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Preliminary study on the distribution of ammonia oxidizers and their contribution to potential ammonia oxidation in the plant-bed/ditch system of a constructed wetland

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Abstract
Purpose Ammonia oxidation—as the rate-limiting step of nitrification—has been found to be performed by both ammonia-oxidizing archaea (AOA) and bacteria (AOB). However, how ammonium content and oxidation–reduction status regulate the distribution of ammonia oxidizers in constructed wetlands and their contribution to potential ammonia oxidation rate are still in dispute. This study aimed to explore the effects of ammonium content and oxidation–reduction status on the abundances of AOA/AOB and examine the contributions of AOA and AOB populations to ammonia oxidation rates in the plant-bed/ditch system of a constructed wetland.
Materials and methods Sampling was carried out in the plant-bed/ditch system of the Shijiuyang Constructed Wetland, China. Three plant-bed soil cores were collected using a soil auger and sampled at depths of 0, 20, and 50 cm in 5-cm increments. Five ditch surface sediments (0–5 cm) were collected along the water flow direction. The abundances of AOA and AOB were investigated by quantitative polymerase chain reaction based on *amoA* genes. The potential ammonia oxidation rate was determined using the chlorate inhibition method.
Results and discussion The results showed that AOA outnumbered AOB in the plant-bed surface soil which had lower ammonium content (4.67–7.63 mg kg^{-1}), but that AOB outnumbered AOA in the ditch surface sediment which had higher ammonium content (14.0–22.9 mg kg^{-1}). Ammonium content was found to be the crucial factor influencing the relative abundances of AOA and AOB in the surface samples of the plant-bed/ditch system. In the deep layers of the plant bed, AOA abundance outnumbered AOB, though much lower oxidation–reduction potential occurred along the water flow direction. Thus, the oxidation–reduction potential may be another factor influencing the distributions of AOA and AOB in the deep layers of the plant bed without significant difference in ammonium content ($p<0.05$). Moreover, the potential ammonia oxidation rate was significantly dominated by AOB rather than AOA in the plant-bed/ditch system.
Conclusions The high ammonium content in the ditch sediment likely favored AOB. AOA seemed to persist more readily even under low oxidation–reduction potential in the deep layers of the plant bed. Ammonium content and the oxidation–reduction potential were important parameters influencing the distribution of AOA and AOB in the plant-bed/ditch system of Shijiuyang Constructed Wetland. AOB contributed more to ammonia oxidation than AOA, both in the plant-bed soils ($r=0.592$, $p=0.0096$) and in the ditch sediments ($r=0.873$, $p=0.0002$).

Keywords Ammonia-oxidizing archaea · Ammonia-oxidizing bacteria · Plant-bed/ditch system · Potential ammonia oxidation rate

1 Introduction

The oxidation of ammonium to nitrite by chemolithotrophic microbes is an important process that determines the fate of nitrogen (N) in aquatic ecosystems. Until recently, it had been attributed to bacteria encoding the α subunit of ammonia monooxygenase (*amoA*) (ammonia-oxidizing bacteria,

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AOB), specifically members of β-Proteobacteria (Head et al. 1993) and γ-Proteobacteria (Ward and O'Mullan 2002). However, recent metagenomic analysis of archaea (Schleper et al. 2005) and the isolation of ammonia-oxidizing Crenarchaeote *Nitrosopumilus maritimus* (Könneke et al. 2005) have proven the existence of ammonia-oxidizing archaea (AOA) and their critical role for contributions to the N cycle based on a series of surveys in marine and terrestrial ecosystems (Francis et al. 2005; Beman and Francis 2006; Leininger et al. 2006; Wuchter et al. 2006; Beman et al. 2007; Coolen et al. 2007; Lam et al. 2007).

Ammonium has been described as among the most important environmental drivers for selection of AOA and AOB, and was characterized well in marine water columns with low ammonium concentration (<0.03–1.0 μM) (Wuchter et al. 2006; Coolen et al. 2007; Lam et al. 2007). The previous studies showed that archaeal *amoA* gene copy numbers were higher than bacterial *amoA* in the North Atlantic and North Sea (Wuchter et al. 2006), Monterey Bay and Hawaii (Mincer et al. 2007), and the Japan Sea (Nakagawa et al. 2007). However, the abundance of AOB was much higher than AOA in wetland sediments with high ammonium content (14.2–147.5 mg kg^{-1}) (Herrmann et al. 2011; Wang et al. 2011). The AOB also dominated numerically over the AOA in a drained peat soil (NH_4^+-N: 18.0–51.0 mg kg^{-1}) (Andert et al. 2011) and in the vertical profile of a natural wetland soil (NH_4^+-N: 18.0–118.0 mg kg^{-1}) (Hofferle et al. 2010).

On the other hand, the range of dissolved oxygen (DO) levels might be among the most important parameters at the sites where AOA and AOB have been detected. Studies have shown that different oxidation–reduction potentials in soils and sediments greatly change the activity of AOB (Bodelier et al. 1996). AOA are considered as being able to tolerate lower DO levels than AOB, and some AOA ecotypes might readily adapt to the low oxygen and oxic–anoxic environments (Erguder et al. 2009).

The relative abundances of AOA and AOB vary considerably in different environments (Leininger et al. 2006; Wuchter et al. 2006; Di et al. 2009; Hofferle et al. 2010; Wang et al. 2011). The consensus is that AOA may be a key factor in the N cycle under unfavorable environmental conditions, e.g., limited nutrient availability, extreme pH/salinity, or sulfide-containing environments (Erguder et al. 2009). The dynamics of these two distinct ammonia-oxidizing groups (i.e., AOA and AOB) are likely to be complex, as they compete for the same substrate and possibly the same ecological niche. Additional studies are needed to discern the specific physical and geochemical conditions under which AOA or AOB are more abundant and/or diverse.

The plant-bed/ditch system of Shijiuyang Constructed Wetland, a drinking water treatment wetland system located in Jiaxing City of the Yangtze River delta, China, provides a heterogeneous field environment in which to examine the niches suitable for the growth of AOA and AOB. Thus, the objective of this study was to distinguish the critical environmental factors influencing the relative abundance of AOA and AOB in the plant-bed/ditch system. Moreover, the contributions of AOA and AOB populations to ammonia oxidation rates were also explored.

2 Materials and methods

2.1 Site description and sample collection

The Shijiuyang Constructed Wetland (SCW) is located in the northwestern corner of Jiaxing City, Zhejiang Province in China, and is the final defensive barrier before the source river water enters the intake of a local drinking water plant. The NH_4^+-N concentration of the inlet water was 0.15–0.36 mM, which was four to ten times higher than the hygienic standard of NH_4^+-N for drinking water (GB 5749-2006) in China. The SCW is composed of a pretreatment zone, a water-level rising and aeration zone, a root-channel purification zone, and a deep purification zone, covering a total area of about 110 ha and a water area of about 60 ha. Among all the functioning zones, the root-channel purification zone is the core reaction site of the SCW, which simulates the natural landscape structure of Baiyangdian Lake, a reed-dominated wetland. The root-channel purification technology utilizes mixed maize and rape straws, of various diameters, as the substrate media of the wetland; the mixed straws were buried in the plant bed to a depth of 20–50 cm. On the top of the plant bed, the reed (*Phragmites australis*) was transplanted as the dominant species because its rhizosphere showed a strong water purification function according to our previous studies (Wang et al. 2002; Wang and Yin 2008). This technology could promote the rapid formation of soil macropores at the initial operation stage of the wetland and has been successfully applied in SCW for over 4 years.

The root-channel purification zone was composed of masses of plant-bed/ditch sub-cells (Fig. 1). In the plant-bed/ditch system, the source water flows through the plant bed under a water-level difference of about 15–20 cm between the high water-level ditch (HD) and the low water-level ditch (LD). The plant bed was designed with a surface width of 16.3 m and a length of 18.5–285 m according to the local topography, while the ditches were designed with a top width of 13.2 m, a bottom width of 1.5 m and a slope of 1:6–1:3.

Sampling of the plant-bed/ditch system was carried out in February 2012. Three soil core collection sites (RB-1, RB-2, and RB-3) were located on the plant bed (Fig. 1). RB-1 and RB-3 were located at the fringes of the plant bed, while RB-2 was located at the middle of the plant bed. In HD, there were three surface sediment collection sites, one located at the

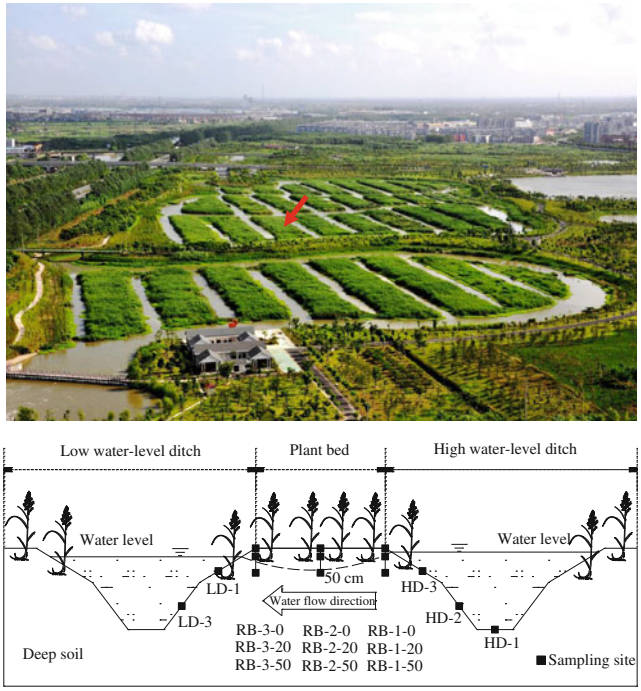

Fig. 1 The view (*upper photograph*) and the transverse sampling section (*lower sketch*) of the plant-bed/ditch system, Shijiuyang Constructed Wetland, China. The specific sampling site is indicated by the red arrow in the photograph

bottom (HD-1) and the other two located on the slope (HD-2 and HD-3). In LD, there were only two surface sediment collection sites, located at the slope (LD-1 and LD-3). Plant-bed soil cores (RB-1, RB-2, and RB-3) were collected using a stainless steel soil auger (inner diameter, 6.5 cm), and sliced into the following depth increments: 0–5 cm, 20–25 cm, and 50–55 cm. Surface sediments (0–5 cm) from the ditches were collected using an organic glass tube (inner diameter, 6.0 cm; length, 2 m) with a rubber stopper at one end. Three individual soil cores or surface sediments from each site were taken within an area of 2 m^2. The soil samples were transferred quickly to self-sealing plastic bags with the air removed, and the surface sediment were sliced with minimal disturbance and transferred quickly into glass jars which were completely filled to exclude oxygen. All the samples were wrapped with aluminum foil, packed with ice packs to keep them cool, and transported to the laboratory. For each site, the samples from the same depth layer were combined into a composite sample by mixing thoroughly in a glove-box with N_2 atmosphere, and stored at 4 °C before analysis. Simultaneously, a subsample for molecular analysis was taken from each mixed sample with a sterile scoop and preserved in a sterile centrifuge tube (5 ml) at −20 °C.

2.2 Chemical analyses of the samples

The pH was determined by shaking 10 g of dried sample with 20 ml de-ionized water on a rotary shaker for 30 min and then measured with an electrode (Mettler Toledo Delta 320) (Blakemore et al. 1987). The contents of NH_4^+-N and NO_2^--N in samples were determined by shaking 5.0 g of fresh sample with 50 ml of 2 M KCl for 2 h. Subsequently, samples were centrifuged at 15,000 rpm for 10 min. The supernatant was sampled and filtered through a 0.45 μm filter for further measurement. Extractable inorganic N contents (in milligram per kilogram dry weight) of the sample were analyzed by the colorimetric method (Anderson and Ingram 1989). The Fe^{2+} and Fe^{3+} contents were determined according to the procedure described by Lovley and Phillips (1987). Three replicated measurements were carried out for each homogenized sample.

2.3 Potential ammonia oxidation activity

The potential ammonia oxidation rate was measured using a chlorate inhibition method with some modifications according to Kurola et al. (2005). Briefly, 5.0 g of fresh sample (transformed to dry weight during calculation) was added to a 50-ml centrifuge tube containing 20 ml in situ water with modified ammonium concentration (1 mM $(NH_4)_2SO_4$). Potassium chlorate with a final concentration of 10 mM was added to inhibit the nitrite oxidation. The suspension was incubated (170 rpm) in the dark at in situ temperature for 6.5 h so as to avoid the impact of ammonium depletion on ammonia oxidation rate. The time zero sample was collected 0.5 h after incubation. Subsequently, at the time points 1.5, 2.5, 4.5, and 6.5 h after incubation the suspensions were collected from the individual time-series centrifuge tubes. The produced nitrite was extracted from the collected samples with 5 ml of 2 M KCl and determined by a spectrophotometer at wavelength of 540 nm with *N*-(1-naphthyl) ethylenediamine dihydrochloride. The potential ammonia oxidation rate was calculated from the linear increase of NO_2^--N concentration in the suspension during the 6.5 h.

2.4 DNA extraction and quantitative PCR assays

DNA was extracted from 0.3 g freeze-dried soil/sediment with the FastDNA Spin Kit for soil (Bio 101, Vista, CA) according to the manufacturer's instructions. The quantity of the DNA extractions was checked with a BioPhotometer (Eppendorf).

AOA and AOB *amoA* gene abundances were quantified by an ABI 7300 quantitative polymerase chain reaction (PCR) instrument (Applied Biosystems, CA, USA) with the fluorescent dye SYBR-Green approach. Amplification was performed in 25-μl reaction mixtures, including 12.5 μl SYBR® Premix Ex Taq™ (Takara, Dalian, China), 1 μl bovine serum albumin (25 mg ml^{-1}), 1 μl each primer (10 μM), and 2 μl tenfold diluted DNA template (1–10 ng). Primer sets

of amoA1F (GGGGTTTCTACTGGTGGT)/amoA2R (CCCCTCKGSAAAGCCTTCTTC) (Rotthauwe et al. 1997) and Arch-amoAF (STAATGGTCTGGCTTAGACG)/Arch-amoAR (GCGGCCATCCATCTGTATGT) (Francis et al. 2005) were applied targeting the AOB and AOA *amoA* gene amplifications, respectively. The thermocycling steps of the quantitative PCR included 50 °C for 2 min, 95 °C for 30 s, followed by 40 cycles of 10 s at 95 °C, 30 s at 53 °C for AOA or 55 °C for AOB, 1 min at 72 °C.

PCR products amplified from extracted DNA with the primers for quantitative PCR assays were gel-purified and ligated into the pGEM-T Easy Vector (Promega, Madison, USA), and the resulting ligation products were transformed into *Escherichia coli* JM109 competent cells following the manufacturer's instructions. After reamplification with the vector-specific primers T7 and SP6, the positive clones were selected to extract plasmid DNA using a GeneJet Plasmid Miniprep Kit (Fermentas MBI, Lithuania) and used as *amoA* gene standards for quantitative analyses. The concentration of plasmid DNA was determined using a Nanodrop ND-1000 UV–Vis Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) for the calculation of *amoA* gene copy number. Tenfold serial dilutions of a known copy number of the plasmid DNA were subjected to quantitative PCR in triplicate to generate an external standard curve.

2.5 Statistical analysis

One-way ANOVA and least significant different multiple comparison tests were carried out using STATISTICA 6.0, and graphing was performed using OriginPro 7.5 software.

3 Results

3.1 Physicochemical parameters of soil/sediment

The contents of NH_4^+-N, NO_2^--N, total nitrogen (TN), organic matter (OM), C/N molar ratio and Fe^{2+}/Fe^{3+} molar ratio showed great spatial differences in the plant-bed/ditch system. The NH_4^+-N contents in plant-bed soils (4.52–7.63 mg kg^{-1}) were significantly lower than those found in ditch sediments (14.0–22.9 mg kg^{-1}) ($p<0.001$), while there was no significant difference of NH_4^+-N content in the deep layers of the plant bed ($p<0.05$) (Table 1). Notably, in the surface soil of the plant bed, the NH_4^+-N contents at the edges (7.12 and 7.63 mg kg^{-1}) were higher than that of the central region (4.67 mg kg^{-1}). The average C/N molar ratios of the plant-bed soil (8.79) and the ditch sediment (7.77) were both higher than 6.625, which indicated that the plant-bed/ditch system was in N deficit.

In the profiles of the plant-bed soil cores, both the contents of TN and OM decreased with depth, while NO_2^--N content showed the reverse pattern (Table 1). The increased Fe^{2+}/Fe^{3+} molar ratio with depth indicated that relatively stronger reduction conditions occurred in the deep layers of the plant bed. On the other hand, in the deep layers of the plant bed, the average Fe^{2+}/Fe^{3+} ratio increased from 0.34 (RB-1-20 and RB-1-50) to 8.60 (RB-3-20 and RB-3-50), suggesting that the reduction condition became stronger along with the water flow direction. Moreover, the average Fe^{2+}/Fe^{3+} ratio of high water-level ditch sediment (2.77) was 2.30 times lower than low water-level ditch sediment.

3.2 Distribution of AOA/AOB abundance and potential ammonia oxidation rate in the surface samples of the plant-bed/ditch system

The abundances of AOA and AOB were investigated by quantitative PCR targeting their *amoA* genes. In the surface soil of the plant bed (NH_4^+-N: 4.67–7.63 mg kg^{-1}), AOA abundance (1.13×10^8 to 8.47×10^8 copies g^{-1}) was one order of magnitude higher than AOB, and AOA/AOB ratio ranged from 1.48 to 14.7 (Fig. 2a). However, in the ditch sediment with relatively high ammonium contents (range 14.0–22.9 mg kg^{-1}), the AOA/AOB ratio ranged from 0.11 to 0.46. As the common substrate for both AOA and AOB, ammonium content may affect the relative abundance of the two ammonia oxidizers in the surface samples of the plant-bed/ditch system.

To investigate the relative contributions of AOA and AOB to ammonia oxidation, the potential ammonia oxidation rates in the surface samples of the plant-bed/ditch system were measured. On average, the potential ammonia oxidation rate of high water-level ditch sediment (136.2±156.7 nmol N g^{-1} h^{-1}) was significantly higher than plant-bed surface soil (14.7±12.2 nmol N g^{-1} h^{-1}) and low water-level ditch sediment (14.1±12.3 nmol N g^{-1} h^{-1}) ($p<0.05$) (Fig. 2b). The highest value was determined at the bottom of high water-level ditch (338.4 nmol N g^{-1} h^{-1}). On the whole, the potential ammonia oxidation rate decreased along the water flow direction in the plant-bed/ditch system. Interestingly, both the potential ammonia oxidation rate and the AOB abundance at the edges of the plant bed were significantly higher than that of the central region ($p<0.05$).

3.3 Distribution of AOA/AOB abundance and potential ammonia oxidation rate in the transversal section of the plant bed

The distributions of potential ammonia oxidation rate, A*amoA* and B*amoA* gene abundances in the transversal section of the plant bed were also explored. The abundance of AOA decreased with depth in all the three soil cores. However, there was no such apparent pattern for AOB (Fig. 3a, b, c). In the deep layers of soil cores RB-2 and

Table 1 The physicochemical parameters of the samples collected from the plant-bed/ditch system

Sample sites	pH	NH_4^+-N (mg kg^{-1})	NO_2^--N (mg kg^{-1})	TN (g kg^{-1})	OM (g kg^{-1})	C/N molar ratio	Fe^{2+} (mg kg^{-1})	Fe^{3+} (mg kg^{-1})	Fe^{2+}/Fe^{3+} molar ratio
HD-1	7.46	14.0	0.43	1.23	17.5	10.5	146.0	92.1	1.59
HD-2	7.55	22.9	0.63	0.63	9.29	8.06	369.4	57.9	6.39
HD-3	7.49	14.3	0.99	0.65	8.94	8.24	21.4	65.1	0.32
RB-1-0	6.14	7.12	0.35	2.29	36.1	11.8	3.69	60.9	0.05
RB-1-20	7.19	6.24	0.72	0.76	10.3	9.93	26.1	114.8	0.23
RB-1-50	7.80	6.11	0.98	0.38	6.19	6.10	12.5	27.7	0.44
RB-2-0	7.14	4.67	0.61	1.21	21.5	11.4	18.0	48.6	0.37
RB-2-20	7.60	6.16	0.73	0.71	11.0	10.2	233.8	79.1	2.96
RB-2-50	7.63	4.52	0.84	0.75	10.7	10.4	266.5	79.3	3.36
RB-3-0	7.16	7.63	0.47	1.04	16.4	10.4	278.5	147.1	1.89
RB-3-20	6.92	6.06	0.60	0.95	15.4	10.3	342.1	98.0	3.49
RB-3-50	7.85	7.43	0.97	0.68	10.7	11.8	213.5	16.4	13.7
LD-1	7.07	16.2	0.59	0.85	16.5	10.6	464.1	56.4	8.63
LD-3	7.33	21.2	0.44	0.73	11.7	7.83	442.1	104.1	4.24

RB-3, the average abundances of AOA ($7.74\pm2.02\times10^7$ copies g^{-1} for RB-2; $7.69\pm2.46\times10^7$ copies g^{-1} for RB-3) were higher than AOB ($2.15\pm1.97\times10^7$ copies g^{-1} for RB-2; $1.54\pm0.42\times10^7$ copies g^{-1} for RB-3). The AOA/AOB ratio varied from 1.81 to 18.8. However, in the deep layers of soil core RB-1 near the high water-level ditch, AOA abundance was lower than AOB with AOA/AOB ratios of 0.20 and 0.40, respectively.

In the soil cores RB-1 and RB-3 located at the fringe of plant bed, the potential ammonia oxidation rate decreased from 24.1 to 9.63 nmol N g^{-1} h^{-1} and from 19.3 to 2.65 nmol N g^{-1} h^{-1} with depth, respectively (Fig. 3d, e, f). However, in the soil core of RB-2 located in the middle of plant bed, the potential ammonia oxidation rate increased from 0.77 to 10.0 nmol N g^{-1} h^{-1} with depth. Overall, the potential ammonia oxidation rate determined from soil core RB-1 was significantly higher than the other two cores ($p<0.01$, $n=3$).

3.4 Correlation between potential ammonia oxidation rate and AOA/AOB abundance

Because the potential ammonia oxidation rate and the AOA/AOB abundance in the heterogeneous plant-bed/ditch system followed non-normal distributions, Spearman's correlation tests were performed with log transformed data. Results showed that the potential ammonia oxidation rate was significant and positively correlated with AOB abundance both in the plant-bed soils ($r=0.592$, $p=0.0096$) and in the ditch sediments ($r=0.873$, $p=0.0002$) (Table 2). However, there was no significant relationship between potential ammonia oxidation rate and AOA abundance in the plant-bed/ditch system. The regression analysis between potential ammonia oxidation rate and the log number of AOA or AOB abundance also displayed a significant relationship between ammonia oxidation rate and AOB abundance ($p<0.05$) (Table S1 in supporting information). The results showed that AOB may contribute more to ammonia oxidation than AOA in the plant-bed/ditch system.

3.5 Cell-specific ammonia oxidation rates

Cell-specific ammonia oxidation rates in the plant-bed/ditch system were inferred from the observed potential ammonia oxidation rates and the *amoA* gene copy numbers (Table 3). For AOB, cell-specific rates of ammonia oxidation ranged from 0.35 to 9.13 fmol NH_3 oxidized $cell^{-1}$ h^{-1}, and was mostly within values reported in the literature, which suggested that bacterial ammonia oxidation was generally sufficiently large to account for the potential ammonia oxidation rate in the plant-bed/ditch system. For AOA, on the other hand, some extraordinarily high cell-specific rates (1.45–31.0 versus 0.08–0.59 fmol NH_3 oxidized $cell^{-1}$ h^{-1} in pure cultures), especially in the high water-level ditch sediments, were observed, suggesting that the AOA community could not account for the nitrification solely and that AOB were indispensable in ammonia oxidation (Wang et al. 2012). Note that the cell-specific rate of archaeal ammonia oxidation is relatively uncertain, because presently only three reports allow for the calculation of such rates (i.e., Könneke et al. 2005; de la Torre et al. 2008; Tourna et al. 2011). The cell-specific rates did not reflect the actual activity because the rates were inferred assuming solely AOA or AOB was functionally involved in ammonia oxidation.

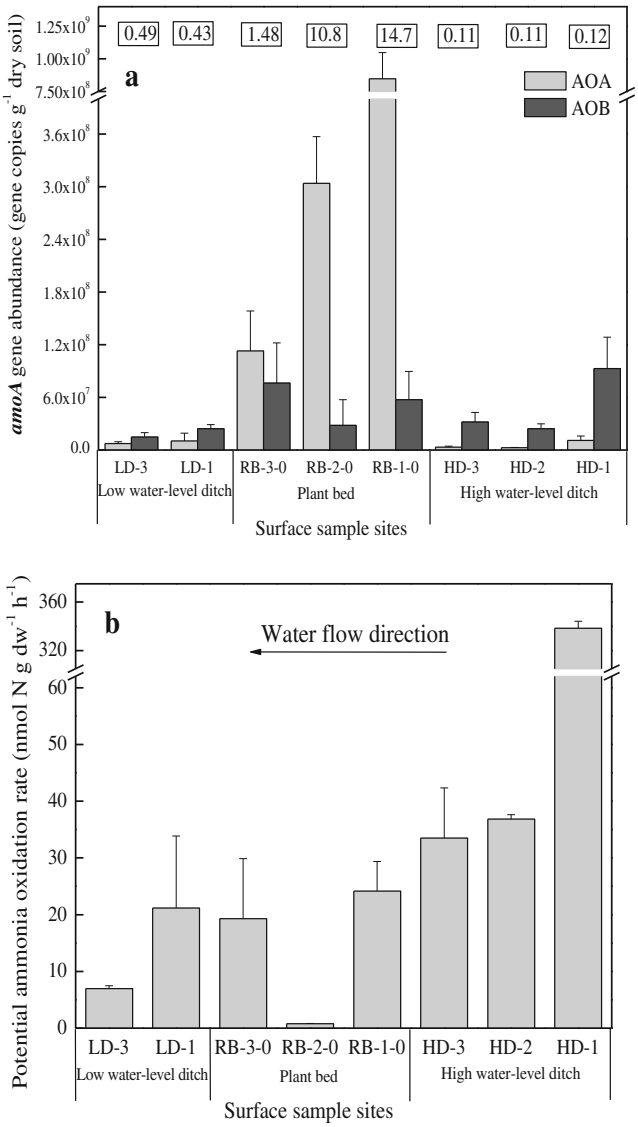

Fig. 2 The archaeal and bacterial *amoA* gene abundances (**a**) and the relevant potential ammonia oxidation rates in the surface samples of the plant-bed/ditch system (**b**). The ratios of AOA to AOB are shown in the *boxes in the upper part of the chart*. *Error bars* indicate one standard deviation (n=3)

4 Discussion

In the present study, the *amoA*-encoding ammonia oxidizer abundance and the potential ammonia oxidation rate were determined along the hydraulic gradient of the plant-bed/ditch system of SCW. High spatial heterogeneities of ammonia oxidizer abundance and potential ammonia oxidation rate were observed. AOA was numerically more dominant than AOB in the plant-bed soil, while the reverse pattern occurred in the ditch sediment. Ammonium content and the oxidation–reduction potential were important factors influencing the relative abundances of AOA and AOB.

Previous studies reported that AOA was predominant among ammonia-oxidizing prokaryotes in soil and marine ecosystems with low ammonium concentrations (Leininger et al. 2006; Wuchter et al. 2006). However, it has also been observed that AOB outnumbered AOA in N-rich sediments and agricultural soils (Wang et al. 2011; 2012; Jia and Conrad 2009). Some environmental factors like pH, salinity, and fertilization have been identified as affecting the distribution of AOA and AOB (Erguder et al. 2009); however, the question of what parameters are effective in their occurrence and abundance remains unclear (Erguder et al. 2009). In the plant-bed/ditch system, the ammonium content seemed to be one of the most decisive factors. In the surface soil of the plant bed with relatively lower ammonium content (4.67–7.63 mg kg^{-1}) compared with that of ditch sediment (14.0–22.9 mg kg^{-1}), AOA outnumbered AOB, while the result was the opposite in the ditch sediment. Verhamme et al. (2011) also showed evidence that ammonium content contributes to the definition of distinct ecological niches of AOA and AOB in soil. The reason that AOA have a preference for low-ammonium environments may be that AOA might possess a much higher affinity for ammonia than known cultured AOB (Taylor and Bottomley 2006; Martens-Habbena et al. 2009). In the present study, with the networks of waterways as the main source of ammonium (0.15–0.36 mM), it provided a suitable substrate for the growth of AOB in the ditch sediment. Though the aboveground part of the reed had been harvested in the sampling season (February 2012) to avoid water recontamination by the decayed plant residue, the underground part was still active and could transmit oxygen as the electron acceptor of ammonia oxidation (Armstrong et al. 1992). Along with water flowing through the plant bed characterized by root channels, the ammonium attenuation was enhanced by certain microbial and physical processes (Wang and Yin 2008). Thus, the niches with relatively low ammonium content suitable for the growth of AOA would form in the plant-bed soil.

On the other hand, relatively higher AOA abundance compared with AOB was detected in the deep layers of the plant bed. In the deep layers of the plant bed with no significant difference in ammonium content (p<0.05), the oxidation–reduction potential may be another parameter influencing the relative abundances of AOA and AOB. The rates of Fe^{2+} abiotic oxidation depend linearly on the oxygen concentration (Vollrath et al. 2012), so the Fe^{2+}/Fe^{3+} ratio showed close a relationship with the oxidation–reduction potential. Given that the Fe^{2+}/Fe^{3+} ratios of RB-2 and RB-3 deep layer soils (2.96–13.7) were much higher than that of RB-1 (0.23–0.44), it could be concluded that the low oxidation–reduction potential formed along with the water flow direction. AOA were found to tolerate a wide range and low oxygen levels in water (Francis et al. 2005; Könneke et al.

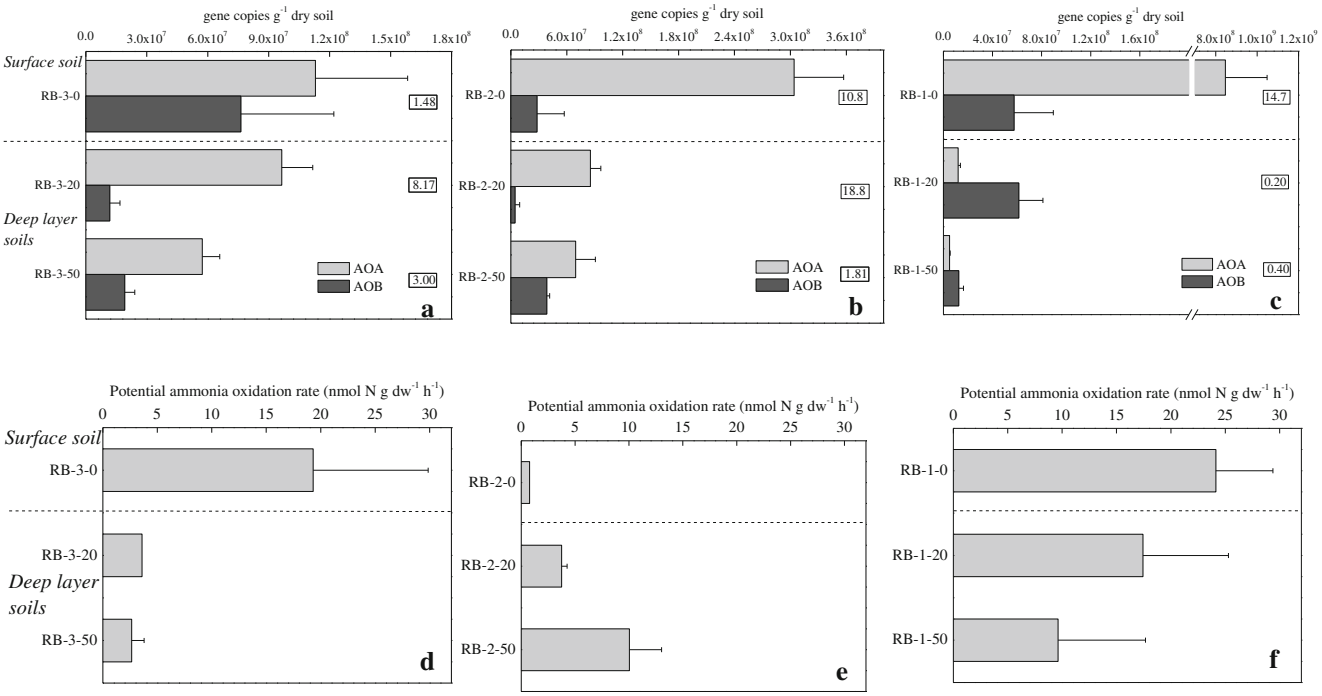

Fig. 3 The archaeal and bacterial *amoA* gene abundances (**a**, **b**, and **c**) and the relevant potential ammonia oxidation rates in the transverse section of the plant-bed (**d**, **e**, and **f**). The ratios of AOA to AOB are shown in the *boxes on the right part of the chart*. *Error bars* indicate one standard deviation ($n=3$)

2005), as low as <1 μM in one case (Coolen et al. 2007), and AOA abundance did not differ at oxygen levels ranging from 0.1-0.2 mM in aquifer sediments (Santoro et al. 2008). However, AOB are generally more sensitive to decreases in oxygen levels (Erguder et al. 2009). It has been proposed that the pathway of ammonia oxidation in AOA may be distinct from the AOB pathway, theoretically requiring only 0.5 O_2 per NH_3 oxidized (Walker et al. 2010). In our study, AOA seemed to persist more readily even under low oxidation–reduction potential in the deep layers of the plant bed. Further studies should be conducted on the environmental factors associated with AOA and AOB distributions to clarify the dynamics of the ammonia oxidization process in the plant-bed/ditch system.

The plant density on the plant bed was thick, with reed as the dominant species and other companion species (such as *Typha orientalis* Presl and *Acorus calamus* L.), reaching up to 72 plant shoots m^{-2} (ranging from 21–113 plant shoots m^{-2}) according to the vegetation investigation in October 2010. So, it could be inferred that the soil cores collected from the plant bed using a soil auger are mostly located at the rhizosphere of wetland plants. In our study, AOA dominated the ammonia oxidizer communities in the plant bed of the constructed wetland, which confirms and extends previous observations of archaeal predominance in the rhizosphere of macrophytes (Chen et al. 2008; Herrmann et al. 2008, 2009; Trias et al. 2012). Moreover, the root exudates of macrophytes may also play a role in the relative distribution of AOA and AOB. *P. australis* can release organic carbon, up to 70 mg g^{-1} (root wet weight) day^{-1}, into the rhizosphere (Toyama et al. 2011), which may include organic compounds that are needed for mixotrophic AOA (Tourna et al. 2011).

The contribution of AOA/AOB to ammonia oxidization remains uncertain and controversial conclusions had been drawn in some aquatic and terrestrial ecosystems (Wuchter et al. 2006; Di et al. 2009; Jia and Conrad 2009). In our study, the abundance of AOB was found to be significantly and positively correlated with potential ammonia oxidation rate

Table 2 Spearman's correlation coefficients describing the relationships between potential ammonia oxidation rate and AOA/AOB abundance

	Comparison	Spearman's correlation coefficient (r)	p
Plant-bed soil	Ammonia oxidation rate vs. AOA	−0.112	0.6586
	Ammonia oxidation rate vs. AOB	0.592	0.0096
Ditch sediment	Ammonia oxidation rate vs. AOA	0.046	0.8860
	Ammonia oxidation rate vs. AOB	0.873	0.0002

Table 3 Estimated cell-specific ammonia oxidation rates of AOA and AOB, and a comparison with relevant studies

Organisms and environments		Cell-specific ammonia oxidation rate (fmol NH_3 oxidized $cell^{-1}$ h^{-1})		References
		AOB[a]	AOA[a]	
Pure cultures	AOB	0.9–83		Belser 1979; Ward et al. 1989
	AOA		0.08–0.59	Könneke et al. 2005; de la Torre et al. 2008; Tourna et al. 2011
Environments	Agricultural soil	0.20–15.6		Okano et al. 2004
	Others, e.g. sludge waste	0.22–12.4		Wagner et al. 1995; Harms et al. 2003
	Agricultural soil (0–20 cm)	0.25	0.002	Jia and Conrad 2009
	Agricultural soil (40–50 cm)	1.78	0.005	
	Littoral sediment (0–10 cm)	0.57	1.25	Wang et al. 2012
	Littoral soil (0–10 cm)	0.91	0.35	
	Littoral soil (30–50 cm)	160.0–166.6	0.12–0.15	
HD-1	Sediment	9.13	31.0	This study
HD-2	Sediment	3.79	13.6	
HD-3	Sediment	2.63	9.91	
RB-1-0	Soil	1.05	0.03	
RB-1-20	Soil	0.71	1.45	
RB-1-50	Soil	1.93	1.95	
RB-2-0	Soil	0.07	0.003	
RB-2-20	Soil	2.06	0.04	
RB-2-50	Soil	0.65	0.14	
RB-3-0	Soil	0.63	0.17	
RB-3-20	Soil	0.76	0.04	
RB-3-50	Soil	0.35	0.05	
LD-1	Sediment	2.20	2.04	
LD-3	Sediment	1.18	0.95	

[a] The cell-specific ammonia oxidation rate of ammonia-oxidizing archaea (AOA) or ammonia-oxidizing bacteria (AOB) cell was calculated by dividing the potential ammonia oxidation rates mentioned before, assuming each cell has equal activity and nitrite produced is solely from either AOA or AOB alone, and each genome of AOA and AOB contains 1.0 and 2.5 *amoA* gene copy, respectively

HD high water-level ditch; *RB* plant bed; *LD* low water-level ditch; *RB-1-0, RB-1-20, and RB-1-50* the soil samples at depths of 0, 20, and 50 cm in plant-bed soil core 1

in both plant-bed soils and ditch sediments. However, there was no significant relationship between potential ammonia oxidation rate and the abundance of AOA in the plant-bed/ditch system. The same result was also obtained by Di et al. (2009) in grassland soils and Bernhard et al. (2010) in estuarine sediments, even though the abundance of AOA was found to be higher than that of AOB. Probably, the phenomenon may result from the water flow state and many other factors still not fully revealed in the plant bed of the constructed wetland. Moreover, the cell-specific ammonia oxidation rate of AOB in the plant-bed/ditch system was comparable with those reported in pure cultures and other environmental samples, which indicated that bacterial ammonia oxidation was generally sufficiently large to account for the potential ammonia oxidation rate in the plant-bed/ditch system (Table 3). In all, the results strongly suggest that AOB functionally dominate the ammonia oxidation in the plant-bed/ditch system of SCW.

5 Conclusions

In the plant-bed/ditch system, the ammonium content of the plant-bed soil was significantly lower than the ditch sediment ($p<0.001$). The abundance of AOA was numerically more dominant than AOB in the plant-bed soil, while AOB outnumbered AOA in the ditch sediment. The high ammonium content in the ditch sediment likely favored AOB. In the deep layers of the plant bed, AOA became more abundant than AOB along the water flow direction, even though the oxidation–reduction potential decreased notably. Ammonium content and the oxidation–reduction potential were important parameters influencing the distributions of AOA and AOB in the plant-bed/ditch system. Moreover, the potential ammonia oxidation rate was significantly dominated by AOB abundance rather than AOA both in the plant-bed soils ($r=0.592$, $p=0.0096$) and in the ditch sediments ($r=0.873$, $p=0.0002$).

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