Ammonia-oxidizing Archaea (AOA) are Winners to Survive in Oxygen-limited Habitat Compared to Ammonia-oxidizing Bacteria (AOB)

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Abstract

Purpose: Both ammonia oxidizing archaea (AOA) and bacteria (AOB) perform the ammonia oxidation together. These two kinds of microbes present a convenient model for studying niche specialization. To date, few surveys concentrated on the influence of oxygen concentration on niche specialization of AOA and AOB in intertidal zones.

Methods: Here, high-throughput sequencing by Illumina MiSeq and qPCR were applied to detect the change of abundance, diversity as well as community structure of both AOA and AOB with 0-60 cm sediments depth in the intertidal zone in Qingdao.

Results and Conclusion: The AOA/AOB *amoA* gene copy numbers and AOA/AOB OTU numbers were all increased as sediment depth went deeper, which indicated that AOA were more adaptive to oxygen-limited niches compared to AOB and oxygen indeed led to the niche specialization of AOA and AOB in intertidal sediments. The dominant AOA and AOB were the *Nitrosopumilus* and *Nitrosospira* clusters, respectively, which indicated an ecological success in intertidal zone. Oxidation-reduction potential (ORP) was significantly positively correlated with AOB abundance and AOB OTU numbers ($P < 0.01$). In addition, both TN ($P < 0.01$) and pH ($P < 0.05$) were significantly and negatively correlated with AOB abundance. TN was also significantly and negatively correlated with AOB OTU numbers ($P < 0.05$).

Introduction

Nitrification, a key role in the ecosystem nitrogen cycle, is the conversion of ammonia to nitrate through nitrite (Beeckman et al., 2018). Three kinds of microorganisms of ammonia oxidant, nitrite oxidizers and complete ammonia oxidizers were involved in the nitrification process (Stein and Klotz, 2016). Ammonia-oxidizing bacteria (AOB) was long thought to the only microorganism that performed ammonia oxidation, the first and rate-limiting step in nitrification. However, the ammonia oxidation theory was changed since the discovery of ammonia-oxidizing archaea (AOA) (Konneke et al., 2005). To date, several strains of AOA such as Candidatus *Nitrososphaera gargensis* (Hatzenpichler et al., 2008), Candidatus *Nitrosocaldus yellowstonii* (de la Torre et al., 2008), Candidatus *Nitrosoarchaeum limnia SFB1* (Blainey et al., 2011) and Candidatus *Nitrosotalea devanaterra* (Laura et al., 2011), were enriched or isolated from various ecosystems. Until now, both AOA and AOB have been shown to perform ammonia-oxidizing (de la Torre et al., 2008; Hatzenpichler et al., 2008; Jia and Conrad, 2009). Various AOA and AOB functional gene abundance, community structures and activity patterns were reported in different biotopes (Wang, et al., 2020). Environmental factors play a key role in the niche differentiation between AOA and AOB. And the AOA and AOB relative contribution to ammonia oxidation in various ecosystems gradually become the hot spots in the field of ammonia oxidation.

As reported previously, a serious of environmental factors may cause the niche specialization between AOA and AOB, such as pH, temperature, salinity, ammonia concentrations and oxygen concentrations. AOA seems to be more adaptive in niches with lower pH and more likely to dominant the process of

ammonia oxidation in acidic niches (Gubry-Rangin et al., 2011; Prosser and Nicol, 2012). AOB were more adaptable than AOA with increasing abundance but no alteration of composition at elevated temperature (Zhang et al., 2019b). Microcosm tests also verified that pH and temperature were key factors that leaded to the niche specialization of AOA and AOB (Aigle et al., 2020). Ammonia, as one of the substrate for ammonia oxidation, could cause niche specialization of AOA and AOB. The concentration of ammonia half saturation constant of some AOB strains (Jung et al., 2011; Kim et al., 2012; Martens-Habbena et al., 2009) were much higher than AOA strains, indicated that AOA had higher affinity for ammonia than AOB. The half-saturation constants of Candidatus *Nitrosoarchaeum koreensis MY1*, Candidatus *Nitrososphaera sp. JG1* and *Nitrosopumilus maritimus* SCM1 were 0.69 μM, 2.15 μM and 133 nM, respectively, which were much lower than that of *Nitrosomonas europaea* (Km = 553uM) (Martens-Habbena et al., 2009). Therefore, AOA is more competitive than AOB in oligotrophic environment (Beman et al. 2008; Verhamme et al. 2011). In the aspects of other substrate for ammonia oxidation, oxygen could also lead to niche specialization of AOA and AOB. The half-saturation constants for oxygen of AOA, such as Candidatus *Nitrosoarchaeum koreensis MY1*, Candidatus *Nitrososphaera sp. JG1* and *Nitrosopumilus maritimus* SCM1 were 10.38, 4.67 and 3.90 μM, separately (Kim et al. 2012; Jung et al. 2011; Martens-Habbena et al. 2009), which were much lower than that of *Nitrosomonas oligotropha* NL7 (76.3 μM) and *Nitrosomonas europaea* C-31 (183.3 μM) (Parket et al. 2010; Park and Noguera 2007). The affinities of AOA for oxygen were much higher than that of AOB, which means that AOA had competitive advantages over AOB in the oxygen-limited environments. The AOA lived better in many oxygen-limited environments. AOA dominated the transcriptome and probably dominated the ammonia oxidation process in the OMZ (Stewart et al. 2012). The increased of AOA OTU number and abundance ratio (AOA: AOB) in flooded soil proved that AOA could better adapt to low oxygen condition (Liu et al., 2015).

The intertidal zone is an ecological crisscross zone with important environmental and ecological functions affected by marine and terrestrial ecosystems (Community structure and organization of tidepools.). Tidal daily rhythm so that sediment is submerged or exposed to air. The Shazikou wharf was built in 1976 and was one of the biggest wharf in Qingdao. Near the wharf, was the biggest fish market in Qingdao. The human activities and the decayed fish provided enough ammonia nitrogen for the ammonia-oxidizing microbes in the intertidal zones nearby. The ecological distribution, including the community structure and of abundance AOA and AOB in intertidal zones have been discussed previously (Hu et al., 2019). However, the relationship between oxygen concentration and niche specialization of AOA and AOB was not investigated. So, the main objectives of this research are: (1) to study the difference of abundances and community structure of AOA and AOB in different layers/depth of the sediments in the intertidal zones, (2) to explore which, AOA or AOB, was the main driver performing ammonia oxidation in different layers/depth of the sediments in intertidal zones, (3) to evaluate how the oxygen concentration influence the niche specialization of AOA and AOB in intertidal zones.

Materials And Methods

Description of sampling sites, collection and physicochemical properties analysis of sediment

The samples of sediment were collected using soil cylindrical sampler from the intertidal zone near the Shazikou wharf in Qingdao, Shandong Province. Three sampling sites (biological triplicates) along the sandy beach were selected. For each site, different depth (0–10, 10–20, 20–30, 30–40, 40–50 and 50–60 cm, namely S_{0-10}, S_{10-20}, S_{20-30}, S_{30-40}, S_{40-50}, S_{50-60}, respectively) were sampled and sediments in the same depth were mixed. Totally six sediment samples were obtained. Each sample was divided into two subsamples: one, stored at 4°C, was used for the physicochemical properties analysis; another, frozen at -80°C, was applied to molecular analysis. The pH and oxidation-reduction potential (ORP) were both measured in situ. The moisture content, inorganic nitrogen contents (including total nitrogen (TN), total inorganic nitrogen (TIN), ammonium (NH_4^+-N), nitrite (NO_2^--N) and nitrate (NO_3^--N)) were measured as described previously (Liu et al., 2013).

DNA extraction and quantitative PCR of amoA genes

We followed the manufacturer's instructions of a Power Soil DNA kit (Mo Bio Laboratories, Carlsbad, California, USA) to extract the DNA using 0.25g soil. The extracted DNA quality was assessed on 1% agarose gel using a nanometer droplet spectrophotometer (ND-1000; Isogen Life Science, Netherlands) to measure the concentration of DNA.

The copy numbers of AOA/AOB *amoA* genes were determined by the two primer sets (amoA-1F/amoA2-R for AOB *amoA* genes and CamoA-19f/CamoA-616r for AOA *amoA* genes). According to the previously described embodiment, the use of real-time iCycleriQ5 thermal cycler and detection system (CA Bio-Rad) for qPCR. (Hu et al., 2014).

High-throughput sequencing and bioinformatic analysisfor the amoA genes

The functional genes (*amoA* genes) of AOB and AOA were amplified using the two primer sets as mentioned above. The protocols used to amplify the two functional genes have been described previously (Shen et al., 2008; Pester et al., 2012). Illumina MiSeq sequencing, carried out by Personalbio (Shanghai, China) was applied to ensure the good coverage of each clone libraries. Bar code oligonucleotides were connected to the ends of two pairs of primers to distinguish *amoA* amplicons of different samples. For each sample, triplicate PCR products were obtained and mixed together. The PCR products length was determined by electrophoresis in a 1.5% agarose gel. Bioinformatic analysis was performed with the Mothur software package (Schloss et al., 2009). After screening, trimming and chimeras checking procedures, the high-quality reads were left for further analysis. Both AOA and AOB *amoA* genes were grouped into OTUs using a 85% similarity as a cut off as previously recommended

(Pester et al., 2012). The diversity indices of ACE, Chao, Shannon and Simpson for each OTU were calculated.

Statistical analysis

BLASTN search was used to examine the representative sequences for all the OTUs of AOA and AOB. Non-AOA and AOB *amoA* gene sequences and OTU were deleted. Pearson correlation analysis at significance level of 0.05 was used to determine the correlation between the AOA/AOB diversities and abundances and environmental factors. The representative sequences of all the OTUs for both AOA and AOB and were imported into MEGA6 to construct alignment files in combination with the sequences of known sequences of AOA and AOB. Phylogenetic analysis of AOA and AOB were performed using MEGA6 software with the neighbor-joining and maximum parsimony methods (Shen et al., 2014).

Results And Discussion

sediment samples physicochemical properties

Determination of physical and chemical properties of sediment samples, including pH, oxidation-reduction potential (ORP), moisture content, ammonium content, nitrite content, nitrate content, total inorganic nitrogen and total nitrogen content (TN) are presented in Table 1. All of the sediments had acidic pH values (i.e., 5.45-6.56). The pH value of the upper layer sediment samples (S_{0-10}, S_{10-20}) were relatively lower than the middle (S_{20-30}, S_{30-40}) and deep layer (S_{40-50}, S_{50-60}), which probably resulted from the higher nitrification rates in the upper layer sediments. The ORP of the upper layer sediments were much higher than the rest of the samples. For the moisture, the middle layer was the highest. The ammonium content of the six sediment samples ranged from 1.32 to 6.21 mg/kg and the peak was found in the sample of S_{30-40}. All the sediment samples had low nitrite content as expected. The nitrate content ranged from 4.99 to 16.89 mg/kg, and the nitrate content in the deep layer were higher than that of the upper layer. As for the TN, the content ranged from 857.77 to 1604.05 mg/kg, and the TN content increased as the depth went deeper.

Table 1
The physiochemical properties of the sediment samples

Sample names	pH	ORP (mV)	Moisture (%)	NH_4^+-N (mg/kg)	NO_2^--N (mg/kg)	NO_3^--N (mg/kg)	TIN (mg/kg)	TN (mg/kg)
S_{0-10}	5.45	149.07	25.12	1.32	0.18	10.75	12.25	857.77
S_{10-20}	5.91	105.88	35.23	1.30	0.18	4.99	6.47	1243.61
S_{20-30}	6.01	76.86	41.44	1.55	0.48	12.73	14.76	1279.37
S_{30-40}	6.56	75.10	44.02	6.21	0.79	16.89	23.90	1521.07
S_{40-50}	6.23	83.17	39.50	3.77	0.67	16.21	20.65	1269.46
S_{50-60}	6.29	89.39	39.56	4.98	0.51	11.61	17.11	1604.05

Abundance of AOA and AOB

In this study, the functional genes (*amoA* gene) of AOA and AOB in six sediment samples were quantitatively analyzed . The number of AOA *amoA* genes of these sediment samples ranged from1.26×10^7 to 2.82×10^8 and the number of AOB *amoA* genes of the sediment samples ranged from 3.33×10^5 to 3.22×10^8 (Table S1). The number of AOA and AOB *amoA* genes observed in this work was similar to previous study in the intertidal zones in Zhoushan, Zhejiang province (Hu et al., 2019). Hu found that the AOA and AOB *amoA* gene copy numbers in the intertidal zone were both ranging from 10^7 to 10^8. The AOB *amoA* gene copy numbers in this study was relatively lower since samples S_{40-50} and S_{50-60} were collected in the deep layers of the sediments. In the intertidal zones in Zhoushan, only surface sediments were collected for analysis. The AOA *amoA* gene copy numbers in the upper layers (S_{0-10}, S_{10-20}) were much lower than that in the middle (S_{20-30}, S_{30-40}) and deep (S_{40-50}, S_{50-60}) layer (Fig. 1). On the contrary to AOA *amoA* genes, the number of AOB *amoA* genes in the upper layers (S_{0-10}, S_{10-20}) were much higher than that in the middle (S_{20-30}, S_{30-40}) and deep (S_{40-50}, S_{50-60}) layer (Fig. 1). The ratio of AOA/AOB *amoA* gene copy numbers ranged from 0.04 to 150.75 in the 6 sediment samples and the ratio increased dramatically from upper layers to deep layers. The ratios of AOA/AOB *amoA* genes in the 6 sediment samples were 0.04 (S_{0-10}), 0.11 (S_{10-20}), 8.20 (S_{20-30}), 7.07(S_{30-40}), 86.77 (S_{40-50}) and 150.75 (S_{50-60}), respectively (Table S1). The ratios of AOA/AOB *amoA* genes increased as the sediments depth went deeper, which indicated that AOA may dominated the ammonia oxidation in the deep layer. This founding was in accordance with the previous work focusing on the ammonia-oxidizing microbes in soils (Leininger et al., 2006). Leininger reported that the ratios of AOA/AOB *amoA* genes increased from 55 to 842 with soil depth went deeper. This phenomenoncould be explained by the higher affinities of AOA for oxygen compared to AOB (Martens-Habbena et al. 2009; Junget al. 2011; Kim et al. 2012). In the upper layers (S_{0-10}, S_{10-20}) where the oxygen was sufficient, AOB won the competition with AOA and may be the

main driver of ammonia oxidation. However, in the middle (S_{20-30}, S_{30-40}) and deep layer (S_{40-50}, S_{50-60}) where the environment became anoxic, the higher affinities of AOA for oxygen could help them to win the competition with AOB. AOA outnumbered AOB in oxygen-limited environment was also previously mentionedin Zhoushan intertidal zones. In the middle tidal zone where the oxygen was relatively sufficient, the number of AOB *amoA* genes was higher than that of AOA. In the subtidal zones where oxygen was limited, AOA outnumbered AOB (Hu et al., 2019). From the point of abundance, oxygen concentration indeed led to the niche specialization of AOA and AOB.

Diversity of AOA and AOB

High-quality AOA and AOB sequences after quality control procedures were applied to diversity analysis. Aa for AOA, a total of 17144 high-quality sequences were obtained for the 6 sediment samples. Using the 15% cut-off, which was recommended previously (Pester et al., 2012), a total of 18 OTUs were obtained for the *amoA* gene of archaeal. The coverages of the 6 AOA *amoA* gene clone libraries were all higher than 99.0%, ranging from 99.80% to 99.96% (Table S2). The AOA OTU numbers of the 6 sediment samples ranged from 10 to 15, with S_{30-40} (10 OTUs) and S_{50-60} (15 OTUs) displayed the lowest and highest diversity, respectively (Table S2). The OTU numbers in the deep layers were higher than that in the upper and middle layer (Fig. 2). Overall, the change rule of the AOA OTU numbers was similar to the AOA abundance, both of them increased with sediment depth. AOA showed higher diversity as oxygen concentration became lower, which was in accordance with previous work in water-level-fluctuating zones in Three Gorges Reservoir (Liu et al., 2015). At higher oxygen concentration of non-flooded areas, AOA OTU were lower value; In the relatively low oxygen concentration in flooded areas, AOA OTU values higher. This indicated that AOA was more adaptive to the oxygen-limited environments. A total of18865 high-quality AOA sequences were obtained form 6 sediment samples. Using 15% as cutoff value (Purkhold et al., 2000), 18865 sequences were assigned to 11 OTUs. The coverages of the 6 AOB *amoA* gene clone libraries were ranging from 99.96% to 100.00% (Table S3). The sample S_{0-10} showed the highest diversity, holding 11 OTUs in total, which was higher than the rest 5 sediment samples (Fig. 2). The OTU numbers in the rest of the 5 samples were all the same, holding 8 OTUs (Table S3). Similar to the change rules of abundance, the AOB OTU numbers decreased as sediment depth went deeper, which indicated that AOB was not tolerant or adaptable to the hypoxic environments. The research in the area of water-level-fluctuating zones in Three Gorges Reservoir also showed the similar pattern. The AOB OTU numbers were always higher in the non-flooded zones where the oxygen concentration was sufficient than that in the flooded zones where the environment was anoxic. The diversity of AOA was always higher than AOB in all the sediment samples (Fig. 2) and the ratio of AOA/AOB OTU numbers increased from 1.09 to 1.88 as the sediment depth went deeper, which was similar to the change pattern of the abundance.

Phylogenetic analysis and community structure of AOA and AOB

After all the quality control procedures, for AOA, totally 17144 high-quality sequences were obtained for the six sediment samples. According to the *amoA* genes classification of Archaea (Pester et al., 2012), the representative sequences of the 18 OTUs were grouped into four different clusters as shown in Fig. 3. Neither *Nitrosotalea* nor *Thermal-related* AOA was found in all the sediment samples. *Nitrosophaera* cluster contained 3 OTUs (OTU 12, OTU15, OTU 18), totally 9 sequences. OTU 12(containing 3 seqs) belonging to *Nitrosophaera* cluster was retrieved from sample S_{20-30} and S_{40-50}, OTU 15 (containing 2 seqs)was detected in sample S_{0-10} and S_{50-60}, and OTU 18 (containing 4 seqs) was only retrieved from deep layer samples (S_{40-50}, S_{50-60}). The rest of the 15 OTUs were all affiliated with *Nitrosopumilus* cluster. Among the 17144 sequences, 17135 sequences were affiliated with *Nitrosopumilus* cluster, accounted for 99.94% of all the sequences obtained. Allthe six sediment samples were composed dominantly by sequences belonging to *Nitrosopumilus* cluster. The dominance of the *Nitrosopumilus* cluster over the *Nitrosophaera* clusterin this study was similar to previous studies in Chongming eastern intertidal sediments and in the intertidal zones in Zhoushan Island (Zheng et al., 2013; Hu et al., 2019). Phylogeny and meta-data analyses of archaeal *amoA* sequences also showed that the most ofAOA belong to the *Nitrosopumilus* cluster (73%), which was nearly twice as much as the *Nitrososphaera* cluster (37%) (Alves et al., 2018). The failure of the *Nitrososphaera* cluster was more likely to form due to their inability to adapt to salty conditions or the fluctuation of intertidal environmental factors. AOB obtained 18865 sequences from 6 sediment samples by the same quality control procedure as AOA Using the 15% cut-off that was recommended in a previous study (Purkhold et al., 2000), 18865 sequences were assigned to 11 OTUs. The representative sequences of the 11 OTUs were grouped into two clusters, with 2 OTUs (OTU 9, OTU 10) clustered into the *Nitrosomonas* cluster and the remaining 9 OTUs clustered into the *Nitrosospira* cluster (Fig. 4). Only 35 sequences were affiliated with the *Nitrosomonas* cluster. All the six AOB communities were primarily composed of *Nitrosospira*-related sequences. The dominance of the *Nitrosospira* cluster over the *Nitrosomonas* cluster in this study was similar to previous studies in the coastal Pearl River estuary (Cao et al., 2011). The lower concentration of ammonia nitrogen (0.03 ~ 0.11 mm NH4 +) in the intertidal zone of Qingdao may help to explain the absolute dominance of nitrosoma and nitrosoma. The *Nitrosomonas* cluster has a lower affinity for the substrate and adapts to higher substrate concentrations. In contrast, the *Nitrosospira* cluster have relatively higher affinity with the substrate and are more likely to survive environments with low substrate concentrations (Zheng et al., 2014; Yu et al., 2016).

Environmental factors that influence the diversity and communities of AOA and AOB

Pearson correlation coefficient was used to analyze the linear relationship between different environmental factors and amoA gene abundance, OTU number, AOA, AOB diversity index, as shown in Table 2.. ORP was significantly positively correlated with AOB abundance and AOB OTU numbers ($P < 0.01$), which mean that AOB preferred the niches with higher oxygen concentrations. On the contrary, ORP was negatively correlated with AOA abundance, which mean that AOA preferred the niches with lower

oxygen concentrations. All this could be explained by the higher affinity of AOA to oxygen compared to AOB. MC was significantly negatively correlated with AOB abundance ($P < 0.01$) and AOB OTU numbers ($P < 0.05$). TN was also found to be significantly negatively correlated with AOB abundance ($P < 0.01$) and AOB OTU numbers ($P < 0.05$). The pH value was negatively correlated with the abundance of AOB ($P < 0.05$).

Table 2
Correlation analysis of environmental factors and AOA, AOB abundance, OTU numbers and diversity index

Environmental factors	Pearson correlation coefficient				
	Relative abundance		Ratio of abundance	Number of OTUs	
	AOA	AOB	AOA:AOB	AOA	AOB
pH	0.300	**-0.020***	0.423	-0.919	-0.055
ORP	-0.184	**0.002****	-0.576	0.976	**0.001****
MC	0.250	**-0.004****	0.605	-0.871	**-0.014***
NH_4^+-N	0.503	-0.178	0.320	-0.887	-0.392
NO_2^--N	0.090	-0.051	0.519	-0.727	-0.242
NO_3^--N	0.083	-0.273	0.676	-0.600	-0.755
TIN	0.142	-0.187	0.516	-0.670	-0.583
TN	0.674	**-0.029***	0.252	0.760	**-0.045***
Significance levels are indicated as follows: **, 0.01; *, 0.05.					

Conclusion

In summary, our results showed the change pattern of the abundance and diversity of AOA and AOB with sediment depth. In the upper layers, the abundance of AOBwas higher than that of AOA, and in the deep layers, AOA outnumbered AOB. Both the ratio of AOA/AOB *amoA* gene copy numbers and AOA/AOB OTU numbers increased as sediment depth went deeper, which indicated that oxygen indeed led to the niche specialization of AOA and AOB and AOA won the competition with AOB in the oxygen-limited niches. AOA may dominant the ammonia oxidation process in the deep layers. The *Nitrosopumilus* and *Nitrosospira* clusters were the absolute dominant AOAand AOB, respectively, indicating an ecological success in the intertidal zone.

Declarations

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Author's contributions

All the authors collaborated for the completion of this work. DP designed and accomplished the first draft. SW, CZ and ZL were involved in the initial writing and editing of the manuscript. DZ provided valuable insights and suggestions for this article.

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Availability of data and materials

Not applicable.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All listed authors consented to the submission of this manuscript for publication.

Competing interests

The authors declare no competing interest.

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Figures

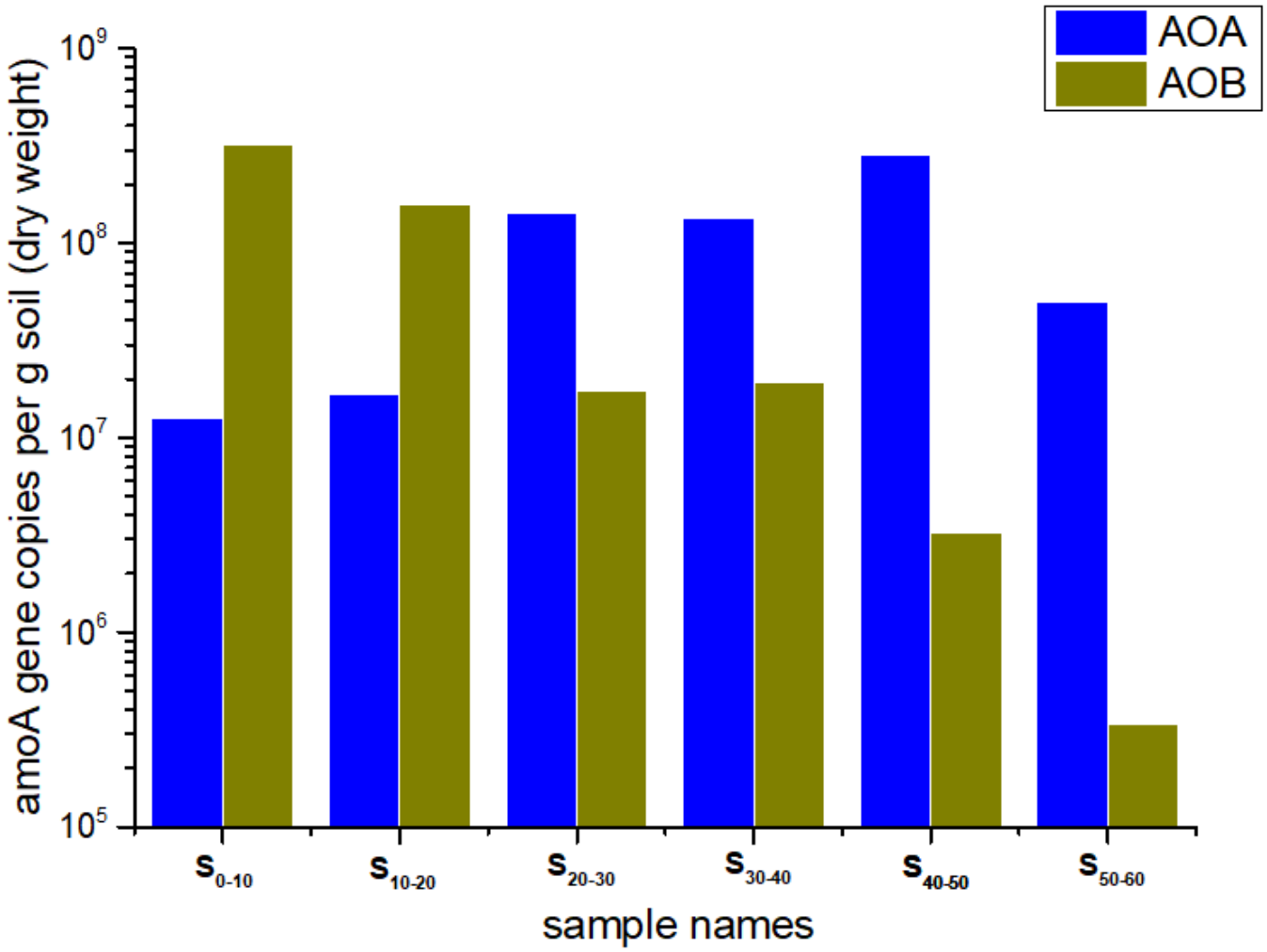

Figure 1

Quantitative analysis of AOA and AOB in the six sediment samples

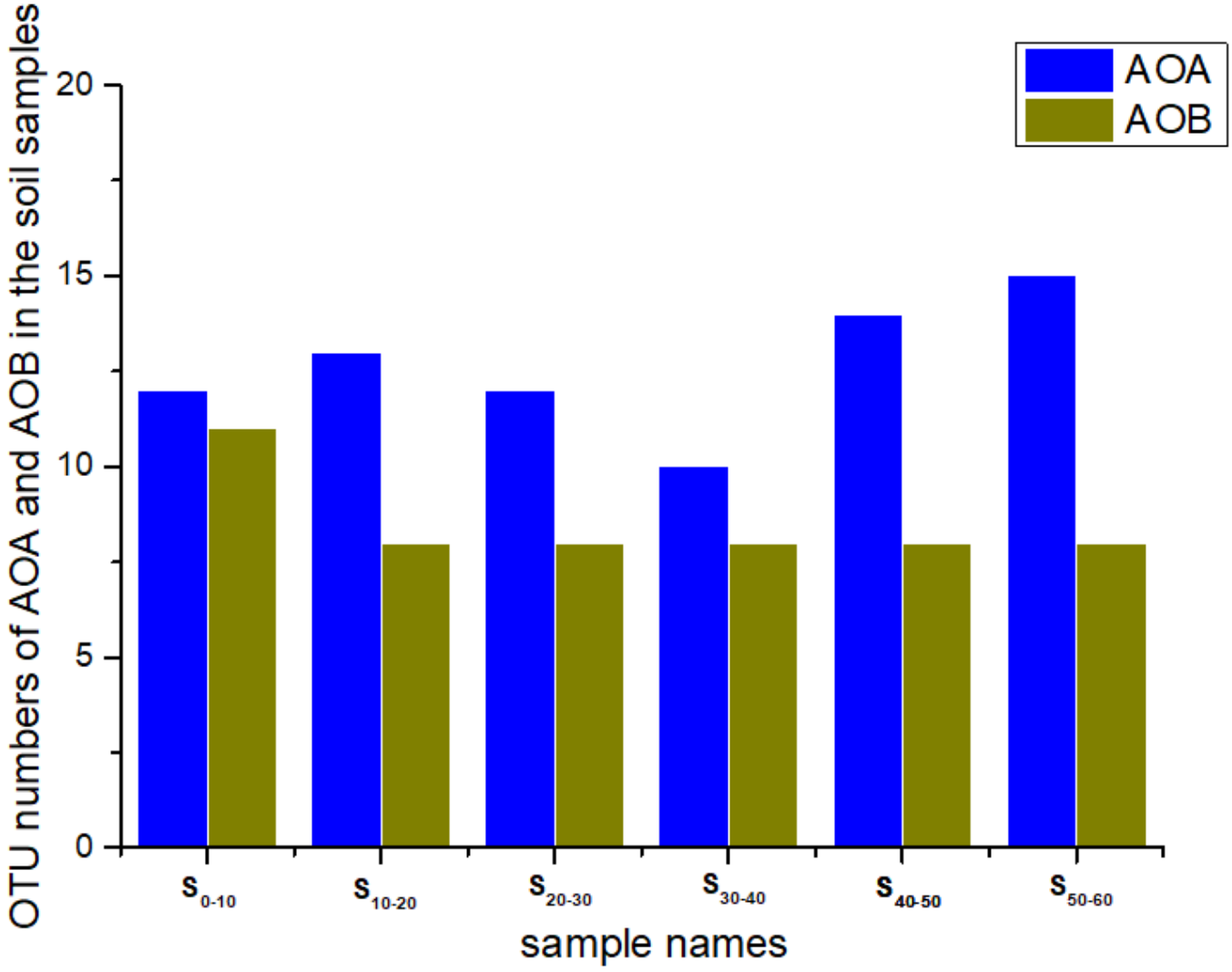

Figure 2

The OTU numbers of AOA and AOB in the six sediment samples

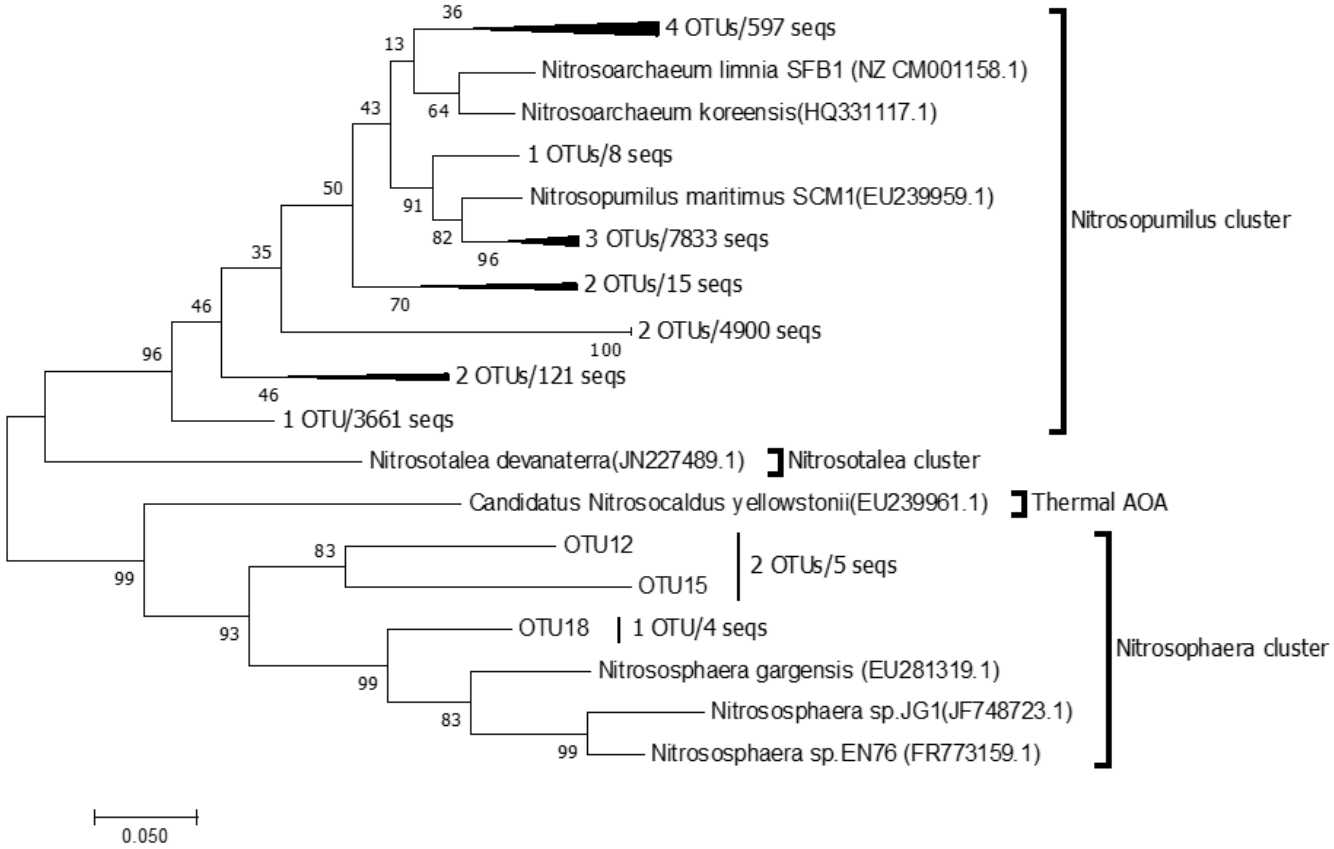

Figure 3

Maximum-Likehood phylogenetic tree showing the phylogenetic affiliations of the AOA sequences recovered from the sediment samples. The numbers at the nodes are percentages that indicate thelevels of bootstrap support from 2000 replicates. The scale barrepresents 0.05 nucleic acid substitutions per nucleotide position.

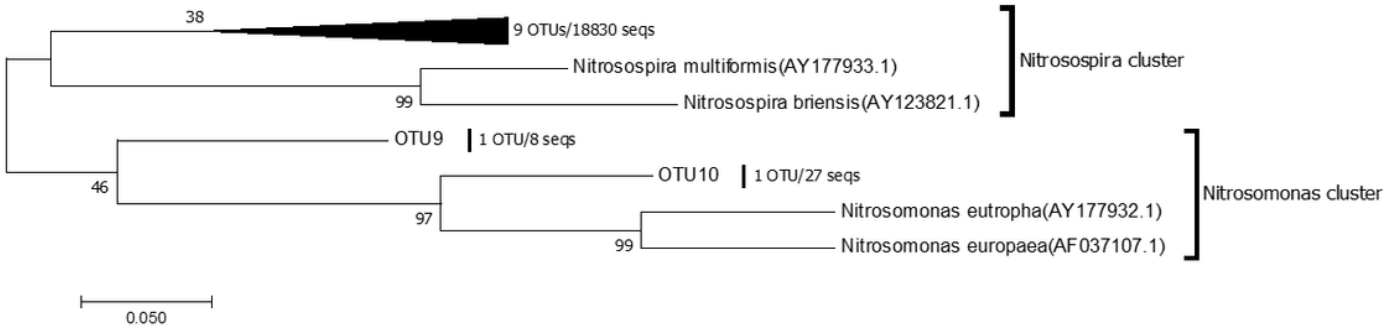

Figure 4

Maximum-Likehood phylogenetic tree showing the phylogenetic affiliations of the AOB sequences recovered from the sediment samples. The numbers at the nodes are percentages that indicate thelevels

of bootstrap support from 2000 replicates. The scale barrepresents 0.05 nucleic acid substitutions per nucleotide position.

Supplementary Files

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