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Differential responses of soil bacterial communities to long-term N and P inputs in a semi-arid steppe

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ABSTRACT

Both nitrogen (N) and phosphorus (P) may limit plant production in steppes and affect plant community structure. However, few studies have explored in detail the differences and similarities in the responses of below-ground microbial communities to long-term N and P inputs. Using a high-throughput Illumina Miseq sequencing platform, we characterized the bacterial communities in a semi-arid steppe subjected to long-term N or P additions. Our results showed that both the Chao richness and Shannon's diversity were negatively correlated to N input rate, while only Chao richness was significantly and negatively correlated to P input rate. Also, both N and P additions altered the bacterial community structure. The bacterial community between plots of the same N or P input rate was much more dissimilar with the higher input level, indicating more severe niche differentiation in pots with higher N or P input. N Inputs significantly increased the relative abundance of the predicted copiotrophic groups (*Proteobacteria* and *Firmicutes*) but reduced the predicted oligotrophic groups (*Acidobacteria*, *Nitrospirae*, *Chloroflexi*), with the order *Rhizobiales* being most affected. P additions significantly affected only two phyla (Armatimonadetes and *Chlorobi*), which were positively correlated with P source. Results from the structural equation modelling (SEM) showed that N additions affected the bacterial community primarily by changing the pH, while P additions did so mainly by improving P availability. Our results suggest that the below-ground bacterial communities are more sensitive to N inputs, but P inputs can also play an important role in bacterial niche differentiation. These findings improve our understanding of bacterial responses to N and P inputs, and their impacts on bacterial-mediated processes, especially in the context of increasing anthropogenic nutrient inputs.

1. Introduction

The Inner Mongolian steppes comprise >20% of the total grassland area in China, in which the plant communities play an important role in livestock farming and ecosystem services (Xu et al., 2014). To maximize biomass production, chronic nutrient additions, often in the form of chemical fertilizers (i.e., NPK) or animal manures, often occur in grasslands to maximize biomass production, this greatly contributes to the release of nutrients for plant uptake and growth (Zhou et al., 2015). Moreover, anthropogenic reactive nitrogen (N) inputs, which mainly originate from fossil-fuel burning and artificial fertilizer application, have increased three- to five-fold over the past century (Galloway et al., 2008) and is expected to continue to increase, especially in Asia (Chen et al., 2015a). In addition, it is generally believed that plant growth is limited by P in many grassland soils, especially calcareous grasslands forming complexes with calcium (Elser et al., 2007; Ford et al., 2016), thereby the continually anthropogenic P inputs are inevitable for maintaining high grass biomass. All of these nutrient inputs may influence the structure of soil microbial communities, which play a pivotal role in regulating soil functioning and maintaining ecosystem sustainability (van der Heijden et al., 2008). Despite the widely acknowledged importance of soil microorganisms, how bacteria ultimately respond to long-term repeated inputs of chemical fertilizers remains poorly understood.

Nitrogen (N) and phosphorus (P) should be the essential nutrients necessary to increase the grass biomass production in Inner Mongolian steppes. Their input often has multiple effects including changes in aboveground primary productivity, biodiversity, species composition, and ecosystem functioning (Bai et al., 2010; Li et al., 2010; Yang et al., 2015). Soil microbiota are recognized as key players in sustaining ecosystem functions and services. Therefore, elucidating the feedbacks of the soil microbiome to N and P inputs is fundamental to understanding the consequences of global changes on ecosystem processes regulated by soil biota. Recent studies on the Inner Mongolian steppe were mainly

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concerned with N-induced soil microbial changes. Significant effects of N addition on bacterial communities were reported and were ascribed to N-induced pH changes, followed by N content and N forms (Zhang et al., 2014a; Chen et al., 2015b; Yang et al., 2015). Other soil chemical changes such as soil total C or P, impacted the microbial communities (Chen et al., 2013). Compared with studies on N addition, only a few studies focused on the effects of P addition on soil microbial biomass and the microbial community, and most of these studies were conducted in agricultural ecosystems (Beauregard et al., 2009; Shi et al., 2013; Tan et al., 2013). Understanding the effects of both N and P addition on soil microbial activities can improve our capacity to predict how the soil microbial community will respond to environmental changes in semi-arid steppe ecosystems. This information will help to develop effective strategies for the management and sustainability of ecosystems under nutrient additions.

Although previous studies focused on the response of the soil microbial community to N addition, only a few studies compared the difference between the impacts of N and P inputs on the bacterial community in Inner Mongolian steppes, China. In this study, soils, collected from a semi-arid steppe located in Inner Mongolia, were subjected to a 16-year N or P addition with gradient rates. We used the high-throughput Illumina Miseq sequencing platform to characterize the bacterial communities' response to the two types of nutrients under varying simulated input rates. The following questions were specifically addressed: (1) Do the bacterial communities have differences and similarities in response to the gradient N and P inputs? and (2) What would be the primary factor resulting from the gradient N or P inputs to impact the soil bacterial communities in the steppes ecosystem?

2. Materials and methods

2.1. Long-term experiment site description

The long-term N and P input experiments were conducted at the Inner Mongolia Grassland Ecosystem Research Station (IMGERS, 43°38′N, 116°42′E) of the Chinese Academy of Sciences, which is located in the Xilin River Basin of Inner Mongolia, China, at an altitude of approximately 1200 m a.s.l. (Bai et al., 2004). Before the experiment began, the site was dominated by *Leymus chinensis*, a widely distributed perennial C_3 rhizome grass in the Eurasian steppe region (Yao et al., 2014). The mean annual precipitation in this area is about 340 mm, with c. 80% rainfall occurring during the growing season from May to September. The average monthly temperature ranges from −21.6 °C in January to 19.0 °C in July. Precipitation mainly falls in the growing season (June–August), which is coincident with high temperatures. The site has a dark chestnut soil (Calcic Chernozem, according to the ISSS Working Group RB, 1998), with a loamy-sand texture (Bai et al., 2010).

2.2. Experimental design and soil sampling

The N and P input experiments were established in the autumn of 1999. An experimental plot was 5 × 5 m in size, arranged in a randomized block design and separated by a 1-m buffer zone (Bai et al., 2010). The plots were not harvested for hay and not grazed. For the N input experiment, five levels of N input rates, 0 $g{\cdot}N{\cdot}m^{-2}\ yr^{-1}$, 1.75 $g{\cdot}N{\cdot}m^{-2}\ yr^{-1}$, 5.25 $g{\cdot}N{\cdot}m^{-2}\ yr^{-1}$, 10.5 $g{\cdot}N{\cdot}m^{-2}\ yr^{-1}$ and 28 $g{\cdot}N{\cdot}m^{-2}\ yr^{-1}$, with pelletized NH_4NO_3 fertilizer were applied to the soil. To ensure that N was the only limiting nutrient, all of the treatments were also supplied with the same amounts of P (10 $g{\cdot}P_2O_5{\cdot}m^{-2}\ yr^{-1}$), S (0.2 $mg{\cdot}m^{-2}\ yr^{-1}$) and trace elements (Zn, 190 $mg{\cdot}m^{-2}\ yr^{-1}$, Mn, 160 $mg{\cdot}m^{-2}\ yr^{-1}$ and B, 31 $mg{\cdot}m^{-2}\ yr^{-1}$) based on local soil census data. For the P input experiment, five levels of P input rates, 0 $g{\cdot}P_2O_5{\cdot}m^{-2}\ yr^{-1}$, 2 $g{\cdot}P_2O_5{\cdot}m^{-2}\ yr^{-1}$, 4 $g{\cdot}P_2O_5{\cdot}m^{-2}\ yr^{-1}$, 8 $g{\cdot}P_2O_5{\cdot}m^{-2}\ yr^{-1}$ and 32 $g{\cdot}P_2O_5{\cdot}m^{-2}\ yr^{-1}$, were applied to the soil with superphosphate fertilizer. To ensure that P was the only limiting nutrient, all of the treatments were also supplied with the same amounts of N (2.4 $g{\cdot}N{\cdot}m^{-2}\ yr^{-1}$) and the same rates of S and trace elements as in the N input experiment. Nutrients were uniformly applied to each plot with manual broadcasting in the mid-growing season (1–5 July) every year, coinciding with high temperatures and precipitation.

Soil samples were collected in the middle of July 2015. Five replicates for each treatment were collected from five individual plots. Six soil cores (2 cm diameter, 0–10 cm deep) were randomly collected from each plot and combined to form one composite soil sample per plot. Soil samples were transported to the laboratory from the experimental site in a constant temperature box with ice. After the soil was gently mixed and roots were removed, the moist soil was passed through a 2-mm-mesh sieve and separated into two parts. One part was directly subjected to DNA extraction. The second part was air-dried for the determination of soil pH, total organic C (TC), total N (TN) and available P (AP). Soil TC and TN were measured with an elemental analyser (Vario MAX; Elementar, Germany), and AP was extracted with 0.5 $M{\cdot}NaHCO_3$ and determined using the ammonium molybdate ascorbic method.

2.3. DNA extraction and MiSeq sequencing of 16S rRNA gene amplicons

Total genomic DNA was extracted using a PowerSoil DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA), according to the manufacturer's instructions. The quality and quantity of the DNA samples was checked by a spectrophotometer (NanoDrop, ND2000, Thermo Scientific, Wilmington, DE, USA) after extraction.

The composition and diversity of bacterial communities were assessed by Illumina MiSeq sequencing analysis of the 16S rRNA gene. The universal primers 515F (5′-GTGCCAGCMGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′) were selected for the PCR amplification of the V4 region. The reverse primer contains a 6-bp error-correcting bar code unique to each sample. Illumina MiSeq sequencing was conducted by the Major Biotechnology Co., Ltd. (Shanghai, China) using an Illumina MiSeq platform. Sequences were submitted to the NCBI database under the accession number SRR3452783 and SRR3452785 for the N input experiment and P input experiment, respectively.

2.4. Sequence data analysis

The raw sequences obtained were processed using the Quantitative Insights Into Microbial Ecology (QIIME) toolkit (Caporaso et al., 2010) and UPARSE pipeline (Edgar, 2013). All sequence reads were trimmed and assigned to each sample based on their barcodes. Multiple steps were required to trim the sequences, such as the removal of sequences <220 bp. For the samples from the N and P input experiments, each sample was rarefied to 14,618 and 14,605 reads, respectively, from the high quality sequences for both alpha-diversity (Chao estimator of richness, observed species and Shannon's diversity index) and beta-diversity (nonmetric multidimensional scaling and NMDS) analyses. The UPARSE pipeline was used to pick the operational taxonomic units (OTUs) to obtain an OTU table at a 97% identity threshold. Taxonomy was assigned using the Ribosomal Database Project classifier (Wang et al., 2007).

2.5. Statistical analysis

All of the changes in the soil microbial communities were evaluated based on the OTU matrix. NMDS plots were used to visualize the structure among the samples based on the Euclidean distance of the OTU matrix in PAST (http://folk.uio.no/ohammer/past/). The statistical significance among the datasets was assessed by PerMANOVA using the Euclidean distance matrix in PAST. The N or P gradient rates were natural logarithm transformed to evaluate the Pearson's correlation coefficients with the Chao estimator of richness, Shannon's diversity index