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High throughput sequencing analysis of biogeographical distribution of bacterial communities in the black soils of northeast China

Junjie Liu [a,1], Yueyu Sui [a,1], Zhenhua Yu [a], Yu Shi [b], Haiyan Chu [b], Jian Jin [a], Xiaobing Liu [a], Guanghua Wang [a,*]

[a] Key Laboratory of Mollisols Agroecology, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin 150081, China
[b] State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China

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ABSTRACT

Black soils (Mollisols) are one of the most important soil resources for maintaining food security of China and are mainly distributed in northeast China. To understand which environmental factors influence the microbial communities and how the communities are distributed in the black soils, we collected 26 soil samples with different soil carbon contents across the black soil zone in northeast China, and the soil bacterial community compositions were estimated using high resolution bar-coded pyrosequencing. A total of 355,813 bacterial 16S rDNA sequences were obtained, which were classified into at least 35 bacterial groups. The dominant groups across all samples (>5% of all sequences) were *Acidobacteria*, *Actinobacteria*, *Proteobacteria*, *Bacteroidetes*, *Chloroflexi*, *Gemmatimonadetes* and *Planctomycetes*. The composition and diversity of the soil bacterial community were dominantly affected by both soil pH and soil total carbon content, and the effect of soil pH was stronger than that of soil carbon content. Variance partitioning analysis showed that geographic distance contributed 14.75% of the bacterial community variation, and soil environmental factors explained approximately 37.52% of the variation. Pairwise analysis showed that a relatively higher diversity of the bacterial community was observed at lower latitudes, suggesting that a latitudinal diversity gradient of the bacterial community might be present in the black soil zone. In general, our results indicated that contemporary factors, such as soil pH and soil carbon content, were more important than the historical factor of geographic distance in shaping the bacterial community in the black soil zone in northeast China.

1. Introduction

The biogeographical distributions of macroorganisms, such as plants and animals, across large scales have been studied for centuries (Levin, 1992). In contrast, similar studies of microorganisms are still limited, although the several efforts to discover the microbial biogeography have been conducted recently (Fierer and Jackson, 2006; Ge et al., 2008; Lauber et al., 2009; Chu et al., 2010). It is known that soil microbial community diversity and composition vary distinctly between different soil ecosystems, and this variation is thought to be related to the changes in a number of soil biotic and abiotic factors (Garbeva et al., 2004; Ramette and Tiedje, 2007; Green et al., 2008). Among these factors, soil pH is often observed as an overarching factor in the dominating overall soil bacterial community (Fierer and Jackson, 2006; Baker et al., 2009; Lauber et al., 2009) and the composition of individual bacterial groups (Nicol et al., 2008; Davis et al., 2009; Jenkins et al., 2009; Jones et al., 2009). However, as stated by Rousk et al. (2010), one limitation of these studies is that we do not know whether pH itself is the factor directly shaping bacterial communities or if it is indirectly integrated with many environmental factors, such as soil nutrition, soil available organic carbon, and vegetation type, in changing the soil bacterial community. Therefore, due to the paucity of the detailed and comprehensive studies of soil bacterial biogeography, particularly across larger spatial scales, our understanding of soil microbial biogeography remains incomplete (Lauber et al., 2009).

Black soils, classified as Dark Chernozemes, also named as Mollisols, are one of the most important soil resources for crop production in China and play a crucial role in food security (Liu et al., 2012). The black soils are primarily distributed in a long and narrow area called the black soil zone, which is approximately 900 km from north to south and 300 km from east to west, in three

* Corresponding author. Tel.: +86 451 86602745; fax: +86 451 86603736.
E-mail addresses: wanggh@neigaehrb.ac.cn, guanghuawang@hotmail.com (G. Wang).
[1] Junjie Liu and Yueyu Sui make an equal contribution to this paper.

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provinces of Heilongjiang, Jilin and Liaoning in northeast China. In the black soil zone, the annual average temperature decreases gradually from south to north, while soil total C, N and P, available N and P (Zhang et al., 2007), soil enzyme activities and microbial biomass (Liu et al., 2008) increase from south to north. Because soil microbes play important roles in nutrient cycling (Ingham et al., 1985), we hypothesized that the soil microbial communities in the black soil zone would also be geographically distributed.

The formation of the microbial biogeographical distribution patterns is determined by two general factors, environmental heterogeneity (contemporary factor) and dispersal limitation (historical factor) (Fierer, 2008). Environmental heterogeneity drives biogeographic patterns by the commonly known hypothesis "everything is everywhere, but the environment selects" (Baas Becking, 1934; De Wit and Bouvier, 2006), suggesting the microbial species are widely distributed and the microbial community compositions are governed by ecological Drift and Selection (Stegan et al., 2013). In contrast, when dispersal limitation is the dominating factor driving the biogeographical patterns, the geographic distance should be the best predictor of genetic divergence between the communities (Fierer, 2008). In a previous study using the polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) method, we preliminary discovered that soil microbial communities in the black soil zone were more similar to their neighboring locations than those at greater distances (Mi et al., 2012). However, the four soil sampling sites and the limited resolution of DGGE fingerprinting that was used in that study were not able to assess the microbial biogeographical distributions and identify which environmental factors exerted the strongest influence on these distributions. Therefore, more sampling sites and a higher resolution technique are essential to study the biogeographical distribution of microbial community in the black soils of northeast China.

Recently, bar-coded pyrosequencing was prevalently used in the analysis of microbial community compositions in broad and fine scales (Acosta-Martínez et al., 2008; Lauber et al., 2009; Chu et al., 2010; Rousk et al., 2010; Xiong et al., 2012). This method can generate one million bp reads with an average length of over 400 bp and can provide a much more detailed description of the microbial communities than traditional methods, such as cloning library, DGGE, PLFA, etc; as such, this method has been shown to be a very powerful technique in microbial ecology research (Roesch et al., 2007). In this study, using the bar-coded pyrosequencing technique, we examined the structure and diversity of the bacterial communities in 26 soils from the black soil zone of northeast China. The objectives of this study were 1) to reveal the bacterial community compositions of the black soils; 2) to examine which environmental factors were important in shaping the distribution of the bacterial community structures; and 3) to compare the differences and similarities of the bacterial communities in 26 black soils.

2. Materials and methods

2.1. Site selection and soil sampling

The soil nutrition, especially soil total C content, gradually changes along the black soil zone of northeast China (Zhang et al., 2007). In this study, based on the database of China Black Soil Ecology (http://www.blackland.csdb.cn), we purposely collected 26 farmland soil samples with different soil carbon contents across a wide range of the black soil zone of northeast China from September 24 to 29, 2012. Briefly, all soils were collected near the maturity growth stage of soybean or maize. At each site, the soil samples were randomly collected from 10 tillage layers (0–20 cm) within an area of approximately 100 m^2. The soil samples were composited and sieved through a 2-mm mesh to thoroughly homogenize and remove the roots, plant residues and stones. A portion of each soil sample was collected in a 50-mL centrifuge tube, placed in an ice-box and transferred to the laboratory. The tubes were archived at −80 °C until soil DNA extraction. The remaining soils were used to measure the microbial biomass (on field-moist soil) and were then air-dried to determine the soil physicochemical properties. The locations of the field sites and their soil properties are shown in Fig. S1 and Table 1, respectively.

2.2. Soil physicochemical property and microbial biomass determination

Soil pH was measured using a pH meter after shaking the soil water (1:5 *w*/*v*) suspension for 30 min. Soil moisture was measured gravimetrically. The soil total carbon (TC) and total nitrogen (TN) were determined using an Elemental analyzer (VarioEL III, Germany). The soil microbial biomass carbon (MBC) was estimated using the chloroform-fumigation-extraction methods, and the organic C in the extracts was determined using an automated TOC Analyzer (Shimadzu, TOC-V_{CPH}, Japan); a K_{EC} of 0.45 was used to convert the difference between the organic C extracted with 0.5 M K_2SO_4 from the chloroform fumigated and unfumigated soil samples (Vance et al., 1987).

2.3. Soil DNA extraction

DNA was extracted from the soil samples (0.5 g wet weight) with E.Z.N.A Soil DNA (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.

2.4. Bar-coded pyrosequencing

An aliquot of the extracted DNA from each sample was used as a template for amplification. The V1–V3 hypervariable regions of the bacterial 16S rRNA gene were amplified with primers 27F and 533R containing the A and B sequencing adapters (454 Life Sciences). The sequence of the forward primer (B-27F) was 5′-*CCT ATC CCC TGT GTG CCT TGG CAG TCC GACT* AGA GTT TGA TCC TGG CTC AG-3′, and the sequence of the reverse primer (A-533R) was 5′-*CCA TCT CAT CCC TGC GTG TCT CCG ACG ACT* NNN NNN NNN NNN TTA CCG CGG CTG CTG GCAC-3′. The sequences of the A and B adapters are shown in italics and are underlined, and the Ns represent a twelve-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}, 1.0–1.5 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: 94 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, followed by an extension at 72 °C for 10 min. Each sample was amplified in triplicate, and the PCR products were pooled and purified using the Agarose Gel DNA purification kit (TaKaRa). An equal amount of the PCR product from each sample was combined in a single tube to be run on a Roche FLX 454 pyrosequencing machine at Majorbio BioPharm Technology Co., Ltd., Shanghai, China.

2.5. Processing the pyrosequencing data

Considering the sources of errors described in previous studies that used 454 sequencing (Dohm et al., 2008), in this study, only sequences >200 bp in length, with an average quality score >25, without ambiguous base calls and with at least an 80% match to a