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Distinct rhizosphere effect on active and total bacterial communities in paddy soils

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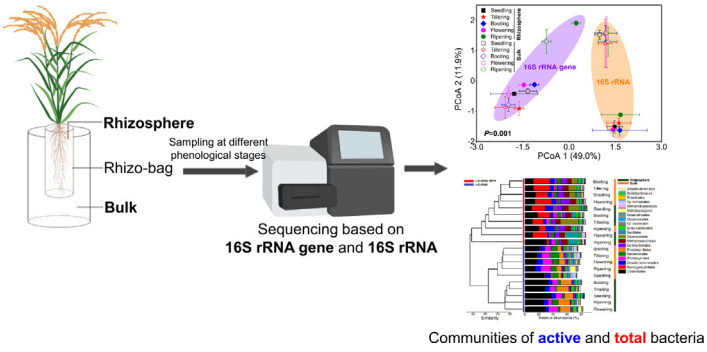
HIGHLIGHTS

- Distinct differences between active and total microbial communities were determined.
- Microbial communities based on 16S rRNA were more accurate.
- Soil compartment contributed more to active microbial communities compared to stages.
- Rhizosphere effect affected active and total microbial communities distinctly.

GRAPHICAL ABSTRACT



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ABSTRACT

Rhizosphere microbes are critical for plant health and biogeochemical cycles. Understanding the diversity of active microorganisms in the rhizosphere is key to enhancing plant growth and productivity. We examined rhizosphere bacterial communities of rice by comparison of the 16S ribosomal subunit amplicons generated from both the total (DNA-based, 16S rRNA gene) and the active (RNA-based, 16S rRNA) soil microbiota. Analysis based on the 16S rRNA gene showed a higher microbial diversity, but with little change in bacterial populations across the growth stages of the plant. Analysis of 16S rRNA recovered much less diversity, demonstrating that much of the 16S signal was derived from free DNA, dead or inactive cells. The rRNA analysis showed a stable microbial population present in the rhizosphere, and this was distinct from that in the bulk soil, which was also stable across the growth period. Root exudates (e.g., acetate, lactate, oxalate and succinate), which are major components contributing to the rhizosphere effect, appeared to shape the bacterial community, with some taxa (e.g., *Oxobacter*, Lachnospiraceae, *Coprococcus* and α-Proteobacteria) being enhanced in the rhizosphere. Soil compartments (rhizosphere vs. bulk) had a greater effect on the bacterial communities than did the plant phenological stages, especially at the rRNA level. These results suggest that the rhizosphere effect plays a key role in structuring the bacterial communities in rhizosphere soils with a distinct effect on active and total bacterial communities.

1. Introduction

Rhizosphere microbes, which are considered to be part of the second genome of plants, play a fundamental role in plant growth and health (Berendsen et al., 2012). The rhizosphere microbiomes' effect on plant

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health is similar to those of microbial communities in the gut on human health (Berendsen et al., 2012). Rhizosphere microorganisms modulate biogeochemical cycles in soils by affecting the rhizosphere processes such as denitrification, nitrification, and respiration (Breidenbach et al., 2016; Philippot et al., 2013). In paddy soils for example, potential iron reducers (e.g., *Geobacter*, *Anaeromyxobacter*) in the rhizosphere environment has been shown to be notably enriched, suggesting that rhizo-microbes affect the iron cycles in the soils significantly (Breidenbach et al., 2016; Zhou et al., 2016). Carrillo et al. (2017) have demonstrated that microbes in rhizosphere soils are important drivers of soil organic matter (SOM) decomposition in a temperate grassland. Beneficial microorganisms in the rhizosphere can enhance growth of plants by enhancing uptake of water, nutrients (Liu et al., 2016), protection from excess heavy metals (Seneviratne et al., 2017), and by promoting induced systemic resistance of plants to many plant pathogens (Kumar et al., 2016).

The rhizosphere bacterial assemblage is taxonomically diverse but redundant, which are essential for maintaining a microbial diversity and community resilience in soil (Berendsen et al., 2012; Egamberdieva et al., 2008). However much still remains unknown about the rhizosphere microbiota, and especially the active bacterial communities and their function for specific processes and turnover of elements. Microorganisms are present in soil at four physiological states, namely active, potentially active, dormant and dead state (Johnsen et al., 2001). The active microorganisms, representing only a fraction of the total bacterial community, participate directly in the ongoing utilization of substrates and biochemical transformations (Blazewicz et al., 2013) and act as primary drivers in soil biogeochemical processes (Blagodatskaya and Kuzyakov, 2013). Consequently, all processes in different ecosystems can be related to the abundance and/or composition of active microorganisms. Investigation of active microorganisms would provide more accurate information about the functional microorganisms in the soil microbiome. However, due to difficulties in extracting RNA from environmental samples, methods such as 16S rRNA gene-based sequencing still remains popular but obtains many redundant information and may miss some important functional microbiota when exploring microbial communities (Kim et al., 2013). Comparison of the active and the total bacterial community could provide important opportunities to better understand the responses of bacteria to altered environmental factors. Previous studies (Ragot et al., 2016; Zhou et al., 2014) have indicated that there are significant differences between the total and the active microbial communities. The microbial community structure changes in relation to the oxygen gradient in unplanted flooded paddy soil microcosms, and the temporal dynamics of the microbial composition can be observed more clearly based on 16S rRNA sequencing compared with 16S rRNA gene sequencing (Noll et al., 2005).

Rhizosphere microbial compositions shows significant differences between plant genotypes (Edwards et al., 2015; Wagner et al., 2016), plant species (Tkacz et al., 2015), soil types (Tkacz et al., 2015), and depend on the phenological stages of plant growth (Breidenbach et al., 2016; Chaparro et al., 2014; Li et al., 2016). Flooded bulk soils with rice are oxygen limited, however, the rhizosphere of the rice is an oxic zone compared to the bulk soil due to the oxygen released by the rice roots, which creates a continual redox and nutrient gradient extending from the root surface to the bulk soils. A recent study has demonstrated that the microbial community in rice rhizospheres changes when it is enriched with bacteria beneficial for plant growth during a long-term green manure amended experiment (Zhang et al., 2017). The abundance and diversity of root exudates, which depend on plant development (Li et al., 2016), affect the redox and nutrient gradients in soils thereby impacting the soil microbiota. Previous studies have reported that the bacterial activity and community involved in biochemical processes are affected by root exudates (Baudoin et al., 2003; Li et al., 2016; Meier et al., 2017). Furthermore, soil compartments (rhizosphere vs. bulk soil) with larger variations in concentration and composition of root exudates (Yang et al., 2015) have a stronger effect on the microbial community than time (Breidenbach et al., 2016). However, only limited studies have focused on exploring the active bacterial community in rice rhizosphere as well as delineating the factors contributing to the dynamics of the active bacterial community.

Although many studies have investigated the microbial community compositions and structures in rhizosphere soils by using the advantage of high-throughput sequencing, comparisons between active and total soil microbes are limited. In this study, we aimed to characterize the differences between the total and the active bacterial community in rice soil. This was done by comparing the 16S ribosomal subunit amplicons generated from both 16S rRNA gene (DNA-based) and 16S rRNA (RNA-based) soil bacterial communities. The phenological growth stage of rice may affect the bacterial community due to changes in soil or pore-water chemical properties as well as root exudates during plant growth. We hypothesized that active bacterial communities would be more sensitive to the change of labile root exudates and soil properties than the total bacterial community would, and thus the rhizosphere effect would be stronger on the active bacterial members than on the total bacterial community. To address this hypothesis, we set up a pot experiment and collected rhizosphere and bulk soil samples at different phenological stages in the rice growth. The bacterial community composition in these samples was investigated at both RNA- and DNA- level by using 16S rRNA gene amplification and Illumina high-throughput sequencing. Root exudates, chemical components of the soils and pore-waters were measured in order to evaluate the contribution of these factors in shaping the bacterial communities in both rhizosphere and bulk soils.

2. Materials and methods

2.1. Plant cultivation, soil and pore water sampling

A paddy soil was collected from Taoyuan city (28°55′15″N, 111°27′35″E), Hunan province, China at 2014. The paddy soil was air-dried, sieved through a 2 mm mesh and stored at room temperature until the beginning of the rice cultivation. Pot experiments were set up using the collected soil, and soil properties were continuously measured during the experiment as described previously (Li et al., 2016). In brief, 3.5 kg of soil were well mixed with a basic fertilizer (urea-N, 250 mg kg^{-1}; superphosphate-P, 60 mg kg^{-1}; KCl-K_2O, 100 mg kg^{-1}) at the beginning of the cultivation. Rice was cultivated under flooded conditions (with standing water in each pot) in a greenhouse with a Rhizo-bag (30-μm nylon mesh, 7.5 cm diameter, 12 cm height) separating rhizosphere and bulk soil (Nie et al., 2015). Rhizosphere soil, bulk soil and pore water were collected at different phenological stages of the rice growth (seedling, day 12; tillering, day 37; booting, day 62; flowering, day 82 and ripening stage, day 122) for chemical property analysis, DNA and RNA extraction. The corresponding pore water was collected for analysis of organic acids and inorganic ions using a sterilized soil moisture sampler (Rhizon SMS, Rhizosphere Research Products, Wageningen, Netherlands). Triplicate pot experiments were set for each phenological stage of rice growth and all replicate samples were subjected to sequencing. The determination of chemical properties and organic acids was conducted within 24 h after sampling. Samples for DNA extraction were stored at −80 °C and samples for RNA extraction were stored at −20 °C after addition of RNA protection solution following the manufacturer's instructions (GENEray Biotechnology, Shanghai, China).

2.2. Chemical property analysis

A detailed analysis of the soil physiochemical properties including pH, moisture, TC_{soil}, TOC_{soil}, TN_{soil}, C/N ratio (C/N_{soil}), ${NH_4}^{+}{}_{soil}$ and ${NO_x}^{-}{}_{soil}$ were performed according to a previous protocol (Li et al., 2016). Determination of LMWOAs in the pore-water (pw) including