Soil type determines the abundance and community structure of ammonia-oxidizing bacteria and archaea in flooded paddy soils

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Abstract

Purpose Ammonia oxidation plays an important role in global nitrogen cycle. However, little information is available on ammonia oxidizers in paddy soils. This study aimed to understand the controlling factors of ammonia oxidizers in the paddy soils.

Materials and methods Three types of paddy soils were collected from southwest [Chongqing City (CQ)], central [Honghu City (HH)], and northeast [Panjin City (PJ)] of China, respectively. The soils were cultured with unplanted and rice-planted pot experiments for 10 weeks. The abundance and composition of ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) of the paddy soil samples were investigated by real-time polymerase chain reaction and denaturing gradient gel electrophoresis approaches based on *amoA* genes.

Results and discussion In this study, minor changes in the abundance and community structure of both AOB and AOA were observed among the treatments of unplanted and rhizosphere and bulk of rice-planted soil samples in the

same type of paddy soil. The AOB population sizes in CQ were lower than those in HH and PJ, while the AOA in CQ and HH were lower than those in PJ. The *amoA* gene copy numbers of AOA were more abundant than those of AOB in all treatments of the three paddy soils. The community structure of both AOB and AOA in all paddy soils was different from each other.

Conclusions The population size and community structure of AOB and AOA in the flooded paddy soils were mainly determined by the soil types, irrespective of rice-planted or unplanted.

Keywords Ammonia-oxidizing archaea · Ammonia-oxidizing bacteria · Denaturing gradient gel electrophoresis · Nitrogen cycle · Paddy soil · Real-time PCR

1 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 (AOB). Until recently, metagenomic studies demonstrated the potential of ammonia oxidation by mesophilic Crenarchaeota and revealed the presence of ammonia monooxygenase gene derived from the Crenarchaeota (Venter et al. 2004; Könneke et al. 2005; Treusch et al. 2005). Ammonia monooxygenase (AMO), the key functional enzyme responsible for the autotrophic ammonia oxidation, is encoded by the genes *amoA*, *B*, and *C*. Increasing evidence showed that ammonia-oxidizing archaea (AOA) can be detected in various habitats, such as marine water columns (Francis et al. 2005; Wuchter et al. 2006), sediments (Beman and

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Francis 2006), estuaries (Mosier and Francis 2008), hot springs (Weidler et al. 2007), and soils (Leininger et al. 2006; He et al. 2007; Shen et al. 2008; Schauss et al. 2009) and may be another major microbial group involved in the ammonia oxidation besides AOB.

Irrigated paddy field is a typical wetland habitat in terrestrial ecosystems, which has served as a useful model for biogeochemistry and microbiology studies. Owing to rice roots releasing O₂, the paddy soil forms a unique environment: the rhizosphere is partially oxic, while the bulk soil is anoxic (Revsbech et al. 1999). This may lead to the different distribution of AOB and AOA communities in rhizosphere and bulk soil. However, limited information is available on ammonia-oxidizing organisms in paddy soils, especially in different types of paddy soils. Some researchers have proposed that soil type is the primary determinant of the bacterial community composition in arable soils (Girvan et al. 2003), but no information is available about the effect of soil types on AOB and AOA abundance and community structure in paddy soils.

In this study, we hypothesized that: (1) rice planting could result in the shifts of AOB and AOA population size and community structure in the flooded paddy soils; (2) different types of paddy soils could have different abundance and community structure of ammonia oxidizers. In order to test these hypotheses, we used three types of paddy soils in pot experiments with three treatments of unplanted, rhizosphere, and bulk of rice-planted. Real-time polymerase chain reaction (qPCR) and denaturing gradient gel electrophoresis (DGGE) approaches based on *amoA* genes were used to characterize the abundance and community composition of AOB and AOA in the paddy soil samples.

2 Materials and methods

2.1 Original soil samples and pot experiment

Original paddy soil samples (0–20 cm) were collected from three different regions of China including purple paddy soil from Chongqing City (CQ, N29.83°, E106.43°), southwestern China; alluvial paddy soil from Honghu City (HH, N29.48°, E113.27°), Hubei Province, central China; and coastal saline paddy soil from Panjin City (PJ, N41.12°, E122.06°), Liaoning Province, north-eastern China. The pH values of these paddy soils were 8.2, 7.6, and 8.1, respectively.

Polyvinyl chloride pots (18 cm diameter, 20 cm height) were used in this experiment. Each soil was prepared for two treatments with three replicates: one treatment was rice-planted and the other unplanted. The

rice-planted treatment was further divided into bulk and rhizosphere compartments 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 air-dried soil including 0.2 kg soil in the root bag. The pots were maintained for waterlogging with deionized water (water depth 3 to 4 cm above the soil surface) until harvest.

Rice seeds (*Oryza sativa* L.) cv. Jiahua-1 were pretreated and germinated as described in previous paper (Chen et al. 2008). After 3 weeks, uniform seedlings were picked out from water culture and transplanted into the pots. Then, the rice plants were cultivated for 10 weeks in a greenhouse where the temperature was kept at 25°C during the day and 16°C during the night, with 14/10 h light/dark cycle and 70% relative humidity.

2.2 Sample collection

The rice plants were harvested after 10 weeks, and soil samples were collected from each treatment. All the samples were divided into two parts: one was stored at 4°C for chemical analyses and the other frozen at –80°C for subsequent DNA extraction and molecular analyses.

2.3 Chemical analyses

Soil pH of the pot experiments was measured in situ with an automatic redox potential device (FJA-3 type, China) before sample collection. Soil ammonium and nitrate contents were extracted from fresh soil samples with 2 M KCl and determined by a continuous flow analyzer (SAN++, Skalar, Holland).

2.4 Molecular analyses

DNA was extracted from 0.5 g frozen paddy soil sample using UltraClean™ Soil DNA Isolation Kit (MoBio Laboratory, USA). The extracted DNA was checked on 0.8% agarose gel, and the concentration was determined with spectrophotometry (NanoDrop, USA).

The quantification of *amoA* gene copies was conducted on an iCycler iQ5 Thermocycler (Bio-Rad, USA) with the fluorescent dye SYBR-Green I (Zhang et al. 2009). Amplification was performed in 25-µl reaction mixtures, including 12.5 µl SYBR® Premix Ex Taq™ (Takara Biotechnology, Japan), 1 µl bovine serum albumin (25 mg ml^{–1}), 0.5 µl each primer (10 µM) listed in Table 1 and 2 µl DNA template (1–10 ng). The standards and the DNA samples were performed on the same plate. The data were analyzed with the iQ™5 software (Bio-Rad, USA).

The PCR for DGGE analysis was performed with the primer pairs containing a 40 base GC-clamp in one primer

Table 1 Primers used for the real-time PCR and PCR-DGGE analyses

Target group	Primer	Sequence(5'-3')	Length of amplicon (bp)	Reference
AOB	amoA1F	GGGGTTTCTACTGGTGGT	491	Rotthauwe et al. 1997
	GC ^a -amoA1F			
AOA	amoA2R	CCCCTCKGSAAAGCCTTCTTC	635	Francis et al. 2005
	Arch-amoAF	STAATGGTCTGGCTTAGACG		
	Arch-amoAR	GCGGCCATCCATCTGTATGT		
	GC ^b -Arch-amoAR			

^a(CGCCCCGCGCGCCCCGCGCCCGTCCCGCGCCCCCGCCCCG)^b(CGCCCCGCGCGCCCCGCGCCCGGCCCGCCCGCCCCGCCCCG)

(see Table 1). The 50- μ l reaction mixtures consisted of 5 μ l 10 $\times$ PCR buffer (Mg²⁺ plus), 4 μ l 2.5 mM dNTPs, 0.5 μ l *Ex Taq* HS polymerase (5U μ l⁻¹, Takara Biotechnology, Japan), 1 μ l of each primer, and 2 μ l DNA template (1–10 ng). A touchdown PCR strategy was used for amplification of bacterial and archaeal *amoA* gene fragments. The thermal cycle parameters of the touchdown PCR employed were as follows: an initial denaturation step at 95°C for 3 min; followed by a touchdown procedure (including 30 s at 95°C, annealing for 45 s at temperatures decreasing from 60°C to 55°C during the first ten cycles, and ending with an extension step at 72°C for 1 min); and then 30 additional cycles of 94°C for 30 s, 55°C for 45 s, 72°C for 1 min; and finally, an extension of 10 min at 72°C.

DGGE analysis was carried out in a DCode universal mutation detection system (Bio-Rad Laboratories, Hercules, USA). The PCR products of bacterial and archaeal *amoA* genes were separated on a 6% (w/v) polyacrylamide gel, using a denaturing gradient of 40–65% and 25–50% (100% denaturant contains 7 M urea and 40% formamide), respectively. After running, the gels were stained with 1:10,000 SYBR Gold nucleic acid gel stain (Invitrogen 20 Molecular Probes, Eugene, USA) for 30 min and then scanned by a GBOX/HR-E-M (Syngene, UK). The results were analyzed using the Quantity One (Bio-Rad Laboratories) software. Correspondence analysis (CA) for community ordination was conducted by CANOCO for

Windows version 4.5 using relative band intensity data from the Quantity One analysis results. The data were log (x+1)-transformed before CA.

2.5 Statistical analysis

The data obtained from this study were statistically analyzed using ANOVA with SPSS for Windows. The significant differences were detected at level of $P<0.05$, and S-N-K test was performed to check for quantitative differences between different treatments.

3 Results

3.1 Soil chemical properties

After 10 weeks of cultivation, pH values in all soils were close to neutral ranging from 7.0 to 7.7 (Table 2). Soil pH values were varied among the treatments from the same paddy soil and found in order of unplanted>bulk rice-planted>rhizosphere rice-planted. With different types of paddy soils, the pH values of the treatments of PJ unplanted and bulk rice-planted soils were higher than those of CQ and HH, but for the rhizosphere rice-planted treatments, there were no significant differences among the soils and the pH values were 7.0. The contents of NH₄⁺-N were

Table 2 Chemical properties of the paddy soils at the end of 10-week pot experiment

Region	Treatment ^a	pH	NH ₄ ⁺ -N (mg kg ⁻¹)	NO ₃ ⁻ -N (mg kg ⁻¹)
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

^a Treatment: n unplanted, r and b rhizosphere and bulk rice-planted samples, respectively. Values are mean±SD ($n=3$). Values within the same column followed by the different letters are significantly different ($P<0.05$)

significantly different among the treatments of the same paddy soil (see Table 2). Likewise, there also existed changes in soil $\text{NH}_4^+\text{-N}$ content among the different types of paddy soils. However, soil $\text{NO}_3^-\text{-N}$ content did not vary much among the treatments except for the PJ paddy soil (see Table 2).

3.2 Abundance of AOB and AOA in paddy soils

The *amoA* gene copy numbers of AOB and AOA were assessed using qPCR. The bacterial *amoA* gene copy numbers in the soils ranged from 1.41×10^4 to 2.87×10^5 copies per gram dry soil, while the archaeal *amoA* gene copy numbers ranged from 3.95×10^6 to 2.87×10^7 copies per gram dry soil (Fig. 1). No significant differences in the abundance of AOB and AOA were detected among the treatments (unplanted, rhizosphere, and bulk of rice-planted) in each paddy soil (see Fig. 1). However, the AOB population size of the CQ soil was lower than those of HH and PJ. The AOA abundance in PJ was higher than those of CQ and HH. The ratios of AOA to AOB *amoA* gene copy numbers ranged from 22.9 to 667 in all treatments, indicating that the AOA population sizes among the treatments in the three paddy soils was higher than those of AOB.

3.3 DGGE profiles of AOB and AOA communities in the three types of paddy soils

The DGGE patterns of AOB and AOA showed little changes in the same type of paddy soil, regardless of the treatments of rice-planted or unplanted (Figs. 2a and 3a). However, some variations were observed among the

different types of paddy soils in the DGGE patterns (see Figs. 2a and 3a). The CA ordination diagram of AOB and AOA communities are shown in Figs. 2b and 3b. The individual points on the two-dimensional biplot represented the communities of the different treatments in three types of paddy soils. The difference between the communities was the distance between the points on the ordination diagram (Yang and Crowley 2000). A comparison of the distances showed that both AOB and AOA communities from the same paddy soil had the most similar structure. However, the communities from the different types of paddy soils were the most dissimilar and separated into three distinct clusters (see Figs. 2b and 3b). The CA supported the visual observation of DGGE patterns, indicating that the different treatments did not influence AOB and AOA composition, while the different types of paddy soils did affect AOB and AOA community structure.

4 Discussion

In this study, we applied qPCR to quantify the abundance of AOB and AOA based on *amoA* genes in different types of paddy soils. Both AOB and AOA were detected in large numbers in all paddy soils of this study. Although rice roots could release oxygen through aerenchymatous tissue and some soil chemical properties in the rice-planted rhizosphere were different from the other treatments, we did not find that the abundance of AOB in the rhizosphere differed from those in the bulk rice-planted and unplanted treatments for the same paddy soil. These results were consistent with those of Chen et al. (2008) findings, but

Fig. 1 The abundances of ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) in different types of paddy soils (CQ, HH, and PJ) with the treatments of unplanted (n), rhizosphere (r), and bulk (b) of rice-planted samples. Error bars indicate standard

deviations ($n=3$). The different letters indicate significant differences ($P<0.05$). Ratios of AOA to AOB *amoA* gene copy numbers are shown at the bottom of each treatment

Fig. 2 (a) Denaturing gradient gel electrophoresis (DGGE) patterns of ammonia-oxidizing bacteria (AOB) with the treatments of unplanted (n), rhizosphere (r) and bulk (b) of rice-planted samples (CQ, HH and PJ). (b) Correspondence analysis (CA) ordination diagram of AOB communities generated by the AOB DGGE patterns

Fig. 3 a Denaturing gradient gel electrophoresis (DGGE) patterns of ammonia-oxidizing archaea (AOA) with the treatments of unplanted (n), rhizosphere (r) and bulk (b) of rice-planted samples (CQ, HH and PJ). b Correspondence analysis (CA) ordination diagram of AOA communities generated by the AOA DGGE patterns

different from those of Nicolaisen et al. (2004) which found the abundance of AOB in rhizosphere was higher than that in the bulk soil. The reason for this inconsistency may be due to the near-neutral pH values of these three paddy soils which would favor the AOB growth. The growth of AOB was considered to be optimal at neutral to alkaline pH values (Prosser 1989). No significant differences were found in archaeal *amoA* gene copy number among the treatments for the same paddy soil despite of the lower oxygen concentrations in the bulk of rice-planted and unplanted treatments. Numerous studies have also reported high copy numbers of archaeal *amoA* gene in low oxygen environment (Park et al. 2006; Lam et al. 2007; Santoro et al. 2008). However, with different types of paddy soils of this study, there were some changes in the population size of both AOB and AOA. These results were similar to the previous study that three different ecological region soils

were characterized by a diverse population of AOB counted with the MPN-Griess method (Yuan et al. 2005). However, little information on the changes of AOA populations in the different soil types is reported. Our study showed that the AOA populations were different in different types of paddy soils, suggesting that soil types affected the abundance of AOA. Likewise, the high ratios of AOA to AOB were found in all samples, which were consistent with many related studies previously conducted in different ecosystems (Wuchter et al. 2006; He et al. 2007; Lam et al. 2007; Mincer et al. 2007; Shen et al. 2008; Ying et al. 2010), providing the evidence of AOA potential contribution to nitrification. However, recent studies also found that AOB *amoA* copy numbers were greater than those of AOA in some estuary and high altitude permafrost soils (Santoro et al. 2008; Zhang et al. 2009), and bacteria rather than archaea functionally dominate ammonia oxidation in

pasture soils (Di et al. 2009, 2010). Thus, it is necessary to further study the function of AOB and AOA in the paddy soils.

The DGGE and CA ordination gave the intuitive results of AOB and AOA community structure. In our study, little difference in the community composition of AOB and AOA was detected among the treatments for each paddy soil. The AOB results were similar to those of Chen et al. (2008), while the AOA results were consistent with the recent research findings of Wang et al. (2009). However, the community shifts were detected among the different paddy soils, suggesting that both AOB and AOA compositions were mainly determined by the soil types. It was found that historical contingencies including soil type were the dominant factor driving variations in soil bacterial diversity (Ge et al. 2008).

In conclusion, our results showed that both AOB and AOA abundance and community structure changed little among the unplanted and rhizosphere and bulk of rice-planted treatments for the same paddy soil, but significant differences were observed among the three paddy soils, suggesting that different soil types affected the population and composition of AOB and AOA communities.

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