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RESEARCH ARTICLE

Long-term rice-rice-green manure rotation changing the microbial communities in typical red paddy soil in South China



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Abstract

On the basis of a long-term (30 years) field experiment that involved four rotation systems, rice-rice-winter fallow (RRF), rice-rice-ryegrass (RRG), rice-rice-rape (RRP), and rice-rice-milk vetch (RRV), this study described the effects of green manure on the microbial communities in the red paddy soils using 454 pyrosequencing for the 16S rRNA gene. The Chao1 richness and non-parametric Shannon's index increased in all soil samples that received green manure treatments. The communities' structures with the green manure applications were significantly dissimilar from that under the winter fallow. Using Metastats tests, many genera in the RRG, RRP and RRV soils were significantly different from those in the RRF soil, including a number of genera that functioned in the nitrogen and sulfur cycles. Analyses of the genera with these functions revealed the shifts in microbial ecosystem functions after long-term green manuring. Changes in the microbial communities increased the ammonium supply and decreased the soil acidification in green-manure-amended soils. Together, these data suggested powerful effects of green manure on both the microbial communities and the biogeochemical cycle driven by the shifts in bacterial functional groups.

Keywords: green manure, community structure, community function, 16S rRNA gene, double rice

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1. Introduction

In China, the utilization of green manure is a traditional method for providing nutrients for crop production and contributes greatly to grain yields from the 1960s to the 1980s, when China ran short of chemical fertilizers. Currently, because

of the deterioration of field environments caused by the overload of chemical fertilizers, the benefits of green manure are receiving attention once again (Cao and Huang 2009). The application of green manures in crop rotation is considered a positive management practice that could improve the agricultural ecosystem by reducing losses of soil organic matter, compaction and soil erosion (MacRae and Mehuys 1985); ameliorating the soil's physical, chemical and biological properties (Tejada *et al.* 2008b); maintaining soil fertility; and increasing the retention of soil carbon and nitrogen (Drinkwater *et al.* 1998). Consequently, green manures could reduce the dependence on chemical fertilizers (Yang *et al.* 2013) and could increase crop yields (Tejada *et al.* 2008a; Macguidwin *et al.* 2012). Soil microbes play an important role in green manures by improving soil systems (Bending *et al.* 2004), and the effect of green manures on soil microbes deserves deeper study. However, previous studies focusing on the microbial effect were conducted only by indirectly assessing microbial biomass, phospholipid fatty acid composition, soil respiration, and soil enzyme activity (Elfstrand *et al.* 2007a, b; Tejada *et al.* 2008a, b; Ye *et al.* 2014). Today, next-generation sequencing techniques (Hayden 2013) provide an opportunity to study the effects of long-term green manure on soil microbial communities in depth.

To evaluate the effects of long-term green manure, we used the long-term trial for cropping system of rice, which was established in 1982 in South China. Previous studies on soil from this experiment have indicated that the long-term planting of green manure reduced the soil's bulk density; enhanced the porosity; increased the soil's water-retention capacity; improved the water-stable aggregate content and aggregate stability (Yang *et al.* 2011); increased the soil's organic matter, active soil organic matter, total organic carbon, total nitrogen, and available nitrogen contents; promoted soil

mineralization and absorption of soil potassium and phosphorus; increased the rice yield and its sustainability index (Gao *et al.* 2011, 2013); increased the microbial population, microbial biomass carbon, microbial biomass nitrogen, soil respiration and the urease, invertase and dehydrogenase activity levels (Yang *et al.* 2011). However, there has been no comprehensive understanding of how the soil microbial communities are affected by long-term green manures. We presumed that different long-term green manures would change the abundance and diversity of the soil microbial communities to different degrees. Therefore, this study focuses on the effect of long-term double rice-green manure rotation on soil microbial communities using the 454 pyrosequencing technique.

2. Results

2.1. Community alpha-diversity

After strict quality control, 154 975 valid reads with an average length of 398 bp and 57 972 operational taxonomic units (OTUs) were obtained from the 12 samples (Table 1). Good's coverage estimations revealed that 54–68% of the OTUs were observed in all the samples (Table 1). The non-parametric Shannon's index, which does not tend to be affected by sampling effort, was consequently selected to describe the community diversity. Among the four treatments, both the Chao1 richness and the non-parametric Shannon's index in the green manure treatments were always higher than those in the RRF treatment on all three sampling dates, except for two samples (i.e., the richness of RRP3 (rice-rice-rape after the early rice harvesting (12 July) in 2012) and RRV3 (rice-rice-milk vetch after the early rice harvesting (12 July) in 2012) (Table 1). Overall, the Chao1

Table 1 Diversity estimates based on operational taxonomic unit definition of 97% similarity for the soil samples

Sample ¹⁾	Reads	Reads retained after quality control/Raw reads (%)	OTUs ²⁾	Chao1 richness	Non-parametric Shannon's index	Good's coverage
RRF1	9 786	56.0	5 239	25 953	8.22	56.1
RRF2	9 850	55.3	4 430	18 219	7.80	64.8
RRF3	16 297	55.7	6 985	29 679	8.10	66.4
RRG1	16 529	52.1	8 189	40 263	8.50	59.8
RRG2	10 204	56.7	5 218	24 237	8.17	59.0
RRG3	14 650	54.3	7 018	32 362	8.27	61.4
RRP1	11 568	54.6	6 309	35 428	8.41	54.6
RRP2	11 978	55.1	5 960	27 237	8.23	60.3
RRP3	15 529	55.6	6 775	28 019	8.13	65.9
RRV1	14 070	53.7	6 745	29 997	8.23	61.2
RRV2	13 384	55.4	5 779	21 498	8.06	67.2
RRV3	11 130	54.9	5 678	23 422	8.25	59.5

¹⁾ RRF, rice-rice-winter fallow (control); RRG, rice-rice-ryegrass; RRP, rice-rice-rape; RRV, rice-rice-milk vetch. 1, 2 and 3, at the full-bloom stage of the green manures (9 April), after the green manure being incorporated and before the early rice transplanting (25 April) and after the early rice harvesting (12 July) in 2012, respectively. The same as below.

²⁾ OTUs, operational taxonomic units.

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