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Influence of 34-years of fertilization on bacterial communities in an intensively cultivated black soil in northeast China

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ABSTRACT

Although the effects of chemical fertilization management on microbial communities in soils have been well studied, few studies have examined such impacts of long-term chemical fertilizations on the microbial community in black soils common to northeast China. We applied high-throughput pyrosequencing and quantitative PCR of the 16S rRNA gene to investigate bacterial communities in a long-term fertilizer experiment started in 1980. The following fertilizer treatments were compared with control plots (no fertilizer): N_1 (low nitrogen fertilizer), N_2 (high nitrogen fertilizer), N_1P_1 (low nitrogen plus low phosphorus fertilizers) and N_2P_2 (high nitrogen plus high phosphorus fertilizers). All fertilization treatments resulted in decreases in soil pH and increases in wheat yield and concentrations of total nitrogen, organic matter and KCl-extractable NO_3^- and NH_4^+. Fertilization also led to a significant decrease in total 16S rRNA gene abundance and bacterial diversity. The phyla Proteobacteria, Acidobacteria and Actinobacteria dominated in all fertilized treatments. There was an increase in relative abundance of Actinobacteria, Proteobacteria, TM7 and Verrucomicrobia across all fertilized treatments compared to unfertilized controls, whereas phyla Acidobacteria and Nitrospirae decreased. The bacterial communities in unfertilized controls and lower-mineral fertilizers (i.e. N_1 and N_1P_1) were predominantly composed of Acidobacteria, Actinobacteria and Proteobacteria, and separated from the communities where more concentrated fertilizer regimes were used (i.e. N_2 and N_2P_2) based on principal coordinates analysis. Soil pH and NO_3^- concentration appeared to be the most important factors in shaping bacterial communities. Our findings suggested that long-term inorganic fertilizer regimes reduced the biodiversity and abundance of bacteria. The influence of more concentrated fertilizer treatments was greater than that of lower concentrations.

1. Introduction

The interaction between plants, soil and microorganisms is considered to be the major driver of ecosystem functions (Suleiman et al., 2013) and microorganisms respond quickly to any modification of vegetation or soil properties (Hallin et al., 2009; Zhao et al., 2014) The diversity and abundance of bacteria community in agroecosystems is critical to maintaining soil quality, productivity and ecological balance in cropland areas (C.H. Li et al., 2014; Y. Li et al., 2014). Over the past 100 years, extensive agricultural intensification has been implemented by high inputs of chemical fertilizers which, in turn, are resulting in serious degradation of soil physicochemical properties (Singh et al., 2014). The nutrient-based alteration and associated microbiota were not surprisingly reflected in significant shifts in nitrogen-fixing bacteria (Berthrong et al., 2014), ammonia-oxidizing bacteria (Ai et al., 2013), methanotrophs (Dai et al., 2013) and denitrifiers (Tang et al., 2010). Understanding the shifts in microbial community structure and composition following long-term fertilization may have significant implications for the development of better fertilizer regimes for

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agroecosystems (Shen et al., 2010). High-throughput sequencing technologies can provide significant insights into species and functional diversity of microbial populations with different fertilizer regimes (Hallin et al., 2009; Ye et al., 2013; Zhao et al., 2014). Recent results suggest that the change of potential denitrification under long-term fertilization regimes was closely related to the shift in denitrifying bacteria communities resulting from the variation of properties in black soil (Yin et al., 2015). In the long term, such modifications may result in shifts in the quality and functionality of the soils (Beauregard et al., 2010).

As different groups of microorganisms can differ in their ability to process various nutrient forms found in soil, fertilization can affect their growth competitiveness, which could make a great difference in the diversity, biomass and activity of the soil microbial community. For example, long-term N application resulted in changes in both the overall bacterial community (Rousk et al., 2010) and the composition of individual bacterial groups, such as ammonia-oxidizing bacteria, Nitrobacter-like bacteria (Wertz et al., 2012), the cellulolytic community (Fan et al., 2012), Actinobacteria (Jenkins et al., 2009) and Acidobacteria (Zhao et al., 2014). Although elevated inputs of N revealed no significant effects on bacterial diversity, the relative abundance of copiotrophic taxa (including members of the Proteobacteria and Bacteroidetes phyla) increased and oligotrophic taxa (mainly Acidobacteria) decreased in the high N plots in two long-term N addition experiments in the USA (Fierer et al., 2012). Furthermore, nitrogen induces a shift in the predominant microbial life-history strategies and inhibits soil microbial respiration rates regardless of the form of nitrogen applied (Ramirez et al., 2010).

In this study, soil samples were collected from 34-year fertilization regimes using N and P fertilizers in an agricultural soil in northeast China. Earlier studies on this site showed that long-term application of chemical fertilizers resulted in a decline in the catabolic activity of fast-growing or eutrophic bacteria as indicated by PCR-Denaturing Gradient Gel Electrophoresis methods (Wei et al., 2008). However, this technique only revealed the dominant bacteria in the soil and there is limited information on the relationship between bacterial community shift and size changes under long-term fertilizer application, as well as the interactions between environmental factors and specific groups of soil bacteria. Here, we hypothesized that the composition of soil bacterial communities would be impacted by long-term inorganic fertilizers inputs in the rich black soils common across Northeastern China; such a shift may directly or indirectly derive from increased soil NO_3^- concentration and decreased pH. Five different fertilizer treatments were employed to evaluate the soil chemical properties, bacteria community structure and their abundance in soils.

2. Materials and methods

2.1. Site description and sampling

The sampling site was located in an experimental field in Harbin city, Heilongjiang Province, China (45°40′N, 126°35′E and altitude 151 m). This region has a temperate continental monsoon climate with an average annual temperature of 3.5 °C and mean annual precipitation of 533 mm. The long-term fertilization experiment started in 1980, comprising five treatments with different applications of inorganic fertilizer: CK (without fertilizer), N_1 (150 kg N ha^{-1} y^{-1}), N_2 (300 kg N ha^{-1} y^{-1}), N_1P_1 (150 kg N plus 75 kg P_2O_5 ha^{-1} y^{-1}) and N_2P_2 (300 kg N plus 150 kg P_2O_5 ha^{-1} y^{-1}). Fertilizer treatments were maintained in the same plot location each year. N fertilizer was applied as urea while P fertilizer was as calcium super phosphate and ammonium hydrogen phosphate. Each treatment had three replicates. The wheat growing period started in early April and ended in late September and we collected soil after wheat harvest in late September 2013. Ten cores (2.5 cm in diameter) were randomly collected from the plough layer of soil (5–25 cm) in each replicate plot with an area of 9 m × 4 m for each plot. The cores from each replicate plot were mixed together, pooled in a sterile plastic bag and transported to the laboratory on ice. The samples were sieved through a 2.0-mm sieve and stored at −80 °C for further molecular analysis.

2.2. Chemical characteristics

For chemical characterization, soil samples were air dried at room temperature and sieved through a 2-mm screen. Soil pH was determined using a glass combination electrode with soil:water of 1:1 (Li et al., 2013). The OM and total nitrogen (TN) were determined according to Strickland and Sollins (1987). Soil KCl-extractable NO_3^- and NH_4^+ were determined by extraction with 2 M KCl, steam distillation and titration (Mulvaney, 1996). Available P was analyzed by resin extraction following a protocol modified from Hedley and Stewart (1982).

2.3. Total community DNA extraction

The total DNA was extracted from 0.25 g of soil using the Power Soil DNA Isolation Kit (MOBIO Laboratories Inc., Carlsbad, CA, USA) according to the manufacturer. To minimize the DNA extraction bias, three successive extractions of microbial DNA were combined and purified using a DNeasy Tissue kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. The total DNA was checked on 1.0% agarose gel and DNA concentration and quality (A260/A280) of the extracts were estimated visually using a NanoDrop ND-1000 UV–vis spectrophotometer (ThermoScientific, Rockwood, TN, USA).

2.4. 16S rRNA gene amplification and sequencing

To amplify the bacterial fragments of the appropriate size for MiSeq, primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACVSGGGTATCTAAT-3′) which target V4 hypervariable regions of bacterial 16S rRNA genes were selected (Caporaso et al., 2012). Both forward and reverse primers were tagged with adapter, pad and linker sequences. Each barcode sequence (12 mer) was added to the reverse primer for pooling multiple samples in one run of MiSeq sequencing. The PCR conditions were 94 °C for 2 min, 30 cycles of 94 °C for 30 s, 50 °C for 30 s and 72 °C for 1 min of extension, followed by 72 °C for 6 min. PCR products were purified and combined in equimolar ratios with the quantitative DNA binding method in order to create a DNA pool that was further used for sequencing from the adaptor. The 16S rRNA gene fragments were sequenced using the Illumina Miseq platform.

2.5. Quantitative PCR (qPCR) analysis

The abundances of bacterial 16S rRNA gene copies were quantified in duplicate using the ABI 7500 Real-Time PCR detection system (USA). The reaction mixture (20 μL) contained FastFire qPCR PreMix (SYBR Green) (Tiangen, China), 10 nM of each primer, ROX Reference Dye, and 1 μL of 1/10 diluted DNA. Bacterial assays used primers 515F and 806R and the following thermal 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 quantity of the 16S rRNA 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). The R^2 of the standard curve was >0.99. The qPCR reactions were run in quadruplicate with the DNA extracted from each soil sample.