



Ecological Success of the *Nitrosopumilus* and *Nitrosospira* Clusters in the Intertidal Zone

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Abstract

The intertidal zone is an important buffer and a nitrogen sink between land and sea. Ammonia oxidation is the rate-limiting step of nitrification, conducted by ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB). However, it remains a debatable issue regarding dominant ammonia oxidizers in this region, and environmental factors driving their spatiotemporal niche differentiation have yet to be identified. In this study, intertidal and subtidal zones of Zhoushan Islands were selected for seasonal sampling. Ammonia-oxidizing activity, quantitative PCR, and 454 high-throughput sequencing were performed to study the nitrification potential, abundance, and community structure of ammonia-oxidizing archaea and bacteria. AOA and AOB *amoA* abundance (10^7 – 10^8 *amoA* gene copies/g dry weight sediment) varied spatiotemporally independently of environmental factors. AOA surpassed AOB in most samples, driven by sediment temperature, moisture, and total nitrogen. The diversity of both AOA and AOB differed spatiotemporally. The *Nitrosopumilus* and *Nitrosospira* clusters accounted for an absolutely dominant percentage of AOA (>99%) and AOB (>99%) respectively, indicating a negligible contribution of other clusters to ammonia oxidation. However, there was no significant correlation between nitrification potential and the abundance of AOA or AOB. Overall, the present study showed that AOA dominated over AOB spatiotemporally in the intertidal zone of Zhoushan Islands due to fluctuations in environmental factors, and the *Nitrosopumilus* and *Nitrosospira* clusters ecologically succeeded in the intertidal zone of Zhoushan Islands.

Keywords Ammonia-oxidizing archaea · Ammonia-oxidizing bacteria · Intertidal zone · Dominance · *Nitrosopumilus* · *Nitrosospira*

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Introduction

Nitrification links the gain and loss of bioavailable nitrogen and thus plays a central part in the nitrogen cycle [1]. The first step of nitrification is mainly conducted by ammonia-oxidizing archaea (AOA) [2] and ammonia-oxidizing bacteria (AOB). Since the discovery of AOA, the coexistence and niche differentiation of AOA and AOB have been intensively studied. However, most studies focused on terrestrial and marine ecosystems (Fig. S1), whereas few studies focused on the intertidal zone, which is in land-sea interaction areas.

Worldwide, large amounts of anthropogenic nitrogen (N) are input into coastal seas through riverine runoff and atmospheric deposition (48 and 8 Tg N/year, respectively) [3]. In land-sea interaction areas, intertidal wetlands and estuarine ecosystems are considered the most important buffer, protecting the open ocean from the impact of anthropogenically derived N [4, 5]. In

sediments of the intertidal zone, microscale redox gradients bolster the cooperation of aerobic and anaerobic N cycling processes [6]. Widely distributed intertidal zones account for up to 70% of all the sedimentary N loss and are therefore perceived as a major sink of fixed N in the oceanic N cycle [3, 5, 7].

Previous studies on AOA and AOB in the intertidal zone reported inconsistent results. The dominant clusters of AOA and AOB reported in previous studies were inconsistent. In sediments near the South Atlantic Gyre, the *Nitrososphaera* cluster dominated over the *Nitrosopumilus* cluster [8], whereas in Chongming eastern intertidal sediments, the *Nitrosopumilus* cluster dominated over the *Nitrososphaera* cluster [9]. Similarly, other studies reported the dominance of the *Nitrosospira* cluster over the *Nitrosomonas* cluster [10] and vice versa [11]. The mutable community composition of AOA and AOB in the intertidal zone could be explained by the specific position and environment of the intertidal zone. On the one hand, the intertidal zone is characterized by highly abundant and diverse microbial communities, converging microorganisms from marine, terrestrial, and freshwater ecosystems [4, 12]. On the other hand, the intertidal zone is a watershed of microorganisms from terrestrial to the marine ecosystem and undergoes a significant change in hydrological, morphological, and chemical conditions [13]. Fluctuations in environmental factors also influence the relative abundance and contribution of AOA and AOB. Unlike most studies in marine and terrestrial systems where AOA generally outnumbered AOB, researchers reported that either AOA outnumbered AOB [10, 14–16] or AOB outnumbered AOA [6, 9, 11, 17–21] in the intertidal zone. Whether AOA or AOB contribute more to ammonium oxidization in the intertidal zone is also under debate. Nitrification potential was attributed to either AOA [19, 22] or AOB [11, 23] alone, or both AOA and AOB [6, 9, 16], or neither AOA nor AOB [17].

The community structure, relative abundance, and nitrification potential of AOA and AOB in the intertidal zone are obscure, varying across research sites [24]. In this study, we sampled sediments from a 100-m zone (from the intertidal zone to the subtidal zone) on Xiaogan Island in Zhoushan, which is located in East China Sea. Quantitative PCR was used to determine *amoA* gene copy numbers. 454 high-throughput sequencing of functional genes were performed to construct the community structure of ammonia-oxidizing microorganisms. Ammonia-oxidizing activity was measured to study the nitrification potential. Specifically, the study aimed to address the following questions: (a) What are the relative abundance and contribution of AOA and AOB? (b) What clusters are the dominant AOA and AOB respectively? (c) What are the possible reasons for the dominance of these clusters of AOA and AOB?

Materials and Methods

Sediment Sampling and Determination of Physicochemical Factors

Sediments were sampled in the intertidal zone (Z1 and Z2, middle tidal zone; Z3 and Z4, low tidal zone) and the subtidal zone (Z5, Z6) on Xiaogan Island, Zhoushan [29° 57' 39" N, 122° 13' (01–03)" E]. The sampling spots were arranged perpendicular to the coastline at intervals of 20 m (Fig. S2), and sediments were collected in triplicate from the upper 2 cm at each spot. The samples were collected in May 2013 (spring), August 2013 (summer), November 2013 (autumn), and February 2014 (winter). Sediments in each season at each spot were homogenized, obtaining a total of 24 samples. Each homogenized sample was later divided into three parts for different experiments: one tested for the determination of nitrification potential, one stored at 4 °C for physicochemical measurements, and one frozen at –20 °C for subsequent molecular analysis [25–27]. The sediment pH, temperature, and oxidation-reduction potential (ORP) were determined in situ using a pH meter (IQ150, IQ Scientific Instruments, Inc., Carlsbad, CA, USA). $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, $\text{NO}_3^-\text{-N}$, and total phosphorus (TP) were determined spectrophotometrically or colorimetrically as previously described [28, 29]. Sediment moisture content was measured before and after each soil sample was dried at 105 °C for 24 h. The total nitrogen (TN) was determined using the FOSS Kjeltec2300 analyzer (FOSS Group, Höganäs, Sweden) [30, 31]. Details are displayed in Table S1.

Determination of Nitrification Potential

Ten grams of sediment sample and 65 mL seawater filtered by 0.22 μm membrane were added into an Erlenmeyer flask, which was placed in a shaker and pre-incubated for 24 h in the dark (30 °C, 150 rpm). Ammonium sulfate (final concentration was 0.5 mM) and potassium chlorate (final concentration was 10 mM) were added then, and flasks continued to be incubated in the shaker for 24 h (30 °C, 150 rpm) [32]. Nitrification potential was measured by the accumulation of nitrite in 24 h after addition of ammonium sulfate [28, 29].

DNA Extraction and Quantitative PCR Amplification

DNA from sediment samples was extracted using the PowerSoil DNA Isolation Kit (Mobio, USA), according to the manufacturer's instructions. DNA quantification was measured using a Nanodrop-Spectrophotometer (Thermo Scientific, Wilmington, USA) as previously described [33]. Quantitative PCR amplification was carried out using iCycler iQ5 (BioRad, CA, USA). Archaeal and bacterial *amoA* genes were quantified by qPCR using *amoA*-specific

primers, CamoA-19f/CamoA-616r and amoA-1F/amoA-2R, respectively [34, 35]. The qPCR reaction system and the standard curves made by plasmids were as previously reported [29, 33] and the reaction conditions were as described in Coci et al. [36] and Pester et al. [35]. For the standard curves, qPCR efficiency was 88–94% and $R^2 > 0.99$.

Cloning Library Construction and 454 High-Throughput Sequencing

The primers used for the construction of the AOA and AOB clone libraries were identical to qPCR, but sequencing adapters and tags were added. The PCR reaction system was performed as previously reported [35, 36]. Amplification results were detected by 1.5% agarose gel electrophoresis. Products were purified using a SanPrep column PCR product purification kit (Sangon Biotech., Shanghai, China). Purified PCR products were analyzed through a NanoDrop photometer (ND-1000, Isogen Life Science, the Netherlands) to determine DNA concentration. 454 high-throughput sequencing was performed on the GS FLX + Titanium platform by Shanghai Majorbio Company. The AOA and AOB *amoA* gene sequences obtained in this study were deposited in NCBI: SRR1046086 and SRR1047950.

Bioinformatics Analysis of High-Throughput Data

The 454 sequencing data of AOA and AOB *amoA* were analyzed using the mothur software (<http://www.mothur.org>) [37], following the main steps as reported before [38]. Especially, the OTUs were clustered as different species depending on the sequence similarity of 85% and 88% for AOA and AOB respectively [8, 35]. The clone coverage and the diversity index (Shannon, Chao, Simpson, ACE) of each sample were calculated. The representative sequences (the default was the longest sequence) of each OTU extracted above were compared in NCBI. Sequences whose *E* value was not 0 were deleted to obtain high-quality OTU representative sequences [38]. High-quality OTU representative sequences were introduced into the MEGA 5.0 software with the known AOA or AOB *amoA* gene sequences [39]. Multiple alignments of the nucleotide sequences were performed, followed by the phylogenetic analysis using the neighbor-joining method, with the step size analysis of 1000 calculations.

Statistic Analysis

OTU clustering results were nondimensionalized and then input into the CANOCO software (Centre for Biometry, Wageningen, the Netherlands). After the detrended correspondence analysis (DCA), the redundancy analysis (RDA) was chosen to analyze the relationship between environmental factors and the AOA or AOB community structure [40]. An ordination principal component analysis (PCA) was also

performed with CANOCO software. Pearson's analysis was conducted in the SPSS 11.0 software.

Results

Site Characteristics

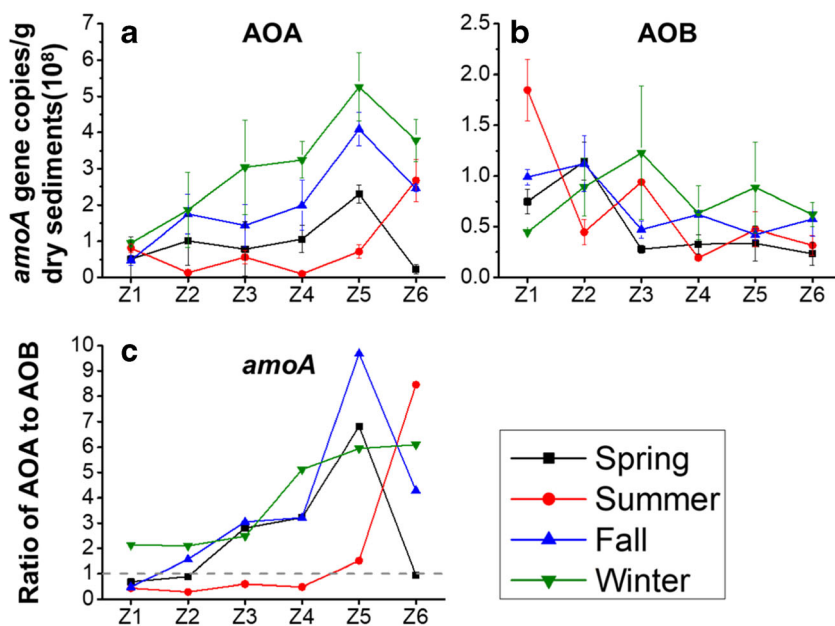
The basic physicochemical characteristics of the sediments are shown in Table S1, with significant spatiotemporal differences. The temperature of the sediments varied with season notably, which was the highest in summer (31.0 °C) and the lowest in winter (9.6 °C). The pH of the sediments was subtly acidic and displayed a propensity to increase from the intertidal zone to the subtidal zone. It was relatively lower in spring (the lowest pH, 5.87) and in winter (the lowest pH, 5.54), and relatively higher in summer (the highest pH, 6.56) and in autumn (the highest pH, 6.76). Sediment moisture tended to increase gradually from the intertidal zone to the subtidal zone. The average moisture of the sediments was 47.00% in spring, 45.06% in summer, 52.88% in autumn, and 43.46% in winter. ORP decreased gradually from the intertidal zone to the subtidal zone, with a drop of 13.9, 11.4, 48.8, and 73.1 mV in spring, summer, autumn, and winter, respectively. Ammonia concentration ($\text{NH}_4^+\text{-N}$) in sediment pore water ranged from 0.26 to 1.12 mg/L and decreased gradually from the intertidal zone to the subtidal zone. On average, ammonia concentration was significantly higher in summer and autumn than in spring and winter. Total nitrogen (TN) of the sediments was between 790.89 and 2353.24 mg/kg and decreased from the intertidal zone to the subtidal zone. TN was higher in summer and autumn than that in spring and winter.

The Abundance of AOA and AOB and the Driving Environmental Factors

AOA and AOB were detected in all 24 samples and had significant seasonal and spatial differentiation (Fig. 1). The abundance of AOA *amoA* reached 10^7 – 10^8 copies/g dry sediment in all seasons, similar to what was reported in some previous studies [11, 16, 18, 20, 41, 42]. The abundance of AOA *amoA* had the general tendency to increase from the intertidal zone to the subtidal zone. The abundance of AOB *amoA* was 1.74×10^7 – 9.33×10^7 , 1.54×10^7 – 1.97×10^8 , 3.36×10^7 – 9.01×10^7 , and 4.84×10^7 – 9.13×10^7 copies/g dry sediment in four seasons, respectively. The abundance of AOB *amoA* in the subtidal zone was only slightly lower than that in the intertidal zone. The ratio of AOA to AOB *amoA* also had significant seasonal and spatial differentiation, with the general tendency to increase from the intertidal zone to the subtidal zone, and ranging from 0.36 to 12.11 in each sampling site temporally (Fig. 1c).

Pearson's correlation analysis showed a significant negative correlation between the AOA abundance and the

Fig. 1 Quantitative analysis of AOA and AOB *amoA* in Zhoushan Islands. The three figures demonstrate the seasonal changes of AOA *amoA* (a), AOB *amoA* (b), and the ratio of AOA to AOB *amoA* (c) from the middle tidal zone Z1 to subtidal zone Z6. The gray dash line corresponds to value 1. Z1 and Z2 were in the middle tidal zone; Z3 and Z4 were in the low tidal zone; Z5 and Z6 were in the subtidal zone. The sampling interval was 20 m



sediment temperature ($p < 0.01$), and a significant positive correlation between the AOA abundance and the sediment moisture ($p < 0.01$). A significant positive correlation was found between the abundance of AOB and the total nitrogen ($p < 0.01$). The ratio of AOA to AOB *amoA* was significantly positively correlated with sediment moisture ($p < 0.01$), and significantly negatively correlated with sediment total nitrogen ($p < 0.01$) (Table 1).

Lines of evidence indicated the independent response of AOA and AOB to environmental factors. Firstly, there was no significant correlation between the abundance of AOA and AOB (Pearson’s correlation; $r = 0.061, p = 0.776$), which was different from previous studies [8]. Secondly, Pearson’s correlation analysis of environmental factors and the abundance of AOA and AOB in different seasons (Table S2) showed different environmental significant factors for AOA and

AOB. Thirdly, seasonality had more significant effects on the abundance of AOA *amoA* than that of AOB (Fig. 1), similar to a previous study [18].

The Community Structure of AOA and AOB and Driving Environmental Factors

A total of 97,667 AOA *amoA* gene sequences were obtained from 24 sediment samples after filtered through mothur. In all 24 sediment samples, the clone coverage of AOA reached a high level ranging from 99.88 to 100.00% (Table S3). All the AOA *amoA* gene sequences were divided into 37 OTUs and the numbers of OTUs per sediment sample ranged from 12 to 20 (Table S3). From the intertidal to the subtidal zone, the OTU number of AOA decreased by 1 and 3 in spring and in autumn and decreased by 7 and 8 in summer and in winter,

Table 1 Pearson’s correlation index describing the relationships of environmental factors, the abundance of AOA and AOB, and nitrification potential in Zhoushan Islands

Factor	Pearson’s correlation index			
	AOA abundance	AOB abundance	<i>amoA</i> ratio of AOA/AOB	Nitrification potential
pH	0.369	-0.035	0.362	-0.013
T	-0.667**	0.001	-0.352	0.745**
ORP	-0.383	0.102	-0.219	0.213
Moisture	0.621**	-0.167	0.623**	-0.243
NH4+-N	0.130	0.290	-0.097	-0.160
NO3--N	-0.375	0.337	-0.105	0.455*
TIN	-0.352	0.375	-0.371	0.429*
TN	-0.564**	0.575**	-0.626**	0.439*
Nitrification potential	-0.329	0.276	-0.547**	/

* $p < 0.05$, ** $p < 0.01$

respectively (Table S3). Pearson's analysis demonstrated no remarkable correlation between environmental factors and the OTU number of AOA (Table S4). Phylogenetic analysis of 37 representative sequences of AOA is shown in Fig. 2a. Thirty OTUs (97,591 sequences, 99.92%) belonged to the *Nitrosopumilus* cluster, 6 OTUs (71 sequences) belonged to the *Nitrososphaera* cluster, and 1 OTU (5 sequences) belonged to the THAOA cluster [43]. In all sediment samples, the proportion of the *Nitrosopumilus* cluster was between 99.73 and 100.00% of AOA sequences. The *Nitrososphaera* cluster was detected in 19 samples, but the proportion was between 0.02 and 0.27%. The THAOA cluster was detected in three samples (SP-Z1, WI-Z1, and WI-Z6), with 0.07%, 0.02%, and 0.02% of AOA sequences, respectively (Table S5).

Eighty thousand five hundred thirty-seven high-quality sequences of AOB *amoA* gene were obtained from 24 sediment samples and clustered into 22 OTUs according to 88% sequence similarity [8]. The OTU number in each sample ranged from 9 to 14, and the clone coverage of AOB was over 99.91% (Table S6). AOB decreased by 1–3 OTUs from the intertidal zone to the subtidal zone (Table S6). Pearson's correlation analysis showed a significant negative correlation between temperature and the OTU number in AOB ($p < 0.01$) (Table S4). Phylogenetic analysis of 22 representative sequences of AOB is shown in Fig. 2b. In the 22 OTUs, 16 OTUs (80,303 sequences, 99.71%) were assigned to the genus *Nitrosospira*, and the remaining 6 OTUs (234 sequences) were assigned to the genus *Nitrosomonas*. In all sediment samples, the proportion of the *Nitrosospira* cluster was between 98.99 and 100.00%. The *Nitrosomonas* cluster was detected in 20 sediment samples with a proportion of 0.01 to 1.01% (Table S5). Pearson's correlation analysis showed that the proportion of the *Nitrosomonas* cluster was positively correlated with ammonium concentration ($p < 0.05$) (Table S7).

PCA analysis showed the compositions of AOA and AOB of different sampling spots as well as different seasons (Fig. 3). The RDA analysis was performed to analyze the influence of environmental factors on the community structure of AOA and AOB (Fig. S3). Seasonality had a more salient influence on the community structure of AOA than that of AOB. The effects of ORP on the community structure of AOA were statistically significant ($p < 0.05$, Monte Carlo test 1000 times, Fig. S3a). No single environmental factors had statistically marked effects on AOB.

Nitrification Potential and the Driving Environmental Factors

Nitrification potential of the intertidal sediments in Zhoushan had obvious spatial and seasonal variations. Nitrification potential tended to decrease from the intertidal zone to the subtidal zone throughout the year (Fig. 4). The average

nitrification potential of 6 sampling sites was 1.73, 1.25, 1.02, and 0.81 $\mu\text{g N/g dry sediment/day}$ in summer, spring, autumn, and winter respectively, with the highest value in summer and the lowest in winter. Pearson's correlation analysis showed that nitrification potential had a significant positive correlation with sediment temperature ($p < 0.01$), nitrate, total inorganic nitrogen (TIN), and TN ($p < 0.05$, Table 1). No significant correlation was found neither between nitrification potential and the abundance of AOA or AOB *amoA* (Table 1) nor between nitrification potential and the abundance of top 9 AOA and AOB OTUs, which had at least 150 reads (Table S8). However, there was a significant positive correlation between nitrification potential and the *amoA* ratio of AOB to AOA ($p < 0.01$, Table 1).

Discussion

Dominance of AOA over AOB Driven by Environmental Factors

According to the *amoA* gene copies, AOA dominated over AOB in two-thirds of the samples (Fig. 1c). In contrast to only one *amoA* gene copy per genome in AOA, there were 3 *amoA* gene copies per genome in AOB of the genus *Nitrosospira* [8]. Since genus *Nitrosospira* occupied 99.71% of all the AOB sequences, it is possible to convert *amoA* abundance into a cell number. According to the cell number, AOA absolutely dominated over AOB in the sampling sites.

The dominance of AOA over AOB might be caused by the competition of AOA and AOB or their adaptation to environmental factors. Several evidences indicated no strong interaction between AOA and AOB. On the one hand, AOA and AOB's lower tide height limits were set by species interactions [44]. There were no lower tide height limits for AOB, similar to previous studies [8, 19]. While the abundance of AOA *amoA* increased from the intertidal zone to the subtidal zone, the abundance of AOB *amoA* in the subtidal zone was only slightly lower than that in the intertidal zone (Fig. 1). On the other hand, there was no intense competition for ammonia by AOA and AOB in the intertidal zone in this study. Pearson's correlation analysis showed that ammonia concentration negatively correlated with the abundance of AOA and positively correlated with the abundance of AOB (Table S2).

In contrast to competition, the independent response of AOA and AOB to environmental factors indicated that the spatiotemporal variation of environmental factors potentially drove the niche differentiation of AOA and AOB. Sediment temperature, moisture, and TN could help explain the dominance of AOA over AOB in this study (Table S2). The negative correlation between the temperature and the abundance of AOA *amoA* indicated that the species of top AOA OTUs were psychrophilic ecotypes, adapting to a lower temperature than

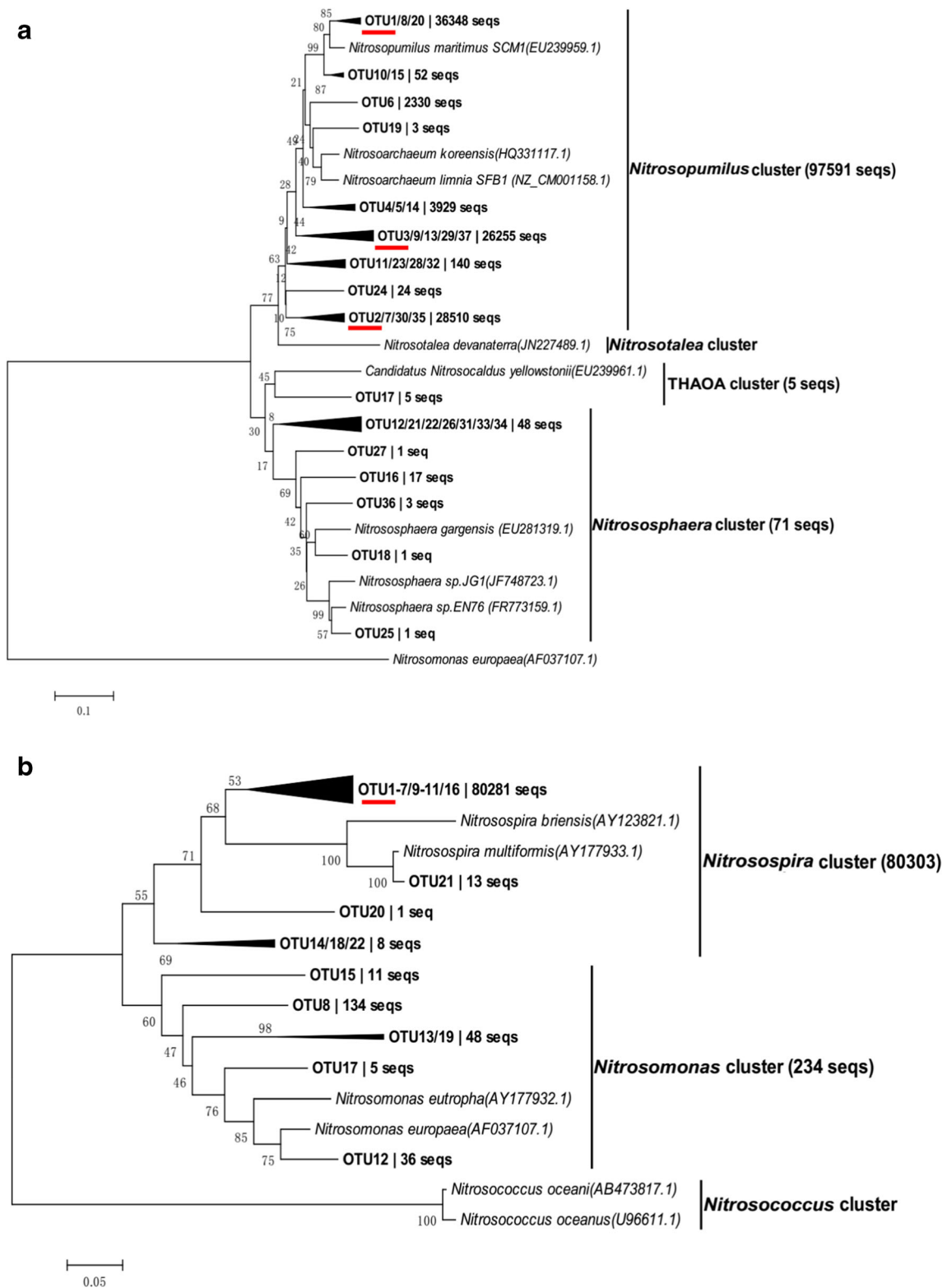


Fig. 2 Neighbor-joining phylogenetic tree showing the phylogenetic affiliations of the representative sequences of AOA (**a**) and AOB (**b**) recovered from Zhoushan Islands. The numbers at the nodes are percentages that indicate the levels of bootstrap support from 1000

replicates. The scale bar represents 0.05 or 0.1 nucleic acid substitutions per nucleotide position. OTUs with red underline were the dominant OTUs in the intertidal zone

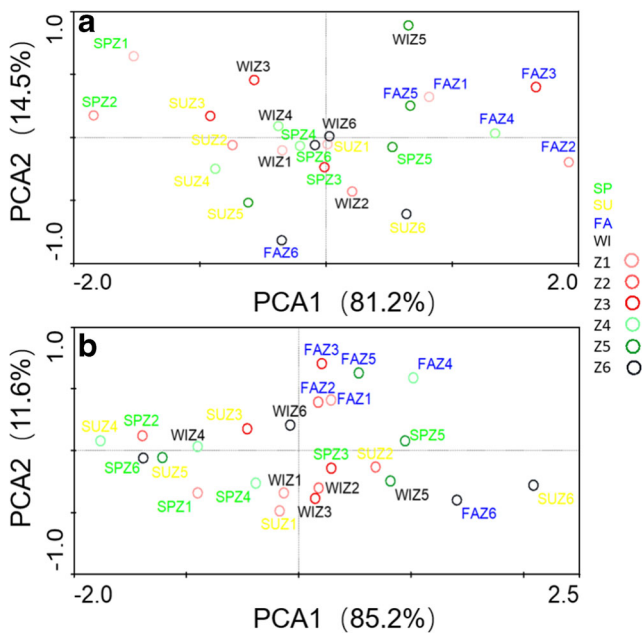


Fig. 3 Principal component analysis (PCA) of AOA (a) and AOB (b) compositions of all 24 samples. Z1 and Z2 were in the middle tidal zone; Z3 and Z4 were in the low tidal zone; Z5 and Z6 were in the subtidal zone. The sampling interval was 20 m. SP spring, SU summer, FA fall, WI winter

AOB in marine environments [10, 13]. Moisture is another significant factor, as indicated in previous studies [16]. The fluctuations in moisture might control the oxygen penetration and thus negatively influencing the oxygen concentration [8, 23]. The low oxygen concentration further limited the growth of AOB and facilitated the growth of AOA, which was also supported by the increase and decrease of the abundance of AOA and AOB respectively from the intertidal zone to the subtidal zone (Fig. 1). In this study, we sampled the typical muddy intertidal sediments from the upper 2 cm, where oxygen rapidly depleted to zero (Fig. S2), similar to previous studies [8, 23]. Compared with AOB, AOA showed a much higher affinity to O_2 [45] and adjusted to low-oxygen and oxic-anoxic environments [46]. ORP is another factor related

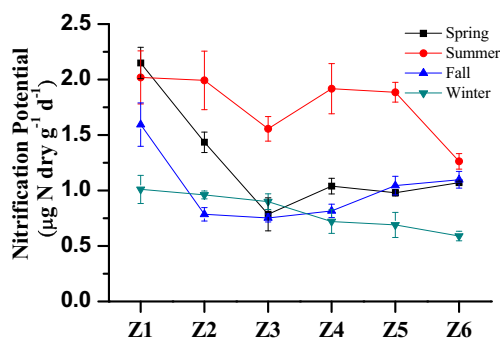


Fig. 4 Nitrification potential in spring, summer, fall, and winter in the intertidal and subtidal zone of Zhoushan Islands. Z1 and Z2 were in the middle tidal zone; Z3 and Z4 were in the low tidal zone; Z5 and Z6 were in the subtidal zone. The sampling interval was 20 m

to the oxygen concentration. However, the concentration of nitrate and sulfate was relatively high in the sampling sites [47, 48] and thus could not represent the oxygen concentration. While temperature and moisture helped explain the dominance of AOA over AOB, TN might be responsible for the low ratio of AOA to AOB which was expected to research 3 magnitudes or even higher as shown in most studies in pelagic or terrestrial systems [24]. TIN was positively correlated with the abundance of AOA and AOB *amoA* (not significant), while TN was negatively correlated with the abundance of AOA *amoA* ($p < 0.01$) and positively correlated with the abundance of AOB *amoA* ($p < 0.01$, Table 1). This implied that organic nitrogen inhibited and promoted the growth of AOA and AOB respectively [49].

Absolute Dominance of the *Nitrosopumilus* Cluster in AOA

The diversity of AOA varied spatiotemporally (Fig. 3a; Table S3), influenced by different environmental factors (Fig. S3a). However, the dominant AOA remained constant (Table S5). To be more specific, OTUs were further classified by frequency (Table S9): persistent OTUs (>85%), intermittent OTUs (35–85%), and transient OTUs (<35%) [50]. All persistent OTUs belonged to the *Nitrosopumilus* cluster, and 3 persistent OTUs accounted for most reads in all 24 samples (Fig. S4a), suggesting the extremely uneven frequency of AOA clades in ecosystems [12]. The only 1 OTU belonging to the THAOA cluster was the transient OTU, most probably dormant/dead cells transported from land [12]. Although 12 OTUs belonged to the *Nitrososphaera* cluster, most of them were transient OTUs. The dominance of the *Nitrosopumilus* cluster over the *Nitrososphaera* cluster in this study was similar to a previous study in Chongming eastern intertidal sediments [9]. Extensive phylogenetic and meta-data analyses of archaeal *amoA* sequences also showed that most estuarine-coastal AOA belong to the *Nitrosopumilus* cluster (73%), nearly twice more than the *Nitrososphaera* cluster (37%) [12]. The *Nitrososphaera* cluster decreased by 1–8 OTUs from the intertidal zone to the subtidal zone in different seasons (Table S3), contained low read numbers, and had no obvious occurrence preference in terms of season and site (Table S9). All of these indicated that the transient *Nitrososphaera* cluster was also composed of dormant/dead cells transported from land [12]. It is not likely that ammonia affinity determined the elimination of the *Nitrososphaera* cluster. Although the *Nitrososphaera* cluster did have a lower ammonia affinity than the *Nitrosopumilus* cluster, it possessed a higher ammonia affinity than *Nitrososphaera* AOB [51], which thrived in the intertidal zone of Zhoushan Islands. Therefore, the failure of the *Nitrososphaera* cluster was more likely to form due to its failure in adapting to chemical conditions or fluctuations in environmental factors in the intertidal zone. In

contrast, the persistent existence of OTUs from the *Nitrosopumilus* cluster and its inconstant correlative environmental factors indicated the adaptation of the *Nitrosopumilus* cluster to the sediment environment in the intertidal zone of Zhoushan Islands [50].

Absolute Dominance of the *Nitrospira* Cluster in AOB

The diversity of AOB varied spatiotemporally (Fig. 3b; Table S6), influenced by different environmental factors (Fig. S3b). However, the dominant AOB remained constant (Table S5). To be more specific, OTUs were further classified by frequency as described above [50]. All persistent OTUs belonged to genus *Nitrospira*. OTU1 dominated in all 24 samples (Fig. S4b) and phylogenetically belonged to the *Nitrospira* cluster 1, which was proposed as exotic inhabitants in marine sediments [52]. The prevalence of the *Nitrospira* cluster 1 in this study supported its active growth in the sediments of the intertidal zone [8]. All OTUs belonging to the *Nitrosomonas* cluster were transient and contained low read numbers, indicating they were dormant/dead cells transported from land like the THAOA and *Nitrososphaera* clusters [12].

It thus raised a question as to why AOB in the intertidal sediments of Zhoushan were mainly composed of *Nitrospira*. The dominance of *Nitrospira* in this study was identical to what was illustrated in a previous study conducted by Cao et al. [10]. However, most previous studies reported the dominance of the *Nitrosomonas* cluster over the *Nitrospira* cluster [11, 18, 19] or a close proportion of the *Nitrosomonas* cluster and the *Nitrospira* cluster [9, 13, 17]. It is also common for the *Nitrosomonas* cluster to outnumber the *Nitrospira* cluster in flooded habitats [53]. Yet where hydrological and chemical conditions fluctuate, it is probable for *Nitrospira* to outnumber *Nitrosomonas*, as shown in a previous study in water-level-fluctuating zones [29]. Low ammonia concentration in the intertidal zone of Zhoushan (0.019–0.08 mM NH_4^+) might help explain the absolute dominance of the *Nitrospira* cluster, according to different physiological characteristics of the *Nitrosomonas* and *Nitrospira* clusters [51]. The dominant AOB detected in this study were phylogenetically close to *Nitrospira briensis*, and *Nitrosomonas* detected in this study were phylogenetically close to *Nitrosomonas europaea*. The growth of *Nitrosomonas europaea*-like AOB ($K_m = \sim 1.5$ mM ammonium, pH 7.5) followed the r strategy [54], whereas the growth of *Nitrospira briensis*-like AOB ($K_m = \sim 40$ μM ammonium, pH 7.8) followed the K strategy [55]. The *Nitrosomonas* cluster has a lower affinity for the substrate and adapts to higher substrate concentrations [19, 56]. In contrast, the *Nitrospira* cluster has a relatively higher affinity with the substrate, preferring to survive in environments with low substrate concentrations [19, 56]. Pearson analysis showed a positive correlation

between the proportion of the *Nitrosomonas* cluster and ammonium concentrations in the sediments ($p < 0.05$, Table S7), supporting the lack of nutrients for the *Nitrosomonas* cluster in the intertidal zone of Zhoushan.

No Significant Correlation Between Nitrification Potential and the Abundance

There was no significant correlation neither between nitrification potential and the abundance of AOA or AOB (Table 1) nor between nitrification potential and the abundance of top 9 AOA and AOB OTUs (Table S8). In contrast, a few previous studies showed a correlation between nitrification potential and either [11, 16, 19, 23] or both of [6, 9] the AOA and AOB *amoA*. There were a few explanations for the irrelevance between nitrification potential and the abundance in this study. (1) The newly discovered complete ammonia oxidizers (comammox) might contribute to this outcome. In a coastal sediment near the sampling sites in this study, comammox *amoA* abundance reached 1×10^6 copies/g dry sediment, indicating a previously overlooked existence of novel nitrogen-cycle microorganisms in the environment [57, 58]. (2) Organic nitrogen might explain the irrelevance between nitrification potential and *amoA* copies. Surprisingly, nitrification potential was positively correlated with the *amoA* ratio of AOB to AOA ($p < 0.01$, Table 1). Furthermore, TN was a significant factor associated with the *amoA* abundance of AOA and AOB, while TIN was not. Organic nitrogen might cause the irrelevance between nitrification potential and the abundance by promoting the AOB *amoA* expression but inhibiting the AOA *amoA* expression [2, 17].

In summary, our results showed that the abundance and diversity of AOA and AOB and nitrification potential in the intertidal zone of Zhoushan showed spatiotemporal changes. The spatiotemporal variation of environmental factors, such as sediment temperature, moisture, and total nitrogen, resulted in the niche differentiation of AOA and AOB, rather than the interaction between AOA and AOB. The *Nitrosopumilus* and *Nitrospira* clusters were the absolute dominant AOA and AOB, respectively, indicating an ecological success in the intertidal zone. The failure of the THAOA and *Nitrososphaera* clusters was likely attributable to the inability to adapt to the environment in the intertidal zone. The dominance of the *Nitrospira* cluster over the *Nitrosomonas* cluster was probably due to their difference of ammonia affinity. To figure out the potential contribution of AOA and AOB to nitrification, an inhibition assay is necessary in the future.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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