Journal of Microbiology (2015) Vol. 53, No. 5, pp. 311–320
DOI 10.1007/s12275-015-4660-0

Communities of ammonia oxidizers at different stages of *Spartina alterniflora* invasion in salt marshes of Yangtze River estuary[§]

Fei Xia[1,2], Jemaneh Zeleke[1,3], Qiang Sheng[2], Ji-Hua Wu[2], and Zhe-Xue Quan[1*]

[1]*Department of Microbiology and Microbial Engineering, School of Life Sciences, Fudan University, Shanghai, P. R. China*
[2]*Ministry of Education Key Laboratory for Biodiversity Science and Ecological Engineering, Department of Ecology and Evolutionary Biology, Fudan University, Shanghai, P. R. China*
[3]*Department of Biology, College of Natural and Computational Sciences, Wolaita Sodo University, Ethiopia*

(Received Nov 20, 2014 / Revised Mar 26, 2015 / Accepted Apr 15, 2015)

Spartina alterniflora*, an aggressive invasive plant species at the estuarine wetlands of China's coasts, has become a major threat to the natural ecosystems. To understand its potential influence on nitrification processes, the community structures and abundance of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) were investigated using 454-pyrosequencing and quantitative real-time PCR (qPCR) in *S. alterniflora* invading salt marsh sediments at the Yangtze River estuary in Chongming island, Shanghai, China. Copy numbers of archaeal and bacterial ammonia monooxygenase subunit A (*amoA*) genes did not show accordant shifts with *S. alterniflora* invasion in the two sampling sites. However, the copy numbers of archaeal *amoA* gene were higher in summer than in spring. Phylogenetic analysis indicated that more than 90% of the archaeal and 92% of the bacterial *amoA* gene sequences were closely related to marine group I.1a and the clusters 13 and 15 in *Nitrosospira* lineage, respectively. The effect of different seasons (spring and summer) was important for the abundance variation of AOA, while different stages of *S. alterniflora* invasion did not show significant effect for both AOA and AOB. Variation of AOA community was significantly related to total carbon (TC) and sulfate concentration ($P < 0.05$), whereas the AOB community was significantly related to sulfate concentration, total nitrogen (TN), TC and pH ($P < 0.05$). In conclusion, the abundance and diversity of ammonia oxidizing microbial communities were not strongly affected by *S. alterniflora* invasion.

Keywords: *Spartina alterniflora*, ammonia oxidizers community, *amoA*, salt marsh

*For correspondence. E-mail: quanzx@fudan.edu.cn; Tel.: +86-21-51630665; Fax: +86-21-55664332
[§]Supplemental material for this article may be found at http://www.springerlink.com/content/120956.

Introduction

Ammonia oxidation, the first step in the microbial oxidation of ammonia to nitrate, plays a key role in the global nitrogen cycling and as source of environmental problems such as greenhouse gas emission and nitrate pollution (Kowalchuk and Stephen, 2001). This rate-limiting step in the nitrification process is performed by aerobic lithotrophic nitrifiers including bacterial and archaeal ammonia oxidizers. For a long time, ammonia-oxidizing bacteria (AOB) that are affiliated to the β-Proteobacteria and γ-Proteobacteria were thought to perform ammonia oxidation exclusively until ammonia-oxidizing archaea (AOA), categorized as Thaumarchaeota (Pester *et al.*, 2011), were discovered from various environments (Venter *et al.*, 2004; Könneke *et al.*, 2005; Treusch *et al.*, 2005; Brochier-Armanet *et al.*, 2008). Since then, the two groups of ammonia oxidizers have been the focus of studies in the nitrogen cycle. Naturally, the ammonia monooxygenase subunit A (*amoA*) gene has been widely used as a molecular marker to characterize AOB and AOA communities in various environmental samples due to its functional significance and conserved phylogeny (Rotthauwe *et al.*, 1997; Francis *et al.*, 2005; Leininger *et al.*, 2006; Caffrey *et al.*, 2007; Tourna *et al.*, 2008; Bernhard and Bollmann, 2010).

Estuary and coastal wetlands, for instance the salt marshes, provide many essential ecosystem services such as regulation, habitat and production (Craft *et al.*, 2008). As a result of decomposition of various plants litter, sediments of salt marshes are often rich in organic and mineral nitrogen and carbon resources. Moreover, runoff from nearby agricultural lands may deposit N-fertilizers in the sediments of the salt marsh. In sediments of estuarine environments, microorganisms involving in the nitrogen cycle, particularly the ammonia oxidizing microbes, may play an important role in minimizing the amount of nitrogen that enters into the sea. As estuarine and coastal wetlands are highly vulnerable for invasion by exotic plant species, their ecosystem functions can be easily altered (Williams and Grosholz, 2008). At present, in salt marshes of the Yangtze River estuary, the native plant (*Phragmites australis*) is being invaded by the aggressive exotic *Spartina alterniflora*, which was introduced into the country intentionally in 1979 for erosion control, soil melioration and dike protection (Quan *et al.*, 2007). As a result of the extensive expansion of *S. alterniflora*, a number of studies have been conducted at this estuarine environment to uncover its threat on the native ecosystem. For instance, its role in altering greenhouse gas emission rates (Cheng *et al.*, 2007) as well as its influence on the aboveground flora and fauna (Jiang *et al.*, 2009) has been reported.

Despite these investigations, very few reports have been made about its influence on microbial communities, in the sediments of the salt marshes at the Yangtze River estuary.

Previous studies have demonstrated that ammonia-oxidizing microbial communities are influenced by many environmental factors such as pH, salinity, carbon, nitrogen and temperature (Bernhard and Bollmann, 2010). The biomass of *S. alterniflora* is denser than the native *P. australis*; hence, its invasion could alter the sediment nutrition due to the organic materials released through root exudates and litter decompositions. Alteration of sediment nutrition may induce a series of changes in various sediment characteristics, which may have several implications for microbial communities inhabiting the sediment (Fenchel, 2002). Although many investigations have been conducted on ammonia-oxidizing communities in Chongming Island of the Yangtze River estuary (Li *et al.*, 2012; Zheng *et al.*, 2012, 2014), as to the best of our knowledge, few ones concerned about the influence of *S. alterniflora* invasion on ammonia-oxidizing communities (Zhang *et al.*, 2011). In recent years, the *S. alterniflora* invasion is getting worse at the Chinese coastline estuaries where the carbon and nitrogen contents of sediments could be altered by the invasion (Turner, 1993; Liao *et al.*, 2007; Page *et al.*, 2010), and yet no information is available about its impact on the nitrogen cycling microorganisms, particularly on the ammonia-oxidizing communities. Despite a study showing the effects *S. alterniflora* invasion on the abundance and composition of denitrifiers at the Jiulong River estuary (Zhang *et al.*, 2011), seasonal variations as well as the dynamic responses of denitrifying microbial communities at different stages of the invasion have not been reported. In addition to the possible variation in the responses of denitrifiers and ammonia oxidizers to *S. alterniflora* invasion, the type of native plants, latitude and geographical conditions of the Yangtze and Jiulong River estuaries are different and hence the influence of *S. alterniflora* invasion on ammonia-oxidizing communities may vary.

Recently, the influence of different *S. alterniflora* invasion stages on the methanogen and sulfate-reducing bacterial communities in salt marshes of the Yangtze River estuary was demonstrated (Zeleke *et al.*, 2013). The invasion of *S. alterniflora* notably increased the abundance of the methanogen and sulfate-reducing microbial communities and decreased the diversity of methanogens, although its effects on the ammonia-oxidizing communities have not been described. In current work, the influence of different stages of *S. alterniflora* invasion on the ammonia-oxidizing communities was investigated seasonally (before full growth and at full growth stage of *S. alterniflora*) using real-time quantitative PCR (qPCR) and 454-pyrosequencing of the *amoA* gene.

Materials and Methods

Sampling and soil parameters

Sediment samples were collected from the salt marsh of Yangtze River estuary in Chongming Island, Shanghai, China (Supplementary data Fig. S1). All detailed descriptions of the sampling locations and processes as well as the characteristics of sediments were reported before (Zeleke *et al.*, 2013). In brief, two replicate locations "a" and "b" with 60 meters apart were selected. For each location, three distinct sampling points with about 20 meters apart covered by *P. australis* only (before invasion), both *P. australis* and *S. alterniflora* (transition area, during invasion) and *S. alterniflora* only (after invasion) were chosen. For each sampling point, five replica sediment samples (from surface to 5 cm depth in about 2 m apart) were collected and then homogenized. Sediment samples were then sealed in polyethylene bags and transported to the laboratory using an ice box for further analysis. Throughout this report, samples from *P. australis* and *S. alterniflora* invaded parts were respectively represented by the letters "P" and "S", whereas samples from the transition area where both plant species were available were represented by the letter "T". On the other hand, "I" and "II" represented the spring and summer samples, respectively, and characters "a" and "b" represented the replicate locations. The concentration of nitrate (NO_3^-) in the sediments were determined from the respective supernatants extracted using 2 M KCl solution (Hou *et al.*, 2003) and measured using an ion chromatograph (ICS-1100; Dionex).

DNA extraction and gene amplification

Total genomic DNA was extracted from sediment samples using PowerSoil DNA isolation Kit (Mo Bio Laboratories) following the manufacturer's instructions. For pyrosequencing, the primer sets that targeted the β-proteobacterial and archaeal *amoA* genes along with the corresponding annealing temperatures are listed in Table 1. The polymerase chain reaction (PCR) for each gene was conducted following the 2-step barcoded PCR method (Berry *et al.*, 2011) with slight modification, as described previously (Zeleke *et al.*, 2013). DNA from each sample was amplified in triplicate, and the PCR products were pooled. Appropriate-sized fragments of the PCR products were separated by agarose gel electrophoresis and purified using commercial gel purification kit (Axygen). The purified DNA fragments were then quantified using ND3300 Fluorospectrometer (NanoDrop Technologies). Finally, the PCR products of each sample were pooled in an equivalent concentration, and 454-pyrosequencing was conducted with Roche/454 (GS FLX Titanium System) according to the manufacturer's protocols.

Table 1. Primers used for pyrosequencing and real-time qPCR

Primer	Sequence (5'→3')	Target	Ta (°C)[a]	Reference
amoA-1F	GGGGTTTCTACTGGTGGT	β-Proteobacterial *amoA* gene (for pyrosequencing[b] and qPCR)	55	Rotthauwe *et al.* (1997)
amoA-2R	CCCCTCKGSAAAGCCTTCTTC			
CrenamoA23f	ATGGTCTGGCTWAGACG	Archaeal *amoA* gene (for pyrosequencing and qPCR)	55	Tourna *et al.* (2008)
CrenamoA616r	GCCATCCATCTGTATGTCCA			

[a] Annealing temperature
[b] For 454-pyrosequencing, a barcode with 8 nucleotides (Parameswaran *et al.*, 2007), adaptor A and B were added for the forward and reverse primers, respectively.

Real-time qPCR

Plasmid DNA containing the corresponding gene fragments were extracted and then linearized with *Eco*RI restriction enzyme (Fermentas) according to the instructions. Then, the correct linear fragments were purified, and concentrations were determined. Linearized plasmid DNA fragments were then diluted serially to generate external standard curves. SYBR Green-I based real-time qPCR was performed on an Mx3000P real-time PCR system (Stratagene) using SYBR® Premix Ex Taq™ (TaKaRa). The primer sets that were used for quantification of the β-proteobacterial or archaeal *amoA* genes are listed in Table 1. Results of qPCR were analyzed using MxPro QPCR software version 3.0 (Stratagene). Consistencies of the results were confirmed by the strong linear relationship between the threshold cycle and the log value of gene copy number (for AOA, $R^2 = 0.99$, amplification efficiency = 103%; for AOB, $R^2 = 0.99$, amplification efficiency = 107%). The abundances of total bacterial and archaeal 16S rRNA genes were determined previously (Zeleke *et al.*, 2013).

Sequence data analyses

Analysis of the *amoA* sequence data was performed as described previously for other functional genes (Zeleke *et al.*, 2013). Briefly, the raw pyrosequencing data of the functional genes were trimmed using the sample-specific barcodes with Mothur software, version 1.8 (Schloss *et al.*, 2009) using the trim.seqs commend. After trimmed with quality score of any base higher than 25 and the fragments length more than 250 bp, 8610 archaeal and 4554 bacterial *amoA* gene sequences were obtained. Then, the denoised sequence reads were translated and corrected by the RDP's functional gene Pipeline tool FrameBot (Wang *et al.*, 2013). After the removal of amino acid sequences with stop codons or uncertain amino acids, a total of 8281 archaeal and 3535 bacterial *amoA* gene sequences with high quality were obtained. Then, the operational taxonomic units (OTUs) were defined at 95% similarity (Francis *et al.*, 2005) and were used for the downstream analyses. After the sequence numbers of each sample were normalized (284 archaeal *amoA* gene sequences and 125 bacterial *amoA* gene sequences), the diversity indices (Shannon-Weiner), richness estimator (Chao1) and the evenness index (ACE) were calculated for each sample using the Mothur. Principal component analysis (PCA) and canonical correspondence analysis (CCA) were performed using R, version 2.15.1 (R Development Core Team, 2004). The OTU representative sequences were edited and translated into amino acid sequences using the BioEdit software (Hall, 1999) and aligned with reference sequences using ClustalX, version 2.1 (Larkin *et al.*, 2007). Then, phylogenetic trees were constructed to determine the phylogenetic positions of the obtained archaeal and bacterial *amoA* genes using MEGA 5.0 software (Tamura *et al.*, 2011) with the neighbor-joining method with p-distance substitutions model and relative confidence of tree topologies was evaluated by performing 1,000 bootstrap replicates. The significance test of abundance of archaeal and bacterial *amoA* genes were performed by SPSS 19.0 package for Windows (SPSS Inc.), where $P < 0.05$ was considered statistically significant.

Sequence accession numbers

The archaeal and bacterial *amoA* gene sequences recovered in this study were deposited in the NCBI GenBank Short Read Archive (SRA) under accession number SRP041169.

Results

Copy numbers of archaeal and bacterial *amoA* genes

The copy numbers of archaeal and bacterial *amoA* genes showed little variation in the different stages of *S. alterniflora* invasion. In the sampling site "a", copy number of archaeal *amoA* genes slightly reduced from $2.04 \pm 0.66 \times 10^5$ to $9.29 \pm 0.80 \times 10^4$ and $3.01 \pm 0.06 \times 10^5$ to $1.58 \pm 0.12 \times 10^5$ copies per g dry matter in spring and summer, respectively. However, this trend was not observed in site "b", where copy numbers appeared to increase through the invasion of *S. alterniflora* (Fig. 1A). In both seasons, ratios of archaeal *amoA* to total archaeal 16S rRNA genes showed reduc-

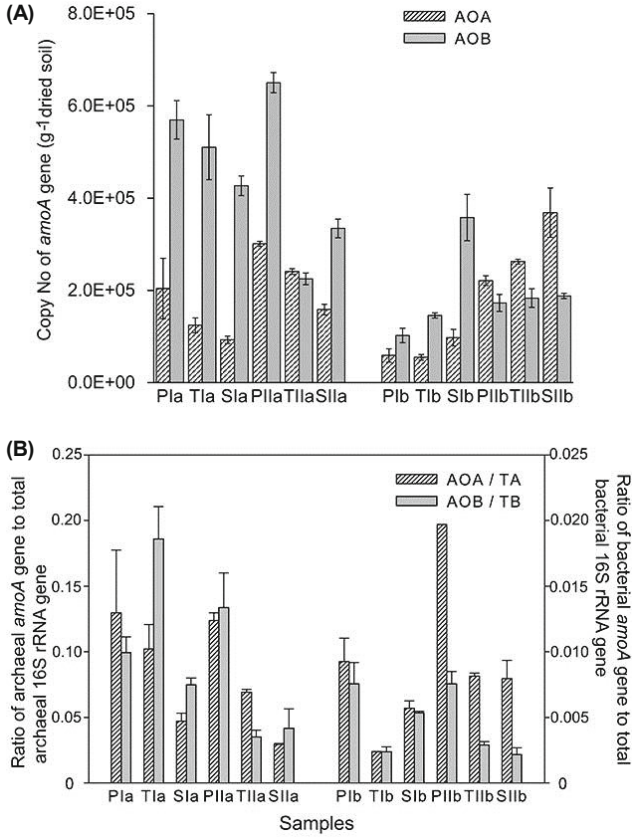

Fig. 1. Copy numbers of archaeal and bacterial *amoA* genes (A) and their ratios to total archaeal and bacterial 16S rRNA genes (B). Error bars indicate standard deviations of the mean (n=3). In the sample names, the capital letters P, S and T represent the samples from *P. australis*, *S. alterniflora* and transition living zone sediments. "I" and "II" represent the spring and summer samples, respectively, and characters "a" and "b" indicate the replicate sampling locations, hereinafter inclusive. AOA and AOB indicate ammonia-oxidizing archaea and bacteria, respectively; TA and TB indicate the total archaea and bacteria, respectively. The copy numbers of 16S rRNA genes of TA and TB were from the work published previously (Zeleke *et al.*, 2013).

tions with *S. alterniflora* invasion (Fig. 1B), although the reduction in site "a" was relatively higher. Consistent with the abundance of archaea reported before for these sites (Zeleke *et al.*, 2013), t-tests indicated that the copy numbers of archaeal *amoA* genes in the two sampling sites were higher in summer than in spring ($P < 0.05$); however, there were no significant shifts across the different stages of *S. alterniflora* invasion ($P > 0.05$).

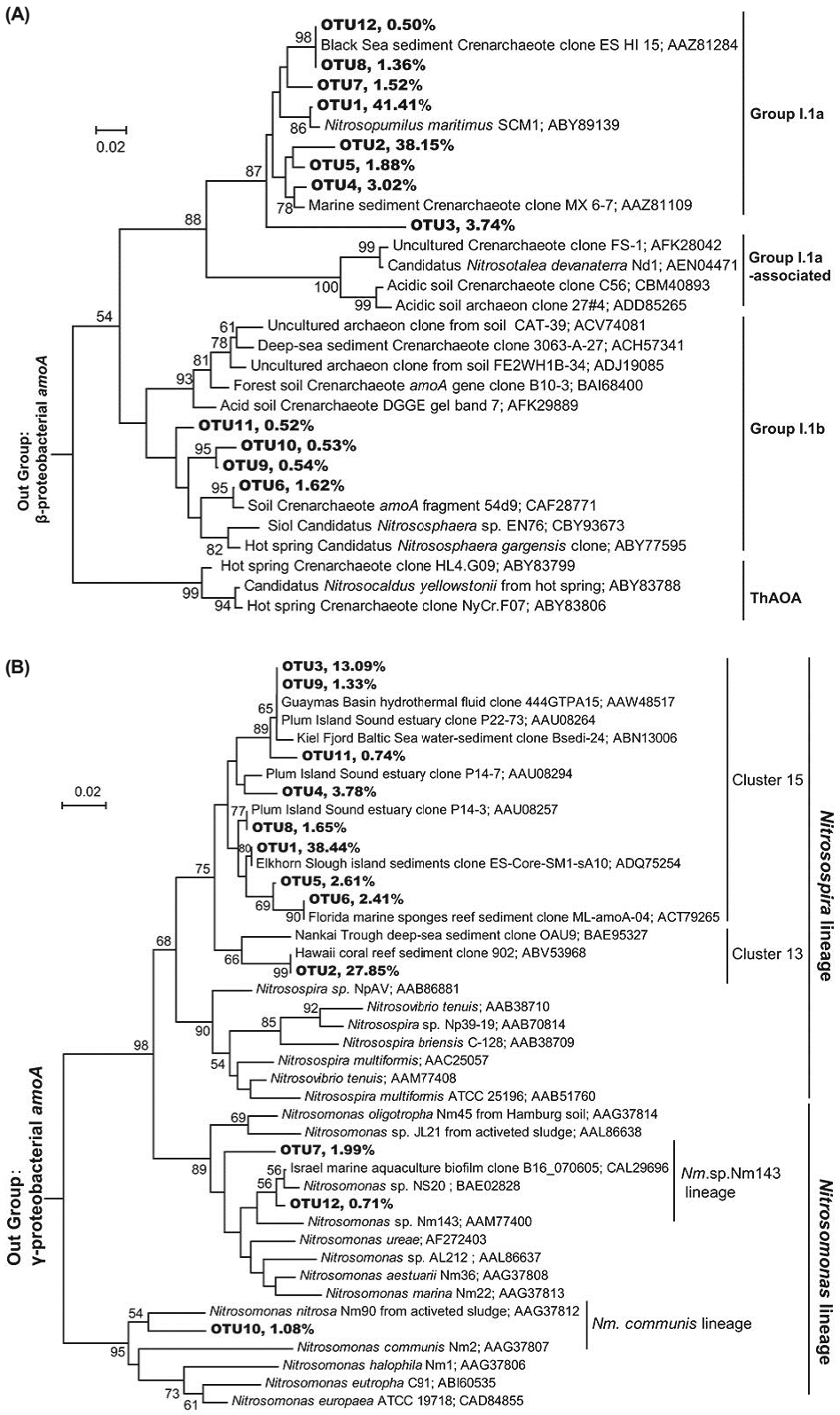

Fig. 2. Neighbor-joining phylogenetic trees of archaeal (A) and β-proteobacterial (B) *amoA* genes. The phylogeny is based on amino acid sequences. Bootstrap values greater than 50% of 1,000 bootstrap replicates are shown near the nodes. The scales indicate the number of amino acids substitutions per site. In Fig. 2B *Nm.* is the abbreviation of *Nitrosomonas*. GenBank accession numbers following the reference sequences are from NCBI and other studies. Percentage numbers following each OTU indicate the percentage of sequences belonging to this OTU from the total recovered sequences. Only the OTUs containing more than 0.5% of the total sequences were shown in the phylogenetic tree.

In site "a", the order of magnitude of bacterial *amoA* gene copy numbers through the invasion appeared constant, although slight reductions were observed from $5.70 \pm 0.42\times10^5$ to $4.27 \pm 0.21\times10^5$ and $6.50 \pm 0.22\times10^5$ to $3.34 \pm 0.20\times10^5$ copies per gram dry matter in spring and summer, respectively. On the other hand, in site "b", copy number of bacterial *amoA* genes increased through the *S. alterniflora* invasion stages in spring and appeared constant in summer. T-test showed that there was no significant difference of bacterial *amoA* gene copies between samples from *P. australis* and *S. alterniflora* invaded areas. The ratios of bacterial *amoA* to total bacterial 16S rRNA genes in the two sampling sites also decreased as the invasion of *S. alterniflora* (Fig. 1B) in both seasons with the exception of the transition area of site "a". Additionally, relatively higher abundance of bacterial than archaeal *amoA* gene copies of the two sampling sites were observed in spring (approximately 2.5 and 4 times higher in the *P. australis* and *S. alterniflora* living zones, respectively), whereas the copy numbers of archaeal and bacterial *amoA* genes in the two sampling sites in summer were variable with no clear trend. However, t-test showed that the ratios of archaeal to bacterial *amoA* gene copies were significantly higher in summer samples than in the spring samples ($P < 0.05$) (Supplementary data Fig. S2). Moreover, the ratio of archaeal to bacterial *amoA* gene copies was not affected by the invasion of *S. alterniflora* ($P > 0.05$) (Supplementary data Fig. S2).

Diversity of AOA and AOB community

Diversity indices were calculated after normalization of the number of *amoA* gene sequences in each sample (284 for archaea and 125 for bacteria). The average number of OTUs defined for the AOA community showed noticeable reduction with the progress of *S. alterniflora* invasion in both seasons (Table 2). For instance, in spring, the average OTU number of AOA community in *P. australis* living sediments was 38, which was decreased to 31 in *S. alterniflora* invaded sediments. Although Shannon-Weiner index of AOA community did not show noticeable variation, Chao1 index was relatively higher at the transition zones in both seasons. On the other hand, the average number of OTUs for the AOB community increased in spring from about 14 in *P. australis* living sediments to about 23 in *S. alterniflora* invaded sediments. In summer, almost no variation was observed in the *S. alterniflora* and *P. australis* covered sediments, although the lowest was observed in the transition zones. Similar to the AOA community, Shannon-Weiner index did not show clear trends for the AOB community. However, the average Chao1 index for AOB community was consistent with the trend in number of OTUs, particularly in summer, where the index values increased from about 22 in *P. australis* living sediments to about 13 in *S. alterniflora* covered sediments (Table 2). The ACE indices of AOA and AOB communities varied with different stages of *S. alterniflora* invasion, seasonal variation and the sampling sites. In general, the ACE indices of the AOA community were relatively higher in spring than in summer, although there was no notable trends were observed for the AOB community.

Phylogenetic analysis of AOA and AOB phylotypes

More than 90% of the total archaeal *amoA* gene sequences were closely related to marine group I.1a (Fig. 2A), suggesting a significant role of marine group I.1a in the salt marsh sediment. Archaeal *amoA* gene sequences closely related to the group I.1b comprised less than 5% of the total sequences. There were no sequences belonging to the ThAOA cluster (Fig. 2A). OTU1 and OTU2, the most abundant OTUs defined for the AOA community, represented about 80% percent of archaeal *amoA* sequences in each sample. With the progress of *S. alterniflora* invasion, there were no significant shifts in the proportions of these main OTUs (Fig. 3A). Representing about 41.4% of the total archaeal *amoA* sequence, OTU1 was closely related to the *Nitrosopumilus maritimus* SCM1 (Könneke *et al.*, 2005), whereas OTU2, OTU4, and OTU5 were clustered together with marine sediment *Crenarchaeota* clone MX 6-7 (Francis *et al.*, 2005).

Table 2. Diversity indices calculated for ammonia-oxidizing microbial communities in the sediments of salt marshes in the Yangtze River estuary

Season	Sample	AOA						AOB					
		No.[a]	Shannon[b]	Simpson	Chao1	ACE	Coverage	No.	Shannon	Simpson	Chao1	ACE	Coverage
Spring	PIa[c]	55	1.5	0.35	43	87	0.95	14	1.7	0.23	11	12	0.98
	PIb	21	1.6	0.33	25	27	0.98	15	1.8	0.26	43	40	0.94
	TIa	40	1.6	0.31	48	105	0.95	23	1.7	0.29	17	19	0.96
	TIb	32	1.7	0.31	67	79	0.94	14	1.7	0.28	17	19	0.96
	SIa	41	1.7	0.28	30	48	0.97	23	1.8	0.22	16	24	0.96
	SIb	21	1.7	0.29	28	41	0.97	24	1.8	0.26	34	58	0.93
Summer	PIIa	41	1.5	0.35	40	57	0.95	24	1.8	0.24	16	17	0.97
	PIIb	19	1.5	0.34	23	28	0.98	19	1.6	0.31	29	43	0.94
	TIIa	20	1.6	0.31	45	38	0.97	21	1.8	0.25	29	59	0.92
	TIIb	24	1.3	0.41	42	39	0.95	11	1.4	0.28	12	17	0.97
	SIIa	27	1.7	0.29	31	38	0.96	19	1.5	0.29	10	11	0.98
	SIIb	23	1.5	0.35	48	59	0.96	24	1.8	0.23	17	19	0.96

[a] Number of OTUs clustered at 5% difference of nucleotide sequence.
[b] The calculation of all the indices was based on the same sequences in each sample with 284 archaeal *amoA* gene sequences and 125 bacterial *amoA* gene sequences in each sample, respectively.
[c] The capital letters P, S and T represent the samples from *P. australis*, *S. alterniflora* and transition zones. "I" and "II" represent the spring and summer samples, respectively, and characters "a" and "b" indicate the replicate locations.

OTU7, OTU8, and OTU12 were closely related to the archaeal *amoA* gene fragment obtained from Black Sea sediment (Francis *et al.*, 2005).

All of the bacterial *amoA* gene sequences recovered in the current study were affiliated to two β-proteobacterial AOB: the *Nitrosomonas* and *Nitrosospira* (Fig. 2B). Interestingly, more than 90% of the sequences were closely related to the *Nitrosospira* lineage, whereas sequences associated to the *Nitrosomonas* lineage represented very small proportion in each sample (Fig. 3B). Sequences belonging to the lineage of *Nitrosospira* were related to clusters 13 and 15, clusters of *Nitrosospira* described elsewhere (Avrahami and Conrad, 2003; Dang *et al.*, 2010). OTU1, OTU3, OTU4, OTU5, OTU6, OTU8, OTU9, and OTU11 (representing approximately 64% of the total sequences) were related to cluster 15 reported from estuarine and coastal sediments (Fig. 2B). OTU2, accounting for approximately 27.9% of the total sequences, was closely related to cluster 13 that was detected in the Hawaii coral reef sediment (Fig. 2B). Only 3.8% of the total sequences were represented by the OTUs that belong to the *Nitrosomonas* lineage with OTU7 and OTU12 were related to *Nitrosomonas* sp. Nm143 lineage and OTU10 was related to *Nitrosomonas communis* lineage (Fig. 2B).

Variations in ammonia-oxidizing microbial communities

Variations in ammonia-oxidizing microbial communities in the sample were analyzed using PCA. For AOA microbial community, the first two components represented 61.63% of the total variance. Although there were no clear clusters of the AOA communities in the samples from different stages of the *S. alterniflora* invasion (Fig. 4A), with few exceptions (PIa, PIb, PIIa, and TIIb), the samples from different seasons were roughly clustered together along PC1 axis (Fig. 4A). This result was also demonstrated by the cluster tree of unweighted pair-group method with arithmetic means (UPGMA) (Supplementary data Fig. S3A). In the UPGMA cluster tree, with the exception of the samples PIIa, SIIa, and PIb, the AOA communities in the samples from different seasons were clustered separately. For the AOB community, the first two components represented 60.94% of the total variance. Similar to the AOA community, AOB communities from the different stages of *S. alterniflora* invasion were not clustered together (Fig. 4B); however, the UPGMA cluster tree (Supplementary data Fig. S3B) showed that the AOB communities in the spring samples were separately clustered from the summer samples, although there were few exception (TIIa, SIIa, PIa, and SIa).

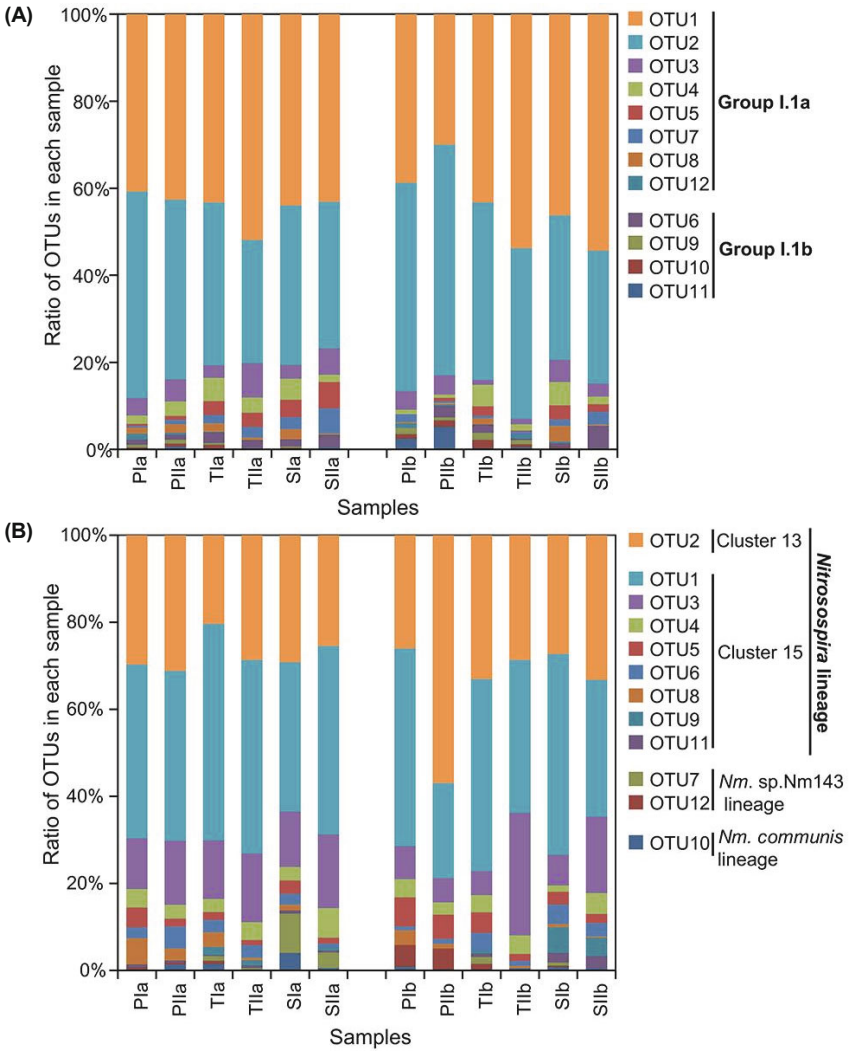

Fig. 3. Proportions of major phylotypes of archaeal (A) and β-proteobacterial *amoA* (B). In Fig. 3B *Nm.* is the abbreviation of *Nitrosomonas* and the OTU numbers were the same with the phylogenetic trees in Fig. 2. The sample names are the same as in Fig. 1.

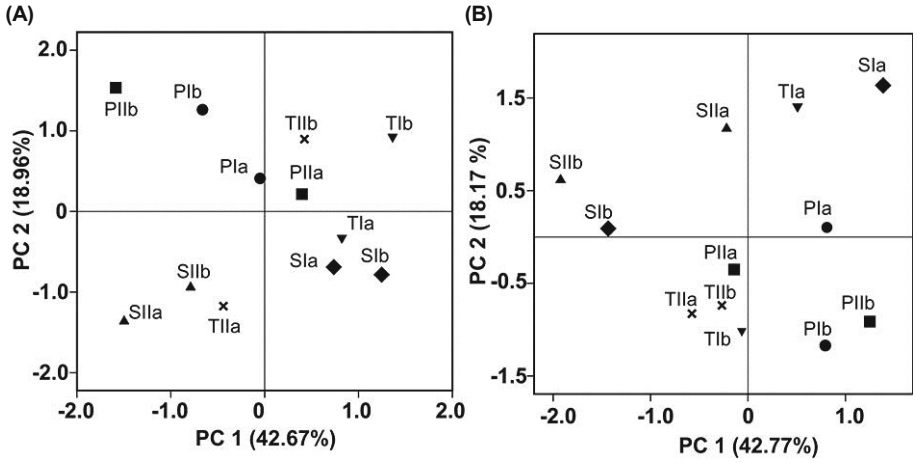

Fig. 4. Principal component analysis (PCA) of AOA (A) and AOB (B) community compositions. Only the first two principal dimensions are shown in the ordination. Numbers on the axes represent the variance explained of the total variance. OTUs that contained more than 0.5% of the total sequences are shown in the ordination plot. Sample names are the same as in Figure 1.

Relationships of ammonia-oxidizing microbial community with different environmental parameters

Correlations of the AOA and AOB communities with various environmental parameters were analyzed via CCA using R software. For AOA community, the first two axes represented 70.89% of the total variance of the relationship of archaeal *amoA* composition and environment (Fig. 5A). Total carbon (TC) and sulfate concentration contributed significantly ($P < 0.05$, 1,000 Monte Carlo permutations) to the relationship between archaeal *amoA* gene sequences composition and the environmental factors. Total nitrogen (TN) and conductivity also contributed to the relationship of archaeal *amoA* genotype and environment (p = 0.06, 1,000 Monte Carlo permutations). For the AOB community, the first two CCA dimensions showed 70.16% of the total variance in the relationship of β-Proteobacterial *amoA* composition and environmental factors (Fig. 5B). Sulfate concentration, TN, TC, and pH were significantly correlated with changes in ammonia oxidizing bacterial community ($P < 0.05$, 1,000 Monte Carlo permutations).

Discussion

The abundance and community composition of AOA and AOB were evaluated to provide insights into the response of ammonia-oxidizing microbial communities as the native *P. australis* was being replaced by the exotic *S. alterniflora* in the salt marshes of Yangtze River estuary. The influence of different *S. alterniflora* invasion stages on the copy numbers of archaeal and bacterial *amoA* genes appeared to be limited in the current study. A previous study conducted at the same sampling sites demonstrated that the abundances of methanogens and sulfate-reducing bacteria were increased with the *S. alterniflora* invasion, likely due to the increase of the sulfate concentration and substrate stimulations of methanogens and sulfate-reducing bacteria in the salt marsh sediments by the exotic *S. alterniflora* (Zeleke *et al.*, 2013). As the concentration of nitrate in the sediments did not vary in the different invasion stages of *S. alterniflora* invasion (Supplementary data Table S1), it might not cause significant variation on ammonia-oxidizing microbial communities.

In both sampling sites, the relatively higher copy numbers of archaeal *amoA* gene in summer than in spring ($P < 0.05$) indicated the effects of seasonal variations on AOA community. This result was consistent with our previous study (Hu *et al.*, 2014) where the greater impacts of seasonal variation on the abundance of AOA than AOB community were demonstrated. The year-round stable abundance of AOA community in subtropical estuarine sediment in Australia where less dramatic seasonal temperature differences occur (Abell *et al.*, 2009) and larger than 100 folds' seasonal variation of the abundance of AOA in the SE USA coastal waters (Hollibaugh *et al.*, 2014) and in salt marshes of Yangtze

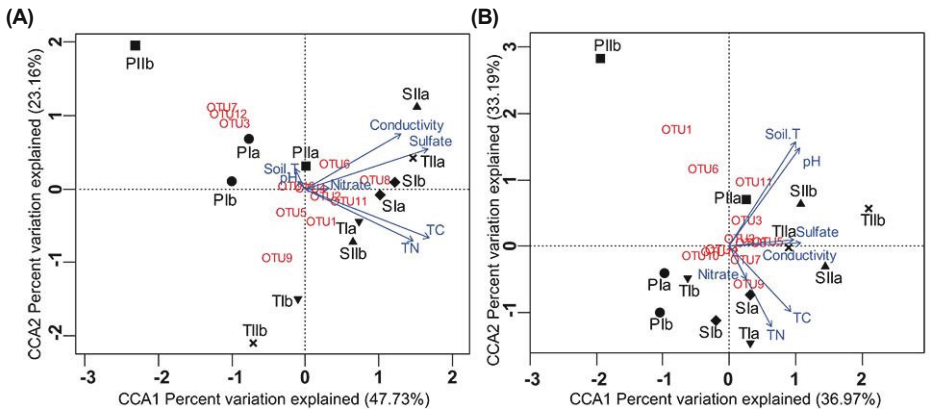

Fig. 5. Canonical correspondence analysis (CCA) plots of the AOA (A) and AOB (B) community compositions with the environmental parameters. Only the first two principal dimensions of the community compositions- environmental parameters relationship are shown in the CCA ordination. Soil. T, TC and TN represent soil temperature, total carbon and total nitrogen, respectively. Numbers on the axes represent the variance explained of the total variance. Only the OTUs that contained more than 0.5% of the total sequences are shown in the ordination plot. The concentrations of nitrate used in the analysis are shown in Supplementary data Table S1. Sample names are the same as in Fig. 1.

River estuary, China (Hu *et al.*, 2014) where seasonal temperature difference is magnificent might suggest temperature to be the primary seasonal factor affecting the abundance of AOA microbial community. The variations in the abundance of archaeal and bacterial *amoA* genes in estuarine sediments have been indicated by a number of studies (Dang *et al.*, 2010; Lage *et al.*, 2010; Zheng *et al.*, 2012). The abundances of archaeal and bacterial *amoA* genes are dependent on physical and chemical characters of sediments (Fenchel, 2002; Yao *et al.*, 2013). In the current study, the variations in the copy numbers of archaeal and bacterial *amoA* genes, even at the two sampling sites of the same plant (e.g. copy number of bacterial *amoA* gene in spring) (Fig. 1A) might signify the effects of microenvironments' variation of different sampling sites.

Most (> 90%) of the archaeal *amoA* gene sequences detected in the present study were related to marine group I.1a. This group was widely detected in the marine water column (Mincer *et al.*, 2007; Beman *et al.*, 2010), marine sediments (Park *et al.*, 2010) and estuarine sediments (Beman and Francis, 2006; Seyler *et al.*, 2014) as well as in salt marsh sediments (Nelson *et al.*, 2009), suggesting the global distribution of this group of AOA. Only a small portion of archaeal *amoA* gene sequences affiliated to the group I.1b, which is considered as a soil group (Hatzenpichler *et al.*, 2008). The group I.1b has been reported from agricultural soil (Kim *et al.*, 2012), grassland soil (Damsté *et al.*, 2012) and forest soil (Hansel *et al.*, 2008; Kim *et al.*, 2014). Indeed, the estuarine sediments could harbor both marine and soil groups of AOA as it was demonstrated by a number of studies (Beman and Francis, 2006; Mosier and Francis, 2008; Bernhard *et al.*, 2010). On the other hand, all of the β-proteobacterial *amoA* gene sequences detected in the current study were grouped within the *Nitrosospira* and *Nitrosomonas* lineages, which have been demonstrated to be the dominant AOB in estuarine sediments (Purkhold *et al.*, 2000; Mosier and Francis, 2008). Containing more than 90% of the sequences detected, the *Nitrosospira* lineage was also reported to be dominant in the salt marsh sediments of estuarine ecosystems (Moin *et al.*, 2009; Lage *et al.*, 2010). Contained in *Nitrosospira* lineages, clusters 13 and 15 were also detected by other studies in meadow soils and estuary sediments (Avrahami and Conrad, 2003; Dang *et al.*, 2010). Cluster 13 may indicate an *amoA* sequence group from coastal and deep sea marine sediments (Hayashi *et al.*, 2007), and Cluster 15, accounting for more than 60% of the total sequences detected in the current study, may indicate an estuarine and coastal group (Bernhard *et al.*, 2005; Wankel *et al.*, 2011). The main OTUs of the AOA and AOB communities seemed not affected by the invasion of *S. alterniflora* (Fig. 3) while the community of sulfate-reducing bacteria was notably affected by the invasion of *S. alterniflora* (Zeleke *et al.*, 2013). The structure of ammonia-oxidizing microbial community was typically regulated by temperature, pH, salinity and organic loading and the net primary productivity (NPP) (Bernhard and Bollmann, 2010), suggesting a multi-factorial drivers of ammonia-oxidizing communities (Yao *et al.*, 2013).

CCA results indicated that the ammonia-oxidizing community structures in the salt marsh were largely affected by sulfate concentration, TN and TC, which was consistent with many other investigations (Zhang *et al.*, 2009; Szukics *et al.*, 2010; Bai *et al.*, 2013). Previous study conducted at the same sampling sites demonstrated that the sulfate concentration was increased with the invasion of *S. alterniflora* in the salt marsh sediments (Zeleke *et al.*, 2013), and sulfate reduction may reduce the oxygen in microenvironments to affect the community of AOA and AOB. The changing of nutrition of the sediments could affect the microbial community structure in soils or sediments (Bais *et al.*, 2006; Haichar *et al.*, 2008), especially for the ammonia-oxidizing community, which has long been considered as the autotrophic organisms. A group of AOA was considered as heterotrophic or mixotrophic catabolism (Ingalls *et al.*, 2006; Jia and Conrad, 2009; Seyler *et al.*, 2014) with some of marine groups were found to have preference for particular carbon sources (Seyler *et al.*, 2014). In the current study, the invasion of *S. alterniflora* increased the TN and TC of the sediments (Zeleke *et al.*, 2013), as a result, the structure of AOA community might be amended where certain group of AOA could be enhanced by the change in the TN and TC contents of sediments in the salt marsh. Similar to AOA, it has been demonstrated that the structure of AOB was also significantly correlated with TC and TN (Jia and Conrad, 2009; Fan *et al.*, 2011; Seyler *et al.*, 2014).

In conclusion, with *S. alterniflora* invasion, the trends of shifts for the abundance of AOA and AOB, as inferred from the respective *amoA* gene copy numbers, were not strongly associated. However, the abundance of AOA was higher in summer than in spring. The archaeal and bacterial *amoA* gene sequences were dominated by sequences closely related to marine group I.1a and the *Nitrosospira* lineage cluster, respectively. TC and sulfate concentrations appeared to be the main factors affecting the structure AOA community as sulfate concentration, TN, TC, and pH were the main factors affecting the AOB community. Overall, seasonal variations and soil parameters appeared to be more important than the effect of *S. alterniflora* invasion for affecting the abundance and structure of ammonia oxidizing microbial communities.

Acknowledgements

This project was partially supported by the National Natural Science Foundation of China (31070097), the National Key Technology R& D Program of China (2010BAK69B14) and the Major Program of Science and Technology Department of Shanghai (10DZ1200700).

References

Abell, G.C.J., Revill, A.T., Smith, C., Bissett, A.P., Volkman, J.K., and Robert, S.S. 2009. Archaeal ammonia oxidizers and *nirS*-type denitrifiers dominate sediment nitrifying and denitrifying populations in a subtropical macrotidal estuary. *ISME J.* **4**, 286–300.

Avrahami, S. and Conrad, R. 2003. Patterns of community change among ammonia oxidizers in meadow soils upon long-term incubation at different temperatures. *Appl. Environ. Microbiol.* **69**, 6152–6164.

Bai, S., Li, J., He, Z., Van Nostrand, J.D., Tian, Y., Lin, G., Zhou, J., and Zheng, T. 2013. Geochip-based analysis of the functional gene diversity and metabolic potential of soil microbial communities of mangroves. *Appl. Microbiol. Biotechnol.* **15**, 1–14.

Bais, H.P., Weir, T.L., Perry, L.G., Gilroy, S., and Vivanco, J.M. 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu. Rev. Plant Biol.* **57**, 233–266.

Beman, J.M. and Francis, C.A. 2006. Diversity of ammonia-oxidizing archaea and bacteria in the sediments of a hypernutrified subtropical estuary: Bahia del Tobari, Mexico. *Appl. Environ. Microbiol.* **72**, 7767–7777.

Beman, J.M., Sachdeva, R., and Fuhrman, J.A. 2010. Population ecology of nitrifying archaea and bacteria in the southern california bight. *Environ. Microbiol.* **12**, 1282–1292.

Bernhard, A.E. and Bollmann, A. 2010. Estuarine nitrifiers: new players, patterns and processes. *Estuar. Coast Shelf. Sci.* **88**, 1–11.

Bernhard, A.E., Donn, T., Giblin, A.E., and Stahl, D.A. 2005. Loss of diversity of ammonia-oxidizing bacteria correlates with increasing salinity in an estuary system. *Environ. Microbiol.* **7**, 1289–1297.

Bernhard, A.E., Landry, Z.C., Blevins, A., José, R., Giblin, A.E., and Stahl, D.A. 2010. Abundance of ammonia-oxidizing archaea and bacteria along an estuarine salinity gradient in relation to potential nitrification rates. *Appl. Environ. Microbiol.* **76**, 1285–1289.

Berry, D., Mahfoudh, K.B., Wagner, M., and Loy, A. 2011. Barcoded primers used in multiplex amplicon pyrosequencing bias amplification. *Appl. Environ. Microbiol.* **77**, 7846–7849.

Brochier-Armanet, C., Boussau, B., Gribaldo, S., and Forterre, P. 2008. Mesophilic crenarchaeota: Proposal for a third archaeal phylum, the Thaumarchaeota. *Nat. Rev. Microbiol.* **6**, 245–252.

Caffrey, J.M., Bano, N., Kalanetra, K., and Hollibaugh, J.T. 2007. Ammonia oxidation and ammonia-oxidizing bacteria and archaea from estuaries with differing histories of hypoxia. *ISME J.* **1**, 660–662.

Cheng, X., Peng, R., Chen, J., Luo, Y., Zhang, Q., An, S., Chen, J., and Li, B. 2007. CH_4 and N_2O emissions from *Spartina alterniflora* and *Phragmites australis* in experimental mesocosms. *Chemosphere* **68**, 420–427.

Craft, C., Clough, J., Ehman, J., Joye, S., Park, R., Pennings, S., Guo, H., and Machmuller, M. 2008. Forecasting the effects of accelerated sea-level rise on tidal marsh ecosystem services. *Front. Ecol. Environ.* **7**, 73–78.

Damsté, J.S.S., Rijpstra, W.I.C., Hopmans, E.C., Jung, M.Y., Kim, J.G., Rhee, S.K., Stieglmeier, M., and Schleper, C. 2012. Intact polar and core glycerol dibiphytanyl glycerol tetraether lipids of group I.1a and I.1b Thaumarchaeota in soil. *Appl. Environ. Microbiol.* **78**, 6866–6874.

Dang, H., Li, J., Chen, R., Wang, L., Guo, L., Zhang, Z., and Klotz, M.G. 2010. Diversity, abundance, and spatial distribution of sediment ammonia-oxidizing betaproteobacteria in response to environmental gradients and coastal eutrophication in Jiaozhou Bay, China. *Appl. Environ. Microbiol.* **76**, 4691–4702.

Fan, F., Yang, Q., Li, Z., Wei, D., Cui, X., and Liang, Y. 2011. Impacts of organic and inorganic fertilizers on nitrification in a cold climate soil are linked to the bacterial ammonia oxidizer community. *Microb. Ecol.* **62**, 982–990.

Fenchel, T. 2002. Microbial behavior in a heterogeneous world. *Science* **296**, 1068–1071.

Francis, C.A., Roberts, K.J., Beman, J.M., Santoro, A.E., and Oakley, B.B. 2005. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. *Proc. Natl. Acad. Sci. USA* **102**, 14683–14688.

Haichar, F.Z., Marol, C., Berge, O., Rangel-Castro, J.I., Prosser, J.I., Balesdent, J., Heulin, T., and Achouak, W. 2008. Plant host habitat and root exudates shape soil bacterial community structure. *ISME J.* **2**, 1221–1230.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* **41**, 95–98.

Hansel, C.M., Fendorf, S., Jardine, P.M., and Francis, C.A. 2008. Changes in bacterial and archaeal community structure and functional diversity along a geochemically variable soil profile. *Appl. Environ. Microbiol.* **74**, 1620–1633.

Hatzenpichler, R., Lebedeva, E.V., Spieck, E., Stoecker, K., Richter, A., Daims, H., and Wagner, M. 2008. A moderately thermophilic ammonia-oxidizing crenarchaeote from a hot spring. *Proc. Natl. Acad. Sci. USA* **105**, 2134–2139.

Hayashi, T., Kaneko, R., Tanahashi, M., and Naganuma, T. 2007. Molecular diversity of the genes encoding ammonia monooxygenase and particulate methane monooxygenase from deep-sea sediments. *Res. J. Microbiol.* **2**, 530–537.

Hollibaugh, J.T., Gifford, S.M., Moran, M.A., Ross, M.J., Sharma, S., and Tolar, B.B. 2014. Seasonal variation in the metratranscriptomes of a Thaumarchaeota population from SE USA coastal waters. *ISME J.* **8**, 685–698.

Hou, L.J., Liu, M., Jiang, H.Y., Xu, S.Y., Ou, D.N., Liu, Q.M., and Zhang, B.L. 2003. Ammonium adsorption by tidal flat surface sediments from the Yangtze estuary. *Environ. Geol.* **45**, 72–78.

Hu, Z., Meng, H., Shi, J.H., Bu, N.S., Fang, C.M., and Quan, Z.X. 2014. Community size and composition of ammonia oxidizers and denitrifiers in an alluvial intertidal wetland ecosystem. *Front. Microbiol.* **5**, 371.

Ingalls, A.E., Shah, S.R., Hansman, R.L., Aluwihare, L.I., Santos, G.M., Druffel, E.R., and Pearson, A. 2006. Quantifying archaeal community autotrophy in the mesopelagic ocean using natural radiocarbon. *Proc. Natl. Acad. Sci. USA* **103**, 6442–6447.

Jia, Z. and Conrad, R. 2009. Bacteria rather than archaea dominate microbial ammonia oxidation in an agricultural soil. *Environ. Microbiol.* **11**, 1658–1671.

Jiang, L.F., Luo, Y.Q., Chen, J.K., and Li, B. 2009. Ecophysiological characteristics of invasive *Spartina alterniflora* and native species in salt marshes of Yangtze River estuary, China. *Estuar. Coast. Shelf. Sci.* **81**, 74–82.

Kim, J., Jung, M., Lee, K., Kim, D., Ko, S., Lee, J., and Rhee, S. 2014. The archaea community associated with lava-formed Gotjawal forest soil in Jeju, Korea. *J. Agr. Chem. Environ.* **3**, 96–102.

Kim, J. , Jung, M. , Lee, K. , Kim, D. , Ko, S. , Lee, J., and Rhee, S. 2014. The Archaea community associated with lava-formed Gotjawal forest soil in Jeju, Korea. *J. Agr. Chem. Environ.* **3**, 96-102.

Kim, J.G., Jung, M.Y., Park, S.J., Rijpstra, W.I.C., Sinninghe Damsté, J.S., Madsen, E.L., Min, D., Kim, J.S., Kim, G.J., and Rhee, S.K. 2012. Cultivation of a highly enriched ammonia-oxidizing archaeon of thaumarchaeotal group I.1b from an agricultural soil. *Environ. Microbiol.* **14**, 1528–1543.

Könneke, M., Bernhard, A.E., de la Torre, J.R., Walker, C.B., Waterbury, J.B., and Stahl, D.A. 2005. Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature* **437**, 543–546.

Kowalchuk, G.A. and Stephen, J.R. 2001. Ammonia-oxidizing bacteria: A model for molecular microbial ecology. *Annu. Rev. Microbiol.* **55**, 485–529.

Lage, M.D., Reed, H.E., Weihe, C., Crain, C.M., and Martiny, J.B. 2010. Nitrogen and phosphorus enrichment alter the composition of ammonia-oxidizing bacteria in salt marsh sediments. *ISME J.* **4**, 933–944.

Larkin, M.A., Blackshields, G., Brown, N., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., and Lopez, R. 2007. Clustal W and clustal X version 2.0. *Bioinformatics* **23**, 2947–2948.

Leininger, S., Urich, T., Schloter, M., Schwark, L., Qi, J., Nicol, G., Prosser, J., Schuster, S., and Schleper, C. 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature* **442**, 806–809.

Li, X.R., Xiao, Y.P., Ren, W.W., Liu, Z.F., Shi, J.H., and Quan, Z.X. 2012. Abundance and composition of ammonia-oxidizing bacteria and archaea in different types of soil in the Yangtze river estuary. *J. Zhejiang. Univ. Sci. B*. **13**, 769–782.

Liao, C., Luo, Y., Jiang, L., Zhou, X., Wu, X., Fang, C., Chen, J., and Li, B. 2007. Invasion of *Spartina alterniflora* enhanced ecosystem carbon and nitrogen stocks in the Yangtze estuary, China. *Ecosystems* **10**, 1351–1361.

Mincer, T.J., Church, M.J., Taylor, L.T., Preston, C., Karl, D.M., and DeLong, E.F. 2007. Quantitative distribution of presumptive archaeal and bacterial nitrifiers in monterey bay and the Monterey Bay and the North Pacific Subtropical Gyre. *Environ. Microbiol.* **9**, 1162–1175.

Moin, N.S., Nelson, K.A., Bush, A., and Bernhard, A.E. 2009. Distribution and diversity of archaeal and bacterial ammonia oxidizers in salt marsh sediments. *Appl. Environ. Microbiol.* **75**, 7461–7468.

Mosier, A.C. and Francis, C.A. 2008. Relative abundance and diversity of ammonia-oxidizing archaea and bacteria in the San Francisco Bay estuary. *Environ. Microbiol.* **10**, 3002–3016.

Nelson, K.A., Moin, N.S., and Bernhard, A.E. 2009. Archaeal diversity and the prevalence of *Crenarchaeota* in salt marsh sediments. *Appl. Environ. Microbiol.* **75**, 4211–4215.

Page, H.M., Lastra, M., Rodil, I.F., Briones, M.J.I., and Garrido, J. 2010. Effects of non-native *Spartina patens* on plant and sediment organic matter carbon incorporation into the local invertebrate community. *Biol. Invasions* **12**, 3825–3838.

Parameswaran, P., Jalili, R., Tao, L., Shokralla, S., Gharizadeh, B., Ronaghi, M., and Fire, A.Z. 2007. A pyrosequencing-tailored nucleotide barcode design unveils opportunities for large-scale sample multiplexing. *Nucleic Acids Res*. **35**, e130.

Park, B.J., Park, S.J., Yoon, D.N., Schouten, S., Sinninghe Damsté, J.S., and Rhee, S.K. 2010. Cultivation of autotrophic ammonia-oxidizing archaea from marine sediments in coculture with sulfur-oxidizing bacteria. *Appl. Environ. Microbiol.* **76**, 7575–7587.

Pester, M., Schleper, C., and Wagner, M. 2011. The Thaumarchaeota: an emerging view of their phylogeny and ecophysiology. *Curr. Opin. Microbiol.* **14**, 300–306.

Purkhold, U., Pommerening-Röser, A., Juretschko, S., Schmid, M.C., Koops, H.P., and Wagner, M. 2000. Phylogeny of all recognized species of ammonia oxidizers based on comparative 16S rRNA and *amoA* sequence analysis: Implications for molecular diversity surveys. *Appl. Environ. Microbiol.* **66**, 5368–5382.

Quan, W., Han, J., Shen, A., Ping, X., Qian, P., Li, C., Shi, L., and Chen, Y. 2007. Uptake and distribution of N, P and heavy metals in three dominant salt marsh macrophytes from Yangtze River estuary, China. *Mar. Environ. Res*. **64**, 21–37.

R Development Core Team. 2004. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria (http://www.r-project.org).

Rotthauwe, J.H., Witzel, K.P., and Liesack, W. 1997. The ammonia monooxygenase structural gene *amoA* as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. *Appl. Environ. Microbiol.* **63**, 4704–4712.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., and Robinson, C.J. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* **75**, 7537–7541.

Seyler, L.M., McGuinness, L.M., and Kerkhof, L.J. 2014. Crenarchaeal heterotrophy in salt marsh sediments. *ISME J*. **8**, 1534–1543.

Szukics, U., Abell, G.C., Hödl, V., Mitter, B., Sessitsch, A., Hackl, E., and Zechmeister-Boltenstern, S. 2010. Nitrifiers and denitrifiers respond rapidly to changed moisture and increasing temperature in a pristine forest soil. *FEMS Microbiol. Ecol.* **72**, 395–406.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. 2011. Mega5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol*. **28**, 2731–2739.

Tourna, M., Freitag, T.E., Nicol, G.W., and Prosser, J.I. 2008. Growth, activity and temperature responses of ammonia-oxidizing archaea and bacteria in soil microcosms. *Environ. Microbiol*. **10**, 1357–1364.

Treusch, A.H., Leininger, S., Kletzin, A., Schuster, S.C., Klenk, H.P., and Schleper, C. 2005. Novel genes for nitrite reductase and amo-related proteins indicate a role of uncultivated mesophilic Crenarchaeota in nitrogen cycling. *Environ. Microbiol.* **7**, 1985–1995.

Turner, R.E. 1993. Carbon, nitrogen, and phosphorus leaching rates from *Spartina alterniflora* salt marshes. *Mar. Ecol. Prog. Ser*. **92**, 135–140.

Venter, J.C., Remington, K., Heidelberg, J.F., Halpern, A.L., Rusch, D., Eisen, J.A., Wu, D., Paulsen, I., Nelson, K.E., and Nelson, W. 2004. Environmental genome shotgun sequencing of the Sargasso Sea. *Science* **304**, 66–74.

Wang, Q., Quensen, J.F., Fish, J.A., Lee, T.K., Sun, Y., Tiedje, J.M., and Cole, J.R. 2013. Ecological patterns of *nifH* genes in four terrestrial climatic zones explored with targeted metagenomics using FrameBot, a new informatics tool. *MBio* **4**, e00592–00513.

Wankel, S.D., Mosier, A.C., Hansel, C.M., Paytan, A., and Francis, C.A. 2011. Spatial variability in nitrification rates and ammonia-oxidizing microbial communities in the agriculturally impacted Elkhorn Slough estuary, California. *Appl. Environ. Microbiol.* **77**, 269–280.

Williams, S.L. and Grosholz, E.D. 2008. The invasive species challenge in estuarine and coastal environments: Marrying management and science. *Estuar. Coast* **31**, 3–20.

Yao, H., Campbell, C.D., Chapman, S.J., Freitag, T.E., Nicol, G.W., and Singh, B.K. 2013. Multi-factorial drivers of ammonia oxidizer communities: evidence from a national soil survey. *Environ. Microbiol*. **15**, 2545–2556.

Zeleke, J., Sheng, Q., Wang, J.G., Huang, M.Y., Xia, F., Wu, J.H., and Quan, Z.X. 2013. Effects of *Spartina alterniflora* invasion on the communities of methanogens and sulfate-reducing bacteria in estuarine marsh sediments. *Front Microbiol*. **4**, 243.

Zhang, Q.F., Peng, J.J., Chen, Q., Li, X.F., Xu, C.Y., Yin, H.B., and Yu, S. 2011. Impacts of *Spartina alterniflora* invasion on abundance and composition of ammonia oxidizers in estuarine sediment. *J. Soils Sediments*. **11**, 1020–1031.

Zhang, L.M., Wang, M., Prosser, J.I., Zheng, Y.M., and He, J.Z. 2009. Altitude ammonia-oxidizing bacteria and archaea in soils of Mount Everest. *FEMS Microbiol. Ecol.* **70**, 208–217.

Zheng, Y., Hou, L., Liu, M., Lu, M., Zhao, H., Yin, G., and Zhou, J. 2012. Diversity, abundance, and activity of ammonia-oxidizing bacteria and archaea in Chongming eastern intertidal sediments. *Appl. Microbiol. Biotechnol*. **97**, 8351–8363.

Zheng, Y., Hou, L., Newell, S., Liu, M., Zhou, J., Zhao, H., You, L., and Cheng, X. 2014. Community dynamics and activity of ammonia-oxidizing prokaryotes in intertidal sediments of the Yangtze estuary. *Appl. Environ. Microbiol*. **80**, 408–419.