Phosphate Enrichment Regulates the Interplay Between Deterministic and Stochastic Processes of Bacterioplankton Community Assembly in a Subtropical Bay Impacted by Thermal Discharge

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Phosphate enrichment regulates the interplay between deterministic and stochastic processes of bacterioplankton community assembly in a subtropical bay impacted by thermal discharge[1]

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[1] Author contributions' statement
LR and XS conceived the study, conducted the fieldwork, analyzed the data, wrote the manuscript, and provided funding. YC, LW, GM, and JG analyzed the data and wrote the manuscript. QLW and BPH contributed to the manuscript revision and editing.

Abstract

Increasing anthropogenic activities has caused intensive environmental issues and undesirable ecological impacts on coastal bay ecosystems. Bacterioplankton play critical roles in ecological functioning of the bay ecosystems, but much remains to be learned regarding the response of bacterioplankton community assembly to heterogeneous environmental issues in the bay ecosystems and its underlying mechanisms. In this study, we used the phylogenetic framework to analyze the bacterioplankton community assembly in the subtropical Daya Bay, where the habitats are connected by water flows and tides and their trophic status are subject to intensive environmental stress induced by human activities (e.g., thermal discharge and nutrient release from aquaculture). We found bacterioplankton community compositions (BCCs) among the sampling habitats in the Daya Bay showed obvious spatial heterogeneity. The spatial distributions of BCCs were significantly shaped by phosphate concentration among the examined environmental factors. We observed that between pairwise sampling habitats, the BCC dissimilarity significantly increased with the differences in seawater phosphate concentration. Compared with stochastic processes, phosphate enrichment imposed stronger effects of environmental filtering in determining bacterioplankton community assembly in the subtropical bay, and bacterioplankton communities tended to be higher phylogenetically clustered in more phosphate-enriched habitats. In summary, we propose that phosphate is a major environmental determinant in the subtropical Daya Bay impacted by thermal discharge and regulates the interplay between deterministic and stochastic processes underlying bacterioplankton community assembly.

Keywords: Phosphate enrichment; bacterioplankton community; phylogenetic assembly; environmental filter; subtropical bay

Introduction

In marine ecosystems, coastal bays are essentially important to human society for various services they provided, such as supporting, regulating, and provisioning services (Barbier et al. 2011; Temmink et al. 2020). However, due to the increasingly and intensively anthropogenic activities around the bay ecosystems, currently, many coastal bay ecosystems are facing heterogeneous environmental issues (Lefcheck et al. 2018; He and Silliman 2019). Existing studies indicate that eutrophication, environmental pollution, and harmful algal bloom have occurred frequently in many coastal bay ecosystems and caused a decrease in biodiversity, a collapse of living organisms, and an ecological imbalance (McLusky and Elliott 2004; Cloern and Jassby 2010; Stauffer et al. 2020). The influence of heterogeneous environmental issues on marine planktonic community has been a hot issue in the field of bay environmental protection (Matear et al. 2019). Bacterioplankton, an integral component of marine planktonic community, play important roles in ecosystem functioning of coastal bays (Falkowski et al. 2008; Wang et al. 2020). Due to the short generation time and frequent ecological drift of bacterioplankton, its community diversity may change synchronously with seawater environmental changes (Luo and Moran 2015) and have influence on the resistance and resilience of other organisms to heterogeneous environmental issues (Bissett et al. 2013). Despite the ecological functioning of bacterioplankton community, much remains to be learned regarding the response of bacterioplankton community assembly to heterogeneous environmental issues in the coastal bay ecosystems and its underlying mechanisms.

It is a longstanding challenge to understand the mechanisms underlying bacterioplankton community assembly in natural aquatic ecosystems (Quattrini et al. 2017; Shen et al. 2018; Aguilar and Sommaruga 2020). One promising approach to

71 understand the mechanisms underlying bacterioplankton community assembly is the
72 use of the phylogenetic framework (Webb 2000; Webb et al. 2002). The changes in
73 the relative influences of deterministic and stochastic processes across heterogeneous
74 environmental issues can be inferred in the phylogenetic framework, by comparing
75 observed community phylogenetic structure with randomization procedures (Webb et
76 al. 2002; Cavender-Bares et al. 2004; Quattrini et al. 2017). Based on the assumption
77 that closely related phylogenetic taxa are more likely to have similar niches in
78 phylogenetic framework, a nonrandom observed phylogenetic structure is expected to
79 suggest the deterministic processes of community assembly (Webb 2000; Wiens et al.
80 2010). Deterministic processes assume that bacterioplankton community structure is
81 controlled by deterministic factors such as environmental heterogeneity and species
82 interactions. Meanwhile, stochastic processes suppose bacterioplankton community
83 structure is shaped by stochastic processes, such as probabilistic dispersal and random
84 birth/death events (Hubbell 2011). Because both deterministic and stochastic
85 processes may play important roles in shaping bacterioplankton community assembly,
86 it comes up a question of that how their relative contributions of stochastic and
87 deterministic processes change with environmental heterogeneity in natural bay
88 ecosystems.

89 Daya Bay is one of the most socially and economically important bay in the
90 northeast part of the Pearl River estuary of China (Wang et al. 2008). As a core region
91 of Pearl River Delta, the coastal area of this bay has been experiencing rapid
92 economic development during the past decades. Since the operation of the first
93 large-capacity commercial nuclear power plant, namely Daya Bay Nuclear Power
94 Station in 1994, more and more agriculture, industries, and urbanization have been
95 carried out around the Daya Bay (Tang et al. 2003; Wang et al. 2011). The

increasingly anthropogenic activities around the bay ecosystems led to undesirable heterogeneous environmental issues and subsequent ecological impacts on the Daya bay ecosystems (Wu et al. 2017). It is showed that the Daya Bay are suffering long-term thermal pollution from the cooling systems of the two nuclear power stations, Daya Bay nuclear power station and Ling'ao nuclear power station (completed in 2002) (Tang et al. 2003; Wei et al. 2013). In addition, the bay is facing long-term eutrophication caused by the excess nutrient input from the domestic sewage and aquaculture (Song et al. 2004; Sun et al. 2006). Long-term thermal pollution and nutrient (e.g., nitrogen and phosphorus) excess might be a "stress" event that acts as an environmental filter selecting bacterioplankton taxa with suitable biological traits and filter out other members from the local species pool (Dai et al. 2017). Besides the deterministic processes of environmental factors, dispersal might simultaneously play important roles in shaping the patterns of the bacterioplankton community assembly in the Daya Bay ecosystem, since frequently exchanges of different bacterioplankton seed banks across different patches through water flows and tides in the bay (Ren et al. 2019). The frequent dispersal of bacterioplankton across different patches might result in the presence of bacterioplankton taxa in places that are less suitable for them and weakening the deterministic effects of environmental factors (Juračka et al. 2016; Wisnoski and Lennon 2021).

In this study, we used a phylogenetic framework to study bacterioplankton community assembly and the underlying determining mechanisms based on 16S rRNA high-throughput sequencing collected from a set of habitats located in the Daya Bay sampled in a wet summer season. We hypothesized that (1) although bacterioplankton seed banks can frequently exchange across different habitats through water flows and tides in a typical subtropical semi-enclosed bay, the patterns of the

bacterioplankton community assembly might be dominantly shaped by deterministic processes of environmental heterogeneity; (2) long-term thermal pollution and nutrient input might act as key environmental filters in determining bacterioplankton community assembly, causing phylogenetically clustered of bacterioplankton communities in the subtropical Daya Bay. Our study might contribute to a broader understanding of the ecological effects of environmental disturbance to subtropical bay and benefit the management of the subtropical bay.

Materials and methods

Study area

Daya Bay is located in the northern South China Sea (114.3°E to 114.5°E, 22.3°N to 22.5°N) between Shenzhen and Huizhou in Guangdong province near Hong Kong (Fig. 1). It is one of the largest and most important semi-closed bays along the southern coast of China with an area around 600 km^2, a depth between 6 and 15 m, and an average annual water temperature from 22.5- 23.5℃. Around the Daya Bay, there are two nuclear power stations (the Daya Bay nuclear power station and Lingao nuclear power station) (Wang et al. 2008). Heated water is released into water body at a unified outlet site away from the intake points of the cooling system of the two nuclear power stations, and the seawater temperature of the area strong affected by thermal pollution is heated over the whole year. Meanwhile, the bay is suffering eutrophication caused by the excess nutrient input from the domestic sewage and aquaculture (Sun et al. 2006).

We set 12 sampling sites in the Daya Bay (S1-S12, Fig. 1), and sampled the seawater in August 2017, including the marine aquaculture area seriously affected by human activities (Fanhe harbour aquaculture area S1; Xiaoguiwan aquaculture area

S5; Dapengao aquaculture area S10), nuclear power station thermal pollution area (S9), petrochemical pollution area (S6), the estuary area (S11 and S12), river inflow area (S2), and site S7 that was less affected by human activities.

Sample collection and environment factor analysis

At each sampling site, we set three replicates. 5 L surface seawater was collected at each replicate site and was filtered through 0.2-m-pore-size Isopore filters (Millipore, Billerica, MA, USA). The filters with microbial biomass were stored at –70°C for further analysis. Water temperature (T), dissolved oxygen (DO), salinity and pH were measured in situ at each replicate site by using multi-parameter water quality analyzer (YSI 6600, Yellow Springs, OH, USA). 500 mL surface seawater was further collected at each replicate site for the measurement of nutrient conditions. According to a standard procedure (GB 17378.4-2007), seawater ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), phosphate (PO_4^{3-}-P), and silicate (SiO_3^{2-}-Si) concentrations were determined using a UV-visible spectrophotometer (UV2450, Shimadzu, Tokyo, Japan).

DNA extraction, PCR amplification, high-throughput sequencing and data processing

Microbial genomic DNA was extracted using PowerWater DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA) and was purified by PowerClean DNA Clean up Kit (Mo Bio Laboratories, Carlsbad, CA, USA). With 60 ng DNA as template, PCR amplification was performed in the bacterial 16S rRNA V3 and V4 regions using the primer with 10 mM barcode and 25 μL2 × PCR Premix Taq. The amplification primers were F341 (5 '-CCTACGGGAGGCAGCAG-3') and R806 (3 '-GGACTACGGGTTCTAAT-5'). PCR reactions included an initial denaturation at

94 °C for 5 min, followed by 30 cycles of 30 s at 94°C, 30 s at 52 °C, and 30 s at 72 °C and the final extension for 10 minutes (72°C). When the PCR amplification was completed, the fragment length and concentration of PCR products were detected by 1% agarose gel electrophoresis and the positive amplicons were quantified using the PicoGreen dsDNA assay kit (Invitrogen Corporation, Carlsbad, CA, USA). Subsequently, the PCR products were equally combined and purified with Zymo's Genomic DNA Clean & Concentrator kit (Zymo Research Corporation, Irvine, CA, USA). Finally, amplicons were sequenced using PE250 on Illumina Hiseq2500 platform.

Raw sequences were processed with the mothur software package (version 1.30.0, 2013) (http://www.mothur.org) according to the MiSeq standard operating procedure (Kozich et al. 2013). Briefly, the raw sequences were combined, denoised, trimmed, quality-filtered, and aligned to the SILVA v132 databases using mother (Kozich et al. 2013). The high-quality sequences were then classified using the SILVA v132 databases at the recommended bootstrap threshold of 80% (Wang et al. 2007). The lineages belonging to chloroplasts, mitochondria, archaea or eukaryotes were removed and the high-quality sequences were then clustered into operational taxonomic units (OTUs) at a 97% similarity level. All singletons of OTUs and OTUs occurring in less than two samples were removed in the further analyses for minimizing bias caused by sequencing depth. Moreover, of the whole sample set, the minimum number of sequences was randomly subsampled to correct for differences in sequencing depth.

Diversity analysis

Taxonomic richness and Shannon-Wiener index of bacterioplankton community were calculated using the "vegan" packages in R. The dissimilarities of

bacterioplanton community compositions (BCCs) between sites were performed based on both of the Bray-Curtis and the UniFrac distance by using the “vegan” packages in R.

Phylogenetic framework

The phylogenetic framework was performed, and both of the mean-nearest-taxon-distance (MNTD) and the standardized-effect size of the MNTD (SES.MNTD) were calculated by using package “picante” in R. The null model was performed by shuffling taxon labels 999 times across the tips of the phylogenetic tree using a given tree topology and branch lengths to randomize phylogenetic relationships among OTUs. By comparing the difference between the observed community and the random community, the main ecological processes of bacterioplankton community assembly was determined in the Daya Bay. SES.MNTD means the differences in MNTD between the observed communities and the mean value of the 999 null communities divided by the standard deviation of the MNTD in the 999 null communities (Webb 2000). When SES.MNTD values greater than 2, it indicates phylogenetic overdispersion (taxa are more distantly related than would be expected random). If SES.MNTD values less than 2, it suggests phylogenetic clustering (taxa are more closely related than would be expected at random). When SES.MNTD values between -2 and 2, it represents that stochastic processes dominate in the bacterioplankton community assembly (Webb 2000; Webb et al. 2002).

Statistical analyses

We performed the analysis of multiple regression tree (MRT) in which the BCCs were the response variable and the environmental variables were the explanatory variable by using the “mvpart” packages in R (version 4.0). The relative abundances

of OTUs of the main lineages or clades of bacterioplankton communities along the phosphate gradient were depicted in a heat map using the pheatmap command in the "pheatmap" package in R. A partial Mantel test with 9,999 permutations was performed to calculate partial matrix correlations between three dissimilar matrices: the BCC matrix, each selected environment variable, and the conditional matrix of the remaining environment variables (Legendre and Legendre 2012). Mantel test was used to test the relationship between environment difference and bacterial sub-community dissimilarity. The correlations among environmental factors (Pearson correlation coefficients) were examined by Pearson regression. The distributions of both taxonomy-based and phylogeny-based BCCs in the Daya Bay were visualized by using nonmetric multidimensional scaling (NMDS) in the vegan package in R.

Accession numbers

The sequence data were submitted to the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) (https://www.ncbi.nlm.nih.gov/sra) under accession number PRJNA765469.

Results

Of the quality sequences, 97.7% were classified at the phylum level and 20 phyla were obtained. The dominant phyla across all sampled habitats were *Alphaproteobacteria*, *Cyanobacteria*, *Bacteroidetes*, and *Actinobacteria*, representing approximately 51.0%, 22.8%, 9.8% and 9.7% of the total sequences, respectively (Fig. S1, Fig.2). In addition, *Gammaproteobacteria* (3.3%), *Planctomycetes* (2.5%), *Deltaproteobacteria* (0.16%), *Firmicutes* (0.13%), and *Acidobacteria* (0.04%) were present in most sampled habitats but with low relative abundance (Fig. S1). We observed that bacterioplankton (sub)phylum compositions at S1 and S3 stations was

similar, and the BCCs was mainly composed of *Alphaproteobacteria* (Fig. S1, Fig. 2e). The relative abundance of *Bacteroides* was much higher in S1, S3, S11, S12 than the other sites, suggesting that *Bacteroides* was more suitable to the habitats of the top and the estuary of the Daya Bay ecosystem (Fig. S1, Fig. 2b). However, *Cyanobacteria* had the highest relative abundance at S7, S8, S9, and S10, where was strongly affected by the thermal pollution from the nuclear power plants (Fig. S1, Fig. 2c). In contrast with other phyla, *Actinobacteria* were mainly found in estuary of the bay (Fig. S1, Fig. 2a).

The average water temperature in the subtropical Daya Bay was 29.78 ± 1.14°C with the highest at site S9 (32.03°C), which was strongly affected by thermal pollution of the cooling system of the nuclear power stations (Fig. S2). The average salinity of the Daya Bay was 28.84 ± 3.03‰, with the highest at site S11 (33.33‰) and the lowest at S1 (22.78‰) (Fig. S2). The concentration of PO_4^{3-}-P, NO_3^--N, NH_4^+-N and SiO_3^{2-} showed a decreased tendency from the top (site S1 and S3) to the estuary (site S11 and S12) of the Daya Bay (Fig. S2). Among the measured environmental factors, we found that seawater phosphate was the best predictor of BCCs in the Daya Bay (Fig. S3). The mantel test also showed that phosphate was significantly correlated with differences in BCCs across different bacterial phyla and subphyla in the water bodies (Fig. 3a). Therefore, we further explored the relationship between the concentration PO_4^{3-}-P and the relative abundance of the dominant phyla. The relative abundances of *Actinobacteria* (Fig. 3b), *Cyanobacteria* (Fig. 3d), *Planctomycetes* (Fig. 3e), and *Gammaprotecteria* (Fig. 3g) decreased linearly with phosphate concentration, while the relative abundances of *Bacteroidetes* (Fig. 3c) and *Alphaproteobacteria* (Fig. 3f) were positively correlated with phosphate concentration. At the site with high phosphate concentration, the main lineage or clades were mostly

composed of the bacteria *Saprospiraceae* and *Croinitomicaceae* of *Bacteriodetes*, and *Rhodobacteraceae*, *Novosphingobium* and *Tabrizicola* of *Alphaproteobacteria* (Fig. 4), while NS4 marine group of *Bacteriodetes*, *Synechococcus* and *Cyanobium* of *Cyanobacteria*, and UBA10353 marine group of *Gammaproteobacteria* dominated in low phosphate concentration (Fig. 4).

The seawater phosphate was a key environmental factor in determining bacterioplankton community structure revealed by redundancy analysis (RDA) followed by temperature and silicate (Fig.S4). Bacterioplankton spatial distributions did not showed obvious geographical patterns (Fig. 5a and 5b), temperature (Fig. S5a and S5b) or silicate (Fig. S5c and S5d) patterns, but was arranged according to the phosphate concentrations as the low concentration (LPC), the medium concentration (MPC), and the high concentration (HPC) (Fig. 5c and 5d). The NMDS plot revealed that the BCCs of different spatial habitats but with the same phosphate concentration tend to be similar, suggesting the existence of strong linkages between phosphate concentration and community structure (Fig. 5c and 5d). Between pairwise sampling habitats, the BCC dissimilarity both based on Bray-Curtis and UniFrac distances significantly increased with the differences in seawater phosphate concentration (Fig. 5e and 5f). Moreover, we found seawater phosphate concentration also had significantly positive correlations with bacterioplankton alpha diversity, such as with OTU richness ($R^2 = 0.15$, $p < 0.05$) and Shannon-Wiener index ($R^2 = 0.12$, $p < 0.05$) (Fig. S6a and S6b).

To understand the mechanisms underlying bacterioplankton community assembly across heterogeneous environmental issues, the phylogenetic framework was performed, and we found a significant negative correlation between the MNTD and the concentration of phosphate (Fig. 6a). The site with the highest phosphate

concentration had the lowest MNTD, indicating that the higher the phosphate concentration, the closer the phylogenetic relationships between the nearest bacterial taxa. Moreover, we observed that, the standardized-effect size of the MNTD (SES.MNTD) was significantly lower than zero and decreased linearly with increasing the phosphate concentration ($R^2 = 0.21$; $p < 0.01$, Fig. 6b).

Discussion

In this study, we examined bacterioplankton community assembly and the underlying mechanisms in the typical subtropical semi-enclosed Daya Bay ecosystem. We found that the spatial pattern of BCCs was dominantly shaped by deterministic processes. Among the environmental factors, phosphate concentration was the key environmental determinant of the assembly patterns of marine bacterioplankton communities. Apparent phosphate concentration-related patterns of bacterioplankton community compositions, diversity, and phylogenetic structure were observed in the Daya Bay impacted by thermal discharge.

The bacterioplankton metacommunity assembly was predominantly shaped by deterministic processes

In metacommunity theory, dispersal plays an important role in determining the assembly of bacterioplankton communities between habitats of aquatic environments (Leibold et al. 2004). High dispersal rates may result in bacterioplankton taxa surviving in habitats that less suitable for their growth, and in contrast, low dispersal rates of bacterioplankton taxa may lead to dispersal limitation and thus lead to significant spatial variations (Juračka et al. 2016; Wisnoski and Lennon 2021). In this study, we found that bacterioplankton dispersal among the sampling habitats by water flows and tides in the Daya Bay did not result in BCC spatial homogeneity. In contrast,

significant heterogeneity in BCCs was found across different sampling sites in the Daya Bay. Among the environmental factors, phosphate concentration was found to be the key environmental determinant of the assembly patterns of marine bacterioplankton communities and apparent phosphate concentration-related patterns of BCC distributions were observed in the Daya Bay. As inferred in previous studies, this finding also suggests that bacterioplankton community assembly in interconnected aquatic habitats was predominantly shaped by deterministic processes of environmental factors, rather than by dispersal (Shen et al. 2018; Ren et al. 2019; Aguilar and Sommaruga 2020). A number of studies have demonstrated that BCCs can rapidly track changes in the environment (Van Der Gucht et al. 2001; Muylaert et al. 2002). Thus, the persistence of newly arrived populations of bacterioplankton migrating through flowing water was more likely to be determined by deterministic processes of heterogeneous habitats, rather than by dispersal (Logue and Lindström 2010).

Phosphate enrichment increased bacterioplankton alpha diversity

The nutrient condition in the Daya Bay has changed from previous N-limited to current P-limited for the “excess nitrogen” input (He et al. 2005; Zhang et al. 2020). Previous studies have revealed the potential phosphorus limitation of phytoplankton growth in Daya Bay (Wang et al. 2008; Wu et al. 2017; Zhang et al. 2018). Phosphorus limitation exists not only for phytoplankton, but also for bacterioplankton. Compared to phytoplankton, bacterioplankton can assimilate phosphorus more effectively because of their larger specific surface area, and they have a lower half-saturation constant for phosphate than phytoplankton (Currie and Kalff 1984). In addition, the N:P ratio of phytoplankton biomass was generally about 16:1, which is much higher than that of heterotrophic bacteria (Kirchman 2000), resulting in a higher

efficiency of phosphorus requirement for bacterioplankton than phytoplankton. It is suggested that bacterial cells were difficult to divide when phosphate concentrations were limit (Zweifel et al. 1993; Yuan et al. 2011). The addition of phosphate in seawater might therefore enhance bacterial cell division and support for higher abundance and diversity of bacterioplankton community (Yuan et al. 2011).

In our study, we found bacterioplankton alpha diversity (the OTU richness and Shannon-Wiener index) were significant increased with phosphate enrichment, and the S1 site with the highest phosphate concentration also had the highest bacterioplankton OTU richness and Shannon-Wiener index. The S1 site in our study was located near the Fanhe harbor aquaculture area. Due to the rapid development of marine aquaculture industry, aquaculture was carried out since the 1990s on mangrove beaches and nutrients were fed into the water body in the form of bait (Wu et al. 2014). It was reported that only about 33% of phosphate was assimilated by fish during cage fish-farming, with the rest being retained in various forms in the cultured environment (Bouwman et al. 2013). It is suggested that sufficient nutrient supply is generally associated with enhanced primary productivity (Whitney et al. 2005). This enhanced primary productivity may lead to increased bacterioplankton alpha diversity for the reduced resource competitive exclusion as “the larger pie can be divided into more pieces” (Brown 1981; Fuhrman et al. 2008).

Heterogeneity of BCCs across phosphate gradients

We found that the sites with higher phosphate concentration were dominated by *Alphaproteobacteria*. *Alphaproteobacteria* are an “opportunistic group” that respond quickly to nutritional pulses, and they are found that dominate in plenty eutrophic water (Pinhassi and Berman 2003). At the lineage or clade level of the top 30 OTUs, we also observed a larger BCC variations at higher phosphate-concentration sites by

compared to the other sites. For higher phosphate-concentration sites, the relative abundance of *Rhodobacteraceae*, *Novosphingobium* and *Saprospiraceae* was obvious increased. We found the OTU with the highest relative abundance belonged to the *Rhodobacteraceae* family. *Rhodobacteraceae* (*Alphaproteobacteria*) were among the nine most widely distributed bacterial lineages in marine habitats (Garrity et al. 2005). Many bacterial taxa of *Rhodobacteraceae* are aerobic heterotrophs and have been identified as key players in organic carbon and sulfur cycling (González et al. 1999; Moran et al. 2003; Newton et al. 2010). It has been reported that *Rhodobacteraceae* often existed in an environment rich in nitrogen/phosphorus nutrients, and it can use the soluble organic matter released by phytoplankton to sustain its own growth (Jones et al. 2007; Beck et al. 2014). Furthermore, recent studies suggest that the *Rhodobacteraceae* showed the highest response to the phosphorus amendment in northwest coastal Mediterranean waters (Sánchez et al. 2017). *Novosphingobium* was another abundant taxon in phosphorus-enriched habitats. Previous study revealed that the main polar lipids of *Novosphingobium* were bisphosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine (Chen et al. 2015). Bacterial taxa belonging to *Novosphingobium* can therefore use surrounding phosphorus efficiently. The enrichment of seawater phosphorus conditions may enhance bacterial growth and increase its environmental relative abundance of *Novosphingobium*. Many studies about *Saprospiraceae* indicated that they were associated with the degradation of organic matter (Schauer et al. 2005; Chen et al. 2014). Shifts in the relative abundance of these bacterial taxa under phosphorus enrichment may alter the bacterioplankton community structure (Lebaron et al. 2001). Moreover, the enrichment of phosphate can stimulate the growth of protozoa (Caron and Countway 2009), which might cause an increase in predation pressure on bacterioplankton taxa, and resulted in an

accelerated variations in the BCCs in phosphate-enriched habitats (Chen et al. 2016).

Bacterioplankton communities turned to be more phylogenetic clustering in higher phosphate-concentration seawater

It is important to elucidate the ecological mechanisms underlying microbial community assembly in community ecology (Webb et al. 2002; Zhou and Ning 2017). In this work, we observed that phylogenetic clustering of the most closely related OTUs increased linearly with phosphate enrichment. This observation raised the following question: why the bacterioplankton community turned to be more phylogenetically clustered at sites with higher phosphate concentrations? In our analyses of the diversity and composition of bacterioplankton, we observed that phosphate was a key environmental factor, which increased the heterogeneity of community compositions and alpha diversity. Therefore, we proposed that the direct or indirect environmental filtering effect may be increased at the sites where the phosphate concentration was high. This result was consistent with the observations of the bacterial communities in some lakes and rivers (Horner-Devine and Bohannan 2006; Mykrä et al. 2016). The phylogenetic clustering has been interpreted as evidence of environmental filtering, where a group of closely related species share a trait, or suite of traits, that allow them to persist in a given habitat (Webb et al. 2002). Our results suggest that environmental filtering may be more important than stochastic processes for the assembly of bacterial communities. Recent studies have shown that long-term coastal nutrient increase significantly alters the composition (Fodelianakis et al. 2014), assembly process (Xiong et al. 2015) and function (Zhang et al. 2015) of the bacterioplankton community. Usually, the differences between bacterial communities are closely related to the nutritional level (Dai et al. 2017; Ren et al. 2017). It is showed that with the increase of nutritional level (long-term and

continuous interference), the determinism of bacterial community assembly exhibited an elevated trend (Dai et al. 2017). They thought that long-term nutrient (phosphate) excess is a "stress" event that acted as an environmental filter, which could select species with suitable biological traits and exclude (filter out) other members from the local species pool. Similarly, in our study, the assembly of bacterioplankton community was dominantly shaped by deterministic processes in the subtropical Daya Bay. Thus, the most closely related OTUs (SES.MNTD) were more phylogenetically clustered in higher phosphate concentration sites.

Conclusion

Our results showed that the bacterioplankton community assembly was predominantly shaped by deterministic processes, and phosphate was a major environmental determinant in the subtropical Daya Bay. We observed that phosphate enrichment not only significantly increased the diversity of bacterioplankton communities, but also largely shifted the community compositions. Compared stochastic processes, phosphate enrichment imposed stronger effects of environmental filtering in determining marine bacterioplankton community assembly in the subtropical Daya Bay, and bacterioplankton communities tended to be higher phylogenetically clustered in more phosphate-enriched habitats. We therefore proposed that among the heterogeneous environmental issues in Daya Bay, phosphate enrichment was a major environmental determinant, regulating the interplay between deterministic and stochastic processes and shaping the patterns of marine bacterioplankton biodiversity. Our research might contribute to a broader understanding of the ecological effects of environmental disturbance to subtropical bay and benefit the management of the subtropical bay, during which, the phosphate emissions should be strictly controlled for their strong deterministic effects in shaping

marine biological communities.

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Author contributions' statement LR and XS conceived the study, conducted the fieldwork, analyzed the data, wrote the manuscript, and provided funding. YC, LW, GM, and JG analyzed the data and wrote the manuscript. QLW and BPH contributed to the manuscript revision and editing.

Conflicts of interest The authors declare no conflict of interest.

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Figure legends

Figure 1 Map of sampling locations in the 12 different habitats in the subtropical Daya Bay, Guangdong, China.

Figure 2 The distribution of dominant bacterial taxa in Daya Bay, *Actinobacteria* (**a**), *Bacteroidetes* (**b**), *Cyanobacteria* (**c**), *Planctomycetes* (**d**), *Alphaproteobacteria* (**e**), and *Gammaproteobacteria* (**f**).

Figure 3 The relationships between environmental factors and their correlations with the community structure of the dominant bacterioplankton (sub)phyla. Edge width means the R values of the Mantel's statistic, and edge color represents the statistical significance based on 9,999 permutations (**a**). The relative abundances of *Actinobacteria* (**b**), *Bacteroidetes* (**c**), *Cyanobacteria* (**d**), *Planctomycetes* (**e**) *Alphaproteobacteria* (**f**), and *Gammaproteobacteria* (**g**) across the phosphate concentration gradient.

Figure 4 The distributions of the top 30 OTUs across the phosphate concentration gradient in Daya Bay.

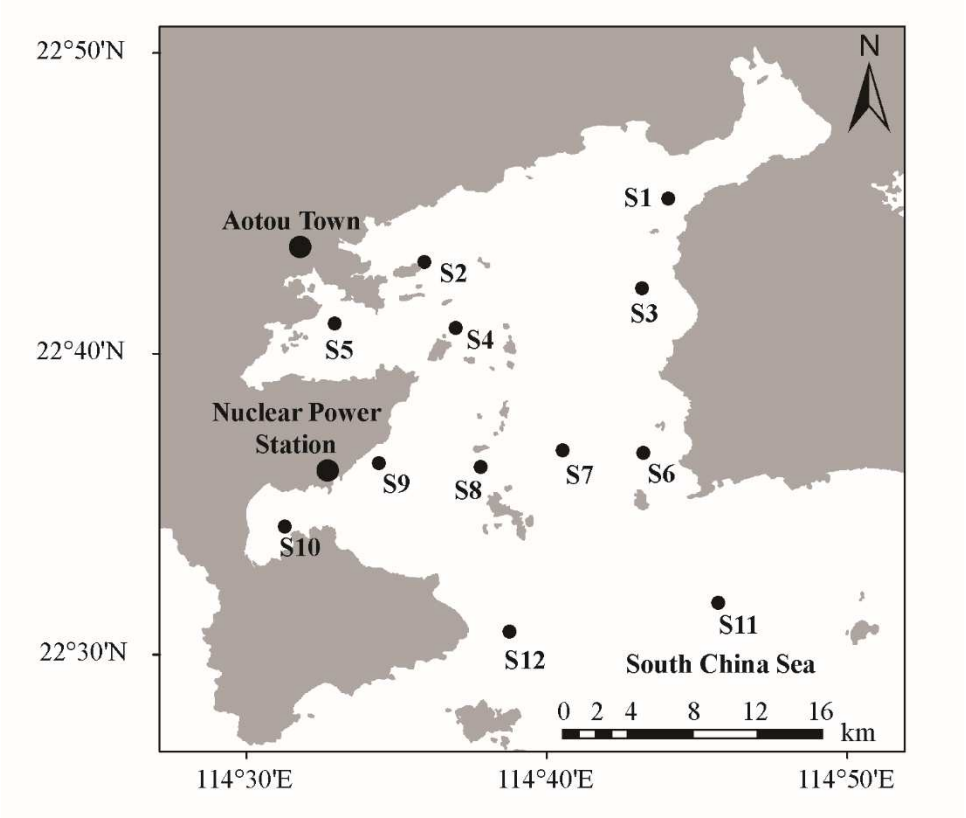
Figure 5 Non-metric multidimensional scaling (NMDS) ordinations showing the distributions of bacterioplankton community compositions (BCCs) in the Daya Bay according to the geographical locations (**a** and **b**) or the phosphate concentration gradient (**c** and **d**) both based on BCC Bray-Curtis dissimilarity (**a** and **c**) and weighted UniFrac dissimilarity (**b** and **d**). Relating BCC Bray-Curtis (**e**) and weighted UniFrac (**f**) dissimilarities with the differences of phosphate concentrations. The

726 formulas, R^2 values, and significances are given in each panel.

727

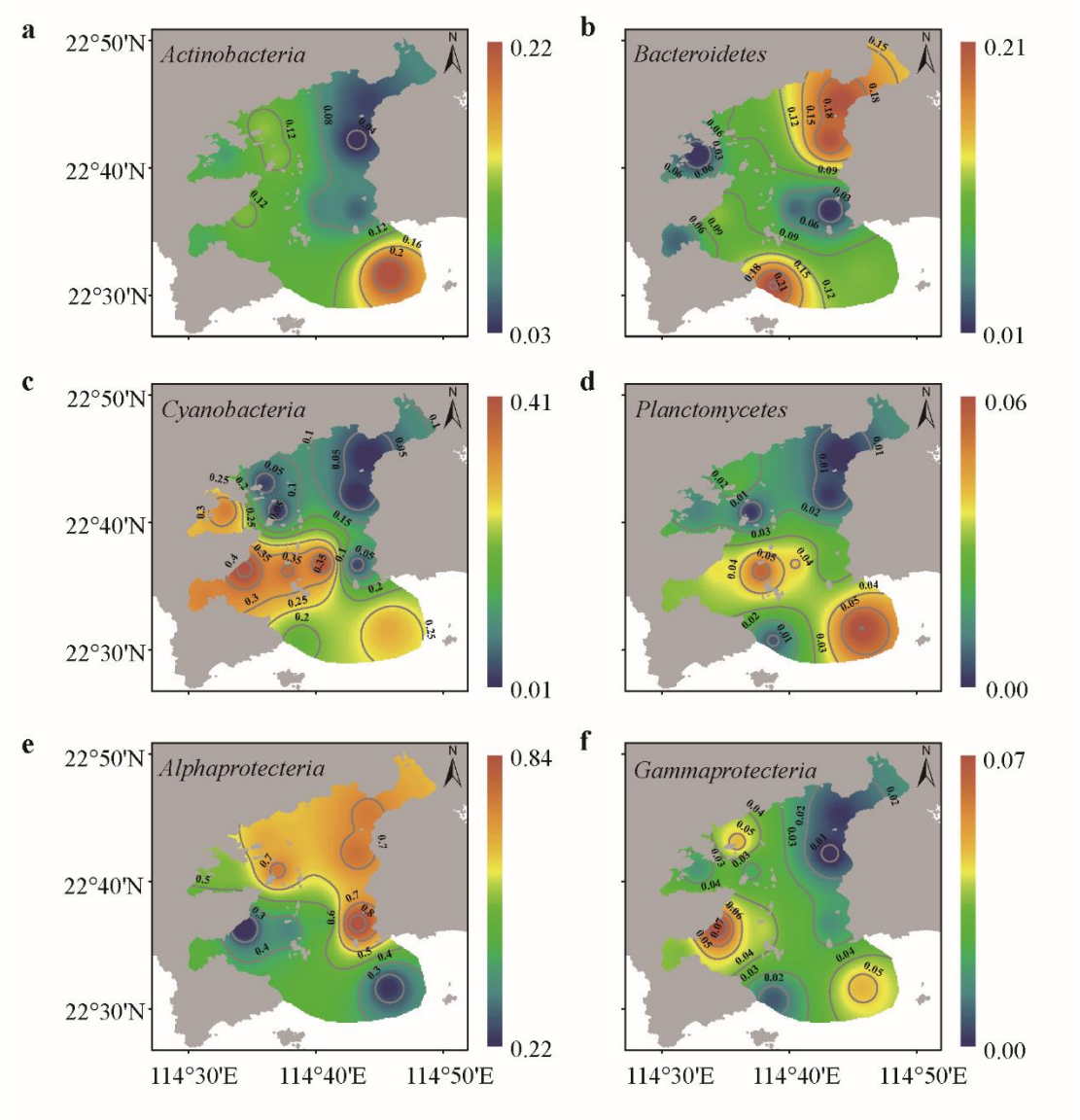
728 **Figure 6** The distributions of the mean nearest taxon distance (MNTD, **a**) and the
729 standardized effect size of the MNTD (SES.MNTD, **b**) of bacterioplankton
730 communities according to the three phosphate categories in the Daya Bay. LPC: low
731 phosphate concentration, MPC: medium phosphate concentration, and HPC: high
732 phosphate concentration.

733 **Figure 1**



734

735 **Figure 2**



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737 **Figure 3**

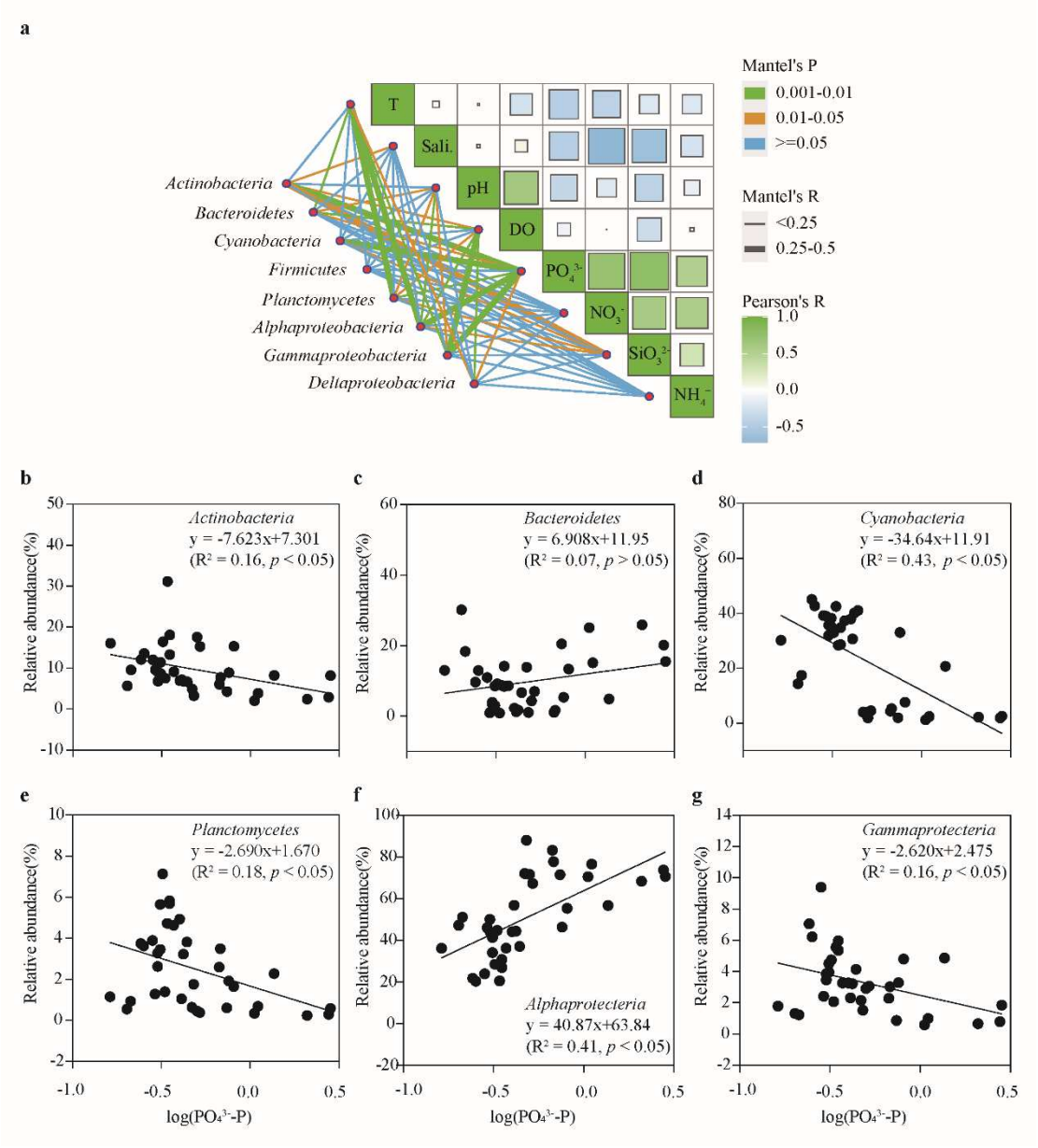

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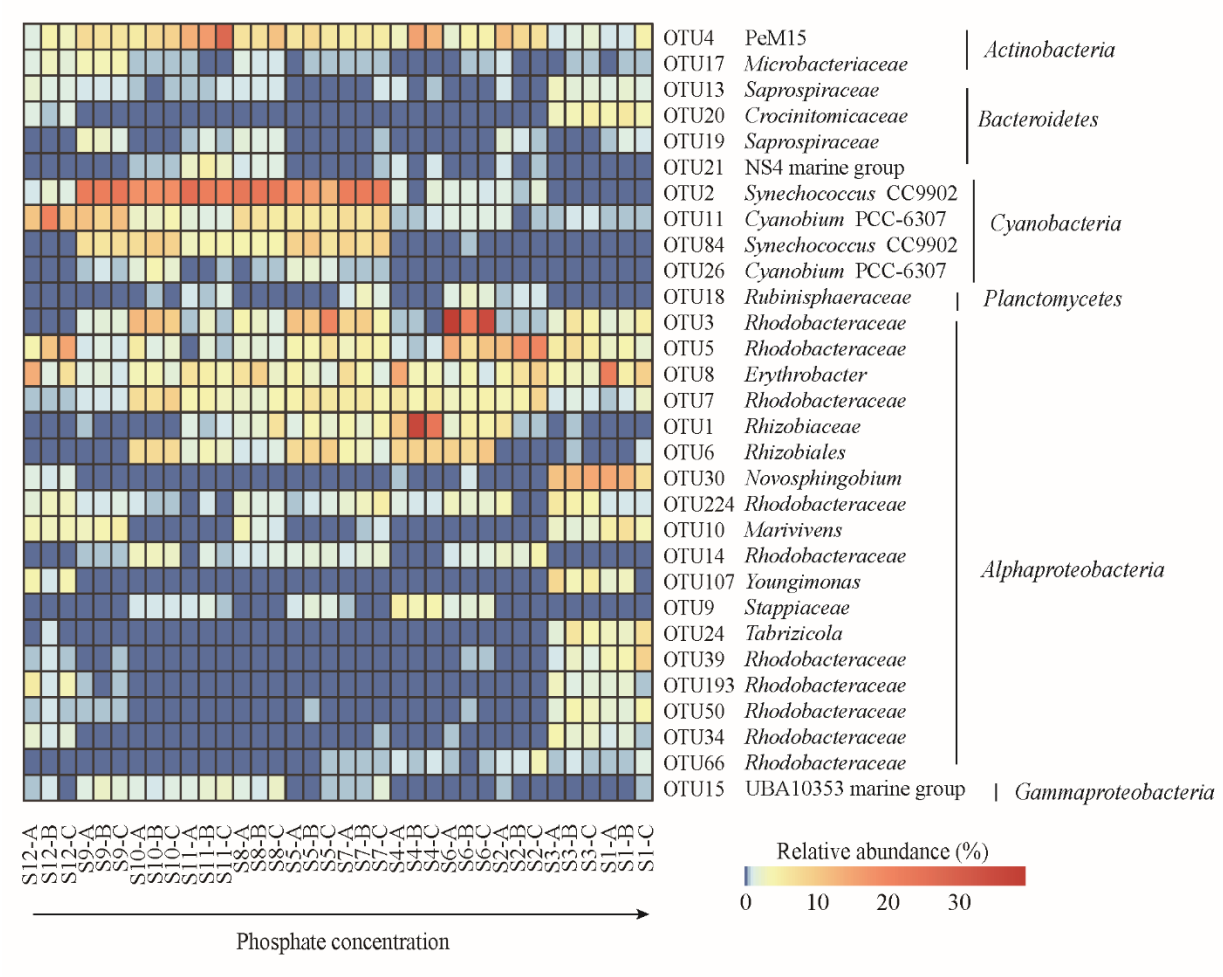
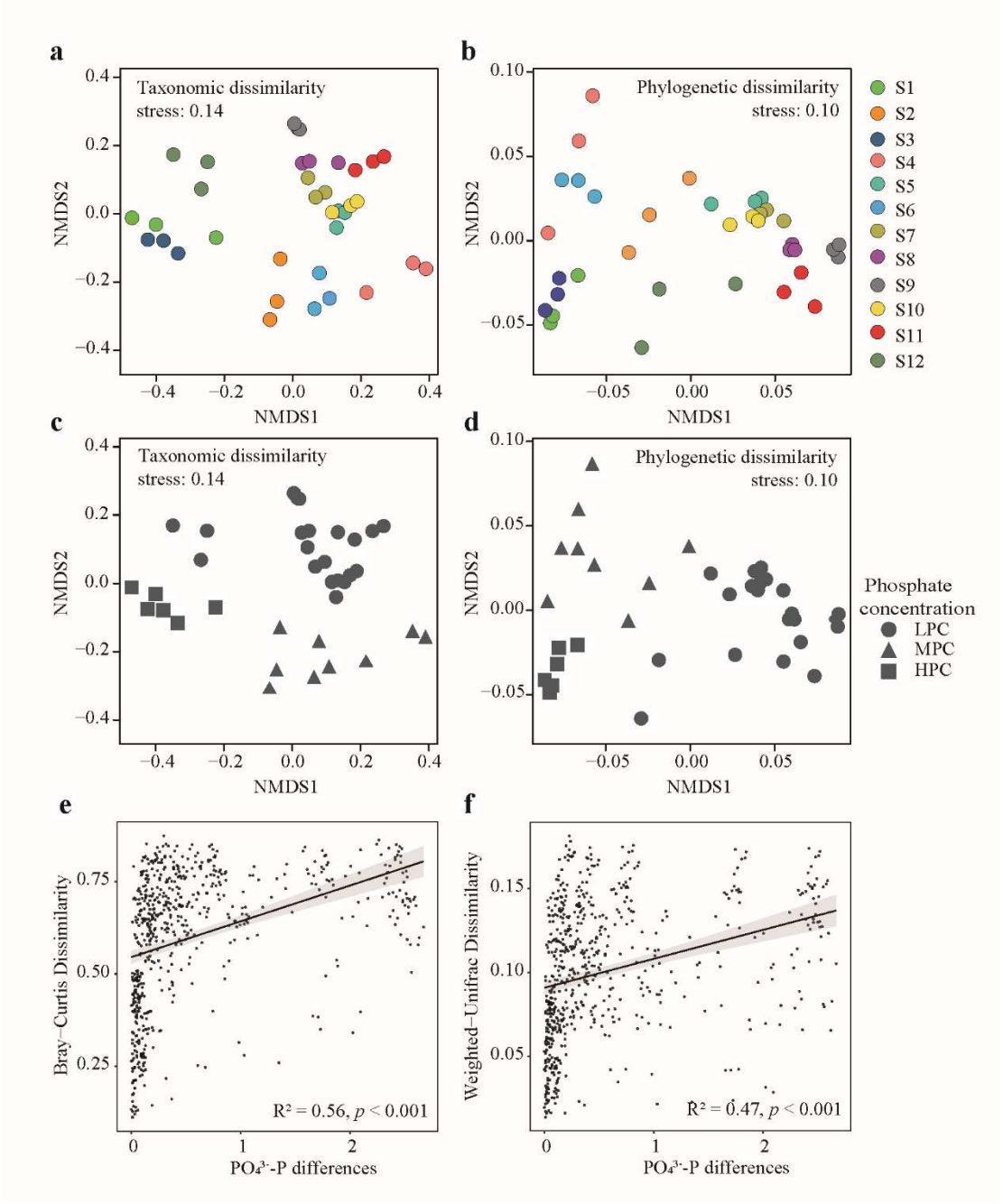
Figure 4


OTU4 PeM15
OTU17 Microbacteriaceae
OTU13 Saprospiraceae
OTU20 Crocinitomicaceae
OTU19 Saprospiraceae
OTU21 NS4 marine group
OTU2 Synechococcus CC9902
OTU11 Cyanobium PCC-6307
OTU84 Synechococcus CC9902
OTU26 Cyanobium PCC-6307
OTU18 Rubinisphaeraceae
OTU3 Rhodobacteraceae
OTU5 Rhodobacteraceae
OTU8 Erythrobacter
OTU7 Rhodobacteraceae
OTU1 Rhizobiaceae
OTU6 Rhizobiales
OTU30 Novosphingobium
OTU224 Rhodobacteraceae
OTU10 Marivivens
OTU14 Rhodobacteraceae
OTU107 Youngimonas
OTU9 Stappiaceae
OTU24 Tabrizicola
OTU39 Rhodobacteraceae
OTU193 Rhodobacteraceae
OTU50 Rhodobacteraceae
OTU34 Rhodobacteraceae
OTU66 Rhodobacteraceae
OTU15 UBA10353 marine group
Actinobacteria
Bacteroidetes
Cyanobacteria
Planctomycetes
Alphaproteobacteria
Gammaproteobacteria
S12-A S12-B S12-C S9-A S9-B S9-C S10-A S10-B S10-C S11-A S11-B S11-C S8-A S8-B S8-C S5-A S5-B S5-C S7-A S7-B S7-C S4-A S4-B S4-C S6-A S6-B S6-C S2-A S2-B S2-C S3-A S3-B S3-C S1-A S1-B S1-C
Phosphate concentration
Relative abundance (%)
0 10 20 30

741 **Figure 5**



742

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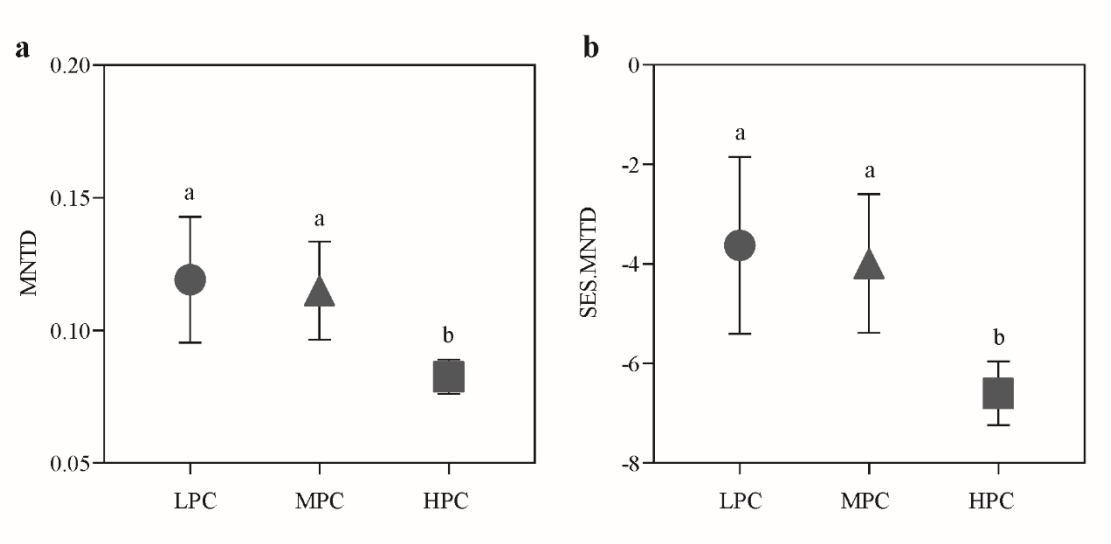

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