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Nitrogen fertilization directly affects soil bacterial diversity and indirectly affects bacterial community composition

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ABSTRACT

Nitrogen (N) deposition influences both above- and below-ground communities and influences ecosystem functioning. However it is not clear about direct or indirect interactions among plants, soils and microbes in response to nitrogen deposition. In this study, the responses of soil bacterial diversity to N enrichment were investigated at surface (0–10 cm) and sub-surface (10–20 cm) soils in a temperate steppe ecosystem. N addition (>120 kg N ha^{-1} yr^{-1}) resulted in a significant shift in bacterial community composition and a decrease in bacterial OTU richness in surface soil, but the effect on the sub-surface layer was far less pronounced, even at the highest addition rate (240 kg N ha^{-1} yr^{-1}). Bacterial OTU richness was significantly correlated with soil and plant characteristics. Hierarchical structural equation modeling showed that soil ammonium availability was responsible for the shift in bacterial richness, whereas the change in bacterial community composition was due to alterations in soil pH and plant composition. These results indicated that N fertilization directly affected soil bacterial richness but indirectly affected bacterial communities through soil acidification and plant community change, indicating distinct controls on soil bacterial diversity and community composition. Our results also suggest that N availability could be a good predictor for the loss of soil bacterial diversity under atmospheric nitrogen deposition.

1. Introduction

Atmospheric nitrogen (N) deposition that mainly originates from anthropogenic activity has increased three to five-fold over the past century (Denman et al., 2007), and the deposition rate of N is predicted to double by 2050 (Galloway et al., 2004; Phoenix et al., 2011). In China, atmospheric N deposition has increased by 8 kg N ha^{-1} since the 1980s (Liu et al., 2013) and has become a matter of great concern to the potential impact on ecosystem structure and function including eutrophication, soil acidification and the loss of biodiversity (Vitousek et al., 1997; Guo et al., 2010). Elevated N inputs usually increase above-ground plant production in terrestrial ecosystems, with corresponding changes in plant community composition and, in most cases, decreased plant diversity, decreased lignin in leaf litter, and influence on N ratio and carbon (C) substrates that directly stimulate heterotrophic respiration (Phoenix et al., 2011). These above-ground alterations cause a ripple of changes that affect the soil C pool that contributes to the global C budget and climate change.

It is well known that many soil microbes are directly related to soil biogeochemical processes and play a prominent role in the soil C cycle (Bardgett et al., 2008). However, the mechanisms underlying the soil microbial feedback in response to global climate change remain elusive. A number of studies have reported negative effects on microbial activity as a result of N amendment, which may culminate in a decrease in the rate of soil respiration and an increase in C sequestration (Liu and Greaver, 2010). The *microbial N mining* hypothesis predicts that soil microbes use labile C to decompose recalcitrant organic matter to facilitate N acquisition, but this process is suppressed by increasing N availability that leads to a decrease in microbial activity (Craine et al., 2007). The *enzyme inhibition* hypothesis proposes that the enzymes involved in

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decomposing recalcitrant C are inhibited by N addition, thereby reducing overall microbial activity (Gallo et al., 2004). Besides changes in microbial activity, N fertilization also induces alteration in soil microbial community composition. Fierer et al. (2007) observed that shifts in the specific community were responsible for the decrease in decomposition rate, that an N addition decreased the relative abundance of oligotrophs that are adept at catabolizing recalcitrant C. However, the microbial responses to elevated N inputs are frequently mixed and lack consistency. For instance, Fierer et al. (2012) found that N enrichment led to significant decrease in bacterial phylotype diversity in an agricultural field but had no effects in grassland, suggesting that the effects of N fertilization on bacterial diversity are likely site-dependent.

The influences of N enrichment on soil microbial community diversity may be caused by direct effects of N as a nutrient, or by indirect changes in soil and plant properties. Soil microbes could promote plant diversity by increasing the diversity of available nutrient pools, whereas plant diversity can promote soil microbial diversity by increasing diversity of food resources and diversity of plant hosts for symbiotic and pathogenic microbes (Klironomos et al., 2011). Ramirez et al. (2010b) reported consistent responses in microbial respiration to N addition regardless of soil type and N form, suggesting that the response was resulted from direct effects by N availability, rather than indirect effects such as soil pH. By contrast, results in Wessén et al. (2010) showed that alteration in bacterial abundance affected by different fertilization regimes (including ammonium sulfate fertilization) was mainly driven by soil pH. Weand et al. (2010) also concluded that the microbial response to N addition was tree species-specific due to qualitative differences in plant-derived C. Therefore, alterations in soil, plant and microbe following N enrichment never occur in isolation, and a whole system approach revealing direct or indirect effects is needed for understanding the mechanisms underlying these ecological responses and feedbacks.

It was shown that a range of edaphic factors including pH, nutrient and O_2 levels varied with soil depth, and these differences result in distinct microbial communities along soil profiles (Fierer et al., 2003; Eilers et al., 2012). N fertilization affects soil parameters in both surface and sub-surface soil layers, and influences the distinct responses of soil microbial communities. Experiments have been conducted on a temperate steppe located at Duolun County in Inner Mongolia, China, in an area that is sensitive to climate change, overgrazing and nutrient supply, but the effects of N deposition were not focused upon (Liu et al., 2011). In the present study, we investigated response of soil bacterial community composition and diversity under different levels of N fertilization, and explore the effects of N enrichment on plant–soil–microbe system and the interactions among these components using a path-relation network and structural equation model. In addition, surface and sub-surface soil samples were collected for comparison of alteration in bacterial diversity between the two soil layers. As a consequence of layer-specific driving forces, it was hypothesized that bacterial composition would vary along the N addition gradient in the two layers.

2. Experimental procedures

2.1. Study sites and experimental design

This study was conducted in a grassland fertilization experimental field at Duolun County in Inner Mongolia, China (DL; temperate steppe; 42°02′ N, 116°17′ E, 1324 m a.s.l.). The climate of the station is a typical temperate continental monsoon, with a warm and dry summer, and long and cold winter. Mean annual temperature is 2.1 °C, and precipitation is 386 mm, with 91% falling from May to October. The experimental field was fenced off to prevent grazing disturbance, and a randomized block design was used, as detailed in Song et al. (2011). The N gradient was established in 2005, at rates of 0, 60, 120 and 240 kg N ha^{-1} yr^{-1} using NH_4NO_3 (except urea was applied in 2005), with five replicate plots in each treatment. N was added to the plots three times for foliar applications in June, July and August.

2.2. Sampling and analysis

Soil samples were collected on 16 June 2011 before N addition, at depths of 0–10 cm (n = 20; designated N0 to N240) and 10–20 cm (n = 20; N0b to N240b). Samples were transported on ice and stored at 4 °C for soil measurements and −40 °C for genomic DNA extraction. Soil pH was measured using a pH meter with a 1:5 (wt/vol) ratio of soil to water following shaking for 30 min. Soil total carbon (TC) and total nitrogen (TN) were measured with a CN Analyzer (Vario Max CN, Elementar, Hanau, Germany). Nitrate (NO_3^-–N), ammonium (NH_4^+–N), dissolved organic carbon (DOC) and dissolved total N (DTN) were extracted from 10 g soil using 2 mol L^{-1} KCl extraction procedures. NO_3^-–N, NH_4^+–N and DTN content was determined using a San^{++} Continuous Flow Analyzer (Skalar, Breda, The Netherlands), and DOC was determined using a Multi N/C 3000 Analyzer (Analytik Jena AG, Thuringia, Germany). Dissolved organic nitrogen (DON) was calculated according to the following formula: DON = DTN − NH_4^+–N − NO_3^-–N.

Plant sampling was performed on the 20th and 21st August in 2011 at maximum sward biomass; all sampling methods except plant biomass determination were performed nondestructively. Plant community coverage and richness were determined by visual estimates. Briefly, one permanent quadrat (1 × 1 m) was established at each subplot, and a frame (1 × 1 m) with 100 equally distributed grids spaced 10 cm apart was placed above the canopy in each quadrat. The percentage cover of each species was estimated visually in all grid cells. Species richness was defined as the number of different species in one quadrat. Above-ground vegetation was sampled by clipping all plant species at the soil surface. A quadrat (1 × 1 m) was placed within each plot randomly, but overlap with the permanent quadrat was avoided, as was placement within 50 cm from the edge of the plot to avoid edge effects. Plant samples were oven dried for 48 h at 65 °C and weighed. Plant N concentration was determined by micro-Kjeldahl digestion and continuous flow stable isotope ratio mass spectrometry (Delta Plus, Finnigan, Pittsburgh, PA, USA). Forbs, legumes and grasses were collected and analyzed as appropriate.

2.3. Barcode pyrosequencing of the 16S rRNA gene

DNA in each sample was extracted from ~0.5 g soil using the FastDNA SPIN Kit for soil (MP Biomedicals, Santa Ana, CA, USA) and purified using the UltraClean Soil DNA Kit (MOBIO Laboratories, Carlsbad, CA, USA) following the manufacturer's instructions. The 16S rRNA gene was amplified in triplicate using a barcoded primer set 515f/907r targeting a region suitable for phylogenetic information (Biddle et al., 2008), and samples were pooled and purified using an EasyPure Quick Gel Extraction Kit (Transgen, Beijing, China). After quantification by NanoDrop ND-100 (Thermo Scientific, Waltham, MA, USA), equimolar concentrations of purified amplicons were pooled into a single tube before sequencing on a Roche FLX 454 pyrosequencing machine (Roche Diagnostics Corporation, Indianapolis, IN, USA).

In order to obtain high-quality sequences, raw sequence data was passed through quality filters to reduce the error rate using MOTHUR v. 1.30.2 (Schloss et al., 2009). Briefly, reads containing ambiguous bases, those shorter than 200 bp, or not perfectly

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