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The diversity (richness and community composition) of ammonia-oxidizing archaea (AOA) and bacteria (AOB) within sediments of the Gulf of Mexico was examined. Using polymerase chain reaction primers designed to specifically target the archaeal ammonia monooxygenase-subunit (*amo*A) gene and bacterial *amo*A gene, we found AOA and AOB to be present in all three sampling sites. Archaeal *amo*A libraries were dominated by a few widely distributed *Nitrosopumilus*-like sequence types, whereas AOB diversity showed significant variation in both richness and community composition. Majority of the bacterial *amo*A sequences recovered belong to *Betaproteobacteria* and very few belong to *Gammaproteobacteria*. Results suggest that water depth and nutrient availability were identified as potential drivers that affected the selection of the AOA and AOB communities. Besides influencing the abundance of individual taxa, these environmental factors also had an impact on the overall richness of the overall AOA and AOB communities. The richness and diversity of AOA and AOB genes were higher at the shallowest sediments (100 m depth) and the deepest sediments (1300 m depth). The reduced diversity in the deepest sediments could be explained by much lower nutrient availability.

Keywords: *amo*A; nitrifiers; sediment nitrification; ammonia oxidation; clone library

1. Introduction

Ammonia-oxidizing microorganisms carry out the first reaction in the oxidative half of the nitrogen cycle, the oxidation of ammonia (NH_3) to nitrite (NO_2^-). As this reaction is almost entirely biologically driven, ammonia-oxidizers have a key role in the biogeochemical cycling of nitrogen compounds. In addition, nearly all of the currently identified ammonia-oxidizing microorganisms are autotrophic and potentially biological sinks for carbon dioxide. The global significance of ammonia oxidation is best characterized through the diversity of ammonia-oxidizing microorganisms and the wide range of environments in which they are active. At present, three major groups of ammonia-oxidizers, which span two domains and three phyla, are currently recognized. These phylogenetic groups include the aerobic ammonia-oxidizing bacteria (AOB), the anaerobic AOB, and the ammonia-oxidizing archaea (AOA). Detailed phylogenetic analyses showed that all recognized AOB are confined to two phylogenetic lineages within the *Gamma*- and *Betaproteobacteria*.[1,2] AOB were long thought to be the sole microorganisms performing the oxidation of ammonia to nitrite. However, the discovery of archaeal *amo*A genes [3–5] and the discovery of AOA cultures [5,6] in natural environments have established the fact that ammonia oxidation is driven by not only members of the domain *Bacteria* but also *Archaea*. Depending on the physiological and metabolic constraints of each group, these organisms have been found in diverse range of environments including soil, activated sludge, freshwater systems geothermal hot springs, and the open ocean.[6–14] Despite the evident importance of nitrification, surprisingly little is known about the microorganisms that mediate this process in the natural environment. Ammonia monooxygenase is the enzyme responsible for the aerobic oxidation of ammonia. AOB and AOA utilize homologous ammonia monooxygenases, which are members of the copper-containing membrane-bound monooxygenase enzyme family [15] in order to activate ammonia and thus both groups carry *amo*-genes in their genomes. In the ocean, archaeal *amo*A genes (coding for the alpha-subunit of the ammonia monooxygenase) outnumber their bacterial counterparts, with both archaeal and bacteria genes being transcribed.[16] AOA prefer low substrate concentration and thus outcompete AOB under low ammonia concentration.[17]

Marine sediments and their associated microbial communities act as biocatalytic filters for the overlying water column in the ocean. Nearly 50% of the biomass produced from primary production is thought to deposit onto the shallow continental shelf seafloor, where active microbial

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communities mineralize the organic matter and release inorganic nutrients back into the water column.[18–20] Nitrogen often limits primary production in the marine environment,[13,21–23] and the predominant loss of nitrogen in the ocean is due to nitrification and denitrification. Understanding the diversity of the bacterial groups associated with nitrification and denitrification is critical to understanding the factors that may influence this important component of the nitrogen cycle in the ocean. Tiquia et al. [23] examined closely the denitrifier microbial community at different depths of the sediment collected from the Gulf of Mexico, with the community found to be very diverse and complex. The study revealed that it is possible to link microbial groups with environmental gradients. Community shifts were evident between surface (oxic) and deepest (anoxic) sediments. The changes in community structure at different depths are possibly driven by oxygen concentration and nutrient availability (nitrate), with lower quality sources of carbon and energy leading to lower diversity.

Although reports of ammonia-oxidizer diversity in the continental shelf sediments have been published,[7,24,25] the database for marine sediments, especially the AOA, remains small. The objective of the present study was to expand the characterization of AOA and AOB community diversity in the shelf sediments across spatial gradients. In order to elucidate the nitrifier communities, the functional gene, *amo*A, was assayed using the polymerase chain reaction (PCR)-based cloning approach. This gene has been used to identify nitrifiers in the sediments. Sediments were obtained from the eastern coast of Mexico in depths between 200 and 1300 m. Our results suggest that water depth and nutrient availability affect the structures of the AOB and AOA communities in the sediments.

2. Materials and methods

2.1. Sample collection and geochemical analysis

Sediment samples for microbial community analyses were collected from the continental margin of the Gulf of Mexico, an oxygenated tropical environment. Sediment samples used in this study were taken from three stations with varying water depths (station 2 = 200 m, station 4 = 100 m, and station 6 = 1300 m) (Figure 1). Sediments were taken using a Soutar box core along with overlying water, and samples for DNA analysis were extruded from sub-cores using 7.5 and 10 cm cast-acrylic tubes. Each section of the core was approximately 0.5 cm in depth. The first 0.0–0.5 cm section, measured from top of the core box, of each Soutar box core was chosen for DNA analysis. Samples were then stored in liquid N_2 for transport to the laboratory. At the laboratory, samples were stored at −20°C until DNA could be extracted.

An oxygen microelectrode was used to determine the pore water profile in the multicore tubes collected. Pore water was separated from the sediments by centrifugation at 7000*g* for 20 min. Using a technique similar to Bender

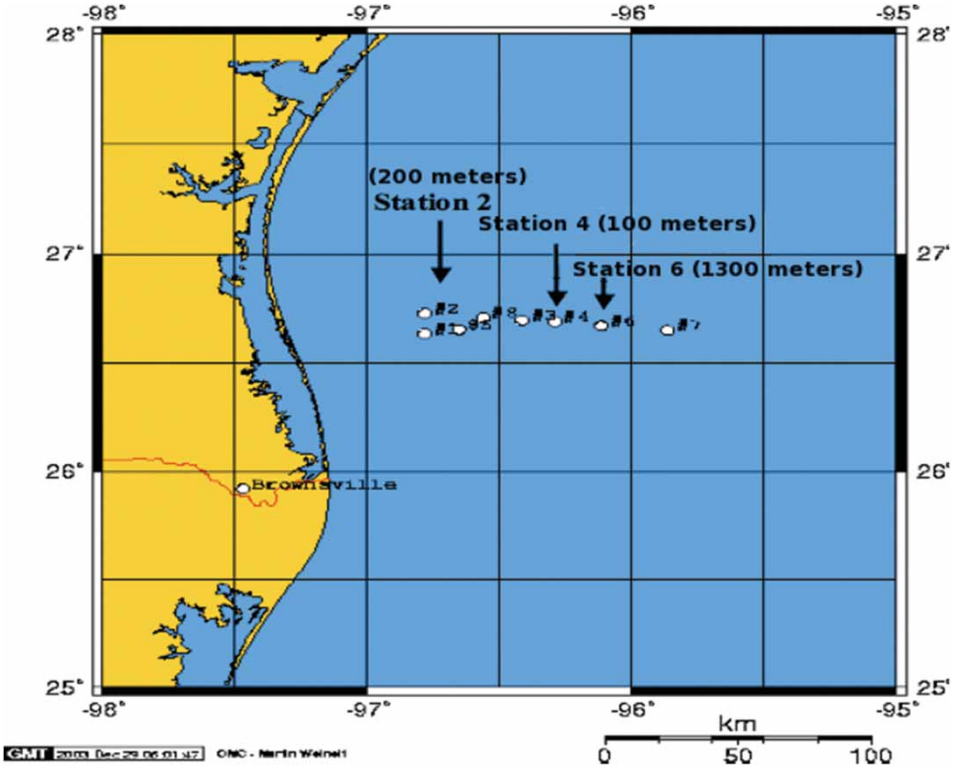

Figure 1. Map of the study area, showing sampling stations 2 (200-m water depth), 4 (100-m water depth) and 6 (1300-m water depth) which were chosen for this study.

et al.,[26] a high-resolution pore water profiles of oxygen and NO_3^- were obtained as described by Brandes and Devol.[27] Nitrate concentrations were measured using the methods of Strickland and Parsons.[28] Using the method of Hedges and Stern [29] with freeze-drying, the total carbon in the sediment samples was analysed using a Leeman Laboratories CHNS elemental analyser.

2.2. Molecular analysis

2.2.1. DNA extraction, PCR amplification, and DNA sequencing

Sediment samples from each site were ground in liquid nitrogen as described previously,[30–32] prior to DNA extraction. Approximately 1.5 g of sediment was ground in liquid nitrogen with lysis matrix B (Qbiogene, Carlsbad, CA, USA) using a mortar a pestle and followed with the use of an UltraClean™ Soil DNA Isolation Kit (Mobio Laboratories, Inc., Carlsbad, CA, USA). Bacterial and archaeal *amo*A genes were amplified from the environmental DNA extracted. The bacterial *amo*A genes were amplified in mixtures containing 50 ng μl^{-1} DNA; Choice™ *Taq* Master mix DNA polymerase (Denville, Metuchen, NJ, USA); 10 μM each of the forward (*amo*A-1F; 5′-GGGGTTTCTACTGGTGGT) and reverse (amoA-2R; 5′-CCCCTCKGSAAAGTTCCTTC) primers (K = T or G and S = C or G) per 25 μl reaction. The following PCR conditions for bacterial *amo*A were applied: initial denaturation at 94 °C for 5 min; followed by 42 cycles of 94 °C for 60 s, annealing for 60 °C for 90 s, extension for 90 s; and completed with a final extension period of 72 °C for 10 min.[33] For the archaeal *amo*A, genes were amplified in mixtures containing 50 ng μl^{-1} DNA; Choice™ *Taq* Master mix DNA polymerase (Denville, Metuchen, NJ, USA); 10 μM each of the forward (Arch-*amo*AF; 5′-STAATGGTCTGGCTTAGACG) and reverse (Arch-*amo*AR; 5′-GCGGCCATCCATCTGTATGT) primers (K = T or G and S = C or G) per 25 μl reaction. The following PCR conditions for archaeal *amo*A were applied: initial denaturation at 95 °C for 5 min; followed by 30 cycles of 94 °C for 45 s, annealing at 53 °C for 60 s, extension at 72 °C for 60 s; and completed with a final extension period of 72 °C for 15 min.[7]

Negative PCR controls without DNA template were run concurrently for each sample. PCR products were visualized in a 1.0% (wt./vol.) agarose Tris-ethyleneaminetetraacetic acid (Tris-EDTA) gel to confirm the size of the product. Five replicate PCR runs were performed for each DNA extract. The size of the bacterial *amo*A amplicon was 491 bp whereas the archaeal *amo*A amplicon was 635 bp.[7,33] The replicate PCR products were combined (125 μl) and loaded into an 0.8% agarose gel, excised, extracted with Qiaquick gel extraction kit (Qiagen, Valencia, CA, USA), and eluted in 30 μl elution buffer (EB) (10 mM Tris–Cl, pH 5.0).

2.2.2. Clone library construction

Purified PCR products were cloned into an Invitrogen TOPO II vector (Invitrogen, Carlsbad, CA, USA), which was then used to transform MAX Efficiency® Stbl2™ Competent Cells (Invitrogen, Carlsbad, CA, USA). Clones were spread plated onto Luria Bertani (LB) agar with 100 μg/ml ampicillin and incubated at 30 °C for 24–36 h. After incubation, transformants were screened and plated on another LB agar with 100 μg ml^{-1} ampicillin and incubated at 30 °C for 24–36 h. Thereafter, the clones were inoculated in a 96-well plate filled with 100 μl LB broth containing 100 μg ml^{-1} ampicillin and were incubated at 30 °C for 24–48 h or until there was visible turbidity. An aliquot (50 μl) of the clones from each well was transferred to a complementary well in a new 96-well plate along with 50 μl of sterile endonuclease free water and then boiled at 100 °C for 10 min. The boiled clones were used as template for a PCR utilizing the *amo*A primers. PCR products were purified using Montage® PCRμ96 and Montage® PCRμ384 plates (Millipore, Bedford, MA, USA). Purified PCR products were concentrated using a Zymo DNA Clean and Concentrator-5 kit and eluted to 30 μl. The DNA concentration for each of the wells was determined through a combination of gel electrophoresis and the use of Nanodrop spectrometrophotometer (Nanodrop 1000, Thermo Scientific, Wilmington, DC, USA) to determine DNA concentration for sequencing. Samples were sequenced by capillary electrophoresis using ABI model 3730 sequencer (Life Technologies, Carlsbad, CA, USA) at the University of Michigan DNA Sequencing Core (http://seqcore.brcf.med.umich.edu/doc/dnaseq/where.html).

2.2.3. DNA sequence analysis and phylogeny

Sequence chromatogram files were analysed using Chromas version 2.33 (Technelysium, Helensvale, Australia) and no-call, miss-calls, and heavily 'busy' regions at the beginning and end of the chromatograms were edited out. Sequence alignments were performed using the Clustal W algorithm in BioEdit version 7.13.[34] Aligned sequences were analysed using VecScreen [35] and segments with strong matches to vectors or of suspect origin were removed. Chimeric sequences were removed after identification using Bellerophon [36] using a 200 bp window and the Huber–Hugenholtz correction.[36] Sequences with homology to sequences of the ammonia monooxygenase-subunit A (*amo*A) from cultured AOB and archaea were identified using nucleotide Basic Local Alignment Sequence Tool (BLAST) (http://www.ncbi.nih.gov/BLAST/). Sequences with unrelated or no homology to those in the GenBanK database were discarded. Separate bacterial and archaeal phylogenetic trees were generated using MEGA 5.2 [37] and constructed with representative sequences from each sediment's unique operational taxonomic units (OTUs) using the Jukes–Cantor model with 10,000 bootstrap

replications. The sequences that were represented in each OTU were determined using MEGA 5.2 distance matrices to find similarities of 97% or greater using the same Jukes–Cantor model. Venn diagrams were also generated from these data to locate 97% similar sequences in each library for every clone to discover if the sequences were universal to all depths or unique to one. The BLAST search was further used to classify sequences by finding the closest match in GenBank and identifying the nucleotide percent similarity.

2.2.4. *Diversity estimates*

Using the computer program Distance-based Operational Taxonomic Unit and Richness (DOTUR) determination, diversity matrices including Shannon–Weirner [H'] index, Simpson's index [$1/D$], and Chao1 index were calculated for each clone library along with rarefaction curves and sample coverage.[38] Distance matrices were first generated from sequence alignment files using BioEdit and then subsequently used to estimate the diversity of the *amo*A communities including the Shannon–Weiner index, Simpson index, and Chao1 index. The Shannon–Weaver index combines diversity and species evenness with higher scores showing greater diversity and species evenness.[39] The Simpson reciprocal index is a measure of diversity and its output is the probability that two sets of individual units from a group are of the same type, so smaller numbers indicate higher diversity.[40] The Chao1 index, another statistical diversity index measuring diversity, is referred to as 'bias corrected' because it tries to take unforeseen species pairs into account. Higher numbers in Chao indicate greater diversity.[41] DOTUR files were used in the construction of rarefaction curves, determination of the number of OTUs present, and the number of clones in each OTU. OTUs were defined as sequences which had <3% difference.[38]

3. Results

3.1. *Site geochemistry*

The overlying ocean conditions for the three sampling stations were similar. The overlying water O_2 concentrations were approximately 150 μM. The overlying water NO_3^- concentrations were 15 μM. There was 1–2% carbon in the water and the average temperature was 12 °C (Table 1).

Table 1. The average overlying water conditions at sampling sites.

Overlying water O_2 (μM)	Overlying water NO_3^- (μM)	%C	Temperature (°C)
~150	15	1–2	12

3.2. *AOA clone libraries*

A total of 374 archaeal *amo*A clones were sequenced; of these 174, 155, and 45 clones were sequenced from stations 2 (200 m water depth), 4 (100 m water depth), and 6 (1300 m water depth), respectively (Table 2). Figure 2 shows the rarefaction curves used to determine sufficient sequencing for each station. All rarefaction curves levelled off and approached a horizontal asymptote, indicating that the majority of the diversity of each station had been sampled within the analysed clones (Figure 2). Levelling off of clone libraries was evident after sequencing140 clones in station 4, 40 clones in station 6, and 160 clones in station 2 (Figure 2).

The diversity of the sampled *amo*A archaeal showed relatively higher diversity in station 4 (shallowest sediments) than in stations 2 and 6 (deeper sediments). Only 8 OTUs were found in stations 2 and 6 whereas 35 OTUs were found in station 4 (Table 2). The diversity indices showed that the archaeal *amo*A clone sequence from station 4 had the highest diversity followed by station 6, with station 2 being the least diverse of the three. Although a large number of clones (174 clones) were sequenced from station 2, it remained the least diverse with only 8 OTUs, a Shannon–Weiner index (H') of 0.472, Simpson's index ($1/D$) of 0.81, and a Chao-1 index of 13. Station 4 had the greatest diversity after sequencing 155 clones with 35 OTUs, a Shannon–Weiner index (H') of 2.94, Simpson's index ($1/D$) of 0.07, and a Chao-1 index of 69.2. Station 6 only had 45 clones sequenced and showed 9 distinct OTUs, a Shannon–Weiner index (H') of 1.65, Simpson's index ($1/D$) of 0.269, and Chao-1 index of 10.5 (Table 2). The OTUs from each library showed no overlap (Figure 3(a)), indicating that these unique clones are endemic to the station in which they were retrieved.

3.3. *AOB clone libraries*

Between 46 and 242 bacterial *amo*A clones were sequenced for each station (Table 3). Rarefaction analysis at ≥97% similarity levels showed that only one station (station 6; 1300 m water depth) reached a horizontal asymptote (Figure 4), which levelled off after only 12 OTUs were detected (Table 3). Rarefaction curves for bacterial clones at stations 2 (200 m water depth) and 4 (100 m water depth) did not reach a horizontal asymptote, suggesting that after sequencing 113–242 clones from each station, complete diversity could not be achieved (Figure 4). No additional clones were sequenced from stations 2 and 4 after 65 and 37 new OTUs were detected from sequencing additional 150 clones from station 2 and 79 clones from station 4. Rarefaction curves of stations 2 and 4 showed a light levelling off after over 100–200 clones were sequenced whereas station 6 showed significant levelling off after only 40 clones were sequenced.

The level of bacterial *amo*A diversity was the highest in station 2 (200 m water depth) followed by station 4 (100 m

Table 2. Diversity estimates of AOA clone libraries.

Station (water depth)	Number of clones sequenced[a]	Number of OTUs[b]	H'[c]	$1/D$[d]	Chao-1[e]
Station 2 (200 m)	174	8	0.47	0.80	13.00
Station 4 (100 m)	155	35	2.93	0.07	69.20
Station 6 (1300 m)	45	9	1.65	0.27	10.50

[a]Number of clones sequenced from each library.
[b]OTUs based on *amo*A gene sequences (≥97% nucleotide sequence similarity).
[c]Shannon–Weiner index; higher number represents higher diversity.
[d]Reciprocal of Simpson's index; higher number represents lower diversity.
[e]Chao-1 diversity index.

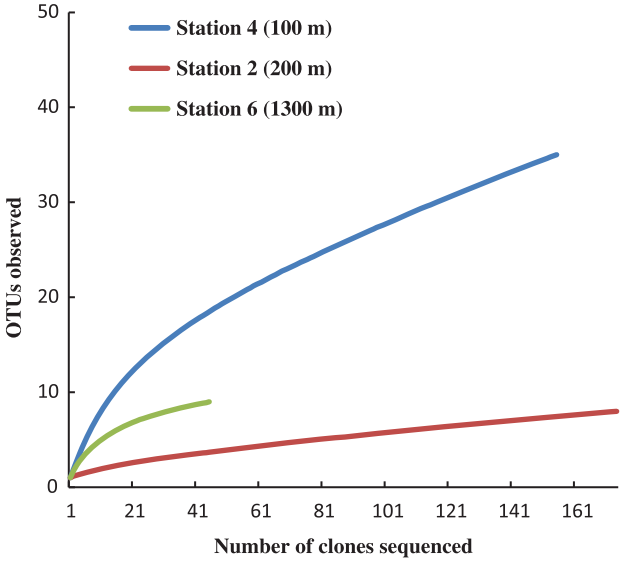

Figure 2. Rarefaction curves of Archaeal *amo*A clone libraries illustrating the relationship between the number of OTUs and the number of clones sequenced. OTUs were defined at ≤97% nucleotide sequence identity.

water depth), with station 6 (1300 m water depth) being the least diverse of the three (Table 3). Station 2, the most diverse of the three stations, generated 113 OTUs from 242 clones sequenced and showed the highest Shannon–Weiner index (3.93), lowest Simpson's index (0.05), and highest Chao-1 index (780.5) values. Station 6 (1300 m), the least diverse of the three stations, produced 12 OTUs from 46 clones sequenced, and showed the lowest Shannon–Weiner index (2.06), lowest Simpson's index (0.17), and highest Chao-1 index of (12.6) values (Table 3). Through a combination of the diversity indices obtained from Table 3 and the rarefaction curve in Figure 4, we can determine that the overall diversity from station 6 is thoroughly represented in these data. The majority of diversity from stations 2 and 4 is also represented here, but there may be some need for further sampling to complete the examination of the complete diversity of these two stations. The OTUs from each library showed little overlap (Figure 3(b)).

3.4. *Phylogeny*

Clone sequences were used to construct a phylogenetic tree of archaeal *amo*A compared to known *amo*A gene

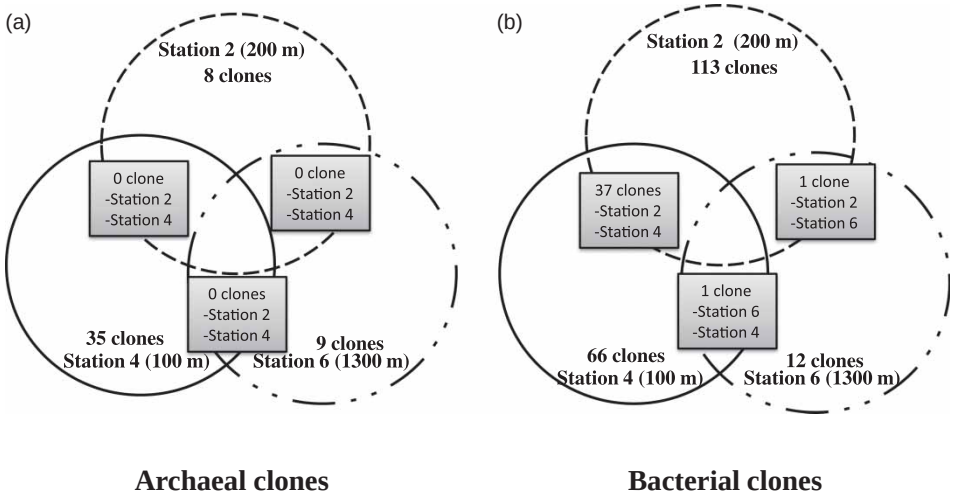

Figure 3. Distribution of overlapping (a) archaeal *amo*A and (b) bacterial *amo*A sequences from marine sediments collected from stations 2 (200-m water depth), 4 (100-m water depth), and 6 (1300-m water depth).

Table 3. Diversity estimates of AOB clone libraries.

Station (water depth)	Number of clones sequenced[a]	Number of OTUs[b]	H'[c]	$1/D$[d]	Chao-1[e]
Station 2 (200 m)	242	113	3.93	0.052	780.5
Station 4 (100 m)	113	66	3.79	0.033	207.7
Station 6 (1300 m)	46	12	2.06	0.168	12.6

[a]Number of clones sequenced from each library.
[b]OTUs based on *amo*A gene sequences (≥97% nucleotide sequence similarity).
[c]Shannon–Weiner index; higher number represents higher diversity.
[d]Reciprocal of Simpson's index; higher number represents lower diversity.
[e]Chao-1 diversity index.

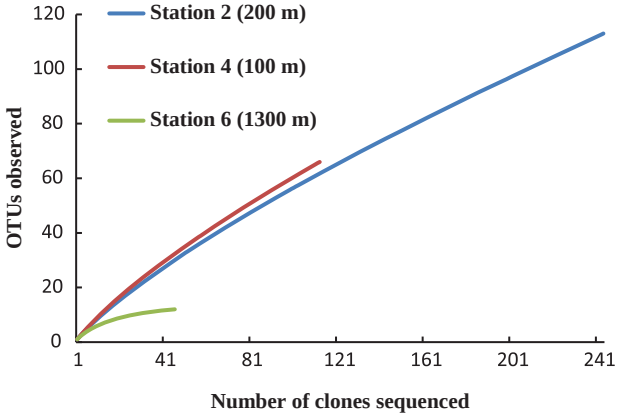

Figure 4. Rarefaction curves of Bacterial *amo*A clone libraries illustrating the relationship between the number of OTUs and the number of clones sequenced. OTUs were defined at ≤97% nucleotide sequence identity.

sequences from known ammonia-oxidizers (Figure 5). Archaeal *amo*A sequences were assembled into 374 full contiguous sequences (61 sequences were not included), of which 313 were determined to be non-chimeric. Chimeric sequences were found in 61 clones from station 4. The sequences were assigned to 40 OTUs. The tree can be visually divided into seven clades (Figure 5). All of the major clades contained at least one sequence from each station except for clade II, which does not contain any sequences from station 6. Sequence 1828366 (station 4) from clade VII is unique because it appears to be more closely related to the bacterial outlier *Nitrosococcus oceani* than it is to the rest of the archaeal *amoA* sequences in the tree and it has the least sequence homology with all of the reference sequences with a range of 54–58% sequence homology with the six reference sequences (Table 4). Clone 1851585 (station 4) from clade II has the highest nucleotide sequence homology with reference sequence at 87% similarity (Table 4). The range of sequence homology of 54–87% similarity between the clones and the reference sequences implies that there are unique *amo*A sequences at stations 2, 4, and 6.

A phylogenetic tree of bacterial *amo*A gene clones compared to known *amo*A gene sequences from known AOB was constructed (Figure 6). The phylogenetic tree contains 189 OTUs which comprise 376 clones that can be segregated into 10 visually distinct clades: clade I consists of 13 *amo*A reference sequences, clade II consists many similar sequences from stations 2, 4, and 6 which have been condensed due to their similarity, clades III and IV comprise clones only from stations 2 and 4, clades V and IX contain clones from stations 2, 4, and 6, clade VI is unique because it only contains the reference sequence, *Nitrosomonas cryotolerans*, and condensed group of 4 OTUs from station 6 which comprise 10 clones, clade VII is unique because it contains 7 OTUs from station 4 and 1 OTU from station 2 comprising 40 sequences and 1 sequence, respectively, clade VIII is unique because it comprises a single clone from station 2, and clade X is also unique because it comprises 3 OTUs from station 2 comprising 3 clones and 8 OTUs from station 4 comprising 8 clones. The presence of clades that do not contain station 6 (clades III, IV, and VII), stations 6 and 4 (clade VIII), or stations 2 and 4 (clade VI) implies that there may be station-specific diversity in the bacterial *amo*A gene. The range of nucleotide sequence homology between the bacterial *amo*A clones and reference sequences was from 0.005% to 85% similarity (Table 5). Clone 1854018 (station 4) from clade V had the highest similarity to a reference sequence with 85% similarity to *Nitrosomonas cryotolerans* (Table 5). The clones 1854084 (station 4, clade X), 1955697 (station 4, clade VII), and 1955624 (station 4, clade X) had the least nucleotide homology to a reference sequence with 0.005% similarity to *Nitrosovibrio* sp. *RY3C* (DQ228466.1), *Nitrosococcus oceanus* (U96611.1) and *Nitrosospira* sp. *9SS1* (DQ228455.1), respectively (Table 5).

3.5. *Comparison between bacterial and archaeal diversity*

The archaeal clone library contained 8, 35, and 9 OTUs for stations 2, 4 and 6, respectively, while the bacterial clone library contained 113, 66 and 12 OTUs for stations 2, 4 and 6, respectively. Each bacterial clone library has more OTUs than its archaeal counterpart. From the bacterial clone library, 25%, 77% and 80% of the OTUs from stations 6, 4, and 2, respectively, consisted of only one clone and 33%,

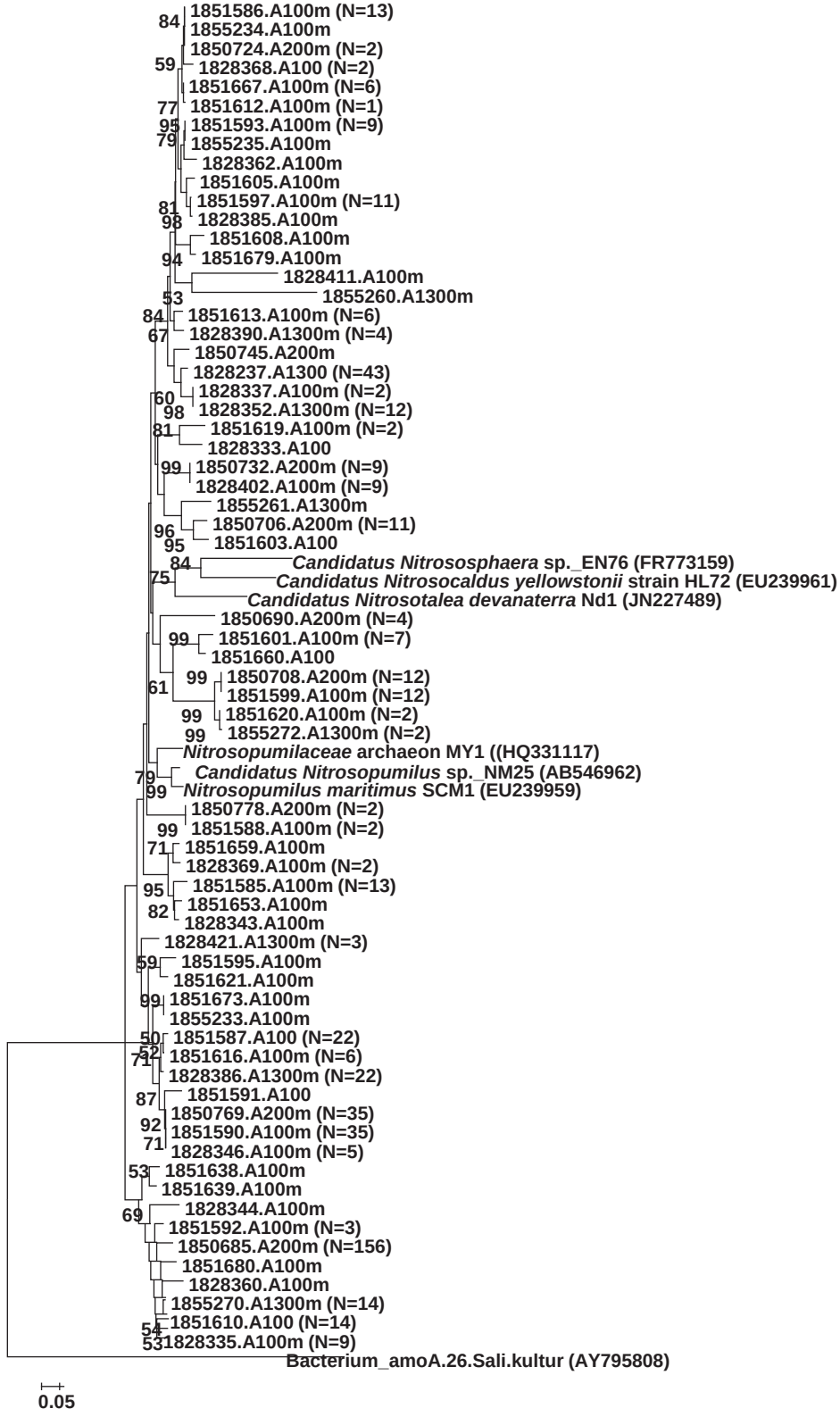

Figure 5. Phylogenetic dendogram showing the relationship of archaeal *amo*A clones with known archaeal *amo*A genes. Nodal values represent bootstrap probabilities based on 10,000 replicates.

Table 4. Clone sequence similarity to known AOA from GenBank.[a]

Known AOA	Closest matched clone sequence	Nucleotide similarity	Most distantly related clone sequence	Nucleotide similarity
Nitrosopumilaceae archaeon (HQ331117.1)	1851604 (station 4) ($n = 10$); 1851585 (station 4) ($n = 3$)	76%	1828411 (station 4) ($n = 1$)	28%
Nitrosopumilus maritimus (EU239959.1)	1851605 (station 4) ($n = 1$)	73%	1828411 (station 4) ($n = 1$)	24%
Nitrosopumilus sp. NM25 (AB546962.1)	1855247 (station 6) ($n = 3$)	73%	1828411 (station 4) ($n = 1$)	25%
Nitrosopumilus maritimus (HM345611.1)	1851585 (station 4) ($n = 3$)	87%	1828411 (station 4) ($n = 1$)	28%
Nitrosopumilus maritimus (HM345610.1)	1851585 (station 4) ($n = 3$)	75%	1828411 (station 4) ($n = 1$)	28%
Nitrosopumilus maritimus (HM345608.1)	1851604 (station 4) ($n = 10$); 1851585 (station 4) ($n = 3$)	76%	1828411 (station 4) ($n = 1$)	28%

[a] Station 4 (100 m depth); station 6 (1300 m depth); n = number of observations.

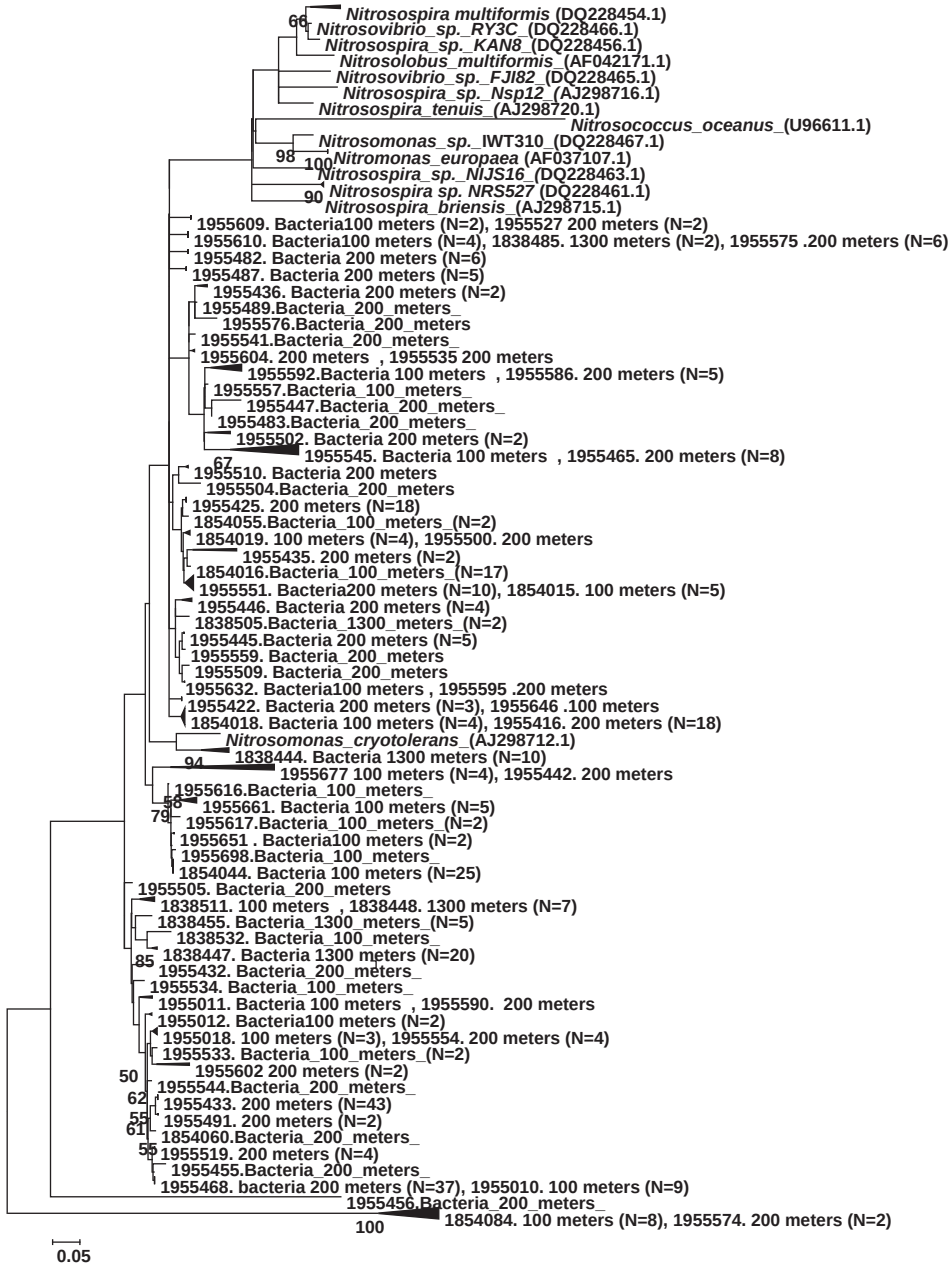

Figure 6. Phylogenetic dendogram showing the relationship of bacterial *amo*A clones with known bacterial *amo*A genes. Nodal values represent bootstrap probabilities based on 10,000 replicates.

Table 5. Clone sequence similarity to known AOB from Genbank.[a]

Known AOB	Closest matched clone sequence	Nucleotide similarity	Most distantly related clone sequence	Nucleotide similarity
Nitrosovibrio sp. RY3C (DQ228466.1)	1955433 (station 2) ($n = 26$)	80%	1854084 (100 m) ($N = 1$)	0.005%
Nitrosovibrio sp. FJI82 (DQ228465.1)	1838485 (station 6) ($n = 2$)	80%	1854084 (100 m) ($N = 1$)	6.7%
Nitrosomonas sp. IWT310 (DQ228467.1)	1955433 (station 2) ($n = 26$)	79%	1854084 (100 m) ($N = 1$)	0.005%
Nitrosomonas cryotolerans (AJ298712.1)	1854018 (station 4) ($n = 4$)	85%	1955459 (200 m) ($N = 1$)	9.6%
Nitrosospira sp. Nsp12 (AJ298716.1)	1955011 (station 4) ($n = 1$)	75%	1955677 (100 m) ($N = 1$)	36%
Nitrosococcus oceanus (U96611.1)	1838532 (station 4) ($n = 1$)	40%	1955697 (100 m) ($N = 1$)	0.005%
Nitrosomonas europaea (AF037107.1)	1955417 (station 2) ($n = 1$)	75%	1955456 (200 m) ($N = 1$)	3.7%
Nitrosomonas europaea (JN099309.1)	1955417 (station 2) ($n = 1$)	75%	1955456 (200 m) ($N = 1$)	3.7%
Nitrosomonas europaea (AB070981.1)	1955417 (station 2) ($n = 1$)	75%	1955456 (200 m) ($N = 1$)	3.7%
Nitrosomonas europaea (AJ298710.1)	1955417 (station 2) ($n = 1$)	75%	1955456 (200 m) ($N = 1$)	3.7%
Nitrosospira sp. NIJS16 (DQ228463.1)	1838499 (station 6) ($n = 1$)	82%	1955456 (200 m) ($N = 1$)	9.6%
Nitrosospira sp. NRS527 (DQ228461.1)	1838499 (station 6) ($n = 1$)	74%	1955456 (200 m) ($N = 1$)	6.7%
Nitrosospira sp. PJA1 (DQ228457.1)	1838489 (station 6) ($n = 2$)	79%	1854084 (100 m) ($N = 1$)	3.7%
Nitrosospira sp. 9SS1 (DQ228455.1)	1955433 (station 2) ($n = 26$)	76%	1955624 (100 m) ($N = 1$)	0.005%
Nitrosospira sp. KAN8 (DQ228456.1)	1955433 (station 2) ($n = 26$)	80%	1854084 (100 m) ($N = 1$)	0.005%
Nitrosospira sp. GS832 (DQ228460.1)	1838499 (station 6) ($n = 1$)	75%	1854084 (100 m) ($N = 1$)	0.005%
Nitrosospira multiformis (DQ228454.1)	1838499 (station 6) ($n = 1$)	79%	1854084 (100 m) ($N = 1$)	3.7%
Nitrosolobus multiformis (AF042171.1)	1838499 (station 6) ($n = 1$)	80%	1854084 (100 m) ($N = 1$)	3.7%
Nitrosospira sp. LT2MFa (AY189145.1)	1838499 (station 6) ($n = 1$)	74%	1955456 (200 m) ($N = 1$)	3.7%
Nitrosospira sp. LT1FMf (AY189144.1)	1838499 (station 6) ($n = 1$)	74%	1955456 (200 m) ($N = 1$)	3.7%
Nitrosospira tenuis (AJ298720.1)	1955546 (station 4) ($n = 3$)	81%	1955456 (200 m) ($N = 1$)	12.3%
Nitrosospira briensis (AJ298715.1)	1838499 (station 6) ($n = 1$)	77%	1955456 (200 m) ($N = 1$)	9.6%

[a]Station 2 (200 m depth); station 4 (100 m depth); station 6 (1300 m depth); n = number of observations.

57%, and 63% of the OTUs from the archaeal clone libraries of stations 6, 4, and 2, respectively, consisted of only one clone. The bacterial clone libraries are more diverse than their archaeal counterparts at each station with respect to the Shannon, Simpson and Chao1 diversity indices (Tables 2 and 3).

4. Discussion

Ammonia oxidation to nitrite is the rate-limiting step in nitrification and hence an important component of the global biogeochemical nitrogen cycle. For more than a century, it has been known that this process can be performed by chemolithoautotrophic bacteria.[42] Detailed phylogenetic analyses showed that all recognized AOB are restricted to two phylogenetic lineages within the *Beta*- and *Gammaproteobacteria*.[1,2] The discovery of AOA revealed that an additional group of microorganisms is able to catalyse this process.[3–5] The present study revealed the presence of AOB and AOB in the sediments. A total of 374 archaeal *amo*A clones and 401 bacterial *amo*A clones were sequenced. However, more unique sequences were observed among AOB clones than AOA clones. About 48% of the AOB clones (191 OTUs) were unique while only 14% of the AOB clones (52 OTUs) were unique to each other. Bacterial and archaeal *amo*A sequences recovered from the sediments were compared to known *amo*A sequences from GenBank. Majority of the bacterial *amo*A sequences recovered belong to *Betaproteobacteria* and very few belong to *Gammaproteobacteria*. *Nitrosovibrio*-, *Nitrosomonas*, *Nitrospira* and *Nitrosolobus*-like sequences, all of which belong to the sub-phylum *Betaproteobacteria* and were recovered from the sediments. *Nitrosomonas*-like sequences are the only ones recovered that belong to the sub-phylum *Gammaproteobacteria*. These sequences have 74–85% nucleotide similarities to the known AOB sequences. The AOB clones were dominated by sequences similar to *Nitrosopumilus* species, with sequence similarities ranging from 83% to 87%. The nearest cultivated organism to the Gulf of Mexico sequences was the isolate *Nitrosopumilus maritimus* (HM365611.1), which shared 83% nucleotide sequences identity and 93% amino acid identity with clones 1851585 (station 4; 100 m depth), 1851608 (station 4; 100 m depth), 1855248 (station 6; 1300 m depth), and 1851607 (station 4; 100 m depth).

There were considerable differences in AOA and AOB diversity and composition between sites. Our results showed that richness and diversity of AOA and AOB genes were higher at the shallowest sediments (100 m depth) and the deepest sediments (1300 m depth). The reduced diversity in the deepest sediments could be explained by much lower nutrient availability due to the poorer substrate available. Kaspari et al. [43] elucidated that the amount of energy available to an ecosystem limits the species richness by limiting the density of its individual taxa. In the AOA clone libraries, unique sequences showed no overlap at all between the three stations, suggesting that that these unique clones are endemic to the station in which they were retrieved. In case of the AOB clone libraries, the unique sequences from each station showed little overlap.

These findings indicate that spatial heterogeneity of AOA and AOB community structure appears to be high. It is known that extant microbial communities are a result of either geochemical conditions that result in selection of a community or founding populations that may be endemic rather than cosmopolitan or both.[21,44,45] Analysis of factors that shape AOA and AOB community structure reveal a strong effect on geographical location.[11,46] Similar results were previously obtained from other groups of microorganisms showing that on a scale of thousands of kilometres, historical separation due to mutation, genetic drift, or differential selective pressures in the past can counteract forces of dispersal and homogenizing effects of environmental factors.[47–49] In the present study, nutrient availability was identified as potential drivers that affected the selection of the AOA and AOB communities. Besides influencing the abundance of individual taxa, nutrient availability also had an impact on the overall richness of the overall AOA and AOB communities.

Despite the discovery of large archaeal population soil, activated sludge, freshwater systems, estuaries, geothermal hot springs and the open ocean,[6–14,50] the drivers of archaeal versus bacteria growth are poorly understood. Based on the distribution of archaeal *amo*A and 16S rRNA genes, it seems that archaea has the potential capacity to oxidize ammonia in the ocean. Astonishingly, estimates based on gene counts (quantitative PCR) indicate that AOA, which have been overlooked for many years, outnumber AOB in most environments, often even by orders of magnitude.[24] Still, our collective knowledge and understanding of the relative role of AOA and AOB in nitrification are still very limited and conflicting. For example, NH_3 oxidation of archaea has been shown to be important for N cycling in the ocean [17] and in soil.[51] On the other hand, Di et al. [52] and Jia and Conrad [53] showed that AOB were functionally more important than AOA in NH_3 oxidation in some agricultural soils. In line with these findings are physiological studies of the only marine-cultivated isolate of AOA, *Nitrosopumilus maritimus*.[17] The organism appears adapted to very low amounts of its substrate ammonia. Both its extremely low threshold and its half saturation constant are unprecedented, but coherent with the conditions in the oligotrophic open ocean. Their study strongly indicates that certain lineages of AOA contribute to a large extent to the nitrogen cycling in the ocean. In the present study, AOB was found to be more diverse in all three stations examined than the AOA. As the primary substrate required for nitrification, NH_4^+ might also be expected to influence AOA community diversity and structure. Sediments from the Gulf of Mexico are characterized by elevated NH_4^+ concentrations [46] caused by hydrologic connection with the Mississippi River Basin,[54] which is highly enriched in NH_4^+. The waters that discharge into the Gulf of Mexico originate in the watersheds of the Mississippi, Ohio and Missouri Rivers, collectively described here as the Mississippi River Basin. With a total watershed of 3 million km^2, this basin encompasses about 40% of the territory of the lower 48 states and accounts for 90% of the freshwater inflow to the Gulf of Mexico.[55] Other factors contributing to the infusion of nutrients into the Gulf include artificial drainage and other hydrologic changes to the landscape, runoff and domestic wastewater discharges from cities and suburbs, and point discharges from feedlots and other sites of intensive agricultural activity.[54]

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