Analysis of aerobic anoxygenic phototrophic bacterial community structure in the different marine functional zones in the Zhoushan Archipelago Sea Area in summer

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Aerobic anoxygenic phototrophic bacteria (AAPB) containing bacteriochlorophyll *a* (BChl *a*) are photoheterotrophic prokaryotes that are a widely distributed functional bacterial group due to their obligately aerobic and facultative photoheterotrophic abilities. Here, we made a comparison of AAPB community structures in different marine functional zones in the surface water of the Zhoushan Archipelago Sea Area using high-throughput sequencing based on the *pufM* gene that encodes the M subunit of the light reaction centre complex. A total of 268,214 clean reads were obtained from the sampling stations. The sequences were divided into 5876 OTUs (97% cut-off value). Proteobacteria, Firmicutes, Bacteroidetes and Actinobacteria phyla were noted in this study. Proteobacteria phylum accounted for the major proportion and existed at all sites. *Roseobacter*-like AAPB was the most abundant genus, in Dongji Island (DJ) and Gouqi Island (GQ). Our results demonstrated that the structure of the AAPB community had different distribution patterns within the Zhoushan Archipelago Sea Area. Furthermore, we found that the diversity of AAPB was controlled by complex environmental factors, which might explain the difficulties encountered when predicting the distribution of total AAPB in aquatic ecosystems.

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Abstract

Aerobic anoxygenic phototrophic bacteria (AAPB) containing bacteriochlorophyll *a* (BChl *a*) are photoheterotrophic prokaryotes that are a widely distributed functional bacterial group due to their obligately aerobic and facultative photoheterotrophic abilities. Here, we made a comparison of AAPB community structures in different marine functional zones in the surface water of the Zhoushan Archipelago Sea Area using high-throughput sequencing based on the *pufM* gene that encodes the M subunit of the light reaction centre complex. A total of 268,214 clean reads were obtained from the sampling stations. The sequences were divided into 5876 OTUs (97% cut-off value). Proteobacteria, Firmicutes, Bacteroidetes and Actinobacteria phyla were noted in this study. Proteobacteria phylum accounted for the major proportion and existed at all sites. *Roseobacter*-like AAPB was the most abundant genus, in Dongji Island (DJ) and Gouqi Island (GQ). Our results demonstrated that the structure of the AAPB community had different distribution patterns within the Zhoushan Archipelago Sea Area. Furthermore, we found that the diversity of AAPB was

controlled by complex environmental factors, which might explain the difficulties encountered when predicting the distribution of total AAPB in aquatic ecosystems.

Key words

aerobic anoxygenic phototrophic bacteria (AAPB), high-throughput sequencing, Zhoushan Archipelago Sea Area, diversity, *pufM*

Introduction

Zhoushan Archipelago, which lies in the northern sea of Zhejiang Province, west in the East China Sea, south of the Yangtze River estuary and east of Hangzhou Bay, is the largest archipelago in the coastal areas of China. According to the status of the national marine functional zoning, combined with its own natural environment and resource conditions. The Zhoushan Archipelago Sea Area can be divided into different functional areas (including port shipping areas, tourist areas, marine engineering areas, marine protected areas, fishing areas, marine aquaculture areas, etc.)(Miao et al. 2011; SOA 2012).

Aerobic anoxygenic phototrophic bacteria (AAPB) containing bacteriochlorophyll *a* (BChl *a*) are a kind of important functional group of marine microbes that utilize a photoheterotrophic metabolism(Kolber et al. 2001). AAPB serve critical functions in carbon and energy cycling in the upper ocean (Beatty 2002; Kolber et al. 2000). These bacteria are widely found in marine plankton communities(Yutin et al. 2007). Moreover, the abundance and distribution of AAPB significantly different between various marine conditions (Cottrell et al. 2006; Lami et al. 2007; Sieracki et al. 2006). AAPB have a relatively larger cell size and a rapid turnover rate in the ocean (Koblízek et al. 2007; Sato-Takabe et al. 2015). They consume dissolved organic matter (DOM) that is generated by the decomposition of phytoplankton and dissolved organic carbon (DOC), and subsequently release recalcitrant DOC into the environment through a range of

biochemical processes, especially the microbial carbon pump(Jiao et al. 2010; Jiao et al. 2007; Zhang & Jiao 2007; Zhang et al. 2006). These photosynthetic microorganisms are players in marine microbial circulation, and are considered to be an important participant in marine carbon cycling(Kolber et al. 2001).

As the genetic materials that codes for the subunits of the light-harvesting (*pufB* and *pufA*) and reaction center (*pufL* and *pufM*) complexes, *pufM* has been frequently used to evaluate the diversity of different aerobic anoxygenic photosynthetic associations(Béjà et al. 2002; Boeuf et al. 2013; Jeanthon et al. 2011; Koh et al. 2011; Schwalbach & Fuhrman 2005). AAPB generally contain diverse members of Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria(Béjà et al. 2002; Boeuf et al. 2013; Jeanthon et al. 2011). *Roseobacter* and *Roseobacter*-like bacteria are the major members of AAPB in oceans(Oz et al. 2005). AAPB not only live in a wide range of aquatic systems, they have also been explored in soils and plant leaves (Atamnaismaeel et al. 2012; Rathgeber et al. 2004). The culture-dependent method has been commonly used to study marine bacterial diversity and community structure(Hantula et al. 1996; Harmsen et al. 1997). Recently, high-throughput sequencing methods have developed quickly, and with time read lengths and accuracy have increased while the cost has decreased(Huse et al. 2007). The methods are currently faster, and have higher throughputs or lower costs than other sequencing methods. The community structure and diversity of marine bacteria have also been analyzed by high-throughput sequencing methods (Abell et al. 2011; Bowman et al. 2012; Comeau et al. 2011). And these methods have been used to analyze studies have been conducted to utilize samples from different kinds of aquatic conditions, such as the Amazon River, the North Pacific subtropical gyre, the Mediterranean coastal lagoons, and a coastal bay of Brazil(Cuadrat et al. 2016; Ghai et al. 2012; Ghai et al. 2011; Konstantinidis et al. 2009). The amplification of the *pufM* gene

has resulted in extremely deep sequencing of bacterial communities(Luna 2015; Zheng et al. 2015). Gradually, these approaches have proven meaningful and the factors that may have impacts on the bacterioplankton community in different sea areas, which allow us to obtain more valuable information.

Previous studies illustrated that the diversity and community structure of AAPB was influenced by various environmental conditions, including salinity, light and nutrient gradients (Ferrera et al. 2014; Jiao et al. 2007; Lehours et al. 2010; Zeng et al. 2009). The latest research has shown that AAPB abundance was significantly positively correlated with pH, and the highest proportion of photoheterotrophs was found in peat-bog lakes with a pH between 6.7 and 7.6 (up to 20% of total bacteria)(Sylwia et al. 2016). But in polar areas, Antarctic AAPB positively correlated with salinity, nitrate, phosphate, and silicate, while the Arctic dominant positively correlated with water temperature (Zeng et al. 2016). The different marine functional zones of AAPB in surface waters have only been investigated in a few studies. Therefore, in this study we used high throughput sequencing to compare the AAPB community structures in the surface waters of eight different marine functional zones in the Zhoushan Archipelago Sea Area, and analyzed the impact of environmental parameters on AAPB distribution. The large amount of data lays a foundation for further biological research in the Zhoushan Archipelago Sea Area.

Materials and Methods

Sample collection

The Zhoushan Archipelago Sea Area surface seawater (0.5 m depth) samples were collected in August 2016. In total 8 sampling sites: Jintang Island, Liuheng Island, Taohua Island, Dongji Island, Gouqi Island, Daishan Island, Shenjiamen and Zhujiajian (Fig. 1). Subsamples (2-3 L) were pre-filtered through 3-μm filters and subsequently filtered onto 0.22-μm-pore-size polycarbonate filters (mixed

cellulose, 47mm diameter, Milipore). These filters were immediately frozen in liquid nitrogen for DNA extraction. DNA was extracted from membranes by the PowerWater® DNA Isolation Kit (MOBIO, USA), according to the manufacturer's instructions using a microcentrifuge (MOBIO, USA), quantified, and stored at -80°C for future use.

Water temperature, salinity, and dissolved oxygen (DO) were measured in situ with our instruments (i.e., salinity and dissolved oxygen meter). The concentration of inorganic nutrients was determined spectrophotometrically following standard procedures(Hoch et al. 1996).

Sequence generation and processing

High throughput sequencing of the *pufM* gene performed in about 200 bp fragments using the Illumina MiSeq™ platform. Partial *pufM* genes were amplified with the primer set: *pufM*-557-F (5′-TACGGSAACCTGTWCTAC-3′) and *pufM*-750-R (5′-CCATSGTCCAGCGCCAGAA-3′)(Achenbach et al. 2001; Mukkata et al. 2016). Polymerase chain reaction (PCR) amplification was conducted using a total volume of 25 µL containing 2 µL of template DNA, 9.5µL of ddH_2O, 12.5µL of 2×Ace Taq Master Mix (Takara), and 0.5µL each primer. Amplification conditions: 95°C for 5 min, and then 30 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s, followed in turn by one 72°C step for 7 min. 10ng purified PCR products (MiniElute PCR purification kit; Qiagen) of each sample were mixed for pyrosequencing on an Illumina MiSeq™ platform.

The generated raw data was processed according to Schloss(Schloss P D 2009). After quality control, a 97% sequence similarity threshold was chosen for clustering sequences into Operational Taxonomic Units (OTUs). Diversity indices were calculated based on OTU abundance using statistical program MOTHUR v.1.32.1 (http://www.mothur.org/wiki/Calculators), and the hierarchical

clustering of all libraries was all determined in the program QIIME. A BLAST search for taxonomic classification was performed using local BLAST in the BioEdit software(Desantis et al. 2006). Redundancy analysis (RDA) of AAPB communities and their relationships to environmental parameters was carried out using Canoco 4.5 software.

Determination of water quality

Phosphate, silicate, nitrate, nitrite, and ammonia were measured with a Smartchem-200 Discrete Auto Analyzer. The dissolved oxygen, temperature and salinity were measured in situ with a dissolved oxygen meter (HACH, USA) and salinometer respectively.

Accession numbers

All the sequences in this study have been submitted to the NCBI-SRA public database (http://www.ncbi.nlm.nih.gov/sra/SRP094672) under the ID: SRP094672 (all the eight samples of the Zhoushan Archipelago Sea Area).

Results

Environmental parameters

The sampling sites were located in different regions of the Zhoushan Archipelago Sea Area (Fig. 1), and represent different functional marine conditions respectively. Samples were all collected from surface waters with temperatures of 26.60~33.50℃ at approximately 0.5 m depth, while the dissolved oxygen (DO) of the eight sampling sites ranged from 6.39 to 10.78 (Table 1). The salinity of the Gouqi Island (GQ) site (30.47) was higher than at other stations. The concentrations of salinity, inorganic nitrogen, phosphate and silicate fluctuated across the different sea areas; they were indispensable parameters in the classification of seawater quality standard. Concrete water quality parameters of each station described in detail in Table 1.

Sequence information and diversity of AAPB

The total number of clean reads from eight stations was 268,214. Libraries with all the sequences were composed of 5876 OTUs (0.03 distance threshold), with the number of OTUs varying from 944 to 1568 OTUs per sample. From these, a total of 52 OTUs were common across all samples; these OTUs took up nearly 85% of the total reads. Based on the OTUs results, diversity indices were calculated for each sample in similar level 97% (Table 2). The Chao (2088~3283) and ACE (3501~6090) indices indicated that community richness of AAPB was high level in all stations.

Community structure

In all the Zhoushan Archipelago Sea Area samples sequencing results, only approximately 13% of the *pufM* sequences were closely related (>97% identity) to known sequences. Table 3 illustrated that Proteobacteria, Firmicutes, Bacteroidetes and Actinobacteria phyla were found in this study. Proteobacteria phylum accounted for the major proportion and existed at all sites. The second phylum was Firmicutes, which was detected in four stations (DS, JT, LH and TH). Besides these, the phyla of Bacteroidetes and Actinobacteria were just recognized in two sites (DJ and LH) and one site (JT), respectively (Table 3).

From the eight environmental sequences obtained from the Zhoushan Archipelago Sea Area in our calculated bar charts, AAPB belonging to the Alphaproteobacteria dominated the columns, followed by Gammaproteobacteria, Betaproteobacteria and Bacilli. The results showed the relative abundance of the top fifteen genera retrieved from the different analyzed metagenomic samples, some were unusual species of AAPB, such as *Pseudohaliea*, *Thauera*, *Brevundimonas*, *Cycloclasticus*, *Allochromatium*, *Alkalibacterium*, *Methylobacterium*, *Ruegeria*, *Bosea*, *Citromicrobium*, *Octadecabacter* and *Thalassobium*, etc (Fig. 2).

When comparing relative abundance distribution patterns in the different stations, various clades obviously preferred one or the other environment (Fig. 2). We could see that the most genera were belonging to Alphaproteobacteria (Proteobacteria phylum). Seven were mainly assigned to the *Roseobacter*-like AAPB and the unique sequence was from SJM, which *Pseudohaliea*-like AAPB accounted for the relative dominance. In DJ, GQ, TH and ZJJ, over 90% of the sequences were clustered into *Roseobacter*-like AAPB. *Pseudohaliea*-like AAPB also took up the second important proportion in JT, LH and DS. *Thauera*-like AAPB just constituted a relatively high proportion in JT and LH. While *Alkalibacterium* constituted only for a very low percentage.

The similarity and difference of AAPB community structure can be seen from the cluster relationship tree among the samples (Fig. 3). It was clear that ZJJ and SJM had the highest similarity, they constituted a small sub-branch, and then gathered into a cluster 3 with TH. The cluster 2 and cluster 3 were more similar and component cluster 4 separately from station DS. Then they all together constituted a larger branch B. The similarity between DJ and GQ formed the cluster 1, and they took up a separate branch A. The similarity between branch A and branch B was the lowest.

RDA analysis

Redundancy analysis (RDA) was used to investigate the relationships between the abundance of AAPB and environmental variables. Forward selection in RDA identified seven environmental variables (i.e., ammonia nitrogen, nitrates, silicates, phosphates, temperature, DO and salinity) that had significant relationships with AAPB abundance ($P \leq 0.05$). RDA performed for microbiological data and environmental parameters showed that the first and second axes explained 99.75 and 99.89% of the cumulative variance, respectively (Fig. 4). There were independent directional variations in the microbiological

AAPB data from the eight sea areas. RDA on microbiological data and environmental parameters showed that ammonia nitrogen, nitrates, salinity and silicates were the four important factors that impacted most bacteria. Most of these bacteria are classified as Proteobacteria, with two representatives of the genus *Thauera* and *Brevundimonas*. DO and phosphates had the strongest relationships with *Sphingomonas*, *Rhodobacter* and *Roseobacter* while temperature and salinity significantly influenced *Pseudohaliea*. The rays of phosphates and ammonia nitrogen were much longer than others that had more significant impacts on the AAPB distribution.

Discussion

This study aimed to demonstrate similarities and/or differences in the composition of AAPB communities between different marine functional zones in the surface waters of the Zhoushan Archipelago Sea Area and examine relationships between AAPB and environmental variables. We hypothesized that the component of AAPB types in Zhoushan Archipelago environments would exhibit both endemism and cosmopolitanism.

All sequences were classified into 5876 OTUs (similar level 97%), and the number of OTUs varied between 944 and 1568 per sample. In sum of 52 OTUs were common between all samples, and possessed nearly 85% of the total reads. Based on the OTUs data, we obtained diversity indices for each sample in a similar level 97% (Table 2). Chao and ACE indices were used to calculate richness, and the Shannon and Simpson indices were used to estimate diversity. Shannon and Simpson indices demonstrated that the community diversities of the LH, GQ, and DS sites were higher than those of other sites. Coverage in all samples suggested that further sequencing would have resulted in no more OTUs, which showed that the sequencing depth was adequate for AAPB community analysis.

In all the Zhoushan Archipelago Sea Area samples sequencing results, the

AAPB community was dominated by Proteobacteria, Firmicutes, Bacteroidetes and Actinobacteria phyla, as well the subclades associated with specific environmental conditions, which accounted for an important proportion in our samples (Table 3). Which was partially confirmed in previous studies (Chen 2014; Hube et al. 2009; MichalKoblížek 2015; Xifu 2011). Proteobacteria phylum took up the significant proportion and distributed in all samples. Then was Firmicutes, which was detected in four stations (DS, JT, LH and TH). In addition, the phyla of Bacteroidetes and Actinobacteria were just identified in two sites (DJ and LH) and one site (JT), respectively (Table 3). Similar to previous results(Lehours & Jeanthon 2015; Zheng et al. 2015), only 13% of the *pufM* sequences were closely related (>97% identity) to clearly classified sequences in our research.

Consistent with previous research (Boeuf et al. 2013; Ritchie & Johnson 2012; Zeng et al. 2016; Zheng et al. 2015), Alphaproteobacteria-like AAPB dominated the OTUs in our samples (Table 3). The representative sequences belonging to Betaproteobacteria and Gammaproteobacteria had also obviously hit with environmental *pufM* sequences in the NCBI nr database. The discovery of Bacilli in this experiment had very important research value. Bacillus has been reported in a graduate thesis (Chen 2014). And genes for proteins with sequence homology to the large subunit of ribulose bisphosphate carboxylase/oxygenase (RuBisCO) had discovered in Bacillus subtilis which meant that some Bacillus might possess photosynthetic capacity(Ashida et al. 2003). Alphaproteobacteria have been extensively studied(Alexandre et al. 2009). They have been colonized on particles under algal bloom conditions, and have been reported to be the dominant group in both open and coastal ocean environments (Alonso-Sáez et al. 2007; Eilers et al. 2001; Pinhassi & Hagström 2000). In recent years, the increased occurrence of red tide at the DJ and GQ has been shown to decrease the relative abundance of Alphaproteobacteria, especially when compared to Gammaproteobacteria(Teira

et al. 2007).

In the Zhoushan Archipelago Sea Area, the diversity of AAPB by pyrosequencing analysis, we found that *Roseobacter*-like and *Pseudohaliea*-like AAPB made up the major proportion in all the samples (Fig. 2). Our results show the predominance of *Roseobacter* in both DJ and GQ, with all approximately 99% of total AAPB in this environment. These results suggest a possible link between the phytoplankton bloom induced by upwelling and the high abundance of *Roseobacter*, as has been earlier shown in the Global Ocean Sampling (GOS) expedition(Yutin et al. 2007). Prior work has shown that the *Roseobacter* genomes encode proteins for the aerobic degradation of the aromatic intermediates gentisate, homoprotocatechuate, homogentisate, benzoate, phenylacetate, and protocatechuate (Moran et al. 2007). However, we still do not understand the role and function of this group in marine environments. Therefore, comprehensive research is required to improve our understanding of the ecological niche for Alphaproteobacteria hydrocarbon degraders in marine environments. The other AAPB genera detected in this study were classified into Alphaproteobacteria. Such as AAPB genera of *Sphingomonas*, *Rhodobacter*, *Brevundimonas*, *Methylobacterium*, *Ruegeria*, *Bosea*, *Citromicrobium*, *Octadecabacter* and *Thalassobium* etc. Interestingly, the genera *Methylobacterium* and *Sphingomonas* were detected in humic acidic conditions and/or the recalcitrant nature in previous freshwater studies (Kml et al. 2010; Salka et al. 2011; Salka et al. 2014). However, our discovery demonstrated that they could also live in the various marine environments.

The AAPB community structure relationship between each sample station could be clearly illustrated by our data (Fig. 3). ZJJ (fishing areas) and SJM (port shipping areas) had the highest similarity, and they gathered into a cluster 3 with TH (tourist areas). It was easy to find that in these sites with relatively frequent

297 human activities had a more obvious impact on the environment. The cluster 1
298 was consist of DJ and GQ, which belonged to two different types of marine
299 aquaculture areas. While their environmental conditions were quite different with
300 other sea areas. Perhaps this was the reason why the tree of similarity was divided
301 into A and B branches.

302 The goal of RDA analysis in this study was to review relationships between
303 AAPB of different functional sea areas and environmental parameters (Fig. 2).
304 We hypothesized that the distribution of AAPB types would follow the patterns
305 of bacterial groups previously determined using the *pufM* gene. Four
306 indispensable factors which positively influenced the most AAPB were ammonia
307 nitrogen, nitrates, salinity and silicates, respectively. Proteobacteria AAPB have
308 been reported to be influenced by changes in salinity in the Delaware
309 Estuary(Waidner & Kirchman 2008) and western Arctic Ocean(Boeuf et al. 2013),
310 we also found the similar relationship in our study. Although salinity varied
311 between 21.31 and 30.47 along transects, these variations are likely accompanied
312 by a range of physiological tolerances across AAPB populations. We hypothesize
313 that in waters with different salinities, photoheterotrophy may have differing
314 physiological roles in warm, high salty waters and in cold, low saline waters when
315 light is plentiful. Furthermore, diversity of *pufM* of the LH site was higher than
316 at the GQ, and DS sites due to different salinities.

317 The diversity of photosynthetic genes of AAPB was also affected by the nutrient
318 level, because the East China Sea belongs to the eutrophic continental shelf. In
319 this research, nitrate and ammonia nitrogen concentrations were higher in the
320 sampling sites of the two marine aquaculture areas (DJ and GQ) than that of other
321 stations. High-density marine aquaculture often results in increased
322 concentrations of nitrogen, phosphorus and other nutrients in the aquatic
323 environment(Degefu et al. 2011). So this may be the reason why these two sites

have relatively high bacterial richness. It has been reported that marine Proteobacteria is associated with the oxidation of ammonia in seawater (Ma et al. 2008). Most of these bacterial groups observed in this study belonged to uncultured bacteria, and their ecological functions were unknown, but the concentration of inorganic nitrogen in non-aquaculture areas were all lower than aquaculture areas, indicating that these Proteobacteria may be associated with The seawater ammonia oxidation related (Fig. 4). *Brevundimonas* and *Thauera* are two representative genera in this study.

Temperature is another important environmental factor that promotes the growth of AAPB (Ferrera et al. 2014; Lew et al. 2015; MichalKoblížek 2015). Data from the Barents Seas show strong linear correlations between AAPB richness and Shannon diversity at higher temperatures(Lehours & Jeanthon 2015). In our study, the abundance of *Roseobacter*-like AAPB at the DJ and GQ were higher than at any other locations, which suggest that temperature is critical for some *Roseobacter*-like AAPB in this environment. Additionally, AAPB are rapidly growing, metabolically active organisms that significantly contribute to the supplementation of dissolved oxygen. We concluded that DO was an important factor that impacts the distribution of many bacteria genera (Fig. 4).

The property of their physiological adaptations and metabolic flexibility needs to be elucidated to understand the selective advantages provided by photoheterotrophy. Physical and/or chemical parameters, as well as synergetic factors, may also contribute to the structure of AAPB communities. Our findings indicate that different AAPB groups were impacted by complex environmental conditions, which may explain the difficulty encountered when estimate the distribution of total AAPB in aquatic ecosystems.

Conclusion

In this study, we selected the sampling stations based on the principles of marine

functional areas scheme and administrative divisions. High-throughput sequencing data of the *pufM* gene was obtained from the advanced Illumina MiSeq™ platform. The result illustrated that diversity of AAPB was controlled by complex environmental factors in the Zhoushan Archipelago Sea Area. Aerobic anoxygenic phototrophic bacteria (AAPB) is a kind of widely distributed functional bacterial group which has potential value in the microbial ecology and biogeochemistry.

Additional Information and Declarations

Competing financial interests

The authors declare no competing financial interest.

Author Contributions

Benxuan Zhao performed the data analysis and drafted the manuscript. Qiang Liu participated in molecular experiments, sample collection and preparation, and helped manuscript writing. Sheng Zhao and Changwen Wu participated in molecular experiments, experimental design, and the discussion. All authors read and approved the final manuscript.

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Figure 1(on next page)

Locationof the study area and sampling stations in the Zhoushan Archipelago Sea Area ofthe East China Sea.

Stations: Dongji Island (DJ), Gouqi Island (GQ), Jintang Island (JT), Liuheng (LH), Shenjiamen (SJM), Taohua Island (TH), Zhujiajian (ZJJ), and Daishan (DS). Samples from stations were collected in August 2016. The figure was generated by ArcGis 10.0 (http://www.esrichina.com.cn/softwareproduct/ArcGIS/).

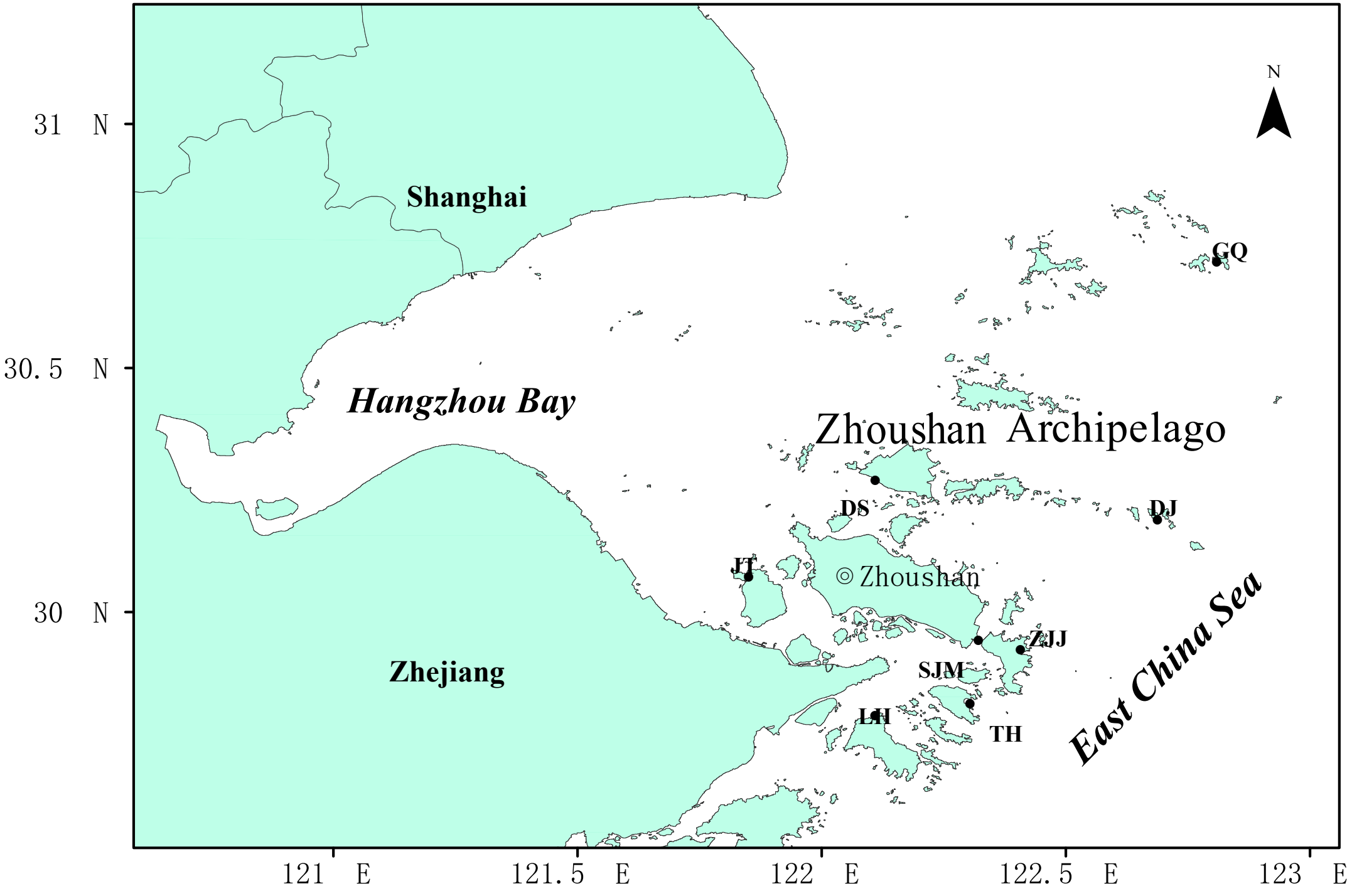

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Figure 2(on next page)

Relative abundance of eachclassified genus retrieved from the analyzed metagenomic samples.

The number of read equivalents for each obtained ORF was counted and percentages were calculated using the classification of the phylogenetic tree generated by MOTHUR. The horizontal axis is the number of each sample and the vertical axis is the relative abundance. Each color corresponds to the name of the species taxonomy, different color widths for different species reflect the relative abundance. Stations: Dongji Island (DJ), Gouqi Island (GQ), Jintang Island (JT), Liuheng (LH), Shenjiamen (SJM), Taohua Island (TH), Zhujiajian (ZJJ), and Daishan (DS). α = Alphaproteobacteria, β = Beta proteobacteria, γ = Gamma proteobacteria, B = Bacilli.

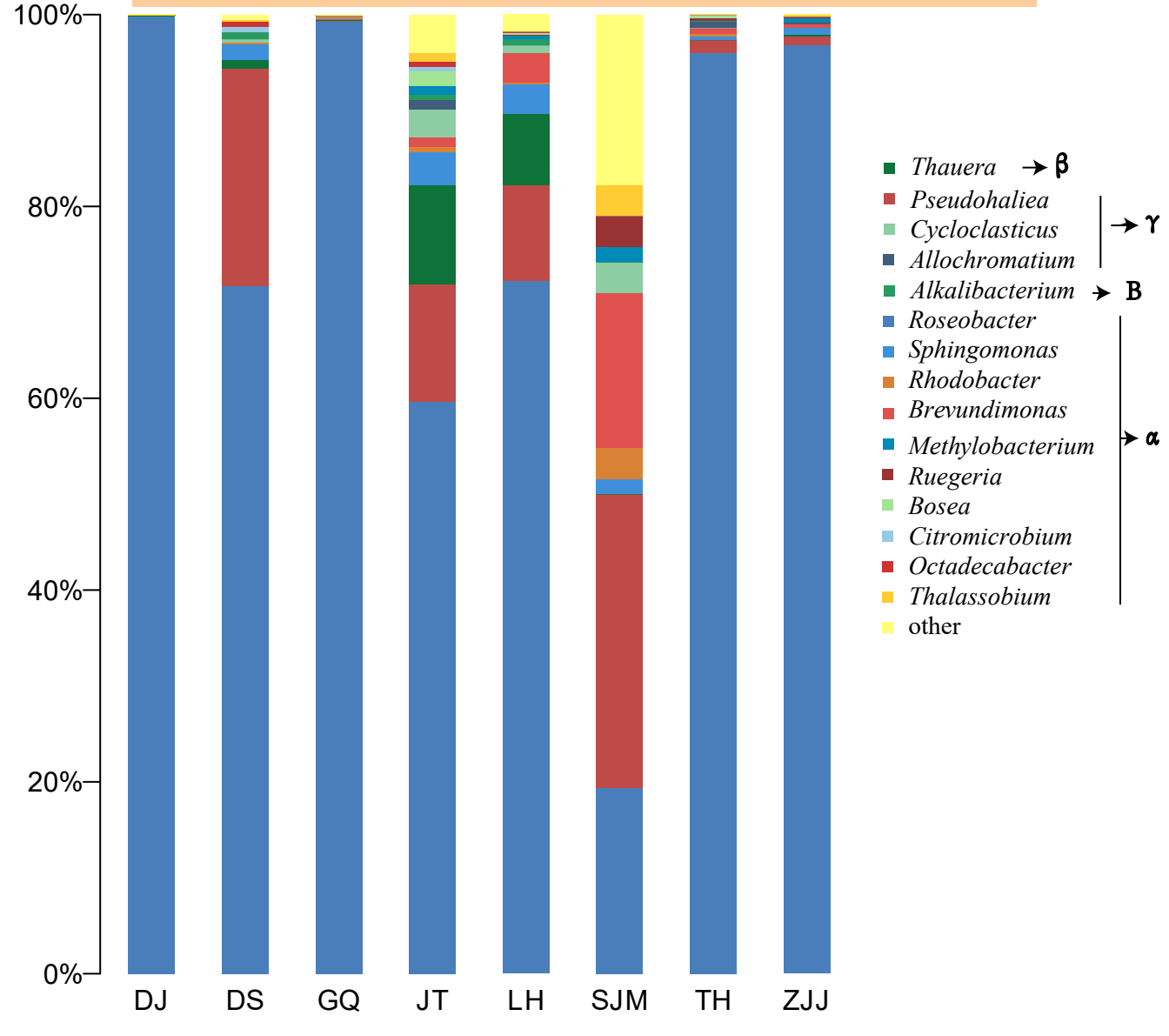

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Figure 3(on next page)

The AAPB community structure relationship tree between each sample station.

Based on the difference in the composition and abundance of each sample, calculating the similarity between the samples and drawing the community structure relationship tree. The legend at the bottom of the figure is the distance scale. The longer the branch distance, the farther the difference is. Stations: Dongji Island (DJ), Gouqi Island (GQ), Jintang Island (JT), Liuheng (LH), Shenjiamen (SJM), Taohua Island (TH), Zhujiajian (ZJJ), and Daishan (DS). The tree is divided into two main branches, branch A and branch B. c 1 = cluster 1, c 2 = cluster 2, c 3 = cluster 3, c 4 = cluster 4.

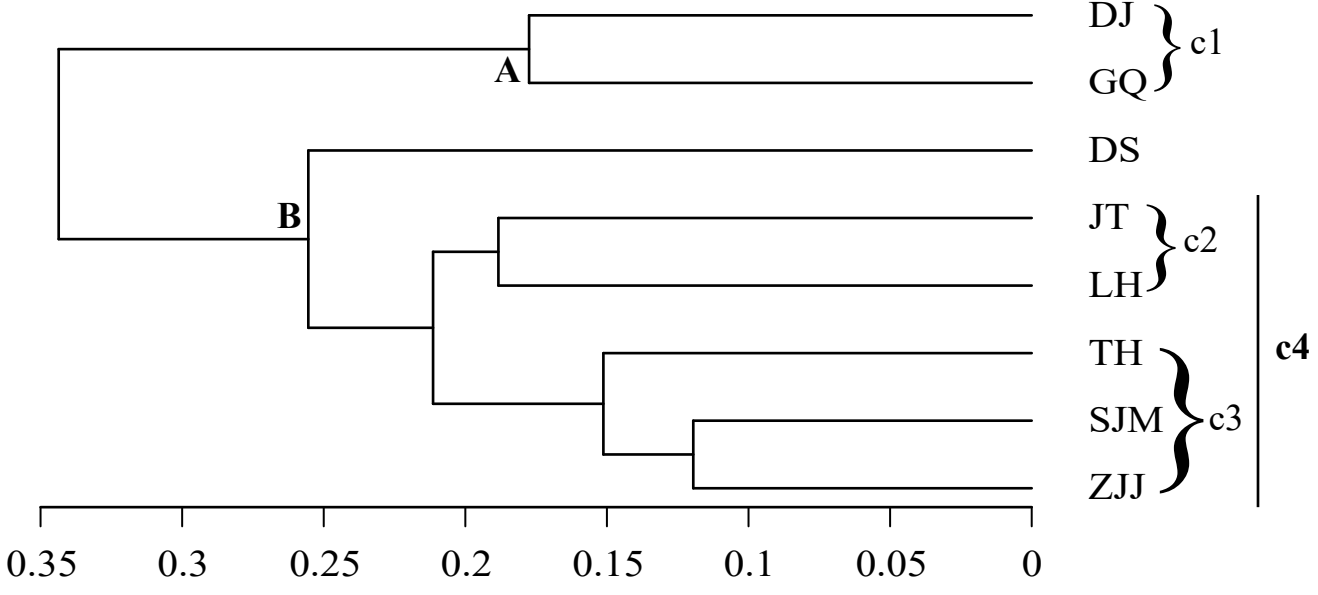

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Figure 4(on next page)

Ordination plot ofredundancy analysis (RDA) for AAPB with environmental variables.

Representative genera of AAPB are indicated in italics. Redundancy analysis (RDA) used CANOCO 4.5. Data were logtransformed, centred and standardized by different populations. RDA axes summary is located in the upper left corner of the graph. Environmental variables: Salinity, dissolved oxygen (DO), ammonia nitrogen (NH_4^+), nitrate (NO_3^-), silicate (SiO_3^{2-}), phosphate (PO_4^{3-}), and temperature . Stations: Dongji Island (DJ), Gouqi Island (GQ), Jintang Island (JT), Liuheng Isand (LH), Shenjiamen (SJM), Taohua Island (TH), Zhujiajian (ZJJ), and Daishan Isand (DS).

RDA axes summary	Axis1	Axis2	Total variance
Eigenvalues:	0.9862	0.0076	
Species-environment correlations:	1.0000	1.0000	
Cumulative percentage variance:			1.0000
of species data :	98. 62	99. 38	
of species-environment relation:	99. 75	99. 89	

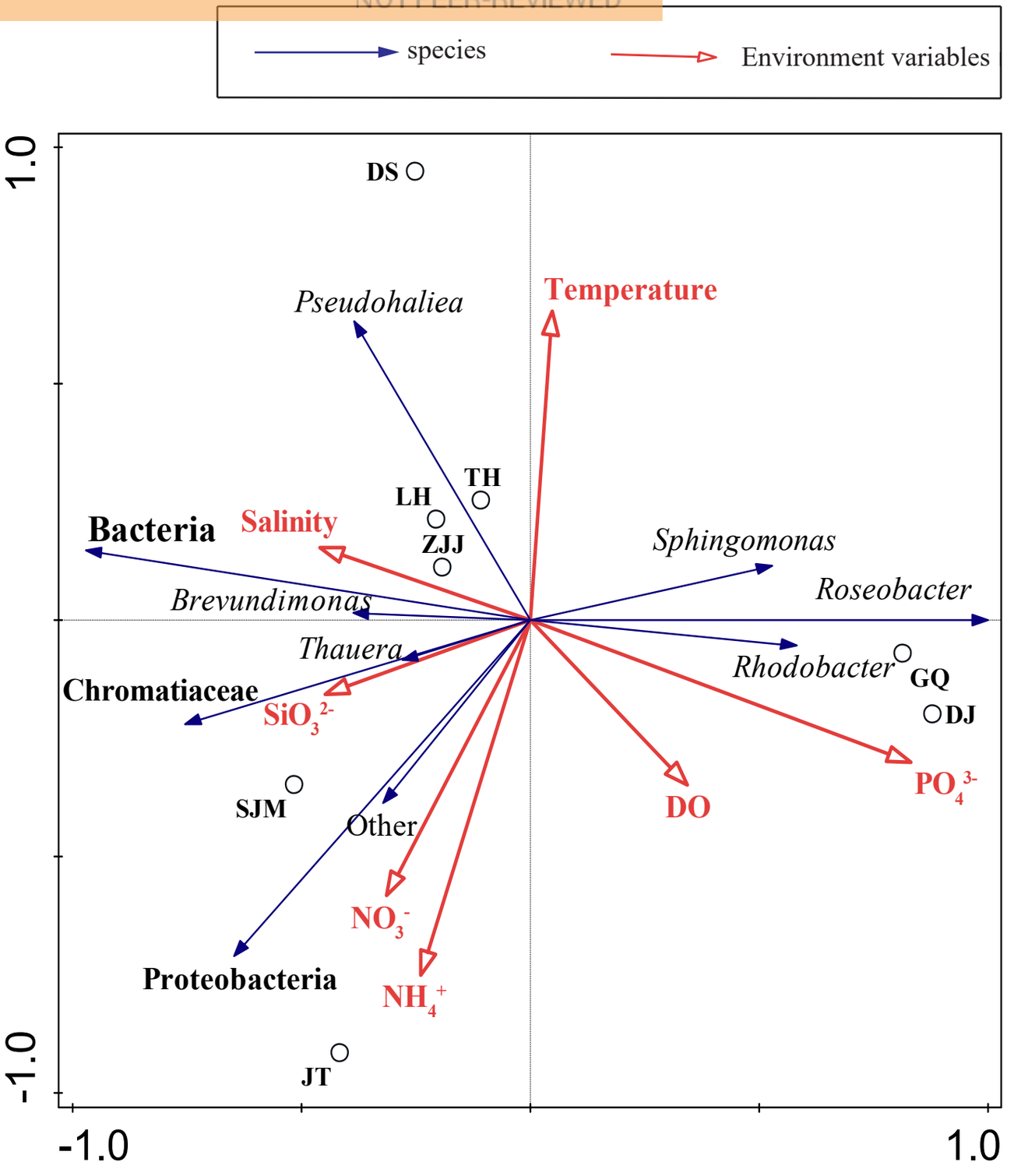

Table 1(on next page)

Information for eachsampling station and environmental variables in the Zhoushan Archipelago SeaArea.

Environmental variables: salinity, dissolved oxygen (DO), ammonia nitrogen (NH_4^+), nitrate (NO_3^-), silicate (SiO_3^{2-}), phosphate (PO_4^{3-}), and temperature (T). Stations: Dongji Island (DJ), Gouqi Island (GQ), Jintang Island (JT), Liuheng Isand (LH), Shenjiamen (SJM), Taohua Island (TH), Zhujiajian (ZJJ), and Daishan Isand (DS).

1

Sampling site	longitude	latitude	Salinity	DO (mg L^{-1})	NH_4^+ (mg L^{-1})	NO_3^- (mg L^{-1})	SiO_3^{2-} (mg L^{-1})	PO_4^{3-} (mg L^{-1})	T (°C)
JT	121.85E	30.07N	21.31	6.95	0.58	0.73	0.64	0.04	28.70
LH	122.11E	29.79N	27.45	7.01	0.54	0.59	1.07	0.02	33.50
TH	122.30E	29.81N	30.13	10.78	0.58	0.63	0.64	0.03	28.20
DJ	122.69E	30.19N	27.65	8.78	0.65	1.00	1.48	0.03	27.00
GQ	122.81E	30.72N	30.47	7.21	0.62	0.9	0.79	0.03	26.90
DS	122.11E	30.27N	29.33	7.26	0.58	0.69	0.64	0.03	27.70
SJM	122.32E	29.94N	29.32	6.39	0.57	0.79	1.17	0.03	26.60
ZJJ	122.41E	29.92N	30.01	8.63	0.59	0.65	0.62	0.03	27.70

2

Table 2(on next page)

The OTUsand diversity indices for each sample at 97% similarity .

Including the ACE index, Chao index, Shannon index, Simpson index, and coverage. Stations: Dongji Island (DJ), Gouqi Island (GQ), Jintang Island (JT), Liuheng Isand (LH), Shenjiamen (SJM), Taohua Island (TH), Zhujiajian (ZJJ), and Daishan Isand (DS).

1

Sampling site	Cutoffs	OTUs	ACE	Chao	Shannon	Simpson	Coverage
DJ	0.03	1300	6090	3038	2.66	0.23	0.98
DS	0.03	970	3501	2088	3.03	0.10	0.98
GQ	0.03	1548	5211	3283	3.16	0.17	0.97
JT	0.03	1227	4216	2725	2.82	0.21	0.98
LH	0.03	1568	4822	3187	4.01	0.08	0.97
SJM	0.03	1152	4313	2582	2.84	0.16	0.98
TH	0.03	1184	5305	3060	2.74	0.18	0.98
ZJJ	0.03	944	5295	3026	2.40	0.20	0.98

2

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Table 3(on next page)

Thesequences taxonomy at the phylum level of AAPB for the eight sites (97% cut-offvalue).

The numbers below DJ, GQ, JT, LH, SJM, TH, and ZJJ indicate the sequences for each site were divided into each phylum. Stations: Dongji Island (DJ), Gouqi Island (GQ), Jintang Island (JT), Liuheng Isand (LH), Shenjiamen (SJM), Taohua Island (TH), Zhujiajian (ZJJ), and Daishan Isand (DS).

1
2

Taxonomy	DJ	DS	GQ	JT	LH	SJM	TH	ZJJ
Proteobacteria	16362	570	13084	393	702	181	843	581
unclassified	18863	33368	20044	31035	30168	32258	33354	32939
Firmicutes	0	4	0	1	4	0	1	0
Bacteroidetes	1	0	0	0	1	0	0	0
Actinobacteria	0	0	0	1	0	0	0	0

3