Spatiotemporal dynamics of ammonium monooxygenase (*amoA*) genes in sediments of the aquaculture area in the Yellow Sea Cold Water Mass

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Abstract

Ammonia oxidation is a fundamental process in the marine nitrogen cycle, driving the transformation of ammonia into nitrate and playing a crucial role in maintaining nitrogen balance in deep-sea ecosystems. Ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) play crucial roles in the ammonia-oxidizing, yet their dynamics in deep-sea aquaculture environments remain poorly understood. This study investigated the spatiotemporal variations in the diversity, community composition, and abundance of archaeal and bacterial *amoA* genes in the sediments of the aquaculture area in the Yellow Sea Cold Water Mass. Results revealed that AOB exhibited higher OTUs, diversity, and richness than AOA. Most AOA and AOB were clustered into the genera *Nitrosopumilus* and *Nitrosomonas*, respectively. The highest abundance of *amoA* gene copies was observed in December. Although bioindicators for both AOA and AOB communities were detected across all sampling sites and times, none of them could be identified as aquaculture-derived indicators. Neutral community model indicated that assembly of the AOB community was primarily driven by stochastic processes (R^2 = 88.6%), whereas the AOA community was more influenced by environmental factors (R^2 = 61.3%). Bottom water temperature and sediment carbon content were the key parameters influencing archaeal *amoA* gene abundance and AOA indicator abundance. These findings highlight the importance of environmental parameters in shaping AOA and AOB communities in the YSCWM. Understanding these microbial dynamics is essential for assessing the stability of nitrogen cycling in deep-sea aquaculture environments and developing sustainable management strategies for marine aquaculture in YSCWM.

Keywords: Ammonia-oxidizing archaea (AOA); Ammonia-oxidizing bacteria (AOB); *amoA* genes; qPCR; Yellow Sea Cold Water Mass; Sediments

1. Introduction

Marine sediments are Earth's largest habitats for microbes to flourish and are particularly important sites for biogeochemical cycles, such as the carbon and nitrogen cycles (Beman et al., 2012; Besaury et al., 2014). Nitrogen is an essential nutrient element for life in the marine ecosystems, influencing primary productivity, organic matter degradation, and trophic dynamics (Luo et al., 2018). However, an excess of inorganic nitrogen, such as ammonia, can trigger eutrophication and pose severe risks to marine biodiversity and sustainability of aquaculture (Shen et al., 2016; Jin et al., 2017). Therefore, understanding nitrification in deep-sea sediments is essential for assessing nitrogen cycling potential, maintaining water quality, and ensuring long-term ecosystem stability (Li et al., 2022).

Nitrification, which involves the sequential oxidation of ammonia (NH_4^+-N) to nitrite (NO_2^--N) and subsequently to nitrate (NO_3^--N), is a crucial process to regulate nitrogen bioavailability (Isnansetyo et al., 2014). The first and rate-limiting step, ammonia oxidation, is catalyzed by ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) (He et al., 2018). Both AOA and AOB carry the *amoA* gene, which encodes the first subunit of ammonia monooxygenase (AMO) (Könneke et al., 2005; Treusch et al., 2005; Ming et al., 2020). To date, *amoA* gene has been a widely used molecular marker to access the diversity and abundance of ammonia-oxidizing microbes across aquatic environments, including estuaries (Mosier and Francis, 2008; Zheng et al., 2014; Ming et al., 2020), freshwater aquaculture ponds (Shen et al., 2016; Yang et al., 2022), and lakes (Hou et al., 2013; Vissers et al., 2013). Extensive studies have been conducted focusing on the relative contribution of AOA and AOB to nitrification (Wang et al., 2014). For example, in river and estuary systems, AOA communities exhibited higher diversity and *amoA* gene abundance than AOB communities (He et al., 2018; Liu et al., 2021). In coastal sediments, the relative contribution of AOA to total potential nitrification rate was greater than that of AOB due to the low salinity and ammonia concentrations in the bottom water (Li et al.,

2022a). In freshwater aquaculture ponds, AOA and AOB distribution was strongly influenced by farming models and physicochemical parameters (Dai et al., 2018; Lu et al., 2019; Yang et al., 2022). Previous studies have shown that seasonal changes in environmental factors, such as pH, temperature, light, dissolved oxygen, salinity, and ammonia concentration, were reflected in the dynamic changes of AOA and AOB community structures and the abundance of functional genes (*amoA*) in diverse aquatic ecosystems (Avrahami et al., 2003; Mosier and Francis, 2008; Jiménez et al., 2011; Merbt et al., 2012; Qin et al., 2017; Lu et al., 2019). These findings highlight the significant influence of environmental conditions on ammonia-oxidizing microbial communities across various aquatic habitats. However, most studies have focused on coastal and freshwater environments, leaving deep-sea aquaculture systems largely unexplored.

Located in the deep sea, the Yellow Sea Cold Water Mass (YSCWM) in China exhibits unique hydrological conditions compared to other ocean environments, such as the costal South Yellow Sea (Dong, 2019; Li et al., 2022b). The formation of thermocline in the area started from May to June, resulting in low bottom seawater temperatures and high dissolved oxygen concentrations in summer, which are suitable for culturing salmonids (*Oncorhynchus mykiss*) (Xin et al., 2015; Li et al., 2022b). In 2017, the first submersible fish cage called “Deep Blue 1” was constructed in the YSCWM to culture Salmonids. Cage farming can significantly impact nitrogen cycling in the sediments through organic matter deposition from residual feed and feces, leading to enhanced nutrients fluxes and microbial nitrogen turnover rates (Rubio-Portillo et al., 2019). Previous studies have shown that aquaculture activities in the YSCWM can alter bacterial community composition and reduce bacterial richness in sediments, with Planctomycetes identified as a biomarker in the aquaculture area (Li et al., 2022b). However, the specific impacts of “Deep Blue 1” on ammonia-oxidizing microbial communities in the YSCWM remain poorly understood.

In this study, we investigated the ammonia-oxidizing microbes in the YSCWM by measuring the gene abundance of archaeal and bacterial *amoA* genes and performing high-throughput sequencing of *amoA*-encoding microorganisms at different culture times. This study represents the first exploration of how aquaculture activities and the unique deep-sea conditions in the YSCWM jointly influence the structure and dynamics of AOA and AOB communities. Our findings offer novel insights into the spatiotemporal dynamics and environmental drivers of these microbial communities in aquaculture sediments. We hypothesize that the distinctive environmental conditions of the YSCWM, combined with aquaculture activities, may significantly shape the community structure and functional gene abundance of ammonia-oxidizing microbes in surface sediments. To address these knowledge gaps, our primary objectives were (1) to explore temporal and spatial variations of the AOA and AOB community structure in the YSCWM, (2) to investigate the potential factors influencing *amoA* gene abundance and community distribution, and (3) to detect the bioindicators in both AOA and AOB communities and evaluate the correlation between the bioindicators and environmental factors.

2. Materials and Methods

2.1. Sample collection and physicochemical properties analysis

In November 2020, more than 40,000 Salmonidae juveniles (average weight of about 150 g) were stocked in the cage named “Deep Blue 1” (perimeter: 180 m, height: 35 m), which is located at 35.21785 N, 122.26140 E . The water depth in this region is around 55 meters (Xu et al., 2024). The sediment in this area was dominated by clay (69.76 % ~ 71.87 %), followed by silt (20.03 % ~ 23.20 %) and sand (5.99 % ~ 10.21 %) (Pan et al., 2025). Based on our previous research, the thermocline in this area is typically found at a depth of around 15 to 22 meters during summer (Nie et al., 2023). The fish were harvested from June 22 to August 06, 2021. On

November 25, 2021, over 100,000 Salmonidae juveniles (average weight of about 1,600 g) were stocked in the same cage and then harvested since June 07, 2022.

Sampling campaigns based on the different culture cycles were as follows: at the end of the first culture cycle (June 24 to 27, 2021), after the first harvest (August 25 to 28, 2021), at the beginning of the second culture cycle (December 03 to 06, 2021), and before the second harvest (April 27 to 30, 2022). Sediment samples were taken from three sampling sites as follows: site E was around the deep-sea fully submersible fish cage "Deep Blue", site C located 500m away from the cage towards the main water flow (140°–320°) under the potential influence of cage culture, and site A located 2000 m away from the cage towards the main water flow, with no apparent anthropogenic influence and fish farming effect (Fig. 1).

The bottom water temperature and the dissolved oxygen concentration were measured in situ with a YSI 650 MDS multi-parameter water quality meter (Yellow springs instruments, USA). Ample surface sediment samples (0–10 cm) in triplicates were collected by a grab sampler at each site, and placed in 50 mL RNase-free centrifuge tubes. All samples were stored and transported at -20 ℃. After immediate delivery to the Key Laboratory of Mariculture, Ocean University of China (Qingdao, China), a portion of the sample was separated for measuring the physicochemical properties of pore water and sediments, and the remaining portion was stored at −80 ℃ for DNA extraction. Concentrations of total ammonia nitrogen (TAN, μg L^{-1}), nitrite (NO_2^--N, μg L^{-1}) and nitrate (NO_3^--N, μg L^{-1}) in the pore water were determined following Boyd (2019). Briefly, TAN was detected by the indophenol blue spectrophotometric method, NO_2^--N was measured using the ethylenediamine dihydrochloride spectrophotometric method, and NO_3^--N was analyzed by the zinc-cadmium reduction ultraviolet spectrophotometric method. For analysis of total nitrogen and carbon content in the sediments, approximately 10 g of sediment was dried to a constant weight in a vacuum freeze-dryer (Alpha1-4LDplus, CHRIST, Germany) at −40°C for 48 h. The dried samples were homogenized and sieved through a 200-mesh

grid before analysis. Total nitrogen and carbon content were quantified using an elemental analyzer (WarsoEL III, ELEMENT, Germany), which operated with two reaction chambers set at 750°C and 500°C, respectively. Data were calibrated against Acetanilide (C: 71.09%, N: 10.36%), with calibration performed twice for every 19 samples.

2.2. DNA extraction and amplicon sequencing

Total genomic DNA was extracted from approximately 0.5g of sediment samples by using MO-BIO PowerSoil DNA Isolation Kit (Carlsbad, CA USA) according to the instructions of the manufacturer. The extracted DNA concentration and purity were measured using a spectrophotometer (ND-2000, Nanodrop, USA), and the integrity of DNA was determined by 1.2% agarose gel.

The archaeal *amoA* gene fragments (256 bp) and bacterial *amoA* gene fragments (491 bp) were amplified using the specific primer pairs as follows: Arch amoA-F (5'-STAATGGTCTGGCTTAGACG-3') and Arch amoA-R (5'-GCGGCCATCCATCTGTATGT-3') for archaeal *amoA* gene and amoA-1F (5'-GGGGTTTCTACTGGTGGT-3') and amoA-2R (5'-CCCCTCKGSAAAGCCTTCTTC-3') for bacterial *amoA* gene (Ming et al., 2020). A two-step PCR amplification was used to create a gene library. All PCR reactions were carried out with 15 μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs), 2 μM of forward and reverse primers, and around 10 ng of template DNA. For AOA *amoA* gene sequencing, the PCR protocol involved an initial denaturation at 98°C for 1 min, followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 56 °C for 30 s, and elongation at 72 °C for 30 s, with a final extension at 72 °C for 5 min. For AOB *amoA* gene sequencing, the PCR conditions were the same, except the annealing temperature was set to 58°C instead of 56°C. The PCR products were run on 2% agarose gel electrophoresis and then purified with AxyPrepDNA Gel Extraction Kit (AXYGEN, USA). Subsequently, amplified PCR products were pooled into a

single tube for sequencing on the Illumina NextSeq 2000 platform (TinyGene Bio-Tech Co., LTD, Shanghai, China).

2.3. Sequence analysis

Raw sequence read were analyzed under specific filtering conditions to obtain the high-quality clean tags using QIIME (V1.9.1, http://qiime.org/scripts/split_libraries_fastq.html). Ambiguous, homologous, and chimera sequences were identified and removed using Mothur (version: 1.39.5, https://mothur.org/wiki/). The clean tags were then clustered into operational taxonomic units (OTUs) at a 97% sequence similarity level using Uparse (Uparse v7.0.1001, http://drive5.com/uparse/). OTUs were aligned to determine the closest reference sequence based on the BLASTn tool (http://www.ncbi.nlm.nih.gov/BLAST) in the latest NCBI database. Raw sequence reads in this study are deposited in the NCBI Sequence Read Archive database with numbers PRJNA1013470 and PRJNA1013414.

2.4. Quantitative PCR

The abundance of archaeal and bacterial *amoA* genes was detected by qPCR using the specific primer pairs that were identical to those used for high-throughput sequencing. All qPCR assays were performed in triplicate on a QuantStudio5 real-time PCR (Applied Biosystems, US) using the SYBR Green I method. The 20 μL reaction volume contained 10 μL of 2 × ChamQ SYBR color qPCR Mater Mix (Vazyme, Nanjing, China), 0.2 μL of each primer (10 mM), 7.6 μL of nuclease-free water, and 10 ng of template DNA. The qPCR amplification started with an initial activation at 95℃ for 30s, followed by 40 cycles of 30 s at 95℃, 1 min at 56℃ and 1 min at 72℃ for AOA, or 40 cycles of 30 s at 95℃, 1 min at 58℃ and 1 min at 72℃ for AOB. After the amplification cycles, a melting stage was added to obtain a melting curve. Standard curves using seven concentrations (10^4-10^{10} copies·μL^{-1} for AOA and 10^3-10^9 copies·μL^{-1} for AOB) of standard plasmids were established. The plasmid was constructed using the pTOPO-TA cloning kit (SparkJade Biotech, China) in chemically competent

E. coli cells (Sangon Biotech, China), following the manufacturer's guidelines. Plasmid DNA was extracted using the SPARKeasy Mini Plasmid Ultra-Fast Kit (SparkJade Biotech, China) following the manufacturer's instructions. The reliability of the qPCR assay with both primers was confirmed by the strong linear inverse relationship between the threshold cycle value (Ct) and the logarithmic value of the gene copy numbers (R^2 > 0.99). Only one observable peak at the melting temperature (77.4℃ for AOA and 79.0℃ for AOB) was found. In addition, no primer-dimer artifacts or other nonspecific PCR products were observed.

2.5. Statistical analysis

The sequences were initially rarefied to the same sampling-depth. Venn diagrams and petal pictures were generated using GraphPad Prism 8 (GraphPad Software, Inc.) to analyze overlapping and OTUs distribution across different sites and sampling times. The α-diversity indices (observed species, Chao1, Shannon, and Good-coverage) were calculated and analyzed using QIIME software (version 1.7.0). Normality of data and homogeneity of variances were checked using the Kolmogorov-Smirnov test and Levene's test, respectively. A two-way analysis of variance (ANOVA) was conducted to test the difference in physicochemical properties, alpha diversity and beta diversity of sediments, and *amoA* gene abundance in different sampling sites over four sampling times. If significant differences were detected, spatial and temporal differences in those parameters were further analyzed using one-way ANOVA, followed by Tukey's multiple comparison test (for homogeneous variances) or Tamhane's T2 multiple comparison test (for inhomogeneous variances). Statistical significance was considered at $P < 0.05$. All statistical analyses were performed by Statistica software (version 6.0, Stat. Soft Inc., USA) and SPSS software (version 19.0, IBM SPSS Statistics, Armonk, NY, USA).

To investigate the changes in community composition, multivariate analyses were performed by R software (version 3.5.1). The OTUs that serve as bioindicators were investigated by a three-step approach described by Rosado et al. (2019) and Lian et al. (2020), which contains the identification of OTUs with significant

spatiotemporal difference using least-squares means (LS means) by the "*lsmeans"* package, selection of OTUs according to the mean decrease in Gini index using random forests by the *"randomForest"* package, and finally intersecting the top 10 dominant species with highest relative abundance. Neutral community model (NCM) was performed with the *"minpack.lm"* package to reveal the relationship between the relative abundance variations of OTUs and their occurrence frequency. Correlation heatmaps, which were adopted to analyze the relationship between relative abundance of bioindicators, gene abundance, and environmental factors, were created with the *"corrplot"* package.

3. Results

3.1. Alpha diversity of ammonia-oxidizing microbial community

A total of 366 and 510 OTUs were obtained for AOA and AOB, respectively. The unique and shared OTUs of the AOA and AOB across each sampling site and time were compared (Fig. 2). The Venn diagram (Fig. 2a & 2c) showed that the highest number of AOA OTUs was observed in April (217), while the largest number of AOB OTUs was found in August (284). Both AOA and AOB had the fewest OTUs in June (AOA: 142, AOB: 253). The number of AOA OTUs shared across four months was 82, accounting for 22.40% of the total, while the number of AOB OTUs shared across four sampling months was 128, representing 25.10% of the total AOB OTUs. Across all sites, 34 core AOA OTUs, which are consistently present across all samples, were identified and accounted for 3.74% of the total (Fig. 2b). For AOB, 81 core OTUs were noted, comprising 6.99% (Fig. 2d).

The Good's coverage values were above 99.90%, indicating that the current sequencing data were sufficient to cover most of the AOA and AOB communities in the marine sediments. Although AOB had more OTUs than AOA, significant temporal and spatial differences were only observed in the number of AOA OTUs (Tab. 1), which was significantly lower at sites C and E than those at site A in August and April (Fig. 3a). The Shannon

indices for both AOA and AOB communities were significantly affected by the sampling site ($P < 0.001$) (Tab. 1). The diversity of both AOA and AOB was significantly lower at site E in August and December, and increased from site A to site E in April (Fig. 3c &d). The Chao1 indices for both AOA and AOB communities were significantly affected by sampling time ($P < 0.05$) (Tab. 1).

3.2. AOA and AOB community composition

A total of six genera were observed in AOA communities and were shown in Fig. 4a. The dominant genera with the highest relative abundance (> 10%) in the sediments were *Nitrosarchaeum* (81.45%) and *Nitrosopumilus* (10.48%). No significant difference was observed in the relative abundance of individual AOA genera among four sampling times ($P < 0.05$). Significant spatial difference was only observed in August and April ($P < 0.05$). In August, the relative abundance of *Nitrosarchaeum* was significantly different among all sampling sites as follows: site E > site A > site C ($P < 0.05$), and the relative abundance of *Nitrosopumilus* and *Candidatus_Nitrosopelagicus* showed the opposite trend. In April, the relative abundance of *Nitrosopumilus* was significantly higher at site E than those at the other sites, and *Candidatus_Nitrosomarinus* was significantly more abundant at site C than those at the other sites ($P < 0.05$).

Only two genera were found in AOB communities, *Nitrosomonas* is the dominant genus with the highest relative abundance (> 0.2%) (Fig. 4b). Sampling time had no significant effect on the relative abundance of AOB genera ($P > 0.05$). Only *Nitrosomonas* showed significant differences among different sampling sites ($P < 0.05$). The relative abundance of *Nitrosomonas* was significantly higher at site E than those at sites A and C in December and was significantly higher at site A than those at sites E and C in April ($P < 0.05$).

3.3. The *amoA* gene abundance

Two-way ANOVA showed that both spatial and temporal factors had significant effects on the abundance of archaeal and bacterial *amoA* genes ($P < 0.05$). The abundance of archaeal *amoA* genes (ranging from 9.03×10^2

to 2.30×10^5 copies·g^{-1}) was always lower than that of bacterial *amoA* genes (ranging from 4.52×10^4 to 2.53×10^5 copies·g^{-1}) in June, August, and April (Fig. 5). Both archaeal and bacterial *amoA* genes showed significant spatial difference in August, December and April ($P < 0.05$). Archaeal *amoA* genes was significantly higher in December than those in the other months, and was lowest in August ($P < 0.05$). Bacterial *amoA* genes was significantly lower in August than those in the other sampling times ($P < 0.05$).

3.4. Bioindicators

In total, we identified nine bioindicators for AOA and seven bioindicators for AOB communities. All AOA bioindicators belonged to the genera *Nitrosarchaeum*, *Nitrosopumilus*, and *Candidatus_Nitrosopelagicus*. OTU1 (average relative abundance: 40.57%) and OTU2 (average relative abundance: 31.74%) which were affiliated with species *Nitrosarchaeum_koreense* were the most abundant (Fig. 6). Only three AOA bioindicators (OTU313, OTU8, and OTU203) showed significant temporal differences, and they were all more abundant in December than those in the other sampling times ($P < 0.05$). Relative abundance of OTU203, OTU275, OTU4, OTU2, and OTU1 differed significantly among sampling sites. OTU1 was significantly more abundant at site E than that at sites C and A, while OTU275, OTU4, and OTU2 had significantly higher relative abundances at site C than that at sites A and E ($P < 0.05$).

Four AOB bioindicators (OTU386, OTU7, OTU202, and OTU5) were significantly different among four sampling times, and the relative abundance of OTU5 and OTU7 was significantly higher in December ($P < 0.05$). Only OTU324 showed significant spatial differences and its relative abundance was significantly higher at site C ($P < 0.05$).

3.5. Neutral community model

In the neutral community model (NCM) (Fig. 7), R squared (Rsqr) indicated the fit to this model, explaining 61.3% and 88.6% of community variance for AOA and AOB communities, respectively. This

suggests that stochastic processes play a more important role in shaping AOB community assembly compared to AOA. In the model, N represents the size of the metacommunity, m quantifies the migration rate within the community, and Nm (Nm=N ×m) integrates these two factors to estimate the level of dispersal within the communities (Sloan et al., 2006). The Nm value was higher for the AOB community (Nm = 5,501) than that for the AOA community (Nm = 164). Since the number of sequences in AOA samples was 54,198, and in AOB samples was 52,281, the m value was estimated to be 0.003 in the AOA community and 0.105 in the AOB community, respectively.

3.6. Pearson relationship between bioindicators, gene abundance and environmental factors

Among all physicochemical parameters, the average bottom water temperature was significantly higher in December, followed by August, June, and April ($P < 0.05$). The average DO in different sampling months followed: April > June > December > August, while TAN concentration in the pore water followed: April > December > August > June. The average carbon content in the sediment was significantly higher in June and August than that in the other two sampling times, whereas the average nitrogen content in the sediment showed the opposite trend (Table S1). Moreover, bottom water temperature, DO, and the concentrations of TAN, NO_2^--N and NO_3^--N in sediment pore water were significantly affected by sample site ($P < 0.05$). Based on Pearson correlation analysis (Fig. 8), temperature of the bottom water had a highly significant positive correlation with the abundance of archaeal *amoA* gene ($P < 0.001$) and significant positive correlation with the relative abundance of an AOA bioindicator (OTU8) ($P < 0.05$). TAN concentration in the pore water was significantly positive correlated with the relative abundance of an AOB bioindicator (OTU5) ($P < 0.05$). Archaeal *amoA* gene abundance was negatively and positively correlated with NO_2^--N and NO_3^--N concentration in the pore water, respectively ($P < 0.05$). The carbon content in the sediment only showed significant correlations with three AOA indicators (OTU203, OTU8, and OTU313) and abundance of the archaeal *amoA* gene, and the correlations were

both positive ($P < 0.05$). Dissolved oxygen concentration of the bottom water and nitrogen content in the sediment showed no significant correlations with the relative abundance of bioindicators and *amoA* gene abundances ($P > 0.05$).

4. Discussion

4.1. Composition of AOA and AOB communities and abundance of *amoA* genes in sediments

In the present study, the number of AOA OTUs shared across different sampling months and sites was consistently lower than that of AOB OTUs, indicating that AOA communities exhibited more pronounced temporal and spatial variability than AOB communities (Chen et al., 2020). Geographical factors significantly contribute to the structures of AOA and AOB communities (Dang et al., 2008; He et al., 2018). In our study, the shared and distinct AOA and AOB OTUs were associated with different sampling sites, consistent with previous reports. In addition, significant temporal and spatial differences were only observed in the number of AOA OTUs, suggesting a more heterogeneous distribution of AOA in the sediments of YSCWM compared to AOB. Contrary to previous studies, the OTUs number of AOB exceeded that of AOA in all YSCWM sediment samples (Shen et al., 2008; He et al., 2018). AOA generally predominated and exhibited more OTUs in various sedimentary environments, such as lakes (Hou et al., 2013) and estuaries (Jin et al., 2011). However, AOB has been found to have more OTUs than AOA in two freshwater aquaculture ponds with moderate ammonia concentrations (Dai et al., 2018). This aligned with our findings, where ammonia concentrations in the YSCWM sediments (30.23–202.94 μg/L) were elevated compared to oligotrophic marine environments. Such moderate ammonia levels may preferentially favor AOB over AOA (Cao et al., 2011; Hou et al., 2014). Additionally, previous studies suggest that AOB may respond more rapidly to nitrogen addition from aquaculture activities, particularly in environments with fluctuating ammonia availability and organic matter deposition. In contrast,

AOA tends to dominate in more stable, low-ammonia conditions (Carey et al., 2016).

Thaumarchaeota, the dominant phylum in the AOA community, played a crucial role in nitrification in marine waters and was frequently identified as the dominant phylum in many aquaculture areas (Veuger et al., 2013). In this study, the AOA community was primarily composed of two genera: *Nitrosarchaeum* and *Nitrosopumilus*. While *Nitrosopumilus* is widely recognized as the dominant genus and demonstrated the potential contribution to nitrification in the marine nitrogen cycle (Krümmel and Harms, 1982; Könneke et al., 2005; He et al., 2018; Pettersen et al., 2022), *Nitrosarchaeum* was rarely identified as the dominant genus in marine sediments. Instead, *Nitrosarchaeum* has been found to dominate in the freshwater systems, such as sediments of the Ganges River, deep lakes, and rivers (Ren and Wang, 2022; Samson et al., 2023). The dominance of *Nitrosarchaeum* in the YSCWM may be attributed to the presence of *Nitrosarchaeum koreensis*, a mesophilic, chemolithoautotrophic, neutrophilic, and aerobic ammonia-oxidizing archaeon, which is well-adapted to the unique environmental conditions of the YSCWM, including relatively oxygenated surface sediments and moderate ammonia concentrations (Kim et al., 2011; Jung et al., 2018)

In the AOB community, Proteobacteria was the dominant phylum, and *Nitrosomonas* was the dominant genus with the highest relative abundance. Previous studies have shown that most ammonia oxidizers in marine sediments were affiliated with Proteobacteria, with the majority of AOB belonging to the genera *Nitrosomonas* and *Nitrosospira* (Kong et al., 2017; Pelikan et al., 2021). For example, in the surface sediments of a grass carp pond, 76.92% and 23.08% of the AOB community were clustered into *Nitrosomonas-like* and *Nitrosospira-like* bacteria, respectively (Lu et al., 2019). In freshwater aquaculture systems, *Nitrosomonas* always dominated the AOB community, whereas *Nitrosospira* might be more adaptable in estuarine areas owing to the increase in salinity of estuarine and coastal environments (Dang et al., 2010). However, studies in deep-sea environments indicate that *Nitrosomonas*-like clusters always predominate in AOB communities and have a high tolerance to

high nutrient and extreme environments, such as wastewater treatment plants and deep-sea hydrothermal plumes (Lam et al., 2004; Geets et al., 2006). In offshore systems, *Nitrosomonas* has also been reported as the predominant AOB, likely due to the substantial amounts of ammonium input from agricultural runoff, which enhances ammonia availability and supports its proliferation (Beman and Francis, 2006). Given the seasonal hydrodynamic changes in the YSCWM, including thermocline formation and organic matter deposition from aquaculture activities, *Nitrosomonas* may have a competitive advantage in ammonia oxidation. In addition, its dominance may be attributed to its adaptability to fluctuating oxygen levels and nutrient inputs, which were affected by seasonal stratification and organic matter mineralization (Urakawa et al., 2008). This adaptability enables *Nitrosomonas-like* bacteria to outperform other AOBs under the environmental conditions in the YSCWM.

The abundance of ammonia oxidizers may indicate the potential nitrification rates in the studied area (Zheng et al., 2014). In this study, the copies of the *amoA* gene in the sediments were most abundant in December, demonstrating that the ammonia oxidation process might be more active during this period. This is aligned with previous studies that AOA and AOB *amoA* gene copy numbers were greater in summer than those in winter due to the higher temperature, and *amoA* gene copy numbers could be consistent with the temperature dynamics within a long term (Beman et al., 2012; Vissers et al., 2013). The elevated bottom water temperature (13.30℃) and carbon content in December likely drove the increased abundance of archaeal *amoA* genes, highlighting the importance of temperature and carbon availability in regulating ammonia-oxidizing communities (Dai et al., 2018; Li et al., 2022a). The abundance of archaeal *amoA* genes in our study was similar to that in grass carp pond sediments in central China and deep-sea sediments in the west Philippine basin, but lower than in lakes, bays, and rivers (Liu et al., 2011; Hou et al., 2013; Lu et al., 2015; He et al., 2018; Lin et al., 2021). Some previous studies reported that the archaeal *amoA* gene was always two to three orders of magnitude

more abundant than the bacterial *amoA* gene in the open ocean (Caffrey et al., 2007; He et al., 2018). In contrast, our results showed a different trend, and this was consistent with the results in the Yangtze Estuary, where the AOB *amoA* copy numbers were also greater than AOA *amoA* copy numbers at most of the sampling sites and times (Zheng et al., 2014), aligning with our finding that AOB tended to dominate in the sedimentary environment of the YSCWM.

The distinct ecological roles and community structures of AOA and AOB suggest that they may differentially influence nitrogen cycling and ecosystem functioning in the YSCWM. *Nitrosomonas*, the dominant genus of AOB, likely plays a key role in ammonia oxidation and nitrogen turnover under relatively elevated ammonia concentrations and eutrophic conditions (Beman and Francis, 2006; Geets et al., 2006). In contrast, the coexistence of AOA genera such as *Nitrosopumilus* and *Nitrosarchaeum* may reflect an ecological buffering capacity, enabling functional stability under fluctuating environmental conditions (Stahl and de la Torre, 2012).

Although we initially expected to observe a significant impact of aquaculture intensity across sampling sites, one-way ANOVA revealed no clear trend in its influence on archaeal and bacterial *amoA* gene abundance. However, environmental factors such as bottom water temperature—known to be influenced by aquaculture practices—showed significant correlations with *amoA* gene abundance. This observation can be explained from two perspectives. First, “Deep Blue 1” is the first and only submersible cage in the studied area, and its impact on the sediment environment is relatively minor compared to the effects of seasonal effects. Second, it suggests that aquaculture activities may indirectly shape the abundance and distribution of ammonia-oxidizing microbes by altering the sedimentary and bottom water environment. Thus, the influence of aquaculture on microbial gene abundance patterns may be indirectly mediated, owing to the complex interactions among environmental factors and sedimentary biogeochemical dynamics. However, this study only qualitatively and quantitatively assessed *amoA* gene abundance without measuring nitrification rates, which limits the mechanistic understanding of

nitrogen turnover in YSCWM. Therefore, future research should focus on correlating *amoA* gene abundance with nitrification rates, and evaluating how shifts in AOA and AOB community structures impact nitrogen cycling, sediment biogeochemistry, and the functional resilience of YSCWM ecosystem under dynamic hydrological conditions.

4.2. Bioindicators and environmental drivers of AOA and AOB communities

Soil microorganisms are the key drivers of nutrient cycling and are widely regarded as strategic bioindicators of soil quality (Vallejos et al., 2022). Genetic profiling of the AOB community has been proposed as a bioindicator method, the presence-absence pattern of which could reflect the changes in community composition (Ritz et al., 2009; Metze et al., 2021). In this study, the bioindicators of AOA communities were affiliated with genera *Nitrosarchaeum*, *Nitrosopumilus*, and *Candidatus_Nitrosopelagicus*. The first two genera were the dominant genera of the AOA community in this study, and *Candidatus_Nitrosopelagicus* was reported to be an important model when assessing the adaptation of archaea to the open ocean (Santoro et al., 2015).

Spirochaeta 2 is reported to be a bioindicator in the highly aquaculture-impacted sediments in the salmon aquaculture area (Kolda et al., 2020). Some OTUs affiliated with class Anaerolineae also have the potential to be bioindicators in the eutrophicated sediment affected by aquaculture (Moncada et al., 2019). However, in the present study, no taxa of AOA and AOB has been reported as an aquaculture-affected bioindicator in the aquaculture area, likely because aquaculture activities in this area have just started with low intensity (Dong, 2019; Li et al., 2022b). Apparent temporal differences in the abundance of *amoA* genes and bioindicators were observed, suggesting that seasonal environmental fluctuations played a greater role than aquaculture in shaping ammonia-oxidizing microbial communities.

Among the key environmental factors, temperature and carbon content were the strongest predictors of AOA *amoA* gene abundance and bioindicator presence. The higher bottom water temperature in December (13.30°C)

was significantly correlated with increased AOA *amoA* gene abundance and the relative abundance of specific AOA bioindicators. This finding aligns with previous studies showing that temperature is a major driver of AOA distribution and metabolic activity (Lee et al., 2011; Zheng et al., 2014; Ming et al., 2020; He et al., 2018). Carbon content has been also identified as a key parameter affecting archaeal *aomA* abundance and AOA indicators. Increasing organic carbon availability can promote AOA community development in aquaculture sediments (Dai et al., 2018; Limpiyakorn et al., 2013). Carbon content was found to be positively correlated with AOA OTUs distribution and the relative abundance of the community based on canonical correlation analysis or correlation analysis in many studies, further supporting our findings (Dai et al., 2018; Ming et al., 2020).

AOB community assembly was largely driven by stochastic processes. A neutral community model (NCM) was used to explain the distribution patterns of bacteria and archaea communities and the relative abundance and occurrence frequency of different taxa (Sloan et al., 2006). The NCM suggested that a stochastic balance between loss and gain (such as stochastic births, deaths, and immigration) was critical in shaping the AOB community assembly, indicating that species dispersal of the AOB community was higher than that of the AOA community. Many studies have also observed importance of stochastic processes in shaping microbial community. For instance, Roguet et al. (2015) found that stochastic processes accounted for a major proportion of variation (R^2 = 76%) in lake bacterial communities. Similarly, Mao et al. (2025) reported that the turnover of unique OTUs across different aquaculture pond types was predominantly driven by stochastic processes—including weak selection, limited dispersal, and ecological drift. These findings underscore the widespread influence of stochastic assembly processes in diverse aquatic microbial communities.

5. Conclusion

This study investigated the spatiotemporal dynamics of ammonia-oxidizing archaeal (AOA) and bacterial

(AOB) communities in the aquaculture area of the Yellow Sea Cold Water Mass. AOB exhibited higher OTU richness and stability, while AOA showed greater temporal and spatial variability, suggesting a higher sensitivity to environmental conditions. AOB community assembly was driven more by stochastic processes, whereas AOA distribution was strongly influenced by environmental factors. Temporal variations were particularly significant in December, with higher bottom water temperatures and sediment carbon content correlating with an increased abundance of archaeal *amoA* genes and AOA bioindicators. This suggests that temperature and carbon content, were the direct key drivers shaping ammonia-oxidizing microbial communities. Despite observing significant spatial variations in *amoA* gene abundance at certain sampling times, no clear trend emerged regarding the influence of aquaculture activities on this abundance. These findings highlight the intricate nature of the interactions among aquaculture, the environment, and microbial communities. This study enhanced our understanding of the ecological roles of AOA and AOB in marine nitrogen cycles and their responses to environmental changes.

CRediT authorship contribution statement

Shuting Li: Investigation, Methodology, Formal analysis, Writing-original draft. **Li Li**: Conceptualization, Validation, Writing-review & editing. **Xiangli Tian**: Methodology, Writing-review & editing. **Qinfeng Gao**: Resources, Writing-review & editing. **Shuanglin Dong**: Project administration, Supervision, Writing-review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All the data generated and analyzed during this study are included in the article.

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