Nitrogen deposition mediates the effects and importance of chance in changing biodiversity

XIMEI ZHANG,*†[1] WEI LIU,†[1] YONGFEI BAI,* GUANGMING ZHANG* and XINGGUO HAN*

State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China, †College of Life Sciences, Beijing Normal University, Beijing 100875, China

Abstract

Nitrogen deposition is changing biodiversity on Earth. We need to understand the underlying mechanisms to conserve biodiversity better. Both selection and chance are potential mechanisms, and they may operate concurrently. Then, what are the respective effects of selection and chance, what is their relative importance and how do they change with increasing nitrogen deposition rate? Here, we performed a 6-year nitrogen addition experiment (0–28 g N/m^2/year) in a typical steppe ecosystem of Inner Mongolia to investigate the community structure of plants, bacteria and ammonia-oxidizing Archaea (AOA). We developed an experimentally based calculation method to first separate the structural variations between plots into the effects of selection (S) and chance (C), and then calculate their relative importance. Our results showed that as nitrogen addition rate increased, S for both plants and bacteria increased, but C for plants first increased and then decreased, and C for bacteria also increased; meanwhile, both S and C for AOA changed nonlinearly. As nitrogen addition rate increased, the importance of chance decreased on the whole for all these communities, but it decreased nonlinearly for plants and bacteria, with a local increase at certain intermediate rates. At all treatments, the importance of chance was <0.5 for plants, but >0.5 for AOA. These results demonstrated that nitrogen deposition changed biodiversity by mediating the effects and importance of chance, implicating different strategies should be adopted in conserving biodiversity according to nitrogen deposition rate and community properties.

Keywords: community structure, ecological drift, immigration, natural selection, neutral theory, niche theory

Received 25 June 2010; revision received 5 October 2010; accepted 14 October 2010

Introduction

The biodiversity on Earth is changing at an unprecedented rate, and nitrogen (N) deposition is predicted to be one of the major drivers of biodiversity change in terrestrial ecosystems (Sala *et al.* 2000). Because of agricultural fertilization and combustion of fossil fuels, N deposition rate has increased from the pre-industrial levels of about 0.1–0.3 to as high as 10 g N/m^2/year in some developed countries (Vitousek *et al.* 1997; Bakker & Berendse 1999; Galloway *et al.* 2004; Stevens *et al.* 2004). And it is predicted that N deposition rate will increase similarly over the next 50 years in many developing countries (Galloway *et al.* 2004). To conserve biodiversity successfully under the increasing pressure of N deposition, it is urgent for us to understand the mechanisms of biodiversity maintenance.

In fact, ecologists have long been interested in the mechanisms of species coexistence within a community (Hutchinson 1959; Chesson 2000). The traditional niche theories propose that different species coexist in a community because they have different niches, e.g. they are restrained by different resources (Pacala & Tilman 1993; Harpole & Tilman 2006, 2007). Within a community, different species have different fitness, and their abundances are consistent with their fitness. As environmental condition changes, their fitness will change inconsistently, resulting in the alteration of their abundances and the community structure. In other words,

Correspondence: Xingguo Han, Fax: +86-10-82595771;
E-mail: xghan@ibcas.ac.cn
[1]These authors contributed equally.

the niche theories focus on the deterministic effects of selection. In recent years, ecologists propose ecological neutral theories, which propose that the changes in the community structure have nothing to do with the differences in fitness of species, but emphasize the chance effects of ecological drift, migration and speciation (Hubbell 1979, 2001; Bell 2001).

In fact, selection and chance are not mutually exclusive, and many experiments and theories have demonstrated that they may operate concurrently (Tilman 2004; Thompson & Townsend 2006; Cadotte 2007; Ellwood *et al.* 2009; Dumbrell *et al.* 2010). On the basis of an analysis of more than 900 plant species from 34 N fertilization experiments across different terrestrial ecosystems, Suding *et al.* (2004) found that perennials, native origin species and species with N-fixing symbionts were often lost, with no respect to their initial abundances, and that rare species were also often lost, regardless of their traits. These findings also indicated that N deposition declined plant richness through both selection and chance. Then, what are the respective effects of selection and chance? What is their relative importance?

Although selection and chance operate concurrently, Travisano *et al.* (1995) successfully disentangled their respective contributions to the fitness of *Escherichia coli* with an innovatively designed experiment. Simply speaking, they cultured three independent populations from a same progenitor population by culturing them for 1000 generations under the ancestral conditions, except with a different carbon source. After measuring the fitness of both the progenitor and three descendants under the current conditions, they attributed the mean difference in fitness between descendant and progenitor to the effects of selection, and attributed the mean differences in fitness among three descendents to the effects of chance.

Inspired by their research (Travisano *et al.* 1995), we performed a 6-year N addition experiment in a typical steppe ecosystem of Inner Mongolia; six N addition rates (nine replicates each) were designed to mimic increasing N deposition rates. To study whether the property of community influences the respective effects of selection and chance and their relative importance, we investigated the community structures of not only plants, but also soil bacteria and ammonia-oxidizing Archaea (AOA). We investigated bacterial community structure with the method of terminal restriction fragment length polymorphism (T-RFLP), which classified all soil bacteria into only tens of operational taxonomic units (OTUs) based not on species trait, but on the enzymes-digested fragment length of 16S rRNA genes (Dunbar *et al.* 2001; Kim & Marsh 2004; Fierer & Jackson 2006). AOA acquires energy through oxidizing ammonia, and N addition changes soil pH and NH_4^+-N content, both of which will affect the survival of AOA. We investigated AOA community structure by sequencing the *amoA* gene, which was critical during oxidation of ammonia (Könneke *et al.* 2005); we obtained 28 sequences of the gene from each plot, which was similar to 28 individuals. After calculating the community structural variations between plots, we will first separate these variations into the respective effects of selection and chance, and then calculate their relative importance.

Because N addition is the single selective force causing structural variation in the experiment, we expect that as N addition rate increases, the effects of selection will increase; because the stochastic forces of ecological drift and migration will not be directly influenced by N addition, we expect that as N addition rate increases, the effects of chance will keep stable; then, we expect that as N addition rate increases, the importance of selection will increase, and the importance of chance will decrease.

Materials and methods

Experimental plots

As details of the experimental design have been described before (Zhang *et al.* 2004; Bai *et al.* 2010), we provide only a brief description here. The study was carried out in a typical steppe ecosystem near the Inner Mongolia Grassland Ecosystem Research Station in China, which lies between 43°26′–44°08′N and 116°04′–117°05′E, with an average elevation of 1200 m. A continental middle temperate semiarid climate dominates the area, characterized by a cold and dry winter but a warm and moist summer. The soil is a dark chest soil; the dominant plant species, which accounted for >80% of plant biomass, include *Leymus chinensis*, *Stipa grandis*, *Agropyron cristatum* and *Achnatherum sibiricum*. The experimental site (400 × 600 m) was built in 1980 with an iron fence to exclude animal grazing. In early July every year from 2000 to 2006, N (NH_4NO_3) was added to plots (5 × 5 m) with a 1-m buffer zone at rates of 0, 1.75, 5.25, 10.5, 17.5 and 28 g N/m²/year, respectively. Each treatment was repeated nine times. We also added phosphorus (10 g P_2O_5/m²/year) and trace elements (Zn: 1.9 mg/m²/year, Mn: 1.9 mg/m²/year, B: 3.12 mg/m²/year) to ensure that other nutrients were non-limiting (Tilman 1987). There were nine control plots which received no nutrients (zero control). All 63 plots were distributed across an area of 55 × 110 m in a randomized block design (Zhang *et al.* 2004; Bai *et al.* 2010).

Sampling

In late August 2006, we harvested all plants in a 0.5 × 1.0 m quadrat from each plot; the plants were

sorted into species, and then oven-dried at 65 °C for 48 h and weighted.

In early September 2006, five soil cores (10 cm depth, 3.5 cm diameter) were taken at five locations from each plot for the measurement of the environmental indexes of soil pH, total N, 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 weight loss after drying for 24 h at 105 °C. NH_4^+-N and NO_3^--N content were determined on a FIAstar 5000 Analyzer (Foss Tecator, Denmark) after extraction of fresh soil with 1 M KCl. Total N was measured with 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 taken and thoroughly mixed to be stored frozen until DNA extraction. We extracted DNA from 0.5 g of mixed soil following the instructions of the FastDNA SPIN kit for soil (Qbiogene, Carlsbad, CA, USA) except that we used 350 μL instead of 50 μL DNA elution solution to elute the DNA in the tenth procedure. For each plot, we extracted two DNA solutions and pooled them. The DNA solution was stored at −20 °C for the measurement of bacterial and AOA community structure.

Investigation of bacterial community structure

For each of the 63 plots, we measured bacterial community structure using the method of T-RFLP, which classified all bacteria into only tens of OTUs (Dunbar *et al.* 2001; Kim & Marsh 2004; Fierer & Jackson 2006). The FAM-labelled primer 27f (5′-GAGTTTGATCMTGGCTC-AG-3′) and unlabelled primer 519r (5′-GWATTACC-GCGGCKGCTG-3′) were used for amplification of bacterial 16S rRNA gene. Each 50-μL PCR mixture contained 25 μL TransTaq PCR SuperMix I (TransGen Biotech, Beijing, China), 0.2 μM of each primer, 20 μg of BSA and 10 ng of template DNA. PCR protocol was 94 °C for 5 min (denature); 28 cycles consisting of 94 °C for 45 s (denature), 52 °C for 45 s (anneal), 72 °C for 90 s (elongate); and 72 °C for 15 min (elongate). Products were combined from three PCRs per DNA sample and size-verified by agarose-gel electrophoresis. After purification by ethanol precipitation, PCR products were digested with restriction endonucleases. Because different enzymes have different sensitivities, we used the two enzymes HhaI and MspI in respective reactions. Digested products were desalted immediately using ethanol precipitation, and then separated by electrophoresis on an ABI 3730 capillary sequencer at SinoGeno-Max Co Ltd (Beijing, China).

Investigation of AOA community structure

For the first five replicates of all treatments (a total of 35 plots), we investigated the community structure of AOA by amplifying and sequencing the *amoA* gene fragment (595 bp) with primers Arch-amoAF (5′-STA-ATGGTCTGGCTTAGACG-3′) and Arch-amoAR (5′-GCGGCCATCCATCTGTATGT-3′) designed in previous research (Francis *et al.* 2005). Each 20-μL PCR mixture contained 10-μL TransTaq PCR SuperMix I (TransGen Biotech, Beijing, China), 0.4 μM of each primer, 8 μg of BSA, and 40 ng of DNA. PCR protocol was 94 °C for 5 min (denature); 15 cycles consisting of 94 °C for 40 s (denature), from 56 to 49 °C decreasing 0.5 °C every cycle for 40 s (anneal), 72 °C for 1 min (elongate) and 72 °C for 10 min (elongate). Triplicate products were pooled and 2 μL of mixture was taken to be the template of the second PCR round, in which the PCR protocol was the same as the first round, except that there were 30 cycles and the annealing temperate was 53 °C. Triplicate PCR products were pooled and cloned with pEASY-T1 Simple cloning vector (TransGen Biotech). White colonies were randomly selected to be grown in a 5-mL tube containing LB broth (with 50 μg/mL kanamycin) for 12 h at 37 °C, and PCR-screened directly for the presence of inserts using primers Arch-amoAF and Arch-amoAR. Positive colonies were sequenced with M13 Forward Primer on Applied Biosystems 3730xl capillary sequencers at Invitrogen Biotechnology Co Ltd (Beijing, China). From each of the 35 clone libraries, 28 clones were randomly sampled and sequenced. Sequences obtained in this study were deposited at GenBank (NCBI) with accession numbers: EU671078–EU672407.

Calculation of AOA genetic diversity

We calculated the genetic diversity of the *amoA* gene for each plot. We first calculated the number of nucleotide differences between every pair of sequences sampled from the plot, and expressed them on a per site basis (Nei & Li 1979), then calculated the average of all pair of sequences to represent the genetic diversity of the *amoA* gene. Then we calculated the mean difference in the AOA genetic diversity between each treatment and the control.

Calculation of structural variations between plots

We calculated the structural variations between every pair of plots. For plants, we calculated the Euclidean distance between plots based on the species percent biomass to represent the community structural variation.

For bacteria, the T-RFLP results were aligned by setting the fragment size tolerance as 0.5 bases. To reduce the influence of PCR, only fragments >50 bp in length and with fluorescence >2% of the total fluorescence in a certain sample were included in the following analysis (Dunbar *et al.* 2001; Kim & Marsh 2004; Fierer & Jackson 2006). We first calculated the percent peak height of each OTU, and extracted the root to represent the OTU's relative abundance, and then calculated the Euclidean distance between plots based on the percent value of each OTU's relative abundance to represent the community structural variation. So, both plant and bacterial community structural variation accounted not only for the presence or absence of species (or OTUs) but also for their relative abundances. For AOA, we first calculated the number of nucleotide differences between any two sequences from different plots, and expressed them on a per site basis (Nei & Li 1979), and then used the average number of all pairs (28 × 28) of sequences from two plots to represent the community structural variation. So, AOA community structural variation accounted for differences in DNA sequence, as well as frequencies of different sequences.

Statistical test of the effects of selection and chance between treatments

To test whether the selection effects of N addition differed between treatments, we calculated the mean structural variation between each treatment plot and all control plots. For all treatment plot (excluding control plots), this kind of mean structural variation was independent, then we used one-way ANOVA to analyse the influence of N addition rate on this kind of mean structural variation. To test whether the chance effects differed between treatments, we tested whether the N addition rate influenced structural variations within a treatment, using the nonparametric permutation analysis method of permutation dispersion (Table S1, Supporting information), following a procedure by Chase (Anderson 2004; Chase 2007).

Separating the respective effects of selection and chance

In our experiment, all plots were within an area of only 55 × 110 m and had the same natural environment (e.g. soil and climate), so N addition was the only different selective force between plots. Because there was no significant influence of spatial factor on structural variation among plots (Data S1, Supporting information), we could assume that there was no systematic variation in the heterogeneity of these communities before the treatments were applied. Therefore, the structural variations between plots were caused by the selection of N

addition and the chance of ecological drift and species migration. Following the line of reasoning of Travisano *et al.* (1995), we could separate these structural variations into the respective effects of selection and chance. The structural variation between the control plots was not caused by N addition, so we could take the mean structural variation between control plots as the base point. Although the structural variations between control plots and treatment plots should be caused by both selection and chance, the selection effects were directional and the chance effects were non-directional. So, for each treatment, we could calculate S = [(mean structural variation between control and treatment) – (base point)], which represented the selection effects of N addition. We used Mantel tests to check the influences of soil total N content, pH, NH_4^+-N and NO_3^--N contents on within-treatment structural variations, the influences were insignificant for almost all treatments (Table S2, Supporting information), which indicated that the selection effects were equal between plots within a treatment, so the structural variations between plots within a treatment should be caused only by chance. So, for each treatment, we could calculate C = [(mean structural variation within treatment) – (base point)], which represented the chance effects after N addition. Both S and C might be positive or negative, corresponding to promoting or restraining the structural changes, respectively; and their absolute values represented the magnitudes of their effects. Then, for each treatment, we could calculate the importance of chance $= \frac{|C|}{|C|+|S|}$ (Table S3, Supporting information). Of course, the importance of selection = 1 – the importance of chance. And, according to our definition, for the control, the importance of chance should be one and the importance of selection should be zero.

Results

The influence of N addition on environmental indexes and biodiversity

For each plot, we sampled five soil cores and measured their respective total N, NH_4^+-N, NO_3^--N contents and pH, then used the mean of the five soil cores to represent the environmental indexes of the plot. N addition significantly increased the total N content (Fig. 1a; randomized block ANOVA: $F = 10.693$, $P < 0.0001$, $n = 54$), NH_4^+-N content (Fig. 1c; randomized block ANOVA: $F = 120.041$, $P < 0.0001$, $n = 54$), NO_3^--N content (Fig. 1d; randomized block ANOVA: $F = 51.007$, $P < 0.0001$, $n = 54$), and decreased pH (Fig. 1b; randomized block ANOVA: $F = 139.026$, $P < 0.0001$, $n = 54$).

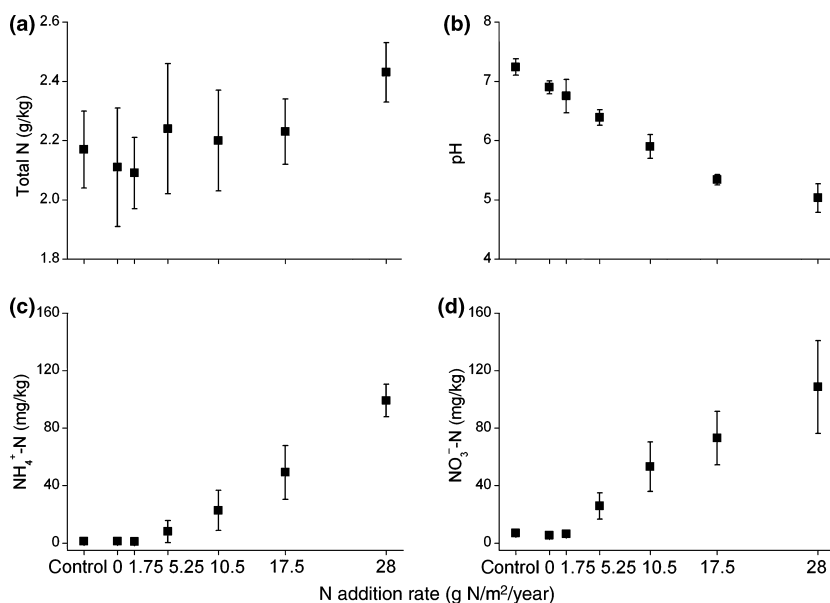N addition significantly decreased plant richness (Fig. 2a; linear regression, slope = −0.144, $F = 24.702$,

Fig. 1 The effects of N addition rate on soil indexes. (a) Total soil N content; (b) Soil pH; (c) NH_4^+-N content; (d) NO_3^--N content. Error bars represent one standard deviation.
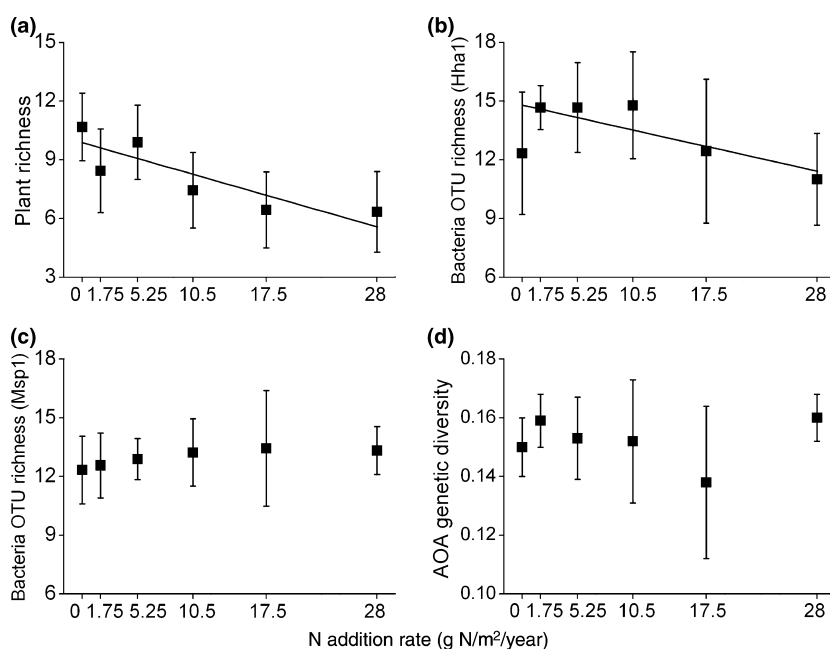

Fig. 2 The effects of N addition rate on biodiversity. (a) Plant richness; (b) Bacterial OTU richness when analysed with Hha1; (c) Bacterial OTU richness when analysed with Msp1; (d) AOA genetic diversity when analysed with *amoA* gene. Error bars represent one standard deviation.

$P < 0.001$, $n = 54$) and significantly decreased bacterial OTU richness when analysed with the enzyme of Hha1 (Fig. 2b; linear regression, slope $= -0.097$, $F = 6.119$, $P = 0.017$, $n = 54$), but the effects on bacterial OTU richness were not significant when analysed with the enzyme of Msp1 (Fig. 2c).

For AOA community, although there were no significant differences in the genetic diversity among all

treatments (Fig. 2d; one-way ANOVA: $F = 1.152$, $P = 0.361$, $n = 30$), there was less genetic diversity at 17.5 than at 28 g N/m²/year (LSD: $P = 0.044$).

The respective effects of selection and chance

For plant communities, the selection effects were significantly different among treatments (one-way ANOVA:
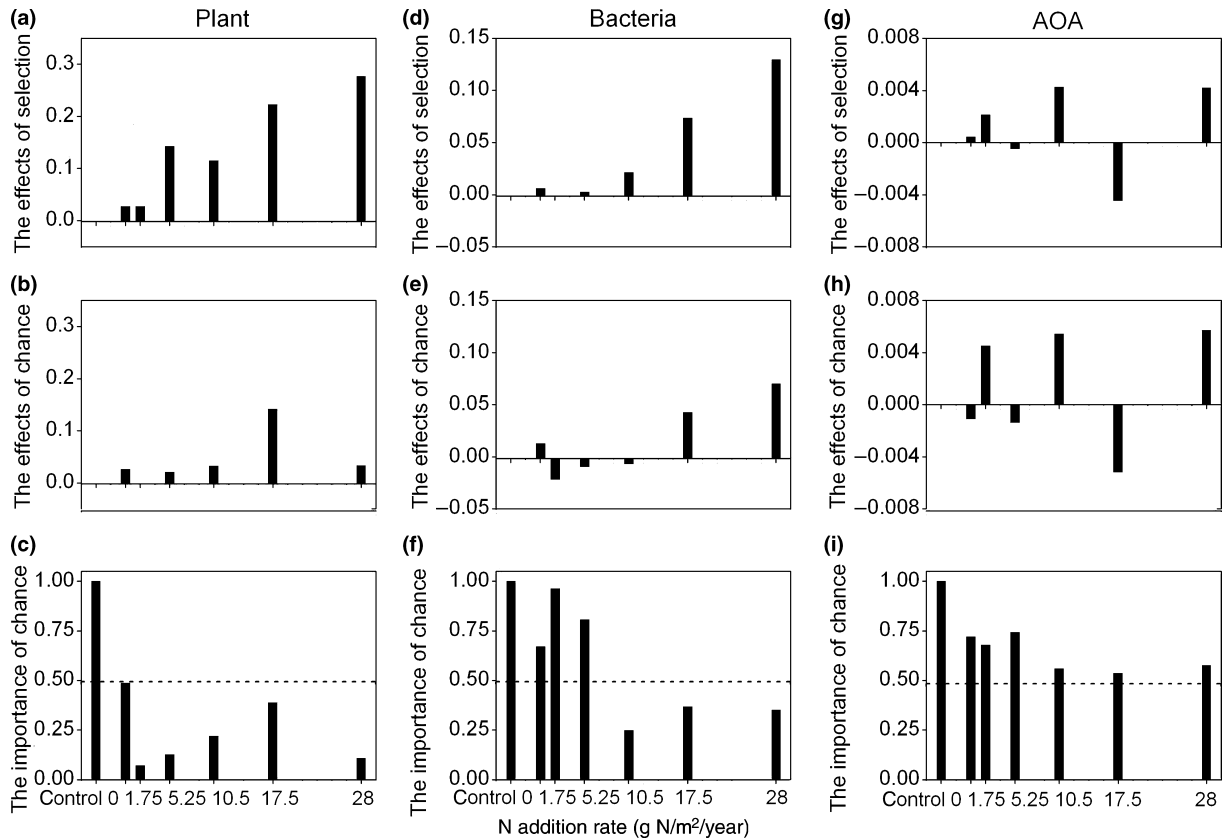
Fig. 3 The influences of N addition rate on the effects of selection, the effects of chance and the importance of chance for plants, bacterial and AOA communities.

$F = 8.347$, $P < 0.001$, $n = 54$). The selection effects were ~0 at N addition rates of 0 and 1.75 g N/m²/year, and increased with increasing rates of 5.25–28 g N/m²/year (Fig. 3a). The chance effects at 17.5 g N/m²/year were larger than those at the other five N addition rates (Table S1), although the differences were not significant among all treatments (permutation dispersion: $F = 1.074$, $P = 0.388$). The chance effects were ~0 at all N addition rates except for the peak at 17.5 g N/m²/year (Fig. 3b).

For bacterial communities, we calculated the integrative results of the two enzymes Hha1 and Msp1 (Table S3). The selection effects were significantly different among treatments (one-way ANOVA: Hha1, $F = 13.762$, $P < 0.001$, $n = 54$; Msp1, $F = 37.261$, $P < 0.001$, $n = 54$). The selection effects were ~0 at 0–10.5 g N/m²/year, and increased with increasing N addition rates of 17.5–28 g N/m²/year (Fig. 3d). The chance effects were also significantly different among treatments when analysed with Hha1 (Permutation dispersion: $F = 9.810$, $P = 0.0001$), and the chance effects at 17.5 and 28 g N/m²/year were greater than those of the other four treatments when analysed with Msp1

(Table S1), although the differences among all treatments were not significant (Permutation dispersion: $F = 1.755$, $P = 0.133$). The chance effects were ~0 at 0–10.5 g N/m²/year, and increased with increasing N addition rates over the range of 17.5–28 g N/m²/year (Fig. 3e).

For AOA communities, the selection effects were less at 17.5 than at 10.5 (LSD: $P = 0.048$) and 28 (LSD: $P = 0.050$) g N/m²/year, although the differences were not significant among all treatments (one-way ANOVA: $F = 1.245$, $P = 0.320$, $n = 30$). The selection effects were ~0 at 0–5.25 g N/m²/year, >0 at 10.5 and 28 g N/m²/year, but <0 at 17.5 g N/m²/year (Fig. 3g). We calculated the mean difference in the AOA genetic diversity between each treatment and the control, and this mean difference was significantly correlated with the selection effects (Pearson correlation: $r = 0.844$, $P = 0.034$, two-tailed). Although the chance effects were not significantly different among treatments (permutation dispersion: $F = 0.707$, $P = 0.626$), they followed the same trends as the selection effects (Pearson correlation: $r = 0.966$, $P = 0.002$, two-tailed; Fig. 3g,h).

The relative importance of selection and chance

According to our definition, the importance of chance was exactly one at the control. In plant community, as N addition rate increased from control to 0 g N/m²/year and then to 1.75–28 g N/m²/year, the importance of chance decreased from 1 to 0.487 and then to <0.400 (Fig. 3c). In bacterial community, as N addition rate increased from control and 0–5.25 to 10.5–28 g N/m²/year, the importance of chance decreased from 1 and ∼0.800 to <0.400 (Fig. 3f). In AOA community, as N addition rate increased from control and 0–5.25 to 10.5–28 g N/m²/year, the importance of chance decreased from 1 and ∼0.700 to ∼0.550 (Fig. 3i). In summary, as N addition rate increased, the importance of chance decreased on the whole.

In plant community, at those N addition rates of 1.75–28 g N/m²/year when the importance of chance was <0.4, as N addition rate increased from 1.75 to 17.5 and then to 28 g N/m²/year, the importance of chance first increased from 0.069 to 0.389, and then decreased to 0.107 (Fig. 3c). In bacterial community, at those N addition rates of 10.5–28 g N/m²/year when the importance of chance was <0.4, as N addition rate increased from 10.5 to 17.5 and then to 28 g N/m²/year, the importance of chance increased from 0.248 to 0.367 and 0.351 (Fig. 3f). In summary, as N addition rate increased, the importance of chance decreased nonlinearly, with a local increase at certain rates for both plants and bacteria.

At all N addition rates from 0 to 28 g N/m²/year, the importance of chance was <0.500 in plant community, but >0.500 in AOA community (Fig. 3c,i).

Discussion

N addition increased soil total N, NH_4^+-N, NO_3^--N contents, and decreased pH (Fig. 1); as selective forces, these environmental changes would alter the structure of plant community. As N addition rate increased, the selection effects increased as we expected (Fig. 3a); but, the chance effects first increased and then decreased rather than kept stable, which was beyond our expectation (Fig. 3b). During the long-term evolutionary history, the plants in the typical steppe ecosystem of Inner Mongolia were adaptive to N-poor and pH-neutral soil (Yuan *et al.* 2006); therefore, as N addition rate increased, more and more species were excluded (Fig. 2a). N addition promoted the dying of some plant individuals (e.g. *Agropyron cristatum*, *Cleistogenes squarrosa* and *Cares korshinskyi*; Table S4, Supporting information; Bai *et al.* 2010), leaving resources (e.g. space) to be taken up by other plant individuals. No matter the resources-acquiring individuals were descendants of pre-existing plants in the plot or immigrants from outside the plot, the resource acquisition process was stochastic. In other words, N addition indirectly promoted the stochastic processes of ecological drift and migration, so the chance effects increased as N addition rate increased from 0 to 17.5 g N/m²/year (Fig. 3b). As N addition rate increased to 28 g N/m²/year, the environmental changes were so sharp that only a few species (e.g. *L. chinensis*, *Chenopodium glaucum* and *Axyris amaranthoides*; Table S4) were adaptive (Xia & Wan 2008; Bai *et al.* 2010), and the selection effects were so powerful that the chance effects turned to decrease (Fig. 3b).

We investigated bacterial community structure with the method of T-RFLP, which classified all soil bacteria into only about 13 OTUs based not on species trait, but on the enzymes-digested fragment length of 16S rRNA genes (Dunbar *et al.* 2001; Kim & Marsh 2004; Fierer & Jackson 2006). In spite of this, as N addition rate increased, the selection effects still showed an increasing trend as we expected (Fig. 3d); the chance effects also increased rather than kept stable (Fig. 3e), which was contrary to our expectation, and we thought that it was because N addition indirectly promoted the stochastic processes of ecological drift and migration, just as for the plants community. There were still two differences between the plant community and the bacterial community: first, the selection effects started to increase at 5.25 g N/m²/year for plants, but at 17.5 g N/m²/year for bacteria; second, at 28 g N/m²/year, the chance effects for plants were decreasing, but for bacteria continued to increase (Fig. 3). If we could investigate the structure of bacterial community based on the real species, perhaps the selection effects would start to increase at a lower N addition rate, and the chance effects also decrease at 28 g N/m²/year.

For AOA community, both the selection effects and the chance effects changed nonlinearly as N addition rate increased (Fig. 3g,h), which were beyond our expectation. Because N addition decreased soil pH and increased NH_4^+-N content (Fig. 1); while the former was unfavourable to AOA's existence, the latter was favourable as a source of energy, and they concurrently influenced the survival of AOA communities. At N addition rates of 0–10.5 g N/m²/year, mainly due to the increased NH_4^+-N content (Fig. 1c), the genetic diversity of the *amoA* gene increased a little (Fig. 2d). At 17.5 g N/m²/year, the soil pH decreased greatly (Fig. 1b), which decreased the genetic diversity of the *amoA* gene (Fig. 2d). When compared with 17.5, at 28 g N/m²/year, the soil pH decreased a little, but the NH_4^+-N content greatly increased (Fig. 1b,c), which increased the genetic diversity of the *amoA* gene again (Fig. 2d). We calculated the mean difference in the genetic diversity between each treatment and control,

and the selection effects changed with the same trend as this mean difference (Pearson correlation: $r = 0.844$, $P = 0.034$, two-tailed). The chance effects also followed the same trend as the selection effects (Pearson correlation: $r = 0.966$, $P = 0.002$, two-tailed; Fig. 3g,h). These results indicate that, for both the selection effects and the chance effects, values >0 means that the increased NH_4^+-N content promotes the genetic diversity, and values <0 mean that the decreased pH restrains the genetic diversity. Of course, the absolute values of both the selection effects and the chance effects represent their magnitudes.

Although as N addition rate increased, the respective effects of selection and chance showed inconsistent changing trends among plants, bacteria and AOA, the importance of chance decreased on the whole for them all (Fig. 3), just as we expected. A recent experiment showed that drought decreased the importance of stochastic community assembly (Chase 2007), in accordance with our results that N addition decreased the importance of chance on the whole. The importance of chance did not decrease linearly, but with a local increase at certain N addition rates, because N addition indirectly promoted the stochastic processes of ecological drift and migration, as we discussed above.

The importance of chance = 0.5 means that 50% of structural variations are driven by the chance of ecological drift and migration, and the remaining 50% are driven by the selection of N addition. Therefore, these results that the importance of chance was always >0.5 in plants community and <0.5 in AOA community indicated that selection always played a more important role than chance in plant community, and that chance always played a more important role than selection in AOA community. Selection drives community assembly based on differences in species fitness; we investigate all plant and the specific functional group of AOA, then the average difference in plant fitness should be larger than that in AOA fitness; so, the importance of selection was larger in plants community than in AOA community, and the importance of chance was larger in AOA community than in plants community. Of course, the relative importance of selection and chance depends not only on the community property but also on the selective intensities. For example, the bacterial community is mainly controlled by chance at 0, 1.75 and 5.25 g N/m²/year, and mainly controlled by selection at 10.5, 17.5 and 28 g N/m²/year (Fig. 3f).

Although scale and scaling were of importance when the structural variations of different communities were to be considered, and N deposition would affect plants and microbes at different spatial scales, it was appropriate for us to analyse plants and microbes at the same spatial scale in this experiment, because spatial factor had no significant influences on structural variations for all of plant, bacteria and AOA (Data S1, Supporting information). It was important for us to analyse the relative importance of selection and chance under the disturbance of N deposition at diverse spatial scales in the future.

In conclusion, by performing a mimic N deposition experiment and developing an experimentally based calculation method, we successfully separated the respective effects of selection and chance and calculated their relative importance in biodiversity maintenance. Our results indicated that the effects of selection and chance and their relative importance are influenced by both N addition rate and the community property. Accounting for these factors, we should adopt different strategies in restoring community structure that has been changed by N deposition. For example, at low N addition rates, both effects of selection and chance are small, the relative abundances of species rather than the species richness are altered, so the community structure may bounce back quickly when N deposition stops. At the intermediate N deposition rates, the chance effects of ecological drift and migration may increase, and then some species from outside the ecosystem may colonize the site. As a means of ecosystem preservation or restoration, strategies of both cutting N deposition rates and eliminating the immigrating species should be adopted. At high N deposition rates, the selection effects are so powerful that many endemic species are excluded, termination of N deposition and eradication of migrating species are inadequate to restore the degraded ecosystem—reintroduction of the endemic species should be considered. In addition to N deposition, there are many other human-induced environmental disturbances (e.g. land-use change, climate change, and elevated carbon dioxide concentration) that are also changing the global biodiversity at an accelerating rate (Sala *et al.* 2000). Our results shed light on the analysis of the underlying mechanisms of ecosystem change induced by anthropogenic disturbances and on the adoption of proper conservation and restoration strategies under different disturbance intensities.

References

Anderson MJ (2004) *Permutation Dispersion: A FORTRAN Computer Program for Permutational Analysis of Multivariate Dispersions (for Any Two-Factor ANOVA Design) Using ermutation Tests*. Department of Statistics, University of Auckland, Aukland, New Zealand.

Bai YF, Jiangguo Wu, Clark CM *et al.* (2010) Tradeoffs and thresholds in the effects of N addition on biodiversity and ecosystem functioning: evidence from inner Mongolia Grasslands. *Global Change Biology*, **16**, 358–372.

Bakker JP, Berendse F (1999) Constraints in the restoration of ecological diversity in grassland and healthland communities. *Trends in Ecology and Evolution*, **14**, 63–68.

Bell G (2001) Neutral macroecology. *Science*, **293**, 2413–2418.

Cadotte MW (2007) Concurrent niche and neutral processes in the competition-colonization model of species coexistence. *Proceedings of the Royal Society B: Biological Sciences*, **274**, 2739–2744.

Chase JM (2007) Drought mediates the importance of stochastic community assembly. *Proceedings of the National Academy of Sciences, USA*, **104**, 17430–17434.

Chesson P (2000) Mechanisms of maintenance of species diversity. *Annual Review of Ecology, Evolution, and Systematics*, **31**, 343–366.

Dumbrell AJ, Nelson M, Helgason T, Dytham C, Fitter AH (2010) Relative roles of niche and neutral processes in structuring a soil microbial community. *The ISME Journal*, **4**, 337–345.

Dunbar J, Ticknor L, Kuske C (2001) Assessment of microbial diversity in four southwestern United States soils by 16S rRNA gene terminal restriction fragment analysis. *Applied and Environmental Microbiology*, **67**, 190–197.

Ellwood MDF, Manica A, Foster WA (2009) Stochastic and deterministic processes jointly structure tropical arthropod communities. *Ecology Letters*, **12**, 277–284.

Fierer N, Jackson RB (2006) The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences, USA*, **103**, 626–631.

Francis CA, Roberts KJ, Beman JM, Santoro AE, Oakley BB (2005) Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. *Proceedings of the National Academy of Sciences, USA*, **102**, 14683–14688.

Galloway JN, Dentener FJ, Capone DG *et al.* (2004) Nitrogen cycles: past, present, and future. *Biogeochemistry*, **70**, 153–226.

Harpole WS, Tilman D (2006) Non-neutral patterns of species abundance in grassland communities. *Ecology Letters*, **9**, 15–23.

Harpole WS, Tilman D (2007) Grassland species loss resulting from reduced niche dimension. *Nature*, **446**, 791–793.

Hubbell SP (1979) Tree dispersion, abundance, and diversity in a tropical dry forest. *Science*, **203**, 1299–1309.

Hubbell SP (2001) *The Unified Neutral Theory of Biodiversity and Biogeography*. Princeton University Press, Princeton.

Hutchinson GE (1959) Homage to Santa Rosalia, or why are there so many kinds of animals? *American Naturalist*, **104**, 501–528.

Kim SH, Marsh TL (2004) The analysis of microbial communities with terminal restriction fragment length polymorphism (T-RFLP). In: *Molecular microbial ecology manual* (eds Kowalchuk GA *et al.*), pp. 789–808. Kluwer Academic Publishers, Dordrecht, the Netherlands.

Könneke M, Bernhard AE, de la Torre JR, Walker CB, Waterbury JB, Stahl DA (2005) Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature*, **437**, 543–546.

Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences, USA*, **76**, 5269–5273.

Pacala SW, Tilman D (1993) Limiting similarity in mechanistic and spatial models of plant competition in heterogeneous environments. *American Naturalist*, **143**, 222–257.

Sala OE, Chapin III FS, Armesto JJ *et al.* (2000) Global biodiversity scenarios for the year 2100. *Science*, **287**, 1170–1174.

Stevens CJ, Duprè C, Dorland E *et al.* (2004) Impact of nitrogen deposition on the species richness of grasslands. *Science*, **303**, 1876–1879.

Suding KN, Collins SL, Gough L *et al.* (2004) Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. *Proceedings of the National Academy of Sciences, USA*, **102**, 4387–4392.

Thompson R, Townsend C (2006) A truce with neutral theory: local deterministic factors, species traits and dispersal limitation together determine patterns of diversity in stream invertebrates. *Journal of Animal Ecology*, **75**, 476–484.

Tilman D (1987) Secondary succession and the pattern of plant dominance along experimental nitrogen gradients. *Ecological Monograph*, **57**, 189–214.

Tilman D (2004) Niche tradeoffs, neutrality, and community structure: a stochastic theory of resource competition, invasion, and community assembly. *Proceedings of the National Academy of Sciences, USA*, **101**, 10854–10861.

Travisano M, Mongold JA, Bennett AF, Lenski RE (1995) Experimental tests of the roles of adaptation, chance, and history in evoution. *Science*, **267**, 87–90.

Vitousek PM, Aber JD, Howarth RW *et al.* (1997) Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Application*, **7**, 737–750.

Xia JY, Wan SQ (2008) Global response patterns of terrestrial plant species to nitrogen addition. *New Phytologist*, **179**, 428–439.

Yuan ZY, Li LH, Han XG *et al.* (2006) Nitrogen response efficiency increased monotonically with decreasing soil resource availability: a case study from a semiarid grassland in northern China. *Oecologia*, **148**, 564–572.

Zhang LX, Bai YF, Han XG (2004) Differential responses of N: P stoichiometry of Leymus chinensis and Carex korshinskyi to N additions in a steppe ecosystem in Nei Mongol. *Acta Botanica Sinica*, **46**, 259–270.

XZ is interested in microbial ecology and evolution. WL is interested in theoretical ecology. YB, GZ and XH are interested in biogeochemistry and biodiversity. This work forms part of XZ's PhD dissertation.

Supporting information

Additional supporting information may be found in the online version of this article.

Data S1 Excluding the influences of spatial distance on community structural variation

Table S1 The differences of within-treatment structural variation between every pair of treatments

Table S2 The influences of environmental indexes on within-treatment structural variations

Table S3 The calculation of the relative importance of selection and chance

Table S4 The effects of N addition on plants species' biomass

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.