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Relationships Between Abundance of Microbial Functional Genes and the Status and Fluxes of Carbon and Nitrogen in Rice Rhizosphere and Bulk Soils*1

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ABSTRACT

Rapid nitrogen (N) transformations and losses occur in the rice rhizosphere through root uptake and microbial activities. However, the relationships between rice roots and rhizosphere microbes for N utilization are still unclear. We analyzed different N forms (NH_4^+, NO_3^-, and dissolved organic N), microbial biomass N and C, dissolved organic C, CH_4 and N_2O emissions, and abundance of microbial functional genes in both rhizosphere and bulk soils after 37-d rice growth in a greenhouse pot experiment. Results showed that the dissolved organic C was significantly higher in the rhizosphere soil than in the non-rhizosphere bulk soil, but microbial biomass C showed no significant difference. The concentrations of NH_4^+, dissolved organic N, and microbial biomass N in the rhizosphere soil were significantly lower than those of the bulk soil, whereas NO_3^- in the rhizosphere soil was comparable to that in the bulk soil. The CH_4 and N_2O fluxes from the rhizosphere soil were much higher than those from the bulk soil. Real-time polymerase chain reaction analysis showed that the abundance of seven selected genes, bacterial and archaeal 16S rRNA genes, *amoA* genes of ammonia-oxidizing archaea and ammonia-oxidizing bacteria, *nosZ* gene, *mcrA* gene, and *pmoA* gene, was lower in the rhizosphere soil than in the bulk soil, which is contrary to the results of previous studies. The lower concentration of N in the rhizosphere soil indicated that the competition for N in the rhizosphere soil was very strong, thus having a negative effect on the numbers of microbes. We concluded that when N was limiting, the growth of rhizosphere microorganisms depended on their competitive abilities with rice roots for N.

Key Words: ammonium-oxidizing archaea, ammonia-oxidizing bacteria, CH_4 emission, microbial biomass, N_2O emission, organic C, organic N

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INTRODUCTION

Nitrogen (N) is usually a limiting nutrient for plant growth in terrestrial ecosystems (Kaye and Hart, 1997). Plant roots and microorganisms interact with, and compete for, nutrients within the rhizosphere (Jackson *et al.*, 1989). Root exudates such as organic acids, sugars, and amino acids may stimulate microbial growth, influencing the C and N biogeochemical cycles (Geisseler *et al.*, 2010). Roots also excrete organic C which additionally serves as an important C source for CH_4 production (Shimoda, 1960). Microorganisms are able to use N from a wide range of organic or inorganic compounds (Geisseler *et al.*, 2010), while plant roots also have the capacity to capture NH_4^+-N, NO_3^--N, or amino acids directly according to plant growth requirements (Shimoda, 1960). Depending on C and N availability, stimulation or retardation of microbial activities can be expected in the rhizosphere of plants due to strong competition for N. Soil microorganisms compete with plant roots for N when their available organic substrate has low N availability (Kaye and Hart, 1997).

Paddy soils are the largest and most highly modified anthropogenic wetlands. Well-defined microscale chemical gradients can be measured in the rhizosphere (Liesack *et al.*, 2000). The oxygen profile seems to govern gradients of electron acceptors (*e.g.*, NO_3^- and Fe^{3+}) and reduced compounds (*e.g.*, NH_4^+ and Fe^{2+}). These gradients modulate the activity and spatial distribution of microbial functional groups (Hirata, 1997). In rice farming, N is the most extensively used fertilizer. However, its use causes environmental problems due to N loss by leaching as NO_3^- or release of greenhouse gas N_2O from soil. Thus, its utilization has implications both for rice productivity and for micro-

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bial C and N transformations (Jackon *et al.*, 1989). Due to the complexity of the rhizosphere and the lack of accurate methods available for collecting rhizosphere soil, very little research data on N behavior in the rhizosphere is available.

The rhizosphere is the major site of coupled nitrification-denitrification in rice-planted soils (Arth *et al.*, 1998). It has been suggested that oxygen supply from the rice roots supports NO_3^- synthesis by nitrification (Raimbault *et al.*, 1977). NO_3^- diffuses rapidly into the adjacent reduced soil and is denitrified to N_2O and N_2 (Reddy and Patrick, 1984). Ammonia oxidation is the first and rate-limiting step of nitrification (Kowalchuk and Stephen, 2001); in the rhizosphere, however, rice roots use NH_4^+ as an N source for plant growth, competing with nitrifying bacteria for NH_4^+. The ammonia monooxygenase enzyme is coded by the *amoA* gene, which has been used extensively as a molecular marker gene for cultivation-independent studies of ammonium-oxidizing bacteria (AOB) and archaea (AOA) in soil (Carney *et al.*, 2004; Leininger *et al.*, 2006). Denitrification is the reduction of NO_3^- through NO_2^- and NO to N_2O and eventually to N_2. Here, we focus on the last step of denitrification which is linked to *nosZ* gene coding for nitrous oxide reductase (Zumft, 1997). However, demonstrating the molecular mechanisms involved in the coupling of denitrification to nitrification in the rhizosphere is still technically difficult.

The objective of this study was to investigate the association between C and N transformations and the abundance of microbial functional genes in rhizosphere and bulk soils. In this study, a greenhouse pot experiment was carried out, using a paddy soil from Quaternary red clay, to distinguish N forms and concentrations and to investigate the difference in the abundance of the key microorganisms involved, in order to provide information on the behavior of N in paddy soils and the impact of rhizodeposition and root competition on microbial growth.

MATERIALS AND METHODS

Soil used and experimental procedure

Soil samples (0–20 cm) used for this study were collected on November 22, 2010, in a field of the Red Soil Ecological Experiment Station (28°12′ N, 116°56′ E), Chinese Academy of Sciences, located in Yingtan City, Jiangxi Province, China. The soil was a paddy soil from Quaternary red clay with 7.72% clay, and the main characteristics of the soil were as follows: pH (H_2O, 1:2.5), 5.24; total organic matter, 20.00 g kg^{-1}; total N, 1.03 g kg^{-1}; available phosphorus, 24.55 mg kg^{-1}; available iron, 131.40 mg kg^{-1}; and available potassium, 54.82 mg kg^{-1}. Soil samples collected were bulked to form a composite sample. Prior to the start of the experiment, the bulked soil was air-dried, homogenized and then sieved (< 2 mm).

Rhizon samplers (Rhizosphere Research Products, Wageningen, The Netherlands) were buried in rhizobags and non-rhizosphere soil for porewater extraction. Rhizobags (30 μm nylon mesh, 7.5 cm diameter, 12 cm height) filled with 475 g sieved soil were placed in the centre of PVC pots (15 cm diameter, 23 cm height), which were then each filled with 3 kg soil (Fig. 1). This allowed the separation of root rhizosphere compartments from the bulk soil compartment (Fig. 1). No N fertilizers were applied during the experiment.

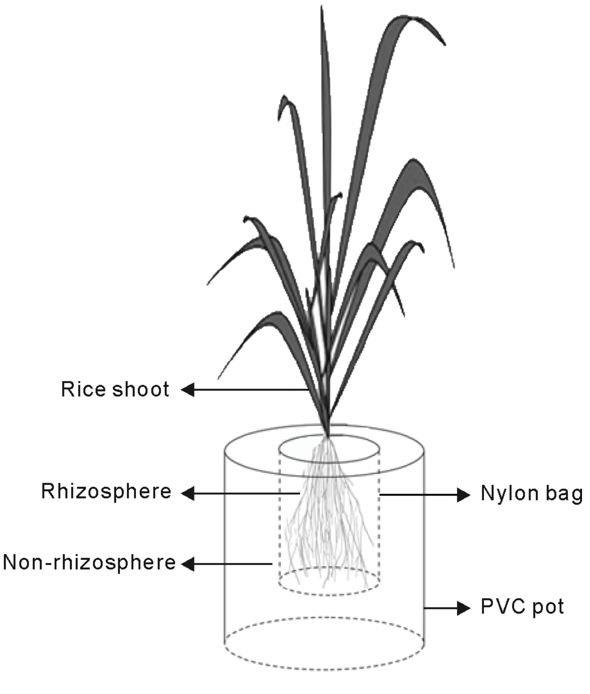

Fig. 1 Schematic structural diagram of a rhizobag for rice growth.

The soil samples were first incubated for one month at 105% water holding capacity (WHC) at 25 °C. Rice seeds (cv. Xiangzaoshan 45) were sterilized in 30% (v/v) H_2O_2 for 10 min, and then thoroughly washed with deionized water. Three days after germination, uniform seedlings were transplanted into the rhizobags. The rice plants were water flooded under 2–3 centimeters during all the growth period. After 37 d of rice growth in a greenhouse (illumination, 1 500 lx (28.3 μmol m^{-2} s^{-1}); ambient temperature, 28 °C during the day, 16 °C at night; ambient humidity, about 90%), porewater, gas fluxes, and rhizosphere and bulk