Responses of plant diversity and soil microbial diversity to nitrogen addition in the desert steppe, China Postprint

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Date: 2024-03-13T00:00:00+00:00

Abstract

Nitrogen (N) deposition is a significant aspect of global change and poses a threat to terrestrial biodiversity. The impact of plant-soil microbe relationships to N deposition has recently attracted considerable attention. Soil microorganisms have been proven to provide nutrients for specific plant growth, especially in nutrient-poor desert steppe ecosystems. However, the effects of N deposition on plant-soil microbial community interactions in such ecosystems remain poorly understood. To investigate these effects, we conducted a 6-year N-addition field experiment in a Stipa breviflora Griseb. desert steppe in Inner Mongolia Autonomous Region, China. Four N treatment levels (N0, N30, N50, and N100, corresponding to 0, 30, 50, and 100 kg N/(hm2 • a), respectively) were applied to simulate atmospheric N deposition. The results showed that N deposition did not significantly affect the aboveground biomass of desert steppe plants. N deposition did not significantly reduce the alfa-diversity of plant and microbial communities in the desert steppe, and low and mediate N additions (N30 and N50) had a promoting effect on them. The variation pattern of plant Shannon index was consistent with that of the soil bacterial Chao1 index. N deposition significantly affected the beta-diversity of plants and soil bacteria, but did not significantly affect fungal communities. In conclusion, N deposition led to coevolution between desert steppe plants and soil bacterial communities, while fungal communities exhibited strong stability and did not undergo significant changes. These findings help clarify atmospheric N deposition effects on the ecological health and function of the desert steppe.

Full Text

Journal of Arid Land (2024) 16(3): 447–459
https://doi.org/10.1007/s40333-024-0008-0
Science Press Springer-Verlag

Responses of plant diversity and soil microorganism diversity to nitrogen addition in the desert steppe, China

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Abstract: Nitrogen (N) deposition is a significant aspect of global change that poses a threat to terrestrial biodiversity. The impact of plant-soil microbe relationships on N deposition has recently attracted considerable attention. Soil microorganisms provide nutrients for specific plant growth, especially in nutrient-poor desert steppe ecosystems. However, the effects of N deposition on plant-soil microbial community interactions in such ecosystems remain poorly understood. To investigate these effects, we conducted a 6-year N-addition field experiment in a *Stipa breviflora* Griseb. desert steppe in Inner Mongolia Autonomous Region, China. Four N treatment levels (N0, N30, N50, and N100, corresponding to 0, 30, 50, and 100 kg N/($hm^2 \cdot a$), respectively) were applied to simulate atmospheric N deposition. The results showed that N deposition did not significantly affect aboveground biomass of desert steppe plants. N deposition also did not significantly reduce alpha-diversity of plant and microbial communities; in fact, low and moderate N additions (N30 and N50) had a promoting effect. Plant Shannon index variation paralleled soil bacterial Chao1 index patterns. N deposition significantly affected beta-diversity of plants and soil bacteria, but did not significantly affect fungal communities. In conclusion, N deposition led to co-evolution between desert steppe plants and soil bacterial communities, while fungal communities exhibited strong stability without significant changes. These findings help clarify atmospheric N deposition effects on ecological health and function of the desert steppe.

Keywords: soil microorganisms; plant-microbial community interaction; plant diversity; nitrogen deposition; desert steppe

Citation: YE He, HONG Mei, XU Xuehui, LIANG Zhiwei, JIANG Na, TU Nare, WU Zhendan. 2024. Responses of plant diversity and soil microorganism diversity to nitrogen addition in the desert steppe, China. *Journal of Arid Land*, 16(3): 447–459. https://doi.org/10.1007/s40333-024-0008-0

1 Introduction

Desert steppes are unique transitional ecotones located between steppe and desert regions in Eurasia [**?**, **?**]. The desert steppe of Inner Mongolia Autonomous Region, China has a simple plant community structure with limited

water resources and poor soil nutrients, making it a fragile ecosystem especially under global climate change [?]. In recent years, climate change has led to increasing atmospheric nitrogen (N) deposition in China [?], with average N deposition estimated at 20.4 ± 2.6 kg N/($hm^2 \cdot a$) during 2011–2015 [?]. N deposition is considered the third greatest threat to global terrestrial biodiversity [?]. Previous studies show that atmospheric N deposition negatively affects plant and soil microbial community diversity in terrestrial ecosystems [?, ?]. Biodiversity is inherently precious and bolsters ecosystem functioning, providing reliable ecosystem services [?, ?, ?]. Understanding biodiversity responses to long-term N deposition in desert steppe ecosystems is therefore urgent [?].

N deposition typically increases plant productivity but decreases plant diversity, with some species becoming rare and even facing extinction [?, ?, ?]. N deposition affects plants through multiple factors, including nutrient imbalances and interspecific competition [?]. Plant community shifts do not occur in isolation; changes in plant composition can influence soil bacterial and fungal communities through cascade effects [?, ?]. Conversely, soil microbial communities influence plant fitness through decomposition, nutrient cycling, and nutrient acquisition [?, ?]. Plant-soil microbial interactions are so intertwined that they can be considered as a single entity subjected to environmental changes and selection [?, ?, ?]. Plant productivity correlates positively with soil microorganism proportions [?]. Main microbial taxa are highly connected, exerting considerable influence on microbiome structure and functioning individually or in guilds, regardless of spatial or temporal abundance [?]. These microbial species often coexist and form clusters in microbial ecological networks that provide nutrients for specific plant growth, especially in nutrient-poor desert steppe ecosystems [?].

N is essential for plant growth, and plants and microbes compete for soil N. Increased competition can decrease soil N availability and microbial activity [?, ?, ?]. For instance, increased N can depress soil microbial activity by altering bacterial community metabolic capabilities [?]. Accumulated evidence suggests N deposition reduces microbial biomass and changes community composition across diverse ecosystems [?, ?]. Increased N availability reduces fungal biomass via changes in plant-specific exudates and altered nutrient competition between plants and rhizosphere microbes [?, ?]. Plant-soil microorganism feedbacks and shifts in feedback effects associated with microbial community composition affect plant coexistence and community composition [?, ?, ?]. Although plant-soil microorganism interactions have been widely studied, little research addresses how belowground microbial communities influence plant biomass in nutrient-poor desert steppes [?, ?, ?, ?]. The mechanisms by which microbial communities affect plant biomass remain unknown. We established a 6-year simulated N deposition experiment in the desert steppe region of Inner Mongolia Autonomous Region, northern China, to evaluate plant-soil-microbiome interactions under N deposition by studying plant community productivity, soil physical-chemical properties, and soil microbial groups and their roles.

2.1 Experimental Design

A field experiment was conducted in Siziwang Banner (41°46 43 N, 111°53 41 E; 1456 m a.s.l.), an arid area in Inner Mongolia Autonomous Region, northern China. Average annual precipitation is 280 mm, with approximately 70% falling during the growing season (May–October). The annual average temperature is 3.4°C. Study area soils have sandy loam texture and are classified as Haplic Calcisols by the FAO soil classification system. The plant community is dominated by *Stipa breviflora* Griseb., *Neopallasia pectinata* (Pall.) Poljak., *Artemisia scoparia* Waldst. et Kit., *Kochia prostrata* (L.) Schrad., and *Cleistogenes songorica* (Roshev.) Ohwi.

Long-term simulated N deposition experiments were established in December 2015 with four treatments: control (N0, no N addition), low N addition (N30, 30 kg N/($hm^2 \cdot a$)), moderate N addition (N50, 50 kg N/($hm^2 \cdot a$)), and high N addition (N100, 100 kg N/($hm^2 \cdot a$)). To mirror natural seasonal N deposition patterns from May to September, we dissolved NH_4NO_3 in purified water (10.0 L per plot; N0 plots received only water) and sprayed it evenly using a sprayer to simulate wet deposition. From October to April, NH_4NO_3 was mixed with soil (1.0 kg sand per plot; N0 plots received only sand) and broadcast by hand to simulate dry deposition. N was applied monthly at the beginning of each month, with application rates determined by the percentage of average monthly precipitation from the previous 5 years relative to total annual precipitation. Experiments followed a randomized block design with four replicate blocks. Each plot measured 7.0 m × 7.0 m, separated by 1-m intervals.

2.2 Plant, Soil, and Soil Microbial Sampling

In August 2021, at peak growing season, we randomly placed three 0.5 m × 0.5 m subplots in each plot, positioned parallel to but at least 1 m from plot edges. We harvested all aboveground plant parts, sorted by species, recorded species richness and abundance (number of individuals), and dried plants at 65°C for 48 h to measure aboveground biomass (AGB) for each species. Soil samples were collected using the five-point method to 10 cm depth. After removing roots and stones and gently mixing, each sample was placed in a sterile plastic bag. One portion was used to determine soil physical and chemical properties including pH, total nitrogen (TN), soil organic carbon (SOC), NH_4^+-N, and NO_3^--N contents [**?**], while the other was used for DNA extraction.

2.3 Soil Microbial DNA Extraction and Sequencing

Microbial community genomic DNA was extracted from 0.5 g soil using a Soil DNA Purification Mini Kit (Omega Bio-Tek, Norcross, USA) following manufacturer instructions. DNA extracts were checked on 1% agarose gel, and concentration and purity were determined with a NanoDrop 2000 UV spectrophotometer (Thermo Scientific, Wilmington, USA).

Soil bacterial and fungal community composition and diversity were determined through amplicon surveys of 16S and ITS (Internal Transcribed Spacer) rRNA. The V3-V4 hypervariable regions of the 16S rRNA gene were amplified using primers 338F (5' -ACTCCTACGGGAGGCAGCAG-3') and 806R (5' -GGACTACHVGGGTWTCTAAT-3'). The ITS1 region of fungal rRNA was amplified using primers ITS1F (5 -CTTGGTCATTTAGAGGAAGTAA-3) and ITS2R (5 -GCTGCGTTCTTCATCGATGC-3).

Polymerase chain reaction (PCR) amplification proceeded as follows: initial denaturation at 95°C for 3 min; 27 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 45 s; final extension at 72°C for 10 min; and terminal hold at 4°C. PCR mixtures contained 5×TransStart FastPfu buffer (4 L), 2.5 mM dNTPs (2 L), 5 M forward primer (0.8 L), 5 M reverse primer (0.8 L), TransStart FastPfu DNA polymerase (0.4 L), 10 ng template DNA, and ddH_2O to 20 L. PCR tests were performed in triplicate. Products were extracted from 2% agarose gel, purified using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, USA), and quantified with a Quantus™ Fluorometer (Promega, Madison, USA).

Purified amplicons were pooled in equimolar amounts and paired-end sequenced on an Illumina MiSeq PE300/NovaSeq PE250 platform (Illumina, San Diego, USA) following standard protocols by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). Raw reads were deposited in the NCBI Sequence Read Archive (SRA) database (PRJNA799672).

Raw 16S and ITS rRNA gene sequencing reads were de-multiplexed and quality-filtered using FASTP v.0.20.0 [**?**] and merged using FLASH v.1.2.7 [**?**] with these criteria: 300-bp reads were truncated at any site with average quality score <20 over a 50-bp sliding window; truncated reads shorter than 50 bp were discarded; reads containing ambiguous characters were discarded; only overlapping sequences longer than 10 bp were assembled with maximum mismatch ratio of 0.2 in the overlapping region; unassembled reads were discarded; samples were distinguished by barcode and primers with exact barcode matching and 2-nucleotide mismatches allowed in primer matching.

Operational taxonomic units (OTUs) were clustered at 97% similarity [**?**, **?**] using UPARSE v.7.1 [**?**], and chimeric sequences were identified and removed. OTU representative sequence taxonomy was analyzed using RDP Classifier v.2.2 [**?**] against 16S and ITS rRNA databases with a confidence threshold of 0.7.

2.4 Statistical Analysis

Microsoft Excel 2019 and R software were used for statistical analyses. All figures were created using Origin 2021 software. Soil microorganism alpha-diversity and redundancy analysis (RDA) were performed using the Majorbio Cloud Platform (https://cloud.majorbio.com/page/tools/). Significant differences among N-addition levels for plant biomass, diversity indices, and soil variables were determined using Tukey' s honestly significant difference test

(P<0.05). Nonmetric multidimensional scaling (NMDS) and permutational multivariate analysis of variance (PERMANOVA) were used to assess N deposition effects on plant and soil microbial community composition between treatments at the genus level based on Bray–Curtis distances.

3.1 Soil Physical-Chemical Properties, Plant Diversity, and Community Composition

N-addition treatments did not affect AGB, while plant community composition was significantly altered (P<0.01; Table 1 ; Fig. 1a [Figure 1: see original paper]). The N100 treatment significantly decreased biomass of perennial grass *S. breviflora* but increased biomass of annual and biennial *N. pectinata* compared with N0 (P<0.05; Table 1). N deposition did not significantly affect other plant biomass (Table 1). Perennial grass biomass gradually decreased with increasing N additions and was replaced by annuals and biennials (Fig. 1a). N deposition did not significantly affect biomass of perennial forbs, shrubs, and semi-shrubs (Fig. 1b).

Plant Shannon index was not significantly affected by N deposition, though it tended to increase under N30 and N50 treatments (Fig. 1c). The Shannon index under N100 showed a decreasing trend compared with N0, but did not reach significance (Fig. 1c). However, Shannon index under N100 was significantly lower than under N30 and N50 (P<0.05; Fig. 1c).

Soil pH tended to decrease gradually with increasing N addition but did not reach significance (Table 2). Conversely, soil SOC showed an increasing trend with N addition amount (Table 2). N addition did not significantly affect soil TN and C/N ratio, but increased soil NH_4^+-N and NO_3^--N contents (Table 2). The N100 treatment significantly increased NH_4^+-N and NO_3^--N contents compared with N0 (P<0.05; Table 2).

3.2 Soil Bacterial and Fungal Community Composition and Diversity

Dominant bacteria across all treatments were Actinobacteriota, Proteobacteria, Acidobacteriota, and Chloroflexi, which together accounted for >83.46% of total sequences (Fig. 2a [Figure 2: see original paper]). Actinobacteriota relative abundance gradually increased with N addition amount (Fig. 2a). Proteobacteria relative abundance gradually decreased from N0 to N50, but was higher under N100 than N0, showing an increasing trend (Fig. 2a). NMDS analysis revealed significant differences in soil bacterial community composition among N treatments (P<0.05; Fig. 2b).

Dominant fungi across all treatments were Ascomycota, Basidiomycota, and Mortierellomycota, which together accounted for >97.26% of total sequences (Fig. 2c). Basidiomycota relative abundance was highest under N30, followed by N50, N100, and N0 (Fig. 2c). Ascomycota relative abundance showed the

opposite pattern, being lowest under N30 (Fig. 2c). N deposition did not significantly affect soil fungal community composition in the desert steppe (P>0.05; Fig. 2d).

N deposition did not significantly affect soil bacterial Shannon index, but significantly affected Chao1 index (Fig. 3a [Figure 3: see original paper] and b). Soil bacterial Chao1 index patterns were consistent with plant Shannon index changes. N deposition had no significant effects on Shannon and Chao1 indices of soil fungal communities (Fig. 3c and d).

3.3 Soil and Plant Properties Associated with Microbial Community Structure

The first axis explained 22.25% and 23.50% of variation in bacterial and fungal community structure influenced by environmental factors, respectively (Fig. 4a [Figure 4: see original paper] and b). In the soil bacteria RDA biplot, soil pH and plant Shannon index were positively correlated with bacterial communities under N0, N30, and N50 (Fig. 4a), but negatively correlated under N100 (Fig. 4a). Soil pH was negatively correlated with Proteobacteria, Gemmatimonadota, and Bacteroidota, but positively correlated with Chloroflexi (Table S1). NH_4^+-N and NO_3^--N were negatively correlated with bacterial communities under N0, N30, and N50 (Fig. 4a), but positively correlated under N100 (Fig. 4a). NH_4^+-N and NO_3^--N were positively correlated with annuals and Actinobacteriota (P<0.05; Table S1).

In the soil fungi RDA biplot, NH_4^+-N, annuals and biennials, and AGB were positively correlated with soil bacterial communities under all N addition treatments (Fig. 4b). NH_4^+-N, annuals and biennials, and AGB were positively correlated with Ascomycota and negatively correlated with Basidiomycota and Mortierellomycota, though none reached significance (Table S2). NH_4^+-N and NO_3^--N were negatively correlated with plant Shannon index and perennial grasses (P<0.05; Table S3), but positively correlated with annuals and biennials (P<0.05; Table S3).

4 Discussion

Our 6-year experiment showed that N deposition did not significantly increase plant AGB but did change plant community composition. Previous studies indicate that aboveground net primary production increases stabilize after approximately 10.5–12.0 g N/($m^2 \cdot a$) addition in desert steppe regions [**?**, **?**]. Our N addition amounts did not exceed this threshold, so the nonsignificant biomass increases may have resulted from water constraints [**?**], consistent with multiple resource co-limitation theory [**?**]. N addition effects on plant communities largely depend on water availability [**?**, **?**]. Although plant AGB did not change significantly, plant community composition did. N addition enhanced AGB of annual *N. pectinata* while detrimentally affecting perennial grass *S. breviflora*. Fast-growing annuals, usually abundant only in early grassland succession stages, can

almost completely replace perennials in mature sites [**?**]. Rapid annual growth is facilitated by species-specific traits including abundant seed production and fast growth. Perennial decline may be attributable to conservative resource-use strategies [**?**]. Additionally, r/K selection theory may be reflected in our results: faster-growing r-strategist forbs increased while slower-growing K-strategist native perennial grasses decreased [**?**]. This may explain the absence of significant changes in plant alpha-diversity. However, NO_3^--N and NH_4^+-N were significantly negatively correlated with plant Shannon index, suggesting prolonged or higher N deposition concentrations may significantly reduce desert steppe plant diversity.

N addition significantly increased soil available N, an important limiting factor for desert steppe microorganisms [**?**]. Low soil N content in desert steppe ecosystems may restrict both microbial proliferation and vegetation growth [**?**]. Our results showed that N addition did not significantly decrease soil microbial alpha-diversity. Besides water restriction, added N rates may be much higher than those to which native inhabitants evolved, as those inhabitants adapted to low N environments [**?**, **?**]. This is supported by the lack of significant plant biomass increase. Soil pH changes caused by N are typically the most important factor affecting microbial diversity responses [**?**]. Most research suggests N deposition decreases soil pH, weakening plant-soil microbe relationships [**?**]. However, our soil pH was not significantly affected, and the main effect came from inorganic N accumulation. NO_3^--N and NH_4^+-N were significantly positively correlated with Actinobacteriota and annual/biennial biomass. N addition increased relative abundances of Actinobacteriota and annuals/biennials, partially explained by copiotrophic bacterial life history strategies [**?**]. Our microbial diversity findings are consistent with other desert steppe regions [**?**, **?**]. However, soil microbial community composition (beta-diversity) was affected by different N-addition amounts. Plant-mediated effects on soil microbial communities may be driven by resource quantity and quality (plant litter and root exudates) or vegetation-microorganism synchrony [**?**]. Plants and soil microbes have direct co-evolutionary relationships that constitute important systems [**?**]. In our study, plant community Shannon index patterns were consistent with soil bacterial Chao1 index patterns. Significant changes in plant community composition (beta-diversity) were accompanied by bacterial composition changes, likely due to plant-microbe co-evolution. Desert steppe soil bacterial communities co-evolved with plant communities, but fungal community structure and diversity were not significantly affected by N deposition. Studies from arid and semi-arid grasslands show drought promotes destabilizing properties of soil bacteria but not fungi, producing prolonged effects on bacterial communities and co-occurrence networks via vegetation composition changes and resultant soil moisture reductions [**?**, **?**, **?**]. These results contribute to comprehensive understanding of atmospheric N deposition effects on biodiversity and mechanisms underlying plant-soil-microbiome interactions in desert steppes.

5 Conclusions

Our data indicate that in nutrient-poor, arid desert steppe ecosystems, plant AGB did not significantly increase after 6 years of N deposition. Simultaneously, N deposition did not decrease alpha-diversity of plants and microorganisms; low N addition had positive effects. However, N deposition significantly affected beta-diversity of plant and bacterial communities, but did not significantly impact fungal communities. Plants and soil bacterial communities coevolved, while fungal communities remained stable. These findings help understand mechanisms of atmospheric N deposition effects on ecological health and function of desert steppes.

Conflict of Interest

The authors declare no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Acknowledgements

This work was funded by the National Natural Science Foundation of China (31860136, 31560156), the Basic Scientific Research Service Fee Project of Colleges and Universities of Inner Mongolia Autonomous Region, and the Graduate Scientific Research Innovation Project of Inner Mongolia Autonomous Region (B20210158Z). We thank the Siziwang Research Station of Inner Mongolia Academy of Agricultural & Animal Husbandry Sciences for support.

Author Contributions

All authors contributed to conceptualization. Methodology, formal analysis, and writing—review and editing: HE Ye, MEI Hong, XU Xuehui, LIANG Zhiwei, JIANG Na, TU Nare, WU Zhendan. Writing—original draft preparation: HE Ye. All authors approved the manuscript.

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References

Angerer J, Han G, Fujisaki I, et al. 2008. Climate change and ecosystems of Asia with emphasis on Inner Mongolia and Mongolia. *Rangelands*, 30(3): 46–51.

Bai Y, Wu J, Clark C M, et al. 2010. Tradeoffs and thresholds in the effects of nitrogen addition on biodiversity and ecosystem functioning: Evidence from inner Mongolia Grasslands. *Global Change Biology*, 16(1): 358–372.

Banerjee S, Schlaeppi K, van der Heijden M G A. 2018. Keystone taxa as drivers of microbiome structure and functioning. *Nature Reviews Microbiology*, 16: 567–576.

Bardgett R D, Mawdsley J L, Edwards S, et al. 1999. Plant species and nitrogen effects on soil biological properties of temperate upland grasslands. *Functional Ecology*, 13(5): 650–660.

Berg G, Smalla K. 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiology Ecology*, 68(1): 1–13.

Bever J D. 2003. Soil community feedback and the coexistence of competitors: Conceptual frameworks and empirical tests. *New Phytologist*, 157(3): 465–473.

Bobbink R, Hicks K, Galloway J, et al. 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: A synthesis. *Ecological Applications*, 20(1): 30–59.

Borer E T, Seabloom E W, Gruner D S, et al. 2014. Herbivores and nutrients control grassland plant diversity via light limitation. *Nature*, 508: 517–520.

Chen S, Zhou Y, Chen Y, et al. 2018. Fastp: An ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*, 34(17): i884–i890.

Chen W, Zhou H, Wu Y, et al. 2021. Plant-mediated effects of long-term warming on soil microorganisms on the Qinghai-Tibet Plateau. *Catena*, 204: 105391, doi: 10.1016/j.catena.2021.105391.

Chen Y, Li J, Ju W, et al. 2017. Quantitative assessments of water-use efficiency in Temperate Eurasian Steppe along an aridity gradient. *PloS ONE*, 12(7): e0179875, doi: 10.1371/journal.pone.0179875.

Contosta A R, Frey S D, Cooper A B. 2015. Soil microbial communities vary as much over time as with chronic warming and nitrogen additions. *Soil Biology and Biochemistry*, 88: 19–24.

Dai Z, Su W, Chen H, et al. 2018. Long-term nitrogen fertilization decreases bacterial diversity and favors the growth of Actinobacteria and Proteobacteria in agro-ecosystems across the globe. *Global Change Biology*, 24(8): 3452–3461.

de Vries F T, Griffiths R I, Bailey M, et al. 2018. Soil bacterial networks are less

stable under drought than fungal networks. *Nature Communications*, 9: 3033, doi: 10.1038/s41467-018-05516-7.

Delgado-Baquerizo M, Maestre F T, Gallardo A, et al. 2013. Decoupling of soil nutrient cycles as a function of aridity in global drylands. *Nature*, 502: 672–676.

Delgado-Baquerizo M, Reich P B, Trivedi C, et al. 2020. Multiple elements of soil biodiversity drive ecosystem functions across biomes. *Nature Ecology & Evolution*, 4: 210–220.

Dunn R M, Mikola J, Bol R, et al. 2006. Influence of microbial activity on plant-microbial competition for organic and inorganic nitrogen. *Plant and Soil*, 289: 321–334.

Edgar R C. 2013. UPARSE: Highly accurate OTU sequences from microbial amplicon reads. *Nature Methods*, 10: 996–998.

Erisman J W, Galloway J N, Seitzinger S, et al. 2013. Consequences of human modification of the global nitrogen cycle. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368(1621): 0116, doi: 10.1098/rstb.2013.0116.

Fan K, Delgado-Baquerizo M, Zhu Y, et al. 2020. Crop production correlates with soil multitrophic communities at the large spatial scale. *Soil Biology and Biochemistry*, 151: 108047, doi: 10.1016/j.soilbio.2020.108047.

Fan M, Li J, Tang Z, et al. 2020. Soil bacterial community succession during desertification in a desert steppe ecosystem. *Land Degradation & Development*, 31(13): 1662–1674.

Gadgil M, Solbrig O T. 1972. The concept of r- and K-selection: Evidence from wild flowers and some theoretical considerations. *The American Naturalist*, 106(947): 14–31.

Harpole W S, Ngai J T, Cleland E E, et al. 2011. Nutrient co-limitation of primary producer communities. *Ecology Letters*, 14(9): 852–862.

Harrington R A, Fownes J H, Vitousek P M. 2001. Production and resource use efficiencies in N- and P-limited tropical forests: A comparison of responses to long-term fertilization. *Ecosystems*, 4: 646–657.

Hautier Y, Niklaus P A, Hector A. 2009. Competition for light causes plant biodiversity loss after eutrophication. *Science*, 324(5927): 636–638.

Hautier Y, Seabloom E W, Borer E T, et al. 2014. Eutrophication weakens stabilizing effects of diversity in natural grasslands. *Nature*, 508: 521–525.

Hortal S, Lozano Y M, Bastida F, et al. 2017. Plant-plant competition outcomes are modulated by plant effects on the soil bacterial community. *Scientific Reports*, 7: 17756, doi: 10.1038/s41598-017-18103-5.

Hu S, Chapin F S, Firestone M K, et al. 2001. Nitrogen limitation of microbial decomposition in a grassland under elevated CO_2. *Nature*, 409: 188-191.

Huang J, Xu Y, Yu H, et al. 2021. Soil prokaryotic community shows no response to 2 years of simulated nitrogen deposition in an arid ecosystem in northwestern China. *Environmental Microbiology*, 23(2): 1222-1237.

Kardol P, Cornips N J, van Kempen M M L, et al. 2007. Microbe-mediated plant-soil feedback causes historical contingency effects in plant community assembly. *Ecological Monographs*, 77(2): 147-162.

Keymer D P, Lankau R A. 2017. Disruption of plant-soil-microbial relationships influences plant growth. *Journal of Ecology*, 105(3): 816-827.

Lau J A, Lennon J T. 2011. Evolutionary ecology of plant-microbe interactions: Soil microbial structure alters selection on plant traits. *New Phytologist*, 192(1): 215-224.

Li H, Xu Z, Yan Q, et al. 2018. Soil microbial beta-diversity is linked with compositional variation in aboveground plant biomass in a semi-arid grassland. *Plant and Soil*, 423: 465-480.

Liu X, Zhang Y, Han W, et al. 2013. Enhanced nitrogen deposition over China. *Nature*, 494: 459-462.

Ma Q, Liu X, Li Y, et al. 2020. Nitrogen deposition magnifies the sensitivity of desert steppe plant communities to large changes in precipitation. *Journal of Ecology*, 108(2): 598-610.

Magoc T, Salzberg S L. 2011. FLASH: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*, 27(21): 2957-2963.

McHugh T A, Morrissey E M, Mueller R C, et al. 2017. Bacterial, fungal, and plant communities exhibit no biomass or compositional response to two years of simulated nitrogen deposition in a semiarid grassland. *Environmental Microbiology*, 19(4): 1600-1611.

Panke-Buisse K, Poole A C, Goodrich J K, et al. 2015. Selection on soil microbiomes reveals reproducible impacts on plant function. *The ISME Journal*, 9: 980-989.

Payne R J, Dise N B, Field C D, et al. 2017. Nitrogen deposition and plant biodiversity: past, present, and future. *Frontiers in Ecology and the Environment*, 15(8): 431-436.

Peng Y, Chen H Y H, Yang Y. 2020. Global pattern and drivers of nitrogen saturation threshold of grassland productivity. *Functional Ecology*, 34(9): 1979-1990.

Ramirez K S, Craine J M, Fierer N. 2012. Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. *Global Change Biology*, 18(6): 1918-1927.

Reynolds H L, Packer A, Bever J D, et al. 2003. Grassroots ecology: Plant-microbe-soil interactions as drivers of plant community structure and dynamics. *Ecology*, 84(9): 2281–2291.

Schlatter D C, Bakker M G, Bradeen J M, et al. 2015. Plant community richness and microbial interactions structure bacterial communities in soil. *Ecology*, 96(1): 134–142.

Sha M, Xu J, Zheng Z, et al. 2021. Enhanced atmospheric nitrogen deposition triggered little change in soil microbial diversity and structure in a desert ecosystem. *Global Ecology and Conservation*, 31: e01879, doi: 10.1016/j.gecco.2021.e01879.

Shao Y, Liu T, Eisenhauer N, et al. 2018. Plants mitigate detrimental nitrogen deposition effects on soil biodiversity. *Soil Biology and Biochemistry*, 127: 178–186.

Stackebrandt E, Goebel B M. 1994. Taxonomic note: A place for DNA-DNA reassociation and 16s rRNA sequence analysis in the present species definition in bacteriology. *International Journal of Systematic and Evolutionary Microbiology*, 44: 846–849.

Stevens C J, Dise N B, Mountford J O, et al. 2004. Impact of nitrogen deposition on the species richness of grasslands. *Science*, 303(5665): 1876–1879.

Su J, Li X R, Li X J, et al. 2013. Effects of additional N on herbaceous species of desertified steppe in arid regions of China: A four-year field study. *Ecological Research*, 28: 21–28.

Tang Z, Deng L, An H, et al. 2017. The effect of nitrogen addition on community structure and productivity in grasslands: A meta-analysis. *Ecological Engineering*, 99: 31–38.

Treseder K K. 2008. Nitrogen additions and microbial biomass: A meta-analysis of ecosystem studies. *Ecology Letters*, 11: 1111–1120.

van der Putten W H. 2017. Belowground drivers of plant diversity. *Science*, 355(6321): 134–135.

Wang Q, Garrity G M, Tiedje J M, et al. 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*, 73(16): 5261–5267.

Wang X, Feng J, Ao G, et al. 2023. Globally nitrogen addition alters soil microbial community structure, but has minor effects on soil microbial diversity and richness. *Soil Biology and Biochemistry*, 179: 108982, doi: 10.1016/j.soilbio.2023.108982.

Wang Z, Na R, Koziol L, et al. 2020. Response of bacterial communities and plant-mediated soil processes to nitrogen deposition and precipitation in a desert steppe. *Plant and Soil*, 448: 277–297.

Wu Q, Ren H, Bisseling T, et al. 2021. Long-term warming and nitrogen addition have contrasting effects on ecosystem carbon exchange in a desert steppe. *Environmental Science & Technology*, 55: 7256–7265.

Yang X, Huang Z, Dong M, et al. 2019. Responses of community structure and diversity to nitrogen deposition and rainfall addition in contrasting steppes are ecosystem-dependent and dwarfed by year-to-year community dynamics. *Annals of Botany*, 124(3): 461–469.

Yu G, Jia Y, He N, et al. 2019. Stabilization of atmospheric nitrogen deposition in China over the past decade. *Nature Geoscience*, 12: 424–429.

Zha T G. 1997. *Soil Physiochemical Analysis*. Beijing: China Forestry Publishing House. (in Chinese)

Zhang T, Chen H Y H, Ruan H. 2018. Global negative effects of nitrogen deposition on soil microbes. *The ISME Journal*, 12: 1817–1825.

Zilber-Rosenberg I, Rosenberg E. 2008. Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS Microbiology Reviews*, 32(5): 723–735.

Appendix

Table S1 Correlation between environmental factors and dominant bacterial phyla

Variable	Actinobacteriota	Proteobacteria	Acidobacteriota	Chloroflexi	Gemmatimonadota	Firmicutes	Bacteroidota	Myxococcota
Shannon index	0.57*	-	-	-	-	-	-	-
Perennial grasses	0.55*	-	-	-	-	-	-	-
Annuals and biennials	-0.52*	0.70**	-0.55*	-0.71**	-	-	-	-

Note: *, P<0.05 level; **, P<0.01 level. AGB, aboveground biomass; TN, total nitrogen; SOC, soil organic carbon; C/N, soil organic carbon/total nitrogen. Abbreviations are the same in following tables.

Table S2 Correlation between environmental factors and dominant fungal phyla

Variable	Ascomycota	Basidiomycota	Mortierellomycota	Chytridiomycota
Shannon index	-0.53*	-	-	-
Perennial grasses	-0.73**	0.62**	-	-
Annuals and biennials	-0.58*	-0.62**	0.55*	-

Table S3 Correlation between soil properties and plant community

Variable	Plant community
Shannon index	-
Perennial grasses	-
Annuals and biennials	-

Note: *, P<0.05 level; **, P<0.01 level.

Note: Figure translations are in progress. See original paper for figures.