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Nicole S. Moin
Ohio State University College of Veterinary Medicine

Katelyn A. Nelson
Columbia University Medical Centre

Alexander Bush
University of Pennsylvania School of Veterinary Medicine

Anne E. Bernhard
Connecticut College, aeber@conncoll.edu

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

Nicole S. Moin,† Katelyn A. Nelson,‡ Alexander Bush,§ and Anne E. Bernhard*
Department of Biology, Connecticut College, New London, Connecticut

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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^-1 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 pSIL2 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 with no unifying patterns or mechanisms that 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...
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 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 conditions: 94°C for 45 s, with a final elongation at 72°C for 5 min. All reactions were started with fluorescence (plus nitrite) was measured as described above. Melt curve analysis was conducted to monitor product specificity. All samples were run 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 × 103 to 2.2 × 106 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 archaean 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 1.1b 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 plasmons containing archaeal 16S rRNA inserts from a previous study (23) that represented Crenarchaeota from a variety of archaeal groups, including the CGL1b and CGL1a groups. The CGL1b primers amplified all the sequences related to the CGL1b group and no sequences that were not in this group. Archaeal 16S rRNA genes amplified with pSL12 were amplified as reported by Mincer et al. (21). We also tested for gammaproteobacterial AOB 16S rRNA 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 NH4Cl) and 60 μM phosphate (as KH2PO4). 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 potential AOB 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 EU3925166 to EU3925374.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5′ to 3′)</th>
<th>Target</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>amoA-1F</td>
<td>GGGGTTTCTACTGGTGT</td>
<td>β-AOB amoA</td>
<td>31</td>
</tr>
<tr>
<td>amoA-2R-TC</td>
<td>CCCCTCAGAAAGCTTCTTAC</td>
<td>β-AOB amoA</td>
<td>25</td>
</tr>
<tr>
<td>ArchamoAF</td>
<td>STAATTGCTGGCTTATAGCC</td>
<td>Archaeal amoA</td>
<td>12</td>
</tr>
<tr>
<td>ArchamoAR</td>
<td>GCGGCCATCCTATGATG</td>
<td>Archaeal amoA</td>
<td>12</td>
</tr>
<tr>
<td>CrenamoAQModF</td>
<td>CARGTHG004ARTCTTAYAA</td>
<td>Archaeal amoA</td>
<td>This study</td>
</tr>
<tr>
<td>GAOB16s-F-20R</td>
<td>GCGTGGGAATCCTGGCTCTA</td>
<td>γ-AOB 16S rRNA</td>
<td>This study</td>
</tr>
<tr>
<td>GAOB16s-R</td>
<td>ATCTGGCTGGTCCACCT</td>
<td>γ-AOB 16S rRNA</td>
<td>This study</td>
</tr>
<tr>
<td>CGL1b-270F</td>
<td>TGGATTGGACTGCGKCCGAT</td>
<td>CGL1b 16S rRNA</td>
<td>27</td>
</tr>
<tr>
<td>CGL1b-750R</td>
<td>GTCGACCGCRTTCTGAGMAG</td>
<td>pSL12 16S rRNA</td>
<td>21</td>
</tr>
<tr>
<td>pSL12_750F</td>
<td>GGTCCRCAGAAGCGGC</td>
<td>pSL12 16S rRNA</td>
<td>21</td>
</tr>
<tr>
<td>pSL12_876R</td>
<td>GTACTCCCCCCAGCGGC</td>
<td>pSL12 16S rRNA</td>
<td>21</td>
</tr>
</tbody>
</table>

* *Bases in bold indicate modifications from those reported by Mincer et al. (21).*
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 *Nitrospira 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 *Nitrospira 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 *Nitrospira tenuis* and *Nitrospira briensis* sequences is consistent with previous studies of β-AOB amoA in other estuarine environments (1, 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-
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 $2.1 \times 10^4$ to $8.2 \times 10^7$ copies per g of dry sediment (or $3.6 \times 10^3$ to $2.6 \times 10^7$ 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. 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.
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 × 10^6 copies per g of dry sediment (or 2.7 × 10^6 to 1.8 × 10^6 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).

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

<table>
<thead>
<tr>
<th>Site</th>
<th>r (P)</th>
<th>All three rRNA genes&lt;sup&gt;a&lt;/sup&gt;</th>
<th>N. maritimus-like 16S rRNA only</th>
<th>All three rRNA genes&lt;sup&gt;a&lt;/sup&gt;</th>
<th>N. maritimus-like 16S rRNA only</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAS</td>
<td>0.29 (0.33)</td>
<td>0.73 (0.0012)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.7 ± 11.3</td>
<td>11.9 ± 2.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.4 ± 0.3</td>
</tr>
<tr>
<td>SAT</td>
<td>0.89 (&lt;0.0001)</td>
<td>0.95 (&lt;0.0001)</td>
<td>2.4 ± 0.3</td>
<td>1.9 ± 0.4</td>
<td>2.9 ± 0.5</td>
</tr>
<tr>
<td>SP</td>
<td>0.75 (0.0006)</td>
<td>0.92 (&lt;0.0001)</td>
<td>2.4 ± 0.3</td>
<td>1.9 ± 0.4</td>
<td>2.9 ± 0.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data for the *N. maritimus*-like 16S rRNA, *Crenarchaeota* group I.1b 16S rRNA, and pSL12 16S rRNA genes.

<sup>b</sup> No pSL12 16S rRNA genes were detected at SAS.
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). Joyce 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.

![Graph showing ratios of AOA to AOB](http://aem.asm.org/)

**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.

Abundances of AOA and β-AOB dominated numerically in coastal systems should provide valuable insights into the physiological tolerances and ecological niches of the different nitrifying populations.

**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**

<table>
<thead>
<tr>
<th>Comparison and site</th>
<th>Rates vs AOA</th>
<th>Rates vs β-AOB</th>
<th>AOA vs β-AOB</th>
</tr>
</thead>
<tbody>
<tr>
<td>All samples</td>
<td>0.08 (0.71)</td>
<td>0.06 (0.79)</td>
<td>0.89 (&lt;0.0001)</td>
</tr>
<tr>
<td>By site</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAS</td>
<td>−0.38 (0.24)</td>
<td>−0.27 (0.42)</td>
<td>0.52 (0.10)</td>
</tr>
<tr>
<td>SAT</td>
<td>0.60 (0.05)</td>
<td>0.60 (0.05)</td>
<td>0.95 (&lt;0.0001)</td>
</tr>
<tr>
<td>SP</td>
<td>0.12 (0.72)</td>
<td>0.27 (0.43)</td>
<td>0.93 (&lt;0.0001)</td>
</tr>
<tr>
<td>By mo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>March</td>
<td>0.77 (0.01)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>April</td>
<td>−0.38 (0.24)</td>
<td>−0.27 (0.42)</td>
<td>0.52 (0.10)</td>
</tr>
<tr>
<td>June</td>
<td>0.18 (0.63)</td>
<td>0.14 (0.71)</td>
<td>0.84 (0.005)</td>
</tr>
<tr>
<td>July</td>
<td>−0.51 (0.16)</td>
<td>−0.50 (0.17)</td>
<td>0.72 (0.03)</td>
</tr>
<tr>
<td>October</td>
<td>0.74 (0.02)</td>
<td>0.79 (0.01)</td>
<td>0.94 (0.0002)</td>
</tr>
</tbody>
</table>

*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.

<table>
<thead>
<tr>
<th>Site</th>
<th>Salinity (ppt)</th>
<th>pH</th>
<th>NH₄⁺ (µM)</th>
<th>NO₃⁻ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAS</td>
<td>23.8–32.7</td>
<td>5.3–6.4</td>
<td>4.0–95.1</td>
<td>6.0–15.2</td>
</tr>
<tr>
<td>SAT</td>
<td>12.0–31.3</td>
<td>6.2–6.5</td>
<td>25.1–258.0</td>
<td>1.5–14.3</td>
</tr>
<tr>
<td>SP</td>
<td>24.0–30.0</td>
<td>5.3–6.3</td>
<td>12.5–111.0</td>
<td>4.6–13.8</td>
</tr>
</tbody>
</table>

*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 NO₃⁻/g [dry weight]/day) compared to 10.8 and 8.6 nmol NO₃⁻/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 is that 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.](http://aem.asm.org/)
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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