Spatial variation of microbial communities in sediments along the environmental gradients from Xiaoqing River to Laizhou Bay

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**A R T I C L E   I N F O**

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**A B S T R A C T**

The Laizhou Bay is famous for aquaculture, but has been subject to eutrophication and contamination for years. High concentrations of nutrients from the Xiaoqing River are considered as the main cause for significant eutrophication in the west of Laizhou Bay. Here we present results of the research on sedimentary microbial assemblages along this spatial gradient between riverine and marine environments by high-throughput sequencing. The predominant phyla/classes of bacteria and fungi clustered the samples into two distinct provinces, while discriminant taxa of each province were strongly associated with spatial factors and inorganic nitrogen. Spatial variation of bacterial communities was mainly related with the distribution of phosphates, suggesting a phosphate-limitation pattern for the bacterial communities. Alpha- and beta-diversity of fungal communities exhibited a significant correlation with water depth. We consider the distinct distributional gradients of bacterial and fungal communities partly explain the different roles in the biogeochemical processes of coastal sediment.

1. Introduction

The Laizhou Bay is an important coastal zone for aquaculture in China, and is surrounded by areas of high population growth and economic development (Zhuang and Gao, 2013). However, the long-term contamination and eutrophication have caused serious ecological destruction and continuous piscatorial decline in Laizhou Bay (Shan et al., 2000; Jin et al., 2013). Around this semi-closed area, there are > 400 chemical enterprises that manufacture over 150 kinds of chemical products (Zhuang et al., 2014). More than a dozen terrestrial runoffs bring riverine suspended matters, industrial waste waters, domestic sewage and agricultural discharges into Laizhou Bay. Large amounts of riverine input have caused high nutrient and contaminant concentrations in Laizhou Bay (Zhong et al., 2011; Zhuang and Gao, 2014; Zhuang et al., 2014). Among the rivers surrounding Laizhou Bay, both the Yellow River and Xiaoqing River are recognized as the most important. Yellow and Xiaoqing River discharge in 2009 was reported at ca. 4650 t and 1536 t of nutrients, respectively, into Laizhou Bay (Wang et al., 2014a). The Xiaoqing River was considered the major source of contamination and eutrophication in western Laizhou Bay, due to smaller runoff volume but higher nutrient and contaminant concentrations compared the Yellow River (Shan et al., 2000; Ma et al., 2004). Zhang et al. (2007) reported that Xiaoqing River waters were extremely eutrophicated in the summer of 2005. Due to the discharge of Xiaoqing River, the measured concentrations of inorganic nitrogen (IN) and inorganic phosphate (IP) were significantly higher in the western than eastern Laizhou Bay (Xia et al., 2009; Liu et al., 2011). Actually, the IN concentrations were very high in the whole Laizhou Bay, whereas phosphorus was relatively low and potentially a limiting factor in this area (Hao et al., 2004; Sun et al., 2006). The lopsided discharge of IN and IP resulted in a dramatic increase of IN/IP ratio in May (the main spawning period of most fishery species) during 1959–2008, which strongly impacted primary and fishery production in Laizhou Bay (Jin et al., 2013). The average N/P ratio reached even 164 during 2006–2009 (Liu et al., 2011), much higher than Redfield’s ratio of 16 (Redfield, 1958). In Laizhou Bay, riverine input was reported to be the main source of IN, while the active phosphate was from riverine input in addition to decomposition and mineralization of organic matter (Xia et al., 2009). Generally, multiple factors could shape spatial and physicochemical gradients (such as temperature, salinity and nutrients) from Xiaoqing River to Laizhou Bay.

Living organisms require the major elements (e.g. carbon, nitrogen, oxygen, and phosphorus) for survival and reproduction, and therefore are involved in the global biogeochemical processes (Grob et al., 2013). Among all living organisms, microbes are recognized as significant engines that drive the cycles of elements (Falkowski et al., 2008). To
understand the ecological function of microorganisms, the composition and distribution patterns of microbial communities must be clarified. In the past two decades, the application of molecular methods has allowed recent studies to reveal the microbial diversity missed by earlier culture-dependent approaches, and provides insight into the mechanisms that drive microbial community organizational changes over space and time (Pommier et al., 2005; van der Gast, 2013; Wang et al., 2015a). According to Martiny et al. (2006), microorganism biogeography was considered to be determined by major factors of contemporary environmental conditions and historical contingencies that result in dispersal limitation. Whereas the relative influence of environmental factors and dispersal limitation on microbial diversity likely depends on spatial scale (Martiny et al., 2011). At the intermediate scales (10–3000 km), both environmental conditions and spatial factors (representing dispersal limitation) were observed to have significant effects on biogeographic variation of microbial communities (Martiny et al., 2006).

Although the relative contribution of environmental conditions and spatial factors is still not clear in connective waters (at intermediate scales), various biotic and abiotic factors have been reported to affect abundance and diversity of microbial communities (Wang et al., 2016). In northern Chinese coastal seas, temperature and dissolved oxygen were reported as significant environmental factors in determining the sedimentary bacterial communities (Liu et al., 2015). The study performed in Bohai Bay showed that sediment texture, water depth, dissolved oxygen, total nitrogen and polycyclic aromatic hydrocarbons (PAHs) regulated the composition of sedimentary bacterial communities (Wang et al., 2015b). However, spatial distribution of microeukaryotes may be driven by different factors. In Bohai and Yellow Seas, microeukaryote biodiversity in surficial sediments were reported to be shaped by water depth, indicating a depth-decay pattern of benthic microeukaryotes (Gong et al., 2015). By contrast, little is known about the biogeography of sedimentary microbes in Laizhou Bay. Here we were inspired to study the spatial distribution of bacterial and fungal communities in sediments along the environmental gradients from Xaoqing River to Laizhou Bay by high-throughput sequencing. Several questions need to be addressed here: (i) Do the dominant taxa of microbial communities vary along the gradients from Xaoqing River to Laizhou Bay? (ii) Do the IN and IP affect the biodiversity of microbial communities in different ways? (iii) Do the spatial factors (geographic distance and depth) contribute more than the environmental factors to the variation of microbial communities at this spatial scale?

2. Materials and methods

2.1. Sites description, samples collection and environmental parameters determination

The Laizhou Bay has a relatively flat and shallow seafloor, with an average depth < 10 m. Since the Laizhou Bay receives high concentrations of nutrients from the Xaoqing River in May, it may form multiple physicochemical gradients from the estuary towards the sea. In addition, the Xaoqing River reaches the lowest annual runoff quantities in April and May, which allows convenient collection of sediments on the riverbed. Thus, nine sites with an average interval of 20 km were determined along the gradient from Xaoqing River to Laizhou Bay (Fig. 1).

The surface sediment samples (top 5 cm) from Laizhou Bay were collected by a stainless steel grab sampler in May 2011, and sediments were subsampled using a 5 mL sterile syringe. Surficial sediments of the Xaoqing River were directly sampled on the riverbed (with water depth 0.1 m) during April 2012. From each site, ten cores of surficial sediments (5 mL) were collected, homogenized, and subdivided for molecular analyses (10 mL) and chemistry determination (40 mL). Subsequently, sedimentary samples were stored in sterile tubes at −20 °C for later analyses.

Environmental parameters were measured as previously described (Li et al., 2013). Temperature, pH, and salinity for the water overlaying surface sediments were recorded in situ with an YSI model 556MPS. Pore-water was extracted by centrifugation at 5000 × g for 10 min. Dissolved inorganic nutrients, including nitrate, nitrite, ammonium and phosphate were measured by a nutrient flow analyzer (Seal, Germany). Total organic carbon (TOC) and total nitrogen (TN) of sediments were determined with a Vario Micro Elemental Analyzer (Elementar, Germany).

2.2. DNA extraction, PCR amplification and Illumina MiSeq sequencing

The total DNA in the sediments was extracted using the FastDNA SPIN Kit for soil (MP BIO, USA) according to the manufacturer’s instruction. The concentration and quality of the DNA extracts were determined by a NanoDrop 2000c spectrophotometer (ThermoFisher, USA) and agarose gel electrophoresis. The V4-V5 region of 16S rRNA genes was amplified using the primer pair 515F (5′-GTG CCA GCC GCG GCC GGT A-3′) and 907R (5′-CCG TCA ATT CCT TTG AGT TT-3′), with a unique 6 nt barcode attached at the end of the forward primer. The PCRs were carried out in triplicate by ABI GeneAmp 9700, using TransGen AP221–02 (TransStart Fastpfu DNA Polymerase) with 20 ng of purified DNA as a template. The PCR cycling started with pre-denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and elongation at 72 °C for 40 s, with a final extension at 72 °C for 7 min. The fungal 18S rRNA genes were amplified in the same way but with fungal primers ITS1-1737F (5′-AGT AAG TGC GGT TCA ATT CCT TTG AGT TT-3′), with a unique 6 nt barcode attached at the end of the forward primer. The PCR products were purified by agarose gel electrophoresis with an AxyPrep DNA Gel Extraction Kit (Axygen, USA), and then quantified with a QuantiFluor-ST Flurometer (Promega, USA). High-throughput sequencing was performed at Majorbio Co., Ltd. (Shanghai, China) using the 250-bp PE Illumina MiSeq sequencing platform. The sequence data of this study were deposited in the Sequence Read Archive of NCBI (https://www.ncbi.nlm.nih.gov/) and are available under accession number SRP095662.

2.3. Data processing and statistical analysis

Raw FASTQ files were processed with QIIME as previously described by Caporaso et al. (2010b). Briefly, the joined-pair reads were filtered with the criteria of Phred quality score (Q) 20 and 75% (per single read length) consecutive high-quality base calls (Bokulich et al.,
3. Results

3.1. Environmental conditions and spatial factors

As expected, physicochemical parameters of nine samples, including 4 samples from Xiaoqing River and 5 samples from Laizhou Bay, showed distinctive characteristics of fresh water and marine environments (Table S1). The Laizhou Bay had deeper waters (2.8–15.2 vs. 0.1 m, P < 0.01), higher pH (8.01–8.14 vs. 7.54–7.74, P < 0.05) and salinity (32.5–34.02 vs. 1.67–5.26 psu, P < 0.001), when compared with the Xiaoqing River. Besides, Pearson’s correlation analysis showed the environmental factors, including temperature, pH, salinity, ammonium and nitrite, had significant correlations with the spatial factors (longitude, latitude, depth and geographic distance) (Table 1; Table S2). The concentration of ammonia increased with longitude and latitude, while the nitrite presented an opposite tendency. Both nitrates and phosphates did not correlate with the spatial factors. Overall, the results roughly confirmed our assumption on the multiple gradients from Xiaoqing River to Laizhou Bay.

3.2. Community composition and spatial variation of benthic microbial communities

Bacterial clean sequences were dominated by thirteen phyla (using class level for the phylum Proteobacteria): Gamma-proteobacteria (28.6%), Bacteroidetes (21.3%), Delta-proteobacteria (8.5%), Acidobacteria (7.2%), Alpha-proteobacteria (6.3%), Beta-proteobacteria (5.0%), Chloroflexi (4.6%), Firmicutes (4.5%), Actinobacteria (3.6%), Planctomycetes (3.2%), Gemmatimonadetes (2.2%), Nitrospirae (1.3%) and Chlorobi (1.0%). About 96.8% of fungal sequences can be classified into seven phyla, including Basidiomycota (55.2%), Ascomycota (23.2%), Entomophthoromycota (7.5%), Chytridiomycota (6.3%), Glomeromycota (2.40%), Zygomycota (2.20%) and Cryptomycota (0.10%). The abundant phyla/classes could cluster the samples into one group for Laizhou Bay (E1, D2, C3 and B4) and two groups for Xiaoqing River (X1 and X2, X3 and X4), indicating two distinctive provinces of microbial communities (Fig. 2). The results of LDA (Fig. 3 and Dataset S1) showed that discriminant taxa in the Laizhou Bay group were associated with bacterial taxa Planctomycetes (including family Pirellulaceae), Deltaproteobacteria (including families Desulfovibulaceae and Desulfuromonadaceae, genus Desulfoococcus) and WS3, families koli13, Flavomycoviraceae, Bacillaceae, Rhodobacteraceae, OM60, Marinillicaceae and Piscirickettsiaceae, and genus Lactococcus. For as the fungal taxa, the Laizhou Bay group was dominated by Eurotiumomyces (including genus Aspergillus), Saccharomyces, Malasseziomycetes (including genus Malassezia), Tremellomyces (including genus Trichosporon), Zygomycota (including genera Lichtheimia and Rhizopus). Dominant bacteria in the Xiaoqing River groups were related to the bacterial taxa Betaproteobacteria (including family Comamonadaceae and genus Thiobacillus), families Nocardioidaceae, Cyclobacteriaceae, Cytophagaceae, Sphingomonadaceae, Alteromonadaceae, Sinobacteria and Xanthomonadaceae. Additionally, abundant fungi in the Xiaoqing River groups were associated with the fungal family Chaetomiaceae, and genera Amanita, Fusulin, Sistotrema, Aurantiporus and Rhizophydium. These bacterial and fungal discriminant taxa from sediments of Laizhou Bay and Xiaoqing River can be well clustered by correlation coefficients with spatial and environmental parameters (Fig. 4). Generally, most of the discriminant taxa from Laizhou Bay and Xiaoqing River exhibited significant positive/negative correlations with longitude, latitude, depth, salinity, ammonium and nitrite (|ρ| > 0.5, P ≤ 0.05); some taxa had significant correlations with temperature, pH, nitrate, phosphate, TN and TOC (|ρ| > 0.5, P ≤ 0.05), but IN/IP were not significantly correlated with any discriminant taxa (P > 0.14; Fig. 4 and Dataset S2). The other dominant taxa, which were ubiquitous in the study area, exhibited similar results (Dataset S4).

3.3. Biodiversity of microbial community associated with the spatial and environmental factors

Bacterial alpha-diversity indexes, including observed OTUs, Shannon-Wiener index and Chao1, did not show significant correlations with the spatial and environmental parameters (P > 0.09, Table S3). For fungi, depth had a strong negative correlation with observed OTUs and Chao1 (ρ < −0.5, P ≤ 0.05), while Chao1 was also significantly correlated with latitude, longitude and salinity (|ρ| > 0.5, P ≤ 0.05).

The PCoA plots (Fig. 5) showed the bacterial and fungal communities exhibited similar clusters but different gradients. The distinction of microbial habitats between Laizhou Bay and Xiaoqing River provided the main explanation for the similar clustering of bacterial and fungal communities. The first two principal coordinates PC1 and PC2 of bacterial PCoA explained 50.05% and 26.03% of total variation. In comparison, the variation of fungal communities was explained less by PC1 and PC2, accounting for 27.50% and 17.05% of total variation. Mantel and partial Mantel tests were used to further explore the effects of geographic distance, depth and environmental variables on
the microbial communities (Table 2 and Table S2). The beta-diversity of the bacterial community based on weighted UniFrac distance was significantly correlated with salinity ($\rho = 0.546$, $P = 0.012$) and phosphate ($\rho = 0.603$, $P = 0.007$). However, the partial Mantel tests showed that the correlation between salinity and bacterial beta-diversity became weak ($|\rho| < 0.5$), indicating a partly dependence on depth ($\rho = 0.416$, $P = 0.024$, Table 2) and geographical distance ($\rho = 0.491$, $P = 0.014$, Table S2). Among all the environmental factors, phosphate had the most important influence on bacterial communities, which was almost immune to the affection from depth ($\rho = 0.601$, $P = 0.009$, Table 2) and geographical distance ($\rho = 0.622$, $P = 0.006$, Table S2). Although the effect of geographical distance on the bacterial beta-diversity appeared insignificant in the Mantel tests ($\rho = 0.322$, $P = 0.059$), correlation was stronger and significant ($\rho = 0.339$, $P = 0.050$; Table 2) after the environmental condition was controlled in the partial Mantel tests. Besides, depth had weak correlations with the bacterial beta-diversity in Mantel ($\rho = 0.398$, $P = 0.022$) and partial Mantel tests (when the environmental condition was controlled, $\rho = 0.429$, $P = 0.031$), while correlation was insignificant when the geographical distance was controlled in the partial Mantel tests ($\rho = 0.252$, $P = 0.077$). By contrast, the beta-diversity of fungal communities based on Bray-Curtis distance had significant correlations with depth in both the Mantel and partial Mantel tests ($P \leq 0.05$). Geographic distance, salinity and pH were also significantly correlated with the fungal beta-diversity ($P < 0.02$, Mantel tests in Table 2), but presented a strong dependence on depth ($P > 0.05$; partial Mantel tests in Table 2). This indicates that depth was the dominant factor impacting the beta-diversity of benthic fungi, overwhelming the effects of geographic distance and environmental conditions.

Since phosphate was not significantly correlated with most of the discriminant bacterial taxa (Dataset S2) but had a strong correlation with bacterial beta-diversity (Table 2), we performed Spearman correlation calculation among all bacterial taxa and then found the components associated with phosphate (Dataset S3). As previously reported, the bacterial taxa Acidimicrobiobla (Jeffries et al., 2015), Cyanobacteria (Janssen et al., 2004), Bacillaceae (Mandic-Mulec et al., 2009), and Opitutus (Lindstrom, 2004) showed strong associations with the biogeochemical processes of the elemental Phosphorus.

The heatmap shows the relative abundance of predominant phyla/classes with color legend. These dominant taxa of bacteria and fungi (relative abundance $> 1\%$ at least in one sample) cluster the sampling sites into distinct provinces. The phyla Proteobacteria, Ascomycota and Basidiomycota are shown at class level.
4. Discussion

4.1. Composition of the microbial communities varied along the gradients from the Xiaoqing River to the Laizhou Bay

The analysis of the environmental factors showed some physicochemical parameters were highly controlled by the spatial factors, shaping the spatial gradients of temperature, pH, salinity, ammonia and nitrite from Xiaoqing River to Laizhou Bay (Table 1; Table S2). Under such environmental conditions, the dominant taxa of bacterial and fungal communities distinguished the province of Xiaoqing River from that of Laizhou Bay (Fig. 2).

Bacterial communities in the Xiaoqing River were characterized by taxa that are closely related with the terrestrial source. For instance, members of beta-Proteobacteria (including the family Comamonadaceae) are commonly retrieved from the eutrophicated freshwater ecosystems, and this phylum was previously reported as having a negative relationship with salinity (Hanson et al., 2014; Wang et al., 2015a). Most genera of the other discriminant taxa, like families Sphingomonadaceae, Nocardioidaceae, Cyclobacteriaceae, Alteromonadaceae and Cytophagaceae, are chemooorganotrophic and aerobic (Glaeser and Kämpfer, 2014; López-Pérez and Rodríguez-Valera, 2014; McBride et al., 2014; Pinnaka and Tanuku, 2014; Tóth and Borsodi, 2014). The presence of these taxa in Xiaoqing River samples might be due to the rich nutrients and shallow water depth (0.1 m). The family Cyclobacteriaceae has the functional groups of nitrate reduction (Pinnaka and Tanuku, 2014). Ecologically, the family Alteromonadaceae is often associated to nutrient-rich environments (López-Pérez and Rodríguez-Valera, 2014). Species of the family Sinobacteraceae mainly occur in soil and freshwater (Zhou et al., 2014). As for the fungal taxa, the family Chaetomiaceae, the genera Rhizophydium, Amanita, Sistotrema, Fistulina and Aurantiporus are typical terrestrial fungi. Members of the Chaetomiaceae are recognized for production of carbohydrate degrading enzymes and antibiotics (Hutchinson et al., 2016). The genus Rhizophydiun primarily occurs as parasites of algae in aquatic systems, while as saprotrophs of pollen in soils (Letcher et al., 2006).

In Laizhou Bay, the families Desulfobulbaceae and Desulfuromonadaceae (including the genus Desulfococcus) were found widely in anoxic environments, playing important roles in the degradation of organic matter and the sulfur cycles (Greene, 2014; Kuever, 2014). The family Rhodobacteraceae frequently thrives in marine environment, and is deeply involved in sulfur and carbon biogeochemical cycles (Youssef and Elshahed, 2014). The family Piscirickettsiaceae (such as Piscirickettsia) and the genus Lactococcus (Streptococcaceae) includes important fish pathogens (Lory, 2014; Marshall et al., 2014), and may be present due to fishery and aquaculture in Laizhou Bay. The
Fig. 4. Correlations between relative abundance of discriminant taxa (in Fig. 3) and environmental factors are shown in this heatmap. Hierarchical clustering was performed based on the Spearman's correlation coefficients (\( \rho \)). The phyla Proteobacteria, Ascomycota and Basidiomycota are shown at class level. TN, total nitrogen; TOC, total organic carbon; IN/IP, ratio of inorganic nitrogen to inorganic phosphorus.

Fig. 5. Principal coordinates analysis (PCoA) presents the spatial variations of bacterial (a) and fungal communities (b) according to the weighted UniFrac distance and Bray-Curtis distance matrices. Samples from the Xiaoqing River are labeled with blue squares, while red dots represent the samples from the Laizhou Bay. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
family Bacillaceae performs fundamental roles in the cycling of organic matter and phosphate solubilization (Mandic-Mulec et al., 2015). The clade OM60 was found positively correlated with salinity, which is consistent with a previous study (Wang et al., 2016). Besides, we found the OM60 clade positively correlated with salinity, which is not only the non-random distribution of microbial composition, but also the spatial gradients of microbial communities.

4.2. Inorganic nitrogen and phosphate determine the composition and diversity of bacterial communities

Our results show that a large portion of dominant taxa correlated with the chemical parameters, ammonium and nitrite, which meanwhile varied along the spatial gradients (Dataset S2 and S4; Table 1). Thus, we consider that the variation of microbial composition coupling with ammonium and nitrite is spatially structured. For instance, the Cyclobacteriaceae lineage had a negative correlation with spatial gradients (Fig. 4). These results indicated not only the non-random distribution of microbial composition, but also the spatial gradients of microbial communities.

4.3. Depth shape the alpha- and beta-diversity of fungal communities

Our results demonstrate a negative correlation between depth and alpha-diversity of benthic fungi (Table S3). Furthermore, Mantel and partial Mantel test show that dissimilarity of fungal communities was significantly affected by geographic distance, depth, and salinity, while phosphate was the most important factor that impacted the beta-diversity (Table 2). This is consistent with our assumption and previous reports (Hao et al., 2004; Sun et al., 2006) that phosphate is the limiting factor of microbial communities in the study area. According to these observations, concentrations of phosphates did not present a spatial gradient (Table 1), but had a higher mean value in Laizhou Bay (3.75 μmol L⁻¹ vs. 3.85 μmol L⁻¹; Table S1). This supports the previous opinion that the active phosphates in Laizhou Bay was mainly originate from the decomposition and mineralization of organic matters in sediments (Xia et al., 2009). Detrital apatite and other inorganic Phosphorus compounds (De–P) account for 55.0–83.6% of total Phosphorus contents in the sediments of Laizhou Bay (Zhuang et al., 2014). Accordingly, we found that the dominant family Bacillaceae and the fungal genus Aspergillus, which dominated in Laizhou Bay (Fig. 4), have the capability of phosphate solubilization. The other bacterial taxa Acidimicrobiaceae, Cyanobacteria, Lactococcus, Stenotrophomonas, and Opitutus also had strong associations with phosphate variability (Dataset S3) (Hoefnagel et al., 2002; Janssen et al., 2004; Lindstrom, 2004; Xiao et al., 2009; Jeffries et al., 2015). This might suggest that bacteria undertake the major responsibility of processing phosphates in the study area.

Table 2
Mantel and partial Mantel tests demonstrate the correlations between depth, geographic distance, environmental condition, individual environmental factors and beta-diversity. The variations of depth, environmental condition and each environmental factor were calculated as Euclidean distance. Significant correlations with $|r| \geq 0.5$ and $P \leq 0.05$ are highlighted with bold type.

| Controlled factor | Bacteria | Fungi |
|-------------------|----------|-------|
| Depth             | $p = 0.398$ | $0.022$ | $0.639$ | $0.005$ |
| Geographic distance | $0.322$ | $0.059$ | $0.501$ | $0.008$ |
| pH                | $0.274$ | $0.059$ | $0.607$ | $0.009$ |
| NH$_4$-N          | $-0.049$ | $0.463$ | $0.214$ | $0.089$ |
| NO$_2$-N          | $0.254$ | $0.172$ | $0.213$ | $0.095$ |
| PO$_4$-P          | $0.063$ | $0.007$ | $-0.081$ | $0.632$ |
| TN                | $-0.026$ | $0.439$ | $0.041$ | $0.322$ |
| IN/IP             | $-0.088$ | $0.560$ | $-0.091$ | $0.654$ |

| Partial Mantel |
|----------------|
| Bacteria | Fungi |
| Controlled factor | $p$ | $P$ | $p$ | $P$ |
| Environmental condition | $0.429$ | $0.031$ | $0.629$ | $0.005$ |
| Geographic distance | $0.252$ | $0.077$ | $0.474$ | $0.023$ |
| Environmental condition | $0.339$ | $0.050$ | $0.490$ | $0.019$ |
| Depth | $-0.054$ | $0.538$ | $-0.143$ | $0.771$ |
| Geographic distance | $-0.121$ | $0.608$ | $0.076$ | $0.303$ |
| Depth | $-0.181$ | $0.729$ | $-0.024$ | $0.519$ |
| Depth | $0.280$ | $0.906$ | $-0.113$ | $0.731$ |
| Depth | $0.416$ | $0.024$ | $0.083$ | $0.262$ |
| Depth | $-0.081$ | $0.649$ | $0.209$ | $0.103$ |
| Depth | $-0.182$ | $0.736$ | $0.047$ | $0.359$ |
| Depth | $0.228$ | $0.190$ | $0.183$ | $0.161$ |
| Depth | $-0.045$ | $0.474$ | $0.278$ | $0.078$ |
| Depth | $0.601$ | $0.009$ | $-0.229$ | $0.915$ |
| Depth | $-0.101$ | $0.628$ | $-0.082$ | $0.642$ |
| Depth | $-0.063$ | $0.493$ | $-0.055$ | $0.623$ |

TN, total nitrogen; IN/IP, ratio of inorganic nitrogen to inorganic phosphorus; the parameter TOC was excluded in this table because of incomplete data.
communities was not limited by the inorganic nutrients. In such eutrophic conditions, dominant taxa of bacteria and fungi were mostly regulated by IN (Fig. 4; Dataset S2), but fungi might not suffer the nutrients pressure as much as bacteria do at the community level. Therefore, the fungal communities formed a distinct biogeographic pattern that was mainly related to the spatial factors.

Water depth appears to be a good proxy for many physicochemical factors in coastal seas. When water depth increases, temperature and pH decrease, while salinity and trace metals increase (Gong et al., 2015). More importantly, sediments represent a sink for particulate organic matter subject to sedimentation from the productive surface waters. Fungi perform very important biogeochemical functions in marine ecosystems, such as decomposing detrital organic matters (Wang et al., 2014b). In coastal zone, the shallower seafloor receives more light and oxygen for primary producers, and more organic matters from the surface water, which means more substrate for benthic fungi. This may explain why the richness and diversity of fungi decreased with the increased depth (Table S3). However, our environmental parameters related to the organic matter are incomplete. In addition, sediment samples of each site were pooled together and homogenized prior to DNA extraction. Therefore, our results indicate a general state of microbial communities but leave the sample variation within one site unknown (Prosser, 2010). Thus, further researches will be very necessary to verify the speculation and to unveil more details on the biogeography of benthic microbes.

5. Conclusions

In the presented study, the bacterial and fungal discriminant taxa exhibited limited distributions in Xaqing River and Laizhou Bay, while the others presented a ubiquitous distribution. Our results support the moderate endemism model for microbes (van der Gast, 2013; van der Gast, 2015). Both spatial and environmental factors significantly affected the composition and biodiversity of benthic microbes. Inorganic nitrogen regulated the major composition of microbial communities in the sediments, while phosphates controlled the beta-diversity of bacterial communities. The fungal communities presented a depth-decay distribution. Geographic distance enhanced the beta-diversity of bacterial communities. The fungal communities presented distinct geographic patterns which might be partly explained by their different function in biogeochemical processes.

However, determination of the specific mechanisms require more than the study of spring samples. To unveil more details, further study is necessary to integrate time-series data and more complete environmental profile.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.marpolbul.2017.04.059.

Conflict of interest

The authors declare no conflict of interest.

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