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Critical transition of soil bacterial diversity and composition triggered by nitrogen enrichment

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Abstract. Soil bacterial communities are pivotal in regulating terrestrial biogeochemical cycles and ecosystem functions. The increase in global nitrogen (N) deposition has impacted various aspects of terrestrial ecosystems, but we still have a rudimentary understanding of whether there is a threshold for N input level beyond which soil bacterial communities will experience critical transitions. Using high-throughput sequencing of the 16S rRNA gene, we examined soil bacterial responses to a long-term (13 yr), multi-level, N addition experiment in a temperate steppe of northern China. We found that plant diversity decreased in a linear fashion with increasing N addition. However, bacterial diversity responded nonlinearly to N addition, such that it was unaffected by N input below 16 g $N \cdot m^{-2} \cdot yr^{-1}$, but decreased substantially when N input exceeded 32 g $N \cdot m^{-2} \cdot yr^{-1}$. A meta-analysis across four N addition experiments in the same study region further confirmed this nonlinear response of bacterial diversity to N inputs. Substantial changes in soil bacterial community structure also occurred between N input levels of 16 to 32 g $N \cdot m^{-2} \cdot yr^{-1}$. Further analysis revealed that the loss of soil bacterial diversity was primarily attributed to the reduction in soil pH, whereas changes in soil bacterial community were driven by the combination of increased N availability, reduced soil pH, and changes in plant community structure. In addition, we found that N addition shifted bacterial communities toward more putatively copiotrophic taxa. Overall, our study identified a threshold of N input level for bacterial diversity and community composition. The nonlinear response of bacterial diversity to N input observed in our study indicates that although bacterial communities are resistant to low levels of N input, further increase in N input could trigger a critical transition, shifting bacterial communities to a low-diversity state.

Key words: acidification; bacterial community composition; bacterial diversity; life history; plant diversity; threshold.

Introduction

Human activities, such as industrialization of fossil fuel combustion and agricultural fertilizer application, have caused increasing nitrogen (N) deposition (Galloway et al. 2004), which poses a serious threat to terrestrial biodiversity (Butchart et al. 2010). This is especially true for grasslands, where N deposition leads to loss of plant diversity across the world (Stevens et al. 2004, Suding et al. 2005, Isbell et al. 2013, Zhang et al. 2019). Soil bacterial communities represent a large proportion of terrestrial biodiversity (Whitman et al. 1998, Delgado-Baquerizo et al. 2017). Both soil physiochemical properties (Fierer and Jackson 2006, Cruz-Martinez et al. 2009, Zhou et al. 2016) and plant community composition (Kowalchuk et al. 2002, Wardle et al. 2004) are known to influence soil bacterial diversity and composition. Yet, it remains largely unknown whether plant and

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soil bacterial communities would respond similarly to elevated N input, or their responses would become decoupled with increasing N input. This represents an important knowledge gap that brings uncertainty for predicting future plant–microbial interactions and thus ecosystem carbon and nutrient dynamics.

Nitrogen inputs could influence bacterial diversity by changing soil environmental conditions such as soil N availability and acidification (Campbell et al. 2010, Zhang and Han 2012). Increasing N availability could result in the extinction of bacterial species that are adapted to nutrient-deficient soils (Zhang and Han 2012, Wang et al. 2018). Both observational and manipulative experiments have identified pH as a key factor shaping the diversity and structure of soil bacterial assemblages (Fierer and Jackson 2006, Rousk et al. 2010, Bartram et al. 2013, Fierer 2017). Since most bacteria grow best around neutral pH, N-induced soil acidification has often been found to cause bacterial diversity loss (Rousk et al. 2010, Ling et al. 2017, Nie et al. 2018). In addition, plants provide metabolic sources for microbes and develop microhabitats around the soil–root interface (Korthals et al. 2001, Wardle et al. 2004). Plant diversity loss under increasing N inputs could decrease the diversity of food resources and microhabitats, and thus might alter microbial diversity (Eisenhauer et al. 2013, Lange et al. 2015).

However, recent studies have revealed inconsistent responses of microbial diversity to nutrient addition. While some studies showed that high levels of N input significantly reduced bacterial diversity (Yao et al. 2014, Wang et al. 2018), others found no effect of N addition on diversity, despite a relatively consistent effect on community composition (Fierer et al. 2012, Ramirez et al. 2012). One possible explanation for the inconsistency among these studies is that the response of bacterial diversity to elevated N input may be nonlinear (Yao et al. 2014), such that bacterial diversity may not necessarily show a discernable decline until a threshold N level is reached. Such thresholds may be more likely to be observed for soil bacterial communities than for their corresponding aboveground plant communities, due to the more complex interactions among microbial species (Shade et al. 2012). Microbial species generally interact with a large number of other co-occurring microbial species, making higher-order interactions potentially common within microbial communities (Bairey et al. 2016, Momeni et al. 2017). If N reaches a critical level that causes the extinction of species embedded in higher-order interactions, an extinction cascade (Bairey et al. 2016, Levine et al. 2017), where the loss of one or a few species cause further extinctions of many other species, could occur.

On the other hand, sufficiently high nutrient input may have the potential to alter the nature of species interactions (Bertness and Callaway 1994, Callaway et al. 2002), which can also have a significant influence on species coexistence and diversity. Recent research demonstrated that the interaction between microbial species could shift from mutualism to competition with increased nutrient supply (Hoek et al. 2016). Given the role of mutualism in promoting diversity (Aschehoug and Callaway 2015, Coyte et al. 2015), the transition from mutualism to competition following sufficient high levels of N inputs may lead to a sharp decrease of species diversity, contributing to the formation of the alternative, low-diversity community state (Scheffer et al. 2012). Despite much recent interest in bacterial community responses to elevated N input, it is still unclear whether such thresholds for the effect of N inputs on bacterial diversity exist.

Here, we investigated the diversity of bacterial communities in a multi-level (0, 1, 2, 4, 8, 16, 32, 64 g $N \cdot m^{-2} \cdot yr^{-1}$), long-term (13 yr), N addition experiment in a temperate steppe in Inner Mongolia, China, in an effort to confirm or refute the presence of an N threshold for soil bacterial diversity. In addition, we also conducted a small-scale meta-analysis by pooling the results from several multi-level N addition experiments across the Mongolia Plateau. Together, we aimed to address two questions. (1) Does soil bacterial diversity respond non-linearly to N addition? We hypothesized that when the N addition level reached a certain level, soil bacterial diversity would cross a threshold and decrease substantially. (2) Will changes in bacterial diversity and community composition be accompanied by parallel changes in plant diversity and community composition under elevated N input? We hypothesized that due to the greater likelihood of high N input altering more complex microbial interactions, microbial communities would be more likely to experience dramatic changes in diversity and composition than the aboveground co-occurring plant communities. Thus, the responses of microbial and plant communities to N input may not necessarily be similar.

Materials and Methods

Study site, experimental design, and sampling

The experimental site is located in a semiarid steppe (42.01′ N, 116.16′ E, and 1,324 m above sea level) in Duolun County, Inner Mongolia, Northern China. Mean annual temperature and precipitation are 2.1°C and 382.3 mm, respectively. The soil type is classified as Haplic Calcisols (FAO classification), with 69.21% ± 0.06% (mean ± SE) sand, 15.60% ± 0.02% silt, and 15.19% ± 0.02% clay. Soil organic C and total N content are 16.94 ± 2.34 and 1.65 ± 0.27 g/kg, respectively. Soil pH is approximately 6.84 ± 0.02. Plant communities are dominated by *Stipa krylovii* Roshev., *Agropyron cristatum* (L.), *Artemisia frigida* Willd, and *Cleistogenes squarrosa* (Trin.) at our experiment site.

Sixty-four plots, each of 10 × 15 m with 5-m buffer zones between adjacent plots, were arranged in eight rows and eight columns. Starting in 2003, each of the eight plots in each row was randomly assigned to one of the eight levels of N fertilization treatments (0, 1, 2, 4, 8, 16, 32, and 64 g $N \cdot m^{-2} \cdot yr^{-1}$). The N addition levels were comparable to those in other N addition experiments in grassland ecosystems (Bai et al. 2010, Dickson and Foster 2011). N fertilization was conducted in July in the form of urea annually. Since 2005, four rows (one in every two rows) were clipped at the end of August once a year.

Soil samples were collected from all non-clipping plots on 15 August 2016. Six randomly located soil cores (15 cm deep and 5 cm in diameter) were taken in 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 lab. Subsamples were stored at 4°C for soil physicochemical analysis and −80°C for DNA extraction, respectively.

Plant species richness and aboveground plant biomass (AGB) were estimated by clipping live biomass during 15–18 August 2016. We counted plant species number using a randomly selected 1 × 1 m quadrat in each plot. All living plant tissues were harvested from a 1 × 1 m quadrat in each plot. All plant samples were oven-dried at 70°C for 48 h and weighed to determine the biomass of each species.

Soil chemical properties

Soil inorganic N (DIN) was extracted with 2 mol/L KCl solution, and concentrations of NH_4^+-N and NO_3^--N in the extracts were measured using a flow injection analyzer (SAN-System, Breda, Netherlands). Soil pH was determined with a glass electrode (soil: water W/V ratio 1:2.5). Air-dried, finely ground and sieved soil subsamples were measured for soil C and N using an elemental analyzer (Analysensysteme, Hanau, Germany). Soil sand, silt, and clay were measured using a laser particle analyzer (Malvern Mastersizer 2000; Malvern, UK).

DNA extraction, amplification, and MiSeq sequencing

Soil DNA was extracted from 0.5 g fresh soil using the PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, California, USA) following the manufacturer's instructions. Purity and quality of the genomic DNA were checked on 0.8% agarose gels. The V3-V4 hypervariable region of bacterial 16S rRNA gene was amplified with the primer sets 338F (5′- ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′- GGACTACHVGGGTWTCTAAT-3′) (Caporaso et al. 2012). To permit multiplexing of samples, a 10-bp barcode unique to each sample was added to the 5' end of the forward and reverse primers. The PCR was carried out on a Mastercycler Gradient (Eppendorf, Hamburg, Germany) using 50-μL reaction volumes, containing 5 μL 10× Ex Taq Buffer (Mg^{2+} plus), 4 μL 12.5 mmol/L dNTP Mix (each), 1.25 U Ex Taq DNA polymerase (TaKaRa), 2 μL template DNA, and 36.75 μL ddH_2O. The thermal-cycling conditions were 94°C for 2 minutes, 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 minutes. Each sample was amplified in triplicates and pooled to mitigate reaction-level PCR biases. The PCR products were purified with the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany), and quantified with Real-Time PCR. Sequencing was performed on a 2× 300 bp paired-end Illumina MiSeq platform (Illumina, San Diego, California, USA).

Bioinformatic analysis

Raw reads were assigned to different samples based on the barcodes and the primers were trimmed through Illumina Analysis Pipeline Version 2.6. The sequences were subsequently processed using QIIME v. 1.8.0 (Caporaso et al. 2010). Paired-end reads were assembled with a minimum overlap of 50 bp using FLASH version 1.0.0 (Magoč and Salzberg 2011). The putative chimeras were detected and filtered by USEARCH (Edgar 2010). The sequences containing ambiguous bases with lengths shorter than 200 bp, as well as all singletons, were deleted. The qualified sequences were clustered into operational taxonomic units (OTUs) at a similarity level of 97% using UPARSE version 7.0 (Edgar 2013). The Ribosomal Database Project (RDP) Classifier tool (Wang et al. 2007) was used to annotate the taxonomic affiliation of all OTUs with a confidence threshold of 0.7 against the Silva 128 database. To standardize survey effort, we randomly resampled 18,877 reads per sample and calculated the diversity indices based on this normalized data set. All sequences have been deposited in SRA of NCBI database under bioproject accession number PRJNA573484.

Statistical analyses

All data were checked for normality using the Shapiro-Wilk test, and log-transformed if necessary. The Levene's test was used to test the homogeneity of variance of bacterial and plant alpha diversity (all $P < 0.05$). One-way ANOVA was performed to assess the effects of N addition on soil bacterial and plant diversity. Tukey HSD test was performed to further explore differences among treatments. Spearman correlations were used to test the relationship between the relative abundance of each bacterial phylum and the N addition level.

To test how N addition affects bacterial community composition, the bacterial community structure was visualized by nonmetric multidimensional scaling (NMDS) ordinations that were based on the Bray-Curtis dissimilarity matrices using the vegan package in R (Oksanen et al. 2013). The permutational multivariate analysis of variance (PERMANOVA) was used to test the effects of N addition on soil bacterial community structure by using the function adonis in the R package vegan. Pairwise comparisons of Bray-Curtis dissimilarity were used to assess the differences in soil bacterial community structure among N addition levels. Moreover, we conducted permutational multivariate homogeneity of group dispersions (PERMDISP2) to test the effects of N addition on the homogeneity of bacterial community dispersion (Anderson et al. 2006) in the R package vegan.

We further assessed bacterial community response to N input level using Threshold Indicator Taxa Analysis (TITAN) with R package TITAN2 (Baker and King 2010). TITAN was used to detect changes in the taxa composition along an environmental gradient, and assess synchrony among taxa change points as evidence for community thresholds (Baker and King 2010). Briefly, each taxon was assigned to an indicator value (IndVal) score that takes into account the frequency of occurrence, the proportional abundance and the direction of taxa changes. IndVal scores were calculated for all species for all possible change points along the N input gradient, and the uncertainty in these scores was assessed by permutation tests. Indicator *z* scores were calculated by standardizing the original IndVal scores using the mean and SD of permuted samples along N levels. The *z* scores distinguished the negative ($z-$) and positive ($z+$) taxa response to N addition. The value of the N level resulting in the maximum (sum($z-$)) represented the community level threshold around which the frequency of occurrence and the abundance of bacterial taxa had the largest aggregate negative changes.

Using the rrnDB database, the rRNA operon copy number of each OTU was searched and estimated according to its closest relatives with known rRNA operon copy (Stoddard et al. 2015). Then, we calculated the abundance-weighted average rRNA operon copy for each soil sample (Wu et al. 2017). To do so, we calculated the product of the estimated operon copy number and the relative abundance of each OTU, and summed these values of all OTUs for each sample. Linear regression analysis was performed to test the relationship between the abundance-weighted average rRNA operon copy number and the N addition level.

Structural equation modeling (SEM) was conducted to examine the causal pathways via which N addition affects soil bacterial richness. Based on our knowledge of the effects of N enrichment on bacterial richness, we developed an a priori model, allowing a hypothesized causal interpretation of the linkages between soil chemical properties, plant productivity, plant richness, and bacterial richness (Appendix S1: Fig. S1). To represent soil N availability, we used the total N concentration and dissolved inorganic N concentration as indicators of the latent variable. N addition levels were ln-transformed before running SEM. The SEM was performed using the AMOS software (IBM SPSS AMOS 20.0.0). We used root mean square error of approximation (RMSEA), and comparative fit index (CFI) to assess model fit (Byrne 2006).

Path analysis can also be used to explore causal connections among relevant factors. We used Mantel path analysis to quantify direct and indirect influences of soil chemical properties and plant community composition on bacterial community composition (Leduc et al. 1992, Hartman et al. 2008). The partial Mantel test was conducted to estimate path coefficients between two variables by keeping all other variables constant. The Bray-Curtis dissimilarity matrix was used for bacterial and plant communities, respectively. The soil chemical variables (total N, DIN, and pH) among samples were Hellinger transformed, and then the distances between samples were calculated on the basis of Euclidean dissimilarity for these variables. The partial Mantel test was performed using ecodist packages in R software (Goslee and Urban 2007).

Meta-analysis for N addition experiments in grasslands in Inner Mongolia

To investigate whether there was a nonlinear response between N inputs and bacterial richness (i.e., OTU numbers) across Inner Mongolia grasslands, we conducted a meta-analysis by searching for N addition studies using Web of Science (Thomson Reuters, New York, New York, USA). The references were screened using the following rules: (1) N manipulative experiments were conducted in grasslands on the Inner Mongolia Plateau; (2) there were at least three levels of N addition treatment; (3) the raw data of 16S rRNA gene sequences could be downloaded from NCBI or obtained from the authors. We finally obtained a total of four experimental studies (including the current study) for the meta-analysis (Appendix S1: Table S1).

We followed the same workflow as in our own experiment to process the sequencing data from these four studies using QIIME v. 1.8.0. OTUs were assigned based on 97% sequence identity to sequences in the Greengenes reference database (McDonald et al. 2012, Shade et al. 2013), and OTU richness was calculated after normalizing the number of sequences at the same depth for all samples. We calculated the response ratio of OTU richness for different N application rates by dividing the mean of the experimental group by the mean of the control group, thus facilitated our comparisons among the four studies. The response ratio was then used as the effect size for the meta-analysis.

We compiled data on the response ratio of OTU richness from the four other N addition experiments in Inner Mongolia grasslands and used smoothing splines to identify the possible threshold of N level (Sherrill et al. 1990, Dodds et al. 2010). We first fit free-knot spines to the data using the function fit.search.numknots in the R package freeknotsplines. The optimum number of knots and the degree of the spline fit were chosen based on the lowest corrected Akaike information criteria (AIC_c) values. In our data, the optimum number of knot was 1. The position of the knot was then determined by using freelsgen in the R package freeknotsplines, which can be interpreted as a threshold value of the N level around which the response ratio of bacterial alpha diversity sharply changes. All statistical analyses were performed with R 3.5.2 (R Development Core Team 2015). $P < 0.05$ was considered to be statistically significant.

Results

Soil bacterial and plant community responses to N addition

Soil bacterial sequences were clustered into a total of 2740 OTUs. Bacterial diversity, including OTU richness, Chao1, Shannon diversity index, and Simpson's diversity index, decreased exponentially with N addition level (Appendix S1: Table S2). Bacterial diversity was not different among the control and N addition treatments up to 16 g $N \cdot m^{-2} \cdot yr^{-1}$ but markedly declined at the two highest N addition levels (32 and 64 g $N \cdot m^{-2} \cdot yr^{-1}$; Fig. 1). Different from bacteria, plant richness ($r^2 = 0.57$, $P < 0.001$), Shannon diversity index ($r^2 = 0.58$, $P < 0.001$), and Simpson's diversity index ($r^2 = 0.53$, $P < 0.001$) decreased linearly with N addition levels (Fig. 1).

The phylum *Proteobacteria* was dominated by *Alphaproteobacteria*, which was not affected by N addition (Fig. 2). The relative abundance of *Bacteroidetes*, *Saccharibacteria*, *Betaproteobacteria*, and *Gammaproteobacteria* increased with increasing N addition, whereas the relative abundance of *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, *Nitrospirae*, and *Deltaproteobacteria* decreased with increasing N addition.

Both bacterial community composition and beta diversity were significantly affected by N addition (PERMANOVA, $r^2 = 0.645$, $F(df=7) = 6.222$, $P = 0.001$; PERMDISP2, $F(df=7) = 3.428$, $P = 0.011$; Fig. 3a, Appendix S1: Table S3). The bacterial communities in the plots receiving 32 and 64 g $N \cdot m^{-2} \cdot yr^{-1}$ were different from the communities receiving N below 16 g $N \cdot m^{-2} \cdot yr^{-1}$ levels. However, there was no detectable difference in bacterial communities between the two highest N addition levels (Appendix S1: Table S3). The result of the TITAN analysis indicated that the threshold for the effect of N inputs on bacterial community structure was between 16 and 32 g $N \cdot m^{-2} \cdot yr^{-1}$ (Fig. 3b).

Linking soil bacterial diversity and community structure to environmental variables and plant communities

The SEM showed that N addition had a negative effect on bacterial richness as it resulted in increased soil N availability, and consequently lower soil pH (Fig. 4; Appendix S1 Table S4). Mantel path analysis (Fig. 5) showed that plant community composition responded most strongly to soil pH after accounting for all other environmental variables ($r_M = 0.470$, $P = 0.001$). Bacterial community composition was influenced not only by soil pH ($r_M = 0.369$, $P = 0.001$) and inorganic N concentration ($r_M = 0.145$, $P = 0.022$), but also by plant community composition ($r_M = 0.228$, $P = 0.001$).

Meta-analysis of N addition effect on bacterial diversity in Inner Mongolia grassland

Bacterial OTU richness responded nonlinearly to increasing N input across the four grassland sites in Inner Mongolia (Fig. 6; Appendix S1: Table S2). When pooling data from all four study sites, we found that the bacterial OTU richness decreased precipitously after the N input reached a threshold of approximately 28 g $N \cdot m^{-2} \cdot yr^{-1}$ (Fig. 6).

Discussion

Nonlinear response of soil bacterial diversity to N addition in temperate steppe

Consistent with our hypothesis, we found that bacterial diversity was not affected by low levels of N input, but sharply declined between 16 and 32 g $N \cdot m^{-2} \cdot yr^{-1}$. This result implies that bacterial diversity responds nonlinearly rather than linearly to N inputs, which is also confirmed by our meta-analysis across four Inner Mongolia grassland sites (Fig. 6). Different from bacterial diversity, plant diversity decreased more gradually with increasing N addition. Compared to plants, bacteria tend to show greater degree of physiological plasticity, higher population densities, and greater dispersal ability (Shade et al. 2012). These individual and population attributes of bacteria could partly explain why bacterial communities respond less to low levels of N input than plant communities. Indeed, another experiment in Inner Mongolia found that compared to bacterial communities, the selective effect of N enrichment on plant communities started to increase at a lower N addition level (Zhang et al. 2011).

Soil pH manipulative experiments found that bacterial diversity decreased with soil acidification (Bartram et al. 2013, Yun et al. 2016), and the largest decrease was observed when soil pH was below 4.5 (Rousk et al. 2010). Accordingly, our SEM suggested that the decrease in bacterial diversity was strongly associated with the decrease in soil pH caused by elevated N inputs (Fig. 4). Furthermore, Mantel path analysis indicated that the environmental effects on bacterial communities depended mainly on changes in soil pH under N enrichment. In our study, the abundance of the two most dominant bacterial taxa (*Acidobacteria* and *Actinobacteria*) decreased sharply when soil pH was decreased below 5 (Fig. 2, Appendix S1: Table S4). On the one hand, the loss of the dominant bacteria taxa may have destructed higher-order interactions within soil bacteria communities, which form complex interspecific interaction networks (Faust and Raes 2012). The loss of higher-order interactions may cause further loss of many other taxa (Bairey et al. 2016, Levine et al. 2017), triggering the shift of bacterial communities to a different, low-

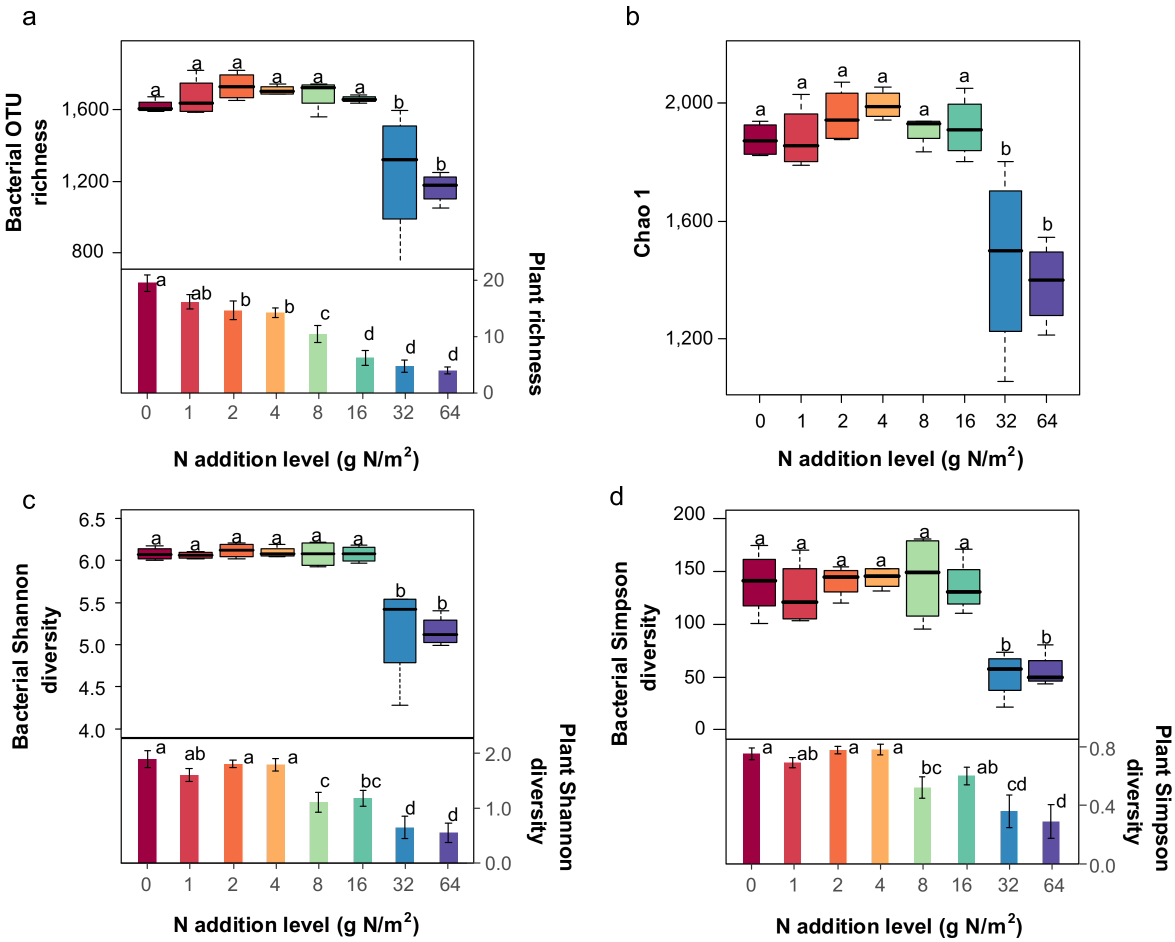

FIG. 1. The effects of N addition on soil bacterial and plant diversity: (a) bacterial operational taxonomic unit (out) richness and plant richness, (b) bacterial Chao 1 diversity index, (c) bacterial and plant Shannon-Wiener diversity index, (d) bacterial and plant Simpson's diversity index. All data are presented as the mean ± SE (n = 4). Box plots show midline, median; box edges, first quartile and third quartile; and whiskers, minimum and maximum. The different letters indicate the significant differences between N addition treatments (P < 0.05).

diversity state. On the other hand, nutrient enrichment may have altered the interaction between microbial species, where the shift from mutualism to competition with increased nutrient supply (Hoek et al. 2016) could also contribute to the formation of the low-diversity community state. Note that the continual increase in N input from 32 to 64 g $N{\cdot}m^{-2}{\cdot}yr^{-1}$ no longer resulted in a significant loss of bacterial diversity, possibly because further increase in N input did not cause significant changes in soil pH (Appendix S1: Table S4). Overall, the large pH gradient (from 6.8 to 4.6), associated with the large range in N input, seems necessary for detecting the nonlinear response of bacterial diversity to N input and the existence of N input threshold.

The linkages between plant and bacterial community composition

Our study revealed that N-induced changes in environmental factors (Appendix S1: Table S4) and plant communities (Appendix S1: Fig. S2) both account for bacterial community composition. The TITAN test indicated that the threshold for N-induced bacterial community structure changes was between 16 and 32 g $N{\cdot}m^{-2}{\cdot}yr^{-1}$. We further found that bacterial community dissimilarity was significantly associated with plant community dissimilarity (r_M = 0.57, P < 0.001). One explanation for this association is that shared environmental factors contribute to relationships between bacterial and plant community composition (Prober et al. 2015), as N-induced changes in soil pH simultaneously influenced the structure of both bacterial (r_M = 0.77, P < 0.001) and plant (r_M = 0.64, P < 0.001) communities. However, the mantel path analysis showed that even after accounting for the effects of abiotic environmental variables, bacterial community composition remained strongly related to plant community composition (Fig. 5), supporting our second hypothesis that changes in bacterial community composition under N enrichment was associated with the changes in plant community

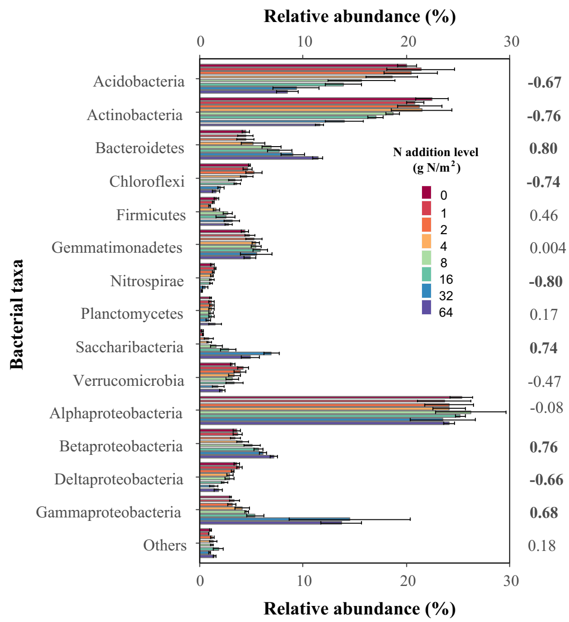

FIG. 2. Changes in the relative abundance of dominant bacterial taxa along with N addition levels. Only taxa with average relative abundances >1% at each N level are shown. All data are presented as the mean ± SE (n = 4). The numbers on the right indicate the correlation (Spearman's r values) between N input rates and relative abundances calculated across all samples.

composition. The contribution of plant community composition to the shift in bacterial community composition under N deposition indicates some covariation between above- and belowground diversity under N deposition. This result presumably reflects the fact that plants provide microhabitats as well as organic substrates for soil bacteria, such that changes in plant community composition lead to changes in both habitats and carbon resources for soil bacteria (Ramirez et al. 2010), translating into changes in soil bacterial communities. To more rigorously distinguish the effects of plant communities and abiotic environments on soil microbial communities, it is necessary to conduct experiments that simultaneously manipulate plant community assembly and abiotic factors.

Shifts in soil bacterial relative abundance along with N addition levels

Our long-term, multi-level N addition created a gradient of soil acidification, with soil pH decreasing from 6.8 to 4.5 with increasingly N addition (Table. S4). We expected that the relative abundance of *Bacteroidetes* would decrease with increasing N due to intolerant of low pH conditions (Lauber et al. 2009). However, the responses of the relative abundance of *Bacteroidetes* were opposite to our expectation (Appendix S1: Fig. S3). Similarly, we expected that the relative abundance of *Nitrospirae*, which performs nitrite oxidation in the second step of nitrification, would be increased by N addition. However, the relative abundance of *Nitrospirae* significantly decreased, which might be caused the increased nitrite concentration and lower soil pH (Ehrich et al. 1995). These results suggest that although changes in soil N supply and changes in soil pH could alter bacterial community structure, the shifts of specific bacterial taxonomic groups could not be simply predicted by changes in soil chemistry (Ramirez et al., 2010).

According to the oligotrophic-copiotrophic concept, copiotrophic bacteria usually have high growth rates under nutrient-rich conditions, while oligotrophic bacteria have lower growth rates but are able to maintain growth under nutrient-poor conditions (Koch 2001). Therefore, increasing N availability should facilitate copiotrophic rather than oligotrophic bacterial growth (Leff et al. 2015). Consistent with previous studies (Fierer et al. 2012, Leff et al. 2015), we found that the

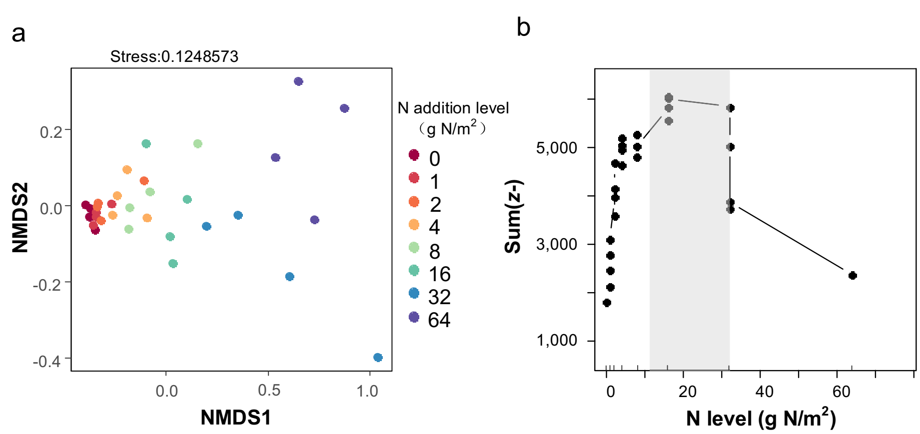

FIG. 3(A). Nonmetric multidimensional scaling (NMDS) analysis of bacterial community structures under increased N inputs. (b) Bacterial community change point for OTUs reduced in abundance along with N addition levels showing community threshold at maximum [sum(z-)] and 5–95% bootstrap percentile range. The sum($z-$) values represent the sum of responses for each possible change point along with N addition levels. The labels marked inside the x-axis represent the specific N addition levels in this study.

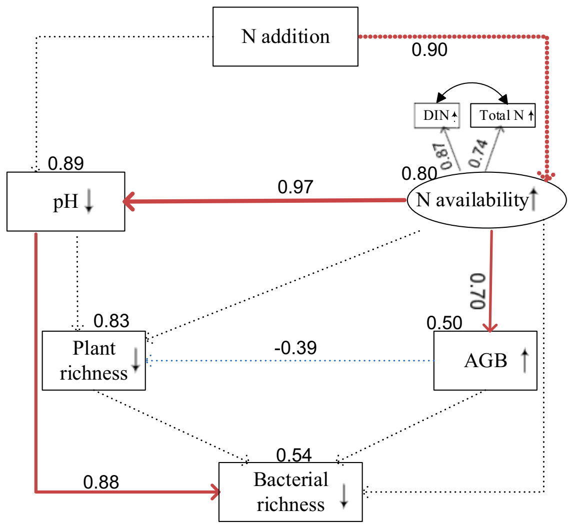

FIG. 4. The structural equation modeling (SEM) analysis of the effect of N addition on soil bacterial richness via the pathways of N addition, soil pH, soil N availability, plant richness, and aboveground biomass (AGB). Here, soil N availability is a latent variable defined by dissolved inorganic N (DIN) and total N concentration. Square boxes represent the measured variables while the oval represents the latent variable. A down arrow indicates a significant decrease upon N addition. Results of the model fitting were λ^2 = 13.513, P = 0.095, df = 8, comparative fit index (CFI) = 0.971, root mean square error of approximation (RMSEA) = 0.054, and n = 32. 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 (R^2). Values associated with the arrows represent standardized path coefficients.

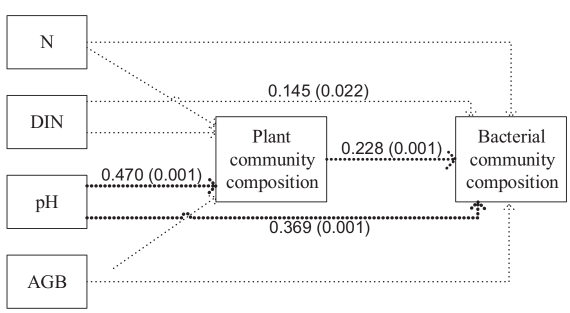

FIG. 5. Mantel path analysis linking taxonomic composition of bacterial communities to soil chemical attributes and plant communities. Values close to paths between boxes present partial Mantel coefficients, similar to regression weights among matrices rather than univariate variables, and demonstrated the magnitude of the relationship. Solid and dashed lines indicate significant and nonsignificant paths, respectively, at P < 0.05. Line width is proportional to the correlation coefficient, and P values are in parentheses.

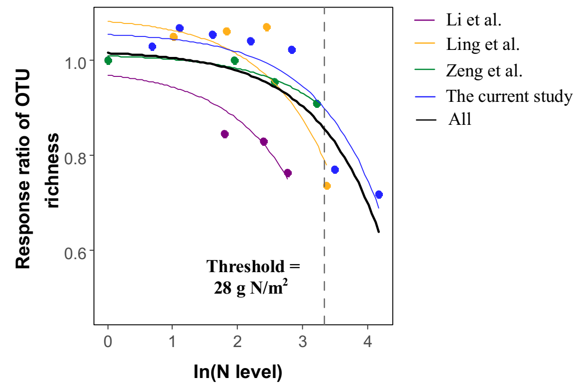

FIG. 6. The relationships between response ratios of bacterial OTU richness to N addition and N addition level across four grassland sites in Inner Mongolia. Papers noted in the color key are Li et al. (2016), Ling et al. (2017), and Zeng et al. (2016).

abundances of *Gammaproteobacteria*, which has often been associated with copiotrophic bacteria (Kurm et al 2017), was stimulated by N addition. At the same time, the abundance of Acidobacteria and Nitrospirae, which have been identified as oligotrophic bacteria, decreased with the increase in N input. In addition, we also found that N addition promoted the abundance of bacteria with higher rRNA operon copy number (Appendix S1: Fig. S4). The rRNA operon copy number correlates with bacterial reproduction rate and response rate to resource availability (Roller et al. 2016, Wu et al. 2017), thus reflects the ecological strategies of bacteria, as higher rRNA operon copy number is associated with the faster-growing copiotrophic bacteria (Roller et al. 2016, Samad et al. 2017). The increased abundance-weighted average rRNA operon copy number under N addition thus provided another line of evidence that elevated N input favored copiotrophic taxa.

Furthermore, compared to oligotrophic bacteria, copiotrophic bacteria prefer to use more labile C substrates (Koch 2001, Eilers et al. 2010), and thus might have a higher C use efficiency (Roller and Schmidt 2015). However, since the substrates under natural conditions are mixtures of organic matters with different qualities, field experiment could not fully explore the linkages between the bacterial life strategy, substrate preference and thus C use efficiency under N enrichment (Ramirez et al. 2012). By using ^{13}C labelled glucose and phenol, our previous laboratory incubation study at the same experimental site found that soil microbial C use efficiency for labile carbon substrate was stimulated by N addition (Liu et al. 2018), which is consistent with the observed increase in the abundance of some copiotrophic bacterial taxa (Fig. 2). Meanwhile, soil microbial C use efficiency for recalcitrant carbon substrate was decreased by N addition (Liu et al. 2018), which is in line with the decrease in oligotrophic bacterial taxa at our site. The shifts in bacterial communities observed in the field and the changes in C use efficiency observed in the lab incubation both indicated that N enrichment shift bacterial communities toward more copiotrophic and away from more oligotrophic taxa. Note that copiotrophic dominating over oligotrophic bacteria is consistent with competition being more important under higher resource input. The shift of specific taxonomic taxa and total community composition indicated that soil bacterial community turnover was a signature of N enrichment more broadly, rather than pH change, which mainly affected bacterial diversity. Further studies will be required to assess the generality of the contrasting mechanism underlying responses of microbial diversity and community composition to N enrichment in other ecosystems.

Conclusions

We found a critical threshold of N input above which soil bacterial diversity declined sharply and bacterial communities shifted substantially in our study temperate grassland. Furthermore, our findings revealed that the decrease in bacterial diversity was associated with N-induced decreases in soil pH, but not N-induced decreases in plant diversity. However, the response of soil bacterial community composition was accompanied by changes in plant communities under N enrichment, though changes in plant diversity were linear rather than threshold-based. In addition, the bacterial community composition at our experimental site shifted toward putatively copiotrophic taxa, possibly responding to increasing N availability and labile C supply. Our findings move a step forward to understand how N deposition-induced changes in biotic and abiotic drivers could interact to regulate the transitions of bacterial communities. Further research is needed to explore how the threshold responses of bacterial communities would feedback to ecosystem structures and functions under future N deposition scenarios.

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LITERATURE CITED

Anderson, M. J., K. E. Ellingsen, and B. H. McArdle. 2006. Multivariate dispersion as a measure of beta diversity. Ecology Letters 9:683–693.

Aschehoug, E. T., and R. M. Callaway. 2015. Diversity increases indirect interactions, attenuates the intensity of competition, and promotes coexistence. American Naturalist 186:452–459.

Bai, Y. F., J. G. Wu, C. M. Clark, S. Naeem, Q. M. Pan, J. H. Huang, L. X. Zhang, and X. G. Han. 2010. Tradeoffs and thresholds in the effects of nitrogen addition on biodiversity and ecosystem functioning: evidence from inner Mongolia Grasslands. Global Change Biology 16:358–372.

Bairey, E., E. D. Kelsic, and R. Kishony. 2016. High-order species interactions shape ecosystem diversity. Nature Communications 7:12285.

Baker, M. E., and R. S. King. 2010. A new method for detecting and interpreting biodiversity and ecological community thresholds. Methods in Ecology and Evolution 1:25–37.

Bartram, A., X. Jiang, M. Lynch, A. Masella, G. Nicol, J. Dushoff, and J. Neufeld. 2013. Exploring links between pH and bacterial community composition in soils from the Craibstone Experimental Farm. FEMS Microbiology Ecology 87:403–415.

Bertness, M. D., and R. Callaway. 1994. Positive interactions in communities. Trends in Ecology & Evolution 9:191–193.

Butchart, S. H. M. et al 2010. Global biodiversity: indicators of recent declines. Science 328:1164–1168.

Byrne, B. M..2006. Structural equation modeling with EQS: Basic concepts, applications, and programming. Second edition. Lawrence Erlbaum Associates Publishers, Mahwah, New Jersey, USA.

Callaway, R. M. et al 2002. Positive interactions among alpine plants increase with stress. Nature 417:844–848.

Campbell, B. J., S. W. Polson, T. E. Hanson, M. C. Mack, and E. A. G. Schuur. 2010. The effect of nutrient deposition on bacterial communities in Arctic tundra soil. Environmental Microbiology 12:1842–1854.

Caporaso, J. G. et al 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7:335–336.

Caporaso, J. et al 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platformsOpen. ISME Journal 6:1621–1624.

Coyte, K. Z., J. Schluter, and K. R. Foster. 2015. The ecology of the microbiome: Networks, competition, and stability. Science 350:663–666.

Cruz-Martinez, K., K. B. Suttle, E. L. Brodie, M. E. Power, G. L. Andersen, and J. F. Banfield. 2009. Despite strong seasonal responses, soil microbial consortia are more resilient to long-term changes in rainfall than overlying grassland. ISME Journal 3:738–744.

Delgado-Baquerizo, M., D. J. Eldridge, V. Ochoa, B. Gozalo, B. K. Singh, and F. T. Maestre. 2017. Soil microbial communities drive the resistance of ecosystem multifunctionality to global change in drylands across the globe. Ecology Letters 20:1295–1305.

Dickson, T. L., and B. L. Foster. 2011. Fertilization decreases plant biodiversity even when light is not limiting. Ecology Letters 14:380–388.

Dodds, W., W. Clements, K. Gido, R. Hilderbrand, and R. King. 2010. Thresholds, breakpoints, and nonlinearity in freshwaters as related to management. Journal of the North American Benthological Society 29:988–997.

Edgar, R. C. 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460–2461.

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

Ehrich, S., D. Behrens, E. Lebedeva, W. Ludwig, and E. Bock. 1995. A new obligately chemolithoautotrophic, nitrite-oxidizing bacterium, *Nitrospira moscoviensis* sp. nov. and its phylogenetic relationship. Archives of Microbiology 164:16–23.

Eilers, K. G., C. L. Lauber, R. Knight, and N. Fierer. 2010. Shifts in bacterial community structure associated with inputs of low molecular weight carbon compounds to soil. Soil Biology & Biochemistry 42:896–903.

Eisenhauer, N., T. Dobies, S. Cesarz, S. E. Hobbie, R. J. Meyer, K. Worm, and P. B. Reich. 2013. Plant diversity effects on soil food webs are stronger than those of elevated CO_2 and N deposition in a long-term grassland experiment. Proceedings of the National Academy of Sciences USA 110:6889–6894.

Faust, K., and J. Raes. 2012. Microbial interactions: from networks to models. Nature Reviews Microbiology 10:538–550.

Fierer, N. 2017. Embracing the unknown: disentangling the complexities of the soil microbiome. Nature Reviews Microbiology 15:579–590.

Fierer, N., and R. B. Jackson. 2006. The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences USA 103:626–631.

Fierer, N., C. L. Lauber, K. S. Ramirez, J. Zaneveld, M. A. Bradford, and R. Knight. 2012. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. ISME Journal 6:1007–1017.

Galloway, J. N. et al 2004. Nitrogen cycles: past, present, and future. Biogeochemistry 70:153–226.

Goslee, S. C., and D. L. Urban. 2007. The ecodist package for dissimilarity-based analysis of ecological data. Journal of Statistical Software 22:1–19.

Hartman, W. H., C. J. Richardson, R. Vilgalys, and G. L. Bruland. 2008. Environmental and anthropogenic controls over bacterial communities in wetland soils. Proceedings of the National Academy of Sciences USA 105:17842–17847.

Hoek, T. A., K. Axelrod, T. Biancalani, E. A. Yurtsev, J. Liu, and J. Gore. 2016. Resource availability modulates the cooperative and competitive nature of a microbial cross-feeding mutualism. PLoS Biology 14:e1002540.

Isbell, F., P. B. Reich, D. Tilman, S. E. Hobbie, S. Polasky, and S. Binder. 2013. Nutrient enrichment, biodiversity loss, and consequent declines in ecosystem productivity. Proceedings of the National Academy of Sciences USA 110:11911–11916.

Koch, A. L. 2001. Oligotrophs versus copiotrophs. BioEssays 23:657–661.

Korthals, G. W., P. Smilauer, C. Van Dijk, and W. H. Van Der Putten. 2001. Linking above- and below-ground biodiversity: abundance and trophic complexity in soil as a response to experimental plant communities on abandoned arable land. Functional Ecology 15:506–514.

Kowalchuk, G., D. Buma, W. de Boer, P. Klinkhamer, and J. Veen. 2002. Effects of above-ground plant species composition and diversity on the diversity of soil-borne micro-organisms. Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology 81:1–4.

Kurm, V., W. H. van der Putten, W. de Boer, S. Naus-Wiezer, and W. H. G. Hol. 2017. Low abundant soil bacteria can be metabolically versatile and fast growing. Ecology 98:555–564.

Lange, M. et al 2015. Plant diversity increases soil microbial activity and soil carbon storage. Nature Communications 6.

Lauber, C. L., M. Hamady, R. Knight, and N. Fierer. 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Applied and Environmental Microbiology 75:5111–5120.

Leduc, A., P. Drapeau, Y. Bergeron, and P. Legendre. 1992. Study of spatial components of forest cover using partial Mantel tests and path analysis. Journal of Vegetation Science 3:69–78.

Leff, J. W. et al 2015. Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. Proceedings of the National Academy of Sciences USA 112:10967–10972.

Levine, J. M., J. Bascompte, P. B. Adler, and S. Allesina. 2017. Beyond pairwise mechanisms of species coexistence in complex communities. Nature 546:56–64.

Li, H. et al 2016. Responses of soil bacterial communities to nitrogen deposition and precipitation increment are closely linked with aboveground community Variation. Microbial Ecology 71:974–989.

Ling, N., D. Chen, H. Guo, J. Wei, Y. Bai, Q. Shen, and S. Hu. 2017. Differential responses of soil bacterial communities to long-term N and P inputs in a semi-arid steppe. Geoderma 292:25–33.

Liu, W., C. Qiao, S. Yang, W. Bai, and L. Liu. 2018. Microbial carbon use efficiency and priming effect regulate soil carbon storage under nitrogen deposition by slowing soil organic matter decomposition. Geoderma 332:37–44.

Magoč, T., and S. L. Salzberg. 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27:2957–2963.

McDonald, D., M. N. Price, J. Goodrich, E. P. Nawrocki, T. Z. DeSantis, A. Probst, G. L. Andersen, R. Knight, and P. Hugenholtz. 2012. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. ISME Journal 6:610–618.

Momeni, B., L. Xie, and W. Shou. 2017. Lotka-Volterra pairwise modeling fails to capture diverse pairwise microbial interactions. eLife 6:e25051.

Nie, Y., M. Wang, W. Zhang, Z. Ni, Y. Hashidoko, and W. Shen. 2018. Ammonium nitrogen content is a dominant predictor of bacterial community composition in an acidic forest soil with exogenous nitrogen enrichment. Science of the Total Environment 624:407–415.

Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. Minchin, R. B. O'Hara, G. Simpson, P. Solymos, M. H. H. Stevenes, and H. Wagner. 2013. Vegan: Community Ecology Package. FR package version 2.0-10. http://CRAN.Rproject.org/package=vegan.

Prober, S. M. et al 2015. Plant diversity predicts beta but not alpha diversity of soil microbes across grasslands worldwide. Ecology Letters 18:85–95.

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

Ramirez, K. S., C. L. Lauber, R. Knight, M. A. Bradford, and N. Fierer. 2010. Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. Ecology 91:3463–3470.

R Development Core Team. 2015. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.

Roller, B., and T. Schmidt. 2015. The physiology and ecological implications of efficient growth. ISME Journal 9:1481–1487.

Roller, B. R. K., S. F. Stoddard, and T. M. Schmidt. 2016. Exploiting rRNA operon copy number to investigate bacterial reproductive strategies. Nature Microbiology 1:16160–16160.

Rousk, J., E. Baath, P. C. Brookes, C. L. Lauber, C. Lozupone, J. G. Caporaso, R. Knight, and N. Fierer. 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME Journal 4:1340–1351.

Samad, M. S., C. Johns, K. G. Richards, G. J. Lanigan, C. A. M. de Klein, T. J. Clough, and S. E. Morales. 2017. Response to nitrogen addition reveals metabolic and ecological strategies of soil bacteria. Molecular Ecology 26:5500–5514.

Scheffer, M. et al 2012. Anticipating critical transitions. Science 338:344–348.

Shade, A. et al 2012. Fundamentals of microbial community resistance and resilience. Frontiers in Microbiology 3:417.

Shade, A., J. G. Caporaso, J. Handelsman, R. Knight, and N. Fierer. 2013. A meta-analysis of changes in bacterial and archaeal communities with time. ISME Journal 7:1493–1506.

Sherrill, D. L., S. J. Anderson, and G. Swanson. 1990. Using smoothing splines for detecting ventilatory thresholds. Medicine & Science in Sports & Exercise 22:684–689.

Stevens, C. J., N. B. Dise, J. O. Mountford, and D. J. Gowing. 2004. Impact of nitrogen deposition on the species richness of grasslands. Science 303:1876–1879.

Stoddard, S. F., B. J. Smith, R. Hein, B. R. K. Roller, and T. M. Schmidt. 2015. rrnDB: improved tools for interpreting rRNA gene abundance in bacteria and archaea and a new foundation for future development. Nucleic Acids Research 43: d593–d598.

Suding, K. N., S. L. Collins, L. Gough, C. Clark, E. E. Cleland, K. L. Gross, D. G. Milchunas, and S. Pennings. 2005. Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. Proceedings of the National Academy of Sciences USA 102:4387–4392.

Wang, C., D. Liu, and E. Bai. 2018. Decreasing soil microbial diversity is associated with decreasing microbial biomass under nitrogen addition. Soil Biology & Biochemistry 120:126–133.

Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole. 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology 73:5261–5267.

Wardle, D. A., R. D. Bardgett, J. N. Klironomos, H. Setala, W. H. van der Putten, and D. H. Wall. 2004. Ecological linkages between aboveground and belowground biota. Science 304:1629–1633.

Whitman, W. B., D. C. Coleman, and W. J. Wiebe. 1998. Prokaryotes: the unseen majority. Proceedings of the National Academy of Sciences USA 95:6578–6583.

Wu, L. et al 2017. Microbial functional trait of rRNA operon copy numbers increases with organic levels in anaerobic digesters. ISME Journal 11:1–5.

Yao, M. et al 2014. Rate-specific responses of prokaryotic diversity and structure to nitrogen deposition in the Leymus chinensis steppe. Soil Biology and Biochemistry 79:81–90.

Yun, Y., H. Wang, B. Man, X. Xiang, J. Zhou, X. Qiu, Y. Duan, and A. S. Engel. 2016. The relationship between pH and bacterial communities in a single karst ecosystem and its implication for soil acidification. Frontiers in Microbiology 7:1955.

Zeng, J., X. Liu, L. Song, X. Lin, H. Zhang, C. Shen, and H. Chu. 2016. Nitrogen fertilization directly affects soil bacterial diversity and indirectly affects bacterial community composition. Soil Biology and Biochemistry 92:41–49.

Zhang, X., and X. Han. 2012. Nitrogen deposition alters soil chemical properties and bacterial communities in the Inner Mongolia grassland. Journal of Environmental Sciences 24:1483–1491.

Zhang, X., W. Liu, Y. Bai, G. Zhang, and X. Han. 2011. Nitrogen deposition mediates the effects and importance of chance in changing biodiversity. Molecular Ecology 20:429–438.

Zhang, Y., J. Feng, M. Loreau, N. He, X. Han, and L. Jiang. 2019. Nitrogen addition does not reduce the role of spatial asynchrony in stabilising grassland communities. Ecology Letters 22:563–571.

Zhou, J. et al 2016. Temperature mediates continental-scale diversity of microbes in forest soils. Nature Communications 7:12083.

Supporting Information

Additional supporting information may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/ecy.3053/suppinfo

Data Availability

The bacterial DNA sequences have been deposited in the NCBI Sequence Read Archive under BioProject accession no. PRJNA573484. The data of meta-synthesis have been deposited in Figshare: http://doi.org/10.6084/m9.figshare.11917593