodiversity and species composition (Ling et al. 2017; Liu et al. 2021; Yang et al. 2015). Numerous studies of the impact of N addition on plant community diversity have yielded mixed results (Liu et al. 2020, 2021), but the responses of grassland ecosystems to P addition are poorly understood (van Dobben et al. 2017). Moreover, significant changes in grassland vegetation communities following nutrient addition should be accompanied by changes in associated soil microbial communities due to the strong interactions among plants, soil characteristics, and microorganisms. Plants primarily regulate the growth and activity of microbial communities through rhizosphere effects (i.e., influences of root exudates on microbial communities) and plant immune system functioning that shape specific soil microbial community structures (Bai et al. 2022; Trivedi et al. 2022). For example, increased input of root exudates with labile substrates into soils often induces rapid growth of microorganisms with higher metabolic efficiencies

(Feng and Wang 2023; Su et al. 2023). In addition, the amount and quality of plant litter input into soils varies depending on the composition and functional traits of different plant communities (Chu et al. 2011; Yang et al. 2018). Fungi decompose more complex organic materials and the ratio of Fungi to Bacteria changes depending on plant litter input (Yao et al. 2018b). Plants subsequently shape soil microbial community compositions through competition for nutrients (especially N) (Feng and Wang 2023) and modification of abiotic soil environments (Guo et al. 2018).

The complex interactions between plants and microorganisms play a significant role in shaping soil microbial communities (Peay et al. 2017; Yang et al. 2018), yet the roles of plants are seldom considered when investigating the factors influencing the structures of grassland soil microbial communities (Liu et al. 2021). This is especially evident when evaluating significant changes in plant community composition after long-term nutrient additions (Chalcraft et al. 2008). Consequently, it is imperative to simultaneously consider both soil and plant factors to comprehensively understand the mechanisms by which nitrogen and phosphorus additions affect soil microbial communities. Here, the responses of plant and soil microbial (bacteria, total fungi, and arbuscular mycorrhizal fungi (AMF)) communities were evaluated after long-term N and P additions. The distinct response mechanisms of plant and soil microbial communities to N and P input were also investigated at the level of diversity changes. Specifically, we hypothesized that (1) higher nutrient availability induced by long-term addition of N and P alters plant diversity and community composition by changing the biomass of specific plant functional groups, and (2) microbial diversity and community composition are strongly influenced by soil properties (e.g., nutrient availability and pH) and plant community characteristics after long-term N and P addition.

## Materials and methods

### Study site

The study was conducted in a typical area of the *Stipa Baicalansis* steppe, within Hulunbeier of Inner Mongolia, China (119°42' E and 48°30' N). The

area experiences a climate characterized by warm summers and cold winters that are influenced by a temperate continental monsoon climate. The mean annual precipitation and mean annual temperature are 329 mm and  $-0.7$  °C, respectively. The natural vegetation in the area comprises grasses dominated by species such as *S. baicalensis* and *Leymus chinensis*, and common species including *Achnatherum sibiricum*, *Filifolium sibiricum*, *Cleistogenes squarrosa*, *Carex duriuscula*, *Astragalus melilotoides*, *Thaichtrum petaloideum*, and *Klasea centauroides* (Yu et al. 2015).

Sixteen 8 m × 8 m plots (4 replicates × 4 treatments) were initiated in the summer of 2010 for a full factorial N and P addition experiment, with the inclusion of a 2 m buffer strip between the plots. Nitrogen (as urea) and P (as triple superphosphate) were each fertilized yearly at a rate of 100 kg ha<sup>-1</sup> year<sup>-1</sup> either alone or together, consistent with the standard protocols of the Nutrient Network (Borer et al. 2014). Unfertilized plots served as controls. Four treatments were included overall, including the control, N fertilization (N), P fertilization (P), and N plus P fertilization (NP).

### Plant and soil sampling and analyses

The aboveground vegetation was collected in mid-August 2021 when annual productivity peaked, and the species in each plot were enumerated using a quadrat measuring 1 m by 1 m. The collected plants were then dried at 65 °C for 48 h and weighed to estimate the aboveground plant biomass in each plot. Following live plant collection, 10 soil cores (3.5 cm diameter) were randomly collected at depths between 0 and 20 cm using a drill. Then 10 cores were thoroughly mixed to construct a single soil sample for each plot, and every sample was sieved using a mesh size of 2 mm. The composite samples were then split into two portions, with one used for measuring soil physicochemical properties by natural drying and the second portion stored at  $-20$  °C for subsequent DNA extraction and microbial community analyses. Soil pH, organic carbon, total N, total P, inorganic N (NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N), and available P were measured according to previously described methods (Bao 2000). The physicochemical measurements are shown in Table S2.

## Soil microbial biomass

Phospholipid fatty acid (PLFA) profiles were analyzed by extracting the biomass of soil microbial functional groups from 3 g of freeze-dried soil samples, following methods described Zhang et al. (2019). PLFAs including 15:0iso, 15:0anteiso, 15:1iso $\omega$ 6c, 16:0iso, 17:0iso, and 17:0anteiso were used to calculate the biomass of Gram-positive (G+) bacteria, and the PLFAs 16:1 $\omega$ 7c, 16:1 $\omega$ 9c, 17:1 $\omega$ 8c, 18:1 $\omega$ 5c, 18:1 $\omega$ 7c, 21:1 $\omega$ 3c, 17:0cyclo $\omega$ 7c, and 19:0cyclo $\omega$ 7c were used to calculate the biomass of Gram-negative (G-) bacteria, as previously described (Yang et al. 2022b). The PLFA 16:1 $\omega$ 5 was used to calculate biomass of AMF biomass, and the PLFAs 18:1 $\omega$ 9c and 18:2 $\omega$ 6c were used to calculate fungal biomass, as previously described (Liang et al. 2016). The PLFAs 10Me16:0, 10Me17:0, 10MeC17:1 $\omega$ 7c, 10Me18:0, and 10MeC18:1 $\omega$ 7c were used to calculate actinomycete biomass (Li et al. 2023b). The PLFAs 14:0, 15:0, 15:0DMA, 16:0, 17:0, 18:0, and 20:0 were used to calculate the biomass of non-specific bacteria (Yang et al. 2022b). Total bacterial biomass was calculated by summing the biomass estimates of G+, G-, and non-specific bacteria. In addition, ratios of G+ to G-, as well as fungi to bacteria, were calculated from their respective biomass levels (Yang et al. 2022b). PLFA concentrations were expressed as nmol g<sup>-1</sup> dry soil.

## DNA extraction and amplicon sequencing

DNA was extracted from soil samples using the FastDNA™ Spin Kit for Soil (116,560–200, MP Biomedicals, USA). DNA concentrations were determined using a NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific, Wilmington, USA), and DNA quality was examined by electrophoresis using a 1% agarose gel. The bacterial V3–V4 hypervariable regions of 16S rRNA genes were amplified using the primer pair 338F (5'-ACTCCTACGGGAGG CAG CAG-3') and 806R (5'-GGACTACHVGGGTWT CTAAT-3'), while fungal ITS1 genes were amplified using the primer pair ITS1F (5'-CTTGGTCATTTA GAGGAAGTAA-3') and ITS2R (5'-GCTGCGTTC TTCATCGATGC-3') (Chen et al. 2021). The V4–V5 hypervariable regions of fungal 18S rRNA genes were amplified using the primer pair AMV4.5NF (5'-AAGCTCGTAGTTGAATTCG-3') and AMDGR

(5'-CCCAACTAT CCCTATTAATCAT-3') (Ma et al. 2022). Detailed information of the PCR protocols and subsequent bioinformatics analysis are provided in the Supplementary material (Appendix A). The sequencing data generated in this study were submitted to the NCBI database under the accession numbers PRJNA928583, PRJNA1057071, and PRJNA1057077.

## Network analysis

Ecological network analysis has been widely used to identify associations among taxa within microbial communities (Ma et al. 2022). Likewise, the exploration of interactions between plants and microorganisms using ecological networks has been increasingly used in recent years. Changes in soil properties resulting from the addition of N and P might modulate plant–microbiota interactions (in 't Zandt et al 2023).

Network analyses were conducted as follows. Prior to constructing the networks, AMF sequences (i.e., OTUs classified as *Glomeromycota*) were excluded from the fungal ITS representative sequence dataset (Ma et al. 2022). Bacterial genera, fungal genera, and AMF genera with relative abundances  $\leq 0.05\%$  were also removed. The plant community data were then filtered to retain species in over six sampling plots, considering the homogeneity of plant communities across the four nutrient addition treatments. Pearson correlation coefficients were calculated using the “corr.test” function of the R psych package to evaluate the significance of correlations among soil properties, microbial genera, and plant species. Correlations meeting the criteria of  $|R| \geq 0.8$  and  $p < 0.05$  were retained. The Cytoscape software program (version 3.9.1) was used to visualize the network.

## Data analysis

Analysis of variance (ANOVA) tests were used to analyze differences in the distributions of soil, plant, and microbial variables. Tukey's honest significant difference (HSD) tests were used to evaluate differences between different nutrient addition treatments. Principal coordinate analysis (PCoA) and PERMANOVA were conducted using the “vegan” package for R. The first two principal component axes were extracted to evaluate as variables related to plant composition. Moreover, linear and quadratic models

were applied to evaluate the relationship direction between soil variables (DIN, pH, and AP) with plant and microbial diversity. In addition, a variance partitioning analysis (VPA) was conducted to assess the relative impacts of vegetation and soil characteristics on the composition of bacterial, fungal, and AMF communities. Mantel path analysis was conducted using the “ecodist” package that quantifies the direct and indirect effects of soil chemical properties and plant community composition on microbial community composition (Liu et al. 2020; Zhou et al. 2022). Models were developed from the measurements and evaluated using structural equation modeling (SEM) using regression analysis and previous insights to identify the effects of soil chemical properties, plant productivity, and plant richness on microbial richness after nutrient addition. The complete model including the hypothetical relationships considered a priori is shown in Fig. S1. The a priori model was constructed according to results from previous studies (Bai et al. 2010; Leff et al. 2015; Li et al. 2023b; Ma et al. 2021; Wang et al. 2023; Xia et al. 2023). Model adequacy was determined using the chi-square ( $\chi^2$ ) test, the comparative fit index (CFI), and the root-mean-square error of approximation (RMSEA). The final model was improved by discarding non-significant pathways

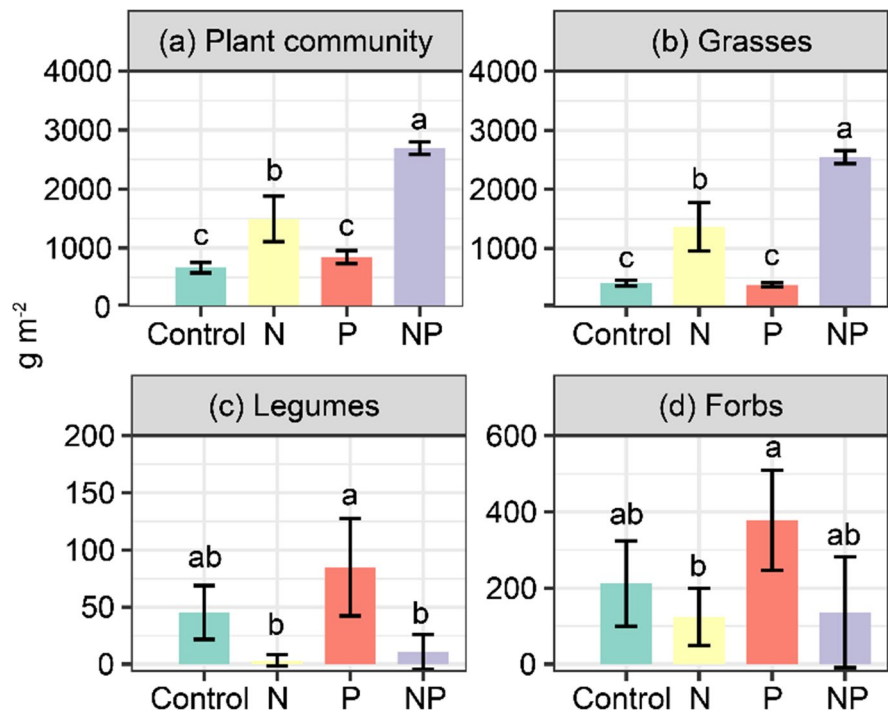
progressively from the *a priori* model based on the above indices. The SEM analysis was performed using the AMOS 22.0 software program (AMOS Development Corporation).

## Results

### Effects of N and P addition on plant aboveground biomass and microbial biomass

The biomass of plant and grass communities significantly increased after N and NP addition treatments when compared with the control, but there was no significant difference between the P addition treatment and the control (Fig. 1a, b; Table S3). In comparison with the control, plant community biomass was enhanced by 124.08% and 403.85% under N and NP addition treatments, respectively. Plant community biomass was closely related to grass biomass (Fig. S2a;  $R^2=0.97$ ,  $p<0.001$ ), and grass biomass contributed an average of 45.81–94.64% to the plant community biomass in different treatments. However, the N and NP addition treatments resulted in reduced legume and forb biomass. Furthermore, P addition treatment led to increased biomass of legumes

**Fig. 1** Effects of N and P addition on plant aboveground biomass. Biomass is shown for (a) plant communities, (b) grasses, (c) legumes, and (d) forbs. Error bars show standard deviations ( $n=4$ ). Different lowercase letters above each bar in the same panel indicate statistically significant differences between different treatments (Tukey’s HSD test,  $p<0.05$ )



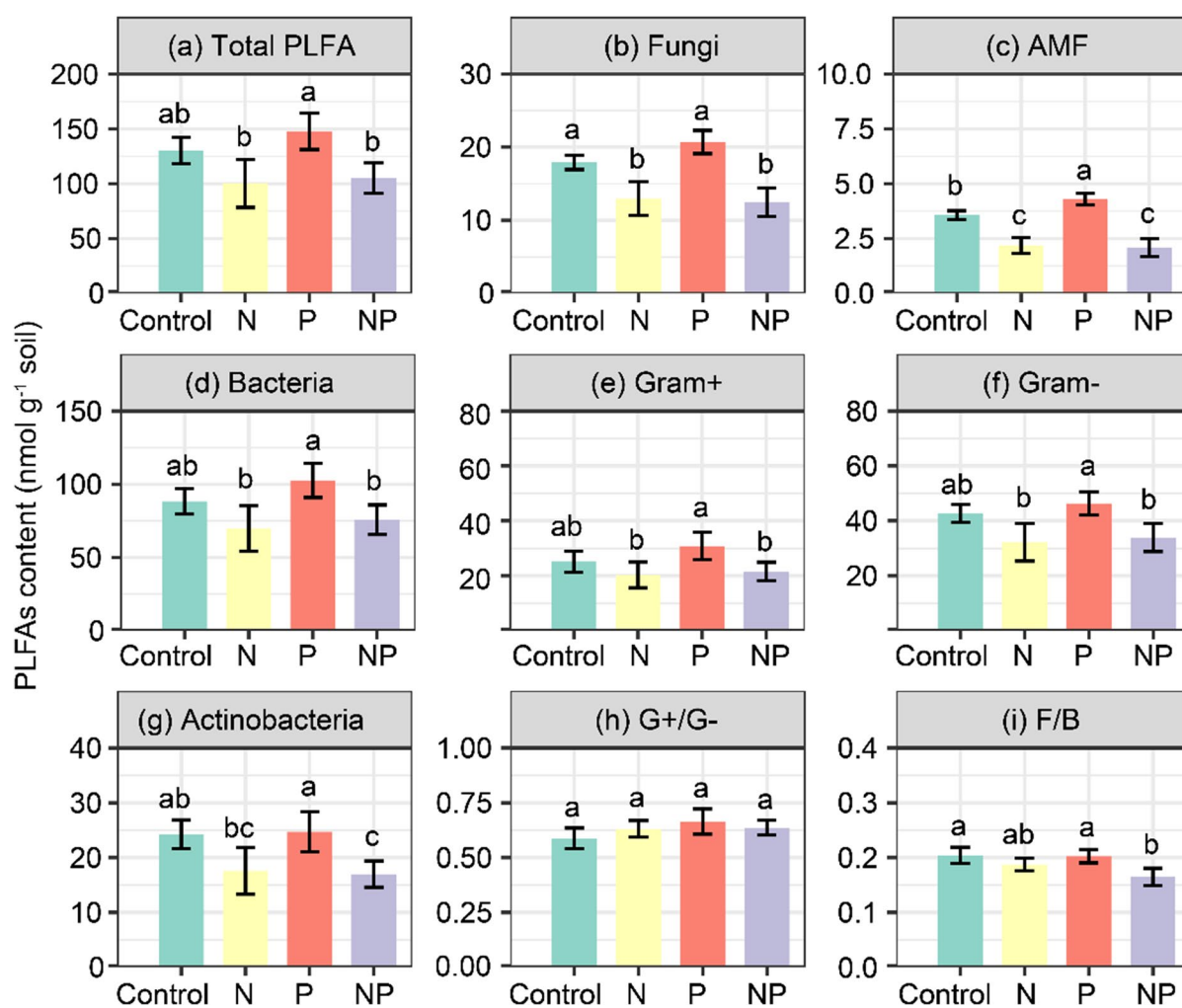
(87.81%) and forbs (78.46%) (Fig. 1c, d). In addition, a negative correlation was observed between plant community biomass and that of legumes (Fig. S2b;  $R^2=0.29$ ,  $p<0.05$ ).

Bacterial biomass, fungal biomass, and total microbial biomass increased significantly after N addition compared with the control, but did not change in the P addition relative to the control (Fig. 2; Table S3). The addition of N and NP also resulted in significantly lower total microbial biomass compared with the addition of P (Fig. 2). Furthermore, the addition of P had a significant positive effect on AMF biomass (Fig. 2c). However, when N was added

together with P, AMF biomass was significantly lowered (Fig. 2c). All nutrient addition treatments did not alter the G+/G- ratio, and the NP addition treatment led to a decreased fungi: bacteria ratio (Fig. 2i).

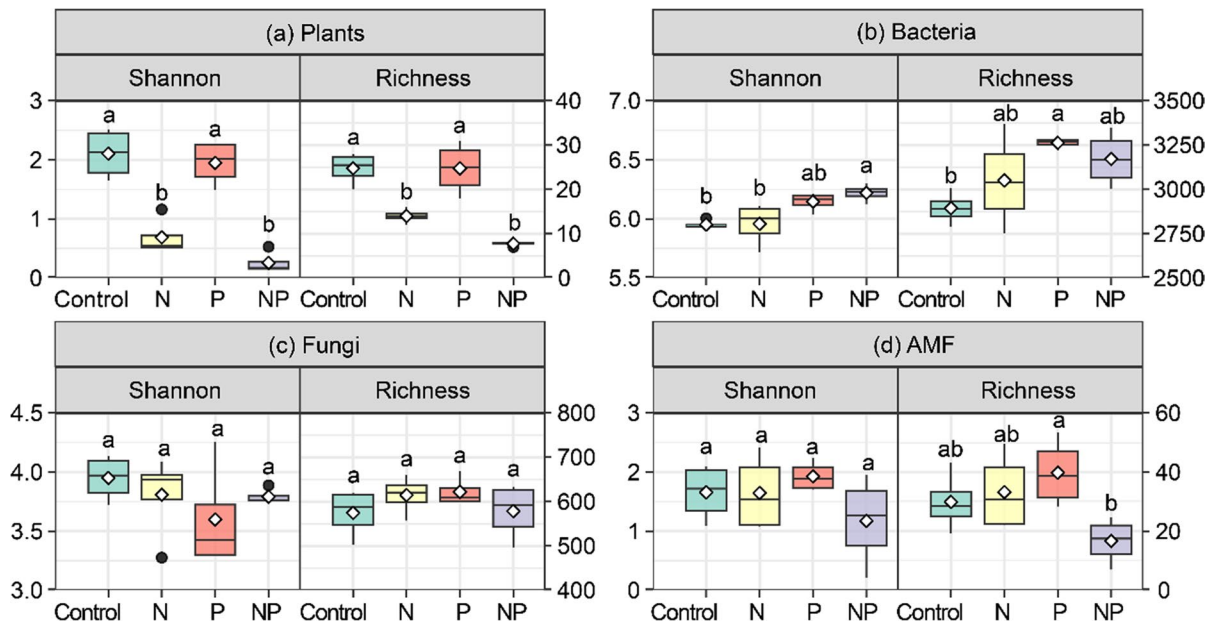
Effects of N and P addition on plant and soil microorganism  $\alpha$ -diversity and community composition

The addition of N and NP significantly decreased plant Shannon and richness indices (Fig. 3a; Table S3), but did not impact microbial diversity, including that of bacterial, fungal, and AMF



**Fig. 2** The effects of N and P addition on microbial biomass (a–g), the G+/G- PLFA ratio (h), and the F/B PLFA ratio (i). Error bars indicate standard deviations ( $n=4$ ). Different lower-

case letters above each bar in the same panel indicate statistically significant differences between treatments (Tukey's HSD test,  $p<0.05$ )



**Fig. 3** The effects of N and P addition on plant and soil microbial diversity. **a** Plant, **b** bacterial, **c** fungal, and **d** AMF diversity levels are shown. Species richness is represented by the Patrick index for plants and the Chao1 index for microbial communities. The box plots show the medians as solid line within boxes and means as hollow diamond within boxes, in

addition to the 25th and 75th percentiles as vertical bars. The upper and lower whiskers denote the maximum and minimum values, respectively. Different lowercase letters above each box in the same panel indicate statistically significant differences between treatments (Tukey's HSD test,  $p < 0.05$ )

communities alone after N addition (Fig. 3b, c, d; Table S3). NP addition also led to a significantly increased bacterial community Shannon index, while P addition alone significantly increased bacterial richness (Fig. 3b). In comparison, the Shannon and richness indices of fungal and AMF communities did not exhibit a significant response to N and P addition but did exhibit such a response to NP addition wherein AMF communities exhibited significantly reduced richness (Fig. 3d;  $p < 0.05$ ). PERMANOVA tests revealed that all nutrient addition treatments (N, P, and NP) altered plant and soil microbial (bacteria, AMF, and fungi) community structures, with the exception of non-significant changes in AMF communities after P addition (Fig. 4; Table S4).

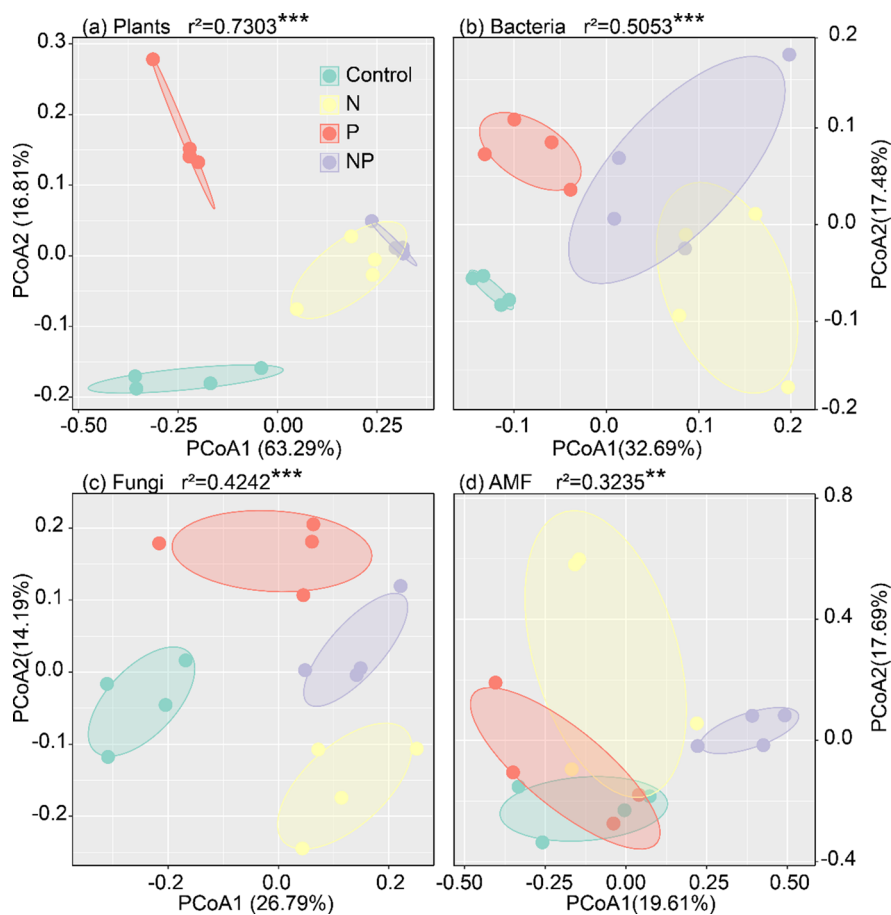
#### Association of soil microbial diversity and community structures with plant communities and soil properties

The relationships among microbial genera, plants, and soil properties were evaluated (Fig. 5; Table S6). Soil pH was the largest node (degree: 20) among

analyzed soil properties and exhibited a significantly negative correlation with multiple bacterial genera, most of which have been identified as acidophilic taxa. *L. chinensis* was the largest node (degree: 10) among all plant species, with half of the genera exhibiting significant correlations being *Proteobacteria*, and all of them exhibiting a positive correlation with *L. chinensis* presence. Overall, positive correlations (85.15%) dominated the network of associations among microbial genera, plants, and soil properties.

The contributions of soil properties and vegetation variables to variation in bacterial, fungal, and AMF community composition were quantified by Mantel and partial-Mantel tests (Fig. 6a). Mantel analysis indicated that AGB exerted the most significant influence on plant ( $r_M = 0.778$ ,  $p = 0.001$ ), fungal ( $r_M = 0.547$ ,  $p = 0.001$ ), and AMF ( $r_M = 0.498$ ,  $p = 0.001$ ) communities. Soil pH ( $r_M = 0.770$ ,  $p = 0.001$ ) was the best predictor of bacterial community composition. In addition, bacterial and fungal community composition were significantly associated with plant community composition after accounting for AGB and all soil properties. VPA demonstrated

**Fig. 4** Beta diversity (community composition) of plant (a), soil bacterial (b), soil fungal (c), and soil AMF communities (d) in response to N, P, and NP addition treatments. Community structural differences were assessed by principal coordinates analysis (PCoA) based on Bray–Curtis dissimilarity distances. The effects of N, P, and NP addition treatments on community structures were assessed using permutational analysis of variance (PERMANOVA) tests. Asterisks (\*, \*\*, and \*\*\*) following  $r^2$  values indicate statistical significance at  $p \leq 0.05$ ,  $\leq 0.01$ , and  $\leq 0.001$ , respectively



that the amount of variation captured by environmental and vegetation variables was 46.9%, 34.9%, and 21.6% for bacterial, fungal and AMF communities, respectively (Fig. 6b). Interactions between soil properties and vegetation variables were significantly associated with bacterial and fungal communities, but not AMF communities (Fig. 6b). In addition, a higher percentage of vegetation (9.39%) than environmental factors (5.42%) explained fungal community variation, while vegetation factors (10.38% and 10.10%) were equally as important as soil properties (11.39% and 11.43%) for bacterial and AMF communities, respectively.

SEM demonstrated that changes in plant and microbial richness caused by N and/or P additions were primarily attributed to changes in nutrient availability and aboveground biomass (Fig. 6c). AGB significantly increased with nutrient availability. Variation in AMF richness was roughly equally associated with AP, DIN and AGB. The addition of P positively

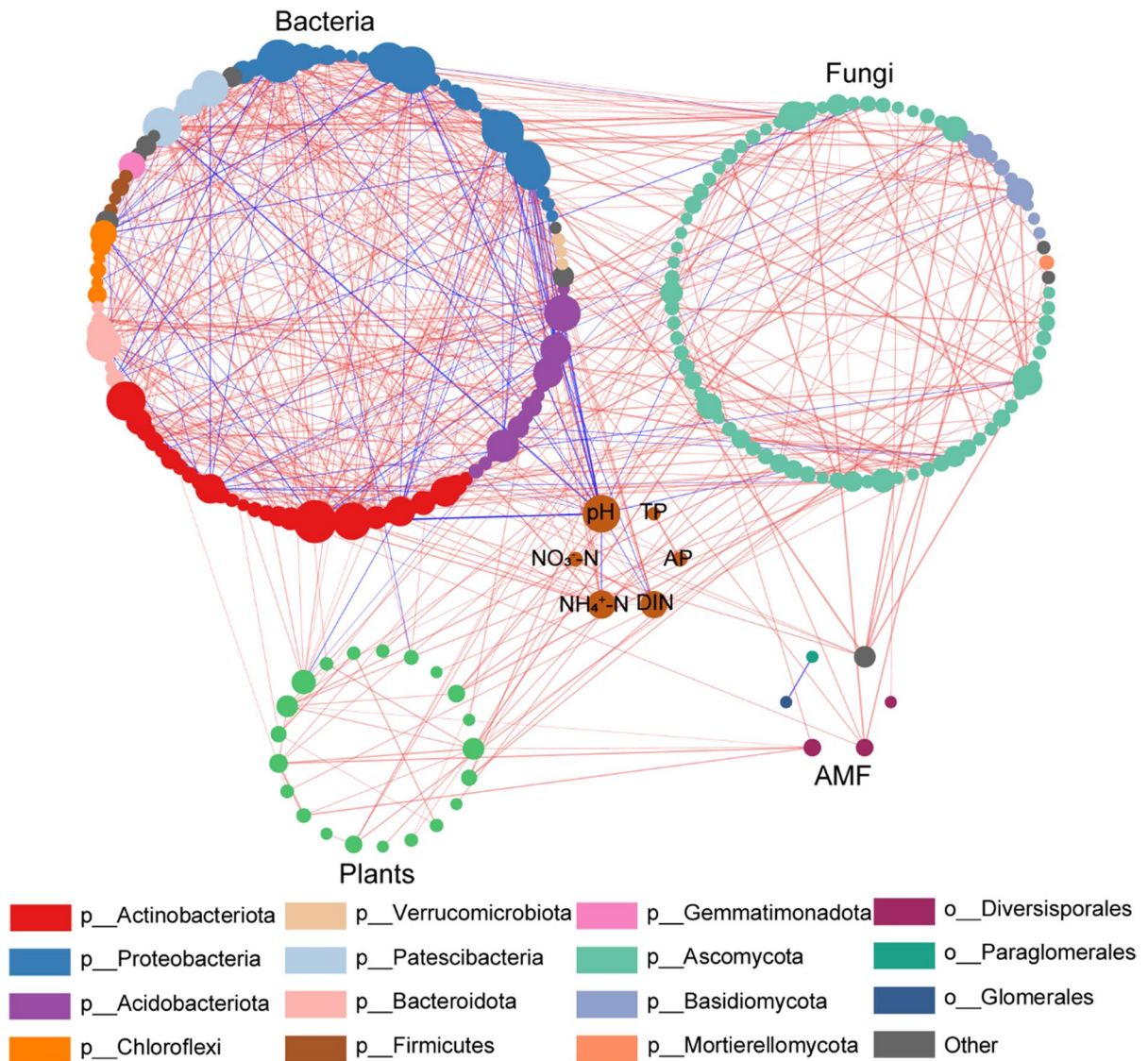
influenced bacterial richness and led to increased soil P availability. In addition, soil fungal richness was primarily associated with DIN.

## Discussion

The effect of N and P addition on plant biomass, diversity, and community structures

Here, N and P addition were shown to shape plant diversity and community structure by leading to increased biomass of specific plant functional groups, consistent with our hypothesis. Long-term N and NP addition over 11 years led to enhanced plant aboveground biomass via increased biomass of grasses like *L. chinensis*, but decreased plant species diversity, leading to reduced biomass of legumes and forbs (Fig. 1; Table S1). These results are consistent with multiple previous studies of temperate



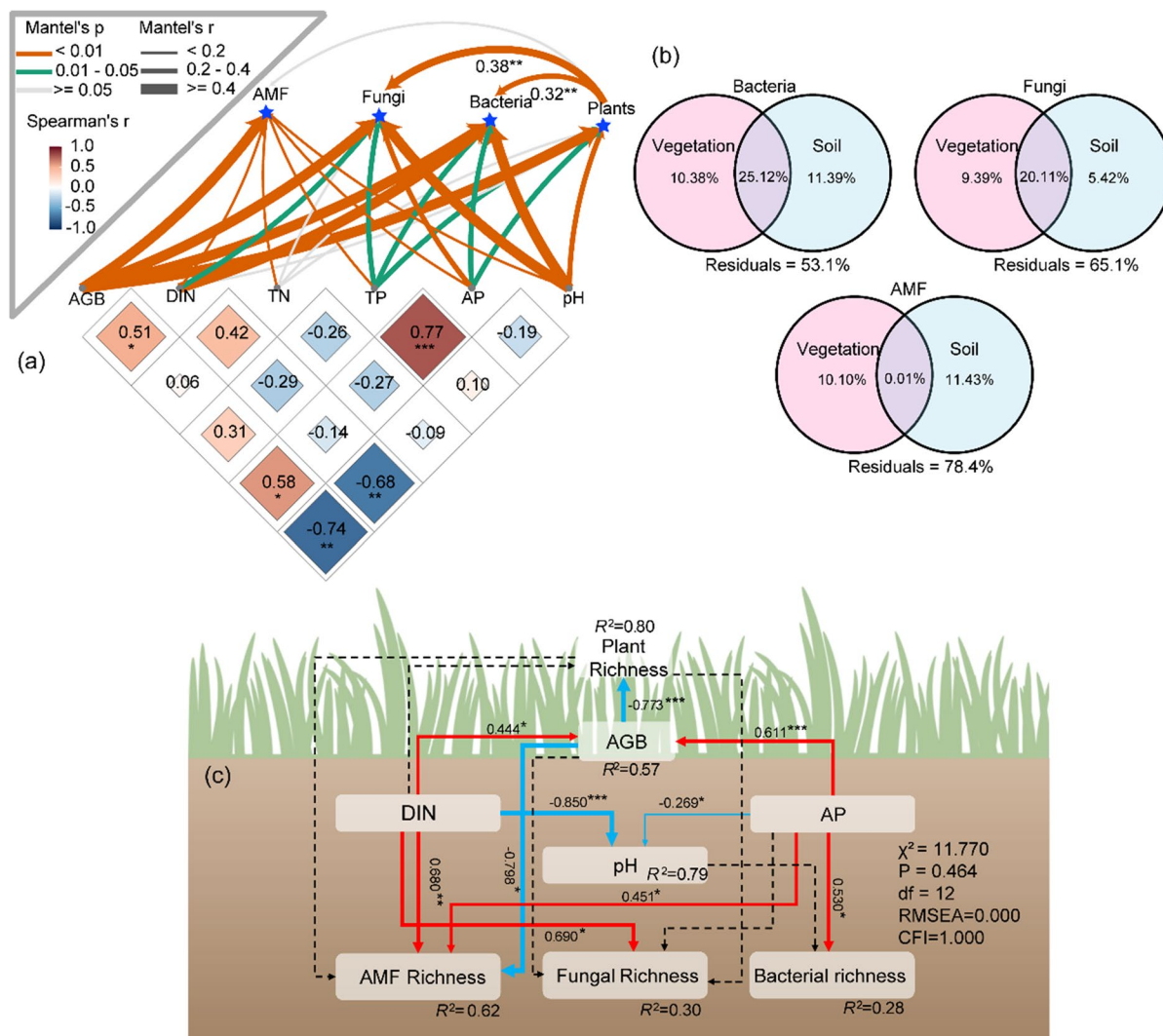


**Fig. 5** Network analysis describing the relationships among the distribution of microbial (bacterial, non-mycorrhizal fungal and AMF) genera, plants species, and soil properties. The overall network was visualized significant associations ( $R^2 > 0.80$ ,  $p < 0.05$ ) of AMF, bacterial, and non-mycorrhizal fungal operational taxonomic units (genera), plant species, and

soil properties. Each node represents a microbial genus or soil property, and the color indicates different microbial taxa, while the node size is proportional to node connectivity. Red lines indicate positive correlations and purple lines indicate negative correlations

steppe ecosystems (Bai et al. 2010; Zhao et al. 2019). Mechanisms underlying the loss of plant diversity induced by N addition have been widely investigated and include soil acidification (Horswill et al. 2008), interspecific differences in resource-use strategies, and shoot competition for light among different plant species (Borer et al. 2014; He et al. 2016; Li et al. 2023a). In a previous experiment conducted in the

same grassland that involved N and P addition, N addition over three years led to significantly increased biomass of multiple grasses (e.g., *L. chinensis* and *S. baicalensis*), while the responses of other species to N addition were mixed (Yu et al. 2015). In this study, the biomass of *L. chinensis* accounted for over 80% of the total aboveground biomass after 11 years of N addition, while many legume and forb species even



**Fig. 6** The effects of environmental factors and plant properties on microbial richness and community structure after nutrient addition. **a** Mantel path analysis linking taxonomic composition of microbial (bacterial, fungal, and AMF) communities to soil chemical attributes and plant communities. **b** VPA was used to identify the relative contributions of soil factors and vegetation properties to microbial (bacterial, fungal, and AMF) communities. **c** Structural equation modeling (SEM) analysis of the effects of N and P addition on soil microbial

(bacterial, fungal, and AMF) richness via pathways related to soil chemical attributes and plant richness. Red and blue solid arrows connecting the boxes represent significant positive and negative effects ( $p < 0.05$ ), respectively. Pathways without a significant effect are indicated by broken lines ( $p > 0.05$ ). Values close to variables refer to the variance accounted for by the model (as  $R^2$  values). Values associated with the arrows represent standardized path coefficients

disappeared (Table S1). Long-term nutrient addition shifted the dominance of the plant community by several plants to single dominance by *L. chinensis*. *L. chinensis* can quickly absorb added N due to its strong root system and high plasticity of N acquisition strategies (Li et al. 2023a) that accelerates the growth of *L. chinensis* during the early stages of N addition

(Zhao et al. 2019). However, the addition of N alleviates nutrient limitation in grassland soils, potentially shifting the limiting factors for plant growth to other resources (e.g., mineral P and light). Consequently, *L. chinensis* was the dominant canopy species within plant communities (Li et al. 2023a) and exhibited significantly increased biomass and cover due to

long-term N addition that reduced light availability for low-lying plants and resulted in further decreases in species diversity (Borer et al. 2014; Hautier et al. 2009; Suding et al. 2005). Moreover, decreased plant species richness may be attributed to acidification induced in the early stages of N addition (Horswill et al. 2008).

The addition of P in this study did not affect grass biomass. A possible reason for this could be that N is the primary limiting nutrient in temperate steppe environments and adding P alone minimally affects grass species, especially *L. chinensis*. P addition also largely increased legume and forb biomass. This effect can be attributed to the improved availability of P resources and enhanced nutrient use efficiencies in legumes and forbs, thereby indirectly increasing their competitive advantage over grasses species (Bi et al. 2019; Ren et al. 2017; Zhao et al. 2019). In addition, this indicates that P is a limiting factor in the temperate steppe and P addition might alleviate P limitation of plant growth (Zhao et al. 2019). The addition of P alone did not exert any influence on plant diversity (Fig. 3a) but resulted in significant shifts of plant community structures (Fig. 4a) that are consistent with an earlier study of 7-year nutrient additions (Yan et al. 2022). P addition alone may shape plant community structure by increasing legume and forb biomass, without altering plant diversity, which is partially consistent with our first hypothesis. Significant changes in plant community structure resulting from P addition alone may be attributed to changes in the biomass of non-dominant plant species like *Astragalus laxmannii*, *Artemisia stechmanniana*, and *Thermopsis lanceolata*. However, the addition of P alone did not alter grass biomass that serves as the canopy species within the communities (Li et al. 2023a). Consequently, changes in height asymmetry would not be generated among plant functional groups (Guo et al. 2022), and influences on species diversity would not be apparent.

Furthermore, the results from this study suggest that above-ground biomass under long-term NP addition treatment was significantly higher than in the N addition alone. *L. chinensis* was the only gramineous plant in the NP addition treatment that contributed to over 90% of the total above-ground biomass. The NP addition treatment also led to decreased biomass of legumes and forbs at a magnitude comparable to that of N addition alone (Fig. 1). The responses of

aboveground biomass to N and P addition were consistent with previous studies, indicating a synergistic effect of NP addition on aboveground biomass in temperate steppe ecosystems (Schleuss et al. 2020; Vázquez et al. 2023). Thus, NP addition enables plants to overcome growth limitations caused by N and P deficiency in temperate steppe ecosystems, further increasing aboveground biomass compared with when only N is added (Vázquez et al. 2023).

#### The effect of N and P addition on microbial diversity

Unlike plants, long-term N addition reduced soil microbial biomass (Fig. 2), although the diversity of bacteria, fungi, and AMF did not decrease (Fig. 3). These results are consistent with those reported by Wang et al. (2023). The latter study proposed a pattern comprising an initial decrease and subsequently increasing responses of microbial richness to N addition over an experimental duration exceeding 10 years, indicating microbial adaptation to long-term N additions.

The addition of phosphorus significantly increased the Shannon and richness indices of soil bacteria in this study (Fig. 3b). This could be due to the crucial role of P in the growth and metabolism of microorganisms, including the synthesis of nucleotides and regulation of enzyme activity (Elser et al. 2003; Xia et al. 2023). Consequently, bacteria with relatively higher growth rates may enhance their competitive advantage over other microorganisms in P-amended grassland systems when facing environmental stress. This supposition is also supported by microbial biomass observations that demonstrate significant decreases in the biomass of both bacteria and fungi after N additions, while the fungi: bacteria ratio significantly decreased after NP addition treatment (Fig. 2). Almost no significant response in soil fungal richness to the addition of nutrients was observed in this study (Fig. 3c), in contrast to results from a previous experiment in a temperate meadow, wherein N and P addition caused increased soil fungal richness (Yan et al. 2022). These trends can be attributed to the temporal variability of fungal richness in response to nutrient additions. An alternative explanation is that the moderate rate of nutrient addition employed in this study, in contrast to others with high nutrient application rates (Kim et al. 2015; Porras-Alfaro et al. 2007), may allow fungal communities to react

to long-term nutrient additions without impacting fungal richness. Moreover, multiple studies have indicated that fungal diversity is less sensitive to nutrient addition compared with soil bacterial diversity (Wang et al. 2023).

Although the total fungal diversity did not significantly change with nutrient addition in this study, AMF diversity was significantly and positively associated with nutrient addition. Multiple different responses of AMF to nutrient additions have been observed, with N and P addition potentially increasing (Camenzind et al. 2016), decreasing (Leff et al. 2015), or having no effect (Duenas et al. 2020) on AMF diversity. The various impacts of N and P addition on AMF diversity may be jointly regulated by soil and plant properties in different ecosystems (Ma et al. 2021; Van Geel et al. 2018). In this study, NP addition significantly reduced AMF richness compared with P addition alone (Fig. 3d), consistent with many previous studies (Egerton-Warburton et al. 2007; Johnson 2010; Lekberg et al. 2021; Ma et al. 2021). The mutualistic symbiotic associations formed by AMF with most plants allow them to uptake carbon (C) from host plants and also facilitate nutrient uptake in host plants (Kiers et al. 2011). In particular, the C-for-P trade is a key aspect of symbiosis between plants and mycorrhizal fungi (Johnson 2010). In this study, the improvement in soil nutrient effectiveness in P-rich soils following NP addition usually led to increased plant biomass. The direct absorption of P from the soil by roots is preferential for plants due to its lower cost compared with AMF uptake, supporting the trade balance model prediction (Johnson 2010; Zheng et al. 2022). In addition, AMF are critical subterranean symbionts associated with plants, and the decrease in diversity and increase in plant biomass under NP addition treatment could result in the “homogenization” of mycorrhizal environments (Hooper et al. 2000; Ma et al. 2021), potentially leading to lower AMF diversity. As suggested by the SEM, AGB was the most important predictor of AMF richness (Fig. 6c).

The linkages between plant and microbial communities under N and P addition treatments

The responses of plants and soil microbial communities to soil nutrient availability are diverse and complex (Liu et al. 2021). Most studies investigating the

impacts of nutrient enrichment on species community composition have primarily focused on aboveground organisms and neglected belowground microbial communities (Chalcraft et al. 2008; Conradi et al. 2017; Seabloom et al. 2021). Here, the impacts of N and P additions on plant and soil microbial community structures were evaluated in addition to assessing the contributions of vegetation and soil properties on changes in soil microbial communities under long-term nutrient addition conditions in temperate steppes for the first time.

Nutrient addition significantly altered the vegetation community structures of grassland. Long-term N and NP addition shifted the dominance of the plant community by multiple species to sole dominance by *L. chinensis*, while long-term P addition increased the importance of legumes and forbs in the grasslands (Bi et al. 2019; Ren et al. 2017). Nutrient addition significantly altered the soil microbial communities in this study, except for when P was added alone, which did not significantly affect soil AMF communities (Fig. 4). Differences in the dominant microbial taxa also serve as an indicator of microbial community changes caused by nutrient additions. Significant shifts have been observed for multiple dominant bacterial taxa after long-term N addition due to the greater sensitivity of bacteria to nutrient enrichment relative to fungi (Leff et al. 2015; Rousk et al. 2010; Wang et al. 2023). For example, N addition promoted the growth of copiotrophic bacteria (e.g., *Proteobacteria*, *Firmicutes*, and *Bacteroidota*), but inhibited the growth of oligotrophic bacteria (e.g., *Acidobacteria*, *Chloroflexi*, and *Verrucomicrobiota*) (Table S5). However, N addition did not significantly affect dominant fungal taxa in soils. In addition, many soil microbial taxa were oppositely effected by P and N additions alone (Leff et al. 2015), although the divergent changes caused by single additions can be alleviated or counterbalanced when N and P are added together.

The compositions of microbial communities can be influenced by both nutrient addition-induced changes in environmental factors and plant communities. Soil nutrient availability and soil pH were significantly associated with the functional and taxonomic groups of both plant and microbial communities. Soil pH is frequently reported to affect microbial community composition, because it directly limits or constrains the physiological activities of microorganisms

(Zeng et al. 2016; Zhou et al. 2020). However, lowering soil pH could potentially elevate the abundance of acidophilic bacteria. This process was supported by the network analysis in this study that demonstrated a significant negative correlation between soil pH and multiple bacterial genera (Table S6), with the majority of these genera being acidophilic bacteria (Högfors-Rönholm et al. 2023; Pankratov and Dedysch 2010; Shu et al. 2023; van den Heuvel et al. 2010; Wolinska et al. 2018; Yang et al. 2022a). Concomitantly, only two fungal genera exhibited significant correlations of their relative abundance with soil pH, suggesting that soil bacteria are more sensitive to changes in pH caused by nutrient additions relative to soil fungi. Partial Mantel tests were also used in this study to evaluate the importance of plant communities in shaping microbial communities. The results suggested that bacterial and non-mycorrhizal fungal communities were still strongly associated with plant community compositions, even when considering the influences of AGB and soil properties (Fig. 6a). This indicates that plant and microbial communities are closely synchronized under long-term nutrient addition (Liu et al. 2020; Zhang et al. 2017). Numerous significant correlations were also observed between plant species and microbial genera based on the network analysis of this study. These results suggest that microbial diversity and community composition may be strongly altered by soil properties (e.g., nutrient availability and pH) and plant community characteristics under long-term N and P addition, which supports our second hypothesis. For example, the distributions of multiple microbial genera were significantly correlated with *L. chinensis* distributions, with genera belonging to the phylum *Proteobacteria* exhibiting significant positive correlations (Fig. 5; Table S6). These results are consistent with a previous study that indicated that the restoration of degraded *L. chinensis* steppe increased the abundance of soil *Proteobacteria* (Yao et al. 2018a). In addition, multiple plant species, including legumes, forbs, and non-dominant grasses, exhibited significant positive correlations solely with fungi (Table S6). These results potentially reflect that plant functional groups and traits mediate changes in microbial microenvironments and carbon resources (Chen et al. 2021; Liu et al. 2020). AMF communities exhibited higher sensitivity to aboveground biomass than to plant community composition. This could be attributed to long-term nutrient addition

enhancing soil nutrient availability, thereby leading to the allocation of more plant biomass aboveground leading to light competition and resulting in changes in the C-for-P trade between plants and AMF, which is the main factor that shapes AMF community structures (Johnson 2010; Zheng et al. 2022). In addition, plant–mycorrhizal interactions were suggested to have crucial roles in plant–microbe correlations, and this impact has been also demonstrated by field-based investigations (Huang et al. 2023; Lastovetsky et al. 2022; Liao et al. 2023).

Overall, plants may mediate soil microbial communities in at least three ways. (1) First, regulation may occur via the synchrony between plant communities and microbial communities (Liu et al. 2020; Zhang et al. 2017). (2) Second, regulation may occur by providing microhabitats and regulating carbon resources (Chen et al. 2021; Liu et al. 2020). (3) Lastly, these interactions may occur via plant–mycorrhizal interactions (Lastovetsky et al. 2022; Li et al. 2020). To rigorously differentiate the effects of plant communities and environmental factors on soil microbial communities, further empirical experiments involving the manipulation of specific host plant species (Schlatter et al. 2015) or plant community assemblies (Liu et al. 2020) under standardized environmental conditions are necessary. Such studies will further highlight the importance of plant–microbe interactions.

## Conclusions

In this study, N addition to a steppe system experimental plot induced changes in plant community structures and diversity by significantly increasing grass biomass, while P addition alone only changed plant community structures primarily due to increases in legume and forb biomass. Meanwhile, NP addition further promoted *L. chinensis* growth but severely undermined the growth advantage of legumes and forbs, thereby exacerbating negative impacts on species diversity. Consequently, long-term N addition shifted the dominance of the plant community by multiple species to a sole *L. chinensis* dominated community. Furthermore, these results revealed a positive relationship between increased bacterial diversity and improved soil P availability. However, changes in bacterial community composition could be largely explained by aboveground biomass and plant

community composition. Despite total fungal diversity was insensitive to N and P addition, increased nutrient availability led to increased aboveground biomass that then influenced the C-for-P trade interactions between plants and mycorrhizal fungi, consequently inducing changes in AMF communities. In addition, the co-occurrence patterns of soil properties, microbial genera distributions, and plants provides insights into the potential relationships between plant functional groups and dominant microbial taxa. Overall, the close synchrony between plant communities and soil microbial communities, as well as the significant relationships between plant functional groups and dominant microbial taxa, emphasizes the important roles of plant-mediated effects on microbial communities and provide new insights into the responses of plant–microbial interactions to long-term N and P deposition.

**Acknowledgements** We thank LetPub ([www.letpub.com](http://www.letpub.com)) for its linguistic assistance during the preparation of this manuscript.

**Funding** This work was supported by the National Key Research and Development Program of China (2023YFF1304102) and National Natural Science Foundation of China (42007046; 41877343).

**Data availability** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

#### Declarations

**Competing interests** The authors have no relevant financial or non-financial interests to disclose.

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