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Nitrogen deposition alters soil chemical properties and bacterial communities in the Inner Mongolia grassland

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Abstract

Nitrogen deposition has dramatically altered biodiversity and ecosystem functioning on the earth; however, its effects on soil bacterial community and the underlying mechanisms of these effects have not been thoroughly examined. Changes in ecosystems caused by nitrogen deposition have traditionally been attributed to increased nitrogen content. In fact, nitrogen deposition not only leads to increased soil total N content, but also changes in the NH_4^+-N content, NO_3^--N content and pH, as well as changes in the heterogeneity of the four indexes. The soil indexes for these four factors, their heterogeneity and even the plant community might be routes through which nitrogen deposition alters the bacterial community. Here, we describe a 6-year nitrogen addition experiment conducted in a typical steppe ecosystem to investigate the ecological mechanism by which nitrogen deposition alters bacterial abundance, diversity and composition. We found that various characteristics of the bacterial community were explained by different environmental factors. Nitrogen deposition decreased bacterial abundance that is positively related to soil pH value. In addition, nitrogen addition decreased bacterial diversity, which is negatively related to soil total N content and positively related to soil NO_3^--N heterogeneity. Finally, nitrogen addition altered bacterial composition that is significantly related to soil NH_4^+-N content. Although nitrogen deposition significantly altered plant biomass, diversity and composition, these characteristics of plant community did not have a significant impact on processes of nitrogen deposition that led to alterations in bacterial abundance, diversity and composition. Therefore, more sensitive molecular technologies should be adopted to detect the subtle shifts of microbial community structure induced by the changes of plant community upon nitrogen deposition.

Key words: environmental heterogeneity; microbial community; microbial diversity; nitrogen enrichment; soil acidification
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Introduction

Anthropogenic activities such as agricultural fertilization and combustion of fossil fuels have increased the rate of atmospheric nitrogen (N) deposition from pre-industrial levels of 0.1–0.3 g N/(m^2·yr), to 0.7 g N/(m^2·yr) in the central and eastern United States, 1.7 g g N/(m^2·yr) in central Europe, and as high as 10 g N/(m^2·yr) in the Netherlands (Vitousek et al., 1997; Bakker and Berendse, 1999; Galloway et al., 2004; Stevens et al., 2004; Clack and Tilman, 2008). The N deposition rate is expected to increase similarly in the future in developing countries of Asia and South America (Galloway et al., 2004). Because N is an important compositional element in organisms and a limiting resource in terrestrial ecosystems (Vitousek and Howarth, 1991), N deposition has dramatically altered biodiversity and ecosystem functioning (Galloway et al., 1995; Jordan and Weller, 1996; Vitousek et al., 1997; Bobbink et al., 2010). To successfully maintain ecosystems under the pressure of N deposition, ecologists have conducted many studies to investigate the mechanisms by which N deposition alters biodiversity and ecosystem functioning; however, most of these studies have focused on the plant community (Suding et al., 2004; Harpole and Tilman, 2007; Hautier et al., 2009; Stevens et al., 2010). Although bacteria are among the most abundant and diverse groups of organisms on earth, and mediate important ecosystem processes, the underlying mechanisms by which N deposition alters the bacterial community have not been thoroughly investigated (Chung et al., 2007; Campbell et al., 2010; Lage et al., 2010; Papanikolaou et al., 2010).

Nitrogen deposition is generally considered to alter biological communities through increases in N content, e.g. eutrophication (Bai et al., 2010; Stevens et al., 2010). Traditional niche theory proposes that different species coexist in a community because they fit different niches; accordingly, N addition leads to a decrease in the niche dimension by providing abundant nutrients, resulting in the loss of biodiversity (Harpole and Tilman, 2007). In fact, N deposition not only increases the total N (TN) content, but also changes the NH_4^+-N and NO_3^--N contents, and

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even alters environmental pH (Bai et al., 2010; Zhang et al., 2011). Each of these changes may lead to alteration of the biological community. For example, soil acidification has been demonstrated to be an important factor during N deposition leading to declining European plant richness (Stevens et al., 2010). Soil pH has also been shown to be important at leading to the structure of the soil bacterial community (Fierer and Jackson, 2006; Rousk et al., 2010). For some special bacterial functional communities, e.g., ammonia-oxidizing bacteria, which acquire energy by oxidizing ammonia, the increase in NH_4^+-N content could also drive changes in their abundance, diversity and composition (Zhang et al., 2011).

In addition to inducing changes in soil TN, NH_4^+-N and NO_3^--N contents, and soil pH, N addition may also change the degree of evenness of the spatial distribution of these compounds. For example, it has been shown that NH_4^+-N evenness and pH evenness in unfertilized soil were lower than in fertilized soil (Webster et al., 2002). Environmental unevenness has traditionally been referred to as environmental heterogeneity in ecological research, and traditional niche theories have assumed that higher environmental heterogeneity represented more niches, which would drive higher biodiversity (Rainey and Travisano, 1998; Horner-Devine et al., 2004; Dufour et al., 2006; Ramette and Tiedje, 2006; Harpole and Tilman, 2007). However, the effect of environmental heterogeneity on biodiversity during the processes of alterations in the biological community in response to N deposition has received little attention.

In addition to the aforementioned soil factors, the plant community is also closely related to the bacterial community. Indeed, bacteria and plants may compete for the same resources, exist in symbiotic relationships, or exist in parasitic relationships. Owing to these close relationships between plants and bacteria, any changes in the plant community (including plant biomass, diversity and composition) caused by N deposition may result in alteration of the bacterial community (Martiny et al., 2006; Chung et al., 2007; Horner-Devine et al., 2007).

In this study, we attempted to study the ecological mechanisms through which N deposition alters the bacterial community. We conducted a long term N deposition experiment in a typical steppe ecosystem in Inner Mongolia, China, in which six N addition rates were investigated (0–28 g N/(m^2·yr)). To evaluate the soil bacterial community, the bacterial abundance, diversity and composition were measured. Additionally, the soil environment was evaluated based on the TN content, NH_4^+-N content, NO_3^--N content, pH and the heterogeneity of these four factors. We also measured the plant biomass, diversity and composition. Specifically, this study was conducted to address the following questions: How does N addition change bacterial abundance, diversity and composition? How does N addition change the environment of bacterial community? Which environmental indexes are primarily responsible for changes in bacterial abundance, diversity and composition?

1 Materials and methods

1.1 Experimental plots

The details of the experimental design have been described before (Zhang et al., 2004; Bai et al., 2010). This study was conducted in a typical steppe ecosystem near the Inner Mongolia Grassland Ecosystem Research Station in China, which lies between 43°26′N–44°08′N and 116°04′E–117°05′E at an average elevation of 1200 m. A continental middle temperate semiarid climate dominates the area, which is characterized by a cold and dry winter and a warm and moist summer. The soil is a dark chest soil; the dominant plant species in the area, which accounted for > 80% of the total plant biomass, are *Leymus chinensis*, *Stipa grandis*, *Agropyron cristatum* and *Achnatherum sibiricum*. The experimental site (400 m × 600 m) was constructed in 1980 and was surrounded by an iron fence to exclude animal grazing. In early July every year from 2000 to 2006, N (NH_4NO_3) was added homogeneously to plots (5 m × 5 m) with a 1-m buffer zone at rates of 0, 1.75, 5.25, 10.5, 17.5 and 28 g N/(m^2·yr), respectively. Each treatment was repeated nine times. Phosphorus (10 g P_2O_5/(m^2·yr)) and trace elements (Zn: 1.9 mg/(m^2·yr), Mn: 1.9 mg/(m^2·yr), B: 3.12 mg/(m^2·yr)) were also added to ensure that other nutrients were non-limiting (Tilman, 1987). All 54 plots were distributed across an area of 55 m × 110 m in a randomized block design (Zhang et al., 2004; Bai et al., 2010).

1.2 Sampling and measurement of plant and soil indexes

In late August 2006, we harvested all plants in a 0.5 m × 1.0 m quadrat from each plot, after which we sorted them into species. We then weighted each species after drying at 65°C in an oven for 48 hr to constant weight.

In early September 2006, five soil cores (10 cm depth, 3.5 cm diameter) were collected at five locations from each plot to measure the environmental indexes of soil pH, TN, NH_4^+-N and NO_3^--N contents. The pH was measured in 1:2.5 (*W*/*V*) suspensions of soil in distilled water. Water content was determined as the weight loss after drying for 24 hr at 105°C. The NH_4^+-N and NO_3^--N contents were determined on a FIAstar 5000 Analyzer (Foss Tecator, Denmark) after extraction of fresh soil with 1 mol/L KCl. Total N was measured using an Alpkem autoanalyzer (Kjektec System 1026 Distilling Unit, Sweden) according to the Kjeldahl acid-digestion method.

From each plot, another five soil cores near to the first five were collected and thoroughly mixed, after which they were frozen until DNA extraction. DNA was extracted from 0.5 g of mixed soil using a FastDNA SPIN kit for soil (Qbiogene, Carlsbad, CA) according to the manufacturer's instructions, except that 350 μL of DNA elution solution was used to elute the DNA in the tenth procedure instead of 50 μL. For each plot, we extracted two DNA solutions and then pooled them. The DNA solution was stored at –20°C to measure the bacterial abundance and diversity.

1.3 Measurement of bacterial abundance

The abundance of bacterial 16S rRNA gene was measured using real time PCR according to a procedure similar to that described by Fierer et al. (2005). Briefly, a standard curve was generated using a 10-fold serial dilution of a plasmid containing a copy of the *Escherichia coli* 16S rRNA gene. The 20 μL PCR reaction mixtures contained 10 μL SYBR Premix (TaKaRa Biotechnology Co., Ltd., Dalian, China), 0.4 μL each of 10 μmol/L forward and reverse primers (Eub338: 5′-ACT CCT ACG GGA GGC AGC AG-3′; Eub518: 5′-ATT ACC GCG GCT GCT GG-3′), 0.4 μL Rox II, 2 μL BSA (10 mg/mL), and 5.8 μL sterile, DNA-free water. Standard and soil DNA samples were added at 1.0 μL per reaction. The reaction was conducted with a Roche LightCycler™ Real-time PCR system using the following program: 95°C for 1 min followed by 40 cycles of 95°C for 5 sec, 55°C for 15 sec and 72°C for 15 sec. Melting curve and gel electrophoresis analyses were conducted to confirm that the amplified products were of the appropriate size. The bacterial 16S rRNA gene copy number was calculated using a regression equation that related the cycle threshold (Ct) value to the known number of copies in the standards. For each soil sample, the qPCR reactions were repeated three times.

We added BSA to the PCR reaction mixes to reduce the inhibitory effects of co-extracted polyphenolic compounds in the soil. Additionally, three rounds of PCR were conducted after adding known amounts of standard plasmid with the soil DNA extract to estimate the possible inhibitory effects of coextracted polyphenolic compounds. The inhibitory effects were found to be negligible.

1.4 Investigation of bacterial community structure

For each of the 54 plots, the bacterial community structure was measured using terminal restriction fragment length polymorphism (T-RFLP), which classified all bacteria into tens of operational taxonomic units (OTUs) (Dunbar et al., 2001; Kim and Marsh, 2004; Fierer and Jackson, 2006). The FAM-labeled primer 27f (5′-GAG TTT GAT CMT GGC TCA G-3′) and unlabeled primer 519r (5′-GWA TTA CCG CGG CKG CTG-3′) were used for amplification of the bacterial 16S rRNA gene. Each 50-μL PCR mixture contained 25 μL of TransTaq PCR SuperMix® (TransGen Biotech, Beijing, China), 0.2 μmol/L of each primer, 20 μg of BSA, and 10 ng of template DNA. The PCR protocol was as follows: 94°C for 5 min (denature), followed by 28 cycles of 94°C for 45 sec (denature), 52°C for 45 sec (anneal), 72°C for 90 sec (elongate); and 72°C for 15 min (elongate). The products of three rounds of PCR per DNA sample were combined and their size was verified by agarose-gel electrophoresis. After purification by ethanol precipitation, the PCR products were digested with HhaI restriction endonuclease. The digested products were desalted immediately using ethanol precipitation, after which they were separated by electrophoresis on an ABI 3730 capillary sequencer at SinoGenoMax Co., Ltd. (Beijing, China).

1.5 Calculation and statistical analyses

To investigate the bacterial community, we measured bacterial abundance, diversity and composition. We calculated the average content of the 16S rRNA gene of three replicates to represent the bacterial abundance of a plot. The T-RFLP results were aligned by setting the fragment size tolerance to 0.5 bases. To reduce the influence of PCR, only fragments > 50 bp in length with a fluorescence > 2% of the total fluorescence in a certain sample were included in the following analysis (Dunbar et al., 2001; Kim and Marsh, 2004; Fierer and Jackson, 2006). First, the percent peak height of each OTU was calculated, after which the root was extracted to represent the OTU's relative abundance. The Shannon-Weiner index was subsequently calculated based on their relative abundance to represent the bacterial diversity. The ordination method of nonmetric multidimensional scaling (NMDS) was used to reveal the relative differences in bacterial composition based on the relative abundance of OTUs using PC-ORD based on the Sorensen distance metric. To use stepwise regression analysis to identify the main factor responsible for the changes in bacterial composition in the following analysis, we used the ordination score of the axis that could explain the most variation in the original data to represent the bacterial composition (Fierer and Jackson, 2006). We used the method of one-way analysis of variance (ANOVA) to analyze whether there were significant differences in bacterial abundance among different N addition rates.

The TN, NH_4^+-N and NO_3^--N contents and pH were measured for each of the five soil samples collected from every plot. The mean of the five samples for each index was calculated to represent the environment index of the plot, and the coefficient of variation (CV) of the five samples for each index was calculated to represent the environmental heterogeneity of the plot (Webster et al., 2002). The relationships between N addition rate and indexes of TN content, pH, NH_4^+-N content, NO_3^--N content, TN heterogeneity, pH heterogeneity, NH_4^+-N heterogeneity and NO_3^--N heterogeneity were analyzed using linear or quadratic regression.

For the plant community, we calculated the plant biomass, diversity and composition. The total dry weight of all plant species in the 0.5 m × 1.0 m quadrat was calculated to represent the plant biomass. The Shannon-Weiner index based on the species' dry weight was calculated to represent the plant diversity. Similar to the bacterial community, we also used the ordination method of NMDS to reveal the relative differences in plant composition based on the biomass of the plant species, while the ordination score of the axis that could explain the most variation in the original data was used to represent the plant composition. To determine if N addition altered the plant biomass, diversity and composition, we used linear or quadratic regression to analyze the relationships between each of the indexes and the N addition rate.

The bacterial community is influenced by both soil and plants. Therefore, the relationship between the bacterial abundance, diversity and composition and TN content,

pH, NH_4^+-N content, NO_3^--N content, TN heterogeneity, pH heterogeneity, NH_4^+-N heterogeneity, NO_3^--N heterogeneity, plant biomass, plant diversity, or plant composition were evaluated. Next, stepwise regression analyses were used to identify the factors that could effectively explain the changes in bacterial abundance from the seven potential factors of TN content, pH, NH_4^+-N content, NO_3^--N content, plant biomass, plant diversity and plant composition, and to identify the factors that could effectively explain the changes in bacterial diversity or composition from the eleven potential factors of TN content, pH, NH_4^+-N content, NO_3^--N content, TN heterogeneity, pH heterogeneity, NH_4^+-N heterogeneity, NO_3^--N heterogeneity, plant biomass, plant diversity and plant composition.

2 Results and discussion

2.1 Effect of N addition on bacterial abundance, diversity and composition

As the N addition rate increased from 0 to 28 g N/(m^2·yr), the bacterial abundance decreased (Fig. 1a). As the N addition rate increased from 0 to 1.75–10.5 and then to 28 g N/(m^2·yr), the bacterial diversity slowly increased, and then sharply decreased (Fig. 1b). Increasing N addition rate also directionally altered bacterial composition (represented by the ordination score of one axis in the NMDS plot, which accounted for 67.0% variation of bacterial composition) (Fig. 1c).

2.2 Effect of N addition on the soil indexes

As the N addition rate increased, all of the soil TN, NH_4^+-N and NO_3^--N contents increased, and the soil pH decreased (Table 1) (Zhang et al., 2011). Additionally, the TN heterogeneity showed no significant change in response to an increasing N addition rate, while the pH heterogeneity first increased, and then stabilized (Fig. 2a, b; Table 1). As the N addition rate increased from 0–1.75 to 5.25–10.5 and then to 28 g N/(m^2·yr), the NH_4^+-N heterogeneity increased and then decreased (Fig. 2c; Table 1). Additionally, increases in the N addition rate from 0 to 1.75–17.5 and then to 28 g N/(m^2·yr) led to an initial increase and subsequent decrease in NO_3^--N heterogeneity (Fig. 2d; Table 1). The heterogeneity of the total C and P and available P were not influenced by the N addition rate (linear regression: $P > 0.05$. data not shown).

The heterogeneity of NH_4^+-N, NO_3^--N and pH represented the degree of their uneven distribution within the plot (Webster et al., 2002). As the N addition rate increased from zero to low levels, the heterogeneity of NH_4^+-N and NO_3^--N increased (Fig. 2c, d), indicating that they were distributed unevenly within the plot. As N addition rate continued to increase, the heterogeneity of both NH_4^+-N and NO_3^--N decreased, indicating that they were distributed more evenly within the plot. Because the steppe ecosystem is characterized by poor N and unevenly distributed plants (Yuan et al., 2006), the addition of small amounts of NH_4^+-N and NO_3^--N homogeneously

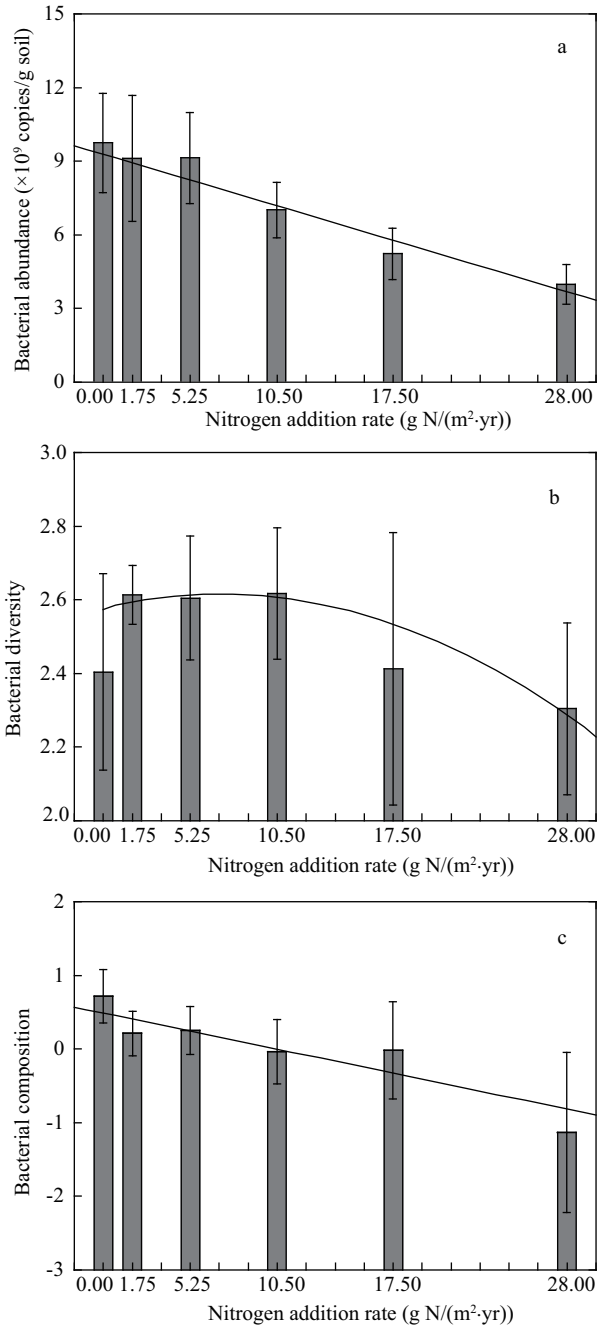

Fig. 1 Effect of N addition rate on (a) bacterial abundance (one-way ANOVA: $F = 17.658$, $P < 0.001$), (b) diversity, and (c) composition. Error bars represent one standard deviation.

to the plots likely resulted in the plants absorbing most of the NH_4^+-N and NO_3^--N nearby areas, leading to the heterogeneous distribution of the compounds. However, when the amount of NH_4NO_3 fertilizer increased, excess N was available, leading to decreased NH_4^+-N and NO_3^--N heterogeneity. In this experiment, as the N addition rate increased from zero to low levels, the pH heterogeneity also increased (Fig. 2b); however, as the N addition rate continued to increase, the pH heterogeneity remained nearly stable. A previous study showed lower NH_4^+-N heterogeneity and pH heterogeneity in fertilized soil than in unfertilized soil (Webster et al., 2002), contrary to the results presented in Fig. 2. They conducted fertilization at a rate of 14 g N/(m^2·yr) for about 9 years, and fertilization

Table 1 Linear or quadratic relationships between soil indexes, plant indexes and N addition rate

Item	Constant	X	X^2	R^2	F	P
Total N content (g/kg soil)	2.110	0.010		0.285	20.718	< 0.001*
pH	6.790	–0.071		0.918	578.475	< 0.001*
NH_4^+-N content (mg/kg soil)	–0.365	1.414	0.077	0.918	285.113	< 0.001*
NO_3^--N content (mg/kg soil)	5.512	3.802		0.830	253.832	< 0.001*
Total N heterogeneity	11.641	–0.062		0.024	1.275	0.264
pH heterogeneity	3.895	0.170	–0.004	0.092	2.576	0.086
NH_4^+-N heterogeneity	52.275	7.146	–0.281	0.346	13.473	< 0.001*
NO_3^--N heterogeneity	44.911	3.156	–0.119	0.150	4.502	0.016*
Plant biomass (g)	69.148	6.571	–0.164	0.524	28.078	< 0.001*
Plant diversity	1.668	–0.030	0.00045	0.289	10.351	< 0.001*
Plant composition	0.525	–0.074	0.001	0.402	17.149	< 0.001*

* Significant at $P < 0.05$.

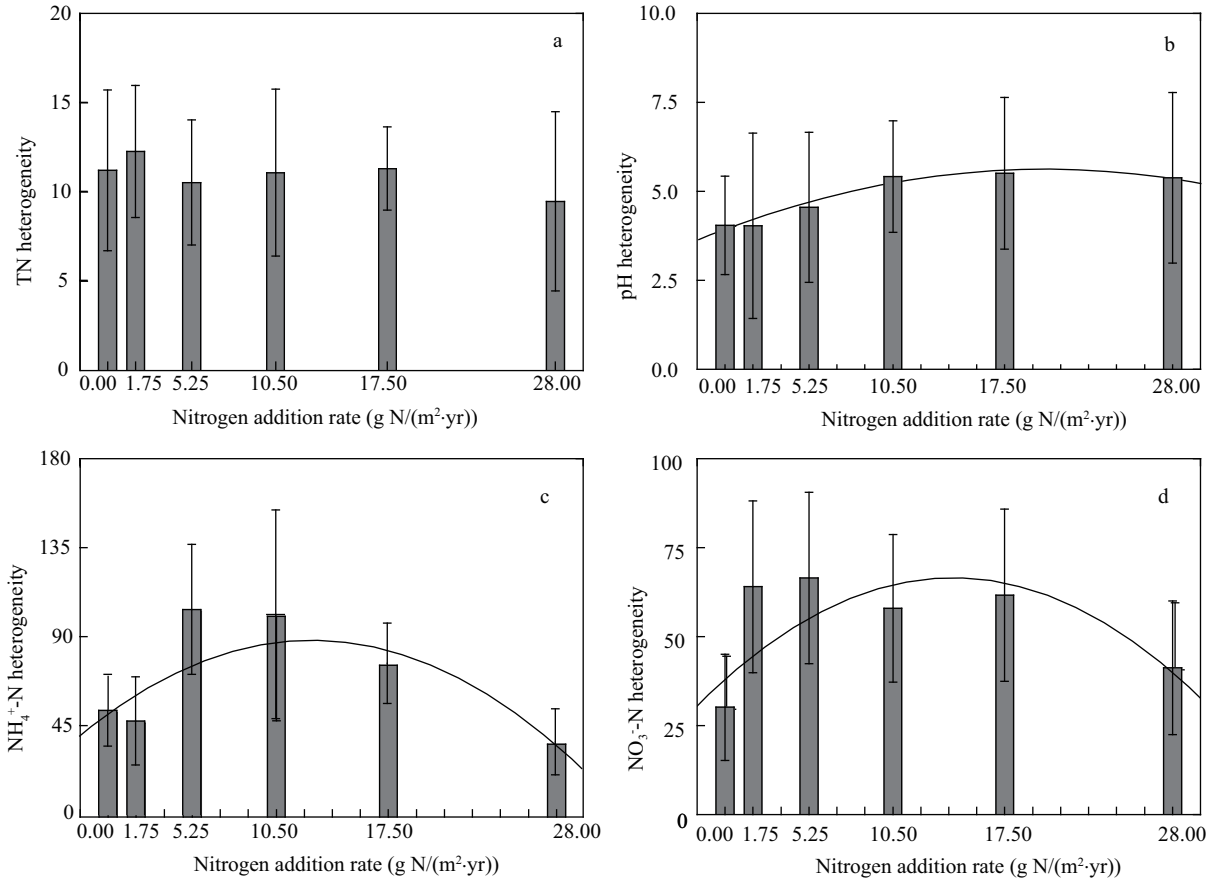

Fig. 2 Effect of N addition rate on TN (a), pH (b), NH_4^+-N (c), and NO_3^--N (d) heterogeneity. Error bars represent one standard deviation. See the statistical results in Table 1.

had an accumulated effect; therefore, if N was added at a higher rate or for more years the environmental heterogeneity may have been smaller.

2.3 Effect of N addition on the plant community

As the N addition rate increased from 0 to 17.5 and then to 28 g N/(m^2·yr), the plant biomass first increased and then stabilized, while the plant diversity first decreased and then stabilized (Table 1). As the N addition rate increased from 0 to 28 g N/(m^2·yr), the plant composition (represented by the ordination score of one axis in the NMDS plot, which accounted for 44.9% variation of the plant composition) also changed directionally (Table 1).

Above-ground plant biomass, which approximates the above-ground net primary productivity (Sala and Austin, 2000; Bai et al., 2004), is often used to represent plant ecosystem functioning. In this study, we used bacterial abundance (the abundance of bacterial 16S rRNA gene) as a similar index to represent ecosystem functioning (Fierer et al., 2005). This was possible because bacterial abundance represents the potential of many ecosystem processes, such as decomposition and nutrient cycling. N addition increased plant biomass (Table 1), but decreased bacterial abundance (Fig. 1a). N addition generally decreased biodiversity and changed the community composition of both plants and bacteria (Fig. 1; Table 1), indicating that fewer organisms could adapt to the N-added environment as the amount added increased. However, some plant species (e.g., *L. chinensis*, *P. bifurca*, *A. amaranthoides* and *C. glaucum*) favored the altered

environment (Bai et al., 2010; Zhang et al., 2011), and they had larger biomass values in the N-added environment than in the pristine environment. However, this was not the case for bacteria, indicating that the species that were present did not favor the N-added environment.

2.4 Identification of the environmental indexes responsible for the changes in bacterial abundance, diversity and composition

Although bacterial abundance was found to be significantly correlated with pH, NH_4^+-N content, NO_3^--N content, pH heterogeneity, plant biomass, plant diversity and plant composition (Table 2), stepwise regression analyses revealed that soil pH alone could explain 62.8% of variation in bacterial abundance (Fig. 3a; Table 3). Although TN content could explain another small part of variation, its coefficient (3.909; Table 3) was positive, indicating that N addition increased bacterial abundance via an increased TN content. These findings are contrary to the decrease in bacterial abundance observed in response to N addition. These results indicate that N addition primarily decreased bacterial abundance via a decrease in soil pH. In accordance with our results, soil pH has been shown to be important to bacterial abundance (Fierer and Jackson, 2006; Rousk et al., 2010). Because the intracellular pH of most organisms was usually within one pH unit of neutral (Madigan et al., 1997), a significant decrease in soil pH caused by N addition would likely impose stress on the organisms, resulting in decreasing bacterial abundance.

Although bacterial diversity was found to be significantly correlated with TN content, pH, NH_4^+-N content, NO_3^--N content and NO_3^--N heterogeneity (Table 2), stepwise regression analyses revealed that the variation was primarily explained by TN content and partially explained by the NO_3^--N heterogeneity (Fig. 3b; Table 3). These findings indicate that N addition primarily altered bacterial diversity through increasing soil TN content and mediating NO_3^--N heterogeneity. According to traditional niche theories, different species existed in an ecosystem by occupying different niches (Harpole and Tilman, 2007). Many land ecosystems are N-limited (Sterner and Elser, 2002), so many species may occupy different N niches. For example, N-mineralizing microbes might fill an N niche based on their N-mineralizing capacity, and N-fixing microbes might exist in an ecosystem owing to their N-fixing capacity. Therefore, the changes in TN content and NO_3^--N heterogeneity represented changes in niche dimensions, and these changes would cause alteration of the bacterial diversity. Different from our results, soil pH has traditionally been found to be the most important factor influencing bacterial diversity (Fierer and Jackson, 2006; Rousk et al., 2010). In our study, all bacteria were classified into only tens of OTUs, and the experimental treatment lasted for only 6 years. Investigation of the bacterial community using a more accurate method such as Roche 454 pyrosequencing or using a longer experimental treatment time may reveal that pH plays an important role in bacterial diversity.

Although bacterial composition was found to be significantly correlated with pH, NH_4^+-N content, NO_3^--N content, plant biomass, plant diversity and plant composition (Table 2), stepwise regression analyses revealed that NH_4^+-N content alone could explain 41.2% of the variation in bacterial composition (Fig. 3c; Table 3). These findings indicate that N addition primarily altered the bacterial composition through an increase in NH_4^+-N content. NH_4^+-N is an available N form for microbes, and different bacterial species may respond differently to the available NH_4^+-N. Therefore, the increased NH_4^+-N content can lead to the changes in overall bacterial composition.

Our results showed that N addition altered different characteristics of bacterial community through changes

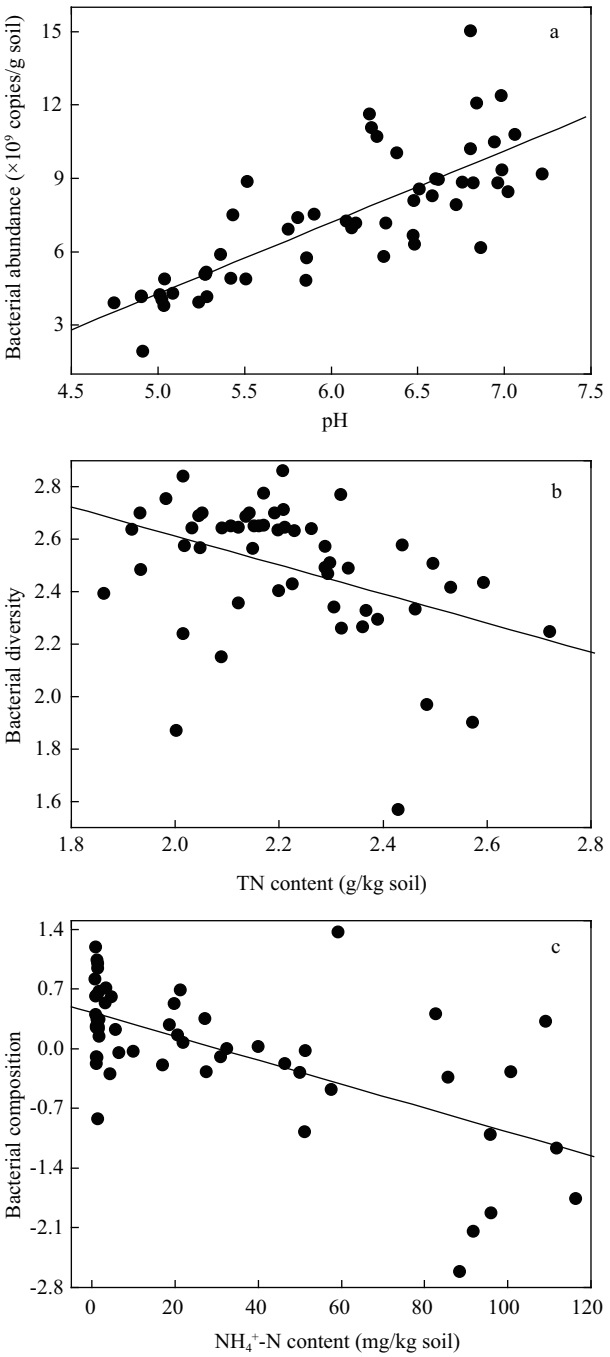

Fig. 3 Linear relationships between bacterial abundance and pH (a), bacterial diversity and TN content (b), and bacterial composition and NH_4^+-N content (c). See the statistical results in Table 3.

Table 2 Pearson correlations between bacterial indexes and soil and plant indexes

Variable	Bacterial abundance			Bacterial diversity			Bacterial composition		
	r	r^2	P	r	r^2	P	r	r^2	P
Total N content (g/kg soil)	–0.245	0.060	0.075	–0.408	0.166	0.002*	–0.233	0.054	0.090
pH	0.792	0.628	< 0.001*	0.284	0.081	0.037*	0.615	0.378	< 0.001*
NH_4^+-N content (mg/kg soil)	–0.750	0.562	< 0.001*	–0.395	0.156	0.003*	–0.642	0.412	< 0.001*
NO_3^--N content (mg/kg soil)	–0.717	0.514	< 0.001*	–0.341	0.116	0.012*	–0.478	0.229	< 0.001*
Total N heterogeneity	0.107	0.011	0.443	0.086	0.007	0.535	0.140	0.020	0.312
pH heterogeneity	–0.274	0.075	0.045*	–0.069	0.005	0.620	–0.117	0.014	0.400
NH_4^+-N heterogeneity	0.016	0.0003	0.909	0.125	0.016	0.368	0.239	0.057	0.082
NO_3^--N heterogeneity	–0.091	0.008	0.514	0.343	0.118	0.011*	–0.060	0.004	0.666
Plant biomass (g)	–0.480	0.230	< 0.001*	–0.059	0.004	0.669	–0.281	0.079	0.040*
Plant diversity	0.428	0.183	0.001*	0.145	0.021	0.294	0.394	0.155	0.003*
Plant composition	0.521	0.272	< 0.001*	0.195	0.038	0.157	0.417	0.174	0.002*

* Significant at $p < 0.05$.

Table 3 Variables responsible for the changes in bacterial abundance, diversity and composition

Dependent variable	Result	r^2	F	P
Bacterial abundance	y = –10.338+2.924pH	0.628	87.650	<0.001
($\times 10^9$ copies/g soil)	y = –22.320+3.473pH+3.909 (TN content)	0.679	53.980	<0.001
Bacterial OTU diversity	y = 3.721 – 0.554 (TN content)	0.166	10.360	0.002
	y = 3.416 – 0.488 (TN content)+0.003 (NO_3^--N heterogeneity)	0.243	8.176	0.001
Bacterial composition	y = 0.424 – 0.014 (NH_4^+-N content)	0.412	36.368	<0.001

in different environmental indexes. Bacterial abundance accounted for the total bacterial community size and not traits of individual bacterial species. Therefore, bacterial abundance was primarily explained by soil pH, which influences the survival of all bacteria, regardless of their species traits. Bacterial diversity accounted for the relative abundances of different OTUs within a community and not community size. Therefore, bacterial diversity was mainly explained by TN content and NO_3^--N heterogeneity, which represented the niche dimensions. Bacterial composition accounted for the relative abundances of different OTUs within a community as well as the differences in the relative abundance of OTUs among communities. Therefore, bacterial composition was mainly explained by NH_4^+-N content, which was the direct N resource of bacteria and changed more rapidly than pH and TN content.

Although N addition changed the pH, NH_4^+-N and NO_3^--N heterogeneity, these factors played little role in maintaining bacterial diversity and composition, except that NO_3^--N heterogeneity played a small role in maintenance of bacterial diversity (Table 3). Similar to our results, a previous study also showed that N addition ultimately decreased plant richness, regardless of whether N was added homogeneously or heterogeneously (Reynolds et al., 2007). Therefore, although environmental heterogeneity drove biodiversity in many ecosystems, we could not attribute any changes in biodiversity to the changes in environmental heterogeneity. For example, the diversity of ammonia-oxidizing bacteria was lower in fertilized soil than in unfertilized soil (Webster et al., 2002), which might have been due to the increased NH_4^+-N content and decreased pH rather than the decreased heterogeneity of both NH_4^+-N and pH.

Although N addition altered plant biomass, diversity and composition, these plant indexes do not influence the processes involved in the changes in bacterial abundance, diversity and composition in response to N addition. However, in this study the entire soil bacterial community has been investigated. If one species or group in the bacterial functional community that is close related to plants is investigated, the role of plants in the processes of N addition that led to alteration of the bacterial community may be elucidated.

3 Conclusions

We systematically investigated the ecological mechanisms of N deposition that led to alterations in bacterial abundance, diversity and composition. We found that N deposition primarily altered bacterial abundance through decreased soil pH, while it mainly altered bacterial diversity through increased soil TN content and changes in NO_3^--N heterogeneity, and altered bacterial composition through increased soil NH_4^+-N content. However, our results are based on statistical analyses; accordingly, additional experimental evidence should be acquired in the future. Moreover, our study included a single steppe ecosystem; therefore, the findings presented here need to be tested in other ecosystems.

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