Acta Oceanol. Sin., 2023, Vol. 42, No. 9, P. 113–124
https://doi.org/10.1007/s13131-023-2153-2
http://www.aosocean.com
E-mail: ocean2@hyxb.org.cn

Prokaryotic diversity and community composition in the surface sediments of the Changjiang River Estuary in summer

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Received 20 December 2021; accepted 21 October 2022

Abstract

Microorganisms are fundamental for the functioning of marine ecosystems and are involved in the decomposition of organic matter, transformation of nutrients and circulation of biologically-important chemicals. Based on the complexity of the natural geographic characteristics of the Changjiang River Estuary, the geographic distribution of sedimentary microorganisms and the causes of this distribution are largely unexplored. In this work, the surface sediment samples from the adjacent sea area of the Changjiang River Estuary were collected. Their prokaryotic diversity was examined by high-throughput sequencing technology, and the environmental factors of the bacterial community were investigated. The results indicated that the distribution of prokaryotic communities in the sediments of the study areas showed obvious spatial heterogeneity. The sampling sequences divided the sample regions into three distinct clusters. Each geographic region had a unique community structure, although Proteobacteria, Bacteroidota, Desulfobacterota, Acidobacteriota, and Actinobacteriota all existed in these three branches. Canonical correspondence analysis demonstrated that prokaryotic diversity and community distribution were significantly correlated with the geographic location of sediment, seawater depth, and in particular, nutrient content (e.g., total phosphorus, total organic carbon and dissolved oxygen). Moreover, it was found for the first time that the metal ions obviously affected the composition and distribution of the prokaryotic community in this area. In general, this work provides new insights into the structural characteristics and driving factors of prokaryotic communities under the background of the ever-changing Changjiang River Estuary.

Key words: prokaryotic diversity, 16S rRNA gene, geophysicochemical factors, high-throughput sequencing (HTS), Changjiang River Estuary

Citation: Qu Changfeng, Wang Xixi, Zhang Liping, Yuan Huamao, Li Xuegang, Li Ning, Chen Fushan, Miao Jinlai. 2023. Prokaryotic diversity and community composition in the surface sediments of the Changjiang River Estuary in summer. Acta Oceanologica Sinica, 42(9): 113–124, doi: 10.1007/s13131-023-2153-2

1 Introduction

Marine sediments occupy almost 70% of the earth's surface, and provide a large habitat for microbes on Earth (Hoshino and Inagaki, 2019). This also makes them an important part of the earth's ecosystem (Hoshino et al., 2020). An enormous number of microorganisms constitute the anaerobic and aerobic microbial ecological system of the submarine environment (Hoshino and Inagaki, 2019; Hoshino et al., 2020). More studies have shown that microorganisms are the basis for the functioning of submarine ecosystems. On the one hand, microorganisms promote the decomposition of sedimentary organic matter, the conversion of nutrients and the circulation of biochemical substances; on the other hand, marine sedimentary organisms have more important ecological and evolutionary significance (Hinrichs and Inagaki, 2012; Hoshino et al., 2020). Due to the great contribution of microbes in marine sediments to global biomass, the microbial species and abundance in various depositional environments have been extensively explored in recent decades (Baldock and Skjemstad, 2000; Hoshino and Inagaki, 2019). However, the microbial communities on the immense ocean floor have not yet been fully exploited (Kallmeyer et al., 2012). Moreover, the factors affecting species diversity in specific habitats and niche differences between habitats have not been well explained (Hoshino et al., 2020; Li et al., 2020).

Foundation item: The National Natural Science Foundation of China under contract Nos 32000074 and 42176130; the Science and Technology Planning Project of Guangxi under contract No. AA21196002; the Natural Science Foundation of Shandong Province under contract No. ZR2021MD044; the Tai Mountain Industry Leading Talent of Shandong under contract No. 2019TSCYCX-06; the Key Research and Development Program of Shandong Province under contract No. 2021TZXD008; the Biosafety Research Program under contract No. 20SWAQX04; the Shandong Program of Pilot National Laboratory for Marine Science and Technology (Qingdao) under contract No. 2022QNLM030003-1.

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The Changjiang River, with a total length of 6 300 km, is the largest river in China and the primary source of terrigenous deposits on the East China Sea shelf (Chai et al., 2009; Zhang et al., 2010; Milliman and Syvitski, 1992). As the main connection between the Asian continent and the Pacific Ocean, massive runoff annually transports a tremendous amount of sediment into the estuary and flows into the East China Sea (Milliman and Syvitski, 1992; Zhang et al., 2010; Beardsley et al., 1985; Wang et al., 2008). This activity results in the transport of plenty of terrestrial nutrients from inland to coastal waters and provides abundant nutrients to the East China Sea (Ludwig et al., 1996; Martin and Meybeck, 1979; Zhang et al., 2007). Therefore, the Changjiang River plays a major role in promoting the biogeochemical cycle of the East China Sea (Milliman and Syvitski, 1992). Equally important, under the combined action of the Kuroshio, Taiwan Warm Current and Yellow Sea Coastal Current, the Changjiang River Estuary and its adjacent waters also play a critical role in the process of land-derived material input from the interior to the western Pacific Ocean.

Prokaryotic diversity and geophysicochemical factors of marine sediments associated with different seasons in the Changjiang River Estuary and its adjacent water have aroused widespread concern and controversy. The Changjiang River not only modifies the hydrological characteristics of the estuary zones but also affects the physical, chemical and biological properties. It is known to disturb the natural biogeochemical cycles of nutrients (carbon, nitrogen, and phosphorus) and metals and may affect the downstream ecosystem of coastal waters. An enormous variation in the quantity of these environmental factors is the core content of construing marine biodiversity and biomass. At a spatio-temporal scale, the result of their action is the change in microorganisms from the cellular level to the ecosystem level. In turn, microorganisms act as vital driving forces for the material circulation, energy flow, ecological regulation and environmental restoration of marine ecosystems. Studies have shown that the microbes in the marine sediment environment are mainly dominated by prokaryotes, and their quantity can reach two-thirds of the total biomass (D'Hondt et al., 2004). Therefore, sedimentary prokaryotes play a pivotal role in the ecosystems of Changjiang River Estuary.

This paper aims to use high-throughput sequencing (HTS) technology to investigate the prokaryotic biodiversity and distribution characteristics of sediments at 15 sampling sites in the adjacent sea area of Changjiang River Estuary in summer. The reason for this investigated area is that the waters of the Changjiang River Estuary have a special geographic location, and form unique seawater physicochemical properties. The local to regional variability of these physicochemical factors controls and affects the ecological environment and biological communities in the sea area and determines that this sea area has distinctive microbial resources. This study will show the ecological distribution characteristics and diversity of microorganisms in the adjacent sea area of the Changjiang River Estuary in summer and make it possible to comprehensively and systematically understand the distribution regularity, influencing factors and significance of the microorganisms in marine ecosystems and biogeochemical cycles.

2 Materials and methods

2.1 *Site locations and sample collection*

All sediment samples were collected from the Changjiang River Estuary adjacent sea area by R/V *Kexue 03* in August, 2020 (Fig. 1). Fifty grams surface sediment samples (depth of 0–2 cm) from 15 sampling stations were obtained using a sediment box corer and immediately placed in sterile plastic tubes (Table 1). For subsequent analysis, all samples were stored in a −196℃ liquid nitrogen tank.

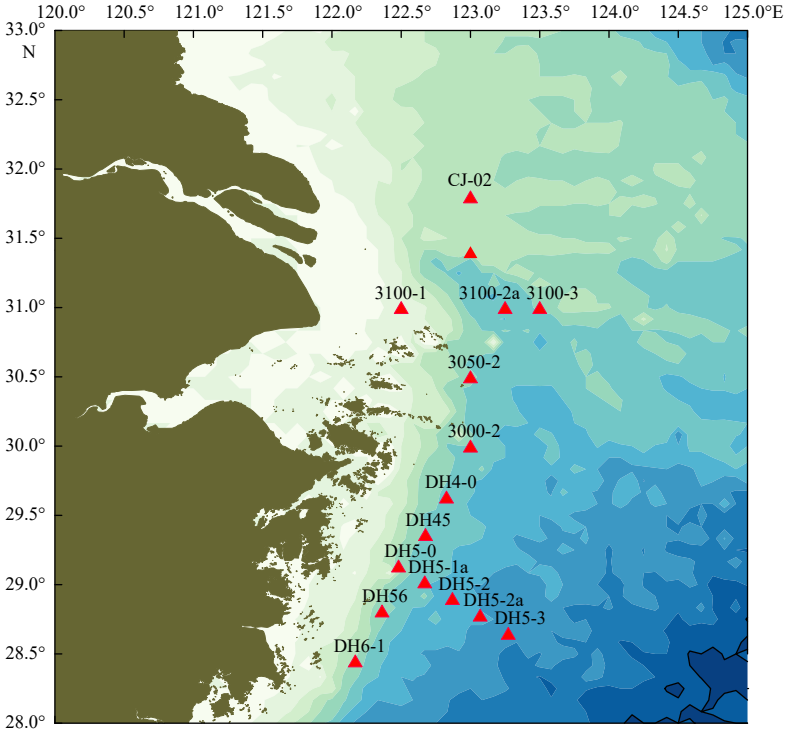

Fig. 1. Sampling sites in the Changjiang River Estuary adjacent sea area.

Table 1. Sample sites description of the 15 surface sediment samples from the Changjiang River Estuary adjacent sea area

Sampling site ID	Sampling site description						
	Latitude	Longitude	Layer/cm	Collected method	Sample amount/g	Water depth/m	Collected time
CJ-02	31.792 86°N	122.996 90°E	0–2	sediment box corer	50	38.6	2020-08-17
3100-1	30.902 66°N	122.452 16°E	0–2	sediment box corer	50	14.5	2020-08-18
3100-2a	31.010 40°N	123.250 10°E	0–2	sediment box corer	50	60.0	2020-08-18
3100-3	31.000 56°N	123.501 38°E	0–2	sediment box corer	50	55.0	2020-08-18
3050-2	30.494 22°N	123.016 68°E	0–2	sediment box corer	50	61.0	2020-08-16
3000-2	30.000 98°N	122.999 94°E	0–2	sediment box corer	50	52.0	2020-08-16
DH4-0	29.631 02°N	122.830 10°E	0–2	sediment box corer	50	51.0	2020-08-14
DH45	29.363 42°N	122.678 72°E	0–2	sediment box corer	50	51.0	2020-08-14
DH5-0	29.136 61°N	122.480 70°E	0–2	sediment box corer	50	44.0	2020-08-14
DH5-1a	29.017 12°N	122.668 46°E	0–2	sediment box corer	50	57.0	2020-08-14
DH5-2	28.897 74°N	122.868 94°E	0–2	sediment box corer	50	64.0	2020-08-15
DH5-2a	28.781 67°N	123.070 78°E	0–2	sediment box corer	50	68.0	2020-08-15
DH5-3	28.650 79°N	123.276 02°E	0–2	sediment box corer	50	72.0	2020-08-15
DH56	28.809 90°N	122.361 22°E	0–2	sediment box corer	50	47.0	2020-08-15
DH6-1	28.450 96°N	122.179 00°E	0–2	sediment box corer	50	43.0	2020-08-15

2.2 *Geochemical analysis of sediments*

A total of 10 g sediment samples from different stations were vacuum dried and ground for subsequent geochemical analysis. The total phosphorus (TP), total organic phosphorus (TOP), total nitrogen (TN), total organic carbon (TOC) and total organic nitrogen (TON) contents of treated samples were detected by a continuous flow analyzer (QuAAtro, SEAL, Norderstedt, Germany) at the Public Technology Service Center, Institute of Oceanology, Chinese Academy of Sciences. Then, the metal elements (Ca, Mg, Al, K, Fe, Mn, Cu, Zn, As, Ba, Pb, Cd) were analyzed using an X-ray fluorescence. The dissolved oxygen (DO) concentrations of the bottom waters were measured using traditional Winkler titration spectrometer according to the method of Dong et al. (2017).

2.3 *Environmental DNA extraction and PCR amplication*

The genomic DNA was extracted from offshore sediment samples using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to manufacturer's protocol. The DNA extract was checked on 1% agarose gel, and DNA concentration and purity were determined with NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA). The hypervariable region V3–V4 of the bacterial 16S rRNA gene was amplified with primer pairs 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) by an ABI GeneAmp® 9700 PCR thermocycler (ABI, CA, USA). The PCR amplification of 16S rRNA gene was performed as follows: initial denaturation at 95℃ for 3 min, followed by 27 cycles of denaturing at 95℃ for 30 s, annealing at 55℃ for 30 s and extension at 72℃ for 45 s, and single extension at 72℃ for 10 min, and end at 4℃. The PCR mixtures contain 5× TransStart Fast Pfu buffer 4 μL, 2.5 mmol/L dNTPs 2 μL, forward primer (5 μmol/L) 0.8 μL, reverse primer (5 μmol/L) 0.8 μL, TransStart Fast Pfu DNA Polymerase 0.4 μL, template DNA 10 ng, and finally ddH_2O up to 20 μL. PCR reactions were performed in triplicate. The PCR product was extracted from 1% agarose gel and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to manufacturer's instructions and quantified using Quantus™ Fluorometer (Promega, Madison, Wisconsin, USA).

2.4 *Sequence data analysis*

The Illumina Miseq PE300/NovaSeq PE250 (Illumina, San Diego, USA) sequencing platform was used for paired-end sequencing of purified amplicons based on the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). In order to make the subsequent analysis more accurate, fastp software (https://github.com/OpenGene/fastp, version 0.20.0) and FLASH software (http://www.cbcb.umd.edu/software/flash, version 1.2.7) were used for quality filtering and merging of the original data (Chen et al., 2018; Magoč and Salzberg, 2011). Operational taxonomic units (OTU) with 97% similarity cutoff were clustered using UPARSE version 7.1, and chimeric sequences were identified and removed (Edgar, 2013; Stackebrandt and Goebel, 1994). The taxonomy of each OTU representative sequence was analyzed by RDP Classifier v (http://rdp.cme.msu.edu/, version 2.2) against the 16S rRNA database (eg., Silva v138) using a confidence threshold of 0.7 (Wang et al., 2007). Diversity indices measure the overall community heterogeneity. Alpha diversity was applied in analyzing the species diversity of total sediments, and Chao1, ACE (Abundance-based Coverage Estimator), Shannon, Simpson, Coverage, Effectivesequence and rarefaction curves were then evaluated using the QIIME software (V1.7.0) (Caporaso et al., 2010). Sediment geochemical factors and prokaryotic diversity were analyzed using the one-way ANOVA method within SPSS19.0 for Windows. Principal Component Analysis (PCA) was performed within the sampling sites where the prokaryotic community structures based on weighted and unweighted UniFrac distance were investigated (Lozupone et al., 2011; Lozupone and Knight, 2005). A Mantel test was detected to reflect the influence of geochemical factors on bacterial community structure (Smouse et al., 1986). Meanwhile, the sediment property matrix between bacterial community structure and environmental factors was constructed using Redundancy analysis (RDA). Linear discriminant analysis effect size (LEfSe) was utilized to describe the microbial taxa of three sampling regions, and linear discriminant analysis influence (LDA) reflected the effect size of each taxon (Segata et al., 2011). Spearman correlation analysis was also used to test the correlations between microbial community diversity and environmental factors using R software (V 2.15.3).

3 Results

3.1 *Environmental characterization of sediments*

The geochemical compounds were measured in all sediment

samples. Generally, TOC and DO (dissolved oxygen) are among the key environmental determinants. The average TOC content of the sampling regions was (4.43 ± 2.0) mg/g. The DO concentrations in the bottom waters were consistently lower than 5 mg/L in most regions. Additionally, the contents of TN and TP in the 15 samples fluctuated greatly, ranging from 171.30 μg/g to 904.97 μg/g and 36.92 μg/g to 246.42 μg/g, respectively (Table 2). Further analysis suggested that the presence of N and P was mainly in organic form.

The analysis of metal components in sediments is an important part of the study of multi-environment factors. Twelve common metal compositions were detected in the sampled sediments (Table 2). The quantity of Mn (475.4–1 154.3 mg/kg), Zn (59.5–121.9 mg/kg), Ba (398–459 mg/kg), and Al (11.03%–16.40%) in surface sediment samples were much higher than other metal components.

3.2 *Prokaryotic diversity analysis*

After quality control of the raw data, a total of 831 374 sequences of 15 collected samples were obtained for subsequent procession, with an average sequence length of 422 bp for each sample (Table 3). Generally, all sequences were divided into operational taxonomic units (OTU), and 44 977 OTU were obtained at 97% identity, with the number of OTU ranging from 2 517 to 3 387 per sample (Table 3). The rarefaction curves can be used to judge whether the data is saturated, and the good's coverage reflects the community coverage. In our results, the curves in all groups were smooth (Fig. S1), and the good's coverage values were ⩾0.96, which together indicated that all sample data were sufficient and covered most species. The Shannon index was used to estimate the microbial diversity in the samples; the larger the Shannon index, the higher the community diversity. The Shannon index of all stations was ⩾6.04, indicating that the microbial species in these areas were quite abundant. In addition, the ACE index also reflected the high species richness and uniform distribution in the adjacent sea area of Changjiang River Estuary.

3.3 *Prokaryotic relative abundance and community composition analysis*

Community analysis indicated that each sample contained a similar community composition at the phylum level, but the relative abundances of prokaryotes were significantly different (Fig. 2a). Histogram showed that the relative abundances of the Top 5 dominant phylum exceeded 65% (66%–72.22%) of the total sequences, which were Proteobacteria, Bacteroidota, Desulfobacterota, Acidobacteriota and Actinobacteriota, respectively. In our work, the sediment environment of inshore coastal water had become more suitable for Proteus species (28.59% ± 2.32%), which formed the primary phylum of prokaryotes. Bacteroidota played a relatively minor role in the level of cluster, comprising approximately 14.26% ± 3.69% of total prokaryotic abundance. The third advantageous phylum was Acidobacteriata, followed by Desulfobacterota and Actinobacteriata, accounting for 9.95% ± 1.84%, 9.45% ± 2.39% and 6.96% ± 1.03% of the samples, respectively.

Hierarchical clustering analysis based on OTU composition showed that 15 sediment samples were grouped into three main categories. The 6 stations far away from the Changjiang River Estuary (CJ-02, 3100-2a, 3100-3 located in the northeast direction, Group A; DH5-2, DH5-2a, DH5-3 located in the southeast direction, Group B) were significantly different from the other stations, which gathered into two clusters; while the coastal stations near the Changjiang River Estuary were divided into one cluster (Group C) (Fig. 2b). PCA revealed that there were significant differences in microbial composition of sediment samples among the three clusters, and the coefficients of PC1 axis and PC2 axis were 38.34% and 19.87%, respectively (Fig. 3). Clearly, there was reasonable agreement between the two sets of the results. Principal Coordinates Analysis also supported this classification result (Fig. S2).

3.4 *Prokaryotic community differences between the three sampling regions*

LEfSe analysis can realize the comparison between multiple groups and also carry out subgroup analysis within the group comparison, so as to find species with significant differences in abundance between groups (Biomaker). Our measurements showed the analysis results of species with significant differences among different groups, and there were 23 characteristic groups with LDA value ⩾4.0 (Fig. 4).

The LDA scores showed that there were differences in biomarkers of microbial communities in three regions (Fig. S3). In Group A, Flavobacteriaceae and Desulfocapsaceae were the main biomarkers of microbial community differences. The abundance of Kiloniellaceae in Group B was high, while it was almost absent in the other two regions. Proteobacteria was a major group, however, in the ecosystem of Group C, including Woeseiaceae and Desulfobulbaceae.

3.5 *Correlation with environmental factors*

Using Variance Inflation Factor to perform a collinearity analysis on all environmental factors, the geophysicochemical factors affecting the composition of prokaryotic community were screened out, including the metal elements (Mg, Ca, and Ba), the depth, the nutrients (TP and TOC) and the DO. Subsequently, nine major elements of geophysicochemical factors in the sediment samples were analyzed using RDA (Fig. 5). Two dimensions of RDA explained the variability of 40.17% and 19.04% in prokaryotic community structure, respectively. In addition, these differences were mainly related to depth, Ba and Ca contents. Mantel test revealed the correlation between environmental factors (metal elements as Mg, Ca, and Ba. depth. nutrients as TP and TOC. and DO) and prokaryotic community (r = 0.427 59, p = 0.003), with specific results as follows: Mg (r = 0.351 50, p = 0.007), Ca (r = 0.392 71, p = 0.006), Ba (r = 0.412 47, p = 0.031), depth (r = 0.321 29, p = 0.008), TOC (r = 0.491 90, p = 0.003), DO (r = 0.427 32, p = 0.003).

3.6 *Effect of environmental factors on prokaryotic diversity and community composition*

Environmental factors are not only related to the abundance of microbial communities but also affect the community composition. Through Spearman analysis, the differences of microbial communities in sediments were obtained, and preliminarily revealed the effects of major environmental factors, especially Mg, Ba and Pb, on the structural characteristics and differences of microbial communities in different groups (Fig. 6). We found that Mg and Ba were significantly positively correlated with the abundance of prokaryotic evolutionary branches such as Campilobacterota, Patescibacteria, MBNT15, Armatimonadota, NKB15, and WPS-2, but significantly negatively related to the abundance of prokaryotic clades such as Actinobacteriota, RCP2-54, Halanaerobiaeota. The difference was that Ca was a significant positive correlation with Hydrogenedentes, Myxococcota and Bdellovi-

Table 2. Geochemical parameters of the 15 surface sediment samples from the Changjiang River Estuary adjacent sea area

Sample ID	Sample geochemical factors characteristic																
	TN/ ($\mu g \cdot g^{-1}$)	TP/ ($\mu g \cdot g^{-1}$)	TON/ ($\mu g \cdot g^{-1}$)	TOP/ ($\mu g \cdot g^{-1}$)	TOC/ ($mg \cdot g^{-1}$)	DO/ ($mg \cdot L^{-1}$)	Mg/%	Al/(%)	K/(%)	Ca/(%)	Fe/%	Mn/ ($mg \cdot kg^{-1}$)	Cu/ ($mg \cdot kg^{-1}$)	Zn/ ($mg \cdot kg^{-1}$)	As/ ($mg \cdot kg^{-1}$)	Ba/ ($mg \cdot kg^{-1}$)	Pb/ ($mg \cdot kg^{-1}$)	Cd/ ($mg \cdot kg^{-1}$)
CJ-02	171.30	214.20	155.71	118.17	3.05	2.29	1.90	11.03	2.23	3.56	3.89	504.1	8.2	48.3	3.1	418	17.8	0.12
3100-1	395.27	245.21	382.54	135.70	4.32	4.71	2.69	14.42	2.65	4.13	5.57	763.5	28.8	91.1	9.4	425	28.1	0.20
3100-2a	400.76	193.65	385.41	149.30	3.43	3.12	2.46	13.78	2.55	4.29	4.24	475.4	12.0	59.9	4.6	400	20.0	0.13
3100-3	362.86	210.42	350.85	116.99	2.25	3.22	2.36	12.51	2.35	5.17	3.98	573.6	11.7	59.5	3.3	398	16.2	0.12
3050-2	437.76	246.53	425.94	85.46	2.38	1.76	2.84	14.95	2.73	4.34	5.16	618.1	18.8	79.0	5.5	428	25.0	0.14
3000-2	416.37	81.16	402.16	58.53	1.99	4.23	2.75	14.65	2.71	4.60	4.93	761.1	15.9	73.8	5.1	426	19.6	0.12
DH4-0	570.39	231.32	555.69	214.63	4.00	4.26	2.90	15.42	2.92	4.11	5.96	624.1	27.3	97.3	7.2	432	31.1	0.18
DH45	719.52	246.42	702.14	161.07	6.66	4.35	3.10	16.35	3.07	3.63	6.62	841.7	35.0	113.9	10.6	441	32.4	0.19
DH5-0	904.97	192.60	889.69	158.24	8.50	4.33	3.13	16.63	3.13	3.37	6.91	1 154.3	37.1	119.1	12.0	458	36.3	0.21
DH5-1a	696.37	202.97	684.89	143.71	4.87	5.14	3.04	16.40	3.08	3.78	6.34	845.8	28.1	106.4	8.8	459	30.2	0.16
DH5-2	766.00	187.82	727.64	125.48	7.20	5.41	2.94	16.39	3.14	4.10	6.54	859.2	25.9	110.9	7.0	450	31.9	0.16
DH5-2a	552.91	36.92	537.40	31.92	3.02	5.16	2.73	15.07	2.83	6.22	5.41	749.9	17.7	85.0	3.7	415	26.1	0.11
DH5-3	550.00	148.94	533.17	138.84	2.21	4.83	2.69	15.18	2.86	5.63	5.32	644.4	17.7	83.1	4.1	417	23.4	0.11
DH56	795.04	216.08	774.52	204.57	7.20	4.18	3.10	16.69	3.22	3.42	7.17	1 012.1	40.2	124.0	11.8	458	38.7	0.21
DH6-1	847.24	87.20	835.40	80.09	5.35	4.99	3.11	16.64	3.16	3.37	7.07	1 179.0	38.0	121.9	12.7	448	37.0	0.21

Note: The contents of Mg、Al、K、Ca and Fe are presented by the percentages of their related oxides (MgO, Al_2O_3, K_2O, CaO and Fe_2O_3) in sediment. TN: total nitrogen; TP: total phosphorus; TON: total organic nitrogen; TOP: total organic phosphorus; TOC: total organic carbon; DO: dissolved oxygen.

Table 3. Biodiversity parameters of the 15 locations of surface Changjiang River Estuary adjacent sea area

Sample ID	Effective sequence	Base number	Operational Taxonomic	Shannon index	ACE estimator	Coverage/%
CJ-02	49 959	21 112 433	2 781	6.31	3 446.88	0.98
3100-1	54 373	22 949 506	2 811	6.21	3 694.29	0.977
3100-2a	44 908	18 883 844	2 517	6.04	3 452.74	0.98
3100-3	53 385	22 541 097	3 003	6.42	3 874.58	0.98
3050-2	66 852	28 235 313	3 296	6.40	4 452.27	0.98
3000-2	55 133	23 327 239	2 768	6.08	3 836.63	0.98
DH4-0	51 194	21 682 724	3 123	6.37	4 336.89	0.97
DH45	60 438	25 475 554	3 096	6.27	4 359.16	0.97
DH5-0	58 946	24 869 661	2 973	6.20	4 330.02	0.97
DH5-1a	41 935	17 703 747	2 757	6.32	4 975.49	0.96
DH5-2	68 649	28 984 752	3 310	6.43	4 490.64	0.98
DH5-2a	54 684	23 052 855	3 138	6.53	4 319.96	0.97
DH5-3	54 545	22 973 489	2 953	6.56	3 721.15	0.98
DH56	52 897	22 363 027	3 064	6.33	4 267.99	0.97
DH6-1	63 476	26 841 604	3 387	6.40	4 688.09	0.97

Note: ACE: Abundance-based Coverage Estimator.

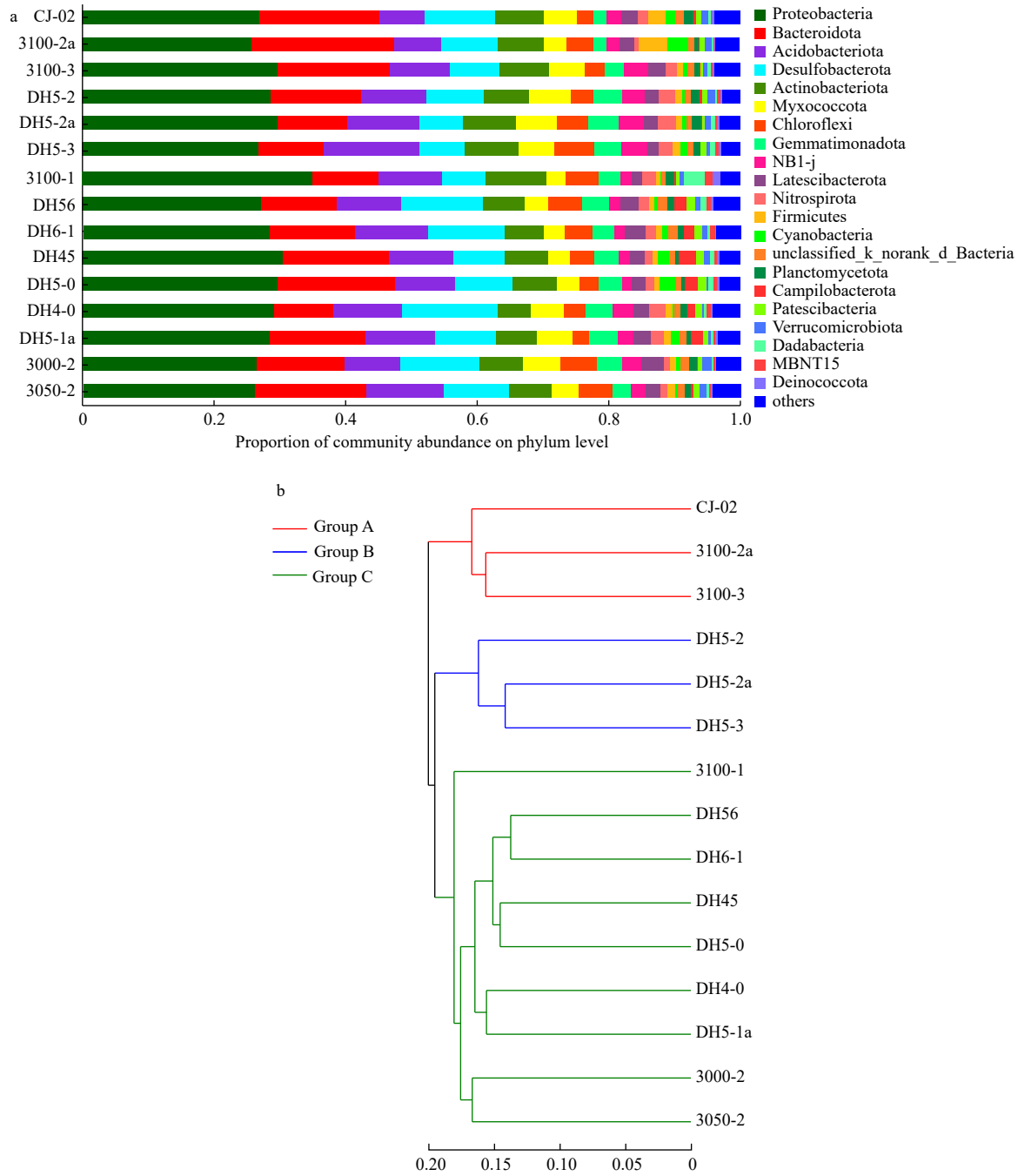

Fig. 2. Clustering of surface sediment prokaryotic communities of sampling sites (a) and of each sample (b) by Unweighted Pair-group Method with Arithmetic Mean.

brionota, but significantly negatively correlated with Spirochaetota, Patescibacteria and Campilobacterota (Fig. 6a). The contents of Mg and Ba were positively correlated with Chao1 and ACE, and negatively correlated with coverage (Fig. 6b). Ca content was related to Shannon, Simpson, ACE, Chao1 and coverage, but some indices were not statistically significant (Fig. 6b). Therefore, the diversity and distribution of prokaryotes are greatly affected by the geophysicochemical factors of the sediments.

4 Discussion

In marine environments, microbial activity has been the most active part in the development of marine ecosystems. However, the taxonomic diversity and spatial distribution of marine microbial communities are severely restricted by geophysicochemical factors on a global scale. Using HTS technology to analyze the differences in biodiversity and community structure in marine sediments can provide a reliable basis for the study of microbial composition, community structure optimization and functional regulation.

The distribution characteristics and control processes of water temperature, salinity and nutrients in the Changjiang River Estuary and its adjacent sea area are unique and complex under the influence of substantial runoff, complex ocean currents and water masses. These differences in the ecological environment directly or indirectly lead to changes in microbial diversity and

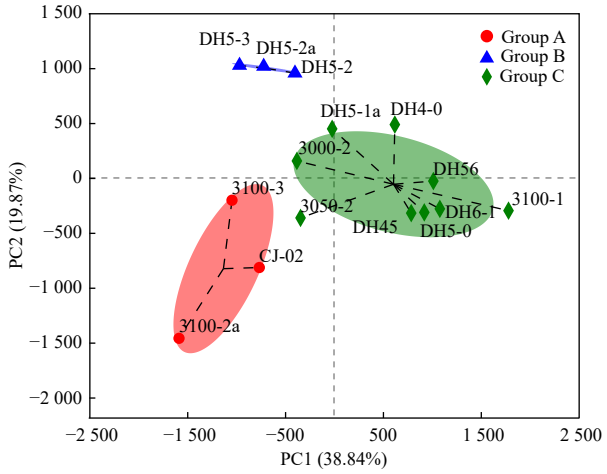

Fig. 3. Principal coordinate analysis plots. The first principal coordinate (PC1) clearly separates the samples according to their collection positions, as indicated by the ovals around pooled samples from the same collection site.

community structure, correlating to a complex distribution trend. Previous studies have suggested that the diversity and distribution of marine microorganisms have obvious biogeographic patterns, and environmental factors (geographic distance, nutrients, temperature and salinity) ultimately influence their distribution through changes in microbial community structure (Green and Bohannan, 2006; Horner-Devine et al., 2004; Martiny et al., 2006). Considering the different geographic locations, organic matter and hydrological conditions in the three sampling areas, the species and abundance of microorganisms in the sediments are regarded as being different. Due to our limited understanding of microbial communities and their functions in these environments, our ability to predict the impact of biogeochemistry on benthic microbial communities in sediments is also limited. Here, we analyzed the correlation between microbial community structure and different environmental factors through the taxonomic composition and abundance distribution of microbial communities at each station. We found that the microbial diversity in the sediments was rich. Microbial diversity in Group B was the highest, while that in Group A and Group C was relatively low, but there was no significant difference. At the same time, the three sampling areas had similar community compositions at the phylum level, but there were significant differences in the relative abundance of prokaryotes, except for the higher relative abundances of Top 5 dominant bacteria. Other bacteria included Myxococcota (4.62% ± 1.15%), Chloroflexi (4.12% ± 1.14%), Gemmatimonadota (3.52% ± 0.85%), Latescibacterota (2.38% ± 0.48%), and Nitrospirota (1.72% ± 0.59%). In addition, these three regions also had bacterial compositions with unique niche adaptations at the family level. Flavobacteriaceae and Desulfocapsaceae were found only in Group A, while Kiloniellaceae related to potential metal-cycling was unique to Group B. Microbes related to organic carbon remineralization (Woeseiaceae) and sulfur oxidizing (Desulfobulbaceae) were more enriched in Group C and were not found elsewhere. Normally, these distinctions could be attributed to environmental heterogeneity and historical events, such as different nutrients and geographic distances, to explain community differences. Previously reported

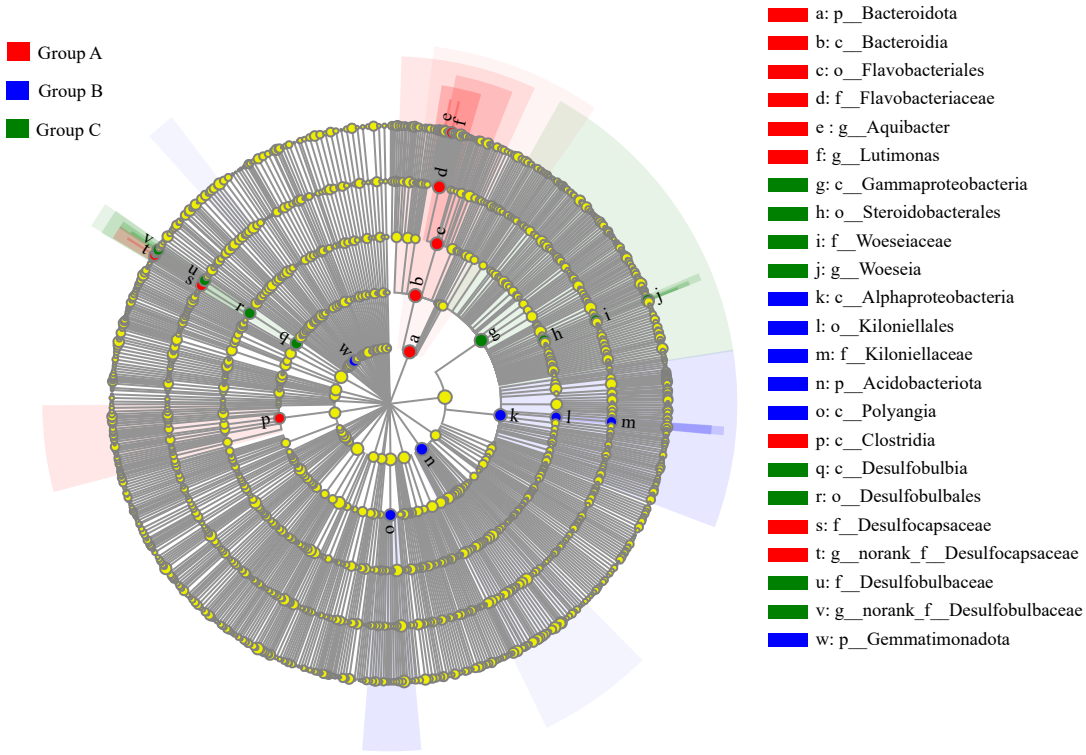

Fig. 4. Identification of the distinct bacterial taxa from different sampling regions using LEfSe. Only bacterial taxa with linear discriminant analysis influence values greater than 4 are displayed in this cladogram. Differences in the most abundant taxa are represented by colors (red, blue, and green indicate Group A, Group B, and Group C, respectively). The diameter of each circle is proportional to the corresponding taxon's abundance. Circles represent phylogenetic levels from phylum to genus. Labels are shown from phylum to genus level.

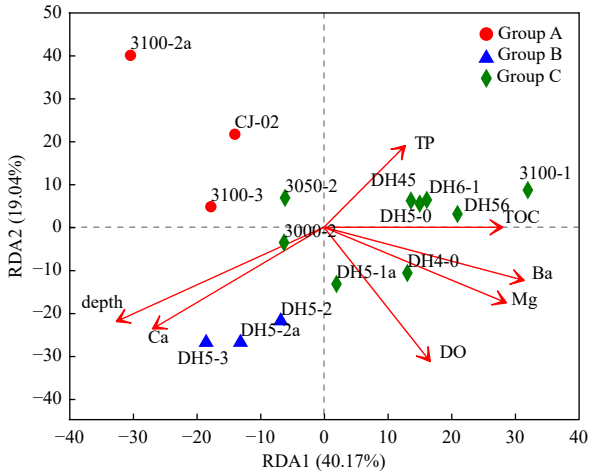

Fig. 5. Canonical correspondence analysis diagram illustrating the relationships between the OTU-level community structures of different sampling sites. The angle between the arrow line and the sorting axis represents the correlation between a certain environmental factor and the sorting axis. The smaller angle represents the higher the correlation is, on the contrary, the lower the correlation is.

differences in microbial assemblages between the two sites may be caused by different biogeochemistry (Hollingsworth et al., 2021). However, the correlation between single environmental variables (such as nitrogen and phosphorus) and the changes in community structure at each station was low in the study, which may be the result of the combined effect of geographic location and environmental factors.

Given the influx of the Changjiang River runoff and the disturbance of Taiwan Warm Current, they undoubtedly provide an organic nutrient flow for bacteria in the surface sediments (Smith et al., 2001; Wang et al., 2008). Microorganisms are important parts of marine ecosystems and play an important role in the biogeochemical cycle, such as organic substance decomposition, nutrient release, and energy transfer (Yergeau et al., 2012). In turn, biogeochemical elements are important sources of energy and nutrition for marine ecosystems and can also affect microbial communities, especially benthic microbes. Variation partitioning analysis (VPA) can quantitatively evaluate the contribution rate of different environmental factors to microbial community differences. The VPA results indicated that sedimentary geophysicochemical factors (environmental heterogeneity), including depth, contributed more to bacterial biogeography than geographic distance (historical events) (Li et al., 2020). Generally, the variability of microbial diversity structures may be attributed to variable organic content (Takebayashi et al., 2007; Bell et al., 2013; Ji et al., 2016). In this paper, the alpha diversity index was not significantly correlated with the organic content. We found that the TOC content differences in the three regions were not entirely consistent with the diversity results. In fact, the bacterial alpha diversity index did not monotonically increase with the increasing TOC. In addition to TOC, TP and depth also affect the relativity and diversity of microbial groups. In addition to organic matter, oxygen is also an important part of marine ecosystems, which profoundly construct bacterial and archaeal communities (Aldunate et al., 2018). Consistent with the diversity results, the DO content in the bottom water of Group B ((5.13 ± 0.2) mg/L) was higher than that of Groups A and C ((2.91 ± 0.54) mg/L and (4.21 ± 0.97) mg/L). However, previous literature has provided evidence that marine deoxygenation may promote bacterial alpha diversity (Sun et al., 2021). It is controversial whether there is no significant correlation between marine dissolved oxygen and bacterial abundance in the Changjiang River Estuary (Zhang, 2011). Considering the unique ecological environmental characteristics of the Changjiang River Estuary, we need further evidence to demonstrate the effect of dissolved oxygen on prokaryotic biodiversity.

Geophysicochemical factors may also have an impact on the population and the quantity of microorganisms in all sampling areas. In the present investigation, we demonstrated the relationship between changes in geophysicochemical factors and the prokaryotic community structure in the sediments of the three regions. Proteobacteria were the most abundant group in both coastal and oceanic surface waters (Yergeau et al., 2012). Similarly, the phylum Proteobacteria was also an important component of the microorganisms in the sediments of the Changjiang River Estuary with a relatively high abundance. By the same token, Desulfobacterota, Acidobacteria and Actinobacteria were also the main groups in the sediments. In grouping taxa, Desulfobacterota was particularly rich in organic-rich coastal and marine sediments, which could reduce sulfate or manganese oxides to obtain energy under anaerobic conditions (Muyzer and Stams, 2008; Thamdrup et al., 2000). In accordance with the results of the sediment geochemical data, there were high contents of organic matter such as nitrogen and phosphorus in the sediments from the adjacent sea area of the Changjiang River Estuary, and each station was located in a traditional low oxygen area, providing a higher reductive and anaerobic environment (Chen et al., 2007; Li et al., 2002; Zhu et al., 2016). Acidobacteria and Actinobacteria, which are distributed globally, are common bacterial groups in marine surface sediments (Orcutt et al., 2011). As a key process in the biogeochemical cycle of marine nutrients, Acidobacteria and Actinobacteria play a crucial role in promoting the marine nitrogen cycle (Fuerst and Sagulenko, 2011; Jetten et al., 2003). A higher Alphaproteobacteria to Acidobacteria ratio also reflected the higher nutrient availability in the sediments (Smit et al., 2001; Thomson et al., 2010). In addition, Gemmatimonadota, Gammaproteobacteria, Deltaproteobacteria, Firmicutes and Bacteroidetes were used as indicative bacteria in these three regions, which can make a valuable contribution to the absorption of carbon, nitrogen, and phosphorus and the biogeochemical cycles in marine surface sediments. Overall, the most abundant microbial groups in the sediments were sulfur bacteria and hypothetical carbon- and nitrogen-fixing microorganisms, which play a key ecological role in the biogeochemical cycle of the habitat.

Another obvious consideration was that some metal elements may be closely related to the taxonomic diversity and spatial distribution of marine sedimentary microbial communities (Dong et al., 2017; Chen et al., 2020). It has been found that metal elements can change the stoichiometry of C, N, P and S through enzymatic decomposition of organic compounds, thus affecting the microbial community structure and enzyme activities (Aponte et al., 2020). Some heavy metals and metalloids in particular change soil pH, clay structure and organic matter content by reducing the activity of various enzymes in the soil, while the last has a significant relationship with the abundance and diversity of microbial communities in the estuarine environment (Aponte et al., 2020). Indeed, Pb, As, Cu and Cd could modulate the activity of various enzymes involved in the C, N, P and S cycles in the soil, according to the most recent study on the topic (Aponte et al., 2020). Consequently, we analyzed the content

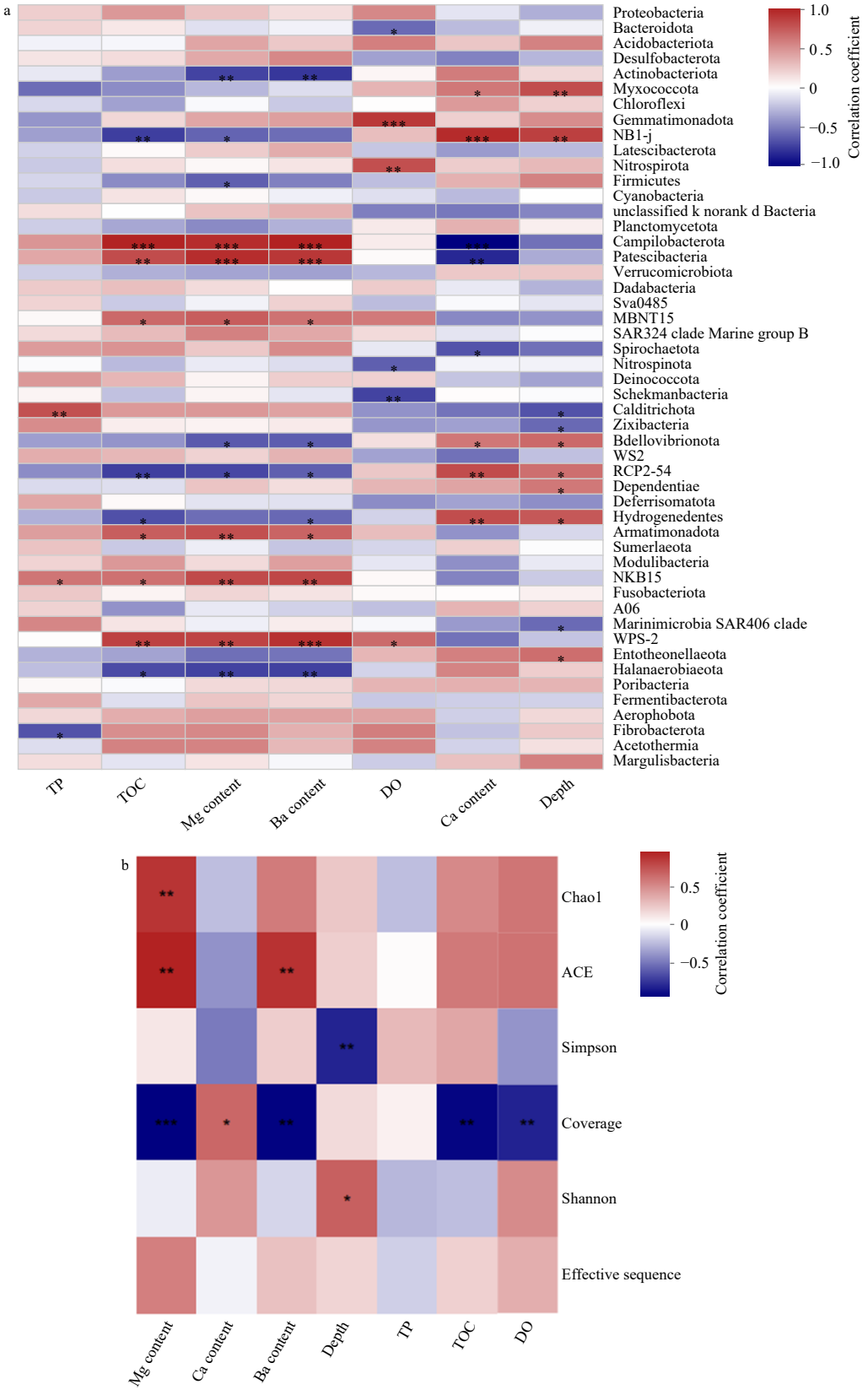

Fig. 6. Spearman analysis of the effects of geochemical parameters on the phylogenetic structure of the prokaryotic communities. a. Cluster analysis between main environmental factor and community abundance; b. cluster analysis between main environmental factor and diversity indices. In Spearman correlation analysis, the intermediate heat map corresponding value is the Spearman correlation coefficient r, $r > 0$ is the positive correlation, $r < 0$ is the negative correlation.* indicates the significance test $p < 0.05$, ** indicates the significance test $p < 0.01$, *** indicates the significance test $p < 0.001$. The contents of the metal elements are represented by symbols Mg, Ca, and Ba, while TP, TON, and DO represent the concentrations of total phosphorus, total organic nitrogen and dissolved oxygen.

variation of different metal elements in the sampling area to explain the correlation between the spatial pattern of bacterial community composition and geophysicochemical factors in the surface sediments. In this report, we examined 12 metal elements at all stations, including metalloids (As), heavy metals (Fe, Mn, Cu, Zn, Ba, Pb, and Cd) and conventional metals (Mg, Al, K, and Ca). The change of metal contents at each sampling site was evident, which could be related to the sampling geographical location in the adjacent sea area of the Changjiang River Estuary. These results, on the other hand, reflected the indirect influence of geographic location on the diversity and community composition of prokaryotes. A Spearman test also showed that there was a degree of significant correlation between the community diversity index and some environmental factors.

In regard to the ocean, the diversity and distribution of prokaryotic organisms and metal elements in the ocean surface sediments need to be comprehensively considered. The microorganisms in the sediments inevitably come into contact and interact with various metal elements. Some metals or metalloids can be used as essential elements to participate in the physiological functions of microorganisms, as well as electron acceptors or donors to be involved in energy metabolism (Ehrlich, 1997). The metal elements in the sediments can also affect the primary productivity of the upper water body (Jeandel et al., 2000; Pfeifer et al., 2001). Here, we used Spearman tests to further analyze the effects of different metals on the structural characteristics and discrepancy of the microbial communities in each group. The relationship between Ba content and variation in prokaryote community structure was discovered in previous studies. Our results showed that Ba was positively correlated with Chao1 and Simpson and significantly positively correlated with ACE. The greater the ACE estimator was, the richer the species were in the environment, and the more evenly the species were distributed. Similar to the previously reported results (Biddle et al., 2006; Orcutt et al., 2011), this study showed that mineralogy was closely related to the microbial community structure. However, the ACE values were present at a high level at all stations, and there were no obvious statistical differences between the three regions. Consistent with the diversity of RDA, we speculated that this could be a link with no significant differences in Ba levels between the different groups. The relative abundance and biodiversity of communities affected by Mg and Ca, in addition to the Ba concentration, still require more evidence to support this hypothesis.

5 Conclusions

Understanding the diversity and functional roles of benthic microbes is vital for marine ecosystems of the Changjiang River Estuary and its adjacent sea area. This work described the spatial distribution and biodiversity of prokaryotic communities in surface sediments in the adjacent sea area of the Changjiang River Estuary, revealing the biogeographic feature shaped by estuary heterogeneity. The spatial pattern of microbial diversity is mainly driven by geophysicochemical factors, especially water depth and nutrients (e.g., TP, TOC, and DO). Moreover, metal factors (Ca, Ba and Mg) also have an obvious effect on prokaryotic diversity and community distribution. Considering the environmental heterogeneity and the dynamic changes in coastal waters, further studies in the Changjiang River Estuary and its adjacent sea area are needed to better understand the correlation between bacterial communities and environmental factors in the rapidly evolving marine ecosystems.

Acknowledgements

We thank the Public Technology Service Center, Institute of Oceanology, Chinese Academy of Sciences for assistance with sample analysis.

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Supplementary information:

Fig. S1. Rarefaction curves of the 15 samples from the Changjiang River Estuary adjacent sea area.

Fig. S2. PCoA of bacterial community compositions based on unweighted UniFrac distance.

Fig. S3. LDA (Linear Discriminant Analysis) value distribution histogram showing bacterial communities with LDA scores greater than 4.