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Soil carbon content drives the biogeographical distribution of fungal communities in the black soil zone of northeast China

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ABSTRACT

Black soils (Mollisols) are one of the most important soil resources for maintaining food security in China, and they are mainly distributed in northeast China. A previous comprehensive study revealed the biogeographical distribution patterns of bacterial communities in the black soil zone. In this study, we used the same soil samples and analyzed the 454 pyrosequencing data for the nuclear ribosomal internal transcribed spacer (ITS) region to examine the fungal communities in these black soils. A total of 220,812 fungal ITS sequences were obtained from 26 soil samples that were collected across the black soil zone. These sequences were classified into at least 5 phyla, 20 classes, greater than 70 orders and over 350 genera, suggesting a high fungal diversity across the black soils. The diversity of fungal communities and distribution of several abundant fungal taxa were significantly related to the soil carbon content. Non-metric multidimensional scaling and canonical correspondence analysis plots indicated that the fungal community composition was most strongly affected by the soil carbon content followed by soil pH. This finding differs from the bacterial community results, which indicated that soil pH was the most important edaphic factor in determining the bacterial community composition of these black soils. A variance partitioning analysis indicated that the geographic distance contributed 20% of the fungal community variation and soil environmental factors that were characterized explained approximately 35%. A pairwise analysis revealed that the diversity of the fungal community was relatively higher at lower latitudes, which is similar to the findings for the bacterial communities in the same region and suggests that a latitudinal gradient of microbial community diversity might occur in the black soil zone. By incorporating our previous findings on the bacterial communities, we can conclude that contemporary factors of soil characteristics are more important than historical factor of geographic distance in shaping the microbial community in the black soil zone of northeast China.

1. Introduction

Biogeography is the study of the distribution of biodiversity over space and time designed to reveal the habitats and abundances of organisms and determine the environmental factors that select or maintain the presence of these organisms (Martiny et al., 2006). Although the biogeographical distribution of macroorganisms, such as plants and animals, across large scales has been studied since the 18th centuries (Levin, 1992), similar studies have not been possible for soil microorganisms because of the high diversity and remarkable abundance of soil microbes as well as limitations related to inadequate sampling and investigation techniques (Ge et al., 2008). Recently, culture-independent molecular techniques, especially high-throughput amplicon sequencing, have been developed to explore bacterial (Lauber et al., 2009; Chu et al., 2010; Rousk et al., 2010; Shen et al., 2013) and fungal community compositions (Polme et al., 2013; Wu et al., 2013; Schmidt et al., 2014) in various environments.

Fungi are critical microbial components in soils that perform a range of important ecological functions, such as decomposition, parasitism, pathogenesis and symbiosis (Christensen, 1989; Buée et al., 2009). Despite their high biodiversity and critical ecological and economical roles, soil fungal communities are still poorly studied relative to soil bacterial communities (Anderson and

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Cairney, 2004; Pautasso, 2013). Soil pH is universally accepted as an overarching factor in determining the biogeographical distribution of soil bacterial communities in various studies (Fierer and Jackson, 2006; Nicol et al., 2008; Baker et al., 2009; Davis et al., 2009; Jenkins et al., 2009; Jones et al., 2009; Lauber et al., 2009; Chu et al., 2010; Shen et al., 2013), although exceptional findings have also indicated that soil parameters other than pH can have a significant influence (Hollister et al., 2010; Sul et al., 2013; Yuan et al., 2014). In contrast, there is no widely accepted conclusion related to the biogeographical distribution of soil fungal communities, and only a limited number of studies have indicated that the biogeographical distribution of soil fungal communities varies with ecosystems. For example, Peay et al. (2013) have reported that in forest ecosystems, soil fungal diversity was strongly correlated with plant diversity. Shi et al. (2014) determined that forest soil fungal community composition was influenced by temperature, latitude and plant diversity and that fungal diversity was higher in temperate forests than in boreal, subtropical and tropical forests. Lauber et al. (2008) stated that in arable soils, soil fungal community composition across land-use types was not influenced by soil pH but was closely associated with soil nutrient contents, especially the soil C:N ratio and extractable P content. A similar result was also reported by Rousk et al. (2010), who investigated fungal communities across the Hoosfield acid strip at the Rothamsted Research site and noted that the relative abundance of fungi was unaffected by soil pH and fungal diversity was only weakly related to soil pH. These findings indicate that biogeographical changes in bacterial and fungal communities are fundamentally different (Brown and Jumpponen, 2014).

Black soils, which are classified as dark Chernozems and also referred to as Mollisols, are one of the most important soil resources for crop production in China and play a crucial role in ensuring national food security (Liu et al., 2012). Black soils are primarily distributed in a long and narrow area called the black soil zone, which is approximately 900 km from the north to south and 300 km from the east to west and stretches across the three provinces of Heilongjiang, Jilin and Liaoning in northeast China. Across the black soil zone, the mean annual temperature decreases gradually from south to north, whereas the soil total C, N and P, available N and P (Zhang et al., 2007), enzyme activities and microbial biomass (Liu et al., 2008) increase from south to north. Our previous study using a 454 pyrosequencing technique revealed that the bacterial communities were geographically distributed across the black soil zone. The composition and diversity of the bacterial community were primarily affected by soil pH and soil carbon content, but the effect of soil pH was stronger than that of soil carbon content (Liu et al., 2014a). In contrast, the biogeographical distribution of fungal communities across the black soil zone was not determined. Because the physiological and ecological roles of bacteria and fungi in soils are fundamentally different (Hendrix et al., 1986; van der Wal et al., 2006; Lauber et al., 2008), we speculated that the biogeographical distribution of fungal communities in black soils would differ from that of bacterial communities and other edaphic factors rather than soil pH may primarily determine the structure and diversity of the fungal community. Therefore, in this study, the same soil samples that were used in the bacterial study were further subjected to 454 pyrosequencing of the internal transcribed spacer (ITS) region of the fungal nuclear ribosomal RNA gene with the goals of 1) assessing the fungal community composition of these black soils; 2) examining the environmental factors that are important in shaping the distribution of the fungal community composition; and 3) comparing the biogeographical distributions of fungal and bacterial communities across the black soil zone.

2. Materials and methods

The methods of soil sampling and the determinations of soil physicochemical properties and microbial biomass carbon (MBC) were described previously (Liu et al., 2014a). Soil DNA was extracted from soil samples (0.5 g wet weight) with E.Z.N.A Soil DNA Kit (OMEGA, USA) according to the manufacturer's instruction. The extracted DNA was diluted in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and stored at −20 °C until use. The locations of the sampled field sites are shown in Fig. S1. The soil physicochemical properties, pyrosequencing fungal data and qPCR assessment of fungal abundance are shown in Table 1.

2.1. Quantitative PCR analysis

The abundances of fungi were quantitatively determined by real-time PCR targeting the fungal ITS1 region of the ribosomal RNA gene using primers ITS1 and ITS2 (White et al., 1990). The standard curves were generated using 10-fold serial dilutions of a plasmid containing the ITS gene insert. Each PCR reaction contained 10 μL of SYBR Premix Ex Taq™ (Takara, Dalian, China), 0.4 μL of 10-μM forward and reverse primers (each), 7.2 μL of sterilized MilliQ water, and 2 μL of standard or extracted soil DNA. The PCR was performed in a LightCycler® 480 (Roche Applied Science) using a program of initial denaturation at 95 °C for 30 s (ramp rate of 4.4 °C/s), followed by 30 cycles of 95 °C for 5 s for denaturation, 60 °C for 30 s for annealing and elongation, and one final cycle at 50 °C for 30 s for cooling. The copy number of fungal ITS1 genes was calculated using a regression equation for converting the cycle threshold (C_t) value to the known number of copies in the standards. All of the real-time PCR reactions were run in triplicate with the DNA extracted from each soil sample.

2.2. Bar-coded pyrosequencing

The fungal community was analyzed using the ITS1/ITS4 primers to amplify the ITS1 region, 5.8S ribosomal DNA, and ITS2 region of the fungal ribosomal DNA (White et al., 1990). This region is usually subjected to pyrosequencing for environmental samples (Bates et al., 2013; Meiser et al., 2014). Briefly, an aliquot of the extracted DNA from each sample was used as a template for amplification. The ITS regions were amplified with primers ITS1 and ITS4 containing the A and B sequencing adaptors (454 Life Sciences). The sequence of the forward primer (A-ITS1) was 5′-*CCT ATC CCC TGT GTG CCT TGG CAG TCC GACT* NNN NNN NNN NN TCC GTA GGT GAA CCT GCG G-3′, and the sequence of the reverse primer (B-ITS4) was 5′-*CCA TCT CAT CCC TGC GTG TCT CCG ACG ACT* TCC TCC GCT TAT TGA TAT GC-3′. The A and B adaptor sequences are shown in italics and underlined, and the Ns represent an eleven-base sample specific barcode sequence. PCR reactions were performed in a 25 μL mixture containing 0.5 μL of each primer at 30 μmol L^{-1}, 1.5 μL of template DNA (10 ng), and 22.5 μL of Platinum PCR SuperMix (Invitrogen, Shanghai, China). The following thermal program was used for amplification: 95 °C for 2 min, 30 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, and an extension step at 72 °C for 5 min. Each sample was amplified in triplicate, and the PCR products were pooled and purified using an Agarose Gel DNA purification kit (TaKaRa, Dalian, China). An equal amount of the PCR product from each sample was combined in a single tube to be run