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Long-term fertilization changes bacterial diversity and bacterial communities in the maize rhizosphere of Chinese Mollisols

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ABSTRACT

The rhizosphere is a dynamic interface in which interactions among a myriad of microorganisms affect plants growth and tolerance to biotic and abiotic stress. Although rhizosphere effects on soil microbial communities have been widely investigated, few studies have evaluated such impacts of long-term fertilization on rhizosphere microbial communities in black soils common to northeast China. Here, we applied quantitative real-time polymerase chain reaction and high-throughput pyrosequencing to characterize rhizosphere and bulk soil bacterial communities in a long-term (36-year) fertilizer experiment. Soils were subjected to six treatments: CK (no fertilizer), N_1 (150 kg urea $ha^{-1} y^{-1}$), N_2 (300 kg urea $ha^{-1} y^{-1}$), M (18,600 kg horse manure $ha^{-1} y^{-1}$), NPK (150 kg urea plus 33 kg P plus 62 kg K $ha^{-1} y^{-1}$), and MNPK (M plus NPK). Inorganic fertilizer, especially N, decreased the 16S rRNA gene copy numbers and bacterial diversity in the rhizosphere and bulk soil, while manure fertilizer increased these values. Moreover, 16S rRNA gene copy numbers were higher and bacterial diversity was lower in the rhizosphere than the bulk soil, indicating that the maize rhizosphere had significant effects on bacterial diversity. The bacterial communities were predominantly composed of Proteobacteria and Acidobacteria in both the rhizosphere and bulk soil, but the rhizosphere and bulk soil communities were distinguished by principal coordinates analysis. Soil pH correlated with bacterial community composition and diversity in both rhizosphere and bulk soil. However, bacterial community composition in rhizosphere was more correlated with soil nutrient concentrations than in bulk soil under long-term fertilization. A redundancy analysis also indicated that soil pH, organic matter and available phosphorus concentrations were the most important factors in shaping bacterial communities in the maize rhizosphere. Our results revealed that long-term fertilization with increasing nutrients availability increased bacterial abundance, decreased biodiversity and changed bacterial composition in the rhizosphere.

1. Introduction

The rhizosphere, a narrow zone of soil that surrounds and is influenced by plant roots, is home to an overwhelming number of microorganisms that are involved in complex biological and ecological processes, and it is considered to be one of the most dynamic interfaces on Earth (Philippot et al., 2013). Plants can shape the rhizosphere bacterial community structure by adjusting the soil pH, improving nutrition levels and reducing competition for beneficial microorganisms by secreting compounds that inhibit pathogenic associations (Bals et al., 2005; Peiffer et al., 2013). The bacteria in the rhizosphere, in turn, provide plants with mineral nutrients (Bais et al., 2006), act as protectants against phytopathogens (Innerebner et al., 2011), help plants withstand salt and heat (Zhang et al., 2008), enhance plant immune systems (Lebeis et al., 2015), and improve plant growth by producing phytohormones (Ali et al., 2009). Studies have shown that microorganisms in the rhizosphere were highly affected by environmental heterogeneity, plant species, genotype and crop management techniques, such as continuous cropping systems and fertilizer (Green et al., 2004; Berg and Smalla, 2009; Aira et al., 2011; Bai et al., 2015). Fertilization can change soil physicochemical properties and alter soil community composition and biodiversity. For example, the studies in our laboratory found that inorganic fertilization can reduce bacterial diversity and abundance (Zhou et al., 2015), while application of

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organic manure supports the development of soil microbial communities with greater biodiversity (Ding et al., 2016) in a long-term fertilized field. The microbial community in the rhizosphere is mainly derived from the surrounding bulk soil community (Yan et al., 2017). Therefore, changes in the bulk soil community are expected to have significant effects on the assembly and final composition of the rhizosphere. Thus, understanding the interaction between microorganisms and their host plants under long-term fertilization may lead to development of better fertilizer regimes and plant breeding methods for heavily fertilized farmland (Peiffer et al., 2013).

Chinese black soil, which was described as Phaeozems according to the World Reference Base (FAO) (IUSS Working Group, 2014) and belongs to the pachic Haploborolls subtype of Haploborolls in the Borolls suborder, is widespread in northeast China (Wen and Liang, 2001). The original black soils are commonly thought to be fertile and productive, even under adverse environmental conditions, because of their high organic matter (5%–8%) and clay content, cation exchange capacity, and their favorable macronutrient status (Han, 2005; Liu et al., 2015). Black soils are one of the most important resources for crop production, comprising 20% of the national arable land and being utilized for cultivation of up to 30% of the national food in China (Han et al., 2013); accordingly, they play a crucial role in ensuring national food security (Liu et al., 2017). However, over the past few decades, high doses of fertilizer have been input into agroecosystems in the pursuit of economic growth and food production, resulting in serious degradation of soil physicochemical properties (Singh et al., 2014). One of the consequences of this appears to be that soil acidification has become an increasingly serious problem in agricultural soil in China (Guo et al., 2010). Soil pH has been shown to affect bulk soil bacterial diversity and community composition in several ecosystems (Rousk et al., 2010; Zhou et al., 2015). However, it is still not clear if soil pH during long-term fertilization is the main driver of the rhizosphere bacterial community structure.

Studies of Chinese black soil conducted to date have shown that long-term application of inorganic and manure fertilizers changed soil microbial communities and biodiversity (Zhou et al., 2015; Ding et al., 2016). However, the composition of microbial communities in the rhizosphere has a greater impact on the growth and health of plants than that in bulk soil (Mendes et al., 2011; Bakker, 2012). Therefore, this study was conducted to: (i) evaluate the effects of long-term fertilization on the bacterial community diversity both in bulk soil and the rhizosphere, (ii) determine the relationships between microbial diversity, dominant groups and soil parameters associated with these changes in the rhizosphere and bulk soil. To accomplish this, we collected soil from areas subjected to 36-year fertilization regimes using inorganic and manure fertilizers in an agricultural soil in northeast China. High-throughput sequencing and quantitative PCR were then used to characterize the bacterial community in the rhizosphere and bulk soil.

2. Materials and methods

2.1. Experimental description and soil sampling

The long-term field experiment was initiated in 1980 in Harbin, Heilongjiang province, China (45°40′N, 126°35′E). The soil at the site was a clay loam, with homogenous soil properties. The basic soil properties (in 1980) were as follows: pH 7.22; organic matter (OM) 26.7 g kg^{-1}; total nitrogen (TN), 1.47 g kg^{-1}; available phosphorus (AP), 51.0 mg kg^{-1}; available potassium (AK), 200 mg kg^{-1}. The experiment had a completely randomized block design with three replications of the following treatments: (i) CK (no fertilization), (ii) N_1 (150 kg urea ha^{-1} y^{-1}), (iii) N_2 (300 kg urea ha^{-1} y^{-1}), (iv) NPK (150 kg urea plus 33 kg P plus 62 kg K ha^{-1} y^{-1}), (v) M (18,600 kg horse manure ha^{-1} y^{-1}), (vi) MNPK (M plus NPK). Fertilizer treatments were maintained in the same plot location each year. N fertilizer was applied as urea, P fertilizer as calcium superphosphate and ammonium hydrogen phosphate, K fertilizer as potassium sulfate, and manure fertilizer as horse manure. All of the fertilizers, N, P, K and manure, were applied once as a basal dressing in the autumn when the crop was harvested.

The maize was planted in rows in early April 2015 with ~170 plants in one plot (9 m × 4 m). All soils were sampled during the reproductive stage of maize in late July of 2015, when the rhizosphere effects (the phenomenon that the soil area was physically, chemically, or biologically altered by the presence of plant roots) tend to be most pronounced (Cheng et al., 2003; Ai et al., 2013). Rhizosphere soil was operationally defined as soil adhering to the total roots after gentle shaking, while bulk soil was defined as unvegetated soil adjacent to the plants (Ai et al., 2013). To obtain enough rhizosphere soil, ten plants with their roots were extracted randomly from each replicated plot with a spade. After shaking off the loosely adhered soil, the adhered soil was carefully collected and combined to form one composite sample. For the bulk soil, ten cores (6 cm in diameter) adjacent to the plants were randomly collected from the plough layer of soil (0–20 cm) in each replicate plot with a drill, and then mixed uniformly to form one composite sample. Thus, six composite samples of each treatment were collected, and pooled in six individual sterile plastic bags. A total of 36 composite fresh samples were placed immediately on ice and transported to the laboratory. All samples were passed through a 2-mm sieve to remove plant roots, and aliquots of the samples were then air dried at room temperature for chemical analysis or stored at −80 °C until molecular analysis.

2.2. Chemical analysis

Soil ammonium nitrogen (NH_4^+) and nitrate nitrogen (NO_3^-) concentrations were determined by extracting the soil with 2M KCl, steam distillation and titration (Mulvaney et al., 1996). TN was analyzed using the Kjeldahl digestion procedure (Bremner and Tabatabai, 1972). OM was oxidized by potassium dichromate and heated to ~180 °C for 5 min, and then excess potassium dichromate was determined by titration with standard 0.2 M ferrous sulfate (Strickland and Sollins, 1987). Soil pH was measured using a glass combination electrode with a 1:1 ratio of soil to water (Li et al., 2012). AP was extracted using 0.5 M sodium bicarbonate and determined colorimetrically by a molybdenum blue procedure (Olsen, 1954). AK was extracted with 1M neutral ammonium acetate and estimated with the flame photometer. Soil moisture content (SM) was determined by drying 15 g of each soil sample at 105 °C until the sample reached a consistent weight (Barrett et al., 2004).

2.3. Soil genomic DNA extraction and quantitative real-time PCR

Soil genomic DNA was extracted from 0.25 g of soil using a Power Soil DNA Isolation Kit (MOBIO Laboratories Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. Six successive extractions of microbial DNA from a replicate soil were combined to minimize the DNA extraction bias (Ding et al., 2016). The DNA was then purified with a DNeasy Tissue kit (Qiagen, Valencia, CA, USA) and checked on 1.0% agarose gel.

Quantitative real-time PCR (qPCR) was used to determine the abundance of 16S rRNA gene copy numbers in soil samples according to Zhou et al. (2015) with the 515F-806R primer set (Peiffer et al., 2013). The reaction was conducted using an ABI Real-Time 7500 system (Applied Biosystems, Waltham, MA, USA) with the following program: 95 °C for 1 min followed by 40 cycles of 94 °C for 15 s, 55 °C for 34 s and 72 °C for 15 s (Lauber et al., 2013). The standard for measuring the 16S rRNA gene quantity was developed from a clone with the correct insert. A plasmid DNA preparation was obtained from the clone using a Miniprep kit (Qiagen, Germantown, MD, USA), sequenced, and then verified using the basic local alignment search tool (BLAST) (http://