

Ammonia-oxidizing bacteria and archaea in different paddy soils of China

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Abstract

Ammonia oxidation plays an important role in global nitrogen cycle. Little information is available on ammonia oxidation microorganisms in paddy soils. The abundance and composition of ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) were investigated by real-time PCR and denaturing gradient gel electrophoresis (DGGE) approaches based on *amoA* genes in selected Chinese soils from Chongqing (CQ, Southwest of China), Honghu (HH, Central China) and Panjin (PJ, Northeast of China) with 10 weeks pot experiments. Little changes in the abundance and community structure of both AOA and AOB were detected among the treatments (without rice, rhizosphere and bulk soil) of the same region paddy soil. The AOB population sizes in CQ paddy soil were lower than those in HH and PJ paddy soils, while the AOA population sizes in CQ and HH paddy soils were lower than those in PJ paddy soil. The *amoA* gene copy numbers of AOA were more than those of AOB in all treatments of the three paddy soils. These results suggested that different region paddy soils determined the population and composition of AOB and AOA.

Key Words

Ammonia oxidizing bacteria and archaea, *amoA* gene, paddy soil, rhizosphere, wetland, nitrogen cycling

Introduction

Ammonia oxidation is the first and rate-limiting step of nitrification and plays a key role in global nitrogen cycle. This process was typically thought to be carried out by ammonia-oxidizing bacteria. Until recently, metagenomic studies demonstrated the potential of ammonia oxidation by mesophilic crenarchaea (Leininger *et al.* 2006; He *et al.* 2007; Prosser and Nicol 2008). Increasing evidences showed that ammonia-oxidizing archaea (AOA) can be detected in wide habitats, such as marine water columns, sediments, estuaries and soils, and may be another major microbial group involved in the ammonia oxidation besides ammonia-oxidizing bacteria (AOB). Extensive researches have paid attention to both AOB and AOA in terrestrial systems, but most of them concern with upland soils, and little information is available in wetland soils. AOB and AOA are aerobes, so it is curious to know their distribution in the flooded anaerobic environment.

Irrigated paddy field is a typical wetland habitat in terrestrial ecosystems. Owing to rice roots releasing O₂, the paddy soil forms a unique environment: the rhizosphere is partially oxic, while the bulk soil is anoxic. The coupling of nitrification and denitrification is favoured by the existence of oxic/anoxic interfaces in paddy soils (Arth *et al.* 1998). As the initial and rate-limiting step of the nitrification and denitrification, ammonia oxidation should even more be studied in paddy fields. China is one of the major rice growers in the world, and has 26% of its total cultivated land growing rice. The suitable environment is provided to study the biogeochemistry and microbiology of the rice paddy. Some studies have shown the abundance and community structure of sulfate reducing prokaryotes, methanotrophs and other important microbial groups (Zheng *et al.* 2008; Liu *et al.* 2009). However, limited information is available on ammonia-oxidizing organisms in paddy soils, especially in different region paddy soils. The aim of this study was to investigate the changes of ammonia-oxidation microbial population size and community structure in different factions (rhizosphere and bulk) of paddy soils collected from different regions of China.

Materials and methods

Soil samples

Paddy soil samples were collected from three different regions of China: CQ, purple paddy soil with wheat-rice rotation from Chongqing city (N29.83°, E106.43°), southwest of China; HH, alluvial paddy soil with rapeseed-rice rotation from Honghu city (N29.48°, E113.27°), Hubei Province, central China; PJ, coastal saline paddy soil with single-season rice from Panjin city (N41.12°, E122.06°), Liaoning Province, northeast of China. Some soil characteristics were listed in Table 1. All soil samples were air dried and sieved through a 2 mm mesh and stored at room temperature for subsequent pot experiments.

Table 1. Selected properties of the original soil samples

Region	pH (H ₂ O)	organic matter (g kg ⁻¹)	alkali-hydrolysable N (mg kg ⁻¹)	available phosphorus (mg kg ⁻¹)	available potassium (mg kg ⁻¹)
CQ	8.2	26.4	173	8.20	224.82
HH	7.6	23.0	171	1.17	220.52
PJ	8.1	13.6	77.0	5.92	221.97

Pot experiment

PVC pots (18 cm diameter, 20 cm height) were used to load the soil samples. Each soil was set up two treatments with triplicate: one treatment was rice planted and the other no rice planted. The treatment growing the rice was divided into bulk soil and rhizosphere with a root bag (37 µm nylon mesh, 5 cm diameter, 10 cm height, and one plant per bag). Each pot was filled with 3 kg of soil, including 0.2 kg soil in the root bag. The pots were maintained waterlogged until harvest. Rice seeds were pretreated and germinated. After three weeks by water culture, uniform seedlings were transplanted into the pots. Then, the rice plants were cultivated for 10 weeks in a greenhouse.

Sample collection

At the tenth week of the pot incubation, rice plants were harvested and meanwhile the rhizosphere and bulk soils were sampled from each treatment. All the samples were divided into two parts: one was stored at 4°C for chemical analysis and the other frozen at -80°C for subsequent DNA extraction and molecular analysis. The soil chemical properties at the end of the pot experiments are listed in Table 2.

Table 2. Selected chemical properties of the paddy soils at the end of the pot experiment

Region	Treatment*	pH	NH ₄ ⁺ -N (mg/kg)	NO ₃ ⁻ -N (mg/kg)
CQ	n-CQ	7.4±0.1 c	370±6 f	0.91±0.01 cd
	r-CQ	7.0±0.0 a	189±27 c	0.91±0.02 cd
	b-CQ	7.3±0.0 b	323±21 e	0.72±0.05 bc
HH	n-HH	7.4±0.0 c	253±12 d	0.87±0.01 cd
	r-HH	7.0±0.1 a	110±16 b	0.88±0.07 cd
	b-HH	7.2±0.1 b	216±23 c	0.82±0.07 cd
PJ	n-PJ	7.7±0.1 d	268±22 d	1.00±0.05 d
	r-PJ	7.0±0.1 a	45.1±13.2 a	0.65±0.16 b
	b-PJ	7.5±0.1 c	187±16 c	0.50±0.12 a

* Treatment: without the rice with n, rhizosphere with r and bulk soil with b. Values are mean±SD (n = 3). Values within the same column followed by the same letter are not significantly different at P < 0.05.

Molecular analyses

DNA was extracted from 0.5 g frozen paddy soil samples using UltraClean™ Soil DNA Isolation Kit following the manufacturer's protocol. The real-time PCR quantification analysis of *amoA* genes was conducted on an iCycler iQ5 Thermocycler, and the PCR-DGGE analysis of *amoA* fragments was performed as described before (He *et al.* 2007; Shen *et al.* 2008).

Results

Abundance of AOB and AOA in paddy soils

The *amoA* gene copy numbers of AOB and AOA were assessed using quantitative PCR. The bacterial *amoA* gene copy numbers in soils ranged from 1.41×10⁴ to 2.87×10⁵ copies per gram dry soil, while the archaeal *amoA* gene copy numbers ranged from 3.95×10⁶ to 2.87×10⁷ copies per gram dry soil (Figure 1). No significant differences in the abundance of AOB and AOA were detected among the treatments (without rice, rhizosphere and bulk soil) in each paddy soil (Figure 1). Within the AOB abundance, the AOB population size in CQ paddy soil was remarkably lower than that in HH and PJ paddy soil. While for the AOA abundance, PJ paddy soil was significantly higher than CQ and HH paddy soils. Ratios of AOA to AOB *amoA* copy numbers were ranged from 22.9 to 667 in all treatments, indicating that the AOA population size among the treatments in three region paddy soils was higher than AOB.

DGGE profiles of AOB and AOA communities in different region paddy soils

The DGGE patterns of AOB indicated clear variations among different region paddy soils. However, the band patterns of AOB were similar in the same region paddy soil, regardless of without rice or rhizosphere or bulk soil. The correspondence analysis (CA) ordination diagram of AOB communities is shown in Figure 2,

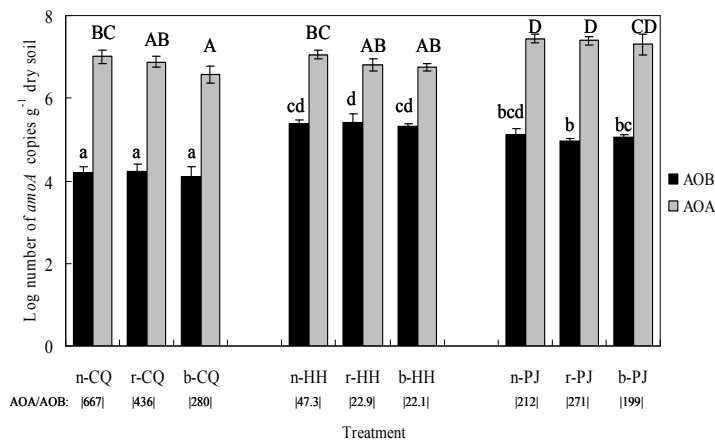


Figure 1. Abundances of AOB and AOA in different region paddy soils (CQ, HH and PJ) with the treatments of without rice (with n), rhizosphere (with r) and bulk (with b). Values are mean±SD (n = 3). Within AOB or AOA *amoA* copy numbers, values followed by the same letter are not significantly different at P < 0.05. Ratios of AOA to AOB *amoA* copy numbers are shown at the bottom with each treatment.

and individual points on the two-dimensional biplot represented AOB communities with different treatment in the three paddy soils. Sixty three percent of the variance in community structure was explained by four eigenvectors. The first and second eigenvectors, plotted on the x and y axes, explained 32% and 18% of the variation, respectively; thus, 50% of the cumulative variation was explained. The difference between the communities with respect to the first and second eigenvectors was the distance between the points representing the communities on the ordination diagram. A comparison of the distances showed that the AOB communities from the same paddy soil had the most similar structure. Take CQ paddy soil as example, the AOB communities associated with the treatments of without rice, rhizosphere and bulk were separated less than 0.5 standard deviation. However, the AOB communities from the different paddy soils were the most dissimilar and were separated by approximately 1 or more than 1 standard deviation. The CA supported visual observation of the DGGE gel, indicating that the different treatments (without rice, rhizosphere and bulk soil) did not influence the species data while the different region paddy soils did affect community structure.

The DGGE results of AOA also revealed that band patterns had little change among the treatments of without rice, rhizosphere and bulk soil in the same region paddy soil. There were also obvious differences among the three region paddy soils in DGGE patterns. From the CA ordination diagram of AOA communities, the treatments from the same paddy soil were clustered together and three distinct clusters were observed according to the three region paddy soils (Figure3). Eighty seven percent of the variance in community structure was explained by four eigenvectors, thus the majority of the changes in community structure can be attributed to the different region paddy soils.

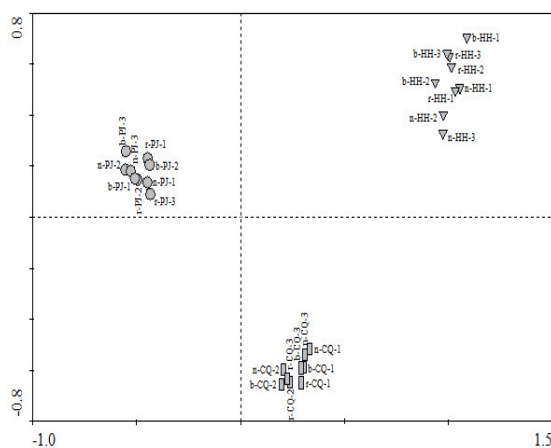


Figure 2. Correspondence analysis (CA) ordination diagram of AOB communities with the treatments of without rice (with n), rhizosphere (with r) and bulk (with b) in different region paddy soils (CQ, HH and PJ) generated by the DGGE profiles. Eigenvectors for first and second ordination axes (x and y axes) explain 32% and 18% of the variation, respectively.

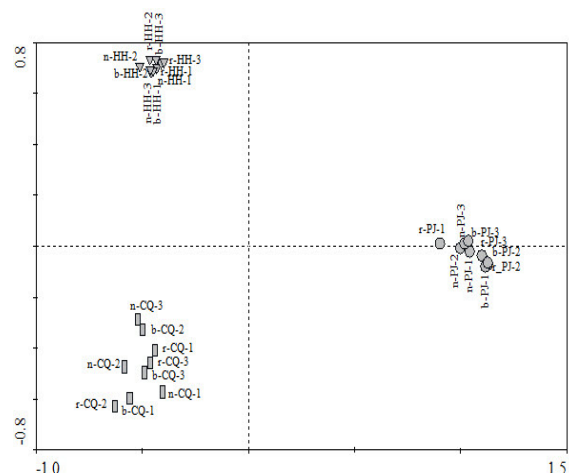


Figure 3. Correspondence analysis (CA) ordination diagram of AOA communities with the treatments of without rice (with n), rhizosphere (with r) and bulk (with b) in different region paddy soils (CQ, HH and PJ) generated by the DGGE profiles. Eigenvectors for first and second ordination axes (x and y axes) explain 51% and 23% of the variation, respectively.

Conclusion

This study found that both AOB and AOA abundance and community composition existed few changes among the treatments (without rice, rhizosphere and bulk soil) in each paddy soil, but distinct differences were observed among the three paddy soils, suggesting different region paddy soils affected the population and composition of AOB and AOA. We also highlighted the need to further study the AOB and AOA growth and activity in paddy soils to assess their contribution in N cycle in paddy soils, together with denitrification and annamox processes.

Acknowledgements

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