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Tillage effects on soil nitrification and the dynamic changes in nitrifying microorganisms in a subtropical rice-based ecosystem: A long-term field study

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ABSTRACT

The dynamics of N mineralization and nitrification may be altered by tillage, which ultimately affects crop growth and yield. This study investigated effects of tillage on soil nitrogen dynamics in a subtropical rice (*Oryza sativa* L.) soil (Hydragric Anthrosols, WRB classification) under combination ridge with no-tillage (RNT) and conventional tillage (CT) for 22 years in Chongqing, China. Soil mineralization, nitrification and changes in abundance of ammonia-oxidizing bacteria (AOB) and archaea (AOA) were investigated to test the hypothesis that RNT decreases nitrification in the rice-based ecosystem. Soil mineralization and nitrification rates decreased after 22 years of RNT compared with CT. Soil pH decreased about 0.5 units after 22 years of RNT, and this difference may be responsible for the decrease of N mineralization and nitrification rates observed under RNT. The nitrification process was best fitted by a first-order kinetic model for both CT and RNT. While the nitrification rate was significantly lower for RNT (2.96 mg N kg^{-1} day^{-1}) than CT (3.40 mg N kg^{-1} day^{-1}), AOB and AOA *amoA* gene copies were significantly higher for RNT than CT. This suggested that the functional expression (*i.e.*, nitrification) may not be closely related to numbers of microorganisms that have the ability to nitrify. The AOA dominated in abundance over the AOB for both CT and RNT paddy soils, with AOA to AOB ratios ranging from 13 to 52, and the AOA abundance showed similar changes with AOB, suggesting that AOA may also play an important role in soil nitrification. Results implied that RNT may increase soil N retention by keeping N longer in the NH_4^+ state, thus decreasing the potential for N loss from leaching.

1. Introduction

China is one of the largest agricultural countries in the world, and consumed 2.38×10^{10} kg N in 2010 (Ministry of Agriculture of China, 2011), which accounted for a large proportion of the world total N fertilizer usage. Application of N fertilizer can lead to soil acidification and generate nitrate through nitrification, which is easily leached from the soil (Kögel-Knabner et al., 2010; Ishii et al., 2011). Therefore, N fertilizer-use efficiency in rice-based ecosystems is usually less than one half of the efficiency typically found in other agricultural systems (Roy and Misra, 2003). In south China, N leaching estimates from paddy fields range from 6.75 to 27.0 kg N ha^{-1} $year^{-1}$ (Xing and Zhu, 2000), which increases the risk of water impairment.

Microbial ammonia oxidation is the first and rate-limiting step of nitrification, a primary process controlling soil nitrate concentrations. Both ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) possess the *amoA* gene for ammonia monooxygenase (AMO), the key enzyme necessary for nitrification, which implies that both are key players in nitrification (Leininger et al., 2006; Lam et al., 2007; Jia and Conrad, 2009; Nugroho et al., 2009). It is important then to understand how these enzymes are affected by tillage management.

Agricultural land management is one of the most significant anthropogenic activities that alter soil characteristics (Sainju et al., 2012). However, any type of paddy management has beneficial and adverse effects on the environment (Kögel-Knabner et al., 2010). For example, Olk et al. (2007) suggested that increasing aeration of rice soils through aerobic decomposition of crop residues or crop rotation improves the soil N supply. On the other hand, increased aeration induces the risk of water losses and crack formation.

A combination of ridge and no-till (RNT) method, which was proposed by Jiang and Xie (2009) in rice-based ecosystems, maybe

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a promising management technique for improving soil fertility and preventing N from leaching (Yuan et al., 2009; Liang et al., 2012). Since NO_3-N is the end-product of the nitrification process and is the most easily leachable N form, it is essential to investigate land management strategies that can slow down nitrification rates and decrease potential for N loss from fields by leaching. We hypothesize that the soil nitrification decreases after 22 years of RNT treatment compared to the conventional tillage. Therefore, the objective of this study was to investigate soil N mineralization and nitrification processes, and the changes of AOB and AOA abundance, for different tillage management in a subtropical rice-based system.

2. Material and methods

2.1. Tillage regimes and soil sampling

The study site was located at Chongqing (30°26′N, 106°26′E), located in the southwest of China. The climate is subtropical with a mean annual temperature of 18.2 °C and a mean annual total rainfall of 1080 mm. The soil at the experimental site is classified as Hydragric Anthrosols (WRB, 2014) that developed from purple mudstone. The field was planted with rape (*Brassica napus* L.) in winter and rice (*Oryza sativa* L.) in summer, and crop residues were returned to the soil at harvest. In 1990, two tillage regimes were imposed: a combination of ridge with no-tillage (RNT) and conventional tillage (CT). The experiment was organized in a randomized complete block design (RCBD) with four replications, and each plot measured 4 by 5 m. The buffer rows between treatments were about 16 m.

Tillage treatments and soil fertility were described in detail by Jiang and Xie (2009). For RNT, ridges were made by manpower in the fields, rice and rape were planted on top of the ridge. The ridges were 25 cm wide at the top and the furrows were 30 cm wide and 35 cm deep. Each plot consisted of five rows. From rice transplantation to vegetative stage, the water surface was kept parallel with ridge top in the field, and during other times of the year, water depth in furrows was 25–30 cm, *i.e.*, ridge top was 5–10 cm above water surface. After the harvest of the rice crop, mud from furrow was artificially stacked on the ridge top to maintain ridge height. Rape was seeded after the field was covered with rice stubble (50–60 cm). During the rape growing season, water levels in the furrow were maintained at a 5–10 cm depth, *i.e.*, ridge top was 20–25 cm above water surface, thus retaining ridge soakage irrigation. After the harvest of the rape crop, rape stubble and weeds were buried in the furrow bottom, and the field was submerged with water up to ridge top for the cultivation of rice. For CT, rice was transplanted in a flooded field with a layer of water until maturation. After rice harvest, the paddy field was drained, ploughed, dried, and harrowed and then rape was seeded. After rape was harvested, the soil was puddled and harrowed twice and rice seedlings were transplanted again. For all treatments, the annual application of fertilizers included 270 kg ha^{-1} urea, 500 kg ha^{-1} calcium superphosphate and 150 kg ha^{-1} potassium chloride. For each plot, five individual soil cores (diameter was 5.5 cm and height was 20 cm) were collected along the plant row in May 2012, air-dried and sieved (<2 mm) with removal of visible plant residues, then pooled and homogenized to reduce heterogeneity.

2.2. Incubation

For each field sample, 50 g soil was added to 48 plastic bottles (250 mL). Distilled water was added to adjust the water content of soil to 60% of water-holding capacity. All bottles were covered with polyethylene film punctured with a needle to create holes to allow gas exchange but minimize evaporation, and were pre-incubated at 28 °C in the dark for 7 days. After pre-incubation, half of the bottles were amended with 264 mg $(NH_4)_2SO_4$ kg^{-1} dry soil to evaluate nitrification, while another group without the additional N supply was prepared to study N mineralization. The loss of water through evaporation was compensated by addition of distilled water every 2 d. At incubation days 0, 1, 7, 14, 21 and 28, four replicate bottles of each treatment were randomly selected for analysis.

For mineral N (NH_4^+-N and NO_3^--N) analysis, duplicate samples were shaken with 100 mL of 2 M KCl for 1 h on a rotational shaker (Keeney and Nelson, 1982), then the soil slurries were filtered and NH_4^+-N and NO_3^--N measured colorimetrically with a SKLAR continuous-flow analyzer. Soil pH was measured using a 1:1 soil to water suspension with a glass electrode. Soil organic carbon (SOC) was determined by acid dichromate wet oxidation as described by Nelson and Sommers (1996). Total N was determined by the micro-Kjeldahl method (Bremner, 1996). Total P in soils was determined by the rapid perchloric acid digestion procedure (Sommers and Nelson, 1972), and total K as described by Jackson (1967). Clay concentration was determined by the standard pipette method (Gee and Bauder, 1986).

2.3. DNA extraction and quantitative PCR assay

At incubation days 0, 14 and 28, four replicate bottles of each treatment were randomly selected to extract DNA and to quantitate *amoA* genes using quantitative PCR (qPCR). The DNA was extracted for three sub-samples from 0.50 g of soil with the FastDNA Spin Kit for soil (MP Biomedicals, United States), according to the protocol of the manufacturer. The quality and quantity of the DNA extracts were assessed using a spectrophotometer (Nanodrop, PeqLab Germany), and were pooled and stored at −20 °C until use. Quantitative PCR of *amoA* genes was performed to estimate the abundance of the ammonia-oxidizing bacteria and archaea communities, respectively. The primers *amoA*-1F (5′-GGGGTTTCTACTGGTGGT-3′) and *amoA*-2R(5′-CCCCTCKGSAAAGCCTTCTTC-3′) were used for ammonia-oxidizing bacteria generating a 491 bp fragment; Arch-*amoA* F (5′-STAATGGTCTGG-CTTAGACG-3′) and Arch-*amoA* R (5′-GCGGCCATC-CATCTGTATGT-3′) were used for ammonia-oxidizing archaea generating a 635 bp fragment (Francis et al., 2005). Quantification was based on the fluorescence intensity of the SYBR green dye and reactions for each sample were carried out in a Bio-Rad CFX-96 thermal cycler. The quantification of *amoA* genes was performed in a total volume of 25 μl reaction mixtures by using 12.5 μl of SYBR Premix Ex Taq™ as described by the suppliers (Takara Bio Otsu, Shiga, Japan), 0.25 μl of each primer (50 μm), 1 μl of soil DNA template, with a final content of 1–10 ng in each reaction mixture, and 11 μl ddH_2O. The fragments for the AOB and AOA were both amplified using an initial denaturation step at 95 °C for 3 min, followed by 35 cycles of 30 s at 95 °C, 30 s at 55 °C, 30 s at 72 °C for AOB, and 45 s at 72 °C for AOA for the collection of fluorescence data. All reactions were finished with a melting curve starting at 65 °C with an increase of 0.5 °C up to 95 °C to verify amplicon specificity. Standard curves for the AOB and AOA were obtained using serial dilutions of linearized plasmids (pGEM-T, Promega) containing cloned *amoA* genes amplified from environmental clones. Ten-fold series dilutions of a known copy number of plasmid of the *amoA* gene clone were generated to produce the standard curve over several orders of magnitude (8.21×10 to 8.21×10^8 copies of template for bacterial *amoA* and 5.46×10^2 to 5.46×10^8 copies of template for archaeal *amoA*) per assay respectively. Amplification efficiencies of 92–107% were obtained for bacterial and archaeal *amoA* quantification with R^2 values of 0.994–0.993 and slopes from −3.2 to −3.5. Data analysis was carried out with Eco™ Software v3.0.16.0.