Ammonia-oxidizing archaea and ammonia-oxidizing bacteria communities respond differently in oxy-gen-limited habitats

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Ammonia-oxidizing archaea (AOA) and bacteria (AOB) are the most important ammonia oxidation functional community, while the coastal environment just provides a different oxygen environment for the ammonia oxidation process. However, few surveys concentrated on the influence of oxygen concentration on the niche specialization of AOA and AOB in the ocean intertidal zones. 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, China. Results showed that the AOA/AOB *amoA* gene copy numbers and AOA/AOB OTU numbers rate increased as sediment depth went more profound, which indicated that AOA was more adaptive to oxygen-limited niches compared to AOB. Oxygen indeed led to the niche specialization of AOA and AOB in intertidal sediments. The dominant AOA and AOB were the clusters of *Nitrosopumilus* and *Nitrosospira*, respectively, which indicated ecological success in the intertidal zone. A significant and positive correlation ($p < 0.01$) between AOB abundance/AOB OTU numbers and Oxidation-reduction potential (ORP) was observed. In addition, both total nitrogen (TN) ($p < 0.01$) and pH ($p < 0.05$) were significantly negatively correlated to AOB abundance. TN was also significantly negatively correlated to AOB OTU numbers ($p < 0.05$). Hence, oxygen led to niche specialization of AOA and AOB, especially under anoxic conditions, AOA played a dominant role in the process of ammonia oxidation. The *Nitrosopumilus* and *Nitrosospira* clusters were the dominant AOA and AOB, respectively, representing an ecological success in the intertidal zone.

KEYWORDS

AOA, AOB, niche specialization, intertidal zone, high-throughput sequencing

Introduction

Nitrification, a vital role in the ecosystem nitrogen cycle, converts ammonia to nitrate through nitrite (Beeckman et al., 2018). Three kinds of microorganisms of ammonia oxidizers, nitrite oxidizers, and complete ammonia oxidizers were involved in the nitrification process (Stein and Klotz, 2016). Ammonia-oxidizing bacteria (AOB) were long thought to be the only microorganism that performed ammonia oxidation, the first rate-limiting step in nitrification. However, the ammonia oxidation theory was changed after discovering 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* (Lehtovirta-Morley 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 crucial role in the niche differentiation between AOA and AOB. Moreover, 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 series 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 dominate the process of ammonia oxidation in acidic niches (Gubry-Rangin et al., 2011; Prosser and Nicol, 2012). AOB was more adaptable than AOA with increasing abundance but no composition alteration at elevated temperature (Zhang et al., 2019). Microcosm test also verified that pH and temperature were vital factors that led to the niche specialization of AOA and AOB (Aigle et al., 2020). Ammonia, as one of the substrates for ammonia oxidation, could cause niche specialization of AOA and AOB. The concentration of ammonia half-saturation constant of some AOB strains (Martens-Habbena et al., 2009; Jung et al., 2011; Kim et al., 2012) was much higher than AOA strains, indicated that AOA had a 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 , 2.15 μM, and 133 nM, respectively, which were much lower than that of *Nitrosomonas europaea* (K_m = 553 μM) (Martens-Habbena et al., 2009). Therefore, AOA is more competitive than AOB in the oligotrophic environment (Beman et al., 2008; Verhamme et al., 2011). In other substrates 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 *Nitrosoarchaeu mkoreensis MY1*, Candidatus *Nitrososphaera* sp. *JG1* and *N. maritimus SCM1* were 10.38, 4.67, and 3.90 μM, separately (Martens-Habbena et al., 2009; Jung et al., 2011; Kim et al., 2012), which were much lower than that of *Nitrosomonas oligotropha NL7* (76.3 μM) and *N. europaea C-31* (183.3 μM) (Park 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 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 oxygen minimum zone (Stewart et al., 2012). The increasing AOA OTU number and abundance ratio (AOA: AOB) in flooded soil proved that AOA could better adapt to low oxygen conditions (Liu et al., 2015).

The intertidal zone is an ecological crisscross zone with significant environmental and ecological functions affected by marine and terrestrial ecosystems (Community structure and organization of tidepools). The daily tidal rhythm made the sediment is submerged or exposed to air. As one of the biggest wharves in Qingdao, the Shazikou wharf is significantly affected by human activities and the decayed fish, which provided enough ammonia nitrogen for the ammonia-oxidizing microbes in nearby intertidal zones. The ecological distribution, including the community structure and AOA/AOB abundance in intertidal zones, have been discussed previously (Bernhard et al., 2005; Zhang et al., 2015; Hu et al., 2019). However, the relationships between oxygen concentration and niche specialization of AOA and AOB have not been investigated, especially, the ecological distribution of AOA and AOB under oxy-gen-limited habitats under different layers/depth of the sediments. 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 influences the niche specialization of AOA and AOB in intertidal zones.

Materials and methods

Description of sampling sites

The sampling sites were distributed in Shazikou wharf coast (36°6′N, 120°33′E). The Shazikou wharf was built in 1976 and was one of the biggest wharves in Qingdao, China. The region is located in the north temperate monsoon region, with a temperate monsoon climate. Regulated by the marine environment, it also

has significant marine climate characteristics. The mean annual temperature is 12.6℃, and the mean annual rainfall is 734.3 mm. The sunshine duration is 2281.4 h, with a frost-free period of 179 days. Coastal lowlands or coastal beaches are typical saline soils.

Collection and physicochemical properties analysis of sediment

The sediment samples were collected using a cylindrical soil sample from the ocean 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 depths (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 the sediments in the same depth were mixed. Totally six sediment samples were obtained. Each sample was divided into two subsamples: one, stored at 4℃, was used to analyze physicochemical properties; another, frozen at −80℃, was applied to molecular analysis. The soil pH was determined at a soil: water ratio of 1:5 with a conductivity meter (FE38-FiveEasyPlus™, Mettler-Toledo, Zurich, Switzerland). The oxidation-reduction potential (ORP) was measured by an ORP meter (ORP-2096, Boqu, China). The content of moisture content (MC) was measured at the same time with soil samples collecting, and the method of oven-drying at 105℃ for 10 h. Total nitrogen (TN) was measured by Kjeldahl method. The ammonium (NH_4^+-N) and nitrate (NO_3^--N) were leached with KCl solution (1 mol L^{-1}), and the filtrate was taken and measured by a continuous flow analyzer (Seal-AA3, Germany). The nitrite (NO_2^--N) was determined by ultraviolet spectrophotometry (Liu et al., 2013).

DNA extraction and quantitative PCR of amoA genes

Total genomic DNA was extracted from the fresh soil samples by employing the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, United States) as the protocols of manufacturer. The final DNA concentration of extracts was determined using a NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, United States). 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.

For the quantification of genes (AOB/AOA amoA) copy numbers, the Abi7300 fluorescent quantitative PCR instrument (Applied Biosystems, United States) was applied to perform real-time PCR. Specific primers were used to amplify target genes. The forward primer sequence of AOA-amoA is STAATGGTCTGGCTTAGACG (5′–3′), and the reverse primer is GCGGCCATCCATCTGTATGT (5′–3′). The forward primer sequence of AOB-amoA is GGGGTTTCTACTGGTGGT (5′–3′), and the reverse primer is CCCCTCKGS AAAGCCTTCTTC (5′–3′) (Park et al., 2008). The reaction system (Supplementary Table S1) and reaction conditions (Supplementary Table S2) are shown in the supporting materials. The constructed plasmid was identified by sequencing. Then the plasmid value was measured at 260 nm by UV spectrophotometer (NanoDrop2000, Thermo Fisher Scientific, United States), and converted into gene copies (copies g^{-1} dry soil) by formula. The standard curve was prepared by 10-fold gradient dilution of the constructed plasmids (10^{-1}–10^{-7} for AOB amoA, 10^{-2}–10^{-8} for AOA amoA) (Hu et al., 2019).

High-throughput sequencing and bioinformatic analysis for the amoA genes

The AOB and AOA amoA genes were amplified using the two primer sets mentioned above, and the protocols used to amplify have been previously reported (Shen et al., 2008; Pester et al., 2012). Illumina MiSeq sequencing, carried out by Personal (Shanghai, China), was applied to ensure the excellent coverage of each clone library. 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 then mixed. PCR products length was subsequently determined by electrophoresis in a 1.5% agarose gel. Bioinformatic analysis was conducted with the Mothur software package (Schloss et al., 2009). After the 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 an 85% similarity as a previously recommended cut-off value (Pester et al., 2012). The obtained sequences were submitted to the RDP pipeline using the FrameBot tool to remove non-amoA sequences (Wang et al., 2013). The OTUs were taxonomically categorized using neighbor-joining phylogenetic trees constructed from representative sequences with the longest length of the amoA genes. AOA amoA representatives were matched with an existing high-quality amoA database (Pester et al., 2012), while AOB amoA representatives were matched with GeneBank reference sequences. The taxonomy-derived reference sequences were combined with the Kimura 2-parameter distance (MEGA 6.0), and the bootstrap values were calculated using 1,000 repetitions (Kumar et al., 2016). In addition, the diversity indices of ACE, Chao1, Shannon, and Simpson for each OTU were also calculated.

TABLE 1 The physiochemical properties of the sediment samples (mean $\pm$ *SE*, *n* = 3).

Sample names	pH	ORP (mV)	MC (%)	NH$_4^+$-N (mg/kg)	NO$_2^-$-N (mg/kg)	NO$_3^-$-N (mg/kg)	TIN (mg/kg)	TN (g/kg)
S$_{0-10}$	5.45 ± 0.09d	149.07 ± 3.13a	25.12 ± 0.41d	1.32 ± 0.09d	0.18 ± 0.01c	10.75 ± 0.17d	12.25 ± 0.49e	0.86 ± 0.01c
S$_{10-20}$	5.91 ± 0.13c	105.88 ± 2.76b	35.23 ± 1.00c	1.30 ± 0.07d	0.18 ± 0.01c	4.99 ± 0.08e	6.47 ± 0.30f	1.24 ± 0.01b
S$_{20-30}$	6.01 ± 0.07c	76.86 ± 1.71d	41.44 ± 1.01ab	1.55 ± 0.10d	0.48 ± 0.03b	12.73 ± 0.15b	14.76 ± 0.77d	1.28 ± 0.02b
S$_{30-40}$	6.56 ± 0.12a	75.10 ± 1.89d	44.02 ± 1.12a	6.21 ± 0.22a	0.79 ± 0.05a	16.89 ± 0.41a	23.90 ± 1.02a	1.52 ± 0.02a
S$_{40-50}$	6.23 ± 0.10bc	83.17 ± 2.31c	39.50 ± 0.89b	3.77 ± 0.11c	0.67 ± 0.03a	16.21 ± 0.22a	20.65 ± 0.84b	1.27 ± 0.02b
S$_{50-60}$	6.29 ± 0.09ab	89.39 ± 2.18c	39.56 ± 0.65b	4.98 ± 0.14b	0.51 ± 0.02b	11.61 ± 0.11c	17.11 ± 0.75c	1.60 ± 0.03a

Note: Different letters in a single column indicate a significant difference between the treatments at $p < 0.05$. S$_{0-10}$, sediment sampled from 0 to 10 cm layer; S$_{10-20}$, sediment sampled from 10 to 20 cm layer; S$_{20-30}$, sediment sampled from 20 to 30 cm layer; S$_{30-40}$, sediment sampled from 30 to 40 cm layer; S$_{40-50}$, sediment sampled from 40 to 50 cm layer; S$_{50-60}$, sediment sampled from 50 to 60 cm layer. ORP, oxidation-reduction potential; MC, moisture content; NH$_4^+$-N, ammonium nitrogen; NO$_2^-$-N, nitrous nitrogen; NO$_3^-$-N, nitrate nitrogen; TIN, total inorganic nitrogen; TN, total nitrogen.

Statistical analysis

Pearson correlation analysis with a 0.05 significance level was used to determine the correlation between the AOA/AOB diversities or abundances and environmental factors. In addition, the representative sequences with the longest length were selected for the following phylogenetic analyses, which were conducted with MEGA 6 through a neighbor-joining tree using Kimura 2-parameter distance with 1,000 bootstrap replicates (Tamura et al., 2013). Redundancy analysis (RDA) was performed to investigate the relationship between AOA/AOB microbial community structure and the environmental factors with CANOCO 5.0 (CANOCO, Microcomputer Power Inc., Ithaca, NY, United States).

Results

Physicochemical properties of sediment samples

Determination of physicochemical properties of sediment samples are showed in Table 1. All the sediments had acidic pH (i.e., 5.45–6.56). The pH value of the upper layer sediment samples (S$_{0-10}$) was relatively lower than the other five layers. Middle and deep layers (S$_{30-40}$, S$_{40-50}$, and S$_{50-60}$) had higher pH values, and S$_{30-40}$ showed the highest pH of 6.56, which probably resulted from the higher nitrification rates in the upper layer sediments. However, the ORP of the upper layer sediments was much higher than the rest of the samples by 40.79%–98.50%. The moisture varied from 25.12% to 44.02%, and the middle layer (S$_{20-30}$, S$_{30-40}$) was the highest of other layer sediments. The NH$_4^+$-N content of the six sediment samples ranged from 1.32 to 6.21 mg kg^{-1}, and the peak was found in the sample of S$_{30-40}$, which was significantly higher than the other five sediment samples by 24.70%–377.70%. All the sediment samples had low NO$_2^-$-N content, and the upper layer sediment samples (S$_{0-10}$, S$_{20-30}$) were the lowest. The NO$_3^-$-N content ranged from 4.99 to 16.89 mg/kg, and the NO$_3^-$-N content in S$_{30-40}$ and S$_{40-50}$ layers was higher than that of the other layers. The TIN of middle and deep layers was higher than the upper layers, and S$_{30-40}$ was higher than the rest samples by 15.74%–269.40%. As for the TN, the content ranged from 857.77 to 1604.05 mg kg^{-1}, and the TN content increased as the depth went deeper.

Abundance of ammonia-oxidizing archaea and ammonia-oxidizing bacteria

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 (Figure 1). On the contrary to AOA *amoA* genes, the number of AOB *amoA* genes in the upper layers (S$_{0-10}$, S$_{10-20}$) was much higher than that in the middle (S$_{20-30}$, S$_{30-40}$) and deep (S$_{40-50}$, S$_{50-60}$) layer (Figure 1). The ratio of AOA/AOB *amoA* gene copy numbers ranged from 0.04 to 150.75 in the six sediment samples, and the ratio increased dramatically from upper layers to deep layers. The ratios of AOA/AOB *amoA* genes in the six 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. In the upper layers (S$_{0-10}$, S$_{10-20}$), where the oxygen was sufficient, AOB won the competition with AOA and maybe 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. In the middle tidal zone, where the oxygen was relatively sufficient, the number of AOB *amoA* genes was higher than 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.
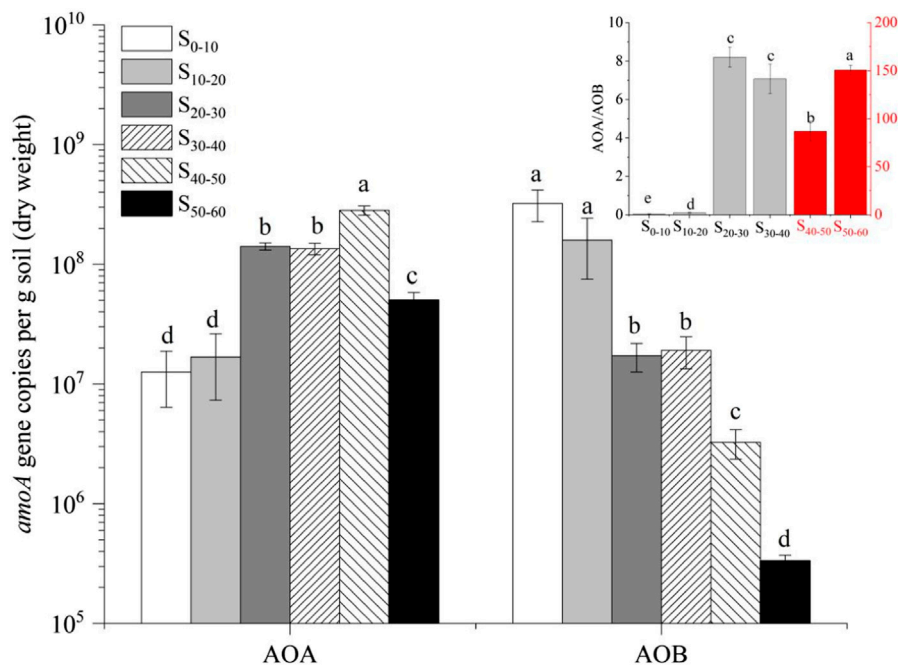
FIGURE 1
Quantitative analysis of AOA and AOB in the six sediment samples (means, *SE*s displayed with errors bars, *n* = 3). S_{0-10}, sediment sampled from 0 to 10 cm layer; S_{10-20}, sediment sampled from 10 to 20 cm layer; S_{20-30}, sediment sampled from 20 to 30 cm layer; S_{30-40}, sediment sampled from 30 to 40 cm layer; S_{40-50}, sediment sampled from 40 to 50 cm layer; S_{50-60}, sediment sampled from 50 to 60 cm layer. AOA, ammonia-oxidizing archaea; AOB, ammonia-oxidizing bacteria. In the figure in the upper right corner, four gray columns corresponded to the principal coordinate axis, and two red columns corresponding to the sub coordinate axis.

TABLE 2 The OTU numbers and the diversity index of AOA in the soil samples (mean $\pm$ *SE*, *n* = 3).

Sample names	Coverage (%)	Shannon	Simpson	Chao	Ace
S_{0-10}	99.91	1.03 ± 0.01d	0.49 ± 0.01a	17.88 ± 0.80c	19.28 ± 0.75c
S_{10-20}	99.94	1.39 ± 0.01b	0.32 ± 0.01b	18.36 ± 0.77c	19.68 ± 0.88c
S_{20-30}	99.90	1.38 ± 0.01b	0.33 ± 0.01b	22.87 ± 0.91b	24.41 ± 1.01b
S_{30-40}	99.96	1.28 ± 0.02c	0.34 ± 0.01b	15.08 ± 0.75c	15.78 ± 0.80d
S_{40-50}	99.85	1.46 ± 0.02a	0.32 ± 0.01b	25.20 ± 0.95b	23.93 ± 0.94b
S_{50-60}	99.80	1.38 ± 0.02b	0.36 ± 0.01b	30.74 ± 1.05a	29.28 ± 1.19a

Note: Different letters in a single column indicate a significant difference between the treatments at $p < 0.05$. S_{0-10}, sediment sampled from 0 to 10 cm layer; S_{10-20}, sediment sampled from 10 to 20 cm layer; S_{20-30}, sediment sampled from 20 to 30 cm layer; S_{30-40}, sediment sampled from 30 to 40 cm layer; S_{40-50}, sediment sampled from 40 to 50 cm layer; S_{50-60}, sediment sampled from 50 to 60 cm layer.

Diversity of ammonia-oxidizing archaea and ammonia-oxidizing bacteria

High-quality sequences of AOA and AOB, after quality control procedures, were applied to the analysis of diversity. In AOA, 17144 high-quality sequences were obtained for the six samples of sediment. The coverages of the six AOA *amoA* gene clone libraries were all higher than 99.0%, ranging from 99.80% to 99.96% among the 18 OTUs obtained from the

AOA *amoA* gene (Table 2). The AOA OTU numbers of the six 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 2). The OTU numbers in deep layers were higher than those in the upper and middle layers (Figure 2). The coverage of the six AOB *amoA* gene clone libraries was ranged from 99.96 to 100.00% (Table 3). The sample S_{0-10} showed the highest diversity, holding 11 OTUs in total, which was higher than the rest five

FIGURE 2
The OTU numbers of AOA and AOB in the six sediment samples (means, *SE* displayed with errors bars, *n* = 3). S_{0-10}, sediment sampled from 0 to 10 cm layer; S_{10-20}, sediment sampled from 10 to 20 cm layer; S_{20-30}, sediment sampled from 20 to 30 cm layer; S_{30-40}, sediment sampled from 30 to 40 cm layer; S_{40-50}, sediment sampled from 40 to 50 cm layer; S_{50-60}, sediment sampled from 50 to 60 cm layer. AOA, ammonia-oxidizing archaea; AOB, ammonia-oxidizing bacteria.

Phylogenetic analysis and community structure of ammonia-oxidizing archaea/ammonia-oxidizing bacteria

For AOA, 17144 high-quality sequences after quality control procedures were obtained for the six sediment samples. As showed in Figure 3, the representative sequences of the 18 OTUs, according to the *amoA* genes classification of AOA (Pester et al., 2012), have been grouped into four different clusters. Neither *Nitrosotalea* nor Thermal-related AOA was found in all the sediment samples. *Nitrosophaera* cluster contained 3 OTUs (OTU 12, OUT 15, OTU 18), a total of 9 sequences. OTU 12 (containing 3 seqs) belonging to *Nitrosophaera* cluster was retrieved from samples 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 all belonged to the *Nitrosopumilus* cluster. Among the 17144 sequences, 17135 accounted for 99.94% of all obtained sequences belonged to the *Nitrosopumilus* cluster. AOB obtained 18865 sequences from 6 sediment samples by the same quality control procedure as AOA, and then these sequences were assigned to 11 OTUs using the 15% cut-off recommended previously (Purkhold et al., 2000). The representative sequences of the 11 OTUs were subsequently grouped into two clusters, as showed in Figure 4. OTU 9 and OTU 10 were clustered into the *Nitrosomonas*, and the remaining 9 OTUs belonged to the *Nitrosospira* cluster. Only 35 sequences belonged to the cluster of *Nitrosomonas*. All six AOB communities were primarily composed of *Nitrosospira*-related sequences.

Environmental factors that influence the diversity and communities of ammonia-oxidizing archaea and ammonia-oxidizing bacteria

As shown in Table 4, the linear relationship between various environmental factors and OTU number, *amoA* gene abundance as

sediment samples. The OTU numbers in the rest of the five samples were all the same, holding 8 OTUs. 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 hypoxic environment. The AOB OTU numbers were higher in the non-flooded zones where the oxygen concentration was sufficient than in the flooded zones where the environment was anoxic. The diversity of AOA was always higher than AOB in all the sediment samples (Table 3), 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 changing pattern of the abundance.

TABLE 3 The OTU numbers and the diversity index of AOB in the soil samples (mean $\pm$ *SE*, *n* = 3).

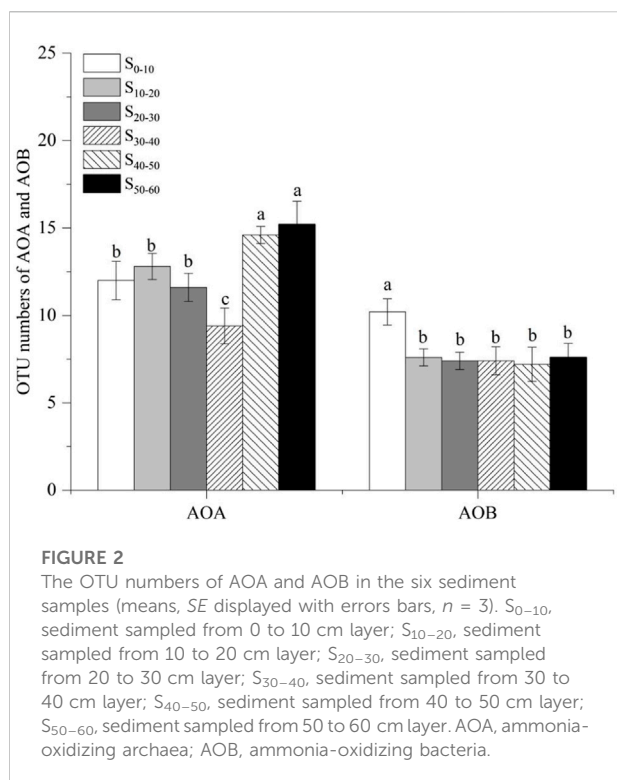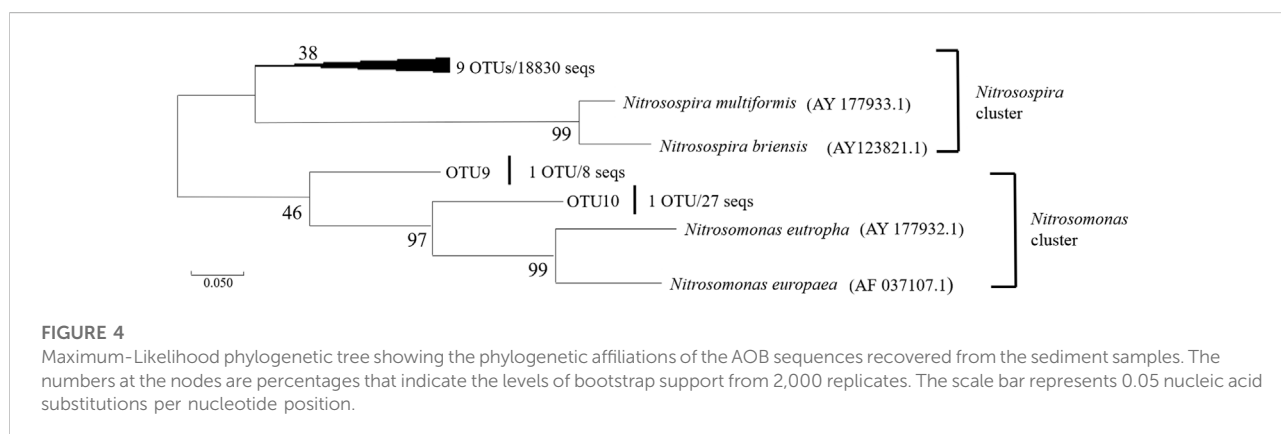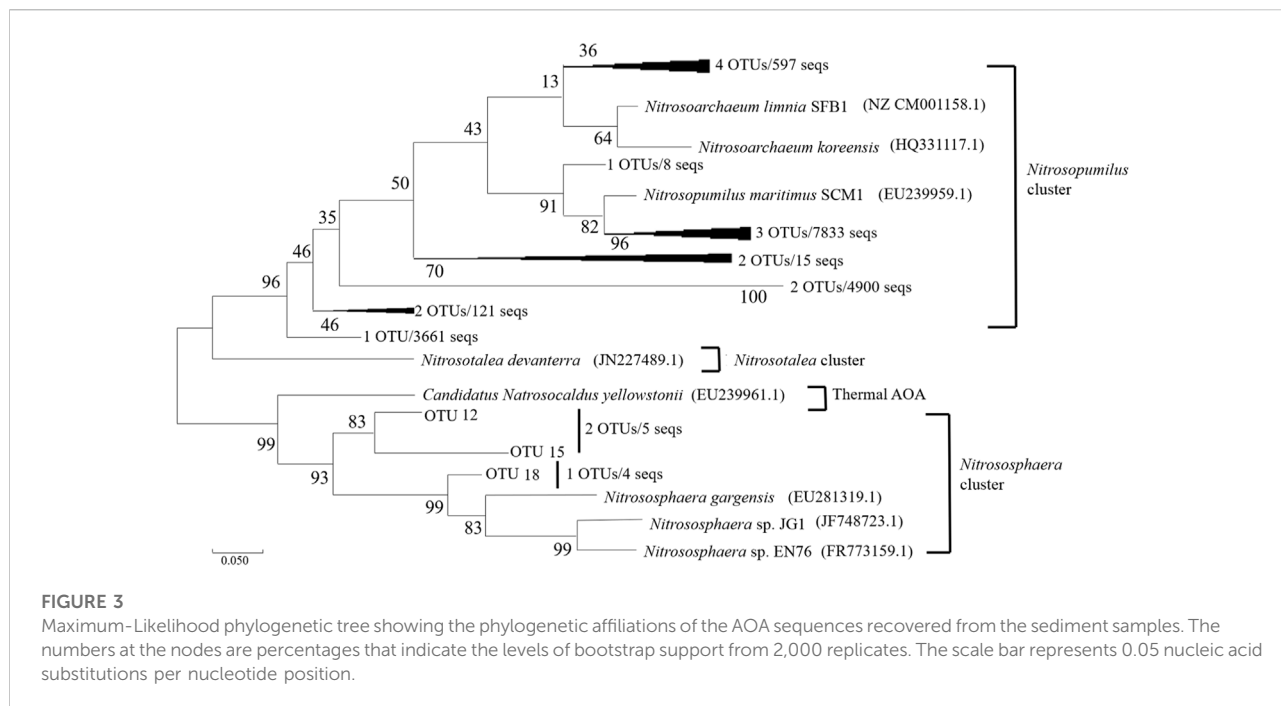| Sample names	Coverage (%)	Shannon	Simpson	Chao	Ace
S_{0-10}	99.98	1.16 ± 0.02ab	0.53 ± 0.01b	20.20 ± 1.00a	22.37 ± 0.52b
S_{10-20}	99.96	1.13 ± 0.01bc	0.55 ± 0.01b	15.30 ± 0.61c	16.57 ± 0.50c
S_{20-30}	99.97	0.94 ± 0.01c	0.63 ± 0.01a	16.79 ± 0.68bc	17.85 ± 0.45c
S_{30-40}	100.00	0.95 ± 0.01c	0.63 ± 0.01a	18.51 ± 0.89ab	24.93 ± 0.73a
S_{40-50}	100.00	1.16 ± 0.02ab	0.54 ± 0.01b	17.85 ± 0.57b	25.28 ± 0.91a
S_{50-60}	100.00	1.30 ± 0.02a	0.52 ± 0.01b	16.96 ± 0.43bc	17.94 ± 0.60c

Note: Different letters in a single column indicate a significant difference between the treatments at *p* < 0.05. S_{0-10}, sediment sampled from 0 to 10 cm layer; S_{10-20}, sediment sampled from 10 to 20 cm layer; S_{20-30}, sediment sampled from 20 to 30 cm layer; S_{30-40}, sediment sampled from 30 to 40 cm layer; S_{40-50}, sediment sampled from 40 to 50 cm layer; S_{50-60}, sediment sampled from 50 to 60 cm layer.

FIGURE 3
Maximum-Likelihood phylogenetic tree showing the phylogenetic affiliations of the AOA sequences recovered from the sediment samples. The numbers at the nodes are percentages that indicate the levels of bootstrap support from 2,000 replicates. The scale bar represents 0.05 nucleic acid substitutions per nucleotide position.

FIGURE 4
Maximum-Likelihood phylogenetic tree showing the phylogenetic affiliations of the AOB sequences recovered from the sediment samples. The numbers at the nodes are percentages that indicate the levels of bootstrap support from 2,000 replicates. The scale bar represents 0.05 nucleic acid substitutions per nucleotide position.

well as AOA/AOB diversity index was determined by the Pearson correlation coefficient. ORP was positively correlated with AOB's abundance and OTU numbers ($p < 0.01$), indicating that AOB preferred the niches with higher oxygen concentrations. On the contrary, a negative correlation between ORP and AOA abundance was observed in Table 4, implying that AOA preferred the niches with lower oxygen concentrations. Moreover, these results could be due to the higher oxygen affinity of AOA than that of AOB. Significantly negative correlation between MC and AOB abundance ($p < 0.01$)/AOB OTU numbers ($p < 0.05$) was also observed. The AOB abundance was significantly negatively correlated to the TN ($p < 0.01$) and pH ($p <$

0.05). In addition, TN was also significantly negatively with AOB OTU numbers ($p < 0.05$).

In order to further study the dominant role of the environmental factors of different sediment site in AOA and AOB community structure, the redundancy analysis (RDA) was used to rank the influencing factors (Figure 5). Figure 5A performed the relationship between AOA community structure and environmental factors. The explanation degree of each factor for AOA community structure distribution on the horizontal axis was 54.06%, and on the vertical axis was 22.25%. Therefore, it mainly

TABLE 4 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*[1]	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*

Note: Significance levels are indicated as follows: **, 0.01; *, 0.05. ORP, oxidation-reduction potential; MC, moisture content; NH_4^+-N, ammonium nitrogen; NO_2^--N, nitrous nitrogen; NO_3^--N, nitrate nitrogen; TIN, total inorganic nitrogen; TN, total nitrogen. AOA, ammonia-oxidizing archaea; AOB, ammonia-oxidizing bacteria.

FIGURE 5
Redundancy analysis between AOA **(A)** and AOB **(B)** flora structure and environmental factors. $S_{0−10}$, sediment sampled from 0 to 10 cm layer; $S_{10−20}$, sediment sampled from 10 to 20 cm layer; $S_{20−30}$, sediment sampled from 20 to 30 cm layer; $S_{30−40}$, sediment sampled from 30 to 40 cm layer; $S_{40−50}$, sediment sampled from 40 to 50 cm layer; $S_{50−60}$, sediment sampled from 50 to 60 cm layer. ORP, oxidation-reduction potential; MC, moisture content; NH_4^+-N, ammonium nitrogen; NO_2^--N, nitrous nitrogen; NO_3^--N, nitrate nitrogen; TIN, total inorganic nitrogen; TN, total nitrogen.

referred to the vector projection length of each factor vector on the horizontal axis. NH_4^+-N, pH, and NO_2^--N were the main factors affected the AOA community structure distribution because of their long projection (>0.05) on the horizontal axis. ORP had the opposite performance with other environmental factors, which was also consistent with the results in Table 4. Figure 5B performed the relationship between AOB community

structure and environmental factors. The explanation degree of each factor for AOB community structure distribution on the horizontal axis was 42.52%, and on the vertical axis was 38.43%. ORP and MC were the important environmental factors that affected the AOB community structure distribution because of their long projection on the horizontal and vertical axis. The results of RDA were consistent with those of correlation analysis

(Table 4) to a certain extent, which confirmed the significant effects of OPR and MC on the distribution of AOB community.

Discussion

The difference of abundances and community structure of ammonia-oxidizing archaea and ammonia-oxidizing bacteria

The number of AOA and AOB *amoA* genes observed in this work was similar toa 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 ranged from 10^7 to 10^8. The AOB *amoA* gene copy numbers in this study were relatively lower since samples S_{40-50} and S_{50-60} were collected in the deep layers of the sediments. The ratios of AOA/AOB *amoA* genes increased as the depth of the sediments went deeper, indicating that AOA may dominate 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 phenomenon could be explained by the higher affinities of AOA for oxygen compared to AOB (Martens-Habbena et al., 2009; Jung et al., 2011; Kim et al., 2012). 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.

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, according to previous work in water-level-fluctuating zones in Three Gorges Reservoir (Liu et al., 2015). At higher oxygen concentrations of non-flooded areas, AOA OTU values were lower. However, AOA OTU values were higher in the relatively low oxygen concentration in flooded areas. This phenomenon represented that AOA was more adaptive to the oxygen-limited environments. A total of 18865 high-quality AOA sequences were obtained from 6 sediment samples, and these sequences were assigned to 11 OTUs using 15% as cut-off value (Purkhold et al., 2000). All the six sediment samples were predominantly composed of sequences that belonged to the *Nitrosopumilus* cluster. The observation of the *Nitrosopumilus* cluster dominance over the *Nitrosophaera* cluster in this research was consistent with the previous reportsin the intertidal zones in Zhoushan Island (Hu et al., 2019) and Chongming eastern intertidal

sediments (Zheng et al., 2013). The most AOA belongs to the cluster of *Nitrosopumilus* (73%) confirmed by the phylogeny and meta-data analyses of AOA amoA sequences, which was nearly twice as much as the *Nitrososphaera* cluster (37%) (Alves et al., 2018). These results of the dominance of the *Nitrosospira* cluster over the *Nitrosomonas* cluster were consistent with the previous report in the coastal Pearl River estuary (Cao et al., 2011). The lower concentration of ammonia nitrogen (0.03–0.11 mm NH_4^+) in the intertidal zone of Qingdao may help to explain the absolute dominance of *Nitrosoma*. The cluster of *Nitrosomonas* has a lower substrate affinity which leads to higher substrate concentration adaption. Conversely, the *Nitrosospira* cluster has relatively higher substrate affinity and is more likely to survive in environments with a low concentration of substrate (Zheng et al., 2014; Yu et al., 2016).

Environmental factors

Ammonia oxidizing microorganisms (AOA and AOB) were the most critical microorganisms involved in nitrification and were the first rate-limiting step in the nitrification process. Studies showed that the abundance of AOA and AOB were significantly related to nitrogen forms and soil pH (Segal et al., 2017). Our results (Figure 5) also showed that NH_4^+-N, pH, and NO_2^--N were the main factors affected the AOA community structure distribution. AOB was the main driver of nitrification in alkaline soils, while AOA was the main driver of nitrification in acidic soils (Hu et al., 2013; Zhang et al., 2012; Lu et al., 2012). Thus, a significantly negative correlation was shown between AOB and pH in our study (Table 4). These results consistently confirmed the characteristics of niche differentiation of AOA and AOB. AOB tended to play a leading role in neutral or alkaline environments with high nitrogen and pH value, while AOA was more suitable to dominate in acidic and low nitrogen soil (Ke et al., 2013; Qin et al., 2013). However, our consequence showed that AOB was significantly negatively correlated with TN and ORP, which was the opposite of some other studies. For example, studies of Di et al. (2010) and Pratscher et al. (2011) showed that a high nutrient environment was more conducive to the growth of AOB, while AOA was more suitable to grow in a low nitrogen environment. Most of these studies were aimed at the upper soil. However, our research range varied from 0 to 60 cm soil layer. The differential changes of ammonia oxidation bacteria in different soil layers might be an important reason for the correlation with environmental factors. Some studies showed that the reduction of ORP was conducive to improve the abundance of AOB (Ke and Lu, 2012), and AOA was easier to fix in a low CO_2 environment (Martens-Habbena et al., 2009). This was contrary to the result that AOB likes niches with high oxygen concentration in our study. On the one hand, it was related to the different pH and salinity of different tested soils

(Min et al., 2016). On the other hand, it was also related to the different flooding conditions between different soil layers (Ke and Lu, 2012). Therefore, it was necessary to study further the effects of multiple environmental factors on ammonia-oxidizing bacteria in the future.

Conclusion

In summary, our results showed the changing pattern of AOA and AOB abundance and diversity with sediment depth. In upper layers, the AOB abundance was higher compared to that of AOA. In contrast, AOA abundance outnumbered AOB in deep layers. Both the AOA/AOB *amoA* gene copy numbers rate and AOA/AOB OTU numbers rate increased as sediment depth went deeper, which indicated that oxygen indeed led to the niche specialization of AOA and AOB. AOA won the competition with AOB in the oxygen-limited niches. AOA may be dominant in the ammonia oxidation process in the deep layers. The *Nitrosopumilus* cluster and *Nitrosospira* cluster were the dominant AOA and AOB, respectively, representing ecological success in the intertidal zone.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Author contributions

Conceptualization, JD; methodology, LM; formal analysis, PC; data curation, MQ; writing—original draft preparation, SC and BZ; writing—review and editing, WS; supervision, XZ. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

Author MQ was employed by Longyan City Company of Fujian Tobacco Company. Author SC was employed by China Tobacco Shandong Industrial Co., Ltd. Author BZ was employed by Fujian Branch of China Tobacco Company.

The remaining 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.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2022.976618/full#supplementary-material

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