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Responses of soil biological traits and bacterial communities to nitrogen fertilization mediate maize yields across three soil types

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ABSTRACT

Although the effects of nitrogen (N) fertilization on soil microflora have been well studied, the effects should be verified across soil types and N-added levels. To understand the impacts of N fertilization on shifts in soil biological traits and bacterial communities and to further explore the coupling mediation of these parameters with respect to crop yields, we sampled soils from three experimental sites (each site received three levels of N fertilization (0, 168 and 312 kg N ha^{-1})) that share the same climatic conditions but have different soil types (clay, alluvial and sandy soils). In clay and sandy soils, total microbial biomass with N fertilization treatments was lower than that with no treatment, and the N and carbon (C) contents of microbial biomass with N fertilization treatments were higher than those without treatment. In alluvial soils, these properties were higher with N fertilization treatment than with no treatment. Together with N addition, bacterial abundance and phylogenetic diversity significantly decreased in alluvial and sandy soils. Soil type had a higher (38.82%, $p < 0.001$) individual impact on bacterial community structure than did N fertilization (18.92%, $p < 0.001$). Interactions between soil type and N fertilization also notably explained 11.08% of the altered community structure. Overall, N fertilization significantly affected soil physicochemical (such as pH, organic C and NO_3–N) and biological properties (microbial biomass C and N), which can affect maize yield directly and indirectly by further mediating soil bacterial abundance, diversity and community structure, with consequences for crop production. Bacterial community structure (path coefficient = 0.64) had the most positive and direct impact on maize yield, followed by organic C (0.37) and available N (0.33). Altogether, these findings suggest that N fertilization affects soil biological traits and bacterial communities across different soil types. Further understanding these soil microbial parameters can contribute to crop yield and may provide deeper insight into predicting the coupling of soil functionality and crop productivity.

1. Introduction

Over the past century, atmospheric nitrogen (N) deposition has increased three- to fivefold compared to preindustrial levels (Alley, 2007), and future global N deposition rates in terrestrial ecosystems are predicted to increase by 200–250% (Davidson, 2009; Phoenix et al., 2011). N is a critical nutrient that controls productivity in many terrestrial ecosystems (Zechmeister-Boltenstern et al., 2011). In particular, crop production largely depends on N availability (Greenwood, 1982). Worldwide, arable soils receive considerable N from anthropogenic activities (Ramirez et al., 2012), which largely have contributed to a stable increase in global cereal production in the past few years (Tilman et al., 2006; Erisman et al., 2008).

In general, long-term N fertilization increases soil organic carbon, total N, and particulate organic matter and decreases soil pH, cation exchange capacity and microbial biomass (Jagadamma et al., 2007; Zhao et al., 2016; Barak et al., 1997). Increased crop yields can be partly explained by N fertilization-induced changes in soil physicochemical properties, including soil texture, available nutrient contents, organic matter and the stability of aggregates (Blanco-Canqui et al., 2006; Wright et al., 1990; Zhao et al., 2016). Additionally, interactions among microbes, soils and crops are considered the main driver of agroecosystem productivity (Sinsabaugh et al., 2015), and microorganisms are particularly sensitive to any modification in soil properties or crops (Hallin et al., 2009; Zhu et al., 2016). Therefore, increased crop yields can also be attributed to N fertilization-induced changes in soil microbial communities and activities. N addition enhances soil ecosystem functionality by potentially impacting the

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composition (Zhou et al., 2015), diversity (Zhou et al., 2015) and activity (Gallo et al., 2004) of microbial communities, which further contribute to soil carbon (C) and N mobilization (Philippot et al., 2013; Fierer et al., 2012). The impacts of N enrichment on these microbial traits may be directly caused by increased soil nutrient availability or indirectly by changes in the properties of soils and crops (Treseder, 2008; Jian et al., 2016).

The responses of soil microbes to elevated N inputs are inconsistent. For example, numerous previous studies have reported that N amendment caused strong reductions in soil microbial activity (Ramirez et al., 2012) because of an increase in C sequestration and/or a decrease in the rate of soil respiration (Ramirez et al., 2012; Liu and Greaver, 2010), and the magnitudes of reduction are strongly associated with the amount and duration of N addition to soil (Treseder, 2008; Ramirez et al., 2012; Janssens et al., 2010). Different responses of microbial diversity and community structure have also been reported, i.e., a decrease, an increase or no net change (Ros et al., 2009; Ma et al., 2016; Allison et al., 2007), which may be ascribed to different durations and amounts of N treatments. Alternatively, different soil types have their own unique set of initial characteristics, including distinct edaphic characteristics and indigenous microbial traits, which may induce different and unquantifiable impacts of N enrichment on soil responses (Zeglin et al., 2007). For example, different soil types show different aeration and water conditions, which can affect the performance of fertilization on soil microbial communities (Bossio et al., 1998; Berg and Smalla, 2009). Müller et al. (2006) indicated that alluvial soils have some unique microorganisms that can tolerate high ammonia concentrations induced by N fertilization. Clay soils with a high clay content typically exhibit greater physical-chemical protection of microbial biomass pools and decreased water stress (Fierer et al., 2012; Kallenbach and Grandy, 2011), which affect the performance of N fertilization on soil microbial communities.

Crop yields and soil productivity are attributed mainly to soil nutrient levels (Lorenz et al., 2007; Agegnehu et al., 2016) and microbial communities (Jin et al., 2009), as well as a favorable soil environment (Agegnehu et al., 2016). All of these parameters impact the temporal availability of soil N for plant uptake (Sabahi et al., 2010) and the immobilization of mineral N (Marinari et al., 2010). Therefore, to comprehensively understand shifts in soil bacterial traits and bacterial communities mediated by N fertilization across different soil types and to further explore the coupling impacts of soil physicochemical and microbiological properties on crop yields, we sampled soils from three different fertilization experimental sites with different levels of N that have the same climatic conditions but different soil types (clay, alluvial and sandy soils). We used permutational multivariate analysis of variance (PERMANOVA) and structural equation modeling (SEM) to synthetically analyze and test the two following main hypotheses. First, we hypothesized that the community composition of soil bacteria would be affected by the application of N fertilizers in three soil types; such a change may derive from decreased pH, decreased total microbial biomass and decreased phylogenetic diversity. Second, if the first hypothesis is true, then changes in these soil properties, either together or individually, further mediate the effect of N fertilization on maize yields.

2. Materials and methods

2.1. Field sites and experiment description

The sampling sites were established in May 2009 in experimental fields at Sankeshu (43°20′N, 124°00′E), Wangjiaqiao (43°15′N, 124°29′E) and Fujiajie (43°21′N, 124°05′E) in Jilin Province, China. The three fertilization experimental sites with different levels of N have the same climatic conditions (a warm temperate, semihumid continental monsoon climate) but different soil types (clay, alluvial and sandy soils). These sites with similar climates experience an annual rainfall of 474 mm and a mean annual temperature of 5.8 °C. On the basis of the World Reference Base (WRB) soil classification system, the three soils are classified as Chernozems, Fluvisols and Arenosols, respectively (Zhang et al., 2014). The basic soil properties measured at the start of the field experiment are shown in Table S1. The experimental design, consisting of three replicates of three different fertilization treatments, i.e., no N (0 kg N ha^{-1}), a low level of N (168 kg N ha^{-1}), and a high level of N (312 kg N ha^{-1}), was applied across 27 plots at the three sites. The application rates, fertilizer types and plot sizes are summarized in Table S2. All plots were arranged in a randomized block design with a plot size of 60 m^2. For each treatment, one-third of the N fertilizer was applied at planting and the other two-thirds were side-dressed at the maize (*Zea mays* L.) six-leaf stage. Maize was planted in May at a target population of 65,000 plants ha^{-1} and harvested in October. All maize residues were removed from the plots after harvest. Weeds were controlled by applying herbicides before seedling emergence.

Soil samples were collected from the plow layer (0–20 cm) in October 2015 after harvest. The collected soil samples were transported to the laboratory on ice, sieved (2 mm) and divided into three subsamples. The samples were stored at 4 °C until biochemical analysis or at −80 °C until DNA extraction was performed. In addition, some samples were air dried to determine the physicochemical properties. Maize yields (15.5% moisture content) were determined by manually harvesting an area of 18 m^2 (six rows with a width of 3.6 m and a length of 5 m) in the middle of each plot.

2.2. Chemical characteristics

Soil microbial biomass C and N contents (MBC and MBN, respectively) were detected using the chloroform fumigation-K_2SO_4 extraction method (Voroney et al., 1993). Soil pH, soil organic C content, soil total N content, available nitrogen (N), available phosphorus (P) and available potassium (K) were measured according to methods described by Yu et al. (2016). Soil NH_4^+ and NO_3^- nitrogen contents extracted with 2 M KCl were determined using a continuous flow analytical system (Santt System; Skalar, Holland).

2.3. Soil DNA extraction and quantitative PCR

Total genomic DNA in sampled soils was extracted using a Power Soil DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, USA) according to the manufacturer's protocol. The DNA was stored at −80°C until analysis.

Quantitative PCR amplifications (q-PCR) were conducted in a total volume of 20 μl in a PRISM thermocycler (MastercycleroRep realpex4, Eppendorf, Real-Time PCR). Each PCR reaction contained 2 μl of the target DNA extract, 10 μl of SYBR Premix EX Taq (2×, TaKaRa, Dalian, China), and 7.2 μl of sterilized water. In addition, 0.4 μl of each primer (347 F (5′-GGAGGCAGCAGTRRGGAAT-3′) and 531R (5′-CTNYGTMTTACCGCGGCTGC-3′)) (Kim et al., 2008; Nossa et al., 2010) targeted the 16S rDNA of bacteria. The thermal cycling profile included a first step at 95 °C (30 s) followed by 40 cycles of 95 °C for 5 s and 60 °C for 30 s, with a final extension at 72 °C for 5 min A standard curve was prepared using serial ten-fold dilutions, and the number of gene copies was calculated by measuring the concentration of the plasmid and the number of base pairs. To evaluate the amplification specificity, melting curve analysis was performed at the end of each PCR run (Větrovský and Baldrian, 2013). Amplification efficiencies of 98% were obtained with R^2 values of 0.9993.

2.4. Illumina MiSeq high-throughput sequencing and data analysis

The community structure and diversity of soil bacteria were evaluated through high-throughput sequencing analysis of the 16S rRNA gene (V3–V4 hypervariable regions) at Shanghai LE AL Tech, Ltd. (Shanghai, China) using the Illumina MiSeq PE250 sequencing platform