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Distribution and Diversity of Archaeal and Bacterial Ammonia Oxidizers in Salt Marsh Sediments[▽]

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Diversity and abundance of ammonia-oxidizing *Betaproteobacteria* (β-AOB) and archaea (AOA) were investigated in a New England salt marsh at sites dominated by short or tall *Spartina alterniflora* (SAS and SAT sites, respectively) or *Spartina patens* (SP site). AOA *amoA* gene richness was higher than β-AOB *amoA* richness at SAT and SP, but AOA and β-AOB richness were similar at SAS. β-AOB *amoA* clone libraries were composed exclusively of *Nitrosospira*-like *amoA* genes. AOA *amoA* genes at SAT and SP were equally distributed between the water column/sediment and soil/sediment clades, while AOA *amoA* sequences at SAS were primarily affiliated with the water column/sediment clade. At all three site types, AOA were always more abundant than β-AOB based on quantitative PCR of *amoA* genes. At some sites, we detected 10^9 AOA *amoA* gene copies g of sediment^{-1}. Ratios of AOA to β-AOB varied over 2 orders of magnitude among sites and sampling dates. Nevertheless, abundances of AOA and β-AOB *amoA* genes were highly correlated. Abundance of 16S rRNA genes affiliated with *Nitrosopumilus maritimus*, *Crenarchaeota* group I.1b, and pSL12 were positively correlated with AOA *amoA* abundance, but ratios of *amoA* to 16S rRNA genes varied among sites. We also observed a significant effect of pH on AOA abundance and a significant salinity effect on both AOA and β-AOB abundance. Our results expand the distribution of AOA to salt marshes, and the high numbers of AOA at some sites suggest that salt marsh sediments serve as an important habitat for AOA.

Nitrification, the sequential oxidation of ammonia to nitrite and nitrate, is a critical step in the nitrogen cycle and is mediated by a suite of phylogenetically and physiologically distinct microorganisms. The recent discovery of ammonia oxidation among *Archaea* (17, 38) has led to a dramatic shift in the current model of nitrification and to new questions of niche differentiation between putative ammonia-oxidizing *Archaea* (AOA) and the more-well-studied ammonia-oxidizing *Betaproteobacteria* (β-AOB). Based on surveys of 16S rRNA genes and archaeal *amoA* genes, it is evident that AOA occupy a wide range of niches (10), suggesting a physiologically diverse group of *Archaea*. Additionally, in studies where AOA and β-AOB were both targeted, AOA were typically more abundant than their bacterial counterparts (19, 21, 42). However, there are reports of β-AOB outnumbering AOA in estuarine systems (6, 33), suggesting a possible shift in competitive dominance under certain conditions.

Patterns of β-AOB diversity in estuaries have been well characterized and appear to be regulated by similar mechanisms within geographically disparate systems (4, 11, 32). However, AOA distribution and their role in nitrification relative to β-AOB remain to be determined. A few studies have begun to address this question in different estuaries, but no unifying patterns or mechanisms have emerged. Although β-AOB have been well studied along estuarine salinity gradients (1, 3, 4, 7, 11, 13, 22, 33, 39) and recent studies have begun to address AOA in estuaries (1, 6, 22, 32, 33), few have investigated β-AOB in salt marshes (9), and none has included AOA.

In this study, we investigated the distribution and abundance of AOA and β-AOB based on the distribution and abundance of *amoA* genes in salt marsh sediments dominated by different types of vegetation. Although we equate the presence of archaeal *amoA* genes with the genetic potential to oxidize ammonia, we acknowledge the possibility that all *Archaea* that have *amoA* genes may not all represent functional ammonia oxidizers. Vegetation patterns of New England salt marshes are strongly correlated with marsh elevation and are controlled by a combination of interspecific competition and tolerance to physico-chemical stress (28). The dominant grasses of New England salt marshes are *Spartina alterniflora* and *Spartina patens*, which typically grow as pure stands. *S. alterniflora* is found in two phenotypically distinct but genetically identical forms, a tall and a short growth form (34). The tall *S. alterniflora* grows to heights of 1 to 2 m and is typically found at the edges of the marsh and along creek banks (SAT sites), while the short-form *S. alterniflora* may reach heights of only 30 cm and is found in sites (SAS sites) slightly higher on the marsh where soil drainage is limited and conditions are more reduced compared to SAT sites (14). Conversely, *S. patens*, due to its lower tolerance of salt and more reduced conditions, is found in sites (SP sites) highest on the marsh, in areas that receive less flooding (5). Because the marsh is subjected to daily tidal fluctuations, most sites experience periods of anoxia, the degree of which depends on the marsh elevation. We hypothesized that ammonia-oxidizing communities in areas dominated

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TABLE 1. Primers used in this study

Primer	Sequence (5′ to 3′)	Target	Reference
amoA-1F	GGGGTTTCTACTGGTGGT	β-AOB *amoA*	31
amoA-2R-TC	CCCCTCTGCAAAGCCTTCTTC	β-AOB *amoA*	25
ArchAmoAF	STAATGGTCTGGCTTAGACG	Archaeal *amoA*	12
ArchAmoAR	GCGGCCATCCATCTGTATGT	Archaeal *amoA*	12
CrenAmoAQModF	CARGT**H**GGN**N**AARTTCTAYAA[a]	Archaeal *amoA*	This study
GAOB16S-F	GCGTGGGAATCTGGCCTCTA	γ-AOB 16S rRNA	This study
GAOB16S-R	CATCGCTGCTTGGCCACCT	γ-AOB 16S rRNA	This study
CGI.1b-270F	TGGATTGGACTGCGKCCGAT	CGI.1b 16S rRNA	27
CGI.1b-750R	GTCGAGCGCRTTCTGGMAAG	CGI.1b 16S rRNA	27
pSL12_750F	GGTCCRCCAGAACGCGC	pSL12 16S rRNA	21
pSL12_876R	GTACTCCCCAGGCGGCAA	pSL12 16S rRNA	21

[a] Bases in bold indicate modifications from those reported by Mincer et al. (21).

by different marsh grasses would reflect the different edaphic conditions associated with each type of grass, due to differences in vertical zonation in the marsh.

MATERIALS AND METHODS

Study site and sample collection. The research was carried out in the Wequetequock-Pawcatuck tidal marsh (locally referred to as Barn Island) of southeastern Connecticut from March to October 2006 (see references 40 and 41 for more complete site descriptions). DNA was extracted from 0 to 2 cm from replicate cores as previously described (23). Pore water salinity, pH, and ammonium levels have been reported elsewhere (23). Pore water nitrate (plus nitrite) was measured by enzymatic reduction of nitrate to nitrite (8), followed by colorimetric determination of nitrite for seawater (35).

Clone library construction. Clone libraries were constructed from samples collected in March 2006. One clone library was constructed from each site for each gene. β-AOB *amoA* genes were amplified as described by Bernhard et al. (3). Archaeal *amoA* genes were amplified using previously published primers (12). Each 20-μl reaction mixture contained 10 μl iQ Supermix (Bio-Rad), a 0.5 μM concentration of each primer, and 1 μl of a 1:10 dilution of DNA (approximately 2 to 10 ng). Reactions were carried out using the following amplification cycle: 95°C for 5 min, followed by 35 cycles of 95°C for 20 s, 54°C for 20 s, and 72°C for 45 s, with a final elongation at 72°C for 5 min. All reactions were performed on an iQ iCycler (Bio-Rad). PCR products were cloned into the pSC vector using the StrataClone PCR cloning kit (Stratagene, Santa Clara, CA) according to the manufacturer's instructions. Transformants were randomly selected and inoculated into 100 μl LB broth with 100 μg ampicillin ml^{-1} in 96-well microtiter plates. All plates were incubated overnight at 37°C. Inserts were amplified from selected clones using the vector-specific primers T3 and T7. PCR products from clones containing the correctly sized insert were sequenced using the T3 primer. All sequencing was performed by High Throughput Sequencing Solutions (Seattle, WA).

Sequence analysis. Sequences were compared to published sequences in GenBank using the Basic Local Alignment Search Tool (BLASTn) to identify related sequences and aligned using the sequence editor and Fast Align in ARB (20). All alignments were checked manually, and regions of ambiguous alignments were excluded from the analysis. All phylogenetic analyses were done with PAUP version 4.0 (36). Phylogenetic relationships were analyzed by using the neighbor-joining and parsimony algorithms. Parsimony analysis was performed using a full heuristic search with random addition sequence. Confidence in tree topology was assessed by 100 bootstrap replicates for both neighbor-joining and parsimony analyses. Sequences were checked for chimeras by comparing phylogenetic placement in trees constructed with the 5′ and the 3′ ends of the sequence. Pair-wise sequence comparisons were calculated in ARB, and operational taxonomic units (OTUs) were defined as sequences sharing ≥95% nucleotide sequence identity.

Real-time PCR of *amoA* genes. Betaproteobacterial *amoA* genes were quantified as described by Bernhard et al. (4). Archaeal *amoA* genes were quantified using a modified version of CrenAmoAQ-F (21) and Arch-amoAR (12) (Table 1). The forward primer was modified to target new archaeal *amoA* genes recovered from estuarine and salt marsh samples that had two to three mismatches with the published CrenAmoAQ-F primer. Specificity of the archaeal *amoA* primers used in this study was confirmed by sequence analysis of clones generated with the same primers. All sequences were archaeal *amoA* genes (data not shown). PCR conditions were the same as above except we used 10 μl of iQ SYBR green I mix instead of iQ Supermix and we ran 50 cycles followed by melt curve analysis (95°C for 1 min, 55°C for 1 min, and then 0.5°C increase every 10 s, with fluorescence read continuously) to monitor product specificity. All samples were run in at least three separate experimental runs and compared to standard curves generated in each experimental run using five standards ranging in DNA concentration from 0.1 fg μl^{-1} to 1 pg μl^{-1}, which is equivalent to 2.2×10^1 to 2.2×10^5 gene copies μl^{-1}. Standards were purified plasmid DNAs from clones generated from archaeal *amoA* genes recovered previously from salt marsh sediments (A. Bernhard, unpublished data). Average PCR efficiencies for archaeal and bacterial *amoA* genes were 81 and 85%, respectively. We tested for inhibitory effects by running each sample at different dilutions (ranging from 1:5 to 1:15) and calculating the slope of the lines. Dilutions ranging from 1:8 to 1:12 gave similar slopes (coefficient of variation, 11.6%), so we used 1:10 dilutions of each sample for final analysis. Additionally, slopes were not significantly different among samples from different sites ($P = 0.46$). Data presented are the means of at least three separate analyses for each sample. Coefficients of variation among runs were 3.5 and 4.0% for β-AOB and AOA *amoA* genes, respectively.

Real-time PCR of 16S rRNA genes. 16S rRNA genes affiliated with *Crenarchaeota* group I.1b were amplified using primers published by Park et al. (27) (Table 1) with the following cycle conditions: 95°C for 10 min, then 50 cycles of 95°C for 15 s, 64°C for 20 s, and 72°C for 30 s. Fluorescence was measured at 86°C to eliminate signals from nonspecific products with lower melting temperatures. Melt curve analysis was conducted to monitor product specificity (95°C for 1 min, 60°C for 1 min, and then 0.5°C increase every 10 s, with fluorescence read continuously). Specificity of primers was tested using DNA isolated from plasmids containing archaeal 16S rRNA inserts from a previous study (23) that represented *Archaea* from a variety of archaeal groups, including the CGI.1b and CGI.1a groups. The CGI.1b primers amplified all the sequences related to the CGI.1b group and no sequences that were not in this group. Archaeal 16S rRNA genes affiliated with pSL12 were amplified as reported by Mincer et al. (21). We also tested for gammaproteobacterial AOB 16SrRNA genes using primers in Table 1. Abundance of archaeal 16S rRNA genes related to *Nitrosopumilus maritimus* were reported previously (23).

Potential nitrification rates. Potential nitrification rate experiments were set up within 4 to 6 h after samples were collected. Two-gram samples of sediment (wet weight) from the 0- to 2-cm horizon were transferred to 50-ml tubes containing 10 ml of artificial seawater (30 ppt) amended with 250 μM ammonium (as NH_4Cl) and 60 μM phosphate (as KH_2PO_4). All samples were incubated at 15°C with shaking to keep oxygen conditions nonlimiting. One subsample from each replicate core was harvested at 0, 24, 48, and 72 h. Samples were centrifuged, filtered through GF/F filters (Whatman), and immediately frozen for nitrate analysis. Nitrate (plus nitrite) was measured as described above. Nitrification rates were calculated based on the change in nitrate (plus nitrite) concentration per gram of dry sediment over time.

Statistical analyses. Multiple comparisons and correlations among quantitative variables were performed with Instat 3.0b (GraphPad Software, Inc.). Potential rate and *amoA* abundance data were log transformed to relieve heteroscedasticity. In cases where normality criteria were not met, data were analyzed by nonparametric tests.

Nucleotide sequence accession numbers. Nucleotide sequences have been deposited in GenBank under accession numbers EU925166 to EU925374.

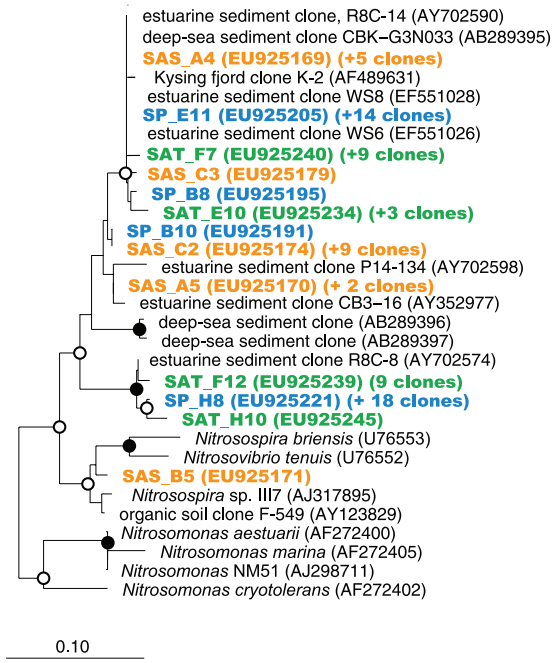

FIG. 1. Phylogenetic relationships among deduced betaproteobacterial AmoA protein sequences. The unrooted neighbor-joining tree was inferred from an alignment of protein sequences with 134 amino acid residues. Bootstrap values (≥50) based on neighbor-joining and parsimony analyses are indicated above and below the nodes, respectively. Sequences with SAS, SAT, or SP as the prefix are from this study.

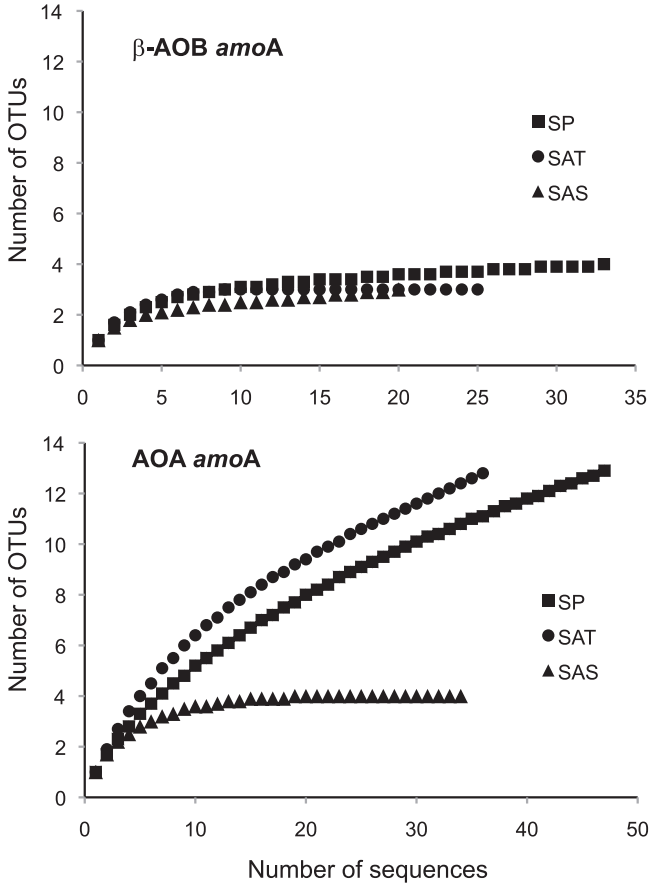

FIG. 2. Rarefaction analyses of betaproteobacterial and archaeal *amoA* genes at the three sites. OTUs were defined as those with ≥95% nucleotide sequence identity.

RESULTS AND DISCUSSION

β-AOB diversity. We analyzed a total of 83 β-AOB *amoA* sequences to compare diversity in salt marsh sediments dominated by different grasses. Eighty-two of the 83 sequences were related to uncultured *Nitrosospira*-like *amoA* sequences recovered from other estuarine and marine environments (1, 3, 39) (Fig. 1). One sequence from SAS was related to *Nitrosospira tenuis* and *Nitrospira briensis*. β-AOB richness was low, with 3 to 4 OTUs detected at each site and only 5 OTUs detected overall (using a 5% cutoff at the nucleotide level) (Fig. 2A).

The low β-AOB richness observed in the salt marsh is similar to β-AOB richness reported from other estuarine sediment environments (1, 3). Additionally, the dominance of *Nitrosospira*-like *amoA* sequences is consistent with previous studies of β-AOB *amoA* in other estuarine environments (3, 11, 13, 39). Unfortunately, there are still no cultured representatives of the dominant β-AOB found in most estuarine and marine systems, so their actual physiological tolerances remain speculative at best.

AOA diversity. All but one of the AOA *amoA* sequences were related to sequences recovered from other marine or estuarine environments (1, 22, 33), with nine sequences most closely related to the *amoA* gene from the ammonia-oxidizing archaeon *N. maritimus* (17) (Fig. 3). One sequence, SAT-B2, was most closely related to a sequence recovered from soil. Topologies of trees constructed from alignments of deduced AmoA protein and *amoA* nucleic acid sequences were highly congruent (data not shown). Additionally, we detected a total of 20 AOA *amoA* OTUs, with 13 OTUs at SAT and 12 at SP but only 4 OTUs at SAS (Fig. 2).

We found approximately equal numbers of AOA *amoA* sequences affiliated with either the water column/sediment or soil/sediment clades at SAT and SP, but at SAS over 70% of the sequences fell within the water column/sediment clade. The recovery of AOA *amoA* genes within both the water column and soil clades is similar to results from other coastal marine or estuarine sites (1, 12, 22, 27). Additionally, some investigators have recovered AOA *amoA* sequences from soil environments that are affiliated with the water column/sediment clade (12, 37).

The richness of AOA *amoA* genes exceeded that of β-AOB *amoA* genes at two of the three sites (Fig. 2). Beman and Francis (1) found 42 AOA *amoA* OTUs in a subtropical estuary, but only 9 OTUs for β-AOB *amoA* genes, when using a 95% cutoff. Using the same 95% cutoff, Mosier and Francis (22) found 67 and 41 AOA and β-AOB OTUs respectively, in the San Francisco Bay. Although the numbers are considerably higher, the pattern is similar. In a subterranean estuary, only 2 β-AOB *amoA* OTUs were recovered, but 52 AOA *amoA* OTUs were found (33). We also found similar differences in richness of AOA and β-AOB *amoA* along a salinity gradient in Plum Island Sound (Bernhard, unpublished). These data sug-

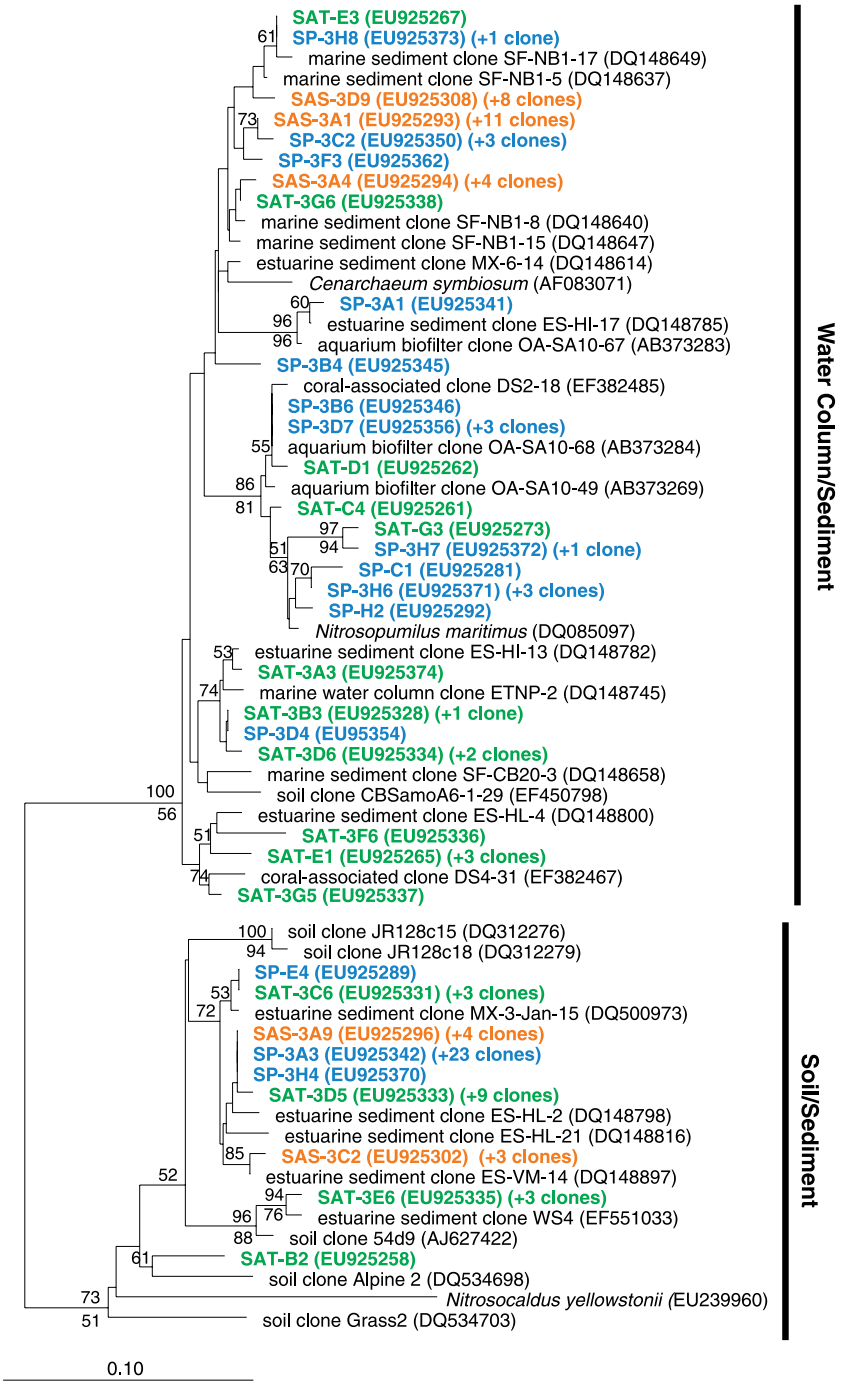

FIG. 3. Phylogenetic relationships among deduced archaeal AmoA protein sequences recovered from the three sites. The unrooted neighbor-joining tree was inferred from an alignment of protein sequences with 191 amino acid residues. Bootstrap support (≥50) based on neighbor-joining and parsimony analyses are indicated above and below the nodes, respectively.

gest a consistent pattern of high AOA *amoA* diversity relative to β-AOB *amoA* diversity in estuarine systems.

AOA and β-AOB *amoA* abundance. Abundance of β-AOB *amoA* genes ranged from 2.1×10^4 to 8.2×10^7 copies per g of dry sediment (or 3.6×10^3 to 2.6×10^7 copies per g of wet sediment) (Fig. 4). β-AOB *amoA* abundance was always lowest at SAS and was highest at SAT in April, June, and July. When data from all sampling dates were combined, β-AOB *amoA* abundance at SP and SAT was significantly greater than at SAS (Kruskal-Wallis, $P < 0.0001$).

β-AOB *amoA* abundance at our sites was similar to abundance measured by real-time PCR in other marine or estuarine environments (4, 22, 27, 30, 33) but about an order of magnitude higher than in a Georgia salt marsh (9). β-AOB *amoA* abundance in the Georgia salt marsh was measured by competitive PCR, which may not be as sensitive as real-time PCR.

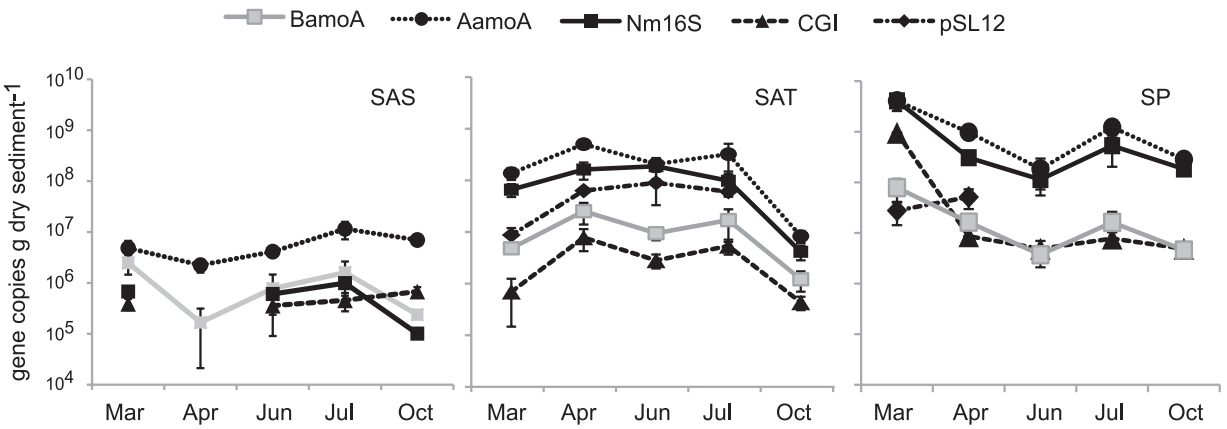

FIG. 4. Abundance of β-AOB and AOA *amoA* genes and 16S rRNA genes affiliated with *N. maritimus*, *Crenarchaeota* group I.1b, and pSL12 at the three study sites: SAS, SAT, and SP. Data are the means of triplicate core samples (except in April, when only duplicate cores were collected). Error bars represent the standard errors (in some cases the error bars are smaller than the symbols). pSL12 was not detected at SAT in October, at SP in June, July, or October, or in any samples at SAS. Additionally, *N. maritimus* and CGI.1b 16S rRNA genes were not detected at SAS in April.

However, similar to our study, Dollhopf et al. (9) also found a significantly higher abundance of β-AOB *amoA* at sites dominated by the tall form compared to sites dominated by the short form of *S. alterniflora*.

AOA *amoA* gene abundance ranged from 1.6×10^6 to 5.8×10^9 copies per g of dry sediment (or 2.7×10^5 to 1.8×10^9 copies per g of wet sediment), was generally highest at the SP site and, similar to β-AOB, was always lowest at the SAS site (Fig. 4). As we found with β-AOB, AOA *amoA* abundance at SP and SAT sites was significantly greater than at the SAS site when all sampling dates were combined (Kruskal-Wallis, $P < 0.0001$). AOA *amoA* abundance at SAS site was similar to AOA abundance reported in other marine and estuarine sediments (22, 27, 33) using similar methods. However, AOA *amoA* abundance at SAT and SP was, on average, at least 10 times higher than levels reported in other studies, and at some sites about 100 times higher, based on comparisons of gene copies per g of wet sediment.

Our AOA *amoA* abundance data are corroborated by previous measurements of 16S rRNA genes related to the ammonia-oxidizing archaeon *N. maritimus*, for which Nelson et al. (23) found numbers as high as 10^9 copies per g of dry sediment at the same sites. Abundance of AOA *amoA* genes and *N. maritimus*-like 16S rRNA genes were highly correlated at SAT and SP ($r = 0.88$ and 0.91, respectively), but less so at SAS ($r = 0.66$).

Ratios of AOA *amoA* genes to *N. maritimus*-like 16S rRNA genes were similar at SAT (2.4 ± 0.3) and SP (2.9 ± 0.5) sites, but the average ratio at SAS was 41.7 ± 11.3. Ratios of *amoA* and 16S rRNA genes between 2 and 3 have been reported in other systems (2, 21). Although the genome of the only cultivated AOA, *N. maritimus*, has only one copy of both the 16S rRNA and *amoA* gene, some cultured β-AOB have as many as three copies of *amoA* (26). Additionally, the primers used by Nelson et al. (23) were designed to target all archaeal 16S rRNA genes related to *N. maritimus* recovered from the marsh, but it is likely that some 16S rRNA genes that represent *Archaea* with *amoA* genes were not targeted by these primers, which would skew our *amoA*/16S rRNA gene ratios.

The unexpectedly high ratios at the SAS site, however, suggest that other *Archaea* at this site harbor the *amoA* gene. To further investigate these ratios, we quantified members of the *Archaea* belonging to the CGI.1b (soil) group, some of which are known to have *amoA* (38), and the pSL12 group, which were previously implicated as potential *amoA*-harboring *Archaea* (21). No pSL12 16S rRNA genes were detected in any samples from SAS, but CGI.1b 16S rRNA genes were sometimes more abundant than *N. maritimus*-like genes (Fig. 4). When these two additional archaeal groups were included in the ratios, there was a slight decrease in ratios at SAT and SP sites, but the average ratio at the SAS site decreased about threefold (Table 2) due to the inclusion of CGI.1b genes. However, the ratios of *amoA* to 16S rRNA genes at SAS were still quite high compared to other reported values, suggesting that *Archaea* not targeted by the suite of 16S rRNA primers used in this study may contribute to the *amoA* abundance at SAS. The majority of archaeal 16S rRNA genes recovered from the SAS site belong to the group I.3b *Crenarchaeota* (23), for which *amoA* genes have not been reported.

In a previous study of archaeal 16S rRNA gene diversity in the same salt marsh, Nelson et al. (23) recovered only one sequence affiliated with the group I.1b *Crenarchaeota*, but about half of our AOA *amoA* genes were affiliated with the soil/sediment cluster designated by Francis et al. (12). Al-

TABLE 2. Pearson correlation coefficients and ratios between AOA *amoA* and 16S rRNA gene abundance levels at the three sites

Site	*r* (*P*)		Ratio	
	N. maritimus-like 16S rRNA only	All three rRNA genes[a]	*N. maritimus*-like 16S rRNA only	All three rRNA genes[a]
SAS	0.29 (0.33)	0.73 (0.0012)[b]	41.7 ± 11.3	11.9 ± 2.7[b]
SAT	0.89 (<0.0001)	0.95 (<0.0001)	2.4 ± 0.3	1.9 ± 0.4
SP	0.75 (0.0006)	0.92 (<0.0001)	2.9 ± 0.5	2.6 ± 0.4

[a] Data for the *N. maritimus*-like 16S rRNA, *Crenarchaeota* group I.1b 16S rRNA, and pSL12 16S rRNA genes.
[b] No pSL12 16S rRNA genes were detected at SAS.

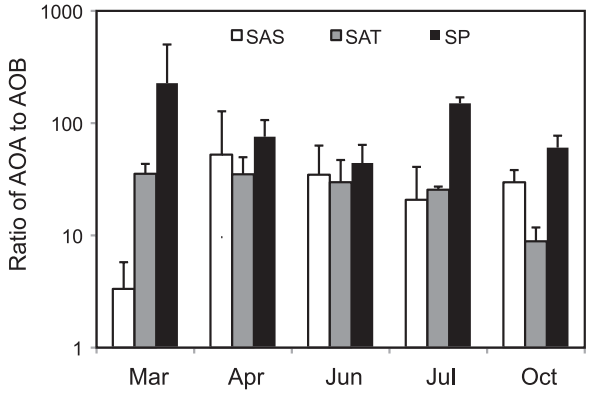

FIG. 5. Ratios of AOA and β-AOB based on abundance of the *amoA* genes in the Barn Island salt marsh. Values represent the means of triplicate sediment cores (except in April, where $n = 2$), and error bars represent 1 standard deviation.

though congruence between the phylogenies of archaeal 16S rRNA and *amoA* genes has been reported in other studies (24, 29, 32), we did not find this to be the case in our samples. The incongruencies we observed, however, may be due to primer biases or, more likely, differences in the AOA communities of the samples. Archaeal 16S rRNA genes reported in Nelson et al. (23) were generated from samples collected in October 2005, but *amoA* gene libraries reported in this study were generated from samples collected in March 2006 due to the high nitrification potentials compared to October. Additionally, the CGI.1b quantitative data indicate fairly high variability, so it is possible that the archaeal communities were quite different in October compared to March.

Similar to other recent studies, AOA *amoA* genes were significantly more abundant than β-AOB *amoA* genes at all three sites (Mann-Whitney, $P < 0.0001$), and the ratio of AOA to β-AOB *amoA* was highly variable (Fig. 5). At the SAS site, AOA *amoA* genes were only about 3 times more abundant than β-AOB in March, but on all other sampling dates at this site AOA *amoA* genes were 30 to 40 times more abundant. At SAT and SP, AOA *amoA* genes were approximately 9 to 215 times more abundant than β-AOB. When all sampling dates were combined, ratios of AOA to β-AOB *amoA* were significantly higher at SP compared to SAS or SAT (analysis of variance, $P < 0.0001$).

In other marine or estuarine sediments, ratios of AOA to β-AOB *amoA* gene abundance range from less than 1 to 80 (6, 22, 27, 33). In open ocean systems, AOA *amoA* genes are often 2 to 3 orders of magnitude greater than β-AOB levels (2, 21, 42). Conversely, β-AOB gene abundance was greater than AOA *amoA* genes in high-salinity areas of a subterranean estuary (33) and San Francisco Bay (22). Caffrey et al. (6) also reported that β-AOB *amoA* genes outnumbered AOA *amoA* genes at two highly sulfidic sites in an Alabama estuary. Such variable results in different systems suggest that the factors regulating marine and estuarine β-AOB and AOA *amoA* gene abundance may be quite complex. Identifying the factors that determine whether AOA or β-AOB dominate numerically in coastal systems should provide valuable insights into the physiological tolerances and ecological niches of the different nitrifying populations.

Abundances of AOA and β-AOB showed similar patterns and were significantly positively correlated (Table 3). However, the correlation of AOA and β-AOB appears to be driven by the strong relationships at SAT and SP sites, since the correlation at the SAS site was not significant when analyzed independently.

It is clear from our results that the diversity and abundance of nitrifiers are consistently lower at SAS relative to SAT and SP sites. Differences in edaphic conditions among the three sites in this study likely contributed to the differences observed. In a previous study of a Georgia salt marsh, Dollhopf et al. (9) also found a lower abundance of β-AOB at SAS sites compared to SAT sites and attributed the differences to enhanced nitrification due to higher concentrations of Fe(III) and macrofauna burrowing activity at the SAT sites. Although we did not measure macrofauna activity or Fe(III) in our study, salt marsh vegetation patterns are highly predictable based on degree of tidal flooding and redox chemistry of the sediments (5, 14), so that the presence of dominant grasses can be used as proxies for prevailing sediment conditions. We think it is likely that the low abundance and diversity of nitrifiers at our SAS site may be a reflection of low redox or high sulfide conditions, which have been reported previously for this site (41). Joye and Hollibaugh (16) reported that sulfide may inhibit nitrification, and this might help explain the higher abundance and richness observed at SP relative to SAS. Unfortunately, redox and sulfide data for SAT are unavailable. However, since the SAT site is along a creek bank, it experiences greater tidal flushing, has a greater range of salinity, and has higher ammonium concentrations than the SP and SAS sites, which are higher on the marsh (Table 4). How these factors directly impact ammonia-oxidizing communities, however, is not clear. Additionally, the reason for the greater abundance of AOA and β-AOB *amoA* genes at the SP site also remains unclear but may be related to differences in plant root exudates between *S. alterniflora* and *S. patens*. Further research that is focused on the impact of plant roots is necessary to address these questions.

TABLE 3. Pearson correlation coefficients between potential nitrification rates and AOA *amoA* and β-AOB *amoA* gene abundance levels for all data combined, by site and by sampling date

Comparison and site or date	r (P) for[a]:		
	Rates vs AOA	Rates vs β-AOB	AOA vs β-AOB
All samples	0.08 (0.71)	0.06 (0.79)	**0.89 (<0.0001)**
By site			
SAS	−0.38 (0.24)	−0.27 (0.42)	0.52 (0.10)
SAT	**0.60 (0.05)**	**0.60 (0.05)**	**0.95 (<0.0001)**
SP	0.12 (0.72)	0.27 (0.43)	**0.93 (<0.0001)**
By mo			
March			**0.77 (0.01)**
April	−0.38 (0.24)	−0.27 (0.42)	0.52 (0.10)
June	0.18 (0.63)	0.14 (0.71)	0.84 (0.005)
July	−0.51 (0.16)	−0.50 (0.17)	**0.72 (0.03)**
October	**0.74 (0.02)**	**0.79 (0.01)**	**0.94 (0.0002)**

[a] Values reported are correlation coefficients (r) followed by the P value in parentheses (correlations that were considered significant are indicated in bold) for the indicated comparison. No nitrification rates ("rates") were measured in March.

TABLE 4. Ranges of pore water salinity, pH, and ammonium and nitrate (plus nitrite) concentrations for sediment samples collected at the three sites from March to October 2006[a]

Site	Salinity (ppt)	pH	NH_4^+ (μM)	NO_3^- (μM)
SAS	23.8–32.7	5.3–6.4	4.0–95.1	6.0–15.2
SAT	12.0–31.3	6.2–6.5	25.1–258.0	1.5–14.3
SP	24.0–30.0	5.3–6.3	12.5–111.0	4.6–13.8

[a] Salinity, pH, and ammonium data are from Nelson et al. (23).

Nitrification potentials. Potential nitrification rates showed a strong seasonal pattern at all three sites, with rates highest during April and decreasing to very low levels by October (Fig. 6). Average rates were highest overall at the SAT site (29.6 nmol NO_3^-/g [dry weight]/day) compared to 10.8 and 8.6 nmol NO_3^-/g (dry weight)/day at SAS and SP sites, respectively, but varied by sampling date. The seasonal patterns of nitrification potentials we report here are similar to those reported for other estuaries (4, 7), suggesting a common mechanism regulating nitrification rates in estuarine systems. One hypothesis for higher rates in April may be a reduced competition for ammonia (30), since it would still be early in the season for algal and plant growth. However, changes in other factors, such as salinity or oxygen, cannot be ruled out. Additionally, significantly lower ammonium concentrations were reported at all three sites in our study in October (23) and may have contributed to the extremely low rates we measured. However, potential nitrification rates and ammonium concentrations were not significantly correlated in this study.

Potential nitrification rates were significantly correlated with AOA and β-AOB abundance only at the SAT site (Table 3), suggesting that the resident AOA and β-AOB at this site are active ammonia oxidizers. When rates and nitrifier abundance were analyzed by sampling date, AOA and β-AOB were significantly positively correlated with rates only in October. In April and July, the correlations were actually negative (but not significant), and in July we did not detect any nitrification activity at the SP site despite the high numbers of AOA and β-AOB *amoA* genes. It is unclear to us why we were unable to detect potential rates at SP in July. Other studies have shown a significant decrease in nitrification potentials during late summer (4, 7). Although rates were still relatively high at SAS and SAT, conditions not conducive to nitrification at SP may have occurred already. We also tested for the presence of AOB belonging to the *Gammaproteobacteria* (γ-AOB), but we were unable to detect γ-AOB 16S rRNA genes in our samples (Bernhard, unpublished), so it is unlikely that potential rates could be attributed to γ-AOB activity, as was recently reported in a pelagic system (18).

Positive correlations between potential rates and β-AOB have been reported for marine sediment microcosms (30), salt marsh sediments (9), and estuarine sediments (4). Others, however, have reported no relationship between potential nitrification rates and β-AOB (6). Additionally, Caffrey et al. (6) reported positive correlations between AOA abundance and potential rates, but the relationship was significant at only two of six sites. Also, a recent study of nitrifiers in agricultural soils reported nitrification activity attributed to AOB and not AOA, despite high numbers of AOA *amoA* genes (15), suggesting that *amoA* gene abundance may not be an appropriate marker for nitrifying potential. It is important to consider that potential nitrification rates do not represent in situ rates and thus may not accurately reflect the nitrifying populations present. It may be that the conditions (such as oxygen, ammonium, or salinity) during the potential rate experiments may not be optimal for all resident nitrifiers. In future studies, it may be helpful to measure gene expression, in addition to abundance, to better quantify the active populations.

Site characteristics and correlation with nitrifying communities. Similar to the results for *N. maritimus* 16S rRNA genes reported by Nelson et al. (23), the largest differences in AOA and β-AOB *amoA* gene abundance were found between samples at SAS and SP, yet there were few differences in pore water chemistry between these sites (Table 4). Differences in gene abundance at these sites are likely due to other environmental variables, such as redox or sulfide, which were previously reported to be significantly different at these sites (41). As has been reported in other estuarine environments (4, 22), salinity was significantly negatively correlated with AOA ($r = -0.47$, $P = 0.02$) and β-AOB ($r = -0.52$, $P = 0.008$) abundance. However, whether the effect on nitrifier abundance is due to a physiological response to salt or to some other factor that covaries with salinity has not been determined. The negative correlation between AOA abundance and pH ($r = -0.46$, $P = 0.02$) in our study also corroborates the results reported in soil samples (24). No significant correlations were detected between ammonium or nitrate concentrations and rates, AOA *amoA* abundance, or β-AOB *amoA* abundance.

Conclusions. We report surprisingly high numbers of AOA *amoA* genes in some salt marsh sediments, suggesting a potentially important role of these *Archaea* in the ecology of the marsh. Additionally, AOA always outnumbered β-AOB at all sites and were considerably more diverse at two of the three sites. Our results also suggest that salinity and pH may be important environmental factors that regulate AOA abundance, as has been suggested by others (22, 24). Interestingly, differences in pore water nitrogen concentrations appeared to have little effect on nitrifier abundance in our study. Although the lack of consistent correlation of rates with nitrifier abundance may be a reflection of methodological limitations, it may also suggest that the relationship among AOA, β-AOB, and

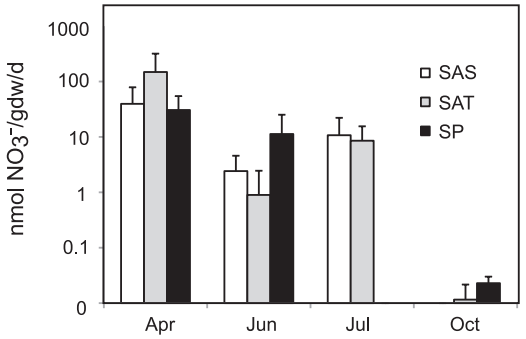

FIG. 6. Potential nitrification rates at the three sites over the growing season. Values represent the means of triplicate core samples (except in April, when $n = 2$), and error bars represent 1 standard deviation. No rates were measured in March.

potential rates is highly complex and warrants further exploration. Measuring levels of *amoA* gene transcripts or AmoA protein levels in situ may help elucidate this relationship under different environmental conditions.

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