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Effects of over 30-year of different fertilization regimes on fungal community compositions in the black soils of northeast China

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ABSTRACT

In this study, we investigated the effects of four long-term fertilization regimes that were performed over 30 years, namely, non-fertilization (NoF), chemical fertilization (CF), manure fertilization (M) and chemical fertilization plus manure (CFM), on a range of soil properties and fungal communities at three locations which located in the northern, middle and southern parts of the black soil region of northeast China. The fungal communities were primarily analyzed by Illumina MiSeq sequencing targeting fungal rRNA operon ITS1 region. The results showed that the fertilizers (organic or inorganic) generally increased the soil nutrient contents and fungal abundances. Principal coordinate analysis (PCoA) revealed that all fungal communities were separated into three groups according to their sampling locations, and the soil pH was the most influential factor in determining the total fungal communities across the three locations. Similar fertilization treatments had inconsistent influences on the fungal community compositions and the most influential soil factor in shaping fungal community structures varied among the three locations. Amending with inorganic fertilizers increased the relative abundances of potentially pathogenic fungi in the southern location, while the addition of manure suppressed possible pathogenic fungal growth and enhanced the growth of beneficial fungi in the three locations. Our findings highlighted that the influences of geographical separation along with fertilization regimes should be considered when examining the responses of fungal communities to fertilization regimes in agricultural management.

1. Introduction

Fertilization is an important agricultural practice. Over the long term, it can directly or indirectly affect the physical, chemical and biological properties of soils, and influences soil fertility and crop productivity (Francioli et al., 2016; Isbell et al., 2013). The soil quality was usually characterized by the activity, biomass, composition of soil microorganisms, and the microbes that are commonly considered as the key components of soil ecosystems, which play important roles in nutrient transformation, soil structure formation and the sustainability of agricultural systems (Schloter et al., 2003; Wardle et al., 1999; Zhang et al., 2015). It has been frequently reported that soil microbial communities are sensitive to the long-term application of fertilizers (Geisseler and Scow, 2014; Su et al., 2015). Thus, understanding the shifts in microbial communities under different fertilization regimes is critical for selecting optimal fertilization practices to improve soil fertility and increase crop yields (Acosta-Martinez et al., 2008; Singh et al., 2009).

Fungi are ubiquitous and critical microorganisms in soil ecosystems due to their enormous abundance and high dispersal rates (Finlay, 2002). Compared with studies on bacterial communities, fewer studies have been conducted on soil fungal communities, although soil fungi play pivotal ecological roles in soils (He et al., 2016). It is well known that different fertilization regimes over the long term will result in significant changes in the soil properties (*e.g.* the soil pH, available P and K), which is normally coupled with shifts in the microbial community structures (Klaubauf et al., 2010; Su et al., 2015; Tian et al., 2015). Among all the changed that occur in the soil parameters, the soil pH is widely accepted as the major predictor of the bacterial community, because the soil pH values normally increase with added organic

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fertilizer and decrease with N fertilizer addition (Sun et al., 2015; Tian et al., 2015; Ding et al., 2016). By contrast, several fertilization studies have indicated that changes in the soil fungal community structures were more strongly associated with the soil nutrient contents rather than the soil pH (He et al., 2016; Jirout et al., 2011). For example, He et al. (2016) reported that adding phosphorus to the soil significantly decreased the fungal species richness and shifted the fungal community composition in an alpine meadow. Wang et al. (2015) noted that N addition had no obvious relationship with the fungal diversity in lichen-dominated crusts, while fungal abundance was promoted by modest N addition but decreased with excess N. It should be noted that most studies that have been focused on investigating the responses of soil fungal communities to long-term fertilization were conducted in forest, pasture and steppe soils (Jirout et al., 2011; Ma et al., 2016; Wallenstein et al., 2006), which restricts our knowledge about the changes in soil fungal communities in agricultural systems. Recently, Zhou et al. (2016) revealed that long-term N and P fertilization decreased the fungal diversity and changed the fungal community composition in a black soil in northeast China. Sun et al. (2016) reported that the addition of organic matter significantly shifted the soil fungal community structure and reduced the relative abundance of potential pathogenic fungi in a typical lime concretion black soil. However, most results related to the changes in fungal communities as influenced by fertilization were conducted in a single location; few reports addressed in multi-locations with different soil types (Antoninka et al., 2011; Chen et al., 2016; Larkin et al., 2006). Therefore, to understand how different fertilizers impact the fungal community in arable soils, a comprehensive investigation of fungal compositions in multi-locations is needed.

Black soils, which are also named as Mollisols, are commonly recognized as inherently fertile and productive soils. Therefore, they are often extensively farmed and increasingly employed for cereal production (Liu et al., 2012). There are four large Mollisol areas distributed around the world, *i.e.*, in North America, in Russia and Ukraine, in South America and in northeast China (Liu et al., 2012). The original black soils in China are fertile, with relatively high cation exchange capacity, epipedon macroaggregate stability and high organic matter content (Xing et al., 2005). However, the productivity and fertility of the black soils have been declining during the past several decades because of extensive farming and long-term improper management practices, in which unbalanced fertilization is one of the serious problems threatening the sustainable agricultural development of this region (Guo et al., 2010; Liu et al., 2003). To date, the detailed information about the influence of long-term fertilizer regimes on fungal communities, especially on soil fungal communities across black soil regions have not been revealed. Therefore, in this study, we collected soil samples from four similar treatments in three long-term fertilization experimental stations across the black soil region of China, and the compositions of the fungal communities were evaluated by the Illumina MiSeq sequencing method. Before conducting this study, we hypothesized that nearly identical fertilizations changed the soil nutrients simultaneously, and similar fungal community compositions across locations should be observed due to their longer and stronger impacts from fertilization. Thus, our study aimed 1) to compare the effects of four fertilization regimes on the soil fungal abundance, diversity and communities in three locations; 2) to evaluate the relationships between fungal community structures and soil properties within individual location or across locations; and 3) to explore the shifts in major fungi that were induced by different fertilization regimes in the black soils.

2. Materials and methods

2.1. Experimental design and soil sampling

Soil samples were collected from three long-term fertilization experimental stations across the black soil region in northeastern China from September 2 to 5 in 2014. The three stations are located in Heihe (50° 15′ N, 127° 27′ E), Mingzhuxiang (45°50′ N, 126°51′ E) and Gongzhuling (43°31′ N, 124°48′ E) in the northern, middle and southern of the black soil region, respectively. Thus, the three sampling locations were coded as NB (north black soil), MB (middle black soil) and SB (south black soil).

The long-term field experiment in the SB was established in 1979 and has since been subjected to monoculture maize. The mean annual precipitation in this location is 530 mm and the mean annual temperature is 4.5 °C. Each treatment plot included 7 rows with 70 cm width and 18 m length, which were arranged in a randomized block design with three replicates. The long-term fertilization experimental field in the MB has been subjected to wheat-maize-soybean rotations since 1980. The mean annual precipitation of the MB is 533 mm and the mean annual temperature is 3.5 °C. Each treatment was randomly arranged into three replicates, and each replicate included 8 rows with 70 cm width and 6 m length. The experimental field under long-term fertilization in the NB has been subjected to wheat-soybean rotation since it was set up in 1979. The mean annual precipitation is 450 mm and the mean annual temperature is −1.5 °C in this location, and each treatment plot covers 220 m^2 with three replicates. The crop grown in the MB and NB in the sampling year (2014) was soybeans.

At each location, four fertilization treatments, *i.e.*, the non-fertilization (NoF), chemical fertilization (CF), manure fertilization (M) and chemical fertilization plus manure (CFM) were selected for this study (Table S1). It should be noted that the amounts of fertilizers had already been fixed on the basis of the local soil characteristics and nutrient contents since the experiments were set up. Thus, the quality and quantity of fertilizers were slightly different among the three locations, but the long-term strategies of no fertilization, single applications of inorganic and organic fertilizers, and combined applications of inorganic and organic fertilizers are suitable for the purpose of this study. 5 individual soil cores within a soil depth of 0–20 cm were randomly collected from each plot and pooled together to minimize within-plot variation. The soil was placed in an individual sterile plastic bag, which was placed inside an ice box and transported to the laboratory immediately. A total of 36 samples (4 treatments × 3 reps × 3 sites) were collected for this study. When the samples arrived to the laboratory, the soils were passed through a 2-mm sieve, and the visible roots, leaves and stones were removed. Each soil sample was divided into two parts: One was stored at −80 °C for DNA extraction, and the other was air-dried at room temperature to determine the soil chemical properties. Detailed information about the four fertilization treatments at the three locations is shown in Table S1.

2.2. Measurements of soil chemical properties

The soil pH was determined with a pH meter using a 1:2.5 (wt/vol) ratio of soil in 0.01 M $CaCl_2$ solution after shaking for 30 min. The soil total carbon (TC) and total nitrogen (TN) were measured (Jones and Willett, 2006) with an elemental analyzer (VarioEL III, Germany). The soil NH_4^+-N and NO_3^--N were extracted from 10 g of soil with 2 M KCl solution (Miranda et al., 2001). The total phosphorus (TP) and available phosphorus (AP) were determined by $HClO_4$-H_2SO_4 digestion and $NaHCO_3$ extraction methods, respectively (Adeloju et al., 1984; Olsen et al., 1954). The NH_4^+-N, NO_3^--N, TP and AP concentrations in the soil were measured with a continuous flow analytical system (SKALAR San + +, Skalar, Holland). The soil total potassium (TK) was estimated by digesting the samples with concentrated hydrofluoric acid (Jackson, 1958) and the available potassium (AK) was extracted by acetic acid and ammonium leaching method (Mehlich, 1984), and then the extracted TK and AK were quantified using inductively coupled plasma-atomic emission spectrometry (ICPS-7500, Shimadze, Japan).

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