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Research Article

Fish and Shellfish Aquaculture Impact on the Sediment Bacterial Communities in Xiangshan Bay, China

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As the increase of demand for seafood supplies, a large amount of high-protein feed has been added to mariculture aeras, which causes hazards for the cultured organisms and environment. Here, we used 16S rRNA gene high-throughput sequencing to evaluate the bacterial communities in the sediments of two aquaculture systems (fish and oyster culture) in Xiangshan Bay, China for four seasons. Analysis of variance results showed that the abundance and biodiversity differed significantly between the culture areas and control areas, and different seasons as well. Proteobacteria, Planctomycetes, and Bacteroidetes were the three most abundant phyla. Gammaproteobacteria, Deltaproteobacteria, and Bacteroidia were the three most abundant classes. Principal co-ordinates analysis showed that sediment bacterial community composition in the fish and oyster culture areas significantly differed from that in the control areas. Analysis of the function prediction of pathways related to the nitrogen cycle in sediments revealed a higher proportion of genes involved in denitrification in aquaculture farms. Nitrate respiration particularly occurred in fish areas, whereas nitrate reduction occurred both in fish and oyster areas, and the proportions in winter were significantly higher than that in other seasons. It also provides insights into the indirect effects of human aquaculture activities on coastal algal blooms.

1. Introduction

Aquaculture is a fast-growing, highly valued, and extremely important sector of the global seafood industry; according to estimation, it will likely account for more than 60% of global seafood production by 2030 [1–3]. While aquaculture provides economic benefits for humans, it is detrimental to aquatic ecosystems. To improve the production, a large amount of high-protein feed is added to mariculture ponds [4]. This alters the aquatic environment, causing a series of environmental problems, and also affects the physical and chemical properties and biological composition of seabed sediments, thereby affecting the material recycling and energy flow of marine ecosystems [5, 6].

Previous studies have shown that the nitrogen utilization rate of feed was approximately 11%–36%. Most of the nitrogen was transferred to the aquaculture environment in the form of residual feed (such as fish mince, rice bran, and corn) and feces, which resulted in eutrophication and oxygen depletion in the surrounding sea areas [6, 7] and released organic nitrogen, ammonia, nitrate, and nitrite during decomposition [8]. In particular, large amounts of organic nitrogen were deposited in the sediments, presenting a long-term hazard to the cultured organisms and the environment [9], and they altered the biogeochemical processes and benthic communities [6, 10, 11]. Additionally, owing to sewage emissions, the nitrogen load pollution from aquaculture exacerbated the eutrophication of the surrounding sea area, which may lead to ecological disturbances such as harmful algal blooms [12]. Though nitrogen like that found in fishmeal and soybeans is not used in oyster farming, oyster farming can potentially influence the availability of oxygen, nitrogen, and carbon in the sediments [13]. Oyster farming can incorporate nutrients via water filtration and direct nutrients to the sediments by

biodeposition to reduce nutrient loads in the water [13, 14]. Oyster biodeposits contain two to three times more carbon and nitrogen than control areas [15]; thus, oysters also alter sediment chemistry. Furthermore, the resuspension of sediment disturbed by aquaculture can enhance the release of internal nutrients and pollutants, which subsequently influences the aquatic environment and plankton community [16, 17].

Bacterial populations can respond quickly to environmental changes, and the community structure shifts with the enrichment of aquaculture farms [10, 18]. Previous studies demonstrated that fish farming drastically reduced bacteria diversity in sediments, and this indicated that the fundamental biological processes of sedimentary bacterial ecosystems have been disrupted [10, 19]. Biological sediments associated with oyster culture may enhance communities of denitrifying bacteria, which convert bioavailable nitrogen to its gaseous form and eventually allow it to leave the system. Considering the increases in coastal nitrogen loads, this global ecosystem service is important [20].

Recently, many molecular biological techniques have been adopted to examine the bacterial component in marine aquaculture ponds. Maximum-likelihood analysis and Bayesian classification were used to predict typical habitats for sediment samples collected at fish farms in the Baltic Sea [21]. High-throughput sequencing and quantitative polymerase chain reaction (PCR) were used to examine the effect of oyster farming on sediment bacterial communities [22]. The communities of nitrifying bacteria in shrimp ponds in Thailand were identified by specific PCR amplification of 16S rDNA [23]. The sediment metagenome of tropical bioaugmented shrimp culture ponds was analyzed to determine the diversity, distribution, and abundance of anammox bacteria [24]. However, the difference of sediment bacterial composition related to the nitrogen cycle between different farming systems and the effect of seasonal dynamics on bacteria communities are still remained to be studied.

Xiangshan Bay (XSB) is a long (approximately 60 km), narrow (around 3–8 km), eutrophic, semienclosed subtropical bay located at the north coast of Zhejiang Province [25]. Its water is sourced mainly from the coastal waters, including the Yangtze River Diluted Water, and the water-residence times are approximately 80, 60, and 15 days in the upper, middle, and lower zones, respectively, for 90% water exchanges [25–28]. It is one of the most important marine aquaculture areas in China. The main species include cultured fish such as large yellow croaker and bass that are farmed in cages, suspended oysters, and kelp. More than 50,000 tons of oysters, crabs, shrimp, and fish are produced in XSB, and more than 68% of aquaculture is concentrated in three inner bays: Tiegang Branch Bay (TG), Huangdonggang Branch Bay (HDG), and Xihugang Branch Bay (XHG) [25]. XSB has been affected by many human activities since the 1980s, including eutrophication driven by industrial and agricultural sewage disposal, tidal flat reclamation, and mariculture expansion [25, 28–30]. The aquaculture areas in XSB in 2016 increased by at least 1.5 times compared with that of 2006, in which suspended oyster farming areas increased by twice times, but fish cage-culture areas decreased gradually.

In this study, we characterized seasonal changes in the sedimentary bacterial community composition and the diversity associated with fish and oyster farming by 16S rDNA Illumina Miseq sequencing in XSB, China, focusing on bacterial taxa involved in the nitrogen cycle. We hypothesized that fish and oyster farming could increase organic matter in the sediments beneath the farming areas, alter the composition of sediment bacterial communities, and even affect the nitrogen cycle of the local area. And the composition of sedimentary bacterial communities would exhibit different patterns in different seasons.

2. Material and Methods

2.1. Study Area and Sampling Methods. Sediment samples were collected from XSB, Zhejiang, China (29°24′–29°46′N, 121°25′–122°00′E) in July (summer) and October (autumn) of 2019 and January (winter) and May (spring) of 2020.

Twelve sampling points were set up from four areas in XSB (Figure 1): the oyster farming zone (Oy) and the nearby control zone (Oc), the fish farming zone (Fi), and the nearby control zone (Fc). Areas approximately 1 km away from the aquaculture areas with similar sedimentary environments were selected as the control areas. During each survey season, sediment samples were collected at each sampling point using three sediment cores (12 cm diameter and 30 cm depth); the sediment of the top 5 cm was then remained and mixed uniformly for each core. Immediately after collection, the subsamples were immediately placed in precleaned polyethylene bags and then stored in liquid nitrogen until they could be transferred to freezers set at −80°C. The sample used for total organic carbon (TOC) analysis was first acidified with excessive $1 \, mol \, l^{-1}$ HCl to remove carbonates, and the TOC content was then determined in an elemental analyzer (PE2400 Series II, PerkinElmer, Norwalk, CT, USA). The TN and TC contents were determined using the same elemental analyzer.

2.2. DNA Extraction, PCR Amplification, and Sequencing Analysis. The total genomic DNA in 0.25 g (wet weight) of each sediment sample was extracted using the TGuide S96 DNA extraction kit (TIANGEN, Beijing, China). DNA extraction, primer synthesis, and PCR were completed at Biomarker Technology Co. Ltd. (Beijing, China). After the total DNA of the sample was extracted, the 16S rRNA gene was amplified using the universal primer 27 F (5′-AGRGTTTGATYNTGGCTCAG-3′) and 1492R (5′-TASGGHTACCTTGTTASGACTT-3′) described by Weisburg et al. [31]. For each PCR reaction, Master Mix was prepared with 15 μl of KOD OneTM (Biolink Technology, Beijing Co., Ltd., China), 10.5 μl of NFW (Biolink Technology, Beijing Co., Ltd., China), 3 μl of barcode primers, and 1.5 μl DNA template in a volume of 30 μl. The PCR program involved an initial denaturation step at 95°C for 2 min; 25 cycles of 98°C for 10 s, 55°C for 30 s, and 72°C for 90 s; and a final 2 min extension at 72°C. The PCR product of each sample was
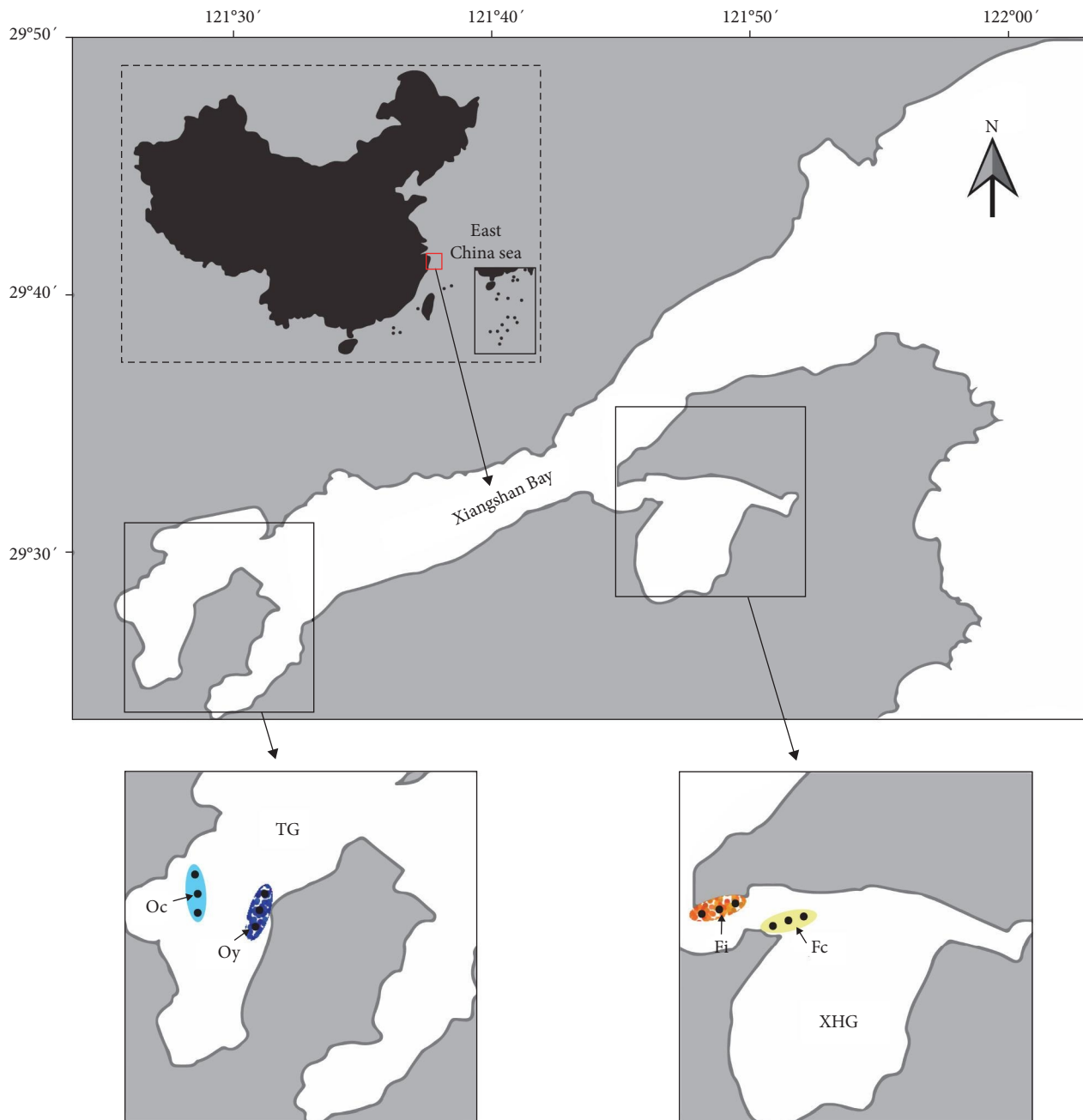
FIGURE 1: Locations of fish cage culture and suspended oyster culture in Xiangshan Bay. Fi: fish cage culture area; Fc: fish control area; Oy: oyster culture area; Oc: oyster control area; TG: Tiegang Branch Bay; XHG: Xihugang Branch Bay.

purified, quantified, and homogenized to form a sequencing library (based on the SMRTbell Template Prep Kit provided by PacBio, USA). The established library was first subjected to library quality inspection, and the library that had passed the quality inspection was sequenced on Sequel II (Pacbio, USA). The sequenced data were in BAM format, and the data of different samples were identified according to the barcode sequence and converted into FASTQ format.

2.3. Assessment of Sequencing Data Quality. Raw paired-end reads were merged using FLASH Version 1.2.11 [32]. The min overlap was 10 bp, and the max error ratio was 0.2.

Trimmomatic Version 0.33 [33] was used to filter quality, exploit the Illumina quality score of each base position to determine where the read should be cut, resulting in the retention of the 5′ portion, while the sequence on the 3′ of the cut point is discarded. The Trimmomatic parameters were as follows: the Sliding Window was 50 bp, and the average quality in the Sliding Window was 20.

UCHIME Version 8.1 [32, 34] was used to identify and remove chimera sequences. The query sequence was divided into four nonoverlapping segments (chunks); the best matches to each chunk were noted, and the two best candidate parents were identified from matches to all chunks. A three-way

multiple alignment of the query to these two candidates was constructed. If a pair of segments extracted from these two candidates had an identity ≥0.8% closer to the query sequence than either candidate alone, a score was computed from the alignment, and a chimera was reported if the score exceeded a predetermined threshold. All the sequencing data had been submitted to NCBI with the accession number PRJNA812702.

2.4. *Data Analysis.* Usearch [35] was used to cluster tags at 97% similarity to get OTUs, and annotate OTU taxonomy based on the Silva (Release132, http://www.arb-silva.de) and UNITE (Release 8.0, https://unite.ut.ee/) databases. By comparing the representative OTU sequences with the bacterial reference database, the species classification information of each OTU was obtained, and the community composition of each sample was calculated at different levels (phylum, class, order, family, genus, species). The species abundance tables at different classification levels are generated by QIIME software [36]. The community structure map of the samples at each taxonomic level was then drawn using the R language tool.

QIIME [36] was used to select the most abundant OTU sequences at the genus level as the representative sequences, perform multiple sequence alignment, and evaluate the alpha diversity index of the samples. PyNAST [37] (Version 1.2.2) and ClustalW2 [38] were used for bacteria to build the phylogenetic tree using the neighbor-joining method; the figure was then drawn using the Python language tool [39]. According to the taxonomic database of existing bacterial species provided by NCBI. The features, Chao1, Shannon diversity, Simpson diversity indices, and principal co-ordinates analysis (PCoA) were calculated by QIIME [36]. The PCoA results were drawn using the R language tool based on the four algorithms obtained from the beta-diversity analysis. Redundancy analysis (RDA) was analyzed by Canoco 5 [40]. Heatmap was analyzed by Origin Pro 2021 (OriginLab, Northampton, MA, USA). Analysis of variance tests was mainly used to detect differences in the mean values of multiple samples (i.e., to assess the significance of seasons and sampling sites).

2.5. *Partial Function Prediction of 16S rRNA Gene.* FAPRO-TAX [41] is a manually constructed database that maps prokaryotic taxa (e.g., genera or species) to metabolic or other ecologically relevant functions (e.g., nitrification, denitrification, or fermentation) based on the literature on cultured representatives. Functions represented in FAPROTAX focus on marine and lake biogeochemistry—particularly sulfur, nitrogen, hydrogen, and carbon cycling—which aligns with our research goals. The conversion of OTU/taxon tables to function tables based on FAPROTAX was performed using Python script (collapse_table.py), and then taxa were affiliated with functional groups by extrapolating the knowledge on a subset of well-studied data to all other organisms based on existing literature.

3. Results

3.1. *Sediment Chemical Variables of XSB.* Figure 2 shows the variation of TN, TC, and TOC in different areas in winter and spring. It was observed that TN, TC, and TOC were all significantly higher in fish cage culture area than oyster culture area in winter ($p < 0.001$). There were more TN, TC, and TOC in oyster culture area in winter compared with oyster control area ($p < 0.01$), and more TN and TOC in spring ($p < 0.001$). There were more TN and TOC in fish cage culture area in spring compared with fish control area ($p < 0.05$).

3.2. *Bacterial Community Composition of XSB.* A total of 282,523 circular consensus sequencing (CCS) sequences were obtained after barcode identification, and an average of 5,886 CCS sequences were generated by each sample. A total of 2,082 OTUs were obtained, and each sample ranged from 402 to 1,264. Figure 3 shows that the Feature, Chao1, and Shannon–Wiener index in the oyster culture area in spring were higher than that in the fish cage culture area ($p < 0.05$), while the Simpson index in the oyster culture area in winter was lower than that in the fish cage culture area ($p < 0.05$). However, no significant differences were found in summer and autumn ($p > 0.05$).

All OTUs were assigned to 39 phyla and 413 species. The compositions of bacteria at phylum level in the two farms, two control areas, and four seasons are shown in Figure 4(a). The most abundant phylum by far was the proteobacteria (45.39% of total sequences), with the maximum proportion (62.35%) in the winter Oc sample and minimum proportion (37.54%) in the summer Fi sample. Other predominant phyla included Planctomycetes (maximum of 19.22% in the autumn Oy sample; minimum of 5.40% in the spring Fc sample), Bacteroidetes (maximum of 16.79% in the spring Fi sample; minimum of 6.04% in the spring Oy sample), some rare bacterial phyla including Acidobacteria (3.18%–7.97%), Gemmatimonadetes (2.05%–5.69%), Verrucomicrobia (0.79%–5.48%), Nitrospirae (1.19%–4.38%), Chloroflexi (0.35%–4.20%), Kiritimatiellacota (1.19%–3.36%), and Cyanobacteria (0.20%–6.34%). Spearman analysis showed that the relative abundance of Verrucomicrobia had a significant positive relationship with TC, while the relative abundance of Proteobacteria had a significant negative relationship with TC (Figure 4(b)).

The community composition of bacterial classes was also investigated (Figure 4(c)). Gammaproteobacteria and Deltaproteobacteria were the two most abundant classes in the Proteobacteria phylum, accounting for 17.48%–40.12% and 9.89%–25.72% of all sequences, and Alphaproteobacteria accounted for 3.77%–10.73%. In the Planctomycetes phylum, Phycisphaerae accounted for 1.15%–6.80%, Planctomycetacia accounted for 1.30%–6.51%, OM190 accounted for 1.31%–3.72%. Other rare bacterial classes, including Bacteroidia (5.17%–16.18%), Verrucomicrobiae (0.79%–5.48%), and Thermoanaerobaculia (0.95%–3.86%). Spearman analysis showed that TC had a significant positive relationship with the relative abundance of Verrucomicrobiae, while it had a significant negative relationship with the relative abundance of Phycisphaerae and Gammaproteobacteria. TN had a significant negative relationship with the relative abundance of Thermoanaerobaculia, and TOC had a significant negative relationship with the relative abundance of Phycisphaerae (Figure 4(d)).
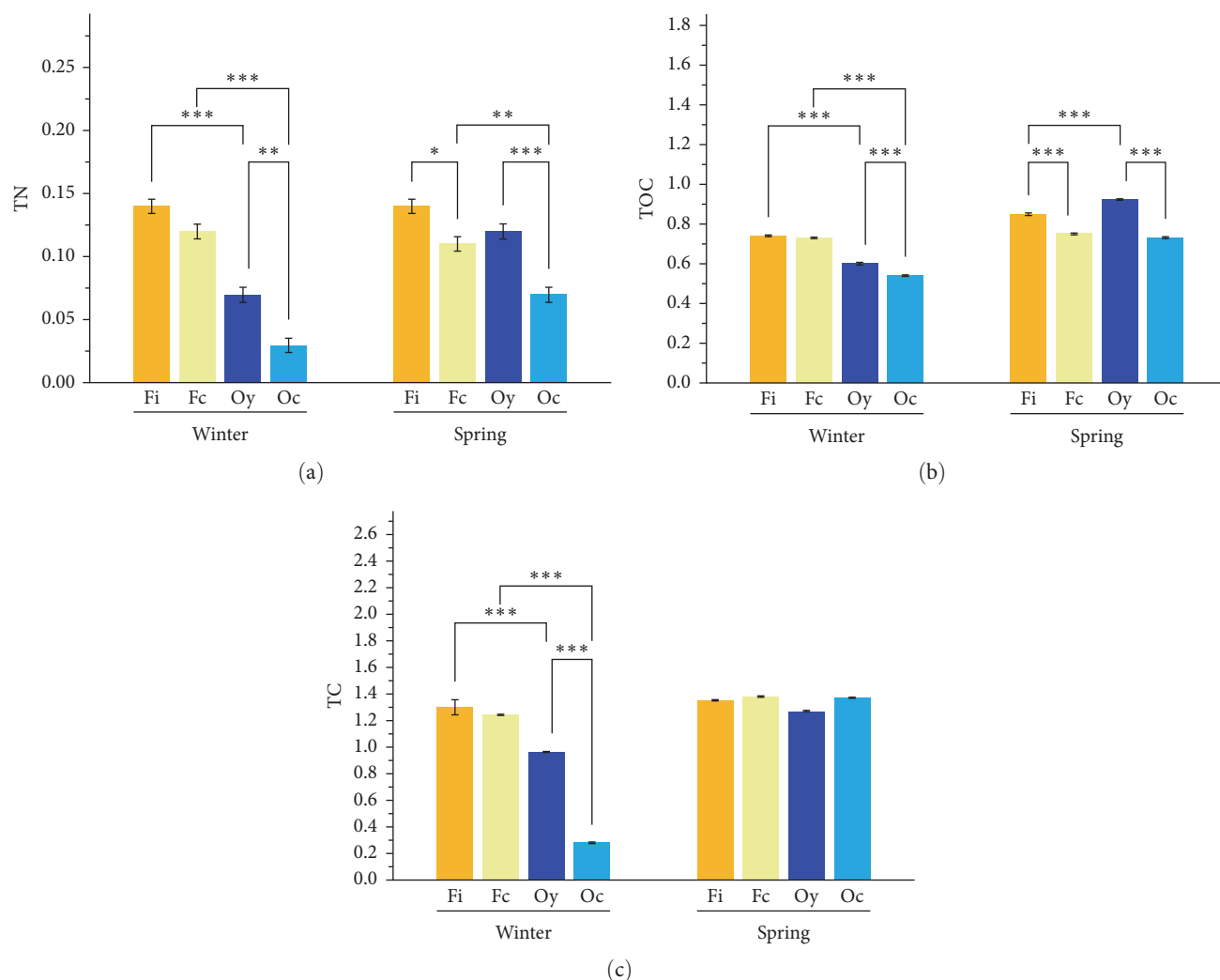
FIGURE 2: Comparison of selected sediment chemical variables in fish cage culture area (Fi), fish control area (Fc), oyster culture area (Oy), and oyster control area (Oc). (a) TN, total nitrogen; (b) TOC, total organic carbon; (c) TC, total carbon. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

3.3. Significant Differences in the Bacterial Community. To explore the distributions of bacterial communities in sediment ecosystems, community structure comparisons were performed by PCoA. In general, most of the samples from different sampling sites and different seasons were well-separated along the first two principal coordinate axes, explaining 44.67% of the total variation in sediment community compositions (Figure 5(a)). All the samples showed a positive relationship with TN, TC, and TOC (Figure 5(b)). Samples from the fish cage culture area showed a stronger positive relationship with TN than other areas, while samples from the fish control area showed a stronger positive relationship with TC and TOC than other areas. Then, RDA was conducted on the bacterial abundance and environmental factors of the 10 most abundant phyla in spring and winter. The results showed that TN had a positive relationship with all phyla except Chloroflexi in spring and had the strongest positive relationship with Bacteroidetes. TC had a positive relationship with Cyanobacteria, Verrucomicrobia, Kiritimatiellaeota, and Bacteroidetes and had the strongest positive relationship with Cyanobacteria. TC had a negative relationship

with Proteobacteria, Planctomycetes, Gemmatimonadetes, Nitrospirae, Acidobacteria, and Chloroflexi and had the strongest negative relationship with Planctomycetes. TOC had a positive relationship with Proteobacteria, Planctomycetes, Gemmatimonadetes, Nitrospirae, Acidobacteria, Bacteroidetes, and Kiritimatiellaeota and had the strongest positive relationship with Proteobacteria. TOC had a negative relationship with Verrucomicrobia, Chloroflexi, and Cyanobacteria and had the strongest negative relationship with Cyanobacteria (Figure 5(c)). In winter, TN, TC, and TOC had a positive relationship with Chloroflexi and Verrucomicrobia, and all had the strongest positive relationship with Chloroflexi. Among them, TN had the strongest negative relationship with Acidobacteria, TC had the strongest negative relationship with Nitrospirae, and TOC had the strongest negative relationship with Bacteroidetes (Figure 5(d)).

3.4. Function Prediction of the Bacteria. Here, we focused on the genes involved in nitrogen cycle (Tables 1 and 2). Among the four sampling sites, we found little difference in the proportion of genes for aerobic ammonia oxidation, aerobic
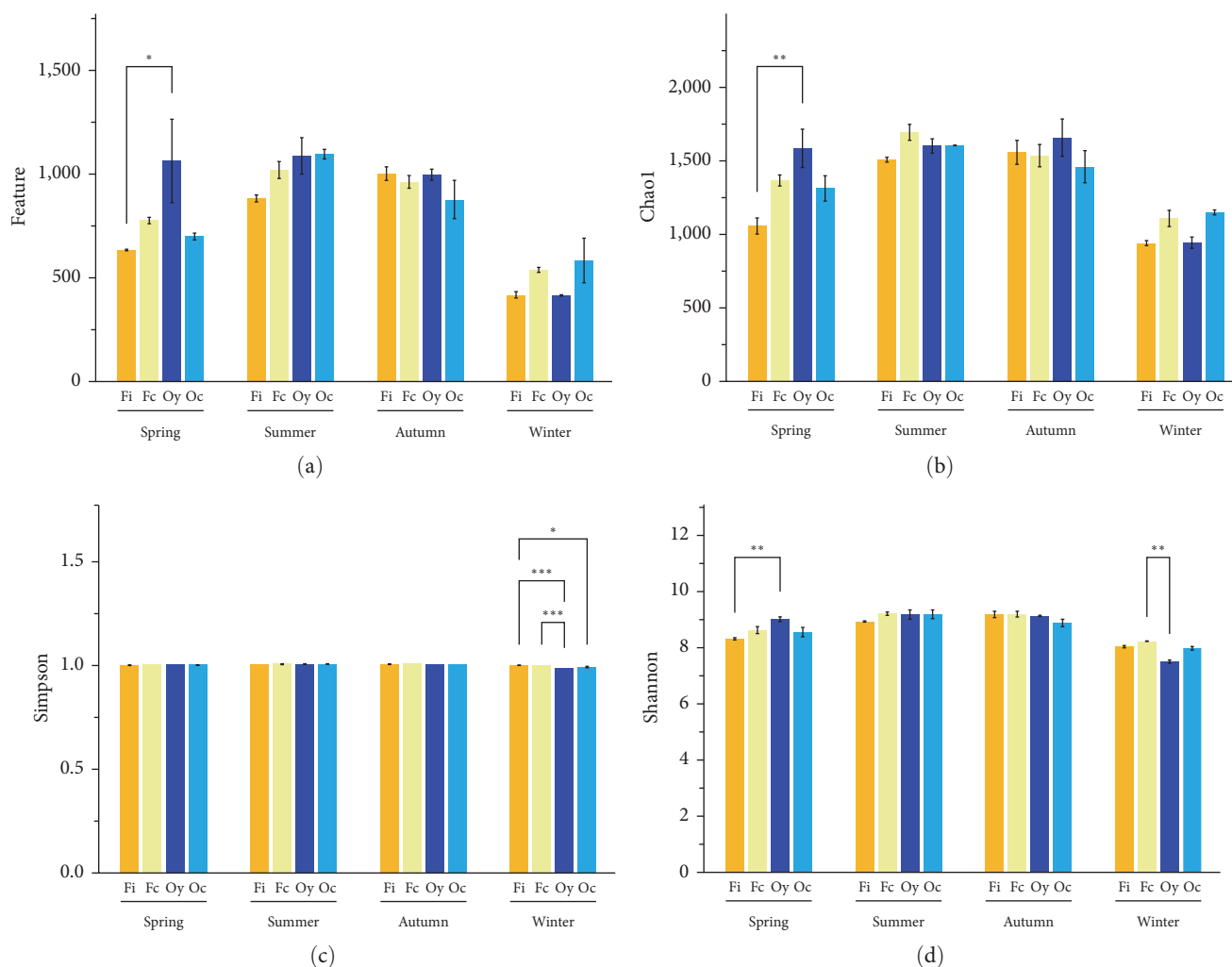
FIGURE 3: Comparison of selected alpha diversity indexes in fish cage culture area (Fi), fish control area (Fc), oyster culture area (Oy), and oyster control area (Oc) in four seasons. (a) Feature = OTUs; (b) Chao1 = Chao1 index; (c) Simpson = Simpson index; (d): Shannon = Shannon index. $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

nitrite oxidation, and nitrification ($p > 0.05$; Table 1), and no significant difference was found between oyster farms and fish farms ($p > 0.05$; Table 1). However, the oyster farms had higher proportions of genes involved in nitrate respiration and nitrate reduction compared with the Oc areas ($p < 0.05$; Table 1). The fish farms showed higher proportions of genes involved in nitrate reduction compared with the Fc areas ($p < 0.05$; Table 1). Otherwise, the proportion of genes for all the pathways involved in nitrate reduction peaked in winter, especially for the genes involved in aerobic ammonia oxidation and nitrification ($p < 0.05$; Table 2). Our results suggest that there were higher proportions of denitrifying bacteria communities in the fish and oyster farms than in the control areas and higher proportions of ammonia oxidation and nitrification bacteria communities in winter than in other seasons.

4. Discussion

4.1. Impacts of Fish and Shellfish Cultures on Environmental Parameters.
Several studies have shown that the physicochemical properties of sediments in the study area are

affected by fish and oyster culture. Karakassis et al. [42] found that the content of organic carbon and nitrogen in sediments near fish farms increased by 1–1.5 as compared with the control areas. Callier et al. [43] reported that the amount of biodeposits produced by mussels was about 32.4–51.5 mg dry wt d^{-1} ind.$^{-1}$, respectively, and the deposition rate in the farms was greater than that in the control areas. In this study, the physicochemical properties of the sediment of XSB showed significant changes under different aquiculture types and seasons (Figure 2). The TN and TOC in culture areas were significantly higher than those in control areas in spring. This result may be because the culture areas were more fertile than control areas. The TN, TC, and TOC in fish cage culture area were significantly higher than those in oyster culture area. It is generally accepted that fish culture induces more nutrient loading into the seawater environment than shellfish culture. Sediment resuspension caused by fish activities can affect the physical and chemical properties of sediment—for example, by increasing the degradation and mineralization of organic matter in water, resulting in high nitrogen and phosphorus [44]. Oysters, however, do
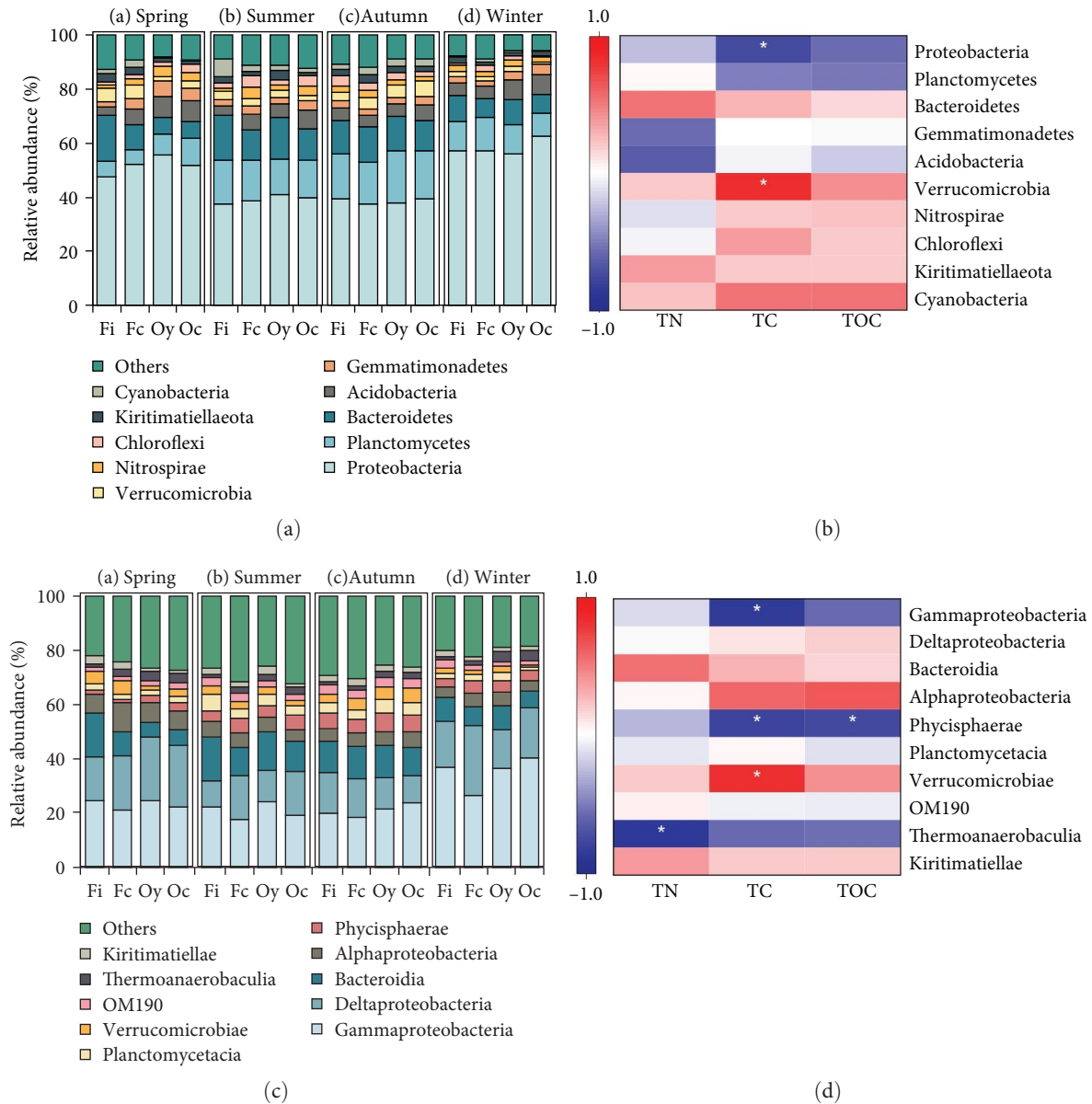
(a)

(b)

(c)

(d)

FIGURE 4: Relative abundance of bacterial communities at the phylum level (a) and class level (c) in sediment samples from Xiangshan Bay in four seasons. Spearman analysis of physicochemical factors and relative abundance of microbiota at the phylum level (b) and class level (d) in sediment samples from Xiangshan Bay in spring and winter. Fi, fish cage culture area; Fc = fish control area; Oy = oyster control area; Oc, oyster control area. TN, total nitrogen; TC, total carbon; TOC, total organic carbon. $^*p < 0.05$.

not need feeds; they absorb nutrients from the water, resulting in the accumulation of nitrogen in sediments through biodeposition [13, 14]. These two different aquaculture modes have different impacts on the local sediment environment.

4.2. Response of Sediment Bacterial Community to Fish and Shellfish Culture. The bacterial community typically responds quickly to environmental changes by regulating its composition and metabolism. Previous studies reported changes in bacterial abundance in sediments beneath aquaculture farms, such as differences in bacterial composition between cultured and uncultured areas [45, 46], the emergence of nitrate-reducing bacteria [47], and a significant reduction in bacterial diversity [48]. Our study focused on the effects of seasonal

dynamics on bacterial communities in sediments of fish farms and oyster farms. We found that the abundances of Proteobacteria, Planctomycetes, and Bacteroides—the three most widely distributed phyla in the sediments of the two farms—changed across seasons and regions. The abundance of Proteobacteria was higher in winter and spring, yet lower in summer and autumn, which is related to the geographical environment of XSB. XSB is located in the inner bay, and it has a lengthy water retention time, abundant nutrients, and relatively high transparency and water column stability, especially during the cold seasons. Past studies have found that the temperature increase caused by the heat emission from a nuclear power plant promoted the growth of phytoplankton and even led to blooms [29, 49, 50]. Beginning with the
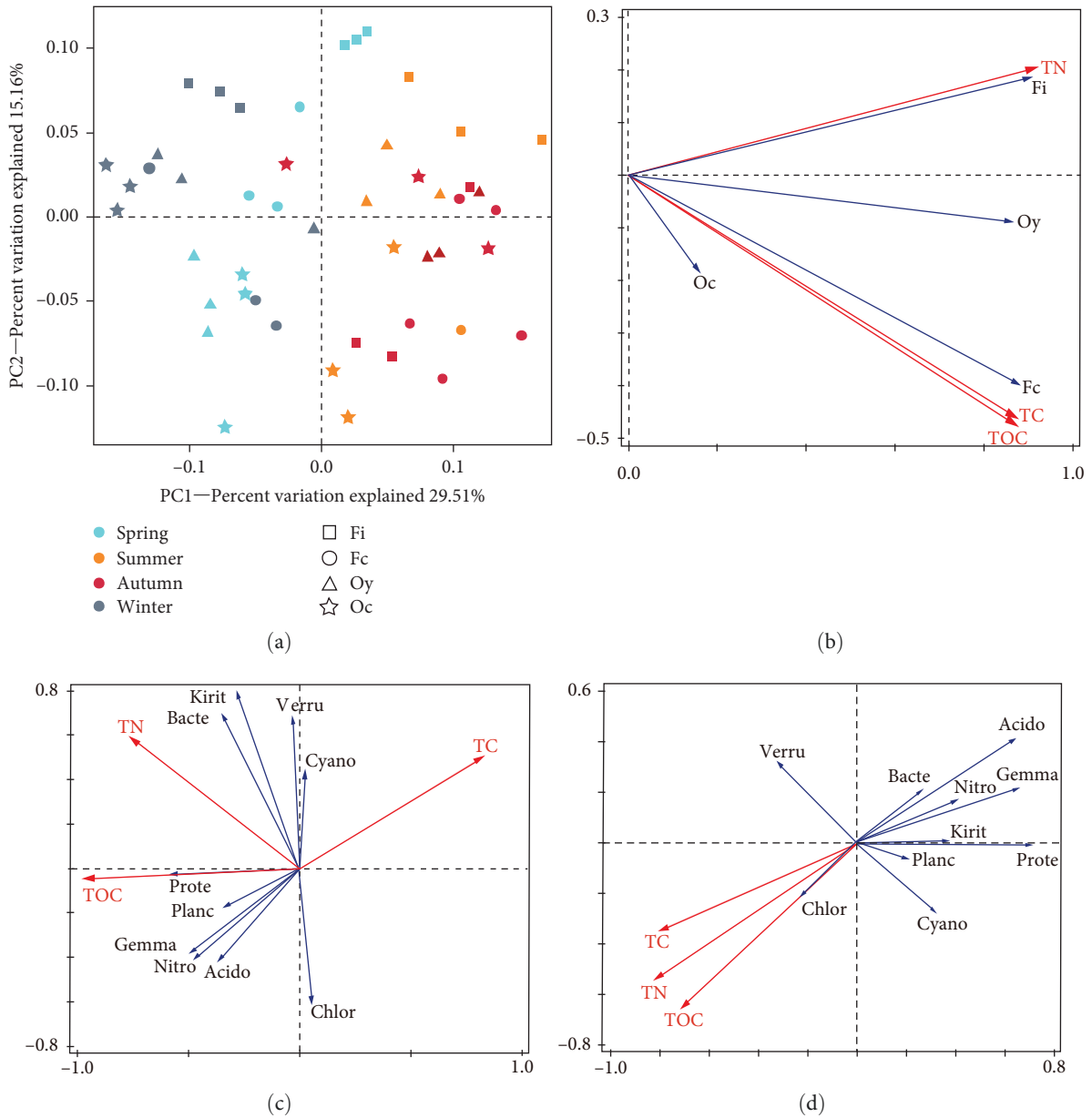
(a)

(b)

(c)

(d)

FIGURE 5: Principal co-ordinates analysis (PCoA) of the weighted UniFrac distances (a), symbols labeled according to sampling sites. Fi, fish cage culture area; Fc, fish control area; Oy, oyster control area; Oc, oyster control area. Redundancy analysis (RDA) of environmental variables with the bacterial community abundance at the sampling sites (b), the single bacterial species abundance at phylum level in spring (c) and winter (d). Fi, fish cage culture area; Fc, fish control area; Oy, oyster control area; Oc, oyster control area. Prote, Proteobacteria; Planc, Planctomycetes; Bacte, Bacteroidetes; Acido, Acidobacteria; Gemma, Gemmatimonadetes; Verru, Verrucomicrobia; Nitro, Nitrospirae; Chlor, Chloroflexi; Kirit, Kiritimatiellaeota; Cyano, Cyanobacteria.

TABLE 1: Proportions of functional genes involved in nitrate reduction between different sampling sites.

Sampling site types	Fi	Fc	Oy	Oc
Aerobic ammonia oxidation (%)	3.07 ± 1.60	2.49 ± 1.02	2.71 ± 0.75	3.53 ± 1.78
Aerobic nitrite oxidation (%)	2.60 ± 1.76	2.49 ± 1.29	2.83 ± 1.01	3.19 ± 1.91
Nitrification (%)	5.68 ± 3.09	4.98 ± 1.78	5.54 ± 1.70	6.72 ± 3.16
Nitrate respiration (%)	0.40 ± 0.44	0.12 ± 0.12	0.22 ± 0.17[b]	0.05 ± 0.07[b]
Nitrate reduction (%)	0.92 ± 0.48[a]	0.30 ± 0.21[a]	0.77 ± 0.49	0.45 ± 0.32

Fi, fish cage culture area; Fc, fish control area; Oy, oyster control area; Oc, oyster control area. Samples labeled with the same letter are significantly different at 0.05 level.

TABLE 2: Proportions of functional genes involved in nitrate reduction between different sampling seasons.

Sampling seasons	Spring	Summer	Autumn	Winter
Aerobic ammonia oxidation (%)	2.25 ± 0.94^a	2.43 ± 0.78^b	2.66 ± 0.60^c	4.46 ± 1.72^{abc}
Aerobic nitrite oxidation (%)	1.84 ± 1.20^{ab}	2.49 ± 1.45	3.41 ± 1.33^a	3.38 ± 1.65^b
Nitrification (%)	4.08 ± 2.04^{ab}	4.92 ± 2.14^c	6.06 ± 1.65^a	7.84 ± 2.79^{bc}
Nitrate respiration (%)	0.21 ± 0.15	0.14 ± 0.18	0.10 ± 0.09	0.34 ± 0.47
Nitrate reduction (%)	0.56 ± 0.37	0.63 ± 0.42^a	0.34 ± 0.14^{ab}	0.90 ± 0.62^b

Samples labeled with the same letter are significantly different at 0.05 level.

commencement of power plant operations in 2006, the seasonality of algal blooms appears to have shifted from spring and early summer to winter [49, 50]. Owing to the appearance of algal blooms in the winter and spring, the content of organic matter and inorganic particles in the environment was relatively high, and it could be effectively degraded by Proteobacteria [51], resulting in the increased Proteobacteria abundance in winter and spring. Because of the high temperature in summer and autumn, the growth and metabolic rates of fish and oysters would decrease. Previous studies showed that the filtration rate of oysters increased with increasing temperature until at ~25°C and then declined and decreased to the minimum when temperatures approach to 30°C in summer [52–54]. According to farmers, reducing feeding at this stage resulted in a low level of organic matter in the water and sediments, and the abundance of Proteobacteria then decreased.

Members of Planctomycetes are considered transient forms between prokaryotes and eukaryotes [55]. Planctomycetes are widely distributed in marine and estuarine environments, especially in sediments, where they may be involved in a series of unique biogeochemical processes such as anaerobic ammonium oxidation [56], methane oxidation, and participation in carbon recycling and sulfur cycles [57, 58]. The abundance of Planctomycetes was relatively high in our study, indicating that the biogeochemical processes in XSB occurred frequently. A different study showed that an increase in stocking density leads to a decline in Planctomycetes abundance [59], given that spring is when fish seedlings are introduced. A sudden increase in stocking density will lead to a lower Planctomycetes abundance in the spring relative to the other three seasons.

Bacteroides in sediments can effectively obtain organic matter from algae, and their distribution and abundance are directly influenced by algae in the aquatic environment. We found a higher abundance of bacteria in fish farms than in Fc areas. This is because the algae in the farms provide a good living habitat for the bacteria [60]. Fish culture is conducive to an increase in cyanobacteria. Highly toxic microcystins are the secondary metabolites of cyanobacteria, and they may cause serious damage and even lead to fish death [61]. We found a higher abundance of cyanobacteria in fish farms than in oyster farms, which suggests that fish farming may increase cyanobacteria abundance. Meanwhile, we also found a lower abundance of Nitrospirae in fish farms than in Fc areas. Nitrospirae are important bacteria involved in nitrification, yet fish culture may lead to a reduction in nitrifying bacteria,

thus threatening fish survival and the aquatic environment overall.

4.3. Effects of Aquaculture on the Sedimentary Nitrogen Cycle and Implications for Ecological Conservation in XSB.

With the development of marine aquaculture, nitrogenous organic compounds contribute most of the nitrogen pool in aquaculture pond sediments [8]. Previous studies have found that increased nutrients in seawater lead to changes in the cycling of nitrogen [5, 6, 18, 62–64]. Our study in XSB showed similar results. Our function prediction results showed that the proportion of genes involved in denitrification in aquaculture areas was significantly higher than that in control areas, especially for nitrate reduction occurring in fish areas ($p < 0.05$, Table 1) and nitrate respiration in oyster areas ($p < 0.05$, Table 1), and that the proportions of genes involved in nitrification, aerobic ammonia oxidation and aerobic nitrite oxidation were significantly higher in winter than in other seasons.

Owing to the long water residence time in XSB, heat emission from nuclear power plants accelerated the warming trend of XSB, leading to algal blooms in the winter [49, 54]. Phytoplankton are the main food source for the culture of filter-feeding bivalves [65–68]. Cultured oysters can remove phytoplankton and other organic and inorganic particles from water [67–69], accumulating nitrogen in sediments through biological deposition [13, 14]. Additionally, denitrifying bacteria in the sediment can transform the nitrogen from oyster biological sedimentation into gaseous nitrogen and ultimately cause it to leave the water system [20]. Our function prediction results indicated that the nitrification and denitrification capabilities in the sediment below the aquaculture farms were higher in farms than in control areas, especially in winter when algal blooms occurred.

Fish activity can cause sediment disturbance, and the nutrient effect caused by sediment resuspension indirectly leads to the exchange of bacterial communities between the water and sediment [17]. Previous studies pointed out that sediment resuspension caused by fish activity can change the release of nitrogen from sediment to water and increase the degradation and mineralization of organic matter in the water column [44], which can then affect the occurrence of autotrophy and heterotrophy in the water. Algal blooms caused by heat emission in XSB in winter lead to eutrophication at fish farms, while the input of artificial feed with high protein content at fish farms may aggravate eutrophication. This would not only increase the burden of the nitrogen cycle in the farming area but also affect the growth and

survival of fish. To keep the aquatic environment healthy, we suggest that the input of food at fish farms be reduced in winter.

The aggravation of human activities leads to the decline of marine resources and the pollution of the marine environment. To ensure the sustainable utilization of XSB's resources in the future, there are relevant suggestions based on our results. At present, there are three aquaculture areas: TG, HDG, and XHG in XSB. The heat emission of nuclear power plants leads to algal blooms in winter in XSB. Our results indicate that aquaculture could promote the occurrence of the nitrification and denitrification in the sediment in XSB in winter. A previous study showed that oyster aquaculture in XSB can reduce the nutrient concentrations and effectively alleviate algal blooms caused by heat emission in the winter [54]. Considering that the input of organic feed in the process of fish culture may cause seawater pollution and aggravation of algae bloom, we suggest that the oyster farming scale could be increased to control algae bloom. However, the geographical location of XSB is closed, and the water exchange rate is low, so it is necessary to further evaluate the potential threat of frequent extreme weather-climate events to the aquaculture and fisheries in XSB in the future.

5. Conclusions

In conclusion, consistent with our hypothesis, the present study demonstrates that aquaculture affects the composition of the bacterial community in the sediment, and different culture types form different patterns. The contents of TN, TC, and TOC in fish culture area were significantly higher than those in oyster area. There were higher proportions of denitrifying bacteria communities in the fish and oyster farms than in the control areas. Biodiversity and abundance of bacterial communities showed significant regional differences in spring and winter. In the winter, there was significantly higher bacterial abundance and proportion of bacteria involved in the nitrogen cycle in the sediments of XSB than in other seasons. However, we cannot ensure that the function prediction results of the 16SrRNA gene apply in the real world, and this can only be overcome by future developments in metagenomics.

Data Availability

Raw data used in this study are available upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

References

[1] B. Campbell and D. Pauly, "Mariculture: a global analysis of production trends since 1950," *Marine Policy*, vol. 39, pp. 94–100, 2013.

[2] WordBank, "Fish to 2030: prospects for fisheries and aquaculture," 2013.

[3] R. R. Gentry, S. E. Lester, C. V. Kappel et al., "Offshore aquaculture: spatial planning principles for sustainable development," *Ecology and Evolution*, vol. 7, no. 2, pp. 733–743, 2017.

[4] A. J. Ray, G. Seaborn, J. W. Leffler, S. B. Wilde, A. Lawson, and C. L. Browdy, "Characterization of microbial communities in minimal-exchange, intensive aquaculture systems and the effects of suspended solids management," *Aquaculture*, vol. 310, no. 1-2, pp. 130–138, 2010.

[5] H. Asami, M. Aida, and K. Watanabe, "Accelerated sulfur cycle in coastal marine sediment beneath areas of intensive shellfish aquaculture," *Applied and Environmental Microbiology*, vol. 71, no. 6, pp. 2925–2933, 2005.

[6] N. Kawahara, K. Shigematsu, T. Miyadai, and R. Kondo, "Comparison of bacterial communities in fish farm sediments along an organic enrichment gradient," *Aquaculture*, vol. 287, no. 1-2, pp. 107–113, 2009.

[7] A. Gyllenhammar and L. Håkanson, "Environmental consequence analyses of fish farm emissions related to different scales and exemplified by data from the Baltic—a review," *Marine Environmental Research*, vol. 60, no. 2, pp. 211–243, 2005.

[8] J. A. Hargreaves, "Nitrogen biogeochemistry of aquaculture ponds," *Aquaculture*, vol. 166, no. 3-4, pp. 181–212, 1998.

[9] C. E. Boyd, M. E. Tanner, M. Madkour, and K. Masuda, "Chemical characteristics of bottom soils from freshwater and brackishwater aquaculture ponds," *Journal of the World Aquaculture Society*, vol. 25, no. 4, pp. 517–534, 1994.

[10] A. Bissett, J. Bowman, and C. Burke, "Bacterial diversity in organically-enriched fish farm sediments," *FEMS Microbiology Ecology*, vol. 55, no. 1, pp. 48–56, 2006.

[11] N. B. Keeley, C. K. Macleod, G. A. Hopkins, and B. M. Forrest, "Spatial and temporal dynamics in macrobenthos during recovery from salmon farm induced organic enrichment: when is recovery complete?" *Marine Pollution Bulletin*, vol. 80, no. 1-2, pp. 250–262, 2014.

[12] L. Cao, R. Naylor, P. Henriksson et al., "China's aquaculture and the world's wild fisheries," *Science*, vol. 347, no. 6218, pp. 133–135, 2015.

[13] R. I. E. Newell, T. R. Fisher, R. R. Holyoke, J. C. Cornwell, R. F. Dame, and S. Olenin, "Influence of eastern oysters on nitrogen and phosphorus regeneration in chesapeake bay, USA," *The Comparative Roles of Suspension-Feeders in Ecosystems*, vol. 47, pp. 93–120, NATO Science Series IV: Earth and Environmental Series, 2005.

[14] C. B. Higgins, K. Stephenson, and B. L. Brown, "Nutrient bioassimilation capacity of aquacultured oysters: quantification of an ecosystem service," *Journal of Environmental Quality*, vol. 40, no. 1, pp. 271–277, 2011.

[15] S. J. Jordan, *Sedimentation and Remineralization Associated with Biodeposition by the American Oyster Crassostrea virginica (Gmelin)*, University of Maryland, College Park, 1987.

[16] K. E. Havens, "Fish-induced sediment resuspension: effects on phytoplankton biomass and community structure in a shallow hypereutrophic lake," *Journal of Plankton Research*, vol. 13, no. 6, pp. 1163–1176, 1991.

[17] T. Garstecki, S. A. Wickham, and H. Arndt, "Effects of experimental sediment resuspension on a coastal planktonic

microbial food web," *Estuarine, Coastal and Shelf Science*, vol. 55, no. 5, pp. 751–762, 2002.

[18] A. E. McCaig, C. J. Phillips, J. R. Stephen et al., "Nitrogen cycling and community structure of proteobacterial β-subgroup ammonia-oxidizing bacteria within polluted marine fish farm sediments," *Applied and Environmental Microbiology*, vol. 65, no. 1, pp. 213–220, 1999.

[19] V. Torsvik, F. L. Daae, R.-A. Sandaa, and L. Øvreås, "Novel techniques for analysing microbial diversity in natural and perturbed environments," *Journal of Biotechnology*, vol. 64, no. 1, pp. 53–62, 1998.

[20] R. W. Howarth, "Coastal nitrogen pollution: a review of sources and trends globally and regionally," *Harmful Algae*, vol. 8, no. 1, pp. 14–20, 2008.

[21] M. Tamminen, A. Karkman, J. Corander, L. Paulin, and M. Virta, "Differences in bacterial community composition in Baltic sea sediment in response to fish farming," *Aquaculture*, vol. 313, no. 1–4, pp. 15–23, 2011.

[22] S. G. Feinman, Y. R. Farah, J. M. Bauer, and J. L. Bowen, "The influence of oyster farming on sediment bacterial communities," *Estuarine and Coast*, vol. 41, pp. 800–814, 2018.

[23] P. Srithep, B. Khinthong, T. Chodanon, S. Powtongsook, W. Pungrasmi, and T. Limpiyakorn, "Communities of ammonia-oxidizing bacteria, ammonia-oxidizing archaea and nitrite-oxidizing bacteria in shrimp ponds," *Annals of Microbiology*, vol. 65, no. 1, pp. 267–278, 2015.

[24] R. R. Nair, B. Rangaswamy, B. S. I. Sarojini, and V. Joseph, "Anaerobic ammonia-oxidizing bacteria in tropical bioaugmented zero water exchange aquaculture ponds," *Environmental Science and Pollution Research*, vol. 27, no. 10, pp. 10541–10552, 2020.

[25] X. Ning and X. Hu, *Aquacultural Ecology and Carrying Capacity Assessment of Fish Cages in Xiangshan Bay*, Ocean Press, Beijing (in Chinese), 2002.

[26] T. Matsuno, J. S. Lee, M. Shimizu, S. H. Kim, and I. C. Pang, "Measurements of the turbulent energy dissipation rate ε and an evaluation of the dispersion process of the Changjiang Diluted Water in the East China Sea," *Journal of Geophysical Research*, vol. 111, no. C11, Article ID C11S09, 2006.

[27] Y. Zhu, J. Ishizaka, S. C. Tripathy et al., "Relationship between light, community composition and the electron requirement for carbon fixation in natural phytoplankton," *Marine Ecology Progress Series*, vol. 580, pp. 83–100, 2017.

[28] Z. Yang, J. Chen, H. Li et al., "Sources of nitrate in Xiangshan Bay (China), as identified using nitrogen and oxygen isotopes," *Estuarine, Coastal and Shelf Science*, vol. 207, pp. 109–118, 2018.

[29] Z.-B. Jiang, Q.-Z. Chen, J.-N. Zeng, Y.-B. Liao, L. Shou, and J. Liu, "Phytoplankton community distribution in relation to environmental parameters in three aquaculture systems in a Chinese subtropical eutrophic bay," *Marine Ecology Progress Series*, vol. 446, pp. 73–89, 2012.

[30] Z. Jiang, Y. Liao, J. Liu et al., "Effects of fish farming on phytoplankton community under the thermal stress caused by a power plant in a eutrophic, semi-enclosed bay: induce toxic dinoflagellate (*Prorocentrum minimum*) blooms in cold seasons," *Marine Pollution Bulletin*, vol. 76, no. 1-2, pp. 315–324, 2013.

[31] W. G. Weisburg, S. M. Barns, D. A. Pelletier, and D. J. Lane, "16S ribosomal DNA amplification for phylogenetic study," *Journal of Bacteriology*, vol. 173, no. 2, pp. 697–703, 1991.

[32] T. Mago and S. L. Salzberg, "FLASH: fast length adjustment of short reads to improve genome assemblies," *Bioinformatics*, vol. 27, no. 21, pp. 2957–2963, 2011.

[33] A. M. Bolger, M. Lohse, and B. Usadel, "Trimmomatic: a flexible trimmer for Illumina sequence data," *Bioinformatics*, vol. 30, no. 15, pp. 2114–2120, 2014.

[34] R. C. Edgar, B. J. Haas, J. C. Clemente, C. Quince, and R. Knight, "UCHIME improves sensitivity and speed of chimera detection," *Bioinformatics*, vol. 27, no. 16, pp. 2194–2200, 2011.

[35] R. C. Edgar, "UPARSE: highly accurate OTU sequences from microbial amplicon reads," *Nature Methods*, vol. 10, pp. 996–998, 2013.

[36] J. Gregory Caporaso, K. Bittinger, F. D. Bushman, T. Z. DeSantis, G. L. Andersen, and R. Knight, "PyNAST: a flexible tool for aligning sequences to a template alignment," *Bioinformatics*, vol. 26, no. 2, pp. 266-267, 2010.

[37] J. Gregory Caporaso, J. Kuczynski, J. Stombaugh et al., "QIIME allows analysis of high-throughput community sequencing data," *Nature Methods*, vol. 7, pp. 335-336, 2010.

[38] M. A. Larkin, G. Blackshields, N. P. Brown et al., "Clustal W and Clustal X version 2.0," *Bioinformatics*, vol. 23, no. 21, pp. 2947-2948, 2007.

[39] M. F. Sanner, "Python: a programming language for software integration and development," *Journal of Molecular Graphics & Modelling*, vol. 17, no. 1, pp. 57–61, 1999.

[40] P. Šmilauer and J. Lepš, *Multivariate Analysis of Ecological Data Using CANOCO 5*, Cambridge University Press, Cambridge, UK, 2014.

[41] S. Louca, L. W. Parfrey, and M. Doebeli, "Decoupling function and taxonomy in the global ocean microbiome," *Science*, vol. 353, no. 6305, pp. 1272–1277, 2016.

[42] I. Karakassis, E. Hatziyanni, M. Tsapakis, and W. Plaiti, "Benthic recovery following cessation of fish farming: a series of successes and catastrophes," *Marine Ecology Progress Series*, vol. 184, pp. 205–218, 1999.

[43] M. D. Callier, A. M. Weise, C. W. McKindsey, and G. Desrosiers, "Sedimentation rates in a suspended mussel farm (Great-Entry Lagoon, Canada): biodeposit production and dispersion," *Marine Ecology Progress Series*, vol. 322, pp. 129–141, 2006.

[44] J. T. Wootton, M. S. Parker, and M. E. Power, "Effects of disturbance on river food webs," *Science*, vol. 273, no. 5281, pp. 1558–1561, 1996.

[45] S. A. Castine, D. G. Bourne, L. A. Trott, and D. A. McKinnon, "Sediment microbial community analysis: establishing impacts of aquaculture on a tropical mangrove ecosystem," *Aquaculture*, vol. 297, no. 1-4, pp. 91–98, 2009.

[46] S. Fodelianakis, N. Papageorgiou, I. Karakassis, and E. D. Ladoukakis, "Community structure changes in sediment bacterial communities along an organic enrichment gradient associated with fish farming," *Annals of Microbiology*, vol. 65, pp. 331–338, 2015.

[47] S. M. S. Santander-de Leon, S. N. Nuñal, H. Wei, T. Yoshikawa, and H. Maeda, "Bacterial community composition shifts in sediments influenced by fish feeds," *Aquaculture Research*, vol. 48, no. 8, pp. 4380–4389, 2017.

[48] G. M. Luna, C. Connaldesi, A. Dell'Anno, A. Pusceddu, and R. Danovaro, "Impact of aquaculture on benthic virus–prokaryote interactions in the Mediterranean Sea," *Water Research*, vol. 47, no. 3, pp. 1156–1168, 2013.

[49] Z. Jiang, P. Du, J. Liu et al., "Phytoplankton biomass and size structure in Xiangshan Bay, China: current state and historical comparison under accelerated eutrophication and warming," *Marine Pollution Bulletin*, vol. 142, pp. 119–128, 2019.

[50] Z. Jiang, Y. Gao, Y. Chen et al., "Spatial heterogeneity of phytoplankton community shaped by a combination of anthropogenic and natural forcings in a long narrow bay in the

East China Sea," *Estuarine, Coastal and Shelf Science*, vol. 217, pp. 250–261, 2019.

[51] C. Beardsley, S. Moss, F. Malfatti, and F. Azam, "Quantitative role of shrimp fecal bacteria in organic matter fluxes in a recirculating shrimp aquaculture system," *FEMS Microbiol Ecology*, vol. 77, no. 1, pp. 134–145, 2011.

[52] J. Gu and J. Li, "Reproductive cycle of Ostrea plicatula Gmelin in Zhejiang Xiang—Shan harbour," *Journal of Ningbo University*, vol. 11, pp. 51–58, 1998.

[53] Z. Yu, Y. Zhou, Z. Qi, P. Luo, and C. Hu, "Temporal dynamics in clearance rate of the Portuguese oyster *Crassostrea angulata* cultivated in Dapeng Cove, southern China," *Aquaculture*, vol. 479, pp. 824–828, 2017.

[54] Z. Jiang, P. Du, Y. Liao et al., "Oyster farming control on phytoplankton bloom promoted by thermal discharge from a power plant in a eutrophic, semi-enclosed bay," *Water Research*, vol. 159, no. 1, pp. 1–9, 2019.

[55] E. G. Reynaud and D. P. Devos, "Transitional forms between the three domains of life and evolutionary implications," *Proceedings of the Royal Society B: Biological Sciences*, vol. 278, no. 1723, pp. 3321–3328, 2011.

[56] S. Neumann, M. C. F. van Teeseling, and L. van Niftrik, "Cell biology of anaerobic ammonium-oxidizing bacteria: unique prokaryotes with an energy-conserving intracellular compartment," in *Planctomycetes: Cell Structure, Origins and Biology*, J. Fuerst, Ed., pp. 89–123, Humana Press, Totowa, NJ, 2013.

[57] D. Woebken, H. Teeling, P. Wecker et al., "Fosmids of novel marine *Planctomycetes* from the Namibian and Oregon coast upwelling systems and their cross-comparison with planctomycete genomes," *The ISME Journal*, vol. 1, pp. 419–435, 2007.

[58] H. Izumi, E. Sagulenko, R. I. Webb, and J. A. Fuerst, "Isolation and diversity of planctomycetes from the sponge *Niphates* sp., seawater, and sediment of Moreton Bay, Australia," *Antonie van Leeuwenhoek*, vol. 104, pp. 533–546, 2013.

[59] L. Fan, K. Barry, G. Hu et al., "Characterizing bacterial communities in tilapia pond surface sediment and their responses to pond differences and temporal variations," *World Journal of Microbiology and Biotechnology*, vol. 33, Article ID 1, 2017.

[60] J. C. Cornwell, M. S. Owens, W. R. Boynton, and L. A. Harris, "Sediment-water nitrogen exchange along the potomac river estuarine salinity gradient," *Journal of Coastal Research*, vol. 32, no. 4, pp. 776–787, 2016.

[61] J. S. Rowan, S. Black, and S. W. Franks, "Sediment fingerprinting as an environmental forensics tool explaining cyanobacteria blooms in lakes," *Applied Geography*, vol. 32, no. 2, pp. 832–843, 2012.

[62] M. Holmer and E. Kristensen, "Seasonality of sulfate reduction and pore water solutes in a marine fish farm sediment: the importance of temperature and sedimentary organic matter," *Biogeochemistry*, vol. 32, pp. 15–39, 1996.

[63] E. Ingall, L. Kolowith, T. Lyons, and M. Hurtgen, "Sediment carbon, nitrogen and phosphorus cycling in an anoxic fjord, Effingham inlet, British Columbia," *American Journal of Science*, vol. 305, no. 3, pp. 240–258, 2005.

[64] P. Chávez-Crooker and J. Obreque-Contreras, "Bioremediation of aquaculture wastes," *Current Opinion in Biotechnology*, vol. 21, no. 3, pp. 313–317, 2010.

[65] J. E. Cloern, "Does the benthos control phytoplankton biomass in South San Francisco Bay?" *Marine Ecology Progress Series*, vol. 9, pp. 191–202, 1982.

[66] P. Souchu, A. Vaquer, Y. Collos, S. Landrein, J.-M. Deslous-Paoli, and B. Bibent, "Influence of shellfish farming activities on the biogeochemical composition of the water column in Thau lagoon," *Marine Ecology Progress Series*, vol. 218, pp. 141–152, 2001.

[67] A. Trottet, S. Roy, E. Tamigneaux, and C. Lovejoy, "Importance of heterotrophic planktonic communities in a mussel culture environment: the Grande Entrée lagoon, Magdalen Islands (Québec, Canada)," *Marine Biology*, vol. 151, pp. 377–392, 2007.

[68] P. Jacobs, R. Riegman, and J. van der Meer, "Impact of the blue mussel *Mytilus* edulis on the microbial food web in the western Wadden Sea, The Netherlands," *Marine Ecology Progress Series*, vol. 527, pp. 119–131, 2015.

[69] C. Dupuy, A. Vaquer, T. Lam-Höai et al., "Feeding rate of the oyster Crassostrea *gigas* in a natural planktonic community of the Mediterranean Thau Lagoon," *Marine Ecology Progress Series*, vol. 205, pp. 171–184, 2000.