Hindawi
Aquaculture Research
Volume 2023, Article ID 1930201, 17 pages
https://doi.org/10.1155/2023/1930201

Research Article

Bivalves Improved Water Quality by Changing Bacterial Composition in Sediment and Water in an IMTA System

Shuo Kong,[1,2] Zhao Chen,[2] Abdallah Ghonimy,[2] Jian Li ,[2] and Fazhen Zhao[2]

[1]*Fisheries College, Ocean University of China, Qingdao 266003, China*
[2]*Key Laboratory for Sustainable Development of Marine Fisheries, Ministry of Agriculture, Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao 266071, China*

Correspondence should be addressed to Jian Li; lijian@ysfri.ac.cn

Received 8 November 2022; Revised 7 March 2023; Accepted 18 March 2023; Published 12 April 2023

Academic Editor: Umar Khan

Integrated multitrophic aquaculture (IMTA) maximises the nitrogen cycle between system components, including bacteria. In order to maximise the bacterial role in nitrogen elimination in an IMTA system, we investigated the effect of bivalve culture on water quality and bacterial community structure in overlying water and sediment in the "shrimp-crab-bivalve-fish" IMTA system. The bacterial composition in overlying water and sediment was measured by Illumina -MiSeq high-throughput sequencing technology. The results show that dissolved oxygen was higher in the bivalve culture area. Ammonia and nitrite in the bivalve culture area were lower than those in the nonbivalve culture area; however, the nitrate and phosphate in the bivalve culture area were higher than those in the nonbivalve culture area. The Chao1, Shannon, and Ace indexes were higher in the bivalve area. More bacteria with nitrification and denitrification functions were detected in bivalve culture areas, such as *Ruegeria* (1.05%–4.79%), *Thalassobius* (0.11%–0.69%), *Limibaculum* (0.07%–0.69%), *HIMB*11 (0.13%–0.21%), and *Rubellimicrobium* (0.01%–0.16%). More Cyanobacteria were detected in bivalve culture areas with higher phosphate concentrations. To sum up, bivalves can release phosphorus through bioturbation, increasing the abundance of Cyanobacteria, which release dissolved oxygen into overlying water through photosynthesis, enhance nitrification (mainly ammonia oxidation), and improve the ammonia nitrogen removal capacity of the system. Meanwhile, bivalves can increase bacterial diversity and abundance by regulating dissolved oxygen. This study provided insight into bivalve interaction with bacterial activity in the IMTA system.

1. Introduction

Aquaculture activity generates massive water drainage with a significant load of nutrients, including nitrogen and phosphorus [1]. A eutrophication phenomenon due to elevated nutrient levels affected the productivity of cultured aquatic organisms and the surrounding water environment [2, 3].

The integrated multitrophic aquaculture (IMTA) system is an eco-friendly and sustainable aquaculture system [3] regarding the nutrient cycle; it causes less water drainage to the natural resources and avoids severe degradation of the ecosystem [4]. IMTA models, which consist of different species [4] that feed at different trophic levels [5, 6], can induce a nitrogen cycle between cultured species; for instance, feed species, such as fish and shrimp, are integrated with extractive species such as autotrophs, filters, and deposit feeders [1]. The feed waste of fed species turns into a nutrient source for extractive species [1]. Bivalves can be used as preferred species for IMTA because they promote the removal of excess nutrients and nitrogen in sediments through bioturbation with remarkable filtration capabilities [7–9]. Besides, bivalves can bury nitrogen in sediments and enhance the denitrification process by increasing microbial activity in bivalve sediments [10–12].

Importantly, a significant nitrogen mass affects the bacterial activity in water bodies and sediments, highlighting the major role of bacteria in the nitrogen cycle [13–16] and

reflecting the water quality to a certain extent [17, 18]. Some heterotrophic bacteria with nitrification and denitrification abilities can assimilate and oxidize nitrogenous waste [19] in water and sediment [20]. By bioturbation, bivalves are not only involved in the nitrogen cycle but also interact with bacterial abundances. Meanwhile, they can remove excess nutrients in sediments by filter feeding and altering bacterial activity [7, 8] and bury nitrogen compounds in sediments, ultimately enhancing the relative abundance of denitrifying bacteria [10–12]. Research work has shown that bivalve farming significantly alters the community in local estuarine habitats, including water and sediment [21].

Bivalves, as an important cultured species in the IMTA system, can change the bacterial community in the environment; however, how they affect the bacterial community structure in the IMTA system and improve water quality need to be studied. In this study, we hypothesised that bivalves could enhance water quality by affecting bacterial abundances in water and sediment through their physical movements in the IMTA system. In this study, we analysed the water chemical indexes and bacteria community structures in overlying water and sediment, performing heatmap analysis between nitrogenous compound levels and bacterial abundances. Our study provides a theoretical basis for maximising the elimination of nitrogenous compounds and improving water quality in the IMTA system.

2. Materials and Methods

2.1. Ethics Statement. This study did not need ethical approval because the experimental materials were water and sediment samples not animals.

2.2. Experimental System. The integrated multitrophic aquaculture (IMTA) system used in this experiment contained *Penaeus chinensis* (shrimp), *Portunus trituberculatus* (crab), *Sinonovacula constricta* (bivalve), and *Cynoglossus semilaevis* (fish). The experiment was conducted in Rizhao Kaihang Fisheries Co., Ltd. (35°31′48″N; 119°41′35″E) in Taoluo Town, Rizhao City, Shandong Province, along the Yellow Sea. The aquaculture pond covered an area of 5333.36 m^2 with two meters of depth. On March 26th, 25 kg *Sinonovacula constricta* (mean weight 5.7 g) were added to the pond. After 15 days, 40,000 *Penaeus chinensis* (mean length≈1 cm) were cultured in the pond. After 20 days, 0.75 kg seeds of *Portunus trituberculatus* (0.05 g per crab) were added. After 34 days, 40 *Cynoglossus semilaevis* (400 g per fish) were added to the pond. The feed quantity of bait was increased by 4 kg per day. Oxygen was provided through nanotubules at the bottom of the pond. After 210 days, the final weight was 22.37 ± 1.58 g for *Penaeus chinensis*, 356.23 ± 22.73 g for *Portunus trituberculatus*, 10.82 ± 0.51 g for *Sinonovacula constricta*, and 270.61 ± 50.28 g for *Cynoglossus semilaevis*.

2.3. Sample Collection. The experimental samples were collected from four sites, including the water inlet, the water outlet, and the areas with and without bivalves (Figure 1). The three samples were collected monthly (September, October, and November) from each location and fully mixed as one sample, where the samples were collected from overlying water and sediment. A water volume of 1000 ml was collected through a plexiglass water collector. A volume of 200 ml of water was filtered through a 0.22 μm acetate fibre membrane, and then the residues on the membranes were used for the microbial community analysis. The remaining 800 ml water was filtered through a 0.45 μm microporous membrane and used to analyse water chemical indexes. Using a plexiglass mud picker, sediment samples were collected at 0–8 cm below the water surface. The sediment sample size was 5 g for the microbial community analysis. The filter membranes and sediment samples were flash-frozen and stored at −80°C until analysis.

2.4. Chemical Indexes in Water. Water temperature, dissolved oxygen (DO), salinity, and pH were measured with a YSI incorporated device (Yellow Springs, OH, USA). Ammonia-N, nitrate-N, and nitrite-N were measured using a QuAAtro nutrient autoanalyser (Seal Analytical Ltd., Germany). The concentrations of three dissolved inorganic nitrogen (DIN) were measured using a water-quality nutrient analyser (SINOHLK-NutriS, Xiamen, China).

2.5. High-Throughput Sequencing of Bacteria and Bioinformatic Analysis. The total DNA of all water and sediment samples was extracted using the TIANamp Bacteria DNA Kit (Tiangen Biotech, Beijing, China), and DNA integrity was confirmed by agarose gel electrophoresis. Using the NanoDrop spectrophotometer (Thermo Scientific, USA), the bacterial DNA concentration was measured. The V3-V4 region of 16SrRNA gene, a specific conserved sequence region of bacterial DNA, with primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) [22], was amplified by polymerase chain reaction using MyCycler™ thermal cycler (BIO-RAD, USA). The bacterial DNA was purified and sequenced by Illumina Miseq by Majorbio. Raw reads were deposited into the NCBI sequence read archive (SRA) database (accession number: PRJNA756424).

2.6. Data Analysis. The statistical analysis of water quality was performed using SPSS programme version 22.0 (SPSS, Chicago, IL, USA), and one-way ANOVA was conducted to compare significant differences between different sampling points on water quality.

Paired-end (PE) reads were spliced by FLASH software [23] according to the overlapping relationship, and Fastp [24] software was used for quality control and to filter original sequencing sequences. After data optimisation, UPARSE [25] software was used for OTU clustering and statistical analysis of biological information for the sequence according to the similarity of 97% [25, 26]. The RDP classifier [27] software package was used for species classification analysis for each sequence. According to the results of the taxonomic analysis, the community structure of the

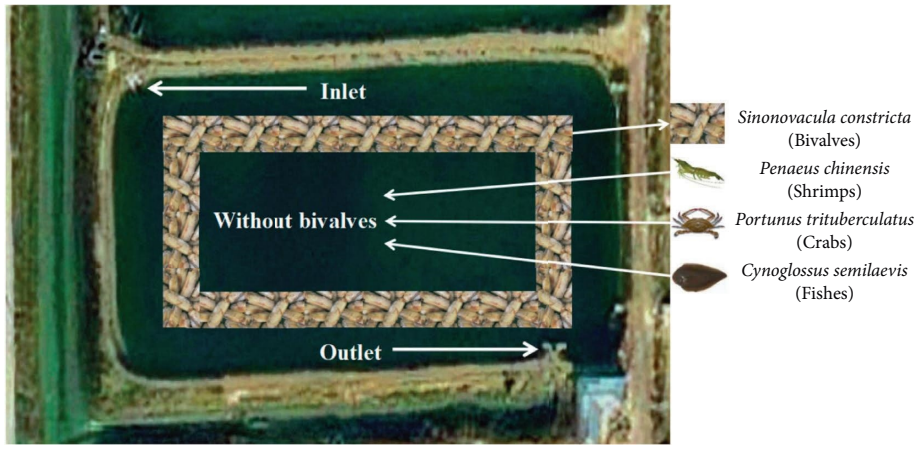


Figure 1: Satellite images of the integrated multitrophic aquaculture (IMTA) ponds, including aquaculture species and sampling sites.

samples at different classification levels was measured by statistical analysis. The alpha diversity was calculated using MOTHUR [28]. The SPSS Statistics 22 was used to analyse data differences; a P value <0.05 was considered statistically significant, and $P < 0.01$ was considered extremely significant [29].

3. Results

3.1. Overlying Water Characteristics

3.1.1. Environmental Conditions. The IMTA system's environmental conditions are summarised in Table 1. Temperature decreased gradually over the experimental time from September to November. Dissolved oxygen (DO) increased over time and was more abundant in aquaculture areas with bivalves than without bivalves. Salinity increased over time. The pH value first decreased and then increased.

3.1.2. Nutrient Contents. Nutrients, including ammonia, nitrite, nitrate, and phosphate in overlying water, are summarised in Table 1. Ammonia and nitrate decreased over time. Nitrite first decreased and then increased over time. Ammonia and nitrite levels were lower in the culture area with bivalves compared to the culture area without bivalves; however, nitrate was higher in the culture area with bivalves. In September, there were significant differences ($P < 0.05$) between ammonia and nitrate in the area with and without bivalves. Phosphate showed a decreased pattern in aquaculture areas over time. The phosphate level showed a lower level in the culture area with bivalves compared to the culture area without bivalves.

3.2. Microbiota Composition and Diversity in the Overlying Water. Among all sampling locations, the microbial composition was similar, but the proportions differed with sampling time; for example, the dominant phylum abundance range was as follows: *Cyanobacteria*, *Proteobacteria*, *Bacteroidota*, and *Actinobacteriota*. These bacteria accounted for about 84% of the total bacteria (Figure 2(a)). The first dominant bacterial groups are shown in Table 2 and Figure 3. Alpha-diversity indexes are shown in Table 3, and the Sobs, Shannon, Ace, and Chao1 values were the highest in November. The indexes representing diversity and abundance were higher in the area with bivalves than without bivalves. In October, the Shannon index in the bivalve area was significantly higher than in the area without bivalves ($P < 0.05$).

The first dominant bacterial composition at the three classification levels was similar between September and October; it was mainly *Cyanobacteria*. These bacteria showed the highest level in September (Inlet) and October (culture with bivalves). In November, the *Proteobacteria* phylum (Figures 2(a) and 3(a)), *Rhodobacteraceae* family (heterotrophic nitrifiers; Figures 2(b) and 3(b)), and *Marivita* genus (photoautotrophic bacteria; Table 2) were the dominant bacteria. Some members of the *Rhodobacteraceae* family and *Marivita* genus are heterotrophic nitrifiers and photoautotrophic bacteria, respectively. These bacteria were the highest in the area with bivalves. Generally, the area with bivalves showed the highest bacterial content of two nitrogen removal pathways, including heterotrophic and phototrophic bacteria.

3.3. Microbiota Composition and Diversity in Sediment. Among all sampling locations, the phyla of *Proteobacteria*, *Bacteroidota*, *Desulfobacterota*, *Chloroflexi*, *Firmicutes*, *Actinobacteriota*, and *Acidobacteriota* were dominant bacteria, accounting for more than 84% of the total bacterial content (Figure 4(a)). Of note, some members of *Proteobacteria*, *Bacteroidota*, *Desulfobacterota*, *Chloroflexi*, and *Firmicutes* are heterotrophic. The area with cultured bivalves was characterised by the highest level of *Desulfobacterota* (heterotrophic denitrifiers; Figure 4(a)) compared to the area without cultured bivalves. The family of *Flavobacteriaceae*, *Woeseiaceae*, *Desulfocapsaceae*, *norank_o__SBR1031*, *Rhodobacteraceae*, *norank_o__SBR1033*, and *Desulfosarcinaceae* were dominant bacteria, accounting for about 37% of the total bacterial content (Figure 4(b)). The first dominant bacterial groups are shown in Table 4 and Figure 5. Alpha-diversity indexes are shown in Table 5, and the Sobs and Shannon values increased over time. Ace and Chao1 indexes

Table 1: Overlying water physical and chemical characteristics.

Sampling time	Sampling sites	Dissolved oxygen (mg/L)	Temperature (°C)	Salinity (ppt)	pH	Ammonia (mg/L)	Nitrite (mg/L)	Nitrate (mg/L)	Phosphate (mg/L)
September	Inlet	6.28 ± 0.06^{b}	30.6 ± 0.01^{a}	28.05 ± 0.03^{ab}	8.13 ± 0.005^{b}	0.25 ± 0.006^{a}	0.18 ± 0.006^{ab}	1.15 ± 0.006^{a}	0.034 ± 0.001^{a}
	Outlet	5.27 ± 0.05^{c}	30.7 ± 0.06^{a}	28.15 ± 0.03^{a}	8.14 ± 0.012^{b}	0.19 ± 0.003^{b}	0.14 ± 0.006^{b}	0.74 ± 0.015^{d}	0.027 ± 0.001^{b}
	Bivalve	7.65 ± 0.09^{a}	30.6 ± 0.06^{a}	27.82 ± 0.06^{c}	8.23 ± 0.005^{a}	0.20 ± 0.003^{b}	0.15 ± 0.003^{b}	1.01 ± 0.006^{b}	0.029 ± 0.001^{ab}
	Nonbivalve	7.35 ± 0.05^{a}	30.7 ± 0.05^{a}	27.77 ± 0.06^{bc}	8.21 ± 0.003^{ab}	0.23 ± 0.006^{a}	0.19 ± 0.003^{a}	0.94 ± 0.009^{c}	0.032 ± 0.001^{a}
October	Inlet	7.92 ± 0.03^{a}	23.7 ± 0.06^{a}	29.77 ± 0.04^{ab}	8.56 ± 0.01^{b}	0.17 ± 0.006^{b}	0.13 ± 0.006^{a}	0.54 ± 0.009^{a}	0.058 ± 0.001^{a}
	Outlet	6.93 ± 0.06^{b}	23.7 ± 0.09^{a}	29.98 ± 0.03^{a}	8.57 ± 0.01^{ab}	0.18 ± 0.003^{abc}	0.11 ± 0.006^{a}	0.34 ± 0.009^{c}	0.013 ± 0.001^{d}
	Bivalve	6.91 ± 0.03^{b}	23.7 ± 0.06^{a}	29.81 ± 0.04^{ab}	8.58 ± 0.01^{a}	0.16 ± 0.012^{bc}	0.13 ± 0.006^{a}	0.48 ± 0.006^{b}	0.031 ± 0.001^{c}
	Nonbivalve	6.92 ± 0.04^{b}	23.7 ± 0.05^{a}	29.54 ± 0.01^{b}	8.57 ± 0.01^{ab}	0.18 ± 0.006^{ac}	0.14 ± 0.006^{a}	0.38 ± 0.006^{c}	0.042 ± 0.001^{b}
November	Inlet	9.29 ± 0.012^{a}	15.0 ± 0.03^{b}	29.30 ± 0.006^{a}	8.80 ± 0.006^{a}	0.15 ± 0.006^{a}	0.12 ± 0.006^{a}	0.46 ± 0.017^{a}	0.021 ± 0.002^{a}
	Outlet	9.25 ± 0.009^{a}	15.2 ± 0.06^{ac}	29.32 ± 0.007^{a}	8.82 ± 0.009^{a}	0.14 ± 0.009^{a}	0.11 ± 0.009^{a}	0.31 ± 0.015^{b}	0.016 ± 0.001^{a}
	Bivalve	9.20 ± 0.006^{b}	15.0 ± 0.05^{b}	29.31 ± 0.005^{a}	8.81 ± 0.006^{a}	0.14 ± 0.008^{a}	0.11 ± 0.006^{a}	0.39 ± 0.009^{ab}	0.016 ± 0.005^{a}
	Nonbivalve	9.20 ± 0.004^{b}	15.1 ± 0.01^{bc}	29.31 ± 0.009^{a}	8.79 ± 0.012^{a}	0.15 ± 0.006^{a}	0.13 ± 0.009^{a}	0.34 ± 0.012^{b}	0.024 ± 0.002^{a}

One-way ANOVA was used to test the significant differences among sampling locations at the same sampling time. [a, b, c, d]: Samples without letters in common indicate significant differences ($P < 0.05$).

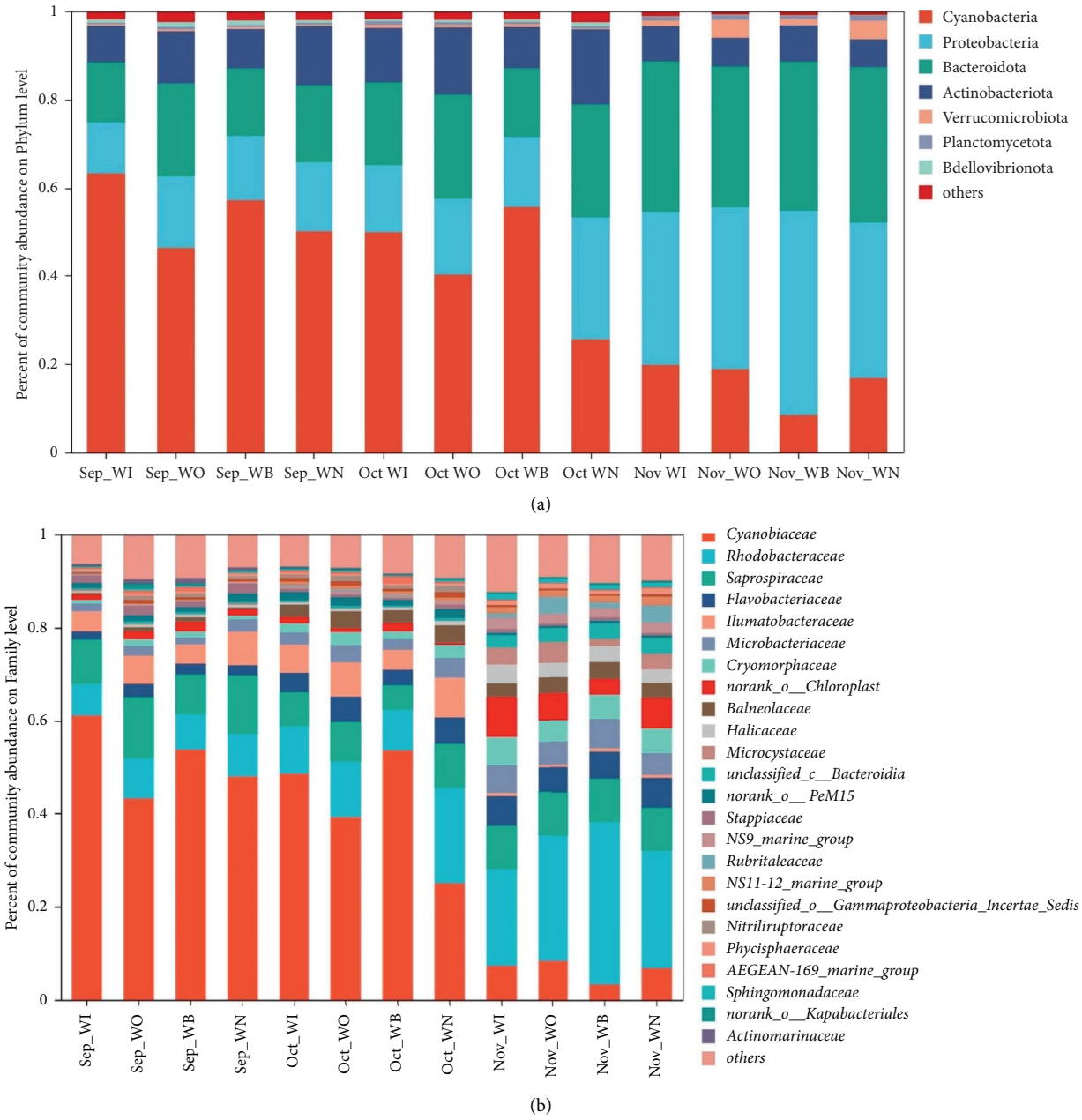


FIGURE 2: Microbiota composition in overlying water at phylum (a) and family (b) levels. *I*: water inlet, *O*: water outlet, *B*: area with bivalves, *N*: area without bivalves, Sep: September, Oct: October, and Nov: November.

increased first and then decreased. All indexes in the area with bivalves were higher than that without bivalves.

Regarding the first bacterial dominant groups, *Proteobacteria* were detected in all locations except for the inlet location in September and October. *Woeseia* (chemoheterotrophic) was detected in the area without bivalves and undetected in the outlet area. In November, these bacteria were detected in all locations except for the outlet. In fact, although the abundance was different, the bacterial composition was similar between the area with bivalves (September) and that without bivalves (September and October). Generally, areas with cultured bivalves showed increased bacterial abundance.

3.4. Correlation Analysis between Microbiota and Overlying Water Characteristics. Spearman correlation analysis shown in Figure 6 was conducted between microbiota and overlying water characteristics, including temperature (*T*), dissolved oxygen (DO), salinity, pH, ammonia (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), and phosphate (PO_4^{3-}) on phylum and family levels.

Cyanobacteria were positively correlated with NH_4^+ ($P = 0.02$), NO_2^- ($P = 0.016$), NO_3^- ($P = 0.007$), and (PO_4^{3-}) ($P = 0.012$). Some bacteria were significantly positively correlated with pH and DO, such as *Flavobacteriaceae* ($P = 0.002$) and *Cryomorphaceae* ($P = 0.0002$). Actinobacteriota was significantly positively correlated with NH_4^+

Table 2: The first dominant bacterial groups in the overlying water.

Location	Classification	Month					
		September	(%)	October	(%)	November	(%)
Inlet	Phylum	Cyanobacteria	63	Cyanobacteria	49	Proteobacteria	35
	Family	*Cyanobiaceae*	61	*Cyanobiaceae*	48	*Rhodobacteraceae*	20
	Genus	*Cyanobium_PCC*-6307	56	*Cyanobium_PCC*-6307	39	*Marivita*	10
Outlet	Phylum	Cyanobacteria	45	Cyanobacteria	39	Proteobacteria	36
	Family	*Cyanobiaceae*	45	*Cyanobiaceae*	38	*Rhodobacteraceae*	26
	Genus	*Cyanobium_PCC*-6307	39	*Cyanobium_PCC*-6307	31	*Marivita*	15
Bivalve	Phylum	Cyanobacteria	57	Cyanobacteria	55	Proteobacteria	46
	Family	*Cyanobiaceae*	53	*Cyanobiaceae*	53	*Rhodobacteraceae*	34
	Genus	*Cyanobium_PCC*-6307	49	*Cyanobium_PCC*-6307	44	*Marivita*	19
Nonbivalve	Phylum	Cyanobacteria	50	Cyanobacteria	25	Bacteroidota	35
	Family	*Cyanobiaceae*	48	*Cyanobiaceae*	24	*Rhodobacteraceae*	24
	Genus	*Cyanobium_PCC*-6307	44	*Cyanobium_PCC*-6307	20	*Marivita*	13

Table 3: Alpha-diversity indexes of the bacterial community in the overlying water.

Time	Site	Sobs	Shannon	Ace	Chao1
September	Inlet	492.33 ± 22^{b}	3.04 ± 0.11^{b}	813.71 ± 7.24^{b}	689.35 ± 12.99^{b}
	Outlet	593 ± 34.39^{a}	3.78 ± 0.17^{a}	814.31 ± 6.39^{b}	869.62 ± 17.62^{a}
	Bivalve	546 ± 24.83^{a}	3.49 ± 0.15^{a}	955.18 ± 14.88^{a}	789.25 ± 17.62^{ab}
	Nonbivalve	530 ± 16.74^{a}	3.46 ± 0.19^{a}	719.78 ± 17.27^{b}	741.67 ± 11.29^{b}
October	Inlet	545.33 ± 14.45^{a}	3.52 ± 0.17^{ab}	866.95 ± 9.03^{a}	815.02 ± 17.04^{a}
	Outlet	527.33 ± 15.3^{a}	3.81 ± 0.11^{ab}	694.62 ± 17.62^{b}	735.56 ± 17.62^{ab}
	Bivalve	542.33 ± 17.65^{a}	4.16 ± 0.08^{a}	706.39 ± 14.19^{b}	709.20 ± 17.35^{b}
	Nonbivalve	535.33 ± 19.64^{a}	3.37 ± 0.12^{b}	683.41 ± 9.84^{b}	707.78 ± 17.36^{b}
November	Inlet	630 ± 17.04^{a}	4.67 ± 0.19^{a}	1185.20 ± 23.39^{a}	946.99 ± 17.62^{ab}
	Outlet	615 ± 17.06^{a}	4.44 ± 0.12^{a}	1235.89 ± 13.87^{a}	1086.12 ± 105.83^{a}
	Bivalve	618.67 ± 12.77^{a}	4.55 ± 0.17^{a}	1163.09 ± 17.62^{a}	1010.74 ± 37.57^{ab}
	Nonbivalve	584 ± 17.62^{a}	4.38 ± 0.12^{a}	975.34 ± 14.9^{b}	850.23 ± 14.73^{b}

One-way ANOVA was used to test the significant differences among sampling locations at the same sampling time. [a, b]: Samples without letters in common indicate significant differences ($P < 0.05$).

($P = 0.02$). Some heterotrophic bacteria, such as Firmicutes ($P = 0.003$) and Chloroflexi ($P = 0.006$), were significantly positively correlated with the NO_2^- level, revealing their possible activity on the nitrification and aerobic denitrification bacterial processes. In this context, bivalves increased nutrient (NH_4^+, NO_2^-, NO_3^-, and PO_4^{3-}) absorption by Cyanobacteria in water, as well as aerobic and heterotrophic bacterial activities (*Flavobacteriaceae*).

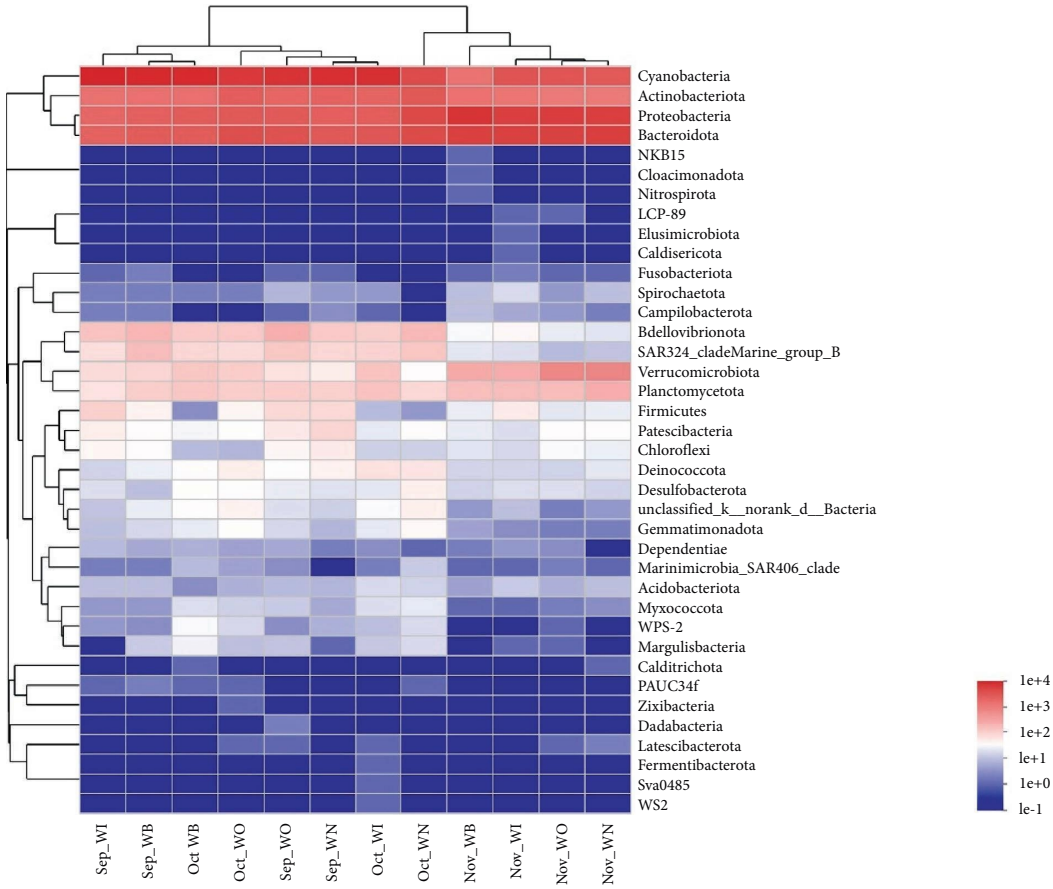
4. Discussion

In this study, the overlying water quality assessment results showed that some water quality factors, such as ammonia and nitrite, which are not conducive to the growth of aquaculture organisms in the bivalve culture area, were lower than those in the nonbivalve culture area. However, the nitrate in the bivalve culture area was higher than in the nonbivalve culture area. Meanwhile, the dissolved oxygen (DO) in the bivalve culture area was higher than in the nonbivalve culture area.

Dissolved oxygen is an essential molecule for ammonia oxidation [30] to promote the growth of nitrifying bacteria. Besides, bivalves can create anoxic microzones through bioprecipitation, eventually promoting the abundance of denitrifying bacteria [7, 31, 32]. In this study, more bacteria could be detected at the genus level in the bivalve culture area than in the area without bivalves in sediment, such as *Ruegeria* (1.05%–4.79%), *Thalassobius* (0.11%–0.69%), *Limibaculum* (0.07%–0.69%), *HIMB11* (0.1%3–0.21%), *Rubellimicrobium* (0.01%–0.16%), and *Ascidiaceihabitan* (0.00%–0.14%), which are aerobic bacteria belonging to the *Rhodobacteraceae* family. Some members of the *Ruegeria* genus have functional denitrification genes, such as the *nosZ* and *nirS* genes [33, 34]. *Thalassobius*, a strictly aerobic, chemo-organotrophic bacterium [35], can reduce nitrate to nitrite [36]. *Limibaculum* [37] and *Rubellimicrobium* [38] are important denitrifying bacteria. *HIMB11* can utilise ammonia, as well as inorganic and organic forms of phosphorus [39]. In addition, some bacteria which can participate in the nitrogen cycle, such as *Nitrosomonas*, *Nitrococcus*, and *Nitrosococcus*, could be detected in the sediment of the bivalve culture area; however, the proportion of each was less than 0.01%. Research has shown that bivalves modulate oxic-anoxic zonation through bioturbation [40] and accelerate the combined process of nitrification and denitrification [41]. Thus, bivalves can enhance nitrification and denitrification to improve water quality. However, in future studies, the abundance of functional genes involved in the nitrogen cycle should be identified to more accurately describe the process and main pathways of the nitrogen cycle.

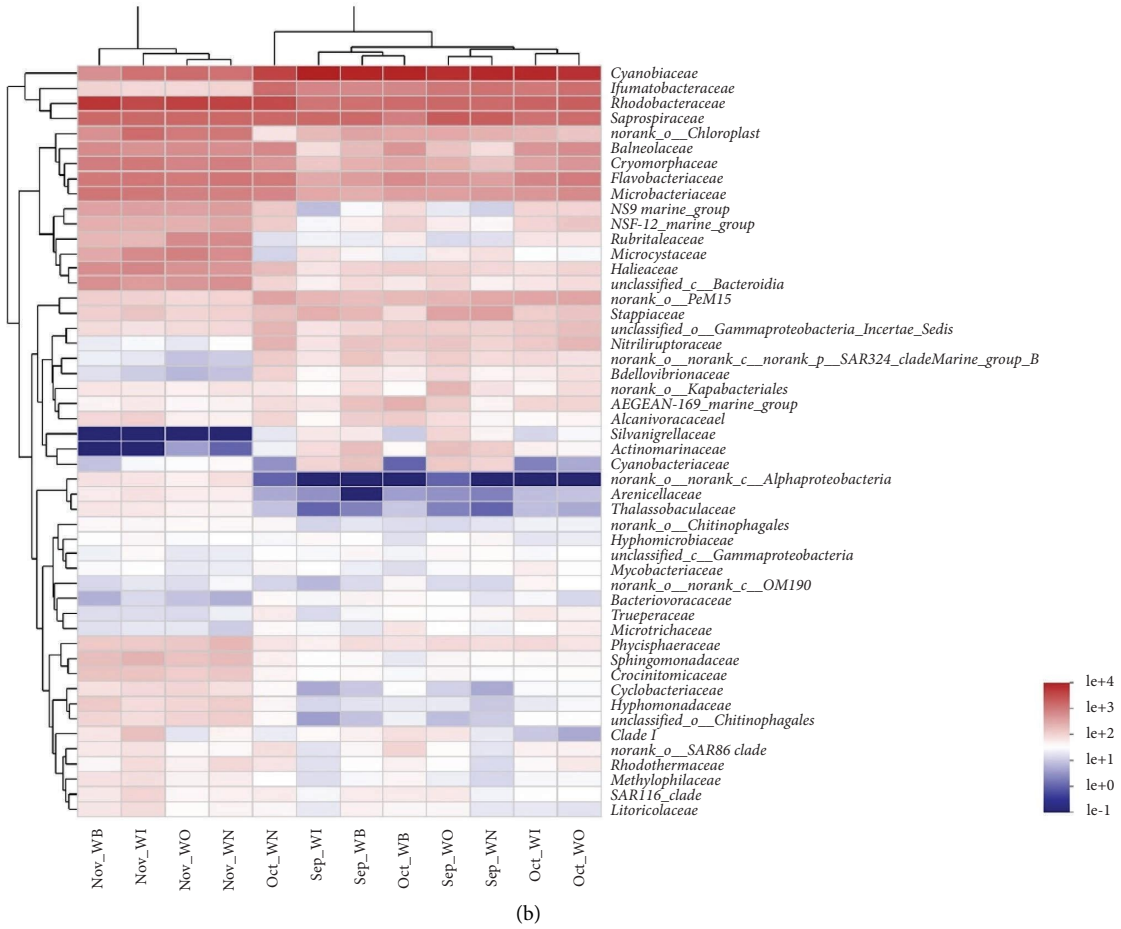
The reason why dissolved oxygen in the bivalve culture area in overlying water was higher is that *Cyanobacteria*, which are oxygenic photoautotrophs [42], were the dominant bacteria in the area with bivalves. However, the benthic filter-feeding bivalves absorb suspended organic particles in the overlying water, which are released by their physical movement over the sediment surface [43] and increased phosphorus release to the overlying water [21, 44], which are essential for the photosynthesis activity in *Cyanobacteria* [45] to influence their growth [46]. Besides, some cyanobacterial species are involved in the nitrogen cycle [47] because *Cyanobacteria* can utilise inorganic nitrogen, atmospheric nitrogen, and some amino acids as nitrogen sources [42]. What is noteworthy is that although dissolved inorganic nutrients are released into the overlying water through bivalve bioturbation, this does not result in an additional nutrient loading due to the rapid nutrient cycling and a net removal of a portion of those nutrients when bivalves are harvested [21].

In general, *Proteobacteria, Bacteroidota*, Firmicutes, and Actinobacteriota are the dominant bacterial phyla in water in IMTA systems [48–51]. In addition, some IMTA systems with different species showed different bacterial abundances; for instance, Actinobacteriota was the predominant phylum in a system containing shrimps, crabs, and bivalves, and *Firmicutes* was the predominant phylum in a system containing shrimps and crabs. The predominated bacterial communities of sediment were Proteobacteria, Acidobacteriota, Chloroflexi, Bacteroidota, Planctomycetota, and Alphaproteobacteria in a bioremediation system with macrobenthos (bivalves and polychaetes) [20]. However, in



(a)

Figure 3: Continued.



(b)

FIGURE 3: Heatmap analysis between bacterial groups, locations, and time in overlying water at phylum (a) and family (b) levels. *I*: water inlet, *O*: water outlet, *B*: area with bivalves, *N*: area without bivalves, Sep: September, Oct: October, and Nov: November.

this study, *Cyanobacteria*, *Proteobacteria*, *Bacteroidota*, and *Acidobacteriota* were dominant bacteria in overlying water, whereas *Proteobacteria*, Desulfobacterota, *Chloroflexi, Bacteroidota*, *Actinobacteriota*, *Firmicutes*, *Acidobacteriota*, and *Cyanobacteria* were the dominant bacterial phyla in sediment. Some members of *Proteobacteria* and *Bacteroidota*, which have a highly stable ability to remove ammonia under aerobic conditions [52], showed a higher proportion in the bivalve area. *Rhodobacteraceae*, which showed higher proportions in the area with bivalves, can use various unstable organic matters [53] to obtain their energy through different energy acquisition mechanisms, including the heterotrophic oxidation of organic matter, photoheterotrophy, and non-obligate chemolithotrophy [54]. In addition, the analysis of species difference among each group was analyzed by one-way ANOVA. The results show that comparing with other sampling locations, the bivalve can significantly increase the *Thiotrichaceae* family and *norank_f__Thiotrichaceae* genus in overlying water and significantly increase *Clostridiaceae* family, *Oceanirhabdus* genus, and *unclassified_f__ Clostridiaceae* genus in sediments, and they have a small proportion of the total bacteria. There was no significant difference ($P > 0.05$) in the dominant bacteria among four groups. These bacteria could explain the effect of bivalves on a bacterial structure through bioturbation, and, subsequently, their role in water quality control, such as nitrogen removal.

In this study, the Shannon and Ace indexes were higher in the water area with bivalves, with significant differences in October and September, respectively. In sediment during the

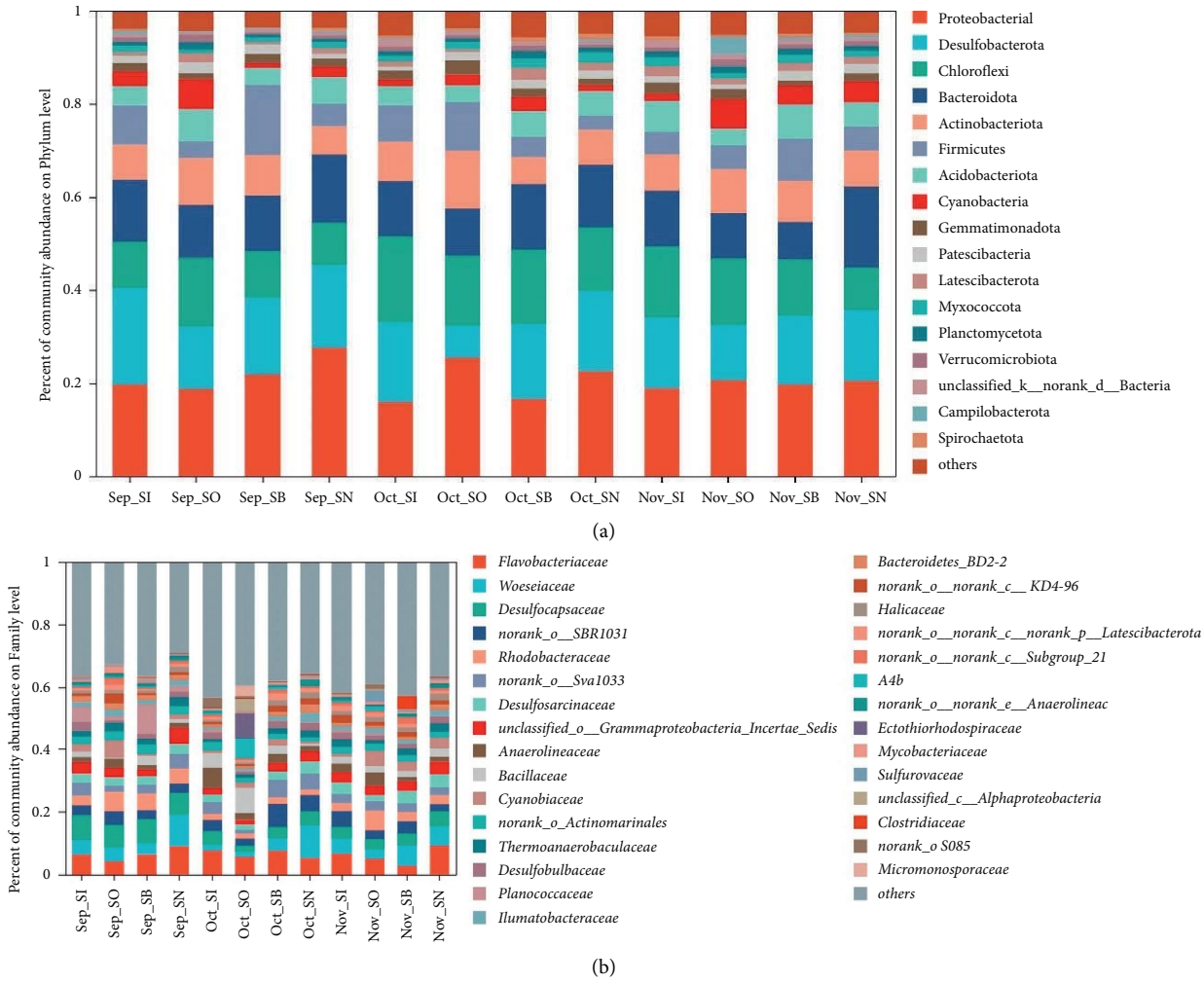


Figure 4: Microbiota composition in sediment at phylum (a) and family (b) levels. *I*: water inlet, *O*: water outlet, *B*: area with bivalves, *N*: area without bivalves, Sep: September, Oct: October, and Nov: November.

whole experiment period, the Ace index was significantly higher in the area with bivalves. The results demonstrated that bivalves could increase the diversity and abundance of bacteria in the system. The possible reason is that bivalves can bioturbate the sediment by moving and feeding, thereby increasing oxygen penetration into the sediment [55],

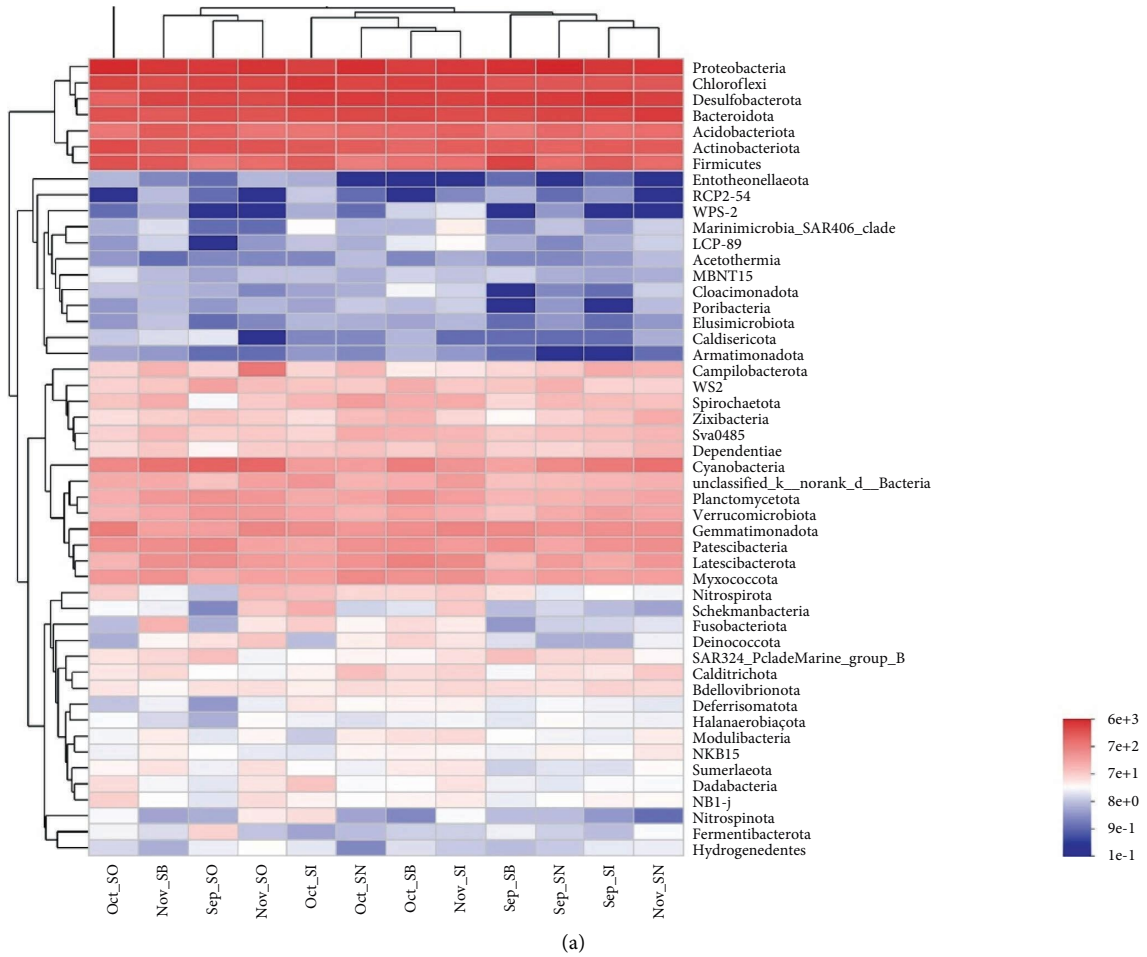
Table 4: The first dominant bacterial groups in sediment.

Location	Classification	Month					
		September	(%)	October	(%)	November	(%)
Inlet	Phylum	Desulfobacterota	20	*Chloroflexi*	18	Proteobacteria	19
	Family	*Desulfocapsaceae*	8	*Flavobacteriaceae*	8	*Flavobacteriaceae*	6
	Genus	*Woeseia*	4	*norank_f__Anaerolineaceae*	5	*Woeseia*	4
Outlet	Phylum	Proteobacteria	18	*Proteobacteria*	25	Proteobacteria	20
	Family	*Desulfocapsaceae*	7	*Ectothiorhodospiraceae*	8	*Rhodobacteraceae*	6
	Genus	*Cyanobium_PCC*-6307	5	*Thiohalomonas*	8	*norank_f__Anaerolineaceae*	4
Bivalve	Phylum	Proteobacteria	22	*Proteobacteria*	16	Proteobacteria	19
	Family	*Planococcaceae*	9	*Flavobacteriaceae*	7	*Woeseiaceae*	6
	Genus	*Planococcus*	4	*norank_f__norank_o__SBR*1031	7	*Woeseia*	6
Nonbivalve	Phylum	Proteobacteria	28	*Proteobacteria*	22	Proteobacteria	20
	Family	*Woeseiaceae*	10	*Woeseiaceae*	10	*Flavobacteriaceae*	9
	Genus	*Woeseia*	10	*Woeseia*	10	*Woeseia*	10

Table 5: Alpha-diversity indexes of the bacterial community in sediment.

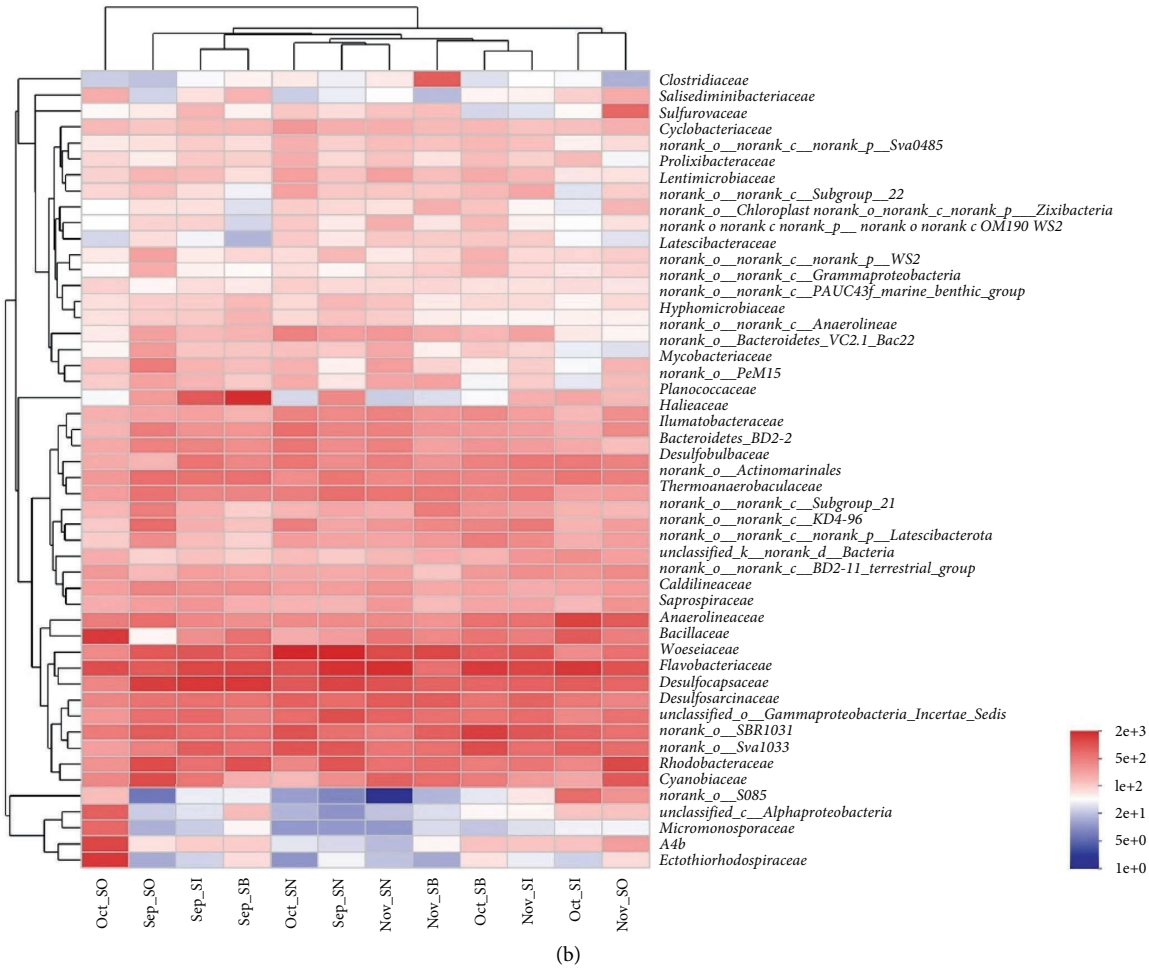
Time	Site	Sobs	Shannon	Ace	Chao1
September	Inlet	2786.33 ± 109.45[a]	6.47 ± 0.07[a]	5470.67 ± 178.65[a]	4361 ± 118.03[a]
	Outlet	2105.33 ± 118.67[a]	6.14 ± 0.07[a]	3938 ± 115.76[c]	3249 ± 108.04[b]
	Bivalve	2664 ± 127.31[a]	6.34 ± 0.04[a]	5351.33 ± 188.57[a]	4285.33 ± 107.63[a]
	Nonbivalve	2302 ± 115.76[a]	6.17 ± 0.07[a]	4744 ± 158.03[b]	3679 ± 109.76[ab]
October	Inlet	2960.33 ± 179.29[a]	6.56 ± 0.12[a]	5767 ± 121.76[a]	4577 ± 115.98[a]
	Outlet	2622.67 ± 118.65[a]	5.98 ± 0.12[a]	5139 ± 115.18[ab]	4084 ± 113.97[a]
	Bivalve	2629 ± 173.49[a]	6.63 ± 0.14[a]	5798 ± 123.76[a]	4680 ± 117.34[a]
	Nonbivalve	2471 ± 133.42[a]	6.32 ± 0.13[a]	4752 ± 115.76[b]	4014 ± 108.95[a]
November	Inlet	3136.67 ± 63.91[a]	6.84 ± 0.06[a]	5704 ± 121.18[a]	4641 ± 116.98[a]
	Outlet	2614 ± 115.76[a]	6.47 ± 0.12[b]	4784 ± 115.76[b]	3858.67 ± 109.99[b]
	Bivalve	2756 ± 116.35[a]	6.67 ± 0.13[ab]	4850 ± 116.94[b]	4002 ± 109.32[ab]
	Nonbivalve	2540 ± 113.85[a]	6.43 ± 0.12[b]	3893 ± 114.97[c]	3865 ± 109.66[b]

One-way ANOVA was used to test the significant differences among sampling locations at the same sampling time. [a, b, c]: Samples without letters in common indicate significant differences ($P < 0.05$).



(a)

Figure 5: Continued.

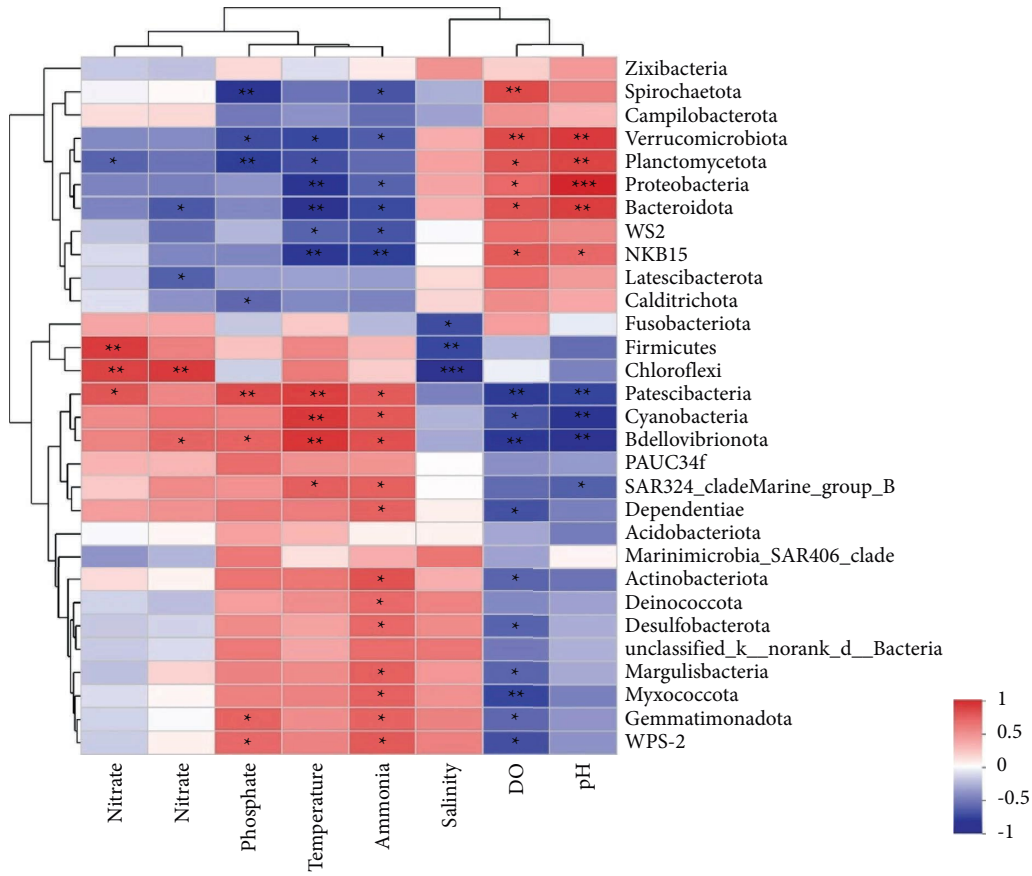


(b)

FIGURE 5: Heatmap analysis between bacterial groups, locations, and time in sediment at phylum (a) and family (b) levels. *I*: water inlet, *O*: water outlet, *B*: area with bivalves, *N*: area without bivalves, Sep: September, Oct: October, and Nov: November.

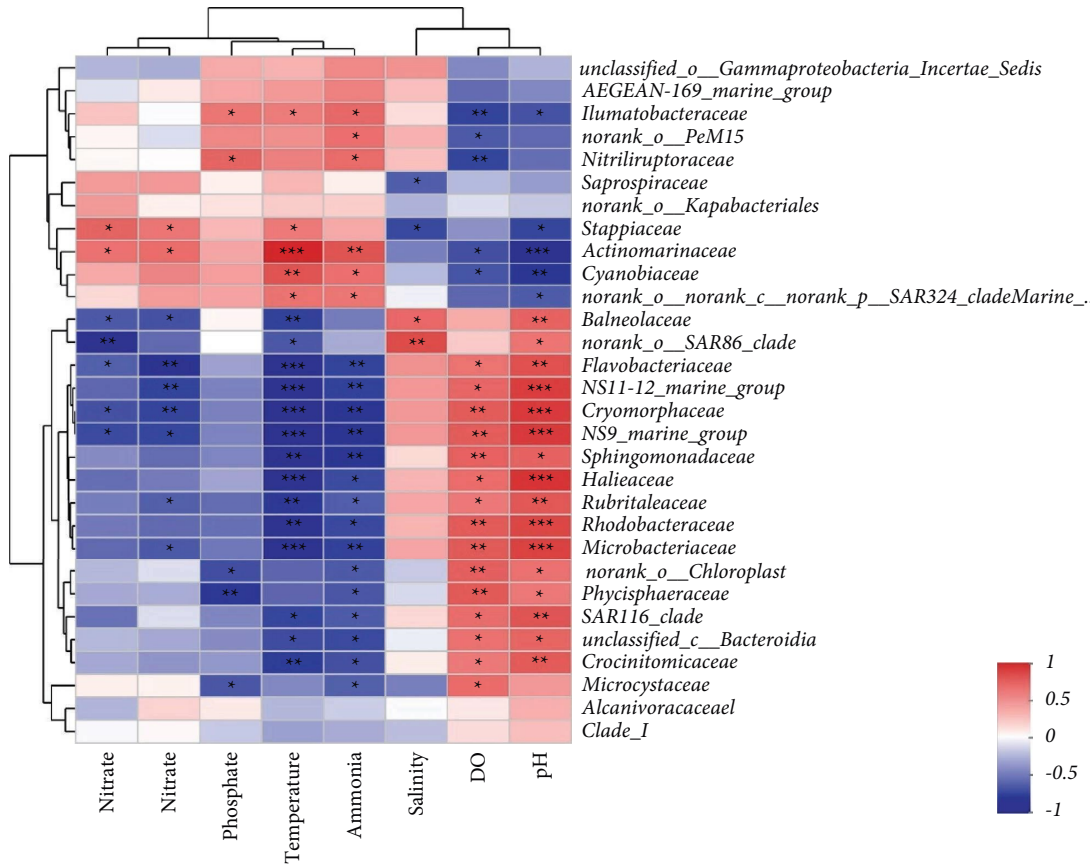
stimulating microbial metabolism [56], and ultimately affecting bacterial community structure, especially increasing the abundance of aerobic bacteria.

However, in sediment, the predominant bacteria in the area with bivalves belonged to the *Planococcaceae* and *Flavobacteriaceae* families, whereas the *Woeseia* genus was predominant in the area without bivalves during the middle and late period of aquaculture. Different bacterial dominance among different studies could be attributed to environmental factors, including nutrient availability, light intensity, temperature, and dietary composition [57, 58], in addition to the aquatic organisms in the IMTA system. Overall, our results suggest that bivalves can change the bacterial community structure of IMTA systems (especially sediments) by releasing nutrients and increasing dissolved oxygen through bioturbation.



(a)

Figure 6: Continued.



(b)

Figure 6: Heatmap analysis between bacterial groups and overlying water chemical characteristics at phylum (a) and family (b) levels. *I*: water inlet, *O*: water outlet, *B*: area with bivalves, *N*: area without bivalves, Sep: September, Oct: October, and Nov: November. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; red means positive correlation, and blue means negative correlation.

5. Conclusions

Bivalves can release phosphorus through bioturbation to increase the abundance of *Cyanobacteria*, which release dissolved oxygen into overlying water through photosynthesis, enhance nitrification (mainly ammonia oxidation), and improve the ammonia nitrogen removal capacity of the system. Furthermore, bivalves can increase bacterial diversity and abundance by regulating dissolved oxygen, especially the abundance of heterotrophic bacteria, which are important for water quality control. This implied the effective role of bivalves in water quality control as an essential aquatic organism in the IMTA system. This study provided insight into the interaction between bivalves and bacterial composition on nitrogen removal in the IMTA system.

Data Availability

All supporting data generated during this study are included within this published article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Shuo Kong. The first draft of the manuscript was written by Shuo Kong. This manuscript was revised by Zhao Chen and Abdallah Ghonimy. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Acknowledgments

The authors acknowledge Zhao Chen and Abdallah Ghonimy for reviewing this manuscript. They specially thank Abdallah Ghonimy for proofreading the article. Jian Li, as the project leader of the project that financed this research, contributed to the exposure planning and execution. The authors acknowledge the sequencing service from the Majorbio Company (Shanghai, China). This study was supported by the National Key Research and Development Program of China (no. 2019YFD0900403); the China Agriculture Research System (no. CARS-48); the National Natural Science Foundation of China (31873039); and the Aoshan Innovation Project of Qingdao National Laboratory for Marine Science and Technology (no. 2015ASKJ02).

References

[1] M. A. J. Nederlof, M. C. J. Verdegem, and A. C. Smaal, "Nutrient retention efficiencies in integrated multi-trophic aquaculture," *Reviews in Aquaculture*, vol. 14, no. 3, pp. 1194–1212, 2022.

[2] M. H. Abreu, R. Pereira, C. Yarish, A. H. Buschmann, and I. Sousa-Pinto, "IMTA with *Gracilaria vermiculophylla*: productivity and nutrient removal performance of the seaweed in a land-based pilot scale system," *Aquaculture*, vol. 312, no. 1–4, pp. 77–87, 2011.

[3] M. Troell, A. Joyce, T. Chopin, A. Neori, A. H. Buschmann, and J. G. Fang, "Ecological engineering in aquaculture — potential for integrated multi-trophic aquaculture (IMTA) in marine offshore systems," *Aquaculture*, vol. 297, no. 1–4, pp. 1–9, 2009.

[4] Z. Q. Chang, A. Neori, Y. Y. He et al., "Development and current state of seawater shrimp farming, with an emphasis on integrated multi-trophic pond aquaculture farms, in China – a review," *Reviews in Aquaculture*, vol. 12, no. 4, pp. 2544–2558, 2020.

[5] A. B. Jones, W. C. Dennison, and N. P. Preston, "Integrated treatment of shrimp effluent by sedimentation, oyster filtration and macroalgal absorption: a laboratory scale study," *Aquaculture*, vol. 193, no. 1-2, pp. 155–178, 2001.

[6] T. M. Samocha, J. Fricker, A. M. Ali, M. Shpigel, and A. Neori, "Growth and nutrient uptake of the macroalga *Gracilaria tikvahiae* cultured with the shrimp *Litopenaeus vannamei* in an Integrated Multi-Trophic Aquaculture (IMTA) system," *Aquaculture*, vol. 446, pp. 263–271, 2015.

[7] D. Gallardi, "Effects of bivalve aquaculture on the environment and their possible mitigation: a review," *Fisheries and Aquaculture Journal*, vol. 05, no. 03, 2014.

[8] B. Lukwambe, L. Zhao, R. Nicholaus, W. Yang, J. Zhu, and Z. Zheng, "Bacterioplankton community in response to biological filters (clam, biofilm, and macrophytes) in an integrated aquaculture wastewater bioremediation system," *Environmental Pollution*, vol. 254, Article ID 113035, 2019.

[9] A. Domingues, I. C. Rosa, J. Pinto da Costa et al., "Potential of the bivalve Corbicula fluminea for the remediation of olive oil wastewaters," *Journal of Cleaner Production*, vol. 252, Article ID 119773, 2020.

[10] P. H. Doering, J. R. Kelly, C. A. Oviatt, and T. Sowers, "Effect of the hard clam *Mercenaria mercenaria* on benthic fluxes of inorganic nutrients and gases," *Marine Biology*, vol. 94, no. 3, pp. 377–383, 1987.

[11] D. Baudinet, E. Alliot, B. Berland et al., "Incidence of mussel culture on biogeochemical fluxes at the sediment-water interface," *Hydrobiologia*, vol. 207, no. 1, pp. 187–196, 1990.

[12] M. L. Kellogg, A. R. Smyth, M. W. Luckenbach et al., "Use of oysters to mitigate eutrophication in coastal waters," *Estuarine, Coastal and Shelf Science*, vol. 151, pp. 156–168, 2014.

[13] J. Lu, Y. Zhang, J. Wu, and J. Wang, "Nitrogen removal in recirculating aquaculture water with high dissolved oxygen conditions using the simultaneous partial nitrification, anammox and denitrification system," *Bioresource Technology*, vol. 305, Article ID 123037, 2020.

[14] S. Suurnäkki, J. T. Pulkkinen, P. C. Lindholm-Lehto, M. Tiirola, and S. L. Aalto, "The effect of peracetic acid on microbial community, water quality, nitrification and rainbow trout (*Oncorhynchus mykiss*) performance in recirculating aquaculture systems," *Aquaculture*, vol. 516, Article ID 734534, 2020.

[15] X. Huang, X. Yang, J. Zhu, and J. Yu, "Microbial interspecific interaction and nitrogen metabolism pathway for the treatment of municipal wastewater by iron carbon based constructed wetland," *Bioresource Technology*, vol. 315, Article ID 123814, 2020.

[16] Q. Guo, J. Liu, L. Yu, H. Korpelainen, and C. Li, "Different sexual impacts of dioecious *Populus euphratica* on microbial communities and nitrogen cycle processes in natural forests," *Forest Ecology and Management*, vol. 496, Article ID 119403, 2021.

[17] M. Labbate, J. R. Seymour, F. Lauro, and M. V. Brown, "Editorial: anthropogenic impacts on the microbial ecology and function of aquatic environments," *Frontiers in Microbiology*, vol. 7, p. 1044, 2016.

[18] W. Dai, J. Zhang, Q. Tu, Y. E. Deng, Q. Qiu, and J. Xiong, "Bacterioplankton assembly and interspecies interaction indicating increasing coastal eutrophication," *Chemosphere*, vol. 177, pp. 317–325, 2017.

[19] Z. Qi, X. H. Zhang, N. Boon, and P. Bossier, "Probiotics in aquaculture of China—current state, problems and prospect," *Aquaculture*, vol. 290, no. 1-2, pp. 15–21, 2009.

[20] Y. Ma, A. Hu, C. P. Yu et al., "Response of microbial communities to bioturbation by artificially introducing macrobenthos to mudflat sediments for in situ bioremediation in a typical semi-enclosed bay, southeast China," *Marine Pollution Bulletin*, vol. 94, no. 1–2, pp. 114–122, 2015.

[21] B. R. Dumbauld, J. L. Ruesink, and S. S. Rumrill, "The ecological role of bivalve shellfish aquaculture in the estuarine environment: a review with application to oyster and clam culture in West Coast (USA) estuaries," *Aquaculture*, vol. 290, no. 3–4, pp. 196–223, 2009.

[22] N. Xu, G. Tan, H. Wang, and X. Gai, "Effect of biochar additions to soil on nitrogen leaching, microbial biomass and bacterial community structure," *European Journal of Soil Biology*, vol. 74, pp. 1–8, 2016.

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

[24] S. Chen, Y. Zhou, Y. Chen, and J. Gu, "fastp: an ultra-fast all-in-one FASTQ preprocessor," *Bioinformatics*, vol. 34, no. 17, pp. i884–i890, 2018.

[25] R. C. Edgar, "UPARSE: highly accurate OTU sequences from microbial amplicon reads," *Nature Methods*, vol. 10, no. 10, pp. 996–998, 2013.

[26] E. Stackebrandt and B. M. Goebel, "Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology," *International Journal of Systematic and Evolutionary Microbiology*, vol. 44, no. 4, pp. 846–849, 1994.

[27] Q. Wang, G. M. Garrity, J. M. Tiedje, and J. R. Cole, "Naïve bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy," *Applied and Environmental Microbiology*, vol. 73, no. 16, pp. 5261–5267, 2007.

[28] P. D. Schloss, S. L. Westcott, T. Ryabin et al., "Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities," *Applied and Environmental Microbiology*, vol. 75, no. 23, pp. 7537–7541, 2009.

[29] T. Liu, A. N. Zhang, J. Wang et al., "Integrated biogeography of planktonic and sedimentary bacterial communities in the Yangtze River," *Microbiome*, vol. 6, no. 1, pp. 16–14, 2018.

[30] L. Wu, M. Shen, J. Li et al., "Cooperation between partial-nitrification, complete ammonia oxidation (comammox), and anaerobic ammonia oxidation (anammox) in sludge digestion

liquid for nitrogen removal," *Environmental Pollution*, vol. 254, Article ID 112965, 2019.

[31] H. F. Kaspar, P. A. Gillespie, I. C. Boyer, and A. L. MacKenzie, "Effects of mussel aquaculture on the nitrogen cycle and benthic communities in Kenepuru Sound, Marlborough Sounds, New Zealand," *Marine Biology*, vol. 85, no. 2, pp. 127–136, 1985.

[32] R. I. E. Newell and E. W. Koch, "Modeling seagrass density and distribution in response to changes in turbidity stemming from bivalve filtration and seagrass sediment stabilization," *Estuaries*, vol. 27, no. 5, pp. 793–806, 2004.

[33] V. Kathiravan and K. K. Krishnani, "Diversity of denitrifying bacteria in the greenwater system of coastal aquaculture," *International Aquatic Research*, vol. 6, no. 3, pp. 135–145, 2014.

[34] A. Choi, H. Cho, S. H. Kim, B. Thamdrup, S. Lee, and J. H. Hyun, "Rates of N_2 production and diversity and abundance of functional genes associated with denitrification and anaerobic ammonium oxidation in the sediment of the Amundsen Sea Polynya, Antarctica," *Deep Sea Research Part II: Topical Studies in Oceanography*, vol. 123, pp. 113–125, 2016.

[35] D. R. Arahal, M. C. Macián, E. Garay, and M. J. Pujalte, "Thalassobius mediterraneus gen. nov., sp. nov., and reclassification of Ruegeria gelatinovorans as Thalassobius gelatinovorus comb. nov," *International Journal of Systematic and Evolutionary Microbiology*, vol. 55, no. 6, pp. 2371–2376, 2005.

[36] S. Park, M. H. Lee, J. S. Lee, T. K. Oh, and J. H. Yoon, "Thalassobius maritimus sp. nov., isolated from seawater," *International Journal of Systematic and Evolutionary Microbiology*, vol. 62, no. 1, pp. 8–12, 2012.

[37] Y. H. Shin, J. H. Kim, A. Suckhoom, D. Kantachote, and W. Kim, "Limibaculum halophilum gen. nov., sp. nov., a new member of the family Rhodobacteraceae," *International Journal of Systematic and Evolutionary Microbiology*, vol. 67, no. 10, pp. 3812–3818, 2017.

[38] G. Fan, J. Zhang, Y. Wang et al., "Microbial community and nitrogen transformation pathway in bioretention system for stormwater treatment in response to formulated soil medium," *Process Safety and Environmental Protection*, vol. 161, pp. 594–602, 2022.

[39] B. P. Durham, J. Grote, K. A. Whittaker et al., "Draft genome sequence of marine alphaproteobacterial strain HIMB11, the first cultivated representative of a unique lineage within the *Roseobacter* clade possessing an unusually small genome," *Standards in Genomic Sciences*, vol. 9, no. 3, pp. 632–645, 2014.

[40] R. C. Aller, "Bioturbation and remineralization of sedimentary organic matter: effects of redox oscillation," *Chemical Geology*, vol. 114, no. 3–4, pp. 331–345, 1994.

[41] B. Lukwambe, W. Yang, Y. Zheng, R. Nicholaus, J. Zhu, and Z. Zheng, "Bioturbation by the razor clam (*Sinonovacula constricta*) on the microbial community and enzymatic activities in the sediment of an ecological aquaculture wastewater treatment system," *Science of the Total Environment*, vol. 643, pp. 1098–1107, 2018.

[42] E. Flores, S. Arévalo, and M. Burnat, "Cyanophycin and arginine metabolism in Cyanobacteria," *Algal Research*, vol. 42, Article ID 101577, 2019.

[43] E. Michaud, G. Desrosiers, F. Mermillod-Blondin, B. Sundby, and G. Stora, "The functional group approach to bioturbation: the effects of biodiffusers and gallery-diffusers of the Macoma balthica community on sediment oxygen uptake," *Journal of Experimental Marine Biology and Ecology*, vol. 326, no. 1, pp. 77–88, 2005.

[44] E. Sinha, A. M. Michalak, and V. Balaji, "Eutrophication will increase during the 21st century as a result of precipitation changes," *Science*, vol. 357, no. 6349, pp. 405–408, 2017.

[45] J. M. Ebeling, M. B. Timmons, and J. J. Bisogni, "Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia–nitrogen in aquaculture systems," *Aquaculture*, vol. 257, no. 1–4, pp. 346–358, 2006.

[46] R. Nicholaus, B. Lukwambe, L. Zhao, W. Yang, J. Zhu, and Z. Zheng, "Bioturbation of blood clam Tegillarca granosa on benthic nutrient fluxes and microbial community in an aquaculture wastewater treatment system," *International Biodeterioration & Biodegradation*, vol. 142, pp. 73–82, 2019.

[47] P. Nandagopal, A. N. Steven, L. W. Chan, Z. Rahmat, H. Jamaluddin, and N. I. Mohd Noh, "Bioactive metabolites produced by Cyanobacteria for growth adaptation and their pharmacological properties," *Biology*, vol. 10, no. 10, p. 1061, 2021.

[48] C. Ying, M. J. Chang, C. H. Hu et al., "The effects of marine farm-scale sequentially integrated multi-trophic aquaculture systems on microbial community composition, prevalence of sulfonamide-resistant bacteria and sulfonamide resistance gene sul1," *Science of the Total Environment*, vol. 643, pp. 681–691, 2018.

[49] G. Califano, M. Kwantes, M. H. Abreu, R. Costa, and T. Wichard, "Cultivating the macroalgal holobiont: effects of integrated multi-trophic aquaculture on the microbiome of *Ulva rigida* (Chlorophyta)," *Frontiers in Marine Science*, vol. 7, p. 52, 2020.

[50] Y. Bao, W. He, S. Zhao et al., "Planktonic and sediment bacterial communities in an integrated mariculture system," *Letters in Applied Microbiology*, vol. 72, no. 3, pp. 341–350, 2021.

[51] M. Q. Zhang, J. L. Yang, X. X. Lai et al., "Effects of integrated multi-trophic aquaculture on microbial communities, antibiotic resistance genes, and cultured species: a case study of four mariculture systems," *Aquaculture*, vol. 557, Article ID 738322, 2022.

[52] Y. Zhao, J. Huang, H. Zhao, and H. Yang, "Microbial community and N removal of aerobic granular sludge at high COD and N loading rates," *Bioresource Technology*, vol. 143, pp. 439–446, 2013.

[53] H. Dang and C. R. Lovell, "Microbial surface colonization and biofilm development in marine environments," *Microbiology and Molecular Biology Reviews*, vol. 80, no. 1, pp. 91–138, 2016.

[54] H. Luo and M. A. Moran, "Evolutionary ecology of the marine *Roseobacter* clade," *Microbiology and Molecular Biology Reviews*, vol. 78, no. 4, pp. 573–587, 2014.

[55] C. C. Vaughn and C. C. Hakenkamp, "The functional role of burrowing bivalves in freshwater ecosystems," *Freshwater Biology*, vol. 46, no. 11, pp. 1431–1446, 2001.

[56] R. Dame, *Ecology of marine Bivalves: An Ecosystem Approach*Routledge, England, UK, 1996.

[57] C. Pereira, L. Santos, A. P. Silva et al., "Seasonal variation of bacterial communities in shellfish harvesting waters: preliminary study before applying phage therapy," *Marine Pollution Bulletin*, vol. 90, no. 1-2, pp. 68–77, 2015.

[58] J. Lin, B. Ju, Y. Yao et al., "Microbial community in a multitrophic aquaculture system of Apostichopus japonicus, Styela clava and microalgae," *Aquaculture International*, vol. 24, no. 4, pp. 1119–1140, 2016.