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Different roles of rhizosphere effect and long-term fertilization in the activity and community structure of ammonia oxidizers in a calcareous fluvo-aquic soil

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ABSTRACT

Ammonia oxidation is a critical step in the soil nitrogen (N) cycle and can be affected by the application of mineral fertilizers or organic manure. However, little is known about the rhizosphere effect on the function and structure of ammonia-oxidizing bacterial (AOB) and archaeal (AOA) communities, the most important organisms responsible for ammonia oxidation in agricultural ecosystems. Here, the potential nitrification activity (PNA), population size and composition of AOB and AOA communities in both the rhizosphere and bulk soil from a long-term (31-year) fertilizer field experiment conducted during two seasons (wheat and maize) were investigated using the shaken slurry method, quantitative real-time polymerase chain reaction and denaturing gradient gel electrophoresis. N fertilization greatly enhanced PNA and AOB abundance, while manure application increased AOA abundance. The community structure of AOB exhibited more obvious shifts than that of AOA after long-term fertilization, resulting in more abundant AOB phylotypes similar to *Nitrosospira* clusters 3 and 4 in the N-fertilized treatments. Moreover, PNA was closely correlated with the abundance and community structure of AOB rather than that of AOA among soils during both seasons, indicating that AOB play an active role in ammonia oxidation. Conversely, the PNA and population sizes of AOB and AOA were typically higher in the rhizosphere than the bulk soil, implying a significant rhizosphere effect on ammonia oxidation. Cluster and redundancy analyses further showed that this rhizosphere effect played a more important role in shaping AOA community structure than long-term fertilization. Overall, the results indicate that AOB rather than AOA functionally dominate ammonia oxidation in the calcareous fluvo-aquic soil, and that rhizosphere effect and fertilization regime play different roles in the activity and community structures of AOB and AOA.

1. Introduction

Microbial ammonia oxidation is the first and rate-limiting step of the nitrification process and is therefore believed to play a key role in the global nitrogen cycle by influencing the availability of fertilizer, nitrogen leaching of NO_3^- and NO_2^-, and release of N_2O and N_2 gas (Kowalchuk and Stephen, 2001). The rhizosphere, which is the volume of soil adjacent to and affected by plant roots (Sørensen, 1997), plays an active role in plant growth and soil fertility (Rovira, 1969). Because soil microbes are often limited by energy in soils, root exudates such as organic acids, sugars and amino acids may stimulate the growth of microbial populations capable of influencing biogeochemical cycling of C, N, P, and S (Fontaine and Barot, 2005; Rovira, 1969). Fertilization, which is widely used to enhance soil fertility and crop yield, strongly influences soil biochemical and biological properties. The effects of fertilization on the activity and community structure of soil ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA), which are ubiquitous in soils and aquatic environments, has recently been emphasized (Cavagnaro et al., 2008; Shen et al., 2008; Verhamme et al., 2011; Wang et al., 2009). However, most investigations have been conducted on a bulk soil scale or in short-term experiments; therefore, there is still little information available regarding rhizosphere effects on ammonia oxidation in agricultural soils subject to long-term fertilization.

Autotrophic AOB have traditionally been considered the exclusive contributors to ammonia oxidation (Prosser, 1990). However, identification of the key gene responsible for ammonia oxidation (ammonia monooxygenase, *amoA*) in *Crenarchaeota* (Venter et al., 2004) and the isolation of *Nitrosopumilus maritimus* (Könneke

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et al., 2005) demonstrated that archaea also have ammonia-oxidizing activity (Francis et al., 2007; Zhang et al., 2010b). Nevertheless, comparative genomic analyses indicate that AOB and AOA may differ greatly in their physiology and metabolic pathways (Park et al., 2010; Walker et al., 2010). These differences imply that environmental factors such as pH, soil nitrogen nutrients, organic C and plant roots may determine the functional importance of both guilds in natural environments, especially anthropogenically disturbed agricultural ecosystems. Jia and Conrad (2009) reported that changes in the activity of ammonia oxidation were coupled with the abundance and community pattern of AOB, but not AOA. In addition, they found that CO_2 applied as a carbon source was mainly assimilated by AOB rather than AOA owing to ammonia oxidation. The results of this and other studies (Glaser et al., 2010; Shen et al., 2008; Wu et al., 2011) seem to suggest that bacteria rather than archaea dominate ammonia oxidation in near-neutral or alkaline agricultural soils. In contrast, AOA play a more important role than AOB in ammonia oxidation in strongly acidic soils (Yao et al., 2011; Zhang et al., 2011). Phylogenetic analyses of the 16S rRNA sequences of AOB have shown that there are at least seven distinct clusters within the β-subclass of proteobacteria (Kowalchuk et al., 2000; Stephen et al., 1996) and that arable soils are dominated by *Nitrosospira* of clusters 2, 3 and 4 (Innerebner et al., 2006; Phillips et al., 2000; Stephen et al., 1996), especially that of cluster 3, which was nearly ubiquitous in soil environments that have been investigated to date (Fierer et al., 2009; Glaser et al., 2010; Shen et al., 2008).

Mineral N fertilizer often leads to a rapid increase in soil potential nitrification activity (PNA) (Chu et al., 2007), which is correlated with soil pH and AOB abundance (Shen et al., 2008; Wu et al., 2011). However, a significant reduction in soil nitrification and abundance of AOB was observed in a Chinese red upland soil following long-term application of inorganic N fertilizer. Fan et al. (2011a) emphasized that the effects of mineral N fertilizer on ammonia oxidizers in soil vary in response to changes in the soil pH induced by fertilization. The effects of inorganic and organic fertilizers on the AOA community are less well studied and appear to be incongruent (Schauss et al., 2009; Shen et al., 2008; Wang et al., 2011), which may in part be due to mixotrophic or heterotrophic metabolism (Walker et al., 2010). Rice plantations have a greater effect on the abundance of the *amoA* gene in the rhizosphere than in the bulk soil, implying a possible rhizosphere effect on the soil nitrification process (Hussain et al., 2011). In another study, increases in AOB community size were commonly stronger in bulk soil than in the rhizosphere following application of $[NH_4]_2SO_4$. Glaser et al. (2010) suggested that there was fierce competition among plants, nitrifiers and other N-assimilating microorganisms for NH_4–N in the rhizosphere. Moreover, suppression of soil nitrification has been found to occur naturally in the rhizosphere via nitrification inhibitors produced by plants (Subbarao et al., 2006, 2007). In the same experimental field tested in this study, rhizosphere effects played an important role in mediation of the degree to which long-term fertilization affects the soil microbial community and extracellular enzyme activities (Ai et al., 2012). However, the specific effects of these factors on the nitrification activity and AOB and AOA communities remain unclear.

Long-term field fertilization experiments may provide profound insight into how anthropogenic disturbances lead to changes in soil properties such as pH, organic C, NH_4^+–N and NO_3^-–N, which in turn influence the function and structure of AOA and AOB communities. The present study was conducted to examine the differences in nitrification activity and AOB and AOA communities between rhizosphere and bulk soil, and how each responds to long-term fertilizations (31-year) during two seasons (wheat and maize). Quantitative real-time polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) were used to estimate AOB and AOA abundance and community structure, respectively. We hypothesized that rhizosphere and bulk soils would have different ammonia oxidizer communities with distinct nitrification activities after long-term fertilization, and that rhizosphere effects would mediate the influence of fertilization on the function and structure of soil AOB and AOA communities.

2. Material and methods

2.1. Field design and sampling

A long-term field fertilizer experiment was initiated in 1979 at Malan Farm (37°55′N, 115°13′E), Hebei Province, China, where wheat-maize rotation is the common cropping system. This region has a temperate and monsoonal type climate with an annual average temperature and precipitation of 12.6 °C and 490 mm, respectively. The experimental field contains calcareous fluvo-aquic soil, which is widespread in the North China Plain. At the beginning of the experiment, the soil had a pH (H_2O) of 7.8, 1.1% organic matter, 1.8 g kg^{-1} total N, and 5.0 and 87.0 mg kg^{-1} of available P and K, respectively. Six treatments (three replicates each) were implemented in 18 plots (12 m × 6.7 m) under a rotation of winter wheat (*Triticum aestivum* L.) and summer maize (*Zea mays* L.) (Ai et al., 2012). Treatments consisted of soil without fertilizer (control, CK), fertilizer N (N), fertilizer N and P (NP), fertilizer N, P and K (NPK), organic manure (M), and organic manure plus fertilizer N, P and K (MNPK). For NPK treatment, fertilizer N, P and K were applied in the form of urea (300 kg N ha^{-1} per year), superphosphate (150 kg P_2O_5 ha^{-1} per year) and potassium chloride (150 kg K_2O ha^{-1} per year), respectively, while no PK or K was applied for the N and NP treatments, respectively. All fertilizer P and K and Manure were applied once as basal dressing during wheat season. Manure and mineral fertilizers were evenly broadcast onto the soil surface and immediately incorporated into the plowed soil (0–20 cm depth) by tillage before sowing. For the N fertilizer, 20% of the urea was used as a basal dressing before sowing wheat, 30% was top-dressed at the reviving stage of wheat, and 50% was top-dressed at the 10-leaf stage of maize. The organic manure (3.75×10^4 kg ha^{-1}) consisted of straw bedding impregnated with liquid and solid horse manure, which had 120 g kg^{-1} organic matter, 5.0 and 2.2 g kg^{-1} total N and P, respectively, and about 50% water content.

Soil samples were collected during the reproductive stages of wheat and maize in early May 2010 and late August 2010, respectively, when the rhizosphere effects tend to be most pronounced (Cheng et al., 2003). Rhizosphere soil was operationally defined as soil adhering to the total roots after gentle shaking, while bulk soil was defined as unvegetated soil adjacent to the plants. The whole plant with their roots was extracted from soil and, after shaking off the loosely adhering soil, the tightly adhering soil (i.e. rhizosphere soil) was carefully collected. The unvegetated soil cores (5 cm diameter) adjacent to the plants (i.e. bulk soil) were sampled at depth 0–20 cm. In order to obtain the enough rhizosphere soil for multiple assays, twenty plants were randomly selected from each plot, and these rhizosphere soils were pooled to form one composite sample. Correspondingly, one composite bulk soil consisting of twenty cores was taken from each plot. Thus, six composite samples of each treatment were collected per sampling time, and a total of 72 composite samples were taken for two consecutive seasons. The fresh samples were placed immediately on ice and transported to the laboratory. Plant roots were removed by passing the sample through a 2-mm mesh sieve, and aliquots of the samples were then stored at room temperature until chemical