

Benthic bacterial communities indicate anthropogenic activity footprints in coastal area under long-term marine spatial planning practice

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Abstract

Marine spatial planning (MSP) is designed to divide the sea area into different types of functional zones, to implement corresponding development activities. However, the long-term impacts of anthropogenic activities associated with MSP practice on the marine microbial biosphere are still unclear. Yalu River Estuary, a coastal region in northeast of China, has been divided into fishery & agricultural (F&A) zone, shipping & port (S&P) zone and marine protected area (MPA) zone by a local MSP guideline that has been run for decades. To examine the effects of long-term executed MSP, benthic bacterial communities from different MSP zones were obtained and compared in this study. The results revealed significant differences in the bacterial community structure and predict functions among different zones. Bacterial genera enriched in different zones were identified, including SBR1031 in MPA, *Woeseia* and Sva0996 in S&P, and *Halioglobus* in F&A. In addition, correlations between some bacterial genera and sediment pollutants were uncovered. Furthermore, bacteria related to sulphide production were more abundant in the F&A zone, which was according to the accumulation of sulphides in this area. Moreover, bacteria associated with chemoheterotrophy and fermentation were more predominant in the S&P zone, consistent with high levels of organic matter and petroleum caused by shipping. Our findings indicated benthic bacterial communities could bring to light the anthropogenic activity footprints by different activities induced by long-term MSP practice.

Key words: bacterial community, structure, predicted function, marine spatial planning, anthropogenic activity

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1 Introduction

Coastal areas are the interface between land and sea, and they are globally characterised by rapid urbanisation and development. Intensive human activities in these areas have given rise to competing requirements for limited marine spatial resources (Tuda et al., 2014). Consequently, many countries are making efforts to manage conflicts between marine spatial resource uses and curb ecosystem degradation (Jayanthi et al., 2019; Maiolo et al., 2020; Wang et al., 2020). However, traditional fragmented sectoral marine administration cannot tackle the ever-increasing issues in marine spatial resource management (Fang et al., 2019). Recently, there has been renewed interest in marine spatial planning (MSP), which could help address complex conflicts in coastal areas (Ansong et al., 2017; Tuda et al., 2014; Yates et al., 2015). Most previous work has focused on the best way to develop MSP in different backgrounds (Calado et al., 2010; Douvere, 2008; Picone et al., 2017), and some studies have evaluated how effective an existing MSP scheme is operating (Carneiro, 2013; Fang et al., 2019). However, few studies have reported on monitoring and evaluating the performance and effectiveness of MSP (Douvere and Ehler, 2011) on the environmental protection, especially from an ecological quality perspective. Marine function-

al zones (MFZs) divided by MSP are generally suffered by completely different anthropogenic activities, such as fishery area is mainly engaged in mariculture and fishing, shipping and industrial zone is mainly engaged in transportation and exploitation, etc. Comprehensive monitoring and evaluation can provide essential information to promote understanding and improve planning and decision-making in adaptive MSP management (Carneiro, 2013).

From the viewpoint of environmental administration, efforts to comprehend the anthropogenic impacts on the health status of coastal areas are essential. However, measuring contamination gradients poses a great challenge in such highly complex and dynamic ecosystems. In most authorities around the world, the implementation of ecological quality monitoring programs relies on the development of appropriate bioindicators (Gadzala-Kopciuch et al., 2004; Asif et al., 2018). Benthic biological communities have specific characteristics including wide distribution, limited mobility, and sensitivity to environmental disturbance, which make them one of the ideal indicators for ecosystem health status (Vassallo et al., 2006; Villnäs and Norkko, 2011). Compared with other biological groups, benthic communities can accurately provide information on site-specific habitat con-

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ditions that integrate multiple disturbances over time (Veríssimo et al., 2012; Simboura et al., 2007). Bacteria are critical to maintaining the ecological functions of marine ecosystems, since they are required to balance both material and energy cycles (Lu et al., 2020; Pita et al., 2018). Human disturbance from marine development is affecting the structures of coastal ecosystems and their associated ecosystem services (Granek et al., 2010; Liu et al., 2021). The development of molecular methods has revealed unprecedented diversity of bacteria with versatile functional groups and metabolic pathways, which provides potential for deeper comprehension of the anthropogenic activity footprints underlying the ecological quality deterioration (Guo et al., 2019; Jeffries et al., 2016; Liu et al., 2015a).

The present study analysed spatial variation in benthic bacterial communities in three MFZs in the Yalu River Estuary. This region has been divided into several different MFZs (Fig. 1a) including fishery & agricultural (F&A) zone, shipping & port (S&P) zone and marine protected area (MPA) zone under a guidance of the local MSP practice for several years. We predicted that differences in human activities in different MFZs will exert different environmental pressures and thereby shape the bacterial communities to form distinct anthropogenic activity footprints. To explore this hypothesis, we collected 15 sediments from different MFZs in the Yalu River Estuary, and benthic bacterial communities were determined by high-throughput sequencing. Differences of benthic bacterial communities between MFZs and their relationships with environmental conditions were investigated. The results will deepen our understanding of the mechanisms underpinning benthic bacterial community differentiation in relation to specific exploitation of the marine spatial resources. The findings may benefit marine biodiversity conservation in other areas experiencing rapid coastal development.

2 Materials and methods

2.1 Study area

The Yalu River Estuary is located in a temperate region of the north western Pacific Ocean. Yalu River is known as the boundary river between China and D.P.R. Korea. The Yalu River Estuary, located to the east of Liaodong Peninsula, is a funnel-shaped

estuary covering an area of 3 435 km². There are many types of marine exploitations in the Yalu River Estuary, such as protection areas for wetland ecosystems, aquaculture, fisheries and shipping. There are over 30 docking berths within the 60 km² port area in the Yalu River Estuary, and cargo throughput was more than 56 million tons in 2019 (<https://www.mot.gov.cn/tongjishuju/gangkouhuowulvketl/>). Moreover, aquaculture and fisheries are other main human activities in the Yalu River Estuary, for which the total production value was 14.72 billion Chinese Yuan in 2013. Besides, the Yalu River wetlands are an important stopover site for migratory shorebirds along the East Asian-Australasian Flyway, which historically supported more than 100 000 Bar-tailed godwits and 55 000 Great knots (Choi et al., 2015). Thus, a national marine protected area was established in the Yalu River Estuary in 1987, with the coastal wetland ecosystem and endangered species as the main conservation targets. Therefore, marine resource development and conservation are conflicting issues in the Yalu River Estuary. An MSP practice has been implemented to balance the conflicts between human activities and environmental conservation. In the Dandong marine spatial plan version 2013 (2013–2020), there are five first class marine functional areas; F&A, S&P, MPA, industrial & urban, and reserved area (Fig. 1).

2.2 Sample collection and processing

In this study, a total of 15 sampling stations were set in the coastal area of Yalu River Estuary (Fig. 1), which covered three main MFZs including F&A, S&P, and MPA, five stations for each zone. Surface sediment samples (0–5 cm) were collected using a grab sampler in September 2020 with triplicate in each sampling station. Six heavy metals in each sample, containing Hg, Pb, Cr, Pd, Cu, and Zn, were analysed using an inductively coupled plasma-mass spectrometry (ICP-MS, Optima 2000 DV, Perkin Elmer, USA) (Li et al., 2017). Total organic carbon (TOC) was achieved by using Vario EL III (Elementar, GER) with 0.25 g sample after removing inorganic carbon with 10% HCl. The concentration of petroleum was measured by gas chromatography according to a previous report (Nasrollahzadeh et al., 2010). Sulfide content in sediments was detected using methylene blue spectrophotometry (Silva et al., 2001).

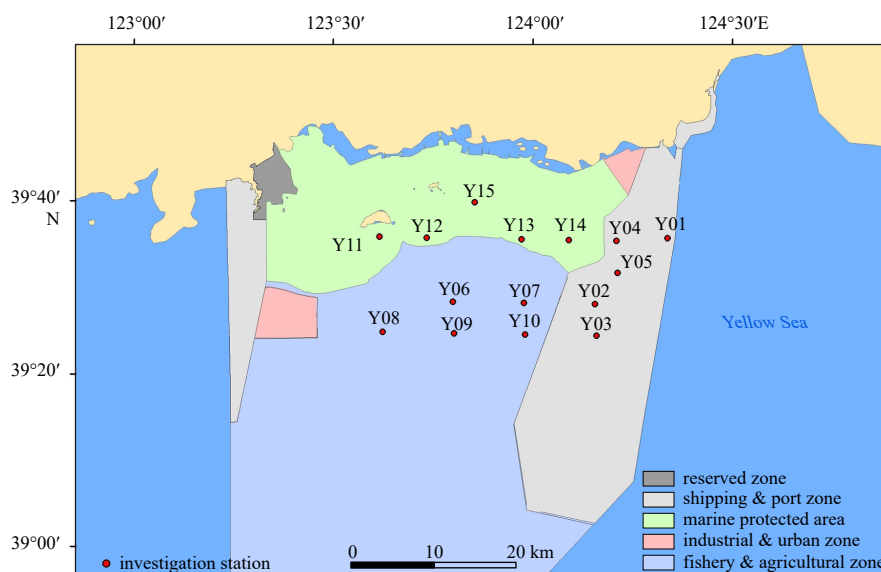


Fig. 1. Sampling stations of this study.

2.3 DNA extraction and bacterial community sequencing

Total DNA from each sediment sample was extracted using the FastDNA SPIN, Kit for Soil (MP Biomedicals, CA) according to the manufacturer's instruction. The concentration and integrity of extracted DNA were determined using NanoDrop, one microvolume ultraviolet-visible spectrophotometer (Thermo Fisher Scientific, Carlsbad, USA), and 2% agarose gel electrophoresis, respectively. All DNA were stored at -20°C for further PCR amplification.

The V3-V4 regions of bacterial 16S rRNA from each sample were amplified using primers 341F-806R (341F: ACTCCTACGG-GAGGCAGCAG, 806R: GGACTACHVGGGTWTCTAAT) (Berg et al., 2012). All PCRs were carried out in 15 μL reactions with 7.5 μL Phusion[®] High-Fidelity PCR Master Mix (New England Biolabs), 1 μL of dNTPs (2.5 mmol/L), 1 μL of forward and reverse primers (10 $\mu\text{mol/L}$), and 1 μL template DNA. Thermal cycling consisted of initial denaturation at 95°C for 2 min, followed by 25 cycles of denaturation at 94°C for 5 s, annealing at 55°C for 30 s, and elongation at 72°C for 30 s. The PCR products of each sample were detected by electrophoresis on 1.5% agarose gel, and all samples were successfully amplified. Then, the obtained PCR products were purified, quantified, and mixed in equal amounts, respectively, to construct the sequencing libraries. Finally, the library quality was determined using Agilent Bioanalyzer 2100 system and Qubit 2.0 Fluorometer (Thermo Scientific), and then all libraries were sequenced on an Illumina NovaSeq 6000 platform.

2.4 Bioinformatic analysis

Reads with an average phred score lower than 30, containing ambiguous bases, homopolymer greater than 6, having mismatches in primers, and sequence length shorter than 150 bp were deleted from the datasets (Bokulich et al., 2013). Remaining high-quality reads were appointed to the samples based on their unique barcodes at the end of reverse primers. Subsequently, reads with overlap longer than 10 bp and without any mismatch were assembled into tags by using FLASH (Magoč and Salzberg, 2011). These tags were then assigned to amplicon sequence variants (ASVs) with the Quantitative Insights Into Microbial Ecology 2 through the Divisive Amplicon Denoising Algorithm 2 method (Bokulich et al., 2018). Representative sequence of each ASV was picked by the default method and appointed to a bacterial taxonomy based on the SILVA release 138 database (Yilmaz et al., 2014). Singletons (number of a specific ASV = 1) were abandoned to improve the efficiency of data analysis. Finally, the bacterial ASV abundance table were normalized using a standard number of tags according to the sample with the least number of tags (36 847).

2.5 Statistical analysis

Alpha diversity indices of benthic bacterial communities, including Chao1, Shannon, Pielou_J, and Pd_faith indices, were calculated using the “vegan” package in R v4.0.2. Differences in environmental variables and bacterial alpha diversity indices among different MFZs were analysed using the Tukey's HSD test. Variations in the benthic bacterial community compositions of samples among different MFZs were evaluated by Principal Coordinate Analysis (PCoA) and Permutational Multivariate Analysis of Variance (PERMANOVA) based on the Bray-Curtis distance using the “vegan” package in R. An analysis of similarities (ANOSIM) was performed to compare within- and between-group similarity of benthic bacterial communities based on the Bray-Curtis distance (Leung et al., 2016). A Venn analysis was

performed using the “VennDiagram” package in R to evaluate the shared bacterial ASVs among different MFZs. The composition and abundance variations of shared bacterial ASVs were exhibited by pie chart and boxplot, respectively. Linear discriminant analysis Effect Size (LEfSe) (Segata et al., 2011) analysis was used to determine the potential biomarkers from bacterial communities of different MFZs. Redundancy analysis (RDA) was conducted to determine the contribution of measured pollutants to the shift of benthic bacterial communities through the “vegan” and “ggplot2” packages in R v4.0.2. Spearman's correlation was applied to assess the relationship between the concentrations of measured pollutants with the abundances of dominant bacterial phyla and genera, and the results were illustrated using the “pheatmap” package in the R v4.0.2. The ecologically relevant functions of the benthic bacterial communities were predicted using FAPROTAX software (Louca et al., 2016) and envisioned using the “ggalluvial” package in the R v4.0.2. Turkey's HSD test was also used to compare whether the total abundances of shared ASVs and main ecological functions in different MFZs were significantly different. Random forest models were constructed for classification the sample sources and identification possible biomarkers based on the relative abundances of bacterial taxa and predicted functions, respectively, using the “RandomForest” package in R v4.0.2.

3 Results

3.1 Sediment pollutant concentration profiles

In this study, the concentrations of six heavy metals in sediments were measured, but no significant differences in heavy metals were found among different MFZs (Turkey's HSD test, $p > 0.05$, Fig. 2). In contrast, significant changes in the concentration of sulphides and petroleum were observed among different MFZs (Turkey's HSD test, $p < 0.05$, Fig. 2). The highest concentrations of sulphides and petroleum were detected in the sediments from F&A and S&P zones, respectively.

3.2 Diversity and composition of benthic bacterial communities

Based on the high-throughput sequencing, a total of 875 495 sequences were obtained, ranging from 36 847 to 69 218 for each sediment. All rarefaction curves were close to the horizontal state (Fig. S1), demonstrating that the amount of sequencing data for each sample was sufficient to reflect the intact bacterial communities. These sequences were clustered into 26 136 ASVs and annotated at phylum to genus levels with 56 phyla, 132 classes, 309 orders, 472 families, and 779 genera. At the phylum level, Proteobacteria was the dominant phylum in all sediments (average relative abundance of 59.76%), followed by Actinobacteria (8.03%), Acidobacteria (7.47%), Bacteroidetes (6.68%) and Chloroflexi (5.94%; Fig. 3a).

Several alpha diversity indices, including Chao1, Pd_faith, Shannon and Pielou_J, were calculated to assess the diversity of benthic bacterial communities in the Yalu River Estuary. The highest Chao1 index was observed for sediments from the MPA, which was significantly higher than that of the S&P (Turkey's HSD test, $p < 0.05$, Fig. 3b). Meanwhile, the highest Pd_faith index was observed for sediment from the F&A, which was significantly higher than that of the S&P (Turkey's HSD test, $p < 0.05$, Fig. 3b). These results suggest that the MPA and F&A had the highest richness and evolutionary diversity of benthic bacterial communities, respectively. By contrast, higher Shannon and Pielou_J indices were also observed for the MPA, but they were not significantly different from other MFZs (Turkey's HSD test, $p > 0.05$,

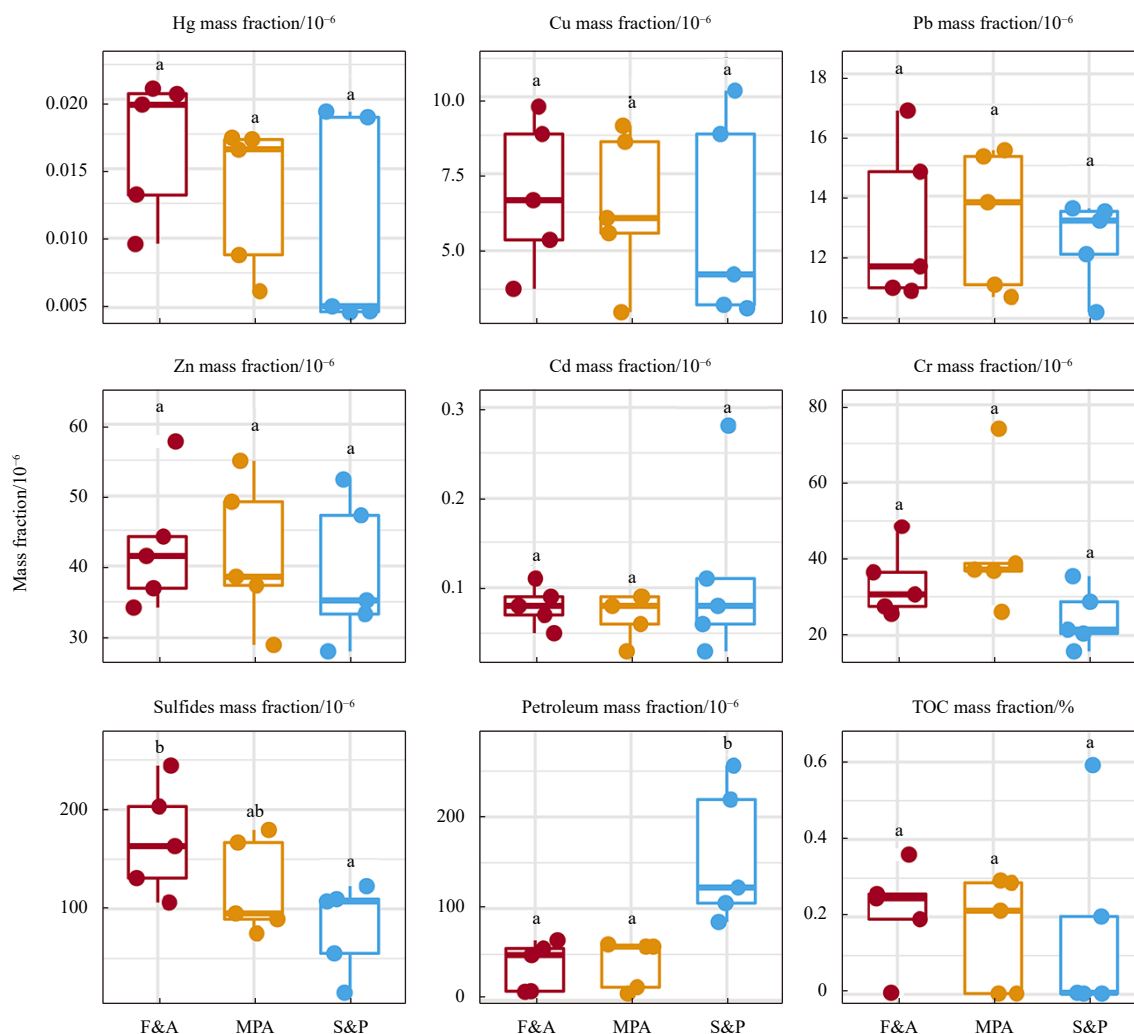


Fig. 2. Environmental factors in sediments of the Yalu River Estuary. Differences in the mass fraction of heavy metals, sulphides, petroleum, and TOC in sediments among different Marine functional zones (MFZs). Different lowercases above each box in the same subfigure represent significant differences between groups (Tukey's HSD test, $p < 0.05$). F&A: fishery & agricultural zone; S&P: shipping & port zone; MPA: marine protected zone.

Fig. 3b). These findings indicate that the diversity and evenness of benthic bacterial communities were consistent among different MFZs.

3.3 Differences in benthic bacterial communities among different MFZs

PCoA was used to evaluate variation in benthic bacterial communities among different MFZs, and the results are shown in Fig. 4a. The first two PCs completely explained 46% of the total variation in benthic bacterial communities, with 32% and 14% for PC1 and PC2, respectively. Benthic bacterial communities from different MFZs were clustered separately, as shown in the PCoA graph. Benthic bacterial communities in the F&A and S&P were quite different, but both overlapped with the MPA to some extent. Moreover, PERMANOVA revealed that benthic bacterial communities were significantly different among different MFZs ($R^2 = 0.275$, $p = 0.012$). Moreover, results of ANOSIM indicated the similarity of benthic bacterial communities within a MFZ was significantly higher than those between different MFZs (ANOSIM, $R = 0.430$, $p = 0.002$).

Venn analysis showed that 2 092 ASVs were shared among the

three different MFZs, whereas 8 217, 7 536 and 8 291 ASVs were unique in the MPA, F&A and S&P zones, respectively (Fig. S2). The taxonomy distributions of these shared ASVs were not in complete agreement with the total bacterial taxonomy composition. Although Proteobacteria was the dominant bacterial phylum between total and shared bacterial communities, the ratios of Acidobacteria and Actinobacteria were increased and decreased, respectively, in shared ASVs compared with total communities (Figs S2 and S3). The total relative abundances of these shared ASVs averaged ~65%, ~60% and ~55% for F&A, MPA and S&P, respectively (Fig. 4b). In addition, the total relative abundance of shared ASVs in the F&A was significantly higher than that in the S&P (Turkey's HSD test, $p < 0.05$, Fig. 4b). The highly unique ASVs and variation in shared ASV ratios also suggested significant variation in benthic bacterial communities among different MFZs.

To further assess differentially abundant bacteria among different MFZs, LEfSe analysis was performed (Fig. 5). The SBR1031 genus, belonging to the Anaerolineae class and the Chloroflex phylum, was enriched in the MPA compared to other MFZs. The *Woeseia* genus, belonging to the Woeseiaceae family and the

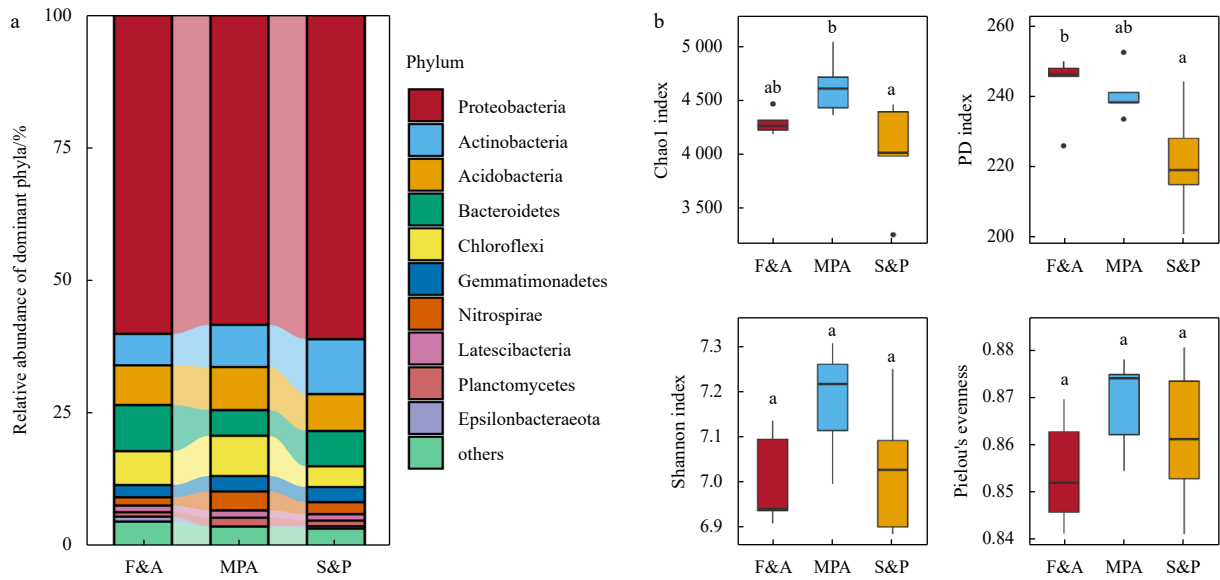


Fig. 3. Relative abundances of dominant bacterial phyla in sediments among different marine functional zones (a). Differences in alpha diversity indices of benthic bacterial communities among different marine functional zones (b). Different lowercases above each box in the same subfigure represent significant differences between groups (Tukey's HSD test, $p < 0.05$). F&A: fishery & agricultural zone; S&P: shipping & port zone; MPA: marine protected zone.

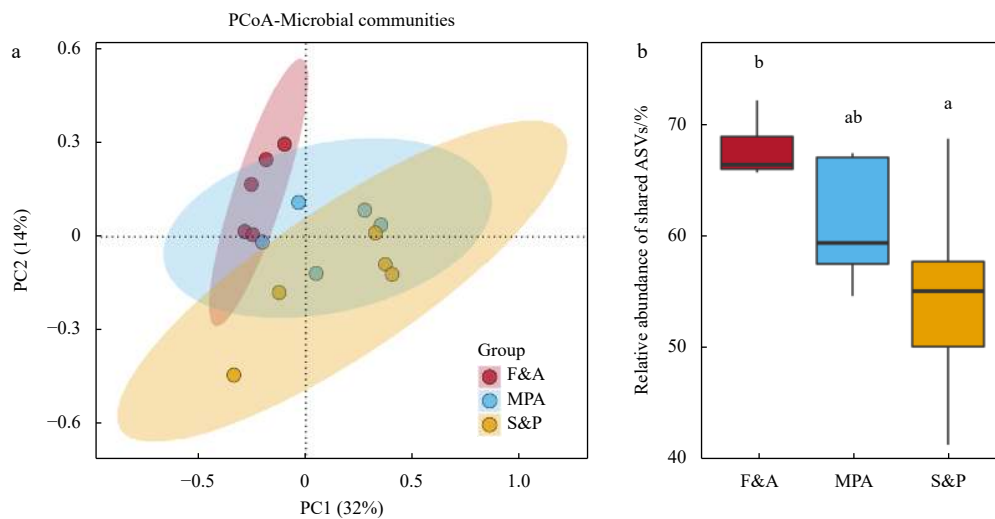


Fig. 4. Principal coordinate analysis (PCoA) of the benthic bacterial communities among different marine functional zones (a). Differences in the total relative abundances of the shared Amplicon Sequence Variants (ASVs) among different marine functional zones (b). Different lowercases above each box in the same subfigure represent significant differences between groups (Tukey's HSD test, $p < 0.05$). F&A: fishery & agricultural zone; S&P: shipping & port zone; MPA: marine protected zone.

Steroidobacterales order, and the Sva0996 marine group belonging to the Microtrichaceae family and the Microtrichales order, the Acidimicrobia class, and the Actinobacteria phylum were more abundant in the S&P than the other zones. Bacteria that were more abundant in the F&A were more diverse, including *Halioglobus* belonging to the Cellvibrionales order, *Bacteroidetes* *BD2_2* belonging to the Bacteroidetes phylum, and several members of the Deltaproteobacteria class (Desulfobulbaceae and Syntrophobacteraceae families and Sva1033 and Sva0485 genera). Besides, random forest models based on the bacterial taxa at different taxonomic levels were constructed to predict the sample sources. Among them, random forest model based on bacterial families had the highest accuracy (66.67%, Fig. 5b). Among bacterial families that were important to classify samples, some were

consistent to biomarkers recognized by LEfSe analysis, including Syntrophobacteraceae, Sva0485, and Bacteroidetes *BD2-2* (Fig. 5c).

3.4 Relationship between environmental pollutants and benthic bacterial communities

Correlations between the environmental pollutants and benthic bacterial communities were assessed by RDA. Results revealed that measured environmental pollutants explained 53.01% variations in the benthic bacterial communities (Fig. 6a). Among all measured environmental pollutants, the concentrations of Hg, Cu, and Zn significantly correlated to the benthic bacterial communities (Fig. 6a). For dominant bacterial phyla in the studied sediments, the concentrations of Hg, Cu, and Zn were

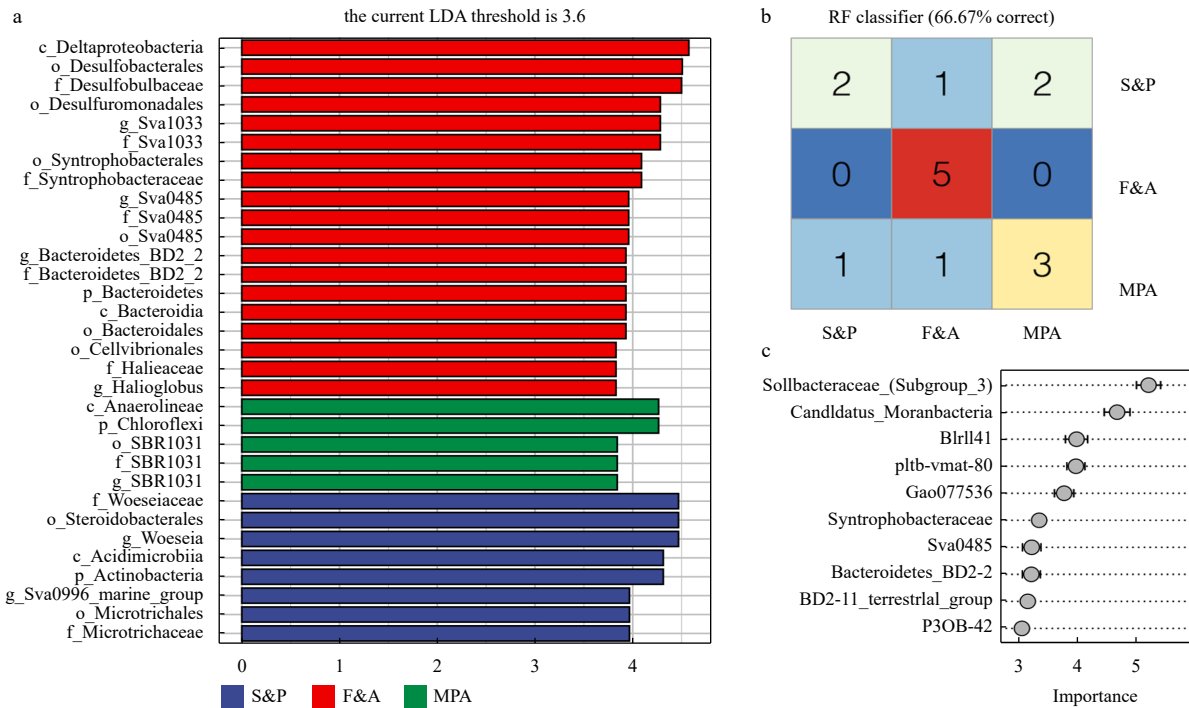


Fig. 5. The LefSe analysis results of benthic bacterial communities among different marine functional zones (a). Predicted accuracy of random forest model based on bacterial families for classification the sample sources (b). Bacterial families are ranked in descending order of importance to the accuracy (c). F&A: fishery and agricultural zone; S&P: shipping and port zone; MPA: marine protected zone; LDA: linear discriminant analysis. RF: random forest.

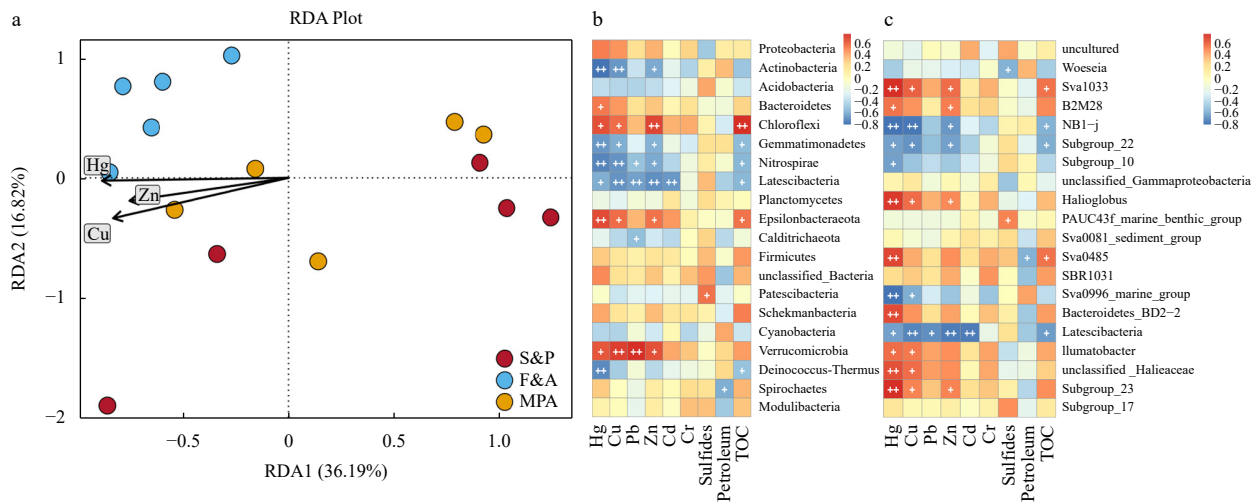


Fig. 6. The redundancy analysis (RDA) evaluated the relationship between the benthic bacterial communities and environmental pollutants (a). Heatmaps of the correlations between dominant bacterial phyla (b) and genera (c) with the concentration of environmental pollutants. + represents p -value < 0.05; ++ represents p -value < 0.01. TOC: total organic carbon; F&A: fishery & agricultural zone; S&P: shipping & port zone; MPA: marine protected zone.

positively and negatively correlated with the relative abundances of Actinobacteria and Chloroflexi, respectively (Spearman correlation, $p < 0.05$, Fig. 6b). In addition, the content of Hg was also positively correlated with the relative abundance of Bacteroidetes (Spearman correlation, $p < 0.05$, Fig. 6b). Sulphides content was positively correlated with Pastecibacteria and petroleum was negatively correlated with Spirochaetes (Spearman correlation, $p < 0.05$, Fig. 6b). For bacterial genera with differences in abundance among different MFZs, Sva1033 and *Halioglobus* were positively correlated with the concentrations of Hg, Cu, and Zn

(Spearman correlation, $p < 0.05$, Fig. 6c). Meanwhile, the Sva0996 marine group and *Latescibacteria* was negatively correlated with levels of Hg and Cu, and *Bacteroidetes DB2-2* was positively correlated with Hg (Spearman correlation, $p < 0.05$, Fig. 6c). Moreover, the PAUC43f marine benthic group and Sva0485 respectively showed positive and negative correlation to the concentrations of sulphides and petroleum (Spearman correlation, $p < 0.05$, Fig. 6c). These results suggest that different anthropogenic activities in different MFZs induced by the long-term MSP practice could affect some key bacteria in the coastal sedi-

ments.

3.5 Prediction of benthic bacterial community functions

The biogeochemical functions of benthic bacterial communities in the Yalu River Estuary were predicted by FAPROTAX. Sulphur cycling were the most dominant functional terms among benthic bacterial communities, followed by chemoheterotrophy and phototrophy-related functional terms (Fig. 7a). We tried to use random forest to discover possible functional indicators. Only 60% accuracy was obtained for random forest model based on the predicted functions for classification the marine functional zones (Fig. 7b). But functional terms related to sulphur cycling, including sulphate respiration, respiration of sulphur compounds, and anoxygenic photoautotrophy Sulphur oxidizing, were identified to be most important to distinguish sample sources

(Fig. 7c). Similar results were also found in comparison among benthic bacterial communities from different marine functional zones (Fig. 7d). Respiration of sulphur compounds and sulphate respiration were more abundant in the F&A than the S&P (Turkey's HSD test, $p < 0.05$, Fig. 7d). By contrast, the abundance of chemoheterotrophy, fermentation, and nitrification terms were significantly higher in the S&P than the F&A (Turkey's HSD test, $p < 0.05$, Fig. 7d). These results indicated that differences in marine use strongly influenced the biogeochemical functions of benthic bacterial communities in the coastal ecosystem.

4 Discussion

In the present study, six heavy metals in the sediments of Yalu River Estuary were detected. The concentration ranges of heavy metals were as follows: Hg, 0.005 1–0.019 6 mg/kg; Cu, 3–10.2

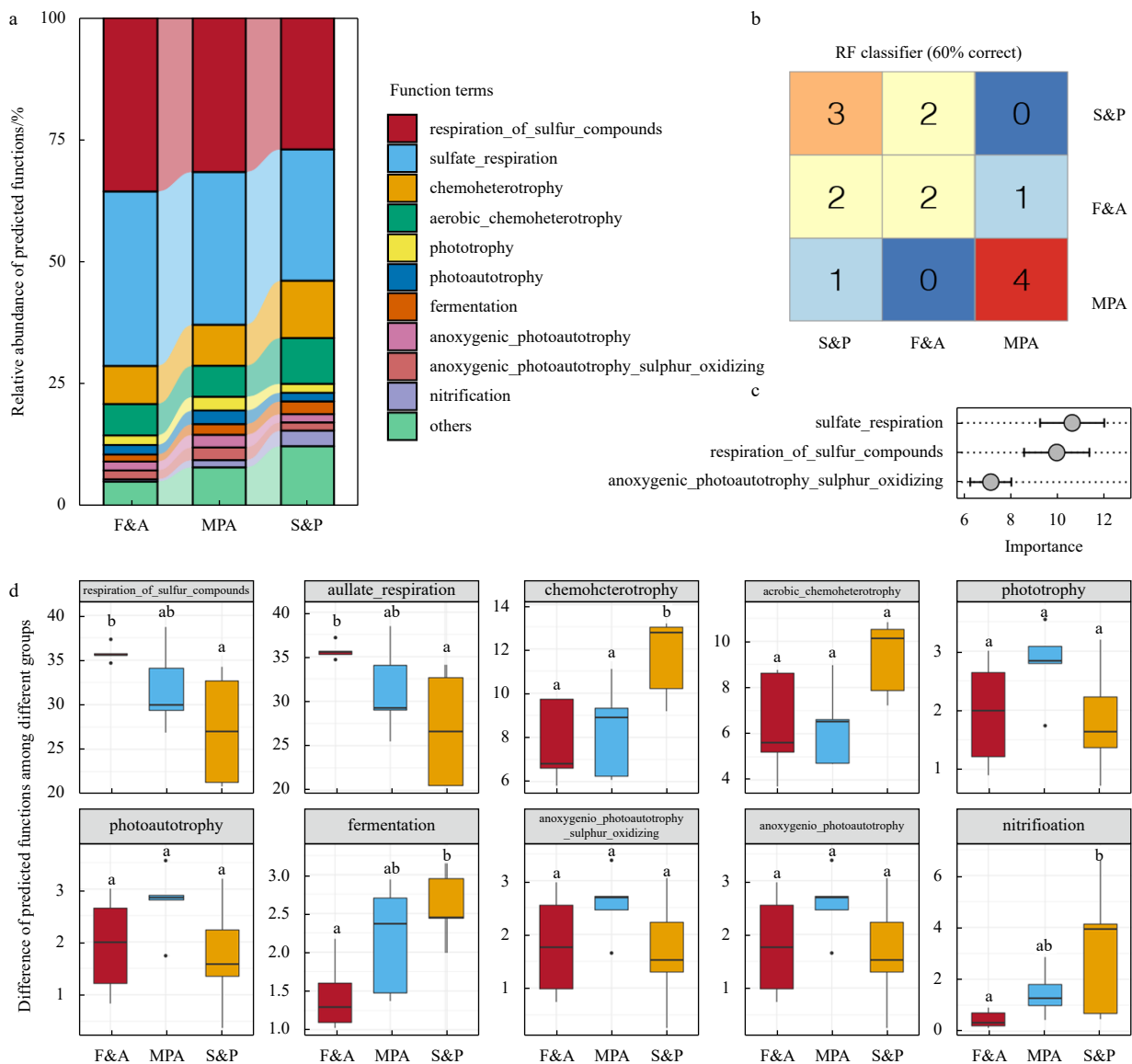


Fig. 7. Relative abundances of the predicted biogeochemical function terms in benthic bacterial communities among different marine functional zones (a). Predicted accuracy of random forest model based on predicted functions for classification the sample sources (b). Biomarkers of predicted functions are ranked in descending order of importance to the accuracy (c). Differences of dominant biogeochemical function terms among different marine functional zones (d). Different lowercases above each box in the same subfigure represent significant differences between groups (Tukey's HSD test, $p < 0.05$). F&A: fishery & agricultural zone; S&P: shipping & port zone; MPA: marine protected zone.

mg/kg; Pb, 10.2–15.5 mg/kg; Zn, 28.1–57.6 mg/kg; Cd, 0.03–0.28 mg/kg; and Cr, 15.9–73.8 mg/kg (Table S1). These results were much lower than those reported in the Zhujiang River Estuary (Liang et al., 2016) and Huanghe River Estuary (Zhao et al., 2017), but were similar to those detected in the Beibu Gulf (Zhao et al., 2022). These results indicated that the Yalu River Estuary may not be a marine area seriously polluted by heavy metals. Moreover, based on high-throughput sequencing of 16S rRNA gene, we determined the variations of benthic bacterial communities in three MFZs under long-term MSP practice in the Yalu River Estuary in this study. Proteobacteria was the most dominant phylum across all samples (Fig. 2a), in agreement with previous studies on bacterial composition in surface waters and sediments of marginal seas in the Northwest Pacific Ocean (Feng et al., 2009; Liu et al., 2015b; Zheng et al., 2014). After the Proteobacteria phylum, Actinobacteria and Acidobacteria were the second and third dominant phyla in this study, respectively. These results were like those of previous studies on coastal sediments from different global areas, despite differences in relative abundance (Behera et al., 2017; Green et al., 2008; Su et al., 2018). In addition, the highest benthic bacterial richness was detected for samples from the MPA (Fig. 2b) in the present study. This result is reasonable since the MPA was established to protect biodiversity and maintain ecosystem stability (Edgar et al., 2014).

Furthermore, significant differences in benthic bacterial communities were obviously observed between S&P and F&A areas in this study (Fig. 3). By contrast, differences between benthic bacterial communities in the MPA were relatively small compared to the S&P and F&A areas (Fig. 3). Bottom trawling, a frequent human activity in the F&A, is used to catch fish and shellfish living on the seabed, and it is regarded as the largest source of human physical disturbance in the marine environment (Hiddink et al., 2020). Previous studies indicated that bottom trawling could lead to reductions in benthic faunal numbers, biomass, and species diversity (Zou et al., 2020). In addition, intensive cage-based and bottom-seeded farming activities were established in the F&A studied in the present work (Li et al., 2017). Nutrient residues from aquaculture and intensively cultured species can also significantly affect benthic bacterial communities (Guan et al., 2020). For the S&P, large numbers of ships moving frequently through relatively narrow areas could severely disturb the inhabitant biome (Jägerbrand et al., 2019). Moreover, shipping activities might also cause petroleum-based pollution (Uddin et al., 2021). The results of the present study suggested that the MPA had more diversified benthic bacterial communities, while different human activities in F&A and S&P exerted different selective pressures on their inhabitant communities.

The identification, assessment, and monitoring of potential bio-indicator species are fundamental for ecological studies in sedimentary environments (De Laet et al., 2019). Several bacterial genera were identified as potential biomarkers for different MFZs in the present work (Fig. 4). The SBR1031 genus was significantly enriched in sediments from the MPA compared with the F&A and S&P, which had been reported as phototrophic and nitrifying bacteria (Li et al., 2020; Ward et al., 2020). *Woeseia* is a facultative, chemoheterotrophic anaerobe that is renowned for its ability to degrade polycyclic aromatic hydrocarbons in deep-sea sediments following oil spills (Bacosa et al., 2018). The Sva0996 marine group is an actinobacterial group that has been detected in both marine sediment and water column habitats, and members possess a strong ability to utilise organic matter (Wang et al., 2018). The significant enrichment of *Woeseia* and the Sva0996 marine group in sediments from the S&P could re-

flect petroleum pollution due to shipping activities. By contrast, *Halioglobus* was found to be more abundant in sediments in the F&A than the other zones, and this was revealed to be the dominant benthic bacteria in other coastal aquaculture regions (Ding et al., 2019). The differentially abundant bacteria identified herein indicated the anthropogenic activity footprints of benthic bacterial communities in different MFZs are distinct, and provided some potential biomarkers for the monitoring of ecological status in the Yalu River Estuary.

Due to long-term human activities in the F&A and S&P, the Yalu River Estuary faces severe pollution problems that threaten the integrity and functions of coastal ecosystems (Suo et al., 2009). FAPROTAX analysis predicted the functional traits of the benthic bacterial communities, implying the impact of anthropogenic activities on the ecological functions of the Yalu River Estuary. The ratio of bacterial responses to the respiration of sulphate and sulphur compounds was significantly higher in sediments from the F&A (Fig. 6b). These bacteria reduce sulphur compounds to hydrogen sulphide (Sorokin et al., 2018), a strongly toxic substance to aquaculture species (Joyner-Matos et al., 2010). These results indicate that excessive hydrogen sulphide could accumulate in sediments of the F&A in the Yalu River Estuary, which could lead to the death of the aquaculture species. Sample collection in this study was performed in summer, the inhibition of sulphur-oxidising bacteria under high temperature conditions in summer induced the excessive hydrogen sulphide accumulation in sediments of coastal aquaculture ponds used to culture sea cucumbers (Zhao et al., 2020). By contrast, bacteria associated with chemoheterotrophy and fermentation were more abundant in sediments of the S&P, consistent with high levels of organic matter and petroleum in this area caused by shipping activities (Wu et al., 2003).

5 Conclusions

This study demonstrated benthic bacterial communities could be used to reveal the anthropogenic activity footprints in different MFZs from a coastal ecosystem under long-term MSP practice. Differentiation in benthic bacterial community structures were observed for MFZs with distinct marine use types. Benthic bacterial communities in the MPA had the highest bacterial richness and a more balanced composition structure. By contrast, distinct human activities, such as frequent fishing and intensive mariculture in the F&A, and ship traffic in the S&P area, can favour specific bacteria and thereby shape distinct benthic bacterial communities. In addition, several bacterial genera, including SBR1031, *Woeseia*, Sva0996 and *Halioglobus*, were identified as potential biomarkers for biomonitoring of different MFZs. Moreover, our findings indicated that the benthic bacterial communities could reveal the risk of sulphides accumulation and petroleum contamination in the F&A and S&P areas, respectively.

Availability of Data and Materials

All raw sequences of sediment bacterial communities studied in this study have been submitted to the NCBI Sequence Read Archive database under the BioProject number PRJNA815376. The metadata of studied sediments were uploaded as supplements (Tables S1–S3).

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Supplementary information:

Fig. S1. Rarefaction curves for benthic bacterial communities from different marine functional zones.

Fig. S2. Venn figure to recognize the shared and unique ASVs among different marine functional zones.

Fig. S3. The ratio of taxon compositions of the shared ASVs at the phylum level.

Table S1. Sediment properties in the samples.

Table S2. Numbers of annotated species at different taxonomic levels

Table S3. Results of functional prediction by FAPROTAX.

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