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Bacterial communities incorporating plant-derived carbon in the soybean rhizosphere in Mollisols that differ in soil organic carbon content

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ABSTRACT

A primary factor regulating the composition of the microbial community in the rhizosphere is carbon (C) efflux from root systems, which fundamentally influences microbial functions in the rhizosphere, such as biodegradation, plant growth, and rhizosphere signalling. However, information regarding the incorporation of plant-C by the bacterial community in the rhizosphere is limited, particularly in soybean. Soybean plants were grown in rhizo-boxes containing low- or high-organic C (C_{org}) Mollisols and labelled with $^{13}CO_2$ at the flowering stage. After soil DNA was extracted from the rhizosphere, ^{13}C-DNA was separated from ^{12}C-DNA using the stable isotope probing method, followed by pyrosequencing analysis. Between soils, significant differences in the abundance of genera incorporating ^{13}C in the rhizosphere were observed, with *Aquincola*, *Dechloromonas*, *Massilia*, *Amycolatopsis*, *Delftia*, *Magnetospirillum*, *Psychrobacter*, *Ochrobactrum*, *Pseudoxanthomonas* and *Niastella* showing greater relative abundances in low-C_{org} soil ($p < 0.05$) compared to high-C_{org} soil. However, the opposite trend was observed for *Enhydrobacter*, *Flavisolibacter*, *Propionibacterium* and *Staphylococcus*. Correspondingly, the number of operational taxonomic units in each genus varied between soils. Soil type greatly affected the flow of plant-C into rhizospheric bacterial community. The plant-C metabolizing bacteria may contribute to the transformation of rhizodeposits in the soil and soil C sequestration.

1. Introduction

The rhizosphere is the interface between plant roots and soil, where plant-microorganism interactions occur (Haase et al., 2008; Philippot et al., 2013). In the rhizosphere, microbial biomass and activity are generally enhanced because compounds are extruded from roots (Sorensen et al., 1996; Raaijmakers et al., 2009). Plant growth-promoting rhizobacteria are examples of rhizosphere microbes that can affect plant growth and fitness (Pil et al., 2015).

The composition of the microbial community in the rhizosphere is specifically affected by the plant species and soil type present (Berg and Smalla, 2009). A number of studies demonstrated the role of plant species in shaping the microbial community in the rhizosphere, such as *Arabidopsis* (Lundberg et al., 2012), rice (Knief et al., 2011), wheat (Donn et al., 2015), and maize (Li et al., 2014). Variations in the chemical composition and quantity of root exudates between plants affect the relative abundance of microorganisms in the rhizosphere (Somers et al., 2004). Particularly, in soybean, the microbial community composition in the rhizosphere differs from that of non-legume species because of its strong ability to form symbiotic relationships with rhizobia and a large amount of protons released into the rhizosphere during N_2 fixation (Tang and Rengel, 2003; Xu et al., 2009; Sugiyama et al., 2014). In addition, soil type strongly influences the composition of the microbial community of the rhizosphere soil (Yang and Crowley, 2000; de Ridder-Duine et al., 2005). For *Carex arenaria* grown at 10 natural sites, the bacterial community structure in the rhizosphere appeared to be largely determined by the bulk soil community composition (de Ridder-Duine et al., 2005). This indicates that indigenous microorganisms likely have measurable effects on the microbial community in the rhizosphere (Shi et al., 2015; Mi et al., 2012). However, little is known regarding how plants select a specific rhizosphere community from the reservoirs of bulk soil populations when plants are grown in different soils.

Compared to bulk soil, in the rhizosphere, the spatial differences in the composition of the microbial community are largely attributed to carbon (C) efflux from roots. Among factors such as pH and nutrients

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regulating the bacterial community in the rhizosphere, C is the most important factor as the soil microbial activity in all soils is limited by labile C and energy (Schimel and Weintraub, 2003; Kuzyakov and Blagodatskaya, 2015). Approximately 12–50% of net photosynthetically fixed C in annual plants is released into the soil (Hütsch et al., 2002). These C compounds such as organic acids, sugars, aliphatics and aromatics, boost the abundance and activity of microorganisms in soil, particularly copiotrophic bacteria (Uren, 2000; Ramirez-Villanueva et al., 2015). The active microbial biomass is 4–20 times greater than that in bulk soil (Blagodatskaya et al., 2009). More rapid microbial responses to C input in the rhizosphere contribute to decomposition and mineralization of rhizodeposits and associated release of ammonium (NH_4^+) and transformation of phosphorus (Jin et al., 2013a; Kuzyakov and Blagodatskaya, 2015). Thus, plant-microorganism interactions in the rhizosphere are very important for soil C sequestration and nutrient cycling in natural ecosystems and agricultural systems (Singh et al., 2004). To understand these interactions, the flow of plant-C to specific microorganisms in the rhizosphere must be determined. However, studies of this topic are limited (Olsson and Johnson, 2005; Strand et al., 2008; Drigo et al., 2010).

^{13}C stable isotope probing (SIP) has been applied to track plant-C fluxes into microbial nucleic acids, and highlight the active microbes that assimilate specific C substrates (Dunford and Neufeld, 2010). This method is important for assigning metabolic functions to diverse communities inhabiting rhizosphere environments. Using $^{13}CO_2$ pulse-labelling on *Trifolium repens* and *Agrostis capillaris*, Vandenkoornhuyse et al. (2007) demonstrated that Burkholderiales affiliated to Proteobacteria were a major microbial group metabolizing root exudates, which encompass known symbionts. However, in crop species such as soybean, the soil microbes that are active and major direct utilisers of photosynthetic C have not been fully identified.

Previous studies of the microbial community in the rhizosphere of soybean investigated the responses of the microbial community to soil type, soybean genotype and growth stage at the phylum level by employing techniques such as denaturing gradient gel electrophoresis and sequencing of 16S rDNA from clone libraries (Xu et al., 2009; Mi et al., 2012). They found that the dominant phyla such as Proteobacteria, Actinobacteria, Bacteroidetes, Nitrospirae, Firmicutes, Verrucomicrobia and Acidobacteria, inhabited in the rhizosphere of soybean, and the abundances of these phyla differed between Mollisols and Alfisols. Recently, SIP combined with the high-throughput sequencing (e.g. Illumina pyrosequencing) of 16S rRNA gene amplicons allows us to evaluate the incorporation of plant-C in microbial communities at the family, genus and operational taxonomic unit (OTU) levels (Drigo et al., 2010; Caporaso et al., 2011; Zhou et al., 2011; Shi et al., 2015) to more accurately predict microbial function in plant-C transformations and specify the relevant eco-functions in the rhizosphere. Such investigations are very important for soil organic C (C_{org}) and crop managements in soybean growing regions.

The major farming region in northeast China is the world's third largest contiguous body of Mollisols where soybean is widely grown (Sui et al., 2009). The content of soil C_{org} greatly differs among different regions of Mollisols, ranging from 0.95% to 5.81%. Moreover, the contribution of photosynthetic C of soybean to soil C_{org} shows geographic variation in Mollisols (Jin et al., 2013b). The different contribution between soils is likely attributed to how the microbes process rhizodeposits. Liu et al. (2014) found that phylogenetic diversity of microbes was significantly associated with soil C_{org} (Liu et al., 2014), and thus soil C_{org} may markedly influence the microbial community metabolizing plant-C in the rhizosphere. Therefore, the aims of this study were to identify the bacterial community in the rhizosphere that incorporated soybean plant-C and to compare the rhizospheric communities in Mollisols differing in C_{org} content. We hypothesised that the bacterial community incorporating plant-C would be preferential in copiotrophic bacteria in low-C_{org} soil compared to high-C_{org} soil. The copiotrophic bacteria may be associated with fast decomposition of rhizodeposits in this soil compared to high-C_{org} soil, as rhizodeposits in low-C_{org} soil was more accessible by microbes due to the specific physiochemical properties in this soil (Jin et al., 2013b).

2. Materials and methods

2.1. Soils and plant materials

Soils were collected in October 2012 from the tillage layer down to a depth of 10 cm at two farming paddocks located in Lishu County in Jilin Province and Beian County in Heilongjiang Province in Northeast China (Table 1). The distance between the two sites is approximately 750 km. The two sites were selected because our previous studies showed that the fate of plant-C and microbial community differed between the two soils (Mi et al., 2011; Jin et al., 2013b). Thirty cores were collected (approximately 6 kg of soil) from each location, which were bulked and sieved through a 4-mm sieve. The soils were classified as Mollisols (USDA soil taxonomy); the chemical and textural characteristics are shown in Table 1. Soil pH was determined using a Thermo Orion 720 pH meter in H_2O (1:5 = w:v). Total soil C and nitrogen (N) were measured using an Elemental III analyser (Hanau, Germany). Labile N was determined using an alkaline hydrolysis method (Cornfield, 1960). To measure total phosphorus (P) and potassium (K), 0.5 g of soil was digested with a mixture of H_2SO_4 and $HClO_4$ (20:1). Labile P and K in the soil was extracted with 0.5 M $NaHCO_3$ (1:30 = w:v) and 1 M CH_3COONH_4 (1:10 = w:v), respectively (Olsen et al., 1954; Wang et al., 2016). Phosphorus and K in extracts were determined by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICPS-7500, Shimadzu, Kyoto, Japan). The C_{org} contents were 1.0% (low-C_{org}) and 5.1% (high-C_{org}) for the two soils, respectively (Table 1). The soils were watered to 50% field capacity and pre-incubated at 25 °C for 15 days to recover microbial activity and function.

The soybean (*Glycine max* L. Merr.) cultivar Suinong 14 (Maturity Group 0) was used in this study. This cultivar is widely grown over 2 million ha, with a total grain yield of 937 million kg in Northeast China since its release in 1996 (Qin et al., 2010).

2.2. Experimental set-up

A rhizo-box experiment was performed at the glasshouse at the Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin, China. The experiment was a completely randomized block design with the two Mollisols as treatments. There were 8 rhizo-boxes in each soil treatment, of which half were for $^{13}CO_2$ labelling and the others were non-labelled controls. The rhizo-box was established as reported by Jin et al. (2013a). Briefly, the Perspex-made rhizo-box (100 mm wide × 150 mm high × 10 mm thick) was filled with 100 g of sieved soil and placed upright on top of a pot containing 2.5 kg of sterilized sand that supplied water to the plants. Basal nutrients were applied at the following rates (mg kg^{-1}): 217, urea; 219, KH_2PO_4; 167, $CaCl_2{\cdot}2H_2O$; 43, $MgSO_4{\cdot}7H_2O$; 9, Fe-EDTA; 6, $ZnSO_4$; 5, $CuSO_4$; 0.7, H_3BO_3; 6.7, $MnSO_4{\cdot}H_2O$; 0.3, $CoSO_4{\cdot}7H_2O$; and 0.2, $Na_2MoO_4{\cdot}2H_2O$. Nutrients were thoroughly mixed with the soil. Five seeds of uniform size were sown into each rhizo-box and were thinned to two on the 10th day after sowing. The greenhouse had a night-time temperature range of 16–20 °C and a daytime temperature range of 24–28 °C. The soil water content was maintained at 80 ± 5% of the field water capacity.

2.3. $^{13}CO_2$ labelling

Plants at the initial flowering stage (R1) were labelled with $^{13}CO_2$ for 10 days before harvest to ensure that most of plant-C metabolizing bacteria in the rhizosphere were labelled (Lu and Conrad, 2005). Four rhizo-boxes from each soil type were randomly selected and subjected to $^{13}CO_2$ labelling. On the labelling day, plants together with rhizo-boxes were transferred into an airtight glass chamber (area