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Ammonia oxidizer abundance in paddy soil profile with different fertilizer regimes

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ABSTRACT

Studies about ammonia-oxidizing bacteria (AOB) and archaea (AOA) are often focused on topsoil, but little is known about their activity and distribution in subsoil. A long-term fertilizer experiment was conducted to assess the effects of different fertilizer treatments on AOB and AOA in vertical soil profiles of paddy soil plots that received no nitrogen fertilizer control (CK), NPK chemical fertilizers (CF), organic–inorganic mixed fertilizer (OIMF) and organic fertilizer (OF). Soil properties, potential nitrification rate (PNR) and *amoA* gene abundance of AOB and AOA were measured and analyzed by two-way ANOVA and correlation analysis. Quantitative PCR analysis of *amoA* genes showed that AOA were more abundant than AOB in all the soil samples. AOB declined sharply with soil depth. Compared with CK and OF treatments, CF and OIMF treatments had higher abundance of AOB throughout the soil profiles. However, AOA tend less responsive to soil depth and fertilizers compared to AOB. This caused the AOA/AOB ratios in subsoil higher than in topsoil, and in CK and OF higher than in CF and OIMF treatments. These results suggest that AOA are more abundant and can be better adapted to nutrient-poor subsoils than AOB, and autotrophic nitrification could likely be determined by a complex suite of environmental factors in vertical profiles of the paddy soil tested.

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1. Introduction

Autotrophic nitrification catalyzes ammonia oxidation to nitrate via nitrite. It is a key process that determines the efficiency of fertilizer use by crops, the pollution of groundwater through soil nitrate leaching, emission of nitrous oxide, and loss of nitrogen (N) fertilizer (Kowalchuk et al., 1997; Zhalnina et al., 2012). Ammonia-oxidizing bacteria (AOB) were once thought to be the major player of this rate-limiting step of nitrification (Arp et al., 2002). However, the discovery of ammonia-oxidizing archaea (AOA) has fundamentally changed our perception of the global nitrogen cycle (Könneke et al., 2005). The widespread presence and extraordinary abundance of AOA are often reported in soil and marine environments (Leininger et al., 2006; Wuchter et al., 2006). It thus, necessitates a re-assessment of the current biogeochemical model of nitrogen cycle on Earth. A growing body of evidences has suggested the distinct lifestyles of AOA and AOB in physiochemically different environments (Prosser and Nicol, 2012). For instance, it is generally accepted that AOA are readily adapted to nutrient-poor environment such as hot-spring (De la Torre et al., 2008) and marine environment (Yan et al., 2012), while AOB favored fertile (especial NH_4^+ -N) and neutral pH environments (Kumar et al., 2004; Prosser and Nicol, 2012). Soil at depth of >20 cm is generally characterized as oligotrophic environment and constitute the majority of microbial communities on the planet (Whitman et al., 1998). Nonetheless, most studies have focused on the 0–20 cm soil, and it remains poorly understood about population dynamics of AOA and AOB in soil vertical profiles (Hofferle et al., 2010).

In the rice field, nitrate (NO_3^-) easily leach into the deeper soil and contaminate the underground water. The application of chemical fertilizers undoubtedly exacerbated this phenomenon,

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moreover, fertilizers managements also have significant effects on soil biology, function, and community structure in rice field soils (Ahn et al., 2012). The problem of mitigating the negative impacts on long term field fertility has evoked researchers' interests (Zhalnina et al., 2012). Recently, a number of studies were conducted on the nitrification in paddy soil. Chen et al. (2008) showed that the AOA predominated among ammonia oxidizing prokaryotes and were more abundant in the rhizosphere than in the bulk soil. Wang et al. (2009) and Wu et al. (2011) showed that different N fertilizer treatments change the AOB but not the AOA community structure in the paddy soil. By comparing different paddy soil types, Chen et al. (2010) reported that the AOA and AOB abundance and community structure were mainly determined by soil types. Most field experiments were focused on 0–20 cm topsoil (Shen et al., 2008; He et al., 2007), however, reports about the influence of soil vertical profile and different fertilizer regimes on ammonia oxidizers are rare (Di et al., 2010). Leininger et al. (2006) evaluated 0–40 cm soil layers and observed that AOB *amoA* gene copy numbers were decreased dramatically with depth, while AOA *amoA* gene copy numbers were slightly decreased. This caused the ratio of AOA to AOB *amoA* gene copies reach to a maximum of 3000 in some treatments with depth. In addition, when comparing the ratios of AOA to AOB *amoA* gene copies in different treatments, they found that the treatment without fertilizer had the highest AOA to AOB *amoA* gene copy ratios throughout the 0–40 cm. Di et al. (2010) found that AOB in the topsoils (0–20 cm) were more abundant than in the subsoils (40–60 cm) in three pasture soils, while AOA in one site of the topsoil was less than in subsoil. AOA community structure in another study was stratified with depth in a wetland soil profiles (Hofferle et al., 2010). These studies suggested that ammonia oxidizers were also available under deeper soils and need further investigations. However, little information is obtainable with respect to niche differentiation of AOA and AOB along a soil vertical profile in response to long-term field fertilization.

Understanding the contribution of AOA and AOB under different fertilizer regimes in soil profiles will help us to better manage the paddy soil. Although Leininger et al. (2006) and Di et al. (2010) had investigated 0–60 cm soil layers, knowledges about AOA and AOB communities in deeper soils are still scarce. The objectives of present study were to understand the effects of different fertilizer regimes on the abundance of AOA and AOB in 0–100 cm soil layers and to determine the environmental factors that might have likely affected soil potential nitrification rate (PNR) and the abundance of AOA and AOB at different soil depths.

2. Materials and methods

2.1. Study site and soil description

This study was carried out in a long-term field experimental site at Changshu, Jiangsu province, China (31°18′N, 120°37′E, 6 m asl), established in 2006. The site has a humid subtropical monsoon climate with average annual rainfall of ≈1063 mm and the annual mean minimum and maximum temperatures of 3.1 °C and 33 °C, respectively (Wang et al., 2012). The field was tilled to an average depth of 15 cm before either sowing wheat or transplanting rice seedlings. Rice plots were flooded with 5 cm of standing water. Subsequent irrigations were given when soil reached the saturation. Rice was transplanted in June using two seedlings per hill at 13 cm × 28 cm spacing. Following the rice harvest, wheat was sown using 150 kg ha^{-1} seed. Crops were harvested manually at ground level by sickle, and the above-ground biomass was removed from the plots. After the rice harvest, plots were ploughed to a depth of 15 cm (Wang et al., 2012).

2.2. Experiment design and soil sampling

The fertilizer experiment was established in a randomized block design including four treatments with three replicates: (1) control without nitrogen fertilizer (CK), (2) NPK chemical fertilizer (CF), (3) organic–inorganic mixed fertilizer (OIMF) and (4) organic fertilizer (OF). The treatments were allocated in plots of 6 m × 7 m in size.

The inorganic fertilizers were applied as urea (46% N), single superphosphate (12% P_2O_5) and muriate of potash (60% K_2O). The organic–inorganic mixed fertilizer was made by mixing inorganic fertilizers and organic fertilizer for final organic C, total N, phosphorus (P_2O_5) and potassium (K_2O) contents of 11.0, 12.0, 4.1 and 4.1% respectively. Chemical fertilizers and organic-inorganic mixed fertilizer were pilled and dried to avoid clumping. Organic fertilizer produced by Tianniang Ltd. (Changshu, Jiangsu, China) was made by composting pig manures and rice straw (9:1 dry weight). The composting process was conducted under aerobic conditions for 30 days. The organic C, total N, phosphorus (P_2O_5) and potassium (K_2O) contents were 26.4, 2.3, 2.9 and 1.3% for the organic fertilizer respectively. The CF and OIMF plots received equal quantities of N (180 kg N ha^{-1}, irrespective the inorganic- or organic-N), P (90 kg P_2O_5 ha^{-1}) and K (110 kg K_2O ha^{-1}). Specifically, the CK plots received 750 kg ha^{-1} superphosphate and 183 kg ha^{-1} muriate of potash only. The CF plots received 391 kg ha^{-1} urea, 750 kg ha^{-1} superphosphate and 183 kg ha^{-1} muriate of potash; OIMF plots received 1500 kg ha^{-1} organic-inorganic mixed fertilizer, with extra application of 28.5 kg ha^{-1} superphosphate and 48.5 kg ha^{-1} muriate of potash. The OF plots received 4500 kg ha^{-1} organic fertilizer only. All fertilizers were applied as basal dose.

For each plot, soil samples were collected from 4 cores (5 cm diameter) of topsoil (0–20 cm), 3 cores of subsoil samples (20–40, 40–60, 60–80 and 80–100 cm) after the rice harvest in October 2012 and then topsoil and subsoil samples were mixed separately and sieved (2 mm) to remove above ground plant materials, roots, and stones. Fresh soils were stored at 4 C° and used for the analysis within two days of sample collection. Sub-samples for DNA extraction were frozen at −80 C°.

2.3. Soil properties and potential nitrification rate

Soil pH was determined with a glass electrode using a soil-to-water ratio of 1:2.5, and soil organic carbon (SOC) was determined by $K_2Cr_2O_7$ oxidation–reduction titration method and Kjeldahl method was used for total nitrogen (TN) estimation. Soil nitrate and ammonium contents were extracted with 2 M KCl and determined by BRAN + LUEBBE AutoAnalyzer 3. Potential nitrification rate (PNR) was measured using the chlorate inhibition method (Kurola et al., 2005) with minor modifications: 5.0 g of fresh soil was added to 50 ml centrifuge tube containing 20 ml of phosphate buffer solution (PBS, g L^{-1}: NaCl, 8.0; KCl, 0.2; Na_2HPO_4, 0.2; NaH_2PO_4, 0.2; pH 7.4) with 1 mM $(NH_4)_2SO_4$. Potassium chlorate with a final concentration of 10 mM was added to the tube to inhibit nitrite oxidation. The suspension was incubated on a rotary shaker at 25 °C and 170 rpm. After 24 h, NO_2^- -N was extracted by 5 ml of 2 M KCl and determined by a spectrophotometrically at 540 nm with *N*-(1-naphthyl) ethylenediamine dihydrochloride.

2.4. DNA extraction

Soil DNA was extracted from 0.25 g fresh soil using the MoBio Powersoil™ DNA Isolation Kits (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. The DNA was stored at −20 °C before analysis.

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