



Reduced dependence of rhizosphere microbiome on plant-derived carbon in 32-year long-term inorganic and organic fertilized soils



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ABSTRACT

Root-derived carbon (C) is considered as critical fuel supporting the interaction between plant and rhizosphere microbiome, but knowledge of how plant–microbe association responds to soil fertility changes in the agroecosystem is lacking. We report an integrative methodology in which stable isotope probing (SIP) and high-throughput pyrosequencing are combined to completely characterize the root-feeding bacterial communities in the rhizosphere of wheat grown in historical soils under three long-term (32-year) fertilization regimes. Wheat root-derived ¹³C was dominantly assimilated by Actinobacteria and Proteobacteria (notably Burkholderiales), accounting for nearly 70% of root-feeding microbiome. In contrast, rhizosphere bacteria utilizing original soil organic matter (SOM) possessed a higher diversity at phylum level. Some microbes (e.g. Bacteroidetes and Chloroflexi) enhancing in the rhizosphere were not actively recruited through selection by rhizodeposits, indicating a limited range of action of root exudates. Inorganic fertilization decreased the dependence of Actinobacteria on root-derived C, but significantly increased its proportion in SOM-feeding microbiome. Furthermore, significantly lower diversity of the root-feeding microbiome, but not the SOM-feeding microbiome, was observed under both organic and inorganic fertilizations. These results revealed that long-term fertilizations with increasing nutrients availability would decrease the preference of rhizosphere microbiome for root-derived substrates, leading to a simpler crop–microbe association.

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1. Introduction

Terrestrial plants impact soil by producing an array of substrates that originate from sloughed-off root materials including cells, tissues and mucilages, as well as active root exudates such as organic acids, sugars, amino acids and phytohormones (Dennis et al., 2010). In general such plant-derived carbon (C) ranges from <10% photosynthetically fixed C to 44% total plant C under nutrient stress (Bais et al., 2006). This has the potential to lead to relationships between plants and various soil microbiota in the rhizosphere, because bacteria are often limited by available C sources in

bulk soils. Microbes inhabiting such niches subsequently impart the plant with beneficial or detrimental traits; accordingly, altering this balance is of great interest in agronomy (Lundberg et al., 2012). However, the role of crop roots in the selection mechanism of the rhizosphere microbiome is less elucidated in agroecosystems (Hirsch and Mauchline, 2012), in part because of a lack of analytical methods (Cardon and Gage, 2006). The core rhizosphere-inhabiting microbiotas in *Arabidopsis thaliana*, soybean and wheat have been recently revealed in great detail (Bulgarelli et al., 2012; Lundberg et al., 2012; Donn et al., 2014; Mendes et al., 2014). These studies indicated that the rhizosphere or endophytic microbiome was a subset of the bulk soil community and dominated by Proteobacteria, Actinobacteria and Bacteroidetes. As a result, considering the less complexity of bacterial groups in the rhizosphere, a higher specialization of rhizosphere bacterial functions would be expected (Mendes et al., 2014). However, evidence of whether these dominant microorganisms thriving in the rhizosphere that is directly

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