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Depth-specific distribution and diversity of nitrite-dependent anaerobic ammonium and methane-oxidizing bacteria in upland-cropping soil under different fertilizer treatments

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ABSTRACT

Anaerobic ammonium oxidation (anammox) and nitrite-dependent anaerobic methane oxidation (n-damo) are two nitrogen-cycle processes that have recently received more attention. However, few studies have been performed on the effects of different fertilizations in upland-cropping soil. Therefore, the objective of this work was to assess the effects of different fertilizations on the distribution and diversity of anammox and n-damo bacteria along the profiles of soil during wheat cropping season of the paddy-upland rotation system. In our study, a five-year fertilization (from 2010 to 2015) was conducted on a paddy-upland rotation field, with four fertilizer treatments: an unfertilized control (CK), chemical fertilizer (CF), pig manure plus 50% chemical fertilizer (PMCF), and rice straw plus 100% chemical fertilizer (SRCF). The quantitative PCR results showed that the total abundance of anammox and n-damo bacteria during the wheat cropping season were lower than those reported previously in paddy soils, and the abundance of anammox bacteria decreased with soil depth, but the abundance of n-damo bacteria remarkably increased. Long-term fertilization reduced the abundance of anammox bacteria in the 0–40 cm layer and that of n-damo bacteria in the 20–60 cm layer. Furthermore, Illumina high-throughput sequencing of the *hzs*B and *pmo*A genes was performed, showing that anammox bacteria were affiliated with "*Candidatus* Brocadia" and n-damo bacteria were related to "*Candidatus* Methylomirabilis oxyfera." Diversity analysis demonstrated that the diversity of anammox bacteria increased with soil depth. Among the fertilizer treatments evaluated, pig manure plus 50% chemical fertilizer (PMCF) yielded the highest abundance and lowest diversity of anammox bacteria in the 20–60 cm layer. Moreover, fertilization diversified the community structure of anammox bacteria in deep soil. For n-damo bacteria, a higher diversity was found under chemical fertilization than under other treatments. Redundancy analysis showed that the community structure of anammox was mainly related to of total nitrogen and soil organic carbon and that of n-damo bacteria was mainly associated with NO_3^- content. Overall, soil depth and fertilization patterns influenced the distribution and diversity of anammox and n-damo bacteria by changing the soil properties.

1. Introduction

Anaerobic ammonium oxidation (anammox) and nitrite-dependent anaerobic methane oxidation (n-damo) are two recently discovered processes in the microbial nitrogen cycle. In the anammox process, ammonium is oxidized with the reduction of nitrite to produce N_2 under anaerobic conditions (Mulder et al., 1995), whereas in the n-damo process methane is oxidized with nitrite reduction to produce N_2 and CO_2 in the absence oxygen (Raghoebarsing et al., 2006). The anammox process plays an important role in the global N cycle as it is

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responsible for high (~50%) N2 removal in marine ecosystems (Thamdrup and Dalsgaard, 2002), contaminated groundwater (18–36%) (Moore et al., 2011), fertilized paddy soil (4–37%) and wetland (~33%) (Zhu et al., 2011a). This process was first patented for wastewater treatment (Mulder et al., 1995) and was later proved to be active in natural environments by Thamdrup and Dalsgaard (2002). Since then, the anammox process has been demonstrated in various habitats, including marine (Dalsgaard et al., 2003), estuarine (Risgaardpetersen et al., 2004; Trimmer et al., 2003), freshwater (Ding et al., 2014; Schubert et al., 2006) and terrestrial environments (Humbert et al., 2010; Shen et al., 2013). Five genera of anammox bacteria have been described to date, including "Candidatus Brocadia" (Kartal et al., 2008; Strous et al., 1999), "Candidatus Kuenenia" (Schmid et al., 2000), "Candidatus Scalindua" (De Vossenberg et al., 2013; Schmid et al., 2003; Woebken et al., 2008), "Candidatus Anammoxoglobus" (Kartal et al., 2007) and "Candidatus Jettenia" (Hu et al., 2012; Quan et al., 2008).

The n-damo process, which is described to be catalyzed by "*Candidatus* Methylomirabilis oxyfera" (Ettwig et al., 2009), creates a unique link between the carbon and nitrogen cycles and might play an important role as a sink for the greenhouse gas methane (Shen et al., 2012, 2015b) by converting it to CO_2. In recent years, the n-damo process has received a great attention and the distribution of n-damo bacteria has been reported in various environments, including freshwater lakes (Kojima et al., 2012), rivers (Shen et al., 2014b), reservoirs (Kojima et al., 2014), wetlands (Hu et al., 2014; Shen et al., 2015b), marine ecosystems (Chen et al., 2014) and paddy soils (Shen et al., 2013; Wang et al., 2012).

As the anammox and n-damo bacteria are active under anaerobic conditions, most studies have focused on the anoxic watery environments (Shen et al., 2014a; Wang et al., 2012). However, few studies have been performed in upland-cropping season of the paddy-upland rotational system, which is an important part of terrestrial ecosystems. In addition, upland-cropping fields have lower soil moisture, soil organic carbon (SOC) and higher oxygen contents than paddy soils even under the same fertilization treatments (Yan et al., 2013). Hence, we hypothesize that the distribution and diversity of both bacterial groups in upland-cropping soils differs from those in paddy soils. As anammox and n-damo bacteria both use nitrite as an electron acceptor, they potentially compete for nitrite (Shen et al., 2015a). It was reported that the concentration of ammonium could affect the competitive relationship in wastewater sludge (Luesken et al., 2011a); thus it would be interesting to unravel the relationship between the anammox and n-damo processes in upland-cropping soil and the major soil traits that may shape the distribution and diversity of both groups of bacteria.

To improve plant nutrition, enhance soil organic matter, and achieve high crop yields, fertilization is an important agricultural practice; however, it also affects soil microbial abundance, activity, and community structure (Gu et al., 2009; Islam et al., 2010). Exploration of the abundance and community structure under different fertilization patterns will help disclosing the effect of fertilizer regimes on N loss and choosing an appropriate fertilization regime for more stable and sustainable agricultural production (Zhao et al., 2014). To date, reports on the influence of fertilizer regimes on anammox bacteria are rare (Zhu et al., 2011b), and for n-damo bacteria, no reports are availabe. As well, it would be interesting to study the vertical distribution of both bacterial groups under different fertilization regimens in upland-cropping fields to evaluate their niche segregation.

Hence, the objectives of the present study were to probe the effects of different fertilization regimes on the distribution and diversity of anammox and n-damo bacteria along soil profiles (0–60 cm layers) and to determine which environmental factors may primarily affect the abundance of both groups at different upland-cropping soil depths.

2. Materials and methods

2.1. Site description and sample collection

This study was carried out in Jintan city, Jiangsu province, China (31°39′N, 119°28′E, 3 m a.s.l). The site has a subtropical monsoon climate, with a mean annual temperature of 15.3 °C and an average annual rainfall of 1063.6 mm (Liu et al., 2015). The soil type is classified as typical Clay loamy Fe-leachic-gleyic-stagnic anthrosol, with a long-term annual winter wheat (*Triticum aestivum* L.)/summer rice (*Oryza sativa* L.) double-cropping system (Liu et al., 2015; Zhao et al., 2014). The field experiment was started in November 2010 and had a completely randomized block design with four replications that had the following fertilizer treatments: an unfertilized control (CK), chemical fertilizer (CF), pig manure plus 50% chemical fertilizer (PMCF), and rice straw plus 100% chemical fertilizer (SRCF). Annually, the CF plots received chemical fertilizers at rates of 240 kg N ha^{-1}, 52.39 kg P ha^{-1}, and 82.98 kg K ha^{-1}, while PMCF plots received chemical fertilizers at rates of 120 kg N ha^{-1}, 26.2 kg P ha^{-1}, and 41.49 kg K ha^{-1}. Pig manure containing 138 kg N ha^{-1}, 78 kg P ha^{-1}, 60 kg K ha^{-1}, 2724 kg organic matter ha^{-1}, and 29.1% moisture was applied in the PMCF treatment. Rice straw in the SRCF treatment contained 113.4 kg N ha^{-1}, 19.8 kg P ha^{-1}, 153 kg K ha^{-1}, 14148 kg organic matter ha^{-1}, and 33.1% moisture (Liu et al., 2015).

Soil sampling was conducted in June 2015 immediately after wheat harvest when the plots were amended with fertilizers for 9 times (2 times per year). In each plot, soil samples were randomly collected from 4 to 5 cores (2.5 cm diameter) of topsoil (0–20 cm) and 3–4 cores of subsoil samples at 2 depths (20–40 cm and 40–60 cm). Soil samples of each layer were homogenized after the removal of plant materials and stones. The collected soil samples were subsequently divided into two portions. One portion was sieved through a 2.0-mm sieve for physicochemical analysis, the other was stored at −80 °C until molecular analysis.

2.2. Chemical analyses

The soil pH was determined after mixing the soil with water at a ratio (soil/water) of 1:2.5; total nitrogen content was determined using the NKD6200 Kjeldahl apparatus (Yihong, Shanghai, China), and organic content was measured using the $K_2Cr_2O_7$ oxidation method. Ammonium (NH_4^+) and nitrate (NO_3^-) concentrations were determined with a flow-injection auto-analyzer (Lachat Instruments, Mequon, WI, USA) after extraction of the soil samples with 2 M KCl. The water content of the soil samples was determined by oven drying overnight at a temperature of 110 °C.

2.3. DNA extraction and PCR amplification

Approximately 0.5 g soil was homogenized (Jxfstprp-24, Shanghai, China) for 300s at 75 hz. DNA was extracted from 0.5 g homogenized soil using a FastDNA spin kit for soil (MP Biomedicals, CA, USA) according to the manufacturer's instructions. The DNA quality was evaluated on a 1% agarose gel and using a bacterial 16S rRNA PCR test. The DNA concentration was measured with a K5600 micro-spectrophotometer (Kaiao, Beijing, China).

As the hydrazine synthase β subunit gene *hzs*B gene has been previously identified as a suitable biomarker for determining anammox bacterial biodiversity and abundance in soils (Wang et al., 2012), we used it in this study. The reported primer pair *hzs*B_396F and *hzs*B_742R was used (Wang et al., 2012). The *pmo*A