Seasonal changes in community composition and diversity of bacterioplankton in an artificial lagoon in China

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Coastal lagoons are highly productive ecosystems. However, coastal lagoons are experiencing the effects of human disturbances at an increasing rate. Bacteria are key ecological players within lagoons, yet little is known about the magnitude, patterns, and drivers of diversity in these transitional environments. In this report, a seasonal study in the Fengxian artificial lagoon (China) was conducted, along with the adjacent sea, to simultaneously explore diversity in different domains and their spatio-temporal variability. Bacterioplankton community structures of surface waters from four sites over the course of four seasons were characterized with Illumina platform sequencing technology. The results showed significant differences in bacterioplankton communities between the four sites. In addition, the results indicated a difference between the enclosed lagoon and offshore waters during the same seasons. Seasonality was shown to be more important than spatial variability in shaping assemblages. The community barplot showed that, with the exception of January which had the same dominant genus in both the enclosed lagoon and offshore water, all other seasons had different genus. Likewise, the heatmap showed that the largest dissimilarity of bacterial species diversity occurred in July between the enclosed lagoon and offshore water, while the highest similarity was in January. This result paralleled genetic studies which also showed that gene expression had not only similar seasonal but spatial changes. Canonical correspondence analysis (CCA) analysis of all water samples showed that environmental indicators of dissolved oxygen, temperature, PO_4, NO_2, and Chemical Oxygen Demand (COD) contributed to the variation. Bacterial communities in the lagoon are affected by temperature, dissolved oxygen and NO_2, while the dominant bacterial communities offshore are affected by COD and PO_4. This study provided evidence for a temporally dynamic structure of bacterial assemblages in lagoons. In this vulnerable ecosystem, there is interplay of seasonally-influenced environmental drivers that works together to shape bacterial assemblages. The goal is to identify beneficial microbial population that improved water quality in an enclosed lagoon in order to provide a new perspective for optimizing the breeding environment.

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Abstract Coastal lagoons are highly productive ecosystems. However, coastal lagoons are experiencing the effects of human disturbances at an increasing rate. Bacteria are key ecological players within lagoons, yet little is known about the magnitude, patterns, and drivers of diversity in these transitional environments. In this report, a seasonal study in the Fengxian artificial lagoon (China) was conducted, along with the adjacent sea, to simultaneously explore diversity in different domains and their spatio-temporal variability. Bacterioplankton community structures of surface waters from four sites over the course of four seasons were characterized with Illumina platform sequencing technology. The results showed significant differences in bacterioplankton communities between the four sites. In addition, the results indicated a difference between the enclosed lagoon and offshore waters during the same seasons. Seasonality was shown to be more important than spatial variability in shaping assemblages. The community barplot showed that, with the exception of January which had the same dominant genus in both the enclosed lagoon and offshore water, all other seasons had different genus. Likewise, the heatmap showed that the largest dissimilarity of bacterial species diversity occurred in July between the enclosed lagoon and offshore water, while the highest similarity was in January. This result paralleled genetic studies which also showed that gene expression had not only similar seasonal but spatial changes. Canonical correspondence analysis (CCA) analysis of all water samples showed that environmental indicators of dissolved oxygen, temperature, PO_4, NO_2, and Chemical Oxygen Demand (COD) contributed to the variation. Bacterial communities in the lagoon are affected by temperature, dissolved oxygen and NO_2, while the dominant bacterial communities offshore are affected by COD and PO_4. This study provided evidence for a temporally dynamic structure of bacterial assemblages in lagoons. In this vulnerable ecosystem, there is interplay of seasonally-influenced environmental drivers that works together to shape bacterial assemblages. The goal is to identify beneficial microbial population that improved water quality in an enclosed lagoon in order to provide a new perspective for optimizing the breeding environment.

Keywords: artificial lagoon, bacterioplankton community, Illumina, environment

Introduction

The coastal natural lagoons are transitional ecosystems at the boundary between land and sea. These vulnerable ecosystems are indirectly connected with the ocean and experience saline intrusions (Schallenberg & Larned et al., 2010). In addition to water exchange with the ocean, the status of a lagoon largely depends on the water quality of all inflowing rivers as well as precipitation. Increased precipitation and river discharge would reduce salinity and enhance eutrophication (Thompson et al., 2009a). Hydrodynamic characteristics are important controls in coastal lagoons as well. Factors such as tidal changes, wind speed, and water density are important drivers of water exchange in the lagoon ecology (Thompson et al., 2009b). Plankton primary production is co-limited by phosphorus and nitrogen levels due to the combined effects of water and high nutrient inputs from the boundary (Béjaoui et al., 2017). Similarly, it has been reported that freshwater surges lead to short-term increases (1–2days) in bacterial production as well as increases in the abundance of bacterioplankton and picoeukaryotes (Fouilland et al., 2017). A study on the bacterioplankton in the Conceição Lagoon, Southern Brazil, was carried out in winter and summer to characterize the bacterial spatiotemporal distribution and heterotrophism. This study indicated that bacterial abundance increased significantly ($p<0.05$) in summer. Principal component analysis showed that salinity, temperature, and light were the abiotic factors that better explained the temporal variability of bacterial assemblages. Spatially, bacterial assemblages were influenced by nutrient gradients and oxygen (Fontes & Abreu, 2010).The Rodrigo de Freitas Lagoon consists of fresh water but has a connection with the ocean through a channel. Thus, research results showed that the lagoon is affected by adjacent fresh water and the structure of the bacterial community had both freshwater and marine characteristics when sampled from within the channel.

Prokaryotes are key components within lagoons, due to their role as primary producers (e.g., photoautotrophic bacteria). Prokaryotes are agents of organic matter remineralization and particles degradation, cycling of biogeochemically relevant elements, pollutants degradation, and transfer of matter and energy to higher trophic levels (Quero et al., 2017).In recent years, bacterioplankton research has been given more attention for these reasons. Researchers have carried out extensive research on coastal waters nationally and internationally. Coastal lagoons are highly productive ecosystems characterized by chemical and physical gradients that make these systems unstable and subject to fluctuating conditions (Manini et al., 2003). They provide diverse ecosystem services, such as flood and erosion control, shoreline stabilization, sediment and nutrient retention, local mitigation of climate change effects and water purification, and they represent a reservoir of biodiversity and biomass (Danovaro, Pusceddu, 2007). At the same time, coastal lagoons are vulnerable to a number of anthropogenic disturbances such as agricultural, industrial, and tourist activities (Ghai et al., 2012a; Ferrarin et al., 2015). They represent at ransition zone between terrestrial, freshwater, and marine interfaces (Newton et al.. 2014) and act either as sinks for organic matter accumulation(Pinhassi, Berman, 2003). In addition, they can act as reservoirs able to fertilize the adjacent sea by exporting organic and inorganic nutrients (Marques et al., 2014a). The balance between export and accumulation depends, in addition to physical and hydrological factors, on degradation and utilization processes by planktonic and benthic microbes. This data demonstrates the unique importance of studying the spatial and temporal dynamics of lagoon microbes.

Shanghai Fengxian Bihaijinsha is located in the south of Hangzhou Bay, near the Yangtze River estuary. As a typical offshore artificial lagoon, it was built in 2005 via coastal reclamation. The sea area is about 2.30 km^2 and the average depth is 15 meters. The water of this artificial lagoon, and its adjacent sea, were characterized by low salinity, muddy water, and large sediment. The industrial waste water receiving area of Hangzhou Bay poses a threat to this environment. The temporal and spatial changes of the microbial community in an industrial effluent receiving area in Hangzhou Bay were investigated by 454 pyrosequencing, and the bacterial community showed that proteobacteria dominated the bacterial communities of all sediment samples tested(Yan et al., 2016).It was found that distribution of microbes in the Yangtze River estuary had obvious seasonal variations. In summer, *Shewanella* and *Pseudomonas* were the dominant species, while the highest abundance in winter was *Acinetobacter* (Cao et al., 2011).Flow cytometry was used to examine the abundance and distribution of different picophytoplankton groups (i.e.,*Synechococcus*, *Prochlorococcus*, and picoeukaryotes).As such, nanophytoplankton, heterotrophic bacteria and viruses were examined in the Yangtze River estuary, China, and adjacent coastal waters during the autumn of 2004. The results showed that picoeukaryotes were the most successful group among picophytoplankton in nearshore eutrophic waters, whereas *Prochlorococcus* surpassed other groups within the pico- and nanophytoplankton communities in the offshore oligotrophic regions of the East China Sea Shelf (Pan , Zhang & Zhang , 2007).

In recent years, the Fengxian artificial lagoon has been mainly used to breed economic shrimp such as *Penaeus monodon* and *Penaeus orientalis*.The water contained in it is restored in March by local tides, and drained off the following January. The water column of the Fengxian artificial lagoon has higher transparency than the adjacent open sea, and is affected by the tide and precipitation levels. Due to aquaculture activities and the characteristics of water exchanges, the water quality of the artificial lagoon is difficult to control. The dynamics and diversity of bacterial communities are important indicators of ecosystem health and function. Changes in microbial community structure can also provide useful information about water environmental assessment and pollution control (Ghai et al., 2012b).In order to explore the relationship between water quality and the microbial communities, the dynamics and diversity of the microbial community in the surface water of the Fengxian artificial lagoon was studied.

Materials and methods

Site description and sampling

The survey was carried out every three months from April 2016 to January 2017, such as April 22, 2016, July 13, 2016, October 16, 2016, and January 15, 2017. Due to rain, sampling times were appropriately delayed. The sampling sites wre located near 121°E 30°W, as shown in Table 1 and Figure 1.Three liters of water from the upper 0.2 m of the sea surface were taken, then transported to the laboratory (on ice).Water samples were pre-filtered through 3µmpore size filters to remove large organisms and particles. Freeliving bacterioplankton cells were collected through 0.22µm polycarbonate filters. Filters were frozen at -80^{o}C until DNA extraction. Water chemistry analysis such as temperature and DO were monitored with a portable dissolved oxygen test (JENCO 9010, USA),while salinity was measured using a salinity meter(HAS-10, Shanghai).NO_2-N, NO_3-N, NH_4^+-N and PO_4^{3-}-P were filtered with 0.22μm cellulose acetate membranes and analyzed with Skalar flow analyzer(Skalar San++, Netherlands) (Londong & Wachtl, 1996).Chemical oxygen demand (COD) was determined by potassium permanganate titration(Tian & Wu SM, 1992).

DNA extraction, PCR and illumine sequencing

After frozen filter membranes were ground in liquid nitrogen, then samples were thoroughly mixed and centrifuged, and the environmental samples were extracted. DNA was quantitated by 1% agarose gel electrophoresis and then subjected to PCR amplification. Amplification of bacterial 16S rRNA gene fragments was conducted using barcode and adaptor added primer 515F (5'-GTGCCAGCMGCCGCGG-3') and 907R (5'-CCGTCAATTCMTTTRAGTTT-3') (Xiong et al, 2012).Barcode sequences were ligated to the sequencing primer during the process of primer synthesis, before PCR was performed. The reaction system consisting of 20 μL was assembled as follows: 5×FastPfu Buffer(4μL), 2.5 mM dNTPs(2 μL), forward primer(5 μM)(0.8 μL), reverse primer(5 μM)(0.8 μL), FastPfu Polymerase(0.4 μl), Template DNA(10 ng).The PCR reaction was performed in triplicate under the following conditions: an initial denaturation at 95°C for 3 min, 25 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s, and then a final extension at 72°C for 5min.After PCR amplification, the resulted PCR products were extracted from a 2% agarose gel and further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor™-ST (Promega, USA) according to the manufacturer's protocol. Finally, the Illumina platform library was constructed and sequencing started. Sequencing was carried out on Illumina platform at Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China.

Sequence quality control and operational taxonomic unit(OTU) assignment

The raw reads were processed following the pipeline of Mothur. According to the overlap relationship between PE reads, the paired reads were merged into a sequence, and the quality of reads and the effect of merge were quality-controlled. According to the sequence information of the two barcode ends and primer sequences, samples were discriminated and a valid sequence was obtained. Filtering parameters were: (i) minimum average quality score of 20; (ii) minimum overlap length of 10 bp; (iii) minimum mismatch rate of stitching sequence of 0.2; (iv) barcode mismatch number of 0 and maximum primer mismatch number of 2. Based on the similarity of the sequences, the sequence was classified as multiple OTUs and these OTUs were analyzed by biological information at 97% similarity levels. Based on the similarity of the sequences, the sequence was classified as multiple OTUs and these OTUs were analyzed by biological information at 97% similarity.

Data analysis

Alpha diversity measures including richness estimator Chao 1 (Chao & Bunge, 2002), diversity index Shannon (Magurran, 1988), and Good's coverage (Good, 1953), were calculated at a 3% dissimilarity level in Mothur. Statistic Package for Social Science (SPSS) software was used to analyze the diversity of the differences between the quarters. A community barplot and heatmap were generated in the Programming Language (R) (version 3.4.0) to compare the community composition of different groups at the Phylum and Genus levels. CCA with the Monte Carlo test was performed to calculate the relationship between bacterial clades and water properties at both the Order and Genus levels.

Results

Environmental characterization

The physical and chemical properties of sampling sites are showed in Figure 2.The temperature changed significantly over the four seasons, the maximum temperature can reach 29.9^{o}C in July, the lowest temperature reached 9.07^{o}C in January. Change in dissolved oxygen (DO) concentration showed an opposite trend with temperature, which reached the lowest value in July and the highest value the following January. The concentration of nutrients on the artificial lagoon water surface also showed a significant gradient. The concentration of PO_4^{3-}, NO_2^-, and NH_4^+ reached the highest value in July, and the concentration of NO_3^- reached the highest value in April. The concentration of COD in each site increased at first and then decreased, with a maximum value PO_4^{3-} in July.

Richness and diversity estimators

The total reads, ranging from 21998–44438 in each sample, were obtained for further analyses. After random resampling, all sequences were fractionated at 3% dissimilarity levels, ranging from 17918–39207.Data were further analyzed for diversity using Chao, Shannon, and Coverage (Table 2).Among these analytical tests, which were grouped by season, there was no significant difference in the Chao of all samples, but there were significant differences between seasons in the closed lagoon. January, April, and July were significantly different ($P<0.05$).In addition, there was no significant difference between the Shannon diversity seasons in all samples, and there was a significant difference between July, April, and October ($P<0.05$) in the closed lagoon. The coverage of all samples was above 99%, indicating that the probability of the sequence being detected in the sample was extremely high and reflected the real situation of microbes in the sample.

Taxonomic assignment

Thirty-four bacterial Phyla were found in this study. Among them, proteobacteria had the highest abundance, followed by cyanobacteria (Figure 3). According to seasonal variations, it was observed that the bacterioplankton community had obvious changes at the phylum level. The bacterioplankton community of the artificial lagoon and the adjacent open sea were significantly different, but no significant change was observed between the sampling sites within the artificial lagoon.

Synechococcus appeared primarily in October and July, and the abundance of the open sea site was significantly lower than that in the artificial lagoon. (Figure 4) The abundance of Betaproteobacterial genes in seasonal abundance was quite different. The abundance of the BAL58 marine group, primarily appearing in April, was higher in the offshore sites. However MWH-UniP1, also appearing in April, was more abundant in the closed lagoon. Comamonadaceae primarily appeared in the offshore samples with the highest abundance in October. In addition, pseudomonas of Gammaproteobacteria was dominant in July in the offshore sample and the abundance was very high. In addition, several genera appeared in Actinomycetes, although the abundance was not high, but the time and space differences were more obvious. The highest abundance of Actinobacteria was from the Hgcl-clade, appearing in July, while the offshore abundance was lower than in the closed lagoon. Several other genes were low in abundance, but there were also seasonal differences. For example, the CL500-29 marine group of Actinobacteria appeared in January, and the *Owenweeksia* and *Flavobacterium* of the Bacteroidetes possessed a high abundance in January and July samples appearing in the lagoon and offshore sites, respectively.

Based on the composition and abundance of each genus, the similarity relationship between the samples was observed as seasonal, and the similarity between the samples was high. Samples were primarily clustered into two groups: January and April, and July and October (Figure 5). There was no obvious aggregation in the four seasons for the offshore samples. The similarity of offshore samples compared to the closed lagoon samples was higher in July, while lowest in July. Significant changes in abundance were observed at the genus level. Genes with similar kinship had similar seasonal and spatial variations. The abundance of Chesapeake-Delaware-Bay and SAR11, which belong to Alphaproteobacteria, showed similarity in space and time, appearing highest in abundance in April. Abundance in the lagoon was significantly higher than that of the open sea. Similarly, Owenweeksia and OM43, which belong to the Bacteroidetes, also showed similar abundance changes.

The relationship between major bacterial clades and environmental factors

The CCA across all samples was conducted to find the determinant environmental parameters shaping bacterial groups (Figure 6). The first axis explained 24.36% of the total variance, while the second axis explained 20.32%.Most of the environmental parameters contributed to the heterogeneous distribution of major bacterial clades. For all samples, salinity played a positive role in the aggregation of many Proteobacteria and Bacteroidetes. The results showed that SAR11, affiliated with Alphaproteobacteria, was positively correlated with dissolved oxygen and negatively correlated with COD. In addition, SAR11 was negatively correlated with temperature and $NH_4^+$and NO_2^-.The HgcI clade of Actinobacteria was negatively correlated with salinity, $NO_{3,}$ and PO_4, but had no correlation with dissolved oxygen. However, CL500-29 was negatively correlated with temperature and COD. Dominant species in the offshore sea showed that Comamonadaceae of Betaproteobacteria, Pseudomonas of Gammaproteobacteria, and *Flavobacterium* of Bacteroidetes were found to be positively related to temperature and various nutrients, showing a higher correlation with NO_2, NH_4, and PO_4.Affiliated with Gammaproteobacteria, *Thiorhodospiral* and Comamonadaceae, as well as *Planktothrix* of Planctomycetes, were found to be negatively related to COD. Cyanobacteria and CL500-29 were negatively correlated with temperature, NO_2, and NH_4.*Synechococcus* was negatively correlated with salinity, although not in an obvious manner, while *Synechococcus* seemed to be negatively correlated with dissolved oxygen.

Discussion

Coastal lagoons are unstable ecosystems characterized by chemical and physical gradients subject to anthropogenic disturbances. They represent a transitional zone between terrestrial, freshwater, and marine environments. The diversity of aquatic prokaryotes is shaped by an array of biotic and abiotic drivers. Advances in studying microbial dynamics have shown that their composition involves complex network interactions (Fuhrman, Cram & Needham, 2015). The importance of seasonality as a structuring factor for coastal bacterioplankton has recently emerged. In previous years, there were few studies on the dynamics of aquatic prokaryotes in the adjacent sea area of the East China Sea. Bacterioplankton production abundance was surveyed in a dilution zone of the Yangtze River estuary. The results showed that the average bacterioplankton production in spring was higher than autumn, and that the production at the surface was higher than the bottom in the surveyed area(Liu et al., 2001).The data presented here provided more information on the diversity of aquatic prokaryotes in the East China Sea and the nearby marine area. Previous microbiological studies in this and other lagoons were restricted to sediment alone (Feng et al., 2009a; Wang et al., 2012). The present study investigated differences in bacterial composition across multiple aquatic ecosystems (lagoon, coastal sea) simultaneously over a seasonal cycle. It was observed that bacterioplankton alpha diversity in July was lower in the lagoon than in the nearby sea. However, the bacterioplankton alpha diversity in both sea areas was higher than in other seasons. In a study that compared lagoons differing in primary productivity, higher bacterial richness was found in the more productive lagoons. We speculate that there is a high diversity of planktonic bacteria in the nearby sea because of the higher concentration of nutrients. Affected by the discharge of land-based sources and man- made activities in Hangzhou Bay, the seawater near the lagoon has maintained a high concentration of nutrients. Due to the impact of aquaculture activities in the lagoon, nutrient concentrations in July were higher. In other months, due to rainfall and large-scale seaweed breeding activities, nutrients had varying degrees of reduction. The CCA plot of lagoon bacterioplankton showed that phosphates and nitrates were also major environmental drivers.

Discovery of the mechanisms and drivers of community assembly is critical to understanding the processes of microbial variation and maintenance, especially in coastal lagoons. The present study showed that within each environment and domain investigated, temporal variations were more important than spatial variations instructuring the assemblages. These results highlighted the fundamental role played by seasonality in structuring coastal bacterioplankton. Feng et al. (Feng et al., 2009b) reported seasonally driven changes in sediment populations in the ChangJiang estuary and the coastal area of the East China Sea, related to the hydrological regime.

Boer et al. (Böer et al., 2009) identified time as the most important factor affecting bacterial diversity in coastal sands. We hypothesize that the seasonal variability observed in lagoon community composition is driven by seasonal changes in environmental and trophic conditions. The heatmap plot of lagoon bacterioplankton revealed a separation among the four seasons. This indicated that different environmental variables could significantly explain the variance in community composition across the different seasons. Proteobacteria was the dominant group, while Alphaproteobacteria was generally abundant in marine waters (Kirchman, Dittel & Cottrell, 2005). It was also observed that as the seasons changed, the abundance of Alphaproteobacteria had a relatively large difference. Among them, the SAR11 population was characterized as oligotrophic. The significant negative correlation between SAR11 and NH_4^+, NO_2^- is shown in Figure 6.

Species and environment correlation analyses are increasingly used to explore large data sets generated by DNA HTS technologies, in order to elucidate potential interactions between microbial taxa (co-occurrence patterns), or between taxa and environmental variables across spatial or temporal scales. The analysis of the network topological parameters indicated that salinity showed positive and negative correlations with many bacterioplankton genera. In the lagoon, negative correlations were found with HgcI. The HgcI clade is common and abundant in a wide range of freshwater habitats (Warnecke, Amann & Pernthaler, 2004), and the salinity of the water was reduced because of rainfall. Therefore, the abundance of the HgcI clade in the summer lagoon was high. In lakes, members of the HgcI clade are often dominant components of the bacterioplankton, where they have a competitive advantage in waters with low DOC concentrations at low temperature (Glã¶Ckner et al., 2000).This clade shows both heterotrophic and autotrophic lifestyles, and a recent single cell genomic study showed that it had a strong genetic ability to consume carbohydrate and N-rich organic compounds. In addition, it also had the potential to utilize sunlight via actinorhodopsin which might promote anaplerotic carbon fixation (Ghylin et al., 2014). However, bacteria in the HgcI clade remain poorly characterized and their functional traits in marine/brackish environments are unknown (Lindh et al., 2015).For

Synechococcus, the influence of salinity was dominant. Research has shown that for stable waters, a decrease in salinity and an increase in rainfall are favorable for a high abundance of *Synechococcus*(R & Mitbavkar, 2013). As such, the high abundance of *Synechococcus* appeared in July and October. Temperature interactions with a variety of taxa were observed, including genera that are dominant in the lagoon (such as *Synechococcus*) and also several minor members, such as CL500-29 and Comamamonadaceae.

Compared with the enclosed lagoon, the dominant species of planktonic bacteria in the offshore waters showed obvious differences(Figure 4).It is worth noting that Comamamonadaceae, affiliated with Comamonadaceae of Betaproteobacteria, and *Pseudomonas*, affiliated with Pseudomonadaceae of Gammaproteobacteria were the two dominant groups having the highest abundance in July in the offshore sea. Studies on the removal of phosphorus from waste water indicated that Comamonadaceae was present when the Bio-P activity was evident (Ge, Batstone & Keller, 2015a). Likewise, FISH analysis combined with DAPI staining showed that bacterial cells of Comamonadaceae were arranged in tetrads contained polyphosphate. These studies identified these species as the key polyphosphate accumulating organisms (Ge, Batstone & Keller, 2015b). Microbial community analysis indicated that organisms classified in the Comamonadaceae were effective denitrifiers widely used in sewage treatment (Long et al., 2017). *Pseudomonas aeruginosa* is a strain of non-fermentative DNPAOs (Denitrifying phosphate-accumulating organisms) with strong nitrogen and phosphorus removal abilities. Studies on the metabolic mechanisms suggested that intracellular PHB of *P.aeruginosa* plays dual roles, supplies energy for phosphorus accumulation, and serves as a major carbon source for nitrification(Liu et al., 2016).Simultaneous removal of nutrients (ammonium and phosphate) and COD was investigated by the co-culture consortium of microalga *Chlorella vulgaris* and bacterium *Pseudomonas putida*. The co-culture system showed higher removal of both nutrients and COD than the each axenic culture, indicating that the nutrient uptake capability of *C. vulgaris* was enhanced in the presence of *P. putida* (Mujtaba, Rizwan & Lee, 2017).Some studies have shown that Gammaproteobacteria has the ability to degrade certain amounts of organic matter in the ocean (Marques et al., 2014b; Brettar, Christen & Höfle, 2006). Similarly, microbial high-throughput sequencing analysis showed that Gammaproteobacteria contain the main PAH degradation genes used in the bioremediation of immobilized bacteria. As such, these organisms could be used to remove complex and structurally related organic compounds in the environment (Tian et al., 2016). *Thiorhodospiral* of Gammaproteobacteria also showed a positive correlation with COD. Compared with the closed lagoon water, the accumulation of dominant bacteria in the offshore waters may be due to the impact of higher concentrations of Hangzhou Bay sewage discharge, nutrients, and COD.

Conclusions

The results of this research showed significant differences in the bacterioplankton community and diversity between the artificial lagoon and the adjacent open sea. This study highlighted the importance of seasonality in modulating planktonic assemblages in coastal lagoons and the adjacent sea. The dominant bacteria in the lagoon were *Synechococcus* and *Cyanobacteria*. The abundance of bacteria varied greatly with the seasons, primarily due to dissolved oxygen, temperature, and salinity. The adjacent sea dominant bacteria such as bacteria in the family Comamamonadaceae and *Pseudomonas* were affected by COD, PO_4, and NO_2.These results indicated a negative impact of different human activities (seawater farming, land-based pollution emissions) on coastal ecosystems. It was also found a dominant microbiota, which can effectively remove nutrients such as nitrogen and phosphorus. These founding provided new methods to improve breeding water quality. In addition, these findings may help to restore the ecosystem in the future using large seaweed beds and local beneficial microorganisms.

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Table 1(on next page)

Location of the sampling site

This table is used to describe the location and depth of sampling points

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Table 1:

Location of the sampling site

site	longitude	latitude	Water depth(m)
1	121°34′00.78″E	30°49′23.87″N	5
2	121°32′46.69″E	30°49′35.48″N	7.8
3	121°31′39.91″E	30°49′10.68″N	5.5
w	121°30′46.32″E	30°48′41.76″N	2.5

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Table 2(on next page)

Comparison of Chao, Shannon diversity, and Coverage of each sample within 3% of the dissimilarity levels

Analysis of the diversity and coverage of each sample

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Table 2:

Comparison of Chao, Shannon diversity, and Coverage of each sample within 3% of the dissimilarity levels

Sample	Optimized reads	OTUs	3%dissimilarity		
			Chao	Shannon	Coverage
1-4	37044	30096	284.17	3.32	0.998
2-4	21998	17918	217.00	3.34	0.997
3-4	36940	29718	292.05	3.43	0.998
w-4	33484	22474	553.61	3.72	0.995
1-7	44073	36588	410.90	3.79	0.998
2-7	41930	37361	313.22	3.48	0.999
3-7	35785	30992	394.29	3.98	0.997
w-7	32051	26188	369.79	3.77	0.997
1-10	42045	32388	364.50	3.39	0.998
2-10	38401	33604	341.2	3.44	0.998
3-10	42471	39207	302.79	3.20	0.999
w-10	36458	20405	576.44	4.50	0.995
1-1	44438	31283	281.2	3.65	0.998
2-1	32865	24084	240.40	3.53	0.998
3-1	41711	29612	253.36	3.61	0.998
w-1	41641	24098	668.51	4.79	0.995

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Figure 1

Location of sampling sites in FengXian

Location of sampling sites in FengXian

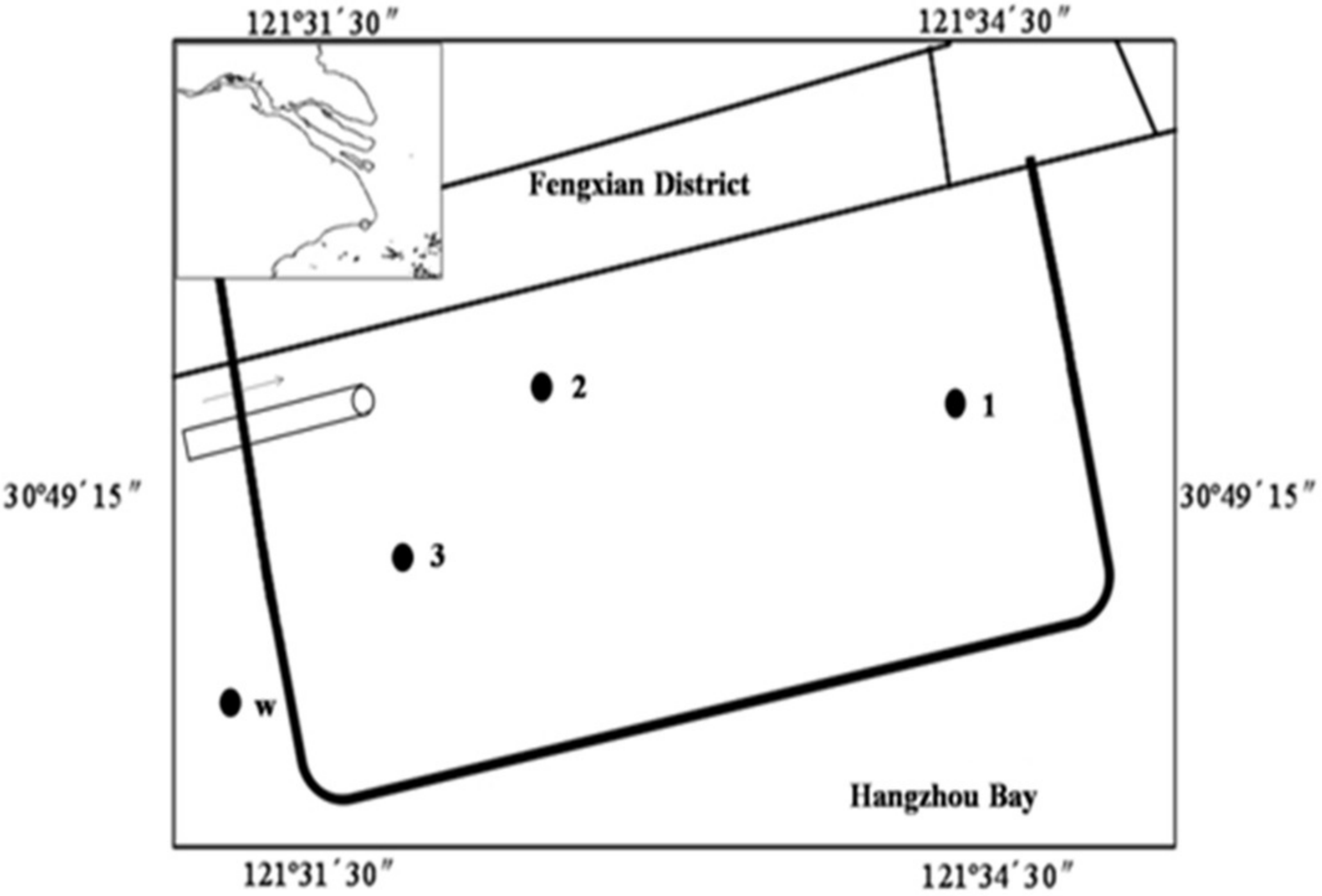

Figure 2

Environmental parameters of water samples.

Y-axis shows the concentration of environmental factors."N" and "W" indicate lagoon and adjacent offshore respectively, and the numbers indicate the sampling month. Y axis, where the main coordinate is a column chart and the second coordinate is a line chart.

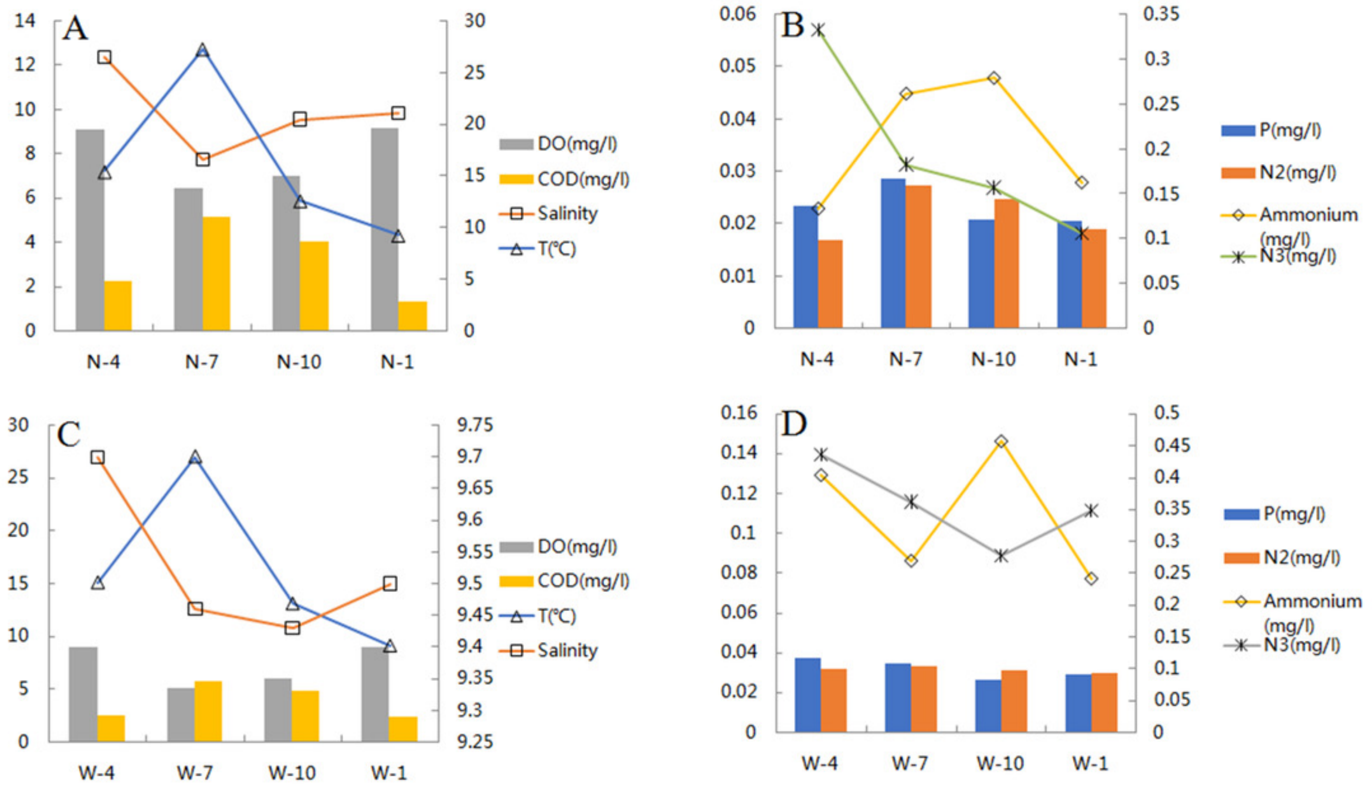

Figure 3

Abundance distribution of the bacterial community at the phylum level.

A description of abundance distribution at the level of bacterial communities in each sample

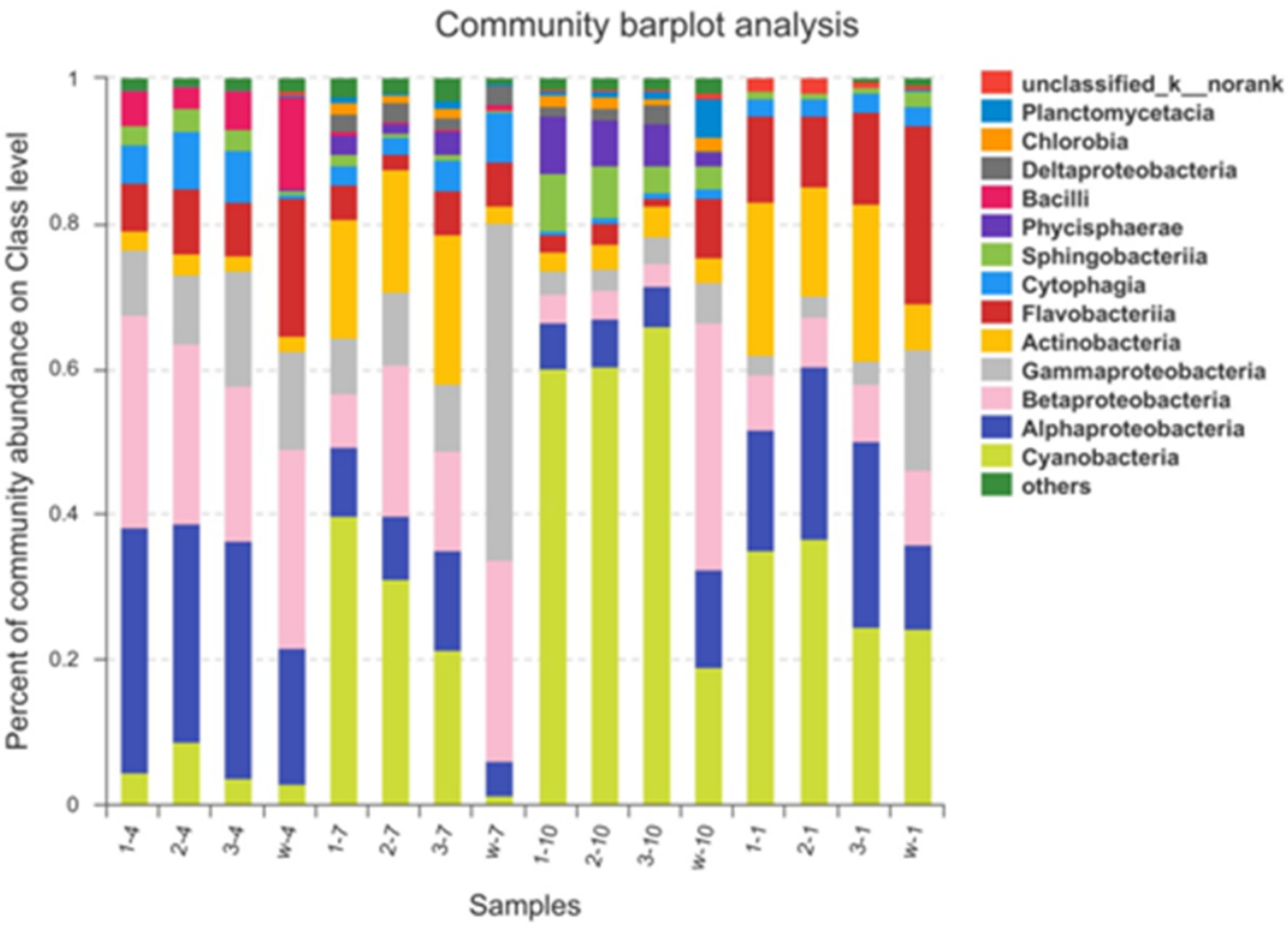

Figure 4

Abundance distribution of the bacterial community at the genes level

Abundance distribution of the bacterial community at the genes level

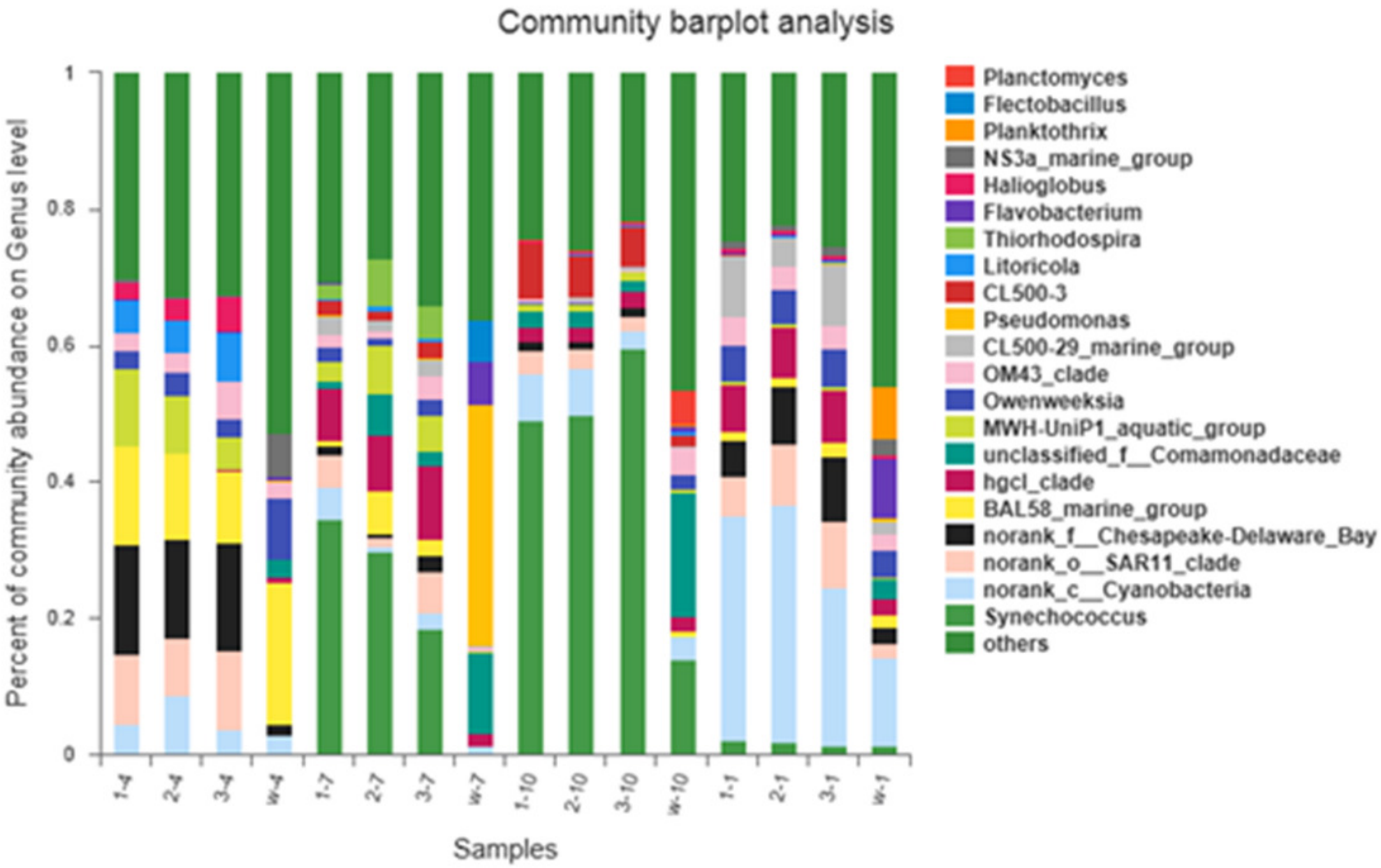

Figure 5

Heatmap of the top 50 genes according to abundance.

Phylogenetic relationships are shown on the right tree. The top tree shows the clustering relationship of the samples.

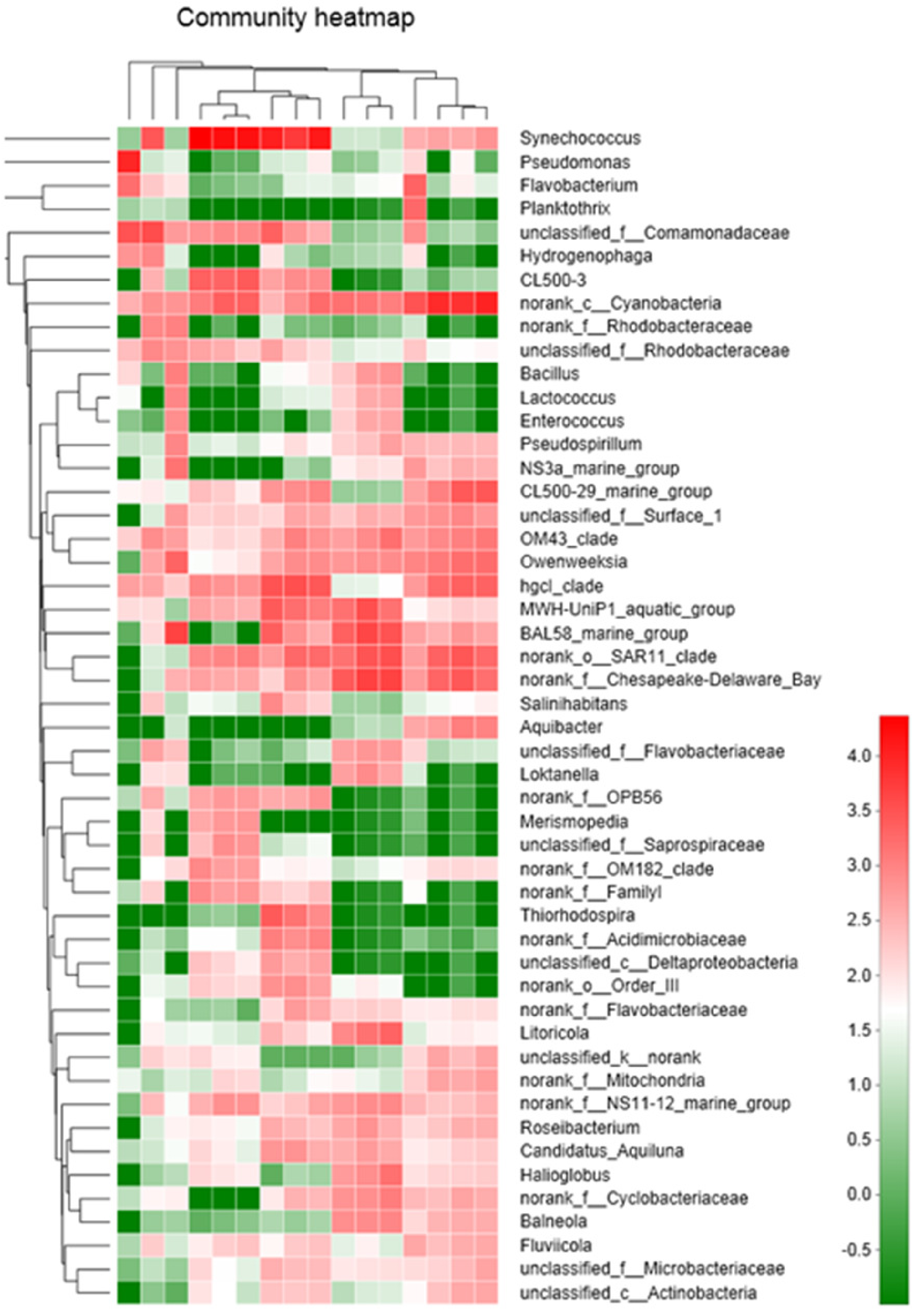
Community heatmap
Synechococcus
Pseudomonas
Flavobacterium
Planktothrix
unclassified_f__Comamonadaceae
Hydrogenophaga
CL500-3
norank_c__Cyanobacteria
norank_f__Rhodobacteraceae
unclassified_f__Rhodobacteraceae
Bacillus
Lactococcus
Enterococcus
Pseudospirillum
NS3a_marine_group
CL500-29_marine_group
unclassified_f__Surface_1
OM43_clade
Owenweeksia
hgcI_clade
MWH-UniP1_aquatic_group
BAL58_marine_group
norank_o__SAR11_clade
norank_f__Chesapeake-Delaware_Bay
Salinihabitans
Aquibacter
unclassified_f__Flavobacteriaceae
Loktanella
norank_f__OPB56
Merismopedia
unclassified_f__Saprospiraceae
norank_f__OM182_clade
norank_f__FamilyI
Thiorhodospira
norank_f__Acidimicrobiaceae
unclassified_c__Deltaproteobacteria
norank_o__Order_III
norank_f__Flavobacteriaceae
Litoricola
unclassified_k__norank
norank_f__Mitochondria
norank_f__NS11-12_marine_group
Roseibacterium
Candidatus_Aquiluna
Halioglobus
norank_f__Cyclobacteriaceae
Balneola
Fluviicola
unclassified_f__Microbacteriaceae
unclassified_c__Actinobacteria
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5

Figure 6

The relationship between major bacterial clades and environmental factors les.

Correlation between bacterial communities and environmental factors in each sample