Differential responses of ammonia-oxidizing archaea and bacteria to long-term fertilization in a New England salt marsh

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INTRODUCTION
Oxidation of ammonia to nitrite is a critical process in nitrogen cycling and is carried out by a suite of distinct microorganisms within the bacterial and archaeal domains. The fate of nitrogen is of particular importance in nitrogen-sensitive coastal systems, such as estuaries and salt marshes, where primary productivity is typically nitrogen-limited (Valiela and Teal, 1974; Howarth, 1988). Nitrogen-cycling processes in salt marshes, therefore, play a significant role in the health and preservation of coastal habitats, yet our understanding of the ecology of the organisms responsible and their responses to perturbations is still evolving.

Over the last decade, researchers have made great progress in describing the diversity and distribution of ammonia-oxidizing bacteria (AOB) in estuaries (see review by Bernhard and Bollmann, 2010) and to a lesser extent in salt marshes (Dollhopf et al., 2005; Moit et al., 2009). Surveys of 16S rRNA genes and ammonia monooxygenase genes (coding for the enzyme responsible for the first step in ammonia oxidation) in estuaries and salt marshes indicate highly similar AOB communities in geographically distinct habitats that are typically dominated by sequences most closely related to *Nitrosomonas* spp., although representative AOB have yet to be obtained in pure culture. Most studies have also reported salinity to be a major factor regulating the distribution and diversity of AOB in these coastal systems (Francis et al., 2003; Bernhard et al., 2005; Ward et al., 2007). However, with the recent discovery of ammonia-oxidizing archaea (AOA; Könneke et al., 2005; Treusch et al., 2005) and their apparent wide distribution in many habitats, the relative importance of AOB to nitrification and their potential interactions with AOA have been brought into question.

The diversity and distribution of AOA have begun to be explored in estuaries and salt marshes, but, unlike the AOB, no consistent pattern or common regulatory factors have been identified. Although archaeal amoA gene sequences from both the water column/sediment and soil/sediment clusters as described in Francis et al. (2005) have been recovered from estuaries and salt marshes, to date, most archaeal amoA genes fall into the water column/sediment cluster, but the diversity of sequence types is quite high (Bernhard and Bollmann, 2010). Furthermore, factors that have been identified as potentially regulating their distribution are many, including salinity, temperature, pH, dissolved oxygen, nitrogen, and net primary productivity (Beman and Francis, 2006;
Sahan and Muyzer, 2008; Santoro et al., 2008; Moin et al., 2009). Thus, our understanding of AOA communities and what regulates their diversity and distribution, and ultimately, their activity in these important coastal ecosystems is still rudimentary.

Manipulating the environment and following changes in communities can be a powerful approach to help tease apart important regulatory factors. Using this approach, Lage et al. (2010) identified changes in AOB communities in relation to short-term N and P additions in a salt marsh. Others have taken a similar approach in soil systems to study changes in AOB communities (Chu et al., 2007, 2008; Shen et al., 2011), and have generally reported significant shifts in activity, community composition, and/or abundance. However, studies of AOA in response to fertilization have yielded conflicting results (Kelly et al., 2011; Verhamme et al., 2011; Wu et al., 2011).

In this study, we explored the diversity and distribution of AOA and AOB in a series of salt marsh plots that have received different levels of commercial fertilizer for nearly 40 years. The plots were originally fertilized to study the capacity of salt marshes to remove and retain nutrients (Valiela et al., 1973). Significant effects of fertilization on other components of the marsh, including vegetation (Valiela et al., 1975, 1976), invertebrates (Meany et al., 1976; Sarda et al., 1996), diatoms (Van Raalte et al., 1976) and nitrogen-cycling processes (Van Raalte et al., 1974; Hamersley and Howes, 2005) have been documented. More recently, Bowen et al. (2011) reported little evidence of fertilization impacts on bacterial and denitrifying communities in the marsh. To further the work of Bowen et al. (2011) on describing effects of fertilization on microbial communities, we report the first investigation of fertilization impacts on nitrifying microorganisms in the marsh.

Two of the questions addressed by our study are: (1) Are there major shifts in AOA and AOB communities that correspond with fertilization treatments? and (2) Do specific populations of AOA or AOB respond predictably to fertilization?

Known effects of experimental enrichment on the nitrogen cycle in salt marsh sediments lead to a prediction that there must be significant responses by ammonia oxidizers to the supply of nitrogen entering salt marsh ecosystems. We know that denitrification rates increase as N supply increases (Hamersley and Howes, 2005), and concentrations of ammonium are consistently higher in fertilized plots relative to those in control plots (Hamersley and Howes, 2005). Since denitrification in the fertilized salt marsh sediments is supported largely by in situ oxidation of ammonium (Hamersley and Howes, 2005), we anticipated an increase in abundance of ammonia oxidizers accompanied by shifts in community composition.

MATERIALS AND METHODS

SITE DESCRIPTION

This study was conducted in the Great Sippewissett Marsh, Falmouth, MA, USA (Figure 1). Circular plots (10 m diameter) of salt marsh vegetation have been fertilized every 2 weeks during the growing season since the early 1970s (Valiela et al., 1973, 1975). Three different levels of mixed NPK fertilizer were applied to each set of two replicate plots: low (LF; 8.4 g/m²/week), high (HF; 25 g/m²/week), and extra high (XF; 75 g/m²/week). The fertilizer is a commercially available sewage sludge fertilizer (10% N, 6% P, 4% K by weight). Forty percent of the N is inorganic, but only 28% is immediately soluble (Valiela et al., 1973). An additional two replicate plots were left unfertilized as controls (C). The rates of fertilization correspond to nitrogen loading rates of 12 (C), 182 (LF), 532 (HF), and 1572 (XF) kg N/ha/year (Fox et al., 2012), and include atmospheric deposition on the marsh surface. The plots are located within an experimental area of 10 ha. Areas within
the plots receiving frequent (daily) tidal inundation and dominated by *Spartina alterniflora* are designated as low marsh habitat. Areas above mean high tide and dominated by *S. patens* (in control plots) or *Distichlis spicata* (in XF plots) are designated as high marsh habitat. More detailed information regarding recent vegetation cover within the plots is reported elsewhere (Fox et al., 2012).

**SAMPLE COLLECTION**

Duplicate sediment cores (6.5 cm in diameter) were collected from low marsh habitat in each of eight experimental plots described above in July 2009 during low tide. Within the C and XF plots, two additional cores were collected from the high marsh habitat. Cores were kept on ice in the dark for several hours during transport to the lab. Salinity was measured by a handheld refractometer from pore water extracted from each core by centrifugation.

**DNA EXTRACTION**

Subsamples (ca. 0.5 g) were taken from sediment cores at a depth of 0–2 cm, and stored at −80°C. DNA was extracted using the PowerSoil DNA Isolation Kit (MO BIO, Carlsbad, CA, USA) according to the manufacturer’s protocol. DNA concentrations were measured spectrophotometrically using a SmartSpec spectrophotometer (Bio-Rad, Hercules, CA, USA) and fluorometrically using a Quant-it fluorometer (Invitrogen, Grand Island, NY, USA). Extracted DNA was also assessed by gel electrophoresis (1% agarose) with 0.1 μl/ml of ethidium bromide. All DNA extractions were performed on duplicate 0.5 g aliquots of sediment and the better of the extraction (assessed by quality and quantity) was selected for downstream analysis.

**TRFLP ANALYSIS**

Archaeal amoA genes were amplified with Arch-AmoAF and Arch-AmoAR (Francis et al., 2009). Betaproteobacterial amoA genes were amplified with amoA-1F and amoA-2R-TC (Rotthauwe et al., 1997; Nicolaisen and Ramsing, 2002). Each 20 μl reaction contained 1× DreamTaq Buffer with KCl and (NH4)2SO4 (Fermentas), 0.4 μ/l of each primer, and approximately 2–10 ng DNA. Each forward primer was labeled at the 5′ with 6-FAM (Eurofins MWG Operon, Huntsville, AL, USA) prior to analyses to alleviate heteroscedasticity. PCR for the DNA extraction volume obtained from a known depth of 0–2 cm, and stored at 101.3 ± 13.3% (betaproteobacterial amoA genes), 107.4 ± 5.2% (archaeal amoA genes), and 91.7 ± 4.5% (bacterial 16S rRNA genes). We calculated copy numbers of amoA genes per gram of sediment by correcting the number of copies detected in each PCR for the DNA extraction volume obtained from a known amount of sediment for each sample. We also normalized amoA gene copies to μg DNA (measured fluorometrically) and to copies of bacterial 16S rRNA genes. We detected no significant differences in amount of DNA among fertilization treatments (ANOVA, P = 0.2).

**REAL-TIME PCR ANALYSES**

Betaproteobacterial amoA genes were quantified as described in Bernhard et al. (2007). Archaeal amoA genes were quantified as described in Moin et al. (2009). Bacterial 16S rRNA genes were quantified as described in Bernhard et al. (2012). Average amplification efficiencies from at least three separate runs were 101.3 ± 13.3% (betaproteobacterial amoA genes), 107.4 ± 5.2% (archaeal amoA genes), and 91.7 ± 4.5% (bacterial 16S rRNA genes). We calculated copy numbers of amoA genes per gram of sediment by correcting the number of copies detected in each PCR for the DNA extraction volume obtained from a known amount of sediment for each sample. We also normalized amoA gene copies to μg DNA (measured fluorometrically) and to copies of bacterial 16S rRNA genes. We detected no significant differences in amount of DNA among fertilization treatments (ANOVA, P = 0.2).

**STATISTICAL ANALYSIS**

ANOVA, t-test, Pearson correlation, and multiple regression analyses were performed in Instat v3.0b (GraphPad, La Jolla, CA, USA). Data from duplicate cores in each plot were averaged prior to statistical analyses. Gene abundance data were log transformed prior to analyses to alleviate heteroscedasticity.

Non-metric multidimensional scaling (NMS) was used to ordinate arsine transformed TRFLP data with Sorenson's distance measure ( Bray-Curtis), using a stability criterion of 0.0001. All ordinations used the Autoplot mode set to slow and thorough, and a suggested iterative optimization procedure (McCune and Mefford, 1999). Final stress, final instability, number of iterations, difference from Monte Carlo test, recommended dimensionality, and final ordination scores were used to evaluate the
results. Ordinations were rotated to maximize the correlation of fertilization rates on axis 1.

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. All alignments were checked manually and regions of ambiguous alignments were excluded from the analysis. Phylogenetic relationships were analyzed by the neighbor-joining algorithm with the Kimura two-parameter correction in ARB. Confidence in tree topology was assessed by 100 bootstrap replicates using PHYML v.3.69. Sequences were checked for chimeras by comparing phylogenetic placement in trees constructed with the 3′ and the 3′ ends of the sequence. Pairwise sequence comparisons were calculated in ARB and operational taxonomic units (OTUs) were defined as sequences sharing ≥95% nucleotide sequence identity using MOTHUR (Schloss et al., 2009). Coverage of each clone library was calculated with the equation: 

\[ C = 1 - (n/N) \]

where \( n \) = number of singleton sequences and \( N \) = total number of sequences analyzed. A total of 526 betaproteobacterial amoA sequences and 310 archaeal amoA sequences were included in the final analysis. Sequence data have been submitted to the GenBank database under accession numbers JX283750–JX284059 (archaeal amoA) and JX306111–JX306636 (betaproteobacterial amoA).

RESULTS

Salinity ranged from 23.6 ± 4.4 in the LF plots to 26.8 ± 1.8 psu in the C plots in the low marsh habitat and was not significantly different among fertilization treatments. In the high marsh plots, however, salinity was significantly lower (\( P = 0.0004 \)) at the XF plots (11.7 ± 2.7 psu) compared to salinity in the control plots (31.0 ± 0.3 psu). Other variables, such as ammonium and sulfide, vary considerably among plots and a summary of these differences can be found elsewhere (Hamersley and Howes, 2005; Bowen et al., 2011).

SHIFTS IN AOB COMMUNITY COMPOSITION

Terminal restriction fragment length polymorphism analysis of betaproteobacterial amoA genes revealed a total of 12 TRFs and an average of 8.3 TRFs per sample. TRF 130 and 192 were the most abundant overall. Only one TRF (472) was not identified in our sequence dataset, but this TRF represents *Nitrosomonas europa* so we included it in our analysis.

Analysis of betaproteobacterial amoA TREFLP patterns using NMS ordinations revealed distinct communities related to fertilization in both the low and high marsh habitats (Figure 2A). In the low marsh, there was generally a linear shift from AOB communities in control plots to AOB communities in XF plots, with AOB communities from LF and HF plots falling in between those from C and XF plots.

Correlation of ordination axis scores with environmental variables indicated that axis 1 was most strongly correlated with fertilization rate (\( r = 0.76, P = 0.004 \)) and N loading rate (\( r = 0.76, P = 0.004 \)), followed by salinity (\( r = -0.61, P = 0.03 \)). Axis 2 was not significantly correlated with any of the variables.

Coverage of the betaproteobacterial amoA gene clone libraries ranged from 89.8 to 100% and we detected a total of 12 OTUs that closely matched the TRFs (Figure 3). Sixty-one percent of the sequences were affiliated with the uncultured *Nitrosospira*-like cluster, with the remainder affiliated with the *Nitrosomonas* cluster.

RESPONSES OF AOB POPULATIONS TO FERTILIZATION

We evaluated relative abundance of each TRF in relation to fertilization rates to characterize responses of specific AOB populations. Four TRFs were significantly correlated with fertilization rates (Table 1), with TRF492 showing the strongest positive response to fertilization. Distribution of sequences among the fertilized treatments corroborated the TRF patterns. For example, all 31 sequences representing TRF 492 in OTUs 9 and 10 were recovered exclusively from HF and XF plots.
We also observed a striking shift in the distribution of Nitrosospira-like and Nitrosomonas-related sequences related to fertilization rates (Figure 4A). Ninety-one percent of the Nitrosospira-like sequences were recovered from C and LF sites, while Nitrosomonas sequences were found mostly in HF and XF sites.

SHIFTS IN AOA COMMUNITY COMPOSITION
A total of 12 AOA TRFs were identified among all plots. TRF 383 was the most abundant overall in both the low and high marsh habitats (23.9 and 39.9% relative abundance, respectively) and 29.8% of the sequences recovered from all the sites had this predicted TRF. This is also one of two TRFs (along with TRF 257) representing sequences most closely related to Nitrosopumilus maritimus (Figure 5). TRF 52 was identified from sequence data (Figure 5), but was not included in the TRFLP profile because we could not discriminate actual signal from background for TRFs smaller than 70 bp. Non-metric multidimensional scaling ordination of archaeal communities based on TRFLP data suggest some effect of
Table 1 | Pearson’s correlation coefficients (r) describing the relationships between abundance of TRFs and fertilization rates.

<table>
<thead>
<tr>
<th>TRF size</th>
<th>Correlation with fertilization rates</th>
<th>Lineage</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRF130</td>
<td>0.50</td>
<td>Nitrospira-like</td>
</tr>
<tr>
<td>TRF403</td>
<td>0.55</td>
<td>Nitrosomonas aestuari/marina</td>
</tr>
<tr>
<td>TRF472</td>
<td>0.42</td>
<td>Nitrosomonas eutropha</td>
</tr>
<tr>
<td>TRF492</td>
<td>0.76</td>
<td>Nitrosomonas ureae/digotapha</td>
</tr>
<tr>
<td>AOA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRF160</td>
<td>0.48</td>
<td>Nitrosopumilus group 1</td>
</tr>
</tbody>
</table>

Only significant correlations (P ≤ 0.05) are shown. Negative correlations are indicated parenthetically.

fertilization treatment, but the patterns were not robust (Figure 2B). There were distinct AOA communities in the control and XF plots in the high marsh sites, but the patterns among the low marsh samples were less convincing. Furthermore, unlike the results for AOB communities, we found no significant correlations of ordination scores for axis 1 (or 2) with fertilization rates, N loading rates, or salinity.

Analysis of archaeal amoA gene sequences revealed 15 OTUs (Figure 5). Although a few of the phylogenetic clusters were characterized by a unique TRF, there was generally less agreement between AOA TRF and OTU distribution compared to AOB. The majority of sequences were affiliated with the water column/sediment cluster as designated by Francis et al. (2005), more recently defined as the Nitrosopumilus cluster described by Pester et al. (2012); Figure 6. Twenty-one sequences fell within the Nitrososphaera cluster as identified by Pester et al.
RESPONSE OF AOA POPULATIONS TO FERTILIZATION

Contrary to patterns observed for AOB, only one TRF showed a significant correlation with fertilization rates (Table 1). However, there were more distinct patterns within the sequence data (Figure 4B). We identified a subcluster of 109 sequences within the Nitrosopumilus cluster that was composed exclusively (with the exception of a single sequence) of sequences recovered from XF plots. We also observed a similar pattern for sequences affiliated with the Nitrososphaera cluster.

AOA AND AOB ABUNDANCE

Abundance of betaproteobacterial amoA genes ranged from $4.5 \times 10^3$ to $1.3 \times 10^7$ gene copies/g sediment, and was one to three orders of magnitude lower than archaeal amoA abundance (Figure 6). Although abundance was highest in the XF plots, the differences among mean values were not significant and linear regression analysis also confirmed no significant fertilization effect.

Abundance of archaeal amoA genes ranged from $2.0 \times 10^5$ to $1.2 \times 10^9$ gene copies/g sediment (wet weight) and were not significantly different among fertilization treatments. Linear regression analysis also confirmed no significant fertilization effect. Ratios of AOA:AOB abundance ranged from 0.5 to $3.1 \times 10^4$ and were highest at the XF plots (Figure 6), although the differences were not significant.
To account for possible differences in DNA extraction efficiencies, we normalized AOB and AOA amoA gene abundances to numbers of bacterial 16S rRNA gene copies and to μg DNA (Table 2). Patterns of abundance for normalized data were virtually identical to patterns based on copy numbers per gram of sediment (data not shown).

**DISCUSSION**

The effects of long-term fertilization in the Great Sippewissett Marsh have been documented for many of the major biological components of the marsh over the past four decades, elucidating fundamental mechanisms driving salt marsh ecology and environmental change. However, studies investigating the effects of fertilization on microbial communities have been lacking. Understanding how microorganisms respond to fertilization is important not only for predicting how microbially mediated ecosystem services may be altered by anthropogenically driven changes to the environment, but also for increasing our understanding of fundamental ecological principles that drive community dynamics. Just recently, Bowen et al. (2011) published the first study on microbial responses in the same plots that were sampled in our study and, rather surprisingly, found no fertilization effect on the composition of total bacterial or denitrifying communities. However, it is possible that there are differential responses among some microbial groups that were not detected in the analysis by Bowen et al. (2011), warranting additional studies within the marsh microbial community, particularly given the significant differences reported for some nitrogen-cycling processes in the marsh (Van Raalte et al., 1974; Hamersley and Howes, 2003). Our investigation of ammonia-oxidizing microorganisms in the marsh suggest, in fact, a complex pattern of responses that provide further insights into mechanisms controlling nitifying populations and communities in coastal ecosystems.

**AOB COMMUNITY COMPOSITION AND RESPONSE TO FERTILIZATION**

We found significant shifts in AOB communities that corresponded to fertilization, which is in agreement with several previous studies of AOB in fertilized soils and sediments (Chiu et al., 2007; Shen et al., 2011; Wu et al., 2011). Our results, however, are somewhat contrary to the recent study by Lage et al. (2010), who found shifts in salt marsh AOB community composition only when a single nutrient (N or P) was applied, but not when both were applied together. Since the fertilizer applied in our study is a complex mix, it is difficult to attribute changes in AOB community composition to a particular nutrient. However, our fertilization rates were 4–12 times higher than those used by Lage et al. (2010), and may account for the differences in community response.

The distribution of sequences we recovered for the betaproteobacterial amoA gene was generally similar to distributions found from other estuarine and salt marsh studies, with the majority of sequences falling into the Nitrosospira-like cluster (see Bernhard and Hollmann, 2010 for review). Sequences within this cluster were recovered from all fertilization treatments as well as both marsh habitats, suggesting that AOB within this cluster are either physiologically diverse or have high physiological plasticity.

Conversely, all of the OTUs within the Nitrosomonas group were restricted by marsh habitat or fertilization treatment, with the exception of OTU 7, suggesting that Nitrosomonas-related AOB in the marsh are more specialized compared to their Nitrosospira counterparts. Particularly striking was the lack of sequences from control or LF sites among OTUs affiliated with the *Nitrosomonas actinarii/marinia* and *Nitrosomonas ureae/eligerotrophia* clusters. Other studies of AOB in fertilized or polluted sediments have also found AOB communities to be dominated by *Nitrosomonas*-related sequences (Beman and Francis, 2006; Cao et al., 2011a,b).

Identification of specific AOB populations that showed consistent response to fertilization may help to better characterize the ecophysiology of uncultured AOB, and may serve as important clues to assist in the ultimate cultivation of these culturally recalcitrant AOB. The differential responses of specific AOB populations also suggest mechanisms for niche-differentiation within this relatively constrained phylogenetic group. Such distinct and reproducible patterns of AOB community composition in relation to fertilization also support the use of AOB as bioindicators, as has been previously suggested (Dang et al., 2010).

**AOA COMMUNITY COMPOSITION AND RESPONSE TO FERTILIZATION**

The lack of a consistent response of the AOA community composition to fertilization was somewhat unexpected given the fertilization history and significant differences in nitrogen-processing rates previously reported. However, results from other studies of AOA community composition in response to fertilization report a similar lack of response. Wu et al. (2011) found that long-term fertilization (32 years) with urea did not alter AOA communities in a paddy soil, and Verhamme et al. (2011) found that AOA communities in soils were not different from controls after short-term (28 days) ammonium additions. We detected distinct AOA communities related to fertilization in the high

**Table 2 | Mean (±SE) betaproteobacterial (AOB) and archaeal amoA (AOA) gene abundances presented as gene copies per μg DNA or normalized to copies of bacterial 16S rRNA genes in plots receiving different levels of fertilization.**

<table>
<thead>
<tr>
<th>Fertilization</th>
<th>AOA/μg DNA</th>
<th>AOB/μg DNA</th>
<th>AOA: bacterial 16S rRNA</th>
<th>AOB: bacterial 16S rRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.9 ± 2.8 × 10^3</td>
<td>5.3 ± 3.5 × 10^3</td>
<td>4.1 ± 2.1 × 10^-2</td>
<td>2.3 ± 1.9 × 10^-5</td>
</tr>
<tr>
<td>LF</td>
<td>2.0 ± 1.1 × 10^5</td>
<td>1.1 ± 6.3 × 10^3</td>
<td>9.2 ± 3.9 × 10^-4</td>
<td>5.1 ± 2.2 × 10^-6</td>
</tr>
<tr>
<td>HF</td>
<td>1.3 ± 6.9 × 10^5</td>
<td>2.1 ± 15 × 10^5</td>
<td>4.1 ± 1.9 × 10^-4</td>
<td>4.7 ± 1.8 × 10^-5</td>
</tr>
<tr>
<td>XF</td>
<td>3.5 ± 15 × 10^7</td>
<td>3.2 ± 17 × 10^5</td>
<td>4.12 ± 14 × 10^-2</td>
<td>2.1 ± 1.1 × 10^-4</td>
</tr>
</tbody>
</table>
Although we did detect a significant fertilization effect for AOA, abundances of AOA and AOB were within the ranges of abundances reported in some estuaries (Sahan and Muyzer, 2008). Although we did not detect robust patterns among the AOA communities related to fertilization rates based on TRFLP data, we identified some patterns among the sequence data suggesting a possible fertilization effect. OTUs 3, 4, 5, and 6 (Nitrosopumilus group 1 in Figure 5) were composed exclusively (with one exception) of sequences recovered from XF sites, suggesting that these AOA may be well-adapted to high nutrient conditions. Unfortunately, some of the TRFs representing OTUs within Nitrosopumilus group 1 were also found in other OTUs, thus obscuring any patterns related to fertilization in the TRFLP analysis. Sequences representing the Nitrososphaera group were also dominant among XF plots, but the number of sequences in this group was relatively small compared to other groups. Additionally, the sequences we recovered from both Nitrosopumilus group 1 and the Nitrososphaera group were closely related to AOA recovered from environments that were not nutrient-enriched, indicating that the sequences in these clusters are not necessarily indicative of high nutrient conditions.

Environmental factors identified as potentially important in regulating AOA community composition are many and include salinity, temperature, pH, nitrite, dissolved oxygen, nutrient productivity, and some heavy metals (reviewed in Erguder et al., 2009). However, common factors regulating AOA communities in coastal systems have not clearly emerged, suggesting that AOA may be a more physiologically diverse group relative to the AOB and nitrogen cycling in the marsh, there was no compelling response of denitrifying communities to long-term fertilization. This leads us to suspect that either the methods we find it notable that in spite of what we know about nitrogen cycling in the marsh, there was no compelling response of AOA or AOB to fertilization in the New England salt marsh and provide some insight into population dynamics, particularly among AOB, and potential regulatory factors of nitrifiers in the marsh. AOB communities showed robust and consistent responses to fertilization, while responses of AOA communities were less clear, possibly reflecting differences in physiological tolerances. However, abundance patterns of AOA and AOB showed minimal responses to fertilization treatments, suggesting that factors other than nutrients, such as redox conditions, may limit abundance of these organisms in the marsh.

**Conclusion**

Our results revealed differential responses of AOA and AOB communities to fertilization in a New England salt marsh. The lack of a compelling change in abundance to differences in fertilization may also be due to the complex nature of the fertilizer applied. Differential responses of AOA and AOB populations to different components of the fertilizer may diminish any effects on overall abundance. Studies that isolate individual environmental factors may be necessary to determine the effects of specific components on the AOA and AOB.

**Acknowledgments**

This work was supported in part by the National Science Foundation award DEB-0814586 (to Anne E. Bernhard). Additional support was provided by the George and Carol Milne Endowment at Connecticut College. We thank the students in Bernhard’s Molecular Ecology class for generating clone libraries of archaeal amoA genes and providing preliminary sequence analysis. The Great Sippewissett Marsh fertilization experiment was originally initiated by Valerie and John Ted. We thank Brian Howes and Dale Goehler for maintaining the experimental plots in recent years.
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Aquatic Microbiology January 2013 | Volume 3 | Article 445 | 10

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 10 August 2012; accepted: 31 December 2012; published online: 22 January 2013.


This article was submitted to Frontiers in Aquatic Microbiology, a specialty of Frontiers in Microbiology.

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