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Ammonia oxidizers and nitrite-oxidizing bacteria respond differently to long-term manure application in four paddy soils of south of China

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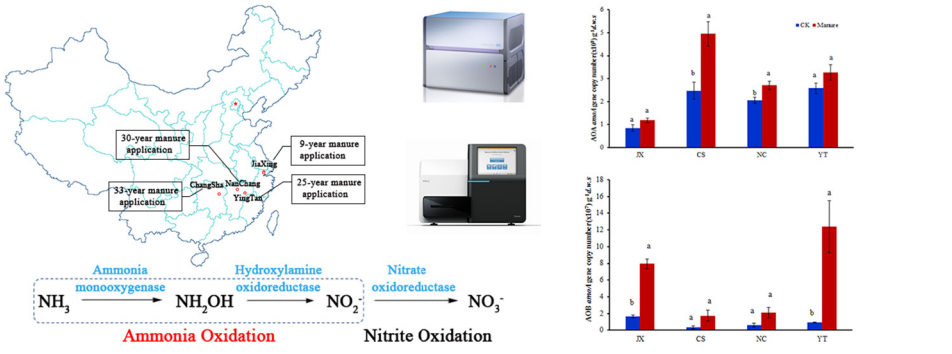
HIGHLIGHTS

- qPCR and Miseq sequencing were used for nitrifier abundance and composition.
- Nitrifiers responded differently to long-term manure application in paddy soils.
- AOA and NOB were more sensitive to long-term manure application.
- pH was the main factor that affected nitrifier abundance and composition.

GRAPHICAL ABSTRACT



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ABSTRACT

Nitrification plays an important role in the soil nitrogen (N) cycle, and fertilizer application may influence soil nitrifiers' abundance and composition. However, the effect of long-term manure application in paddy soils on nitrifying populations is poorly understood. We chose four long-term manure experimental fields in the south of China to study how the abundance and community structure of nitrifiers would change in response to long-term manure application using quantitative PCR and Miseq sequencing analyses. Our results showed that manure application significantly increased ammonia oxidizing archaea (AOA) abundance at the ChangSha (CS) and NanChang (NC) sites, while the abundance of ammonia oxidizing bacteria (AOB) represented 4.8- and 12.8- fold increases at the JiaXing (JX) and YingTan (YT) sites, respectively. Miseq sequencing of 16S rRNA genes indicated that manure application altered the community structure of nitrifying populations, especially at the NC and YT sites. The application of manure significantly changed AOA and nitrite oxidizing bacteria (NOB) community structures but not those of AOB, suggesting that AOA and NOB may be more sensitive to manures. Variation partitioning analysis (VPA) and redundancy analysis (RDA) indicated that soil pH, TN, NO_3^--N and water content were the main factors in shaping nitrifying communities. These findings suggest that nitrifiers respond diversely to manure application, and soil physiochemical properties play an important role in determining nitrifiers' abundance and communities with long-term manure addition.

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1. Introduction

Conventional nitrification, performed by two phylogenetically distinct microbe groups, can be divided into two steps: (i) ammonia oxidation by autotrophic ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA); and (ii) nitrite oxidation by nitrite-oxidizing bacteria (NOB) (Gruber and Galloway, 2008). Ammonia oxidizers are a focus of attention as they drive the first and rate-limiting step of nitrification. It is widely accepted that both AOA and AOB are key players in soil ammonia oxidation (Jia and Conrad, 2009; Offre et al., 2009). AOA and AOB may respond differently to the complex soil environment given that they belong to distinct domains and differ in physiology and metabolic pathways (Hallam et al., 2006; Martens-Habbena et al., 2009; Walker et al., 2010). Factors including soil pH, ammonia substrate and organic matter influence their growth, activity, geographical distribution and communities (He et al., 2012; Prosser and Nicol, 2012). For example, AOB dominate nitrification in nitrogen-rich soils (Di et al., 2009; Jia and Conrad, 2009), while AOA are favored in low pH and low ammonia soil environments (Zhang et al., 2010, 2012). NOB are more diverse than AOA and AOB, and include six genera, namely *Nitrospira*, *Nitrobacter*, *Nitrococcus*, *Nitrotoga*, *Nitrospina* and *Nitrolancetus* (Bock et al., 2005; Alawi et al., 2007; Sorokin et al., 2012).

Rice (*Oryza sativa* L.) is one of the most important stable food crops grown on almost 155 million ha in the world, and rice is used to feed more than half of the world's population (Zhu et al., 2011). With multiple alternations of drying and wetting (Kimura et al., 2000), rice paddy soils provide unique environments for soil microbes and biochemical cycles. Ammonium-based fertilizers are usually used for rice production as rice prefers NH_4^+ to NO_3^- as the nitrogen source (Sasakawa and Yamamoto, 1978; Kiuchi, 1980). The application of organic manure or crop straw has been widely recommended to improve soil fertility, increase crop yield and reduce nutrient leaching (Eneji et al., 2001; Burger and Jackson, 2003).

In south China, acidic rice paddy soil accounts for a large proportion due to the soil parent material and the large application of N fertilizer or other factors. To manage soil acidification, organic fertilizers are used to increase soil pH (Saiful Alam et al., 2013; Zhong et al., 2010; Li et al., 2017). Chemical and organic fertilizers can affect nitrification and the abundance and communities of ammonia oxidizers (Chu et al., 2007, 2008; He et al., 2007; Shen et al., 2008; Wang et al., 2015; Zhou et al., 2015). Some studies showed that long term fertilization (chemical and/or organic) altered AOA abundance and communities in acidic soils (Chen et al., 2011; He et al., 2007). However, others indicated that it was AOB not AOA that dominated in neutral and alkaline soils after long-term fertilization (Chu et al., 2007, 2008; Shen et al., 2008; Wu et al., 2011; Wang et al., 2014). In addition, a recent $^{13}CO_2$ stable-isotope study on an acidic soil showed that AOA dominated in no fertilizer and chemical-fertilizer treated soils, and AOB dominated in organic manure fertilized soil (Wang et al., 2015). On the contrary, another study suggested that AOA might affect nitrification in manure treatments, and AOB were more important in chemical fertilizer treatments (Zhou et al., 2015). How soil pH and fertilizer type affect soil ammonia oxidizers is still a poorly understood topic. Research so far on nitrifier responses to long- term fertilization focused on single soil types with different levels of chemical and/or organic fertilizer application rates (Zhou et al., 2015; Wang et al., 2015). The effect of long-term manure fertilization on nitrifying communities in paddy soils with different pH has hardly been studied.

We chose four long-term manure-treated paddy fields with three acidic- and one neutral-pH in the south of China. We hypothesized that long-term manure fertilization could result in the shifts of nitrifiers' abundance and community structure in paddy soils and such shifts may be partly caused by changes in soil physicochemical properties following long-term manure application.

2. Materials and methods

2.1. Soil sampling

The soil samples used in this study were collected from four different paddy soils with long-term manure application after crop harvest between May and July 2014. The four experimental fields are in JiaXing (JX), Zhejiang province (30°50′N,120°40′E), ChangSha (CS), Hunan province (28°37′N, 112°80′E), NanChang (NC) (28°34′N, 115°34′E) and YingTan (YT) (28°15′N, 116°55′E), Jiangxi province. The total applied manures were 12,800 kg^{-1} $ha^{-1}y^{-1}$dw at the JX site for 9 years, 3480 kg^{-1} $ha^{-1}y^{-1}$dw at the CS site for 33 years, 4200 kg^{-1} $ha^{-1}y^{-1}$dw at the NC site for 30 years and 4500 kg^{-1} $ha^{-1}y^{-1}$dw at the YT site for 25 years, respectively. The soils at the four sites were fertilized twice a year to follow local farming practice (Tang et al., 2015). The manure treatments and unfertilize treatments of the four fields were arranged in a randomized complete block design with each treatment having three replicates. Soil samples were collected form a depth of 0–5 cm at five random locations of each site and were mixed as one soil sample for each replicate. All the soil samples were transported to laboratory immediately on ice. A portion of the soils was used to analyze the physicochemical properties and a sub-sample was used for DNA extraction for molecular analysis and high-throughput sequencing. The properties of soil samples are shown in Table 1.

2.2. Soil properties

Soil pH was determined with a 1:2.5 soil to water ratio using a Satorius basic pH meter (Sartorius Scientific Instruments Co. Ltd., Beijing, China). The total C and N were measured using an elemental analyzer (Elementar Analysensysteme GmbH., Germany). Soil available phosphorus (P) was extracted using sodium bicarbonate and measured by the molybdenum blue method (Olsen, 1954). Soil available potassium (K) was extracted using ammonium acetate and determined with flame photometry (nova 300, Analytic Jena, Germany) (Carson, 1980). Soil inorganic N (NH_4^+ and NO_3^-) was extracted from soil samples with 1 M KCl and measured by a flow injection analyzer (SAN++, Skalar, Netherlands). Soil NH_3 concentration was calculated on the basis of Hendersone Hasselbalch equation (pKa of 9.25 at 25 °C) (Lu et al., 2012).

2.3. DNA extraction and quantitative PCR (qPCR) analysis

Soil nucleic acid extraction was performed using 0.5 g soil by the FastDNA spin kit for soil (MP Biomedicals, OH, USA) following the manufacturer's protocol. DNA quality and concentration were measured by gel electrophoresis and a Nanodrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), respectively, and stored at −20 °C. The primers and conditions used for qPCR are shown in Table S1. AOA and AOB *amoA* genes were quantified by the primers of Arch-amoAF/Arch-amoAR and amoA-1F/amoA-2R, respectively. The qPCR was carried out on Roche LightCycler 480 Real-Time PCR Machine (Roche Applied Science). The reactions were performed in 20-μl containing 10 μl SYBR Premix Ex Taq (TaKaRa, Dalian, China), 400 nM of each primer, 1 μl of DNA template and Milli-Q water to the final volume. The plasmids containing each target gene were diluted by 10 times successively with the spanning of 10^8–10^1 and ten-fold serial dilutions were used as standard curves. The plasmids were extracted using a MiniBEST Plasmid Purification Kit (TaKaRa, Janpan) after the purified PCR products were cloned onto the pGEM-T Easy Vector (Promega, Madison, WI, USA) and transferred into *Escherichia coli* JM109 competent cells (Promega, Madison, WI, USA). The concentration of plasmids was measured by a Nanodrop® ND-2000 UV–vis and the standard copy numbers were calculated. The amplification efficiency ranged from 92% to 95% with the R^2 values ranging between 0.998 and 0.999.