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Rhizosphere-associated bacterial network structure and spatial distribution differ significantly from bulk soil in wheat crop fields

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ABSTRACT

The spatial distribution of bacteria in bulk soil has been well studied, but little is known about the bacterial biogeography in the rhizosphere of crops. Here, we investigated bacterial distribution in bulk soil, loosely- and tightly-bound soils, from wheat fields distributed across 800,000 km^2 of the North China Plain. Bacterial community composition differed dramatically among bulk and rhizospheric soils, and bacterial diversity decreased with the root proximity. Soil pH correlated with bacterial community composition and diversity in three compartments. Bacterial community in tightly bound soil formed a hub-based network topology with higher transitivity and greater number of central nodes compared with loosely bound and bulk soils, potentially as a result of more direct ecological interactions between the members of the tightly bound soil compartment. Bulk and rhizospheric soils maintained similar compositional distance decay patterns (with equal decay rates), but distinct phylogenetic distance decay patterns (with steeper slope of tightly bound soil). Geographical distance described a relatively greater proportion of bacterial spatial distribution in tightly bound soil, compared with loosely bound soil and bulk soil. Deterministic processes dominated the assemblage of bacterial communities in all soil compartments, while phylogenetic clustering was weaker in tightly bound soil. Taken together, our results suggest distinct bacterial network structure and distribution patterns among bulk soil, loosely bound soil and tightly bound rhizospheric soil, which could possibly result in potential functional differentiation.

1. Introduction

Soil bacterial distribution has been widely investigated in natural ecosystems (Fierer and Jackson, 2006) and agricultural ecosystem (Carlson et al., 2012; Gumiere et al., 2016), however, bacterial biogeographic patterns in the rhizosphere of crop systems remain relatively unexplored. Soil pH correlates with bacterial distribution in many ecosystems, including forests (Nacke et al., 2011), grasslands (Will et al., 2010) and tundra (Chu et al., 2010; Shen et al., 2013). However, soil carbon content (Chu et al., 2016), soil carbon to nitrogen ratio (Högberg et al., 2007) and temperature (Zhou et al., 2016) also correlate with the distribution of bacterial communities in certain ecosystems. These differences may be in part due to the different study designs and methods used to generate the microbial profiles, and historical contingencies or spatial distances have also been shown to influence the distribution of microbial community at regional (Griffiths et al., 2011), continental (Martiny et al., 2011), and global (Caruso et al., 2011) scales. Agricultural ecosystems are often more homogeneous across spatial scales, with lower plant diversity and frequent human disturbance when compared to natural environments (Kennedy and Smith, 1995). While soil pH has been identified as a key factor correlating with microbial communities in agricultural soils in

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China (Liu et al., 2014, 2016), the type of history of agricultural activity for a given site has been shown to describe a greater degree of variance in microbial distribution when compared to pH (Ge et al., 2008). Importantly, while the biogeography of bulk agricultural soil has been extensively investigated (Ge et al., 2008; Liu et al., 2014), the factors driving bacterial community distribution patterns in rhizospheric soil remain relatively unknown (Bulgarelli et al., 2015).

The rhizosphere is a biological hotspot whose physicochemical properties differ substantially from the surrounding bulk soil. Root exudation of low-molecular-weight organic acids can fundamentally alter the biogeochemistry of this environment (Wang et al., 2002). It is hypothesized that plants secrete these organic compounds to support microbial activity near the roots, which, in turn, can provide beneficial services to the plant, including enhanced mineral acquisition and pathogen protection (Dennis et al., 2010; Turner et al., 2013). Bacterial community composition has been shown to differ substantially among different rhizosphere compartments, with the diversity of these communities decreasing from the bulk soil towards the root (Donn et al., 2015). A similar pattern has also been observed in rice, which supports a multistep model for root microbiome assembly from soil (Edwards et al., 2015). In grapevines, the root and plant-tissue associated communities have been shown to be a subset of the bulk soil community (Zarraonaindia et al., 2015). A less complex network structure has also been found in rhizosphere when comparing with bulk soil in a short-term plantation system (Mendes et al., 2014). Most of these studies were limited to a small number of spatially proximal sites, or confined to a greenhouse or short-term plantation systems; therefore, the structures of root-associated compartments in typical farmland remain unclear.

Although the properties of the rhizosphere soil are modified by a range of processes occurring during plant growth (Philippot et al., 2013), distance decay relationships can still capture the variance in bacterial community composition and phylogeny across spatial scales. Microbial community structure is shaped mainly by deterministic factors (such as competition, niche differentiation) and neutral processes (Ofiţeru et al., 2010). A recent short-term (1–5 years) soybean cultivation study demonstrated that microbial community selection in the rhizosphere occurred via niche filtering, while the bulk soil composition and structure seemed to be regulated by neutral processes (Mendes et al., 2014). Some studies have shown that both deterministic and neutral processes operate in structuring the same microbial community (Dumbrell et al., 2010; Caruso et al., 2011; Ferrenberg et al., 2013).

In this study, we combined bulk and rhizospheric soils to investigate bacterial distribution patterns in agricultural ecosystem. We further collected the soils from three compartments namely bulk soil, loosely bound soil and tightly bound soil according to the root proximity, and tightly bound soil was always regarded as rhizosphere soil (Donn et al., 2015). The sampling sites were located in wheat fields on the North China Plain, which is an important agricultural area in China, with a long-term (about 40 years) wheat-maize rotation system (Chen et al., 2004). Wheat (*Triticumaestivum* L.) is one of the main grain crops globally, but productivity increases per year have slowed to 0.9% (Fischer and Edmeades, 2010). It is possible that targeted manipulation of soil processes, including those involving microbial biogeochemistry, could help to accelerate crop productivity again. The rhizosphere is intriguingly complex and dynamic (Philippot et al., 2013), and understanding the microbial distribution patterns therein is key to developing strategies for enhancing plant productivity and ecosystem function (Brink, 2016). We addressed three hypotheses in the current study. First, the physicochemical properties that correlate with bacterial community structure are different in different compartments. Second, the network co-association properties within bacterial communities differ significantly between soil compartments. Third, the biogeographic distribution patterns across spatial distance (compositional and phylogenetic distance decay) and processes of bacterial community assembly vary among soil compartments.

2. Materials and methods

2.1. Field survey and sample collection

Wheat field survey data of North China Plain were collected from the Chinese agriculture web system (http://english.agri.gov.cn/). We also used GIS (Geographic Information System) map to choose the sampling sites under same wheat-maize rotation system, and determine plot locations based on the following approach. Nine study sites, each ~100 km^2 square region, were identified across typical wheat planting fields over a broad area (~800,000 km^2) (32°N~38°N; 110°E~118°E) of the North China Plain (Fig. S1A) during the wheat filling stage (Soils from the wheat field in the nine sites were collected on 22nd–28th of the May, 2015). Within each of the nine sampling sites, five locations (one in each corner and one in the center) were chosen for repeat sampling (Fig. S1B). At each of the five repeat locations, six groups of plants (four to six plants in each group) were extracted by digging around the groups to collect loosely bound soil and tightly bound soil (Donn et al., 2015). Plants were lightly shaken by holding with shoots to get the loosely bound soil and then soils remain attached to the root surface were brushed down as tightly bound soil which was defined as rhizosphere soil (Philippot et al., 2013). The topsoil (0–15 cm), beside each group ~50 cm without plants, were collected by drill as bulk soil (BS). At each sampling time, the drill and brush were washed with sterile water and then air dried. Samples at each of the five repeat locations were pooled into a single composite sample for BS, LS and TS, respectively. All the samples were packed into polyethylene bags and immediately transported on ice packs to the laboratory. The soils were sieved through 2 mm meshes to remove visible roots, residues and stones. Each composite sample was then divided into two parts: one was stored at 4 °C for the soil biogeochemical properties analyses and the other one was stored at −20 °C for DNA extraction within two weeks.

2.2. Soil chemical analysis

There were 135 samples in total: 45 samples for each compartment (bulk soil, loosely bound soil and tightly bound soil). The soil type of most sampling locations were Typic Ochri-Aquic Cambosols and Typic Hapli-Udic Argosols; sandy loam soil and clay loam soil dominated the soil texture (Table S1). For each soil sample, soil moisture was measured for 12 times using QS-SFY, and soil pH was determined with a fresh soil to water ratio of 1:5 using a pH monitor (Thermo Orion-868). Soil was air dried and sieved (1 mm mesh), and total carbon (TC), total nitrogen (TN), total phosphorus (TP) and total potassium (TK) were determined by combustion (CNS-2000; LECO, St. Joseph, MI, USA).

2.3. PCR amplification and high throughput sequencing

A total of 0.5 g of fresh soil was used for DNA extraction using the Power Soil DNA kit (MO BIO Laboratories, Carlsbad, CA, USA) following the manufacturer's instructions. Primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 907R (5′-CCGTCAATTCCTTTGAGTTT-3′) (Biddle et al., 2008), which targeting the bacterial 16S rRNA V4-V5 region, were used to amplify the 16S rRNA gene