

Long-term nitrogen input alters plant and soil bacterial, but not fungal beta diversity in a semiarid grassland

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Abstract

Anthropogenic nitrogen (N) input is known to alter plant and microbial α -diversity, but how N enrichment influences β -diversity of plant and microbial communities remains poorly understood. Using a long-term multilevel N addition experiment in a temperate steppe, we show that plant, soil bacterial and fungal communities exhibited different responses in their β -diversity to N input. Plant β -diversity decreased linearly as N addition increased, as a result of increased directional environmental filtering, where soil environmental properties largely explained variation in plant β -diversity. Soil bacterial β -diversity first increased then decreased with increasing N input, which was best explained by corresponding changes in soil environmental heterogeneity. Soil fungal β -diversity, however, remained largely unchanged across the N gradient, with plant β -diversity, soil environmental properties, and heterogeneity together explaining an insignificant fraction of variation in fungal β -diversity, reflecting the importance of stochastic community assembly. Our study demonstrates the divergent effect of N enrichment on the assembly of plant, soil bacterial and fungal communities, emphasizing the need to examine closely associated fundamental components (i.e., plants and microorganisms) of ecosystems to gain a more complete understanding of ecological consequences of anthropogenic N enrichment.

KEYWORDS

community assembly, deterministic processes, environmental heterogeneity, N deposition, stochastic processes, β -diversity

1 | INTRODUCTION

Anthropogenic nitrogen (N) input is recognized as one of the major threats to biodiversity (Sala et al., 2000; Stevens et al., 2004; Vellend et al., 2017). A substantial body of literature has documented erosion in local plant (Midolo et al., 2019; Stevens et al., 2004) and microbial (Dai et al., 2018; Wang et al., 2018) diversity (i.e., α -diversity loss) following N enrichment. By comparison, much less is known about how N deposition influences compositional differences between local communities (i.e., β -diversity), an essential component of biodiversity. The few investigations of N fertilization effect on plant β -diversity have produced mixed results (Chalcraft et al., 2008; Conradi et al., 2017; Houseman et al., 2008; Yang et al., 2019; Zhang et al., 2019). Moreover, little work has been done to explore the role of N input in regulating microbial β -diversity (Zhang et al., 2016). Therefore, general patterns and mechanisms of plant and microbial β -diversity responses to N input remain elusive. Elucidating β -diversity changes under N enrichment, however, is essential for understanding how biodiversity changes at the regional scale (Chalcraft et al., 2008), and for advancing our knowledge on community assembly mechanisms under anthropogenic influences (Mori et al., 2018).

Both deterministic (Chase & Leibold, 2003) and stochastic (Bell, 2001; Hubbell, 2001) processes are known to operate in natural communities (Vellend, 2010). Deterministic processes are important in regulating ecological communities when environmental filtering and/or species interactions shape community assembly by selecting species with certain traits, whereas stochastic processes are important in regulating ecological communities when chance events associated with birth, death, and migration contribute to community assembly. N enrichment could potentially influence the relative importance of deterministic and stochastic processes, and therefore, the trajectories of community assembly. For example, intensified N enrichment may increase the role of directional environmental filtering by favoring species that are better resource competitors (Hautier et al., 2009; Tilman, 1982) and tolerant of low pH (Duprè et al., 2010; Sala et al., 2000) and heavy metal toxicity (Bai et al., 2015; Tian et al., 2016). This would strengthen the importance of deterministic processes in shaping community assembly, resulting in enhanced convergence (i.e., reduction in β -diversity) in the structure of the assembling communities (Chalcraft et al., 2008; Conradi et al., 2017). Moreover, N enrichment may further reduce β -diversity if it decreases spatial heterogeneity in environmental conditions among localities (Chalcraft et al., 2008; Donohue et al., 2009; Passy & Blanchet, 2007). On the other hand, N enrichment may often result in increased ecosystem productivity known to promote the importance of stochastic assembly processes (Chase, 2010) and, in turn, the greater likelihood of communities attaining alternative states (Houseman et al., 2008; Yang et al., 2019). The relative importance of the aforementioned mechanisms would determine how N enrichment influences β -diversity and the assembly trajectories of the affected communities.

Within the context of community assembly under N enrichment, one important, yet unresolved question is whether the closely

associated soil microbial and plant communities would exhibit similar β -diversity responses to N enrichment. On the one hand, responses of soil microbial communities may be expected to be coupled with those of plant communities (Leff et al., 2015; Prober et al., 2015), as soil microbes depend largely on plants for habitat and substrate provision (van der Putten et al., 2013), and symbiotic and pathogenic microbes rely on their plant hosts for survival (Chaloner et al., 2020). On the other hand, differences in the physiology, life history, and metabolic strategies of plants and microbes may cause their β -diversity responses to be decoupled from each other. For example, microbes are generally less constrained by dispersal limitation than plants (Finlay, 2002). Stochastic dispersal thus may have greater opportunities to shape microbial community assembly (Nemergut et al., 2013). Moreover, the structural and functional differences between plants and microbes may potentially translate into their different responses to environmental changes associated with N enrichment. For example, plants and microbes may perceive soil environmental heterogeneity differently, such that soil microbes, characterized by small body size and high metabolic diversity, may be more sensitive to changes in small-scale soil heterogeneity associated with N enrichment than plants (Portell et al., 2018).

Much of what we know about anthropogenic environmental change effect on β -diversity came from grassland-based research (Chalcraft et al., 2008; Conradi et al., 2017; Houseman et al., 2008; Yang et al., 2019; Zhang et al., 2019). Continuing this tradition, here we report, for the first time to our knowledge, a grassland experiment simultaneously exploring the effect of increased N input on plant, soil bacterial and fungal β -diversity. This experiment was conducted in a temperate steppe in northern China, where anthropogenic N deposition is projected to continue to increase (Zhang, Xu, et al., 2017). Our study aimed at answering two main questions. First, how does N input influence β -diversity of plants, soil bacteria, and fungi? Second, what mechanisms underlie the differential responses (if any) of plant, soil bacterial and fungal β -diversity to N input? Within this question, we also explored whether changes in plant β -diversity contribute to changes in soil bacterial and fungal β -diversity.

2 | MATERIALS AND METHODS

2.1 | Experimental site and design

Our experimental site is located in a semiarid steppe (42.01°N, 116.16°E and 1324 m a.s.l) in Duolun County, Inner Mongolia, China. The region has a dry, monsoon-influenced continental climate, characterized by cold, dry winters and warm, humid summers. Mean annual temperature is 2.1°C, with average monthly temperatures ranging from -17.5°C (January) to 18.9°C (July); mean annual precipitation is 382.3 mm, with approximately 50% falling in July and August. The soil type is classified as Haplic Calcisols according to the Food and Agriculture Organization of the United Nations, with 69.21 ± 0.06% sand, 15.60 ± 0.02% silt,

and $15.19 \pm 0.02\%$ clay. Soil organic C and total N concentrations are 16.94 ± 2.34 and 1.65 ± 0.27 g kg⁻¹, respectively. Soil pH is 6.84 ± 0.02 . Ambient N deposition is approximately 1.6 g N m⁻² (Liu et al., 2018). Plant communities at our experimental site are dominated by perennial grasses and forbs, including *Stipa krylovii* Roshev., *Agropyron cristatum* (L.), *Artemisia frigida* Willd., and *Cleistogenes squarrosa* (Trin.).

Sixty-four 10 × 15 m experimental plots were arranged in eight blocks, with eight rows in each block in a Latin square design. There were 5-m buffer zones between adjacent plots. Starting from 2003, each of the eight plots per row was randomly assigned to one of the eight levels of N fertilization treatments (0, 1, 2, 4, 8, 16, 32, and 64 g N m⁻² year⁻¹). The N addition levels are comparable to those used in several other grassland N addition experiments. This large N gradient facilitates the understanding of the N dose–biodiversity relationships, which, if nonlinear, may be difficult to detect using smaller ranges of N levels. N was applied in the form of urea in early July each year. Since 2005, four rows (one out of every two rows) were mowed using a rotary flail mower at the ground level in late August each year; all hay was removed from the plots afterward. We used the data from the nonmowing plots in this study.

2.2 | Plant survey and soil chemical properties

We surveyed plant communities at the peak biomass in a permanent 1 × 1 m quadrat of each plot in August 2016, using the point intercept method. Plant species richness and coverage were estimated by placing a 1 × 1 m frame with 100 equally distributed 10 × 10 cm grids on the permanent quadrat. Plot-level richness was recorded as the number of plant species present in the quadrat; plant cover was summed across species to give plot-level coverage. We also measured soil moisture in each plot three times per month from May to September, using a portable soil moisture device (Diviner 2000; Sentek Pty. Ltd.).

Soil samples were collected from each plot on August 15, 2016. Six randomly located soil cores (15 cm deep and 5 cm in diameter) were taken from each plot and combined into one composite sample. After removing roots and stones by sieving through 2-mm mesh, soil samples were stored on ice and transferred to the laboratory. Subsamples were stored at 4°C for soil physicochemical analysis and –80°C for DNA extraction. Soil total carbon (C) and N were measured using elemental analysis (Elementar Analysensysteme GmbH) in the laboratory. Soil pH values were measured using a glass electrode in the soil suspension (soil:deionized water W/V ratio 1:2.5) of each sample. Soil inorganic N was extracted with 2 M KCl solution; concentrations of NH₄⁺-N and NO₃⁻-N were measured using a flow injection analyzer (SAN-System). Soil exchangeable Mn²⁺ was extracted with 20 ml of extractant consisting of 0.005 M diethylenetriaminepentaacetic acid, 0.1 M triethanolamine, and 0.01 M CaCl₂ at the pH of 7.3 (Lindsay & Norvell, 1978), and exchangeable Al³⁺ was extracted with 50 ml of 0.1 M BaCl₂. The concentrations of exchangeable Mn²⁺ and Al³⁺ were then measured using inductively

coupled plasma optical emission spectroscopy (iCAP 6300; Thermo Scientific).

2.3 | DNA extraction, amplification, and MiSeq sequencing

DNA was extracted from 0.5 g fresh soil of each sample using the PowerSoil DNA Isolation Kit (MoBio Laboratories), following the manufacturer's instructions. To profile soil bacterial communities, we amplified the V3–V4 hypervariable region of 16S rRNA gene with the primer sets 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'; Caporaso et al., 2012). For fungal communities, we amplified the ITS1 region with the primers sets ITS1-F (5'-CTTGGTCATTAGAGGAAGTAA-3') and ITS2 (5'-TGCCTTCTTCATCGATGC-3'; McGuire et al., 2013). To permit multiplexing of samples, a 10-bp barcode unique to each sample was attached to the 5' end of primers. Polymerase chain reaction (PCR) was performed with 50 µl reaction volumes: 5 µl 10×Ex Taq Buffer (Mg²⁺ plus), 4 µl 12.5 mM dNTP Mix, 1.25 U Ex Taq DNA polymerase (TaKaRa), 2 µl template DNA, and 36.75 µl ddH₂O. For the 16S rRNA gene, the PCR thermal cycling condition was 94°C for 2 min, followed by 30 cycles of 94°C for 30 s, 57°C for 30 s and 72°C for 30 s with a final extension at 72°C for 10 min. For the ITS1 region, the thermal cycling condition was 95°C for 5 min, followed by 35 cycles of 95°C for 45 s, 55°C for 50 s, and 72°C for 45 s with a final extension at 72°C for 10 min. PCR for each sample was performed in triplicate and negative controls were included in each batch of PCR. PCR products were pooled in equimolar concentrations and purified with the QIAquick Gel Extraction Kit (Qiagen). The purified PCR products were sequenced on the Illumina MiSeq platform (Illumina).

2.4 | Bioinformatic analysis

Raw reads were trimmed at the 3' end to remove low-quality (Phred score < 20) bases. Then, high-quality reads were assembled using the FLASH software (version 1.0.0; Magoč & Salzberg, 2011). Assembled sequences were assigned to samples according to their unique barcodes, and then the barcodes and primers were removed from these sequences using the MOTHUR software (Schloss et al., 2009). The sequences containing ambiguous bases, with lengths shorter than 200 bp, as well as all singletons, were removed. The remaining sequences were clustered into operational taxonomic units (OTUs) at a 97% similarity level using the UPARSE algorithm (Edgar, 2013), and chimeras were eliminated during this procedure. Taxonomic annotations of OTUs were determined using the Ribosomal Database Project Classifier tool (Wang et al., 2007) with a confidence threshold of 0.7 against the Silva 128 database (Quast et al., 2012) for bacteria and UNITE 7.2 database (Nilsson et al., 2018; Tedersoo et al., 2018) for fungi. OTUs that were not classified into bacteria and fungi were removed before subsequent analyses. To standardize sampling effort, we rarefied

all samples to an even number of sequences per sample for bacteria (18,877) and fungi (24,945). All sequences have been deposited in NCBI's SRA database under project accession number PRJNA573484 and PRJNA573488.

2.5 | Data analyses

We used plant species richness and microbial OTU richness as the metrics of plant and microbial α -diversity, respectively. We calculated the abundance-based Bray–Curtis dissimilarity and incidence-based Jaccard dissimilarity, as the metrics of β -diversity, to quantify community compositional difference between replicate plots of the same treatment. As β -diversity often depends on community size (α -diversity; Chase et al., 2011; Kraft et al., 2011; Myers et al., 2015), we also calculated the standard effective size of β -diversity (i.e., β -deviation) by comparing observed β -diversity values to those generated by null models. We generated 999 null local communities within each N treatment, by randomly placing individuals into each local community with probabilities proportional to regional relative cover/abundance of the species, while preserving local α -diversity (Ning et al., 2019, 2020). The standardized effect size (β -deviation for Bray–Curtis and Jaccard dissimilarity) was calculated as the difference between the observed β -diversity and mean β -diversity of the null communities divided by the standard deviation of β -diversity of the null communities (Kraft et al., 2011; Ning et al., 2019, 2020). β -Deviation values close to 0 indicate that β -diversity does not deviate from stochastic expectations, whereas positive and negative β -deviation values indicate higher and lower β -diversity than expected by chance, respectively. β -Diversity and β -deviation were calculated for plants, bacteria, and fungi, separately, at each N addition level. We further directly quantified the magnitude of stochastic processes shaping plant, bacterial, and fungal communities using the normalized stochasticity ratio (Ning et al., 2019). This proposed approach assumes that the community is a combination of species which are driven by two ecological processes separately: some species are operated by completely deterministic assembly and the other are operated by completely stochastic assembly. The stochasticity levels are predetermined by assigning different ratios of stochastic species. Stochasticity ratio is calculated based on relative difference between observed and null dissimilarity, and normalized stochasticity ratio derived from stochasticity ratio is to better assess the relative position of observed value between the extremes under pure deterministic and pure stochastic assembly. Hence, the normalized stochasticity ratio explicitly measures the importance of stochastic relative to deterministic assembly, with the contribution of the two processes adding up to 100%. This ratio thus ranges from 0, indicating complete determinism, to 100%, indicating complete stochasticity.

We used bivariate regressions to examine the relationships between α - and β -diversity/deviation of plant, bacterial, and fungal communities and N input level; Exponential and polynomial regressions were used over linear models if they provided significantly

better fits to the data. Bivariate regressions were also used to delineate how environmental heterogeneity changed with increasing N addition. Environmental heterogeneity was quantified as the total pairwise Euclidean distance among the four replicate plots of the same treatment, based on the seven soil physicochemical variables (soil moisture, soil pH, soil total C, total N, inorganic N concentration, and exchangeable Al^{3+} and Mn^{2+} concentrations). Specifically, soil moisture is an indicator of soil microclimate; total C is an indicator of soil fertility; total N and inorganic N concentration are the indicators of soil N availability; and soil pH, exchangeable Al^{3+} and Mn^{2+} concentrations are the indicators of soil biogeochemical conditions. These seven soil physicochemical variables were standardized using Z-score before calculation. Soil moisture values were averaged across the growing season for each plot prior to standardization. We assessed the relative importance of soil physicochemical conditions and environmental heterogeneity for β -deviation of plant and soil microbial communities, using the Lindeman–Merenda–Gold method. Prior to the analyses, variance inflation factors were calculated to assess the multicollinearity of predictor variables. This led to the removal of soil total C, total N, Al^{3+} , and Mn^{2+} , leaving soil moisture, dissolved inorganic N concentration, soil pH, and environmental heterogeneity as predictor variables with low collinearity. For soil bacterial and fungal β -deviation, we also considered plant β -deviation as an independent predictor.

All statistical analyses were performed in R 3.5.2 (R Core Team, 2015). β -Diversity was calculated using the “vegdist” function in the package “vegan” (Oksanen et al., 2012). Environmental Euclidean distance was calculated using the function “dist” in the package “stats.” The null communities were generated using the function “commsim” in the package “vegan” (Oksanen et al., 2012). Normalized stochasticity ratio and relative importance values were calculated with the packages “NST” (Ning et al., 2019, 2020) and “relaimpo,” respectively.

3 | RESULTS

Plants, soil bacteria, and fungi differed substantially in their α -diversity responses to N addition (Figure 1). While plant α -diversity decreased monotonically with increasing N addition, soil bacterial α -diversity (OTU richness) did not decline until N addition reached 32 g N m^{-2} . Soil fungal α -diversity (OTU richness) was unaffected by N addition.

Plants, soil bacteria, and fungi also exhibited divergent responses in their β -diversity to N addition. While plant β -diversity decreased monotonically with increasing N addition (Figure 2a), soil bacteria exhibited a significant nonlinear response, where β -diversity first increased then decreased with increasing N addition (Figure 2b; Table S1), and soil fungal β -diversity showed little response to increasing N addition (Figure 2c). Similar patterns emerged when β -deviation, which accounted for difference in α -diversity among the compared communities, was examined (Figure 2d–f). These patterns remained unchanged when Jaccard dissimilarity (Figure S1; Table S1), rather than Bray–Curtis dissimilarity, was used.

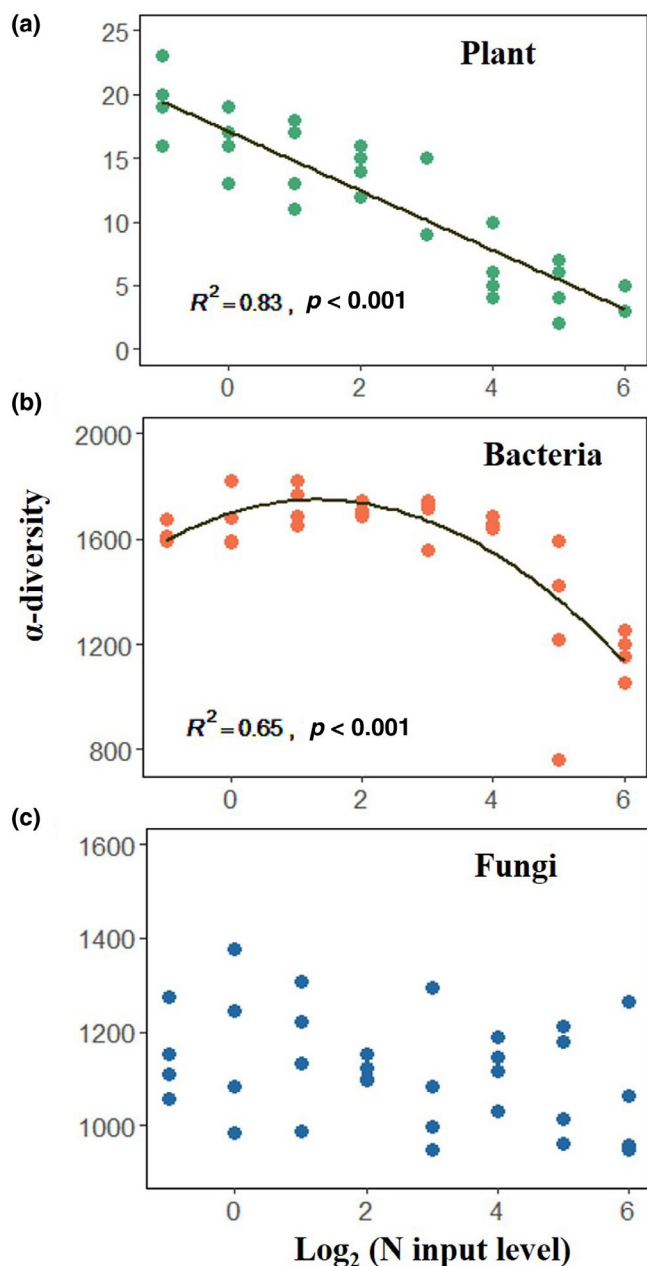


FIGURE 1 α -Diversity of plant (a), bacterial (b), and fungal (c) communities with increasing N input. Significant regression lines are shown

Nitrogen addition differentially affected the role of stochasticity in governing plant, soil bacterial and fungal communities (Figure 3), which mirrored β -diversity patterns. Specifically, as N input increased, the magnitude of stochasticity declined (from 82.6% to 21.8%) for plant communities, showed a hump-shaped response (ranging from 38.3% to 64.7%) for soil bacterial communities, and remained relatively high with little trend (between 57.8% and 71.2%) for soil fungal communities. Similar results were obtained when stochasticity estimation was based on Jaccard dissimilarity (Figure S2), rather than Bray–Curtis dissimilarity.

Soil environmental heterogeneity initially increased with N input, but eventually declined at high N levels (Figure 4; Table S1).

Plant β -diversity was unaffected by environmental heterogeneity, whereas plant β -deviation declined as environmental heterogeneity increased (Figure S3). In contrast, both soil bacterial β -diversity and β -deviation exhibited significantly positive relationships with environmental heterogeneity (Figure S3). Neither β -diversity nor β -deviation of fungal communities were related to environmental heterogeneity (Figure S3). Results based on Jaccard dissimilarity (Figure S4) were qualitatively similar.

The relative importance analysis showed that soil moisture, inorganic N concentration, soil pH, and environmental heterogeneity together explained 69.06% of the variation in plant β -deviation, where soil N availability and pH were identified as the two most important predictors (Figure 5a). By contrast, all the predictor variables, including plant β -deviation, explained only 17.89% of variance in soil bacterial β -deviation, with soil environmental heterogeneity being the overwhelmingly important predictor (Figure 5b). The same model, however, accounted for only 2.88% of variance in soil fungal β -deviation, with each of the predictor variables explaining little variation (Figure 5c).

4 | DISCUSSION

Our study demonstrated the previously unrecognized, strikingly different response patterns of plant and soil microbial β -diversity to increasing N input. As N input increased, plant β -diversity declined, soil bacterial β -diversity exhibited a unimodal response, while fungal β -diversity remained unchanged in our study grassland. These divergent results, combined with those of stochasticity and relative importance analyses, point to the difference in the relative importance of deterministic and stochastic processes in regulating the assembly of plant, soil bacterial and fungal communities in the face of increasing N enrichment (Figure 6).

Much of the research on N enrichment effects on β -diversity has focused on plants, which has produced mixed results (Chalcraft et al., 2008; Conradi et al., 2017; Houseman et al., 2008; Yang et al., 2019; Zhang et al., 2019). In particular, it has been reported that while N enrichment had an overall positive effect on plant β -diversity across North American grasslands, N enrichment tended to increase plant β -diversity at low productivity sites and reduce plant β -diversity at high productivity sites (Chalcraft et al., 2008). The negative N enrichment effect on plant β -diversity in our experiment, however, does not follow this pattern, as our experimental site is characterized by low productivity (Xu et al., 2018). In our experiment, the application of urea as the N fertilizer, which led to increased N availability and lower soil pH (Figure S5), may have imposed strong environmental filtering on plant communities. Consistent with this idea, we identified soil pH and inorganic N concentration as the two key predictors of plant β -diversity in our experiment (Figure 5a). This result, coupled with the sharp decline in the role of stochasticity in regulating plant communities under greater N input, points to increased directional environmental filtering as the driver of lower plant β -diversity at higher N levels. In our experiment, such directional environmental

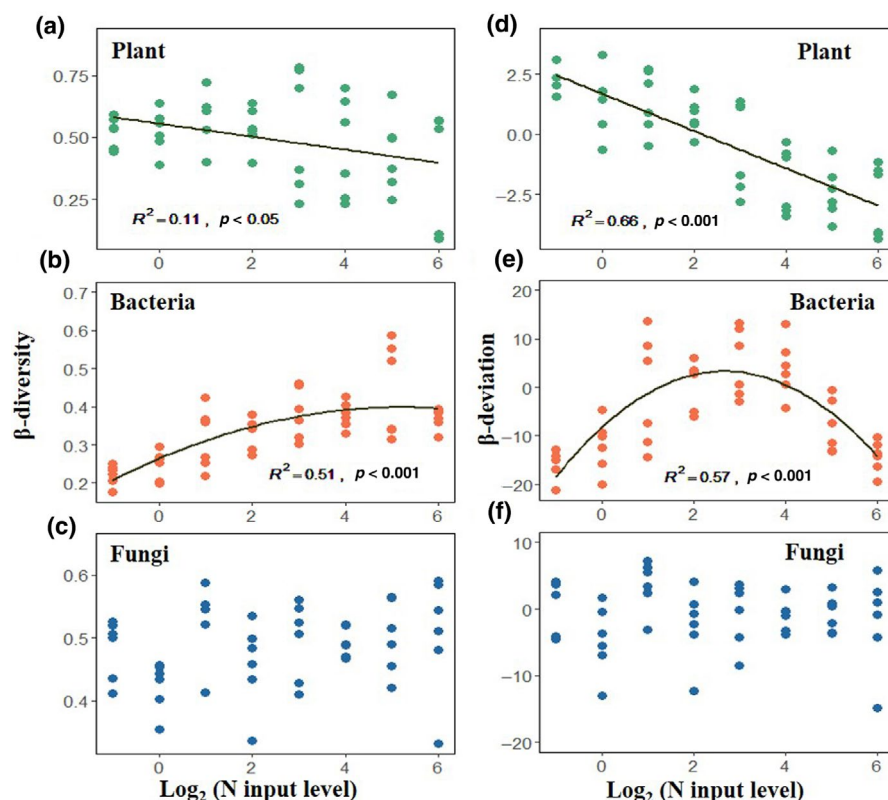


FIGURE 2 β -Diversity (a–c) and β -deviation (d–f) of plant, bacterial, and fungal communities among replicate plots within each N input treatment. β -Diversity is measured by Bray–Curtis dissimilarity. β -Deviation is the standardized effect size of β -diversity, calculated by comparing the observed β -diversity to the null models. Negative β -deviation values indicate lower β -diversity than expected by chance, whereas positive β -deviation values indicate the opposite. Significant regression lines are shown

filtering led to the increased dominance of grasses, particularly tall-statured ones, over forbs in all replicate plots of higher N treatments (Figure S6). This homogenization of plant communities arose as N enrichment tends to reduce root length and biomass of forbs, which weakens their water absorption ability (Bai et al., 2015), and soil acidification tends to cause elevated soil Al^{3+} and Mn^{2+} concentrations (Figure S7), which are more toxic to forbs than grasses (Bai et al., 2015; Tian et al., 2016, 2020). Note that competition for light, which becomes more important with greater plant biomass under N enrichment (Craine & Dybzinski, 2013; Hautier et al., 2009), may have contributed to the dominance of tall-statured grasses (e.g., *Leymus chinensis*) over low-statured ones (e.g., *Cleistogenes Chinese*) at our site (Figure S6). Unfortunately, we did not quantify light availability in our experiment, which precludes us from directly assessing the role of light limitation in regulating plant community assembly.

It has been increasingly recognized that plants can strongly influence soil microbiota. For example, changes in the structure of plant communities, due either to natural variation in soil environmental conditions (Prober et al., 2015; Ranjard et al., 2013) or resource amendment (Leff et al., 2015; Li et al., 2018; Zeng et al., 2016), are known to incur changes in the structure of soil bacterial communities. Soil pH is also widely recognized as an important predictor of soil bacterial community composition (Fierer, 2017; Lauber et al., 2009; Maestre et al., 2015; Wei et al., 2013; Zhang, Shen, et al., 2017). In particular, soil acidification has been found to act as a strong environmental filter, resulting in increased determinism in soil bacterial community assembly (Tripathi et al., 2018). Consequently, we expected that N-induced convergence of plant communities and soil acidification would lead to reduced bacterial

β -diversity in our experiment. Contrary to our expectation, bacterial β -diversity showed a unimodal response to increasing N input, indicating that bacterial communities in fact became more divergent in their structure after low N input. While somewhat surprising, this positive response of bacterial β -diversity to low N input is consistent with the idea that increasing resource availability tends to promote stochastic community assembly via strengthening priority effects (Chase, 2003), where stochasticity associated with the order and timing of species arrival influences species interactions, and consequently, community structure (Fukami, 2015). This idea has received most support from studies of macroorganisms (Chase, 2010; Houseman et al., 2008; Yang et al., 2019). An alternative, but not mutually exclusive explanation for the increased divergence in bacterial communities following low N input is that it may have been driven by soil environmental heterogeneity, which also exhibited a unimodal response to N input (Figure 4). In fact, soil environmental heterogeneity, but not plant β -diversity or soil pH, accounted for a significant fraction of the variation in bacterial β -diversity in our experiment (Figure 5; Figures S3, S4, S8, and S9). This result suggests that deterministic processes, as a result of soil heterogeneity that existed between replicate plots within the same N treatments, may have contributed to the observed divergence patterns in soil bacterial communities. Note that although the role of environmental heterogeneity in structuring ecological communities is well appreciated (Amarasekare, 2003; Hutchings et al., 2003; Martiny et al., 2011), within-treatment environmental heterogeneity has received little attention (Gilliam, 2006). Our result thus emphasizes the need to quantify the magnitude of within-treatment soil heterogeneity (akin, conceptually, to the need to consider intraspecific trait variation)

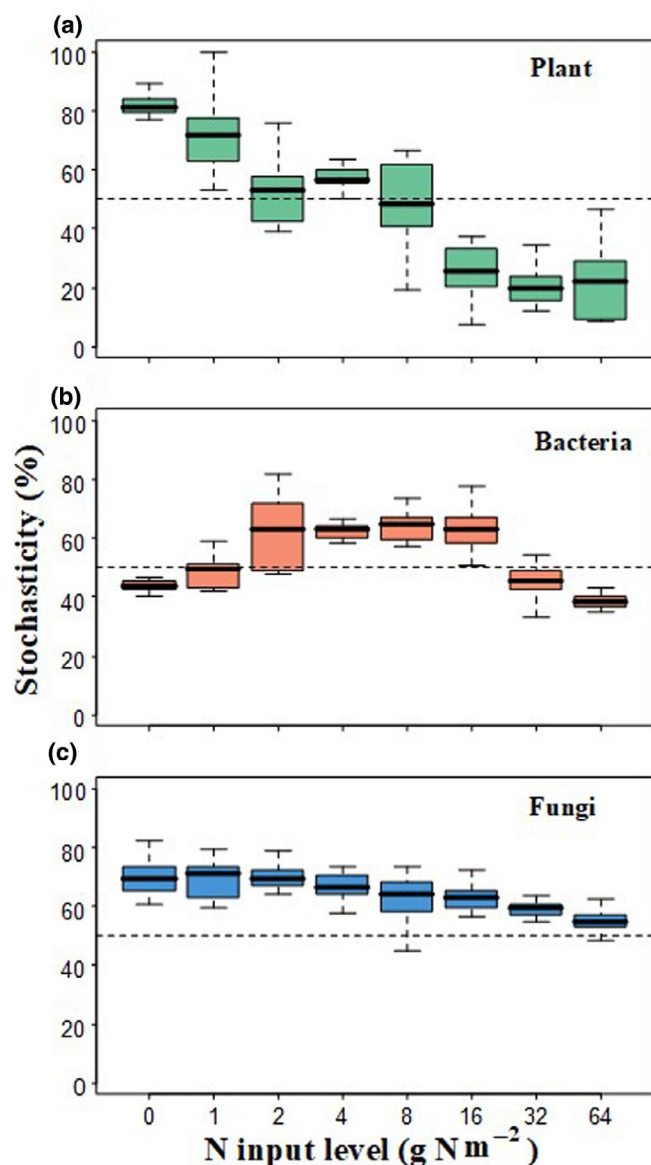


FIGURE 3 The magnitude of stochasticity, quantified as the normalized stochasticity ratio, in regulating plant (a), bacterial (b), and fungal (c) communities with increasing N input. The values were calculated based on Bray–Curtis dissimilarity. Box plots show the median (midline), first quartile and third quartile (box edges), and minimum and maximum (whiskers)

and its role in community assembly. Finally, we note that a major fraction of variation in bacterial β -diversity in our experiment remains unexplained, suggesting that stochasticity may have played a more important role in structuring bacterial, than plant communities (Figure 5b).

Compared with bacteria, soil fungal communities are often thought to be even more strongly coupled with plant communities due to their greater reliance on plants for habitats and substrates (Chen et al., 2018; Peay et al., 2013; van der Linde et al., 2018; Yang et al., 2017). Soil pH has also been identified as a driver of soil fungal composition (Glassman et al., 2017; Leff et al., 2015; Maestre et al.,

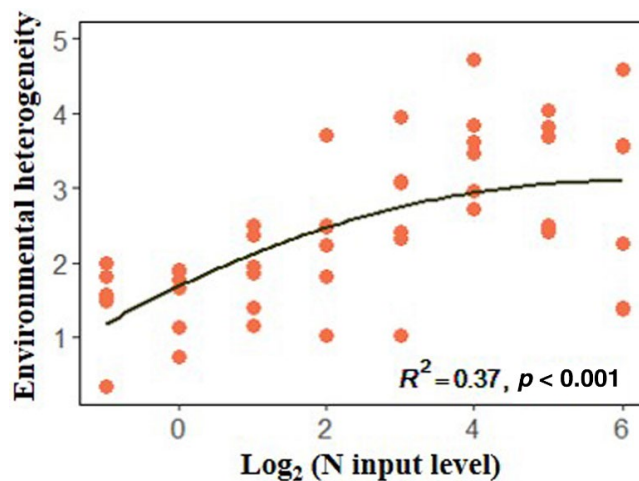


FIGURE 4 Soil environmental heterogeneity as a function of N input. Environmental heterogeneity is calculated as the pairwise Euclidean distance in the seven standardized soil physicochemical variables (soil moisture, soil pH, soil total C, total N, inorganic N concentration, and exchangeable Al³⁺ and Mn²⁺ concentrations) among the four replicate plots of the same treatment

2015). Nevertheless, we found that soil fungal β -diversity was not affected by plant β -diversity (Figures S8 and S9). In fact, soil fungal β -diversity remained roughly constant (Figure 2c) and the magnitude of stochasticity remained uniformly high (Figure 3c) across the N gradient. Two fungal characteristics may have contributed to this pattern. First, compared to bacteria, the dispersal of fungal spores is typically constrained to much shorter distances (Adams et al., 2013; Norros et al., 2012; Peay et al., 2010), offering greater opportunities for dispersal limitation to shape fungal community structure (Li et al., 2020; Peay et al., 2010; Wang et al., 2019). Our result is thus consistent with recent studies reporting that stochastic assembly, driven by dispersal limitation, characterized the soil fungal communities across Scotland (Powell et al., 2015) and on a group of land bridge islands in subtropical China (Wang et al., 2019). Second, compared to bacteria, fungi tend to be more tolerant of adverse environmental conditions, such as soil acidification (Herold et al., 2012; Rousk et al., 2009, 2010) and high metal concentration (Rajapaksha et al., 2004; Stefanowicz et al., 2008). This relative insensitivity to changes in environmental conditions may be the reason why none of the soil environmental variables, including their heterogeneity, failed to explain fungal β -diversity in our experiment (Figure 5c; Figures S3 and S4).

In our study, plant and microbial β -diversity responses to N addition bear some resemblance to their corresponding α -diversity responses to N addition, raising the question whether changes in α -diversity drove the observed changes in β -diversity. However, β -deviation, which accounts for variation in α -diversity, exhibited similar trends as β -diversity, suggesting the independence of β -diversity responses from α -diversity responses. Indeed, bacterial α - and β -diversity were best predicted by soil pH (Liu et al., 2020) and soil environmental heterogeneity (this study), respectively, indicating that they were regulated by different mechanisms. On the other

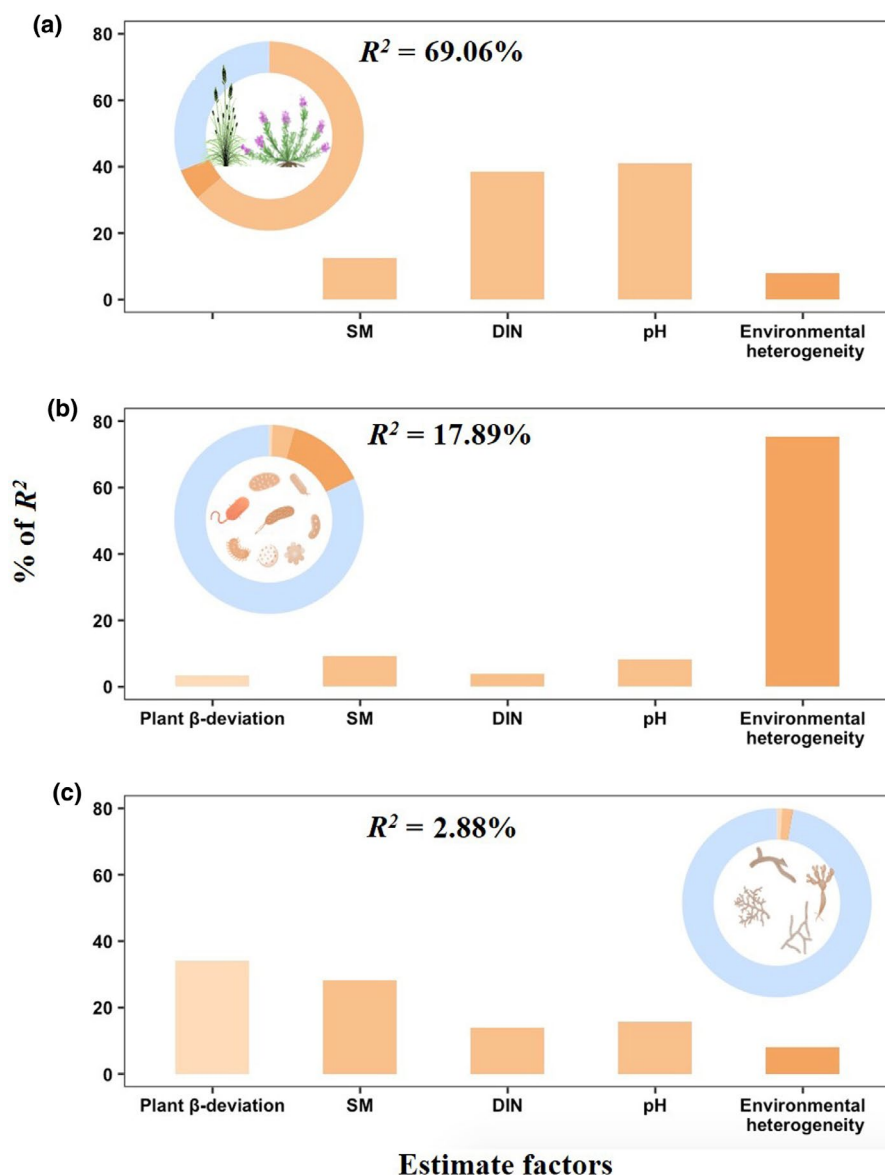


FIGURE 5 Relative importance of different predictors of plant (a), bacterial (b), and fungal (c) β-deviation. For bacteria and fungi, plant β-deviation, in addition to soil moisture (SM), soil dissolved inorganic N concentration (DIN), soil pH, and environmental heterogeneity were used as predictors of their β-deviation. R^2 indicates the total explanation. The importance values of all predictor variables were normalized to sum to 100%. The orange portion of the circle indicates the fraction of variation explained by the predictors, whereas the blue portion indicates the fraction of unexplained variation

hand, our result of lower plant α- and β-diversity under greater N enrichment (i.e., greater directional environmental filtering) is consistent with previous report that deterministic species extinction reduced both plant diversity components in Mediterranean grasslands (Segre et al., 2014). Note that the continuous decline in plant α- and β-diversity with increasing N input is particularly concerning, as it suggests the erosion of plant regional diversity in our study grassland if anthropogenic N input is left uncurbed. Given that N input tends to reduce plant α-diversity in terrestrial habitats (Midolo et al., 2019), understanding how plant β-diversity responds to N input in various ecosystems would help understand how anthropogenic N input influences plant diversity at regional and global scales.

Our study provided, to our knowledge, the first empirical evidence on the divergent responses of plant, bacterial, and fungal β-diversity to increased N input, indicating the operation of different community assembly mechanisms for the three co-occurring taxonomic groups (Figure 6). Consistent with classic niche theory, deterministic processes

dominated plant community assembly, lowering plant β-diversity under higher N input. By contrast, stochastic processes strongly shaped soil fungal community assembly, resulting in high fungal β-diversity across the N gradient. Both deterministic and stochastic processes appeared important for soil bacterial community assembly, where a unimodal response to N input emerged for bacterial β-diversity. Plant-soil interactions are known to be important for ecosystem functions (van der Putten, 2017; Wagg et al., 2014). It remains to be seen how the decoupling of plant and microbial spatial diversity revealed in our study would translate into alterations in grassland plant-microbe interactions and ecosystem functions.

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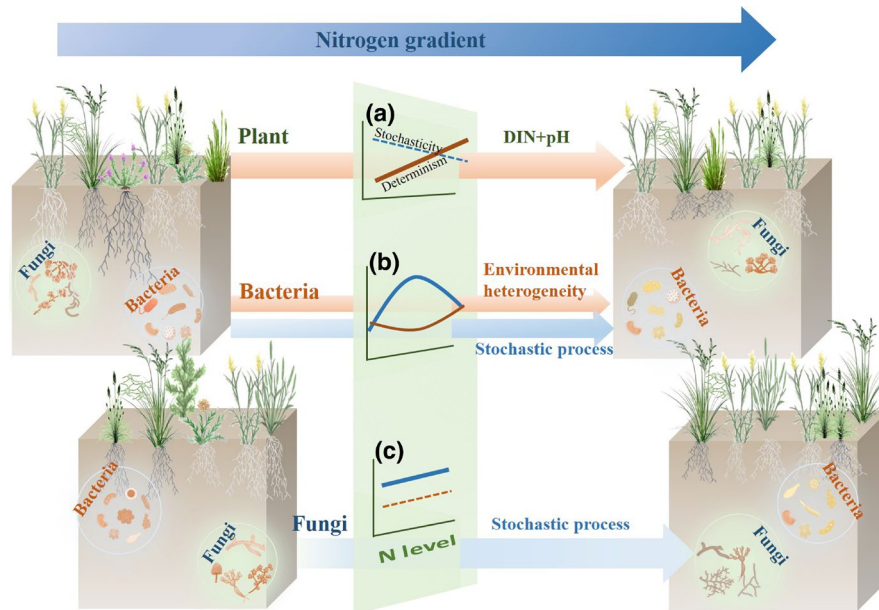


FIGURE 6 A conceptual diagram illustrating the difference in the relative importance of deterministic (represented by the orange lines and arrows) and stochastic (represented by the blue lines and arrows) processes in regulating plant, soil bacterial and fungal communities along a gradient of increasing N availability. (a) Plant community assembly is increasingly governed by deterministic processes with increasing N input; (b) Soil bacterial community assembly is mediated by both stochastic and deterministic processes, with the latter driven by environmental heterogeneity; (c) Soil fungal community assembly is predominantly regulated by stochastic processes. Solid lines represent the driving processes in community assembly

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

AUTHOR CONTRIBUTION

Lin Jiang, Lingli Liu, and Weixing Liu designed the research. Weixing Liu performed the study. Weixing Liu and Xian Yang analyzed the data. Weixing Liu, Xian Yang, Lingli Liu, and Lin Jiang wrote the first draft of the manuscript. All the authors contributed substantially to the manuscript revision.

DATA AVAILABILITY STATEMENT

The bacterial and fungal DNA sequences generated during this study are available from the National Center for Biotechnology Information's GenBank database under project accession number PRJNA573484 and PRJNA573488, respectively. The beta diversity of plant, soil bacterial and fungal communities is available in Figshare: <http://doi.org/10.6084/m9.figshare.14569824>.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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