



## Characteristics of microbial community indicate anthropogenic impact on the sediments along the Yangtze Estuary and its coastal area, China



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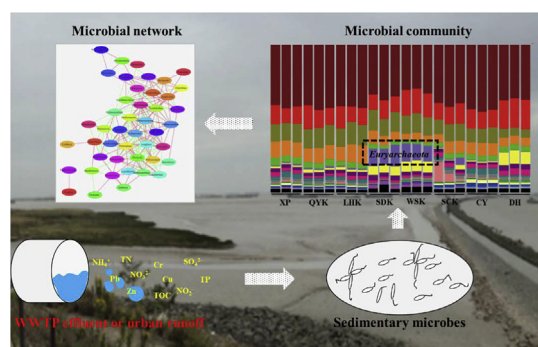
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### HIGHLIGHTS

- Microbial community was characterized in sediments of the Yangtze Estuary.
- Microbial communities varied spatially, especially for Euryarchaeota.
- Euryarchaeota were mainly composed of methanogens.
- Anthropogenic nutrients and metals regulated methanogen and methanotroph distribution.
- Methanogens and methanotrophs could be potential bio-indicators to human activities.

### GRAPHICAL ABSTRACT



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### ABSTRACT

In the contaminated coastal sediments, variations of microbial community can reflect the impact of anthropogenic activities. The identification, evaluation and monitoring of the potential bio-indicator species and bio-marker communities are vital for the ecological studies in sedimentary environments. Based on the high-throughput sequencing, the microbial communities were characterized in the sediments along the Yangtze Estuary and its coastal area. The results showed that the structure and composition of microbial communities varied greatly among different sampling sites at the phyla level, especially for *Euryarchaeota*. Metabolic pathway and quantitative PCR analyses suggested that the methane metabolism-related microbes were mainly included in the phylum of *Euryarchaeota*. Elevated abundances of methane metabolism-related microbes were found at Shidongkou (SDK) and Wusongkou (WSK), where microbes were seriously impacted by the wastewater treatment plant (WWTP) effluent and urban runoff. By comparing with the *Euryarchaeota* in WWTP sludge, the relatively high abundance of *Euryarchaeota* in sediment at SDK may be mainly related to the massive growth of indigenous species, promoted by anthropogenic nutrients. Moreover, redundancy discriminant analysis and correlation analysis revealed that methanogens and methanotrophs mainly respond to the nutrients and metals, such as total organic carbon, total phosphorus, total nitrogen,  $\text{SO}_4^{2-}$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$ , Cr, and Zn, which were often related to human activities. Network analyses showed that the species related to the metabolism of methane may play a vital role in the interassociation among different microbial communities. Therefore, methanogens,

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methanotrophs and their community compositions could be considered as potential bio-indicator species and biomarker communities, indicating anthropogenic activities in the sediments along the Yangtze Estuary and its coastal area.

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## 1. Introduction

Estuarine ecosystems are the ecotone between river and marine environment, and usually support the development of the neighbouring cities. They also receive amounts of pollutants from land-based sources of cities via river runoffs and sewage outfalls. In estuarine environments, the fine-grained and organic rich sediments act as reservoirs for pollutants (Ducrottoy et al., 2010), such as nutrients (Liu et al., 2003), heavy metals (Lin et al., 2002), antibiotics (Shi et al., 2014), polycyclic aromatic hydrocarbons (Liu et al., 2001), pesticides (Liu et al., 2008), and antibiotic resistance genes (Guo et al., 2018a). Owing to their persistence and continuous discharge into the environment, these contaminants pose a potential risk to the sedimentary biota, especially for microbial communities in the estuarine systems (Wang et al., 2016; Guo et al., 2018b). To adapt to different kinds of habitats, microorganisms often form some specific community structures to cope with the various environmental stresses (Feng et al., 2009; Campbell and Kirchman, 2013). Therefore, the characterization of the microbial communities in contaminated sediments can provide opportunities to identify potential bio-indicator species and biomarker communities responding to specific contaminants (Ducrottoy et al., 2010).

Microbial communities play a vital role in the biogeochemical cycles in estuarine ecosystems (Piccini and Garcíaalonso, 2015; Hou et al., 2013), and the occurrence of these cyclic processes are often related to some obligated microbial-community compositions (Lyautey et al., 2005; Despland et al., 2012; Guo et al., 2018b), such as anaerobic ammonium oxidation bacteria and denitrifiers to remove nitrogen (Thamdrup and Dalsgaard, 2002; Hou et al., 2013; Y.L. Zheng et al., 2014; B.H. Zheng et al., 2014), sulphate-reducing prokaryotes to fix sulphate (Muyzer and Stams, 2008; He et al., 2015), and methanogens to degrade organic matters (Conrad, 2007; She et al., 2016). Moreover, to a certain extent, the changes in dominant bacterial groups and their interactions with other bacterial communities usually affect the structure, function and stability of microbial ecosystems (Shannon et al., 2003). In addition, these changes in contaminated environments are usually caused by human activities either directly or indirectly. For example, polycyclic aromatic hydrocarbons contamination can regulate the composition of the bacterial communities (Tian et al., 2008); heavy metals can shift the benthic estuarine microbial communities and their metabolic functions (Echavarri-Bravo et al., 2015), and antibiotics can change the richness and diversity of antibiotic resistance bacterial communities (Fernandes et al., 2015). Thus, understanding the composition and dominant groups of bacterial communities as well as monitoring their variations, especially in sedimentary environments are essential for environmental and ecological studies.

The Yangtze Estuary is located at the mouth of the Yangtze River into the East China Sea, and it may be one of the most heavily polluted regions because large amounts of nutrients from the Yangtze River and lots of domestic sewage from the Yangtze River delta urban belt are discharged into the estuarine and intertidal environment (Zheng et al., 2016). Several studies on the functional bacterial communities have been conducted in recent years (Hou et al., 2013; Y.L. Zheng et al., 2014; B.H. Zheng et al., 2014; Zheng et al., 2016). However, research on the whole bacterial community and their response to environment factors is limited (Guo et al., 2018b). Thus, the specific objectives of the present study were as follows: (1) to explore the microbial community structures and compositions in the sediments along the Yangtze Estuary and its coastal area using the 16S rRNA gene-based Illumina Miseq

sequencing method; (2) to find some potential biomarkers of microbial communities related to the specific contaminants from human activities by metabolic function prediction in KEGG and real-time quantitative PCR (qPCR); and (3) to reveal the possible environmental factors influencing the abundance, diversity and composition of the microbial communities.

## 2. Materials and methods

### 2.1. Sample collection

Eight sites along the Yangtze Estuary and its coastal area were selected for sediment sampling (Fig. S1). The Xupu (XP) and Donghainongchang (DH) are situated at the intertidal flat where it is influenced by the water of the Yangtze River and the tide of the East China Sea. Four sites, including Qiyakou (QYK), Liuhekou (LHK), Sanchakou (SCK), Wusongkou (WSK) and Chaoyangnongchang (CY), are at the junction of the Yangtze River and urban river, which are mainly affected by urban river runoff and the estuary tide. The Shidongkou (SDK) site is located at the downstream of a wastewater treatment plant (WWTP), and this place often receives large amounts of domestic sewage and sludge. In July 2016, three sediment samples (0–5 cm deep) at each site were collected using sterile shovels, and the activated sludge was collected from the WWTP, which is located at the upstream of the SDK site. All of the samples were immediately pooled into sterile plastic bags, subsequently stored and transported to the laboratory on ice. After homogenizing the samples, one part of the sediment samples and the sludge samples were stored in sterile tubes at  $-20^{\circ}\text{C}$  for DNA extraction, and the remaining sediment samples were stored at  $4^{\circ}\text{C}$  for the physicochemical properties analysis.

### 2.2. Physicochemical properties analyses

The measured environmental parameters, including salinity, pH, grain size, total organic carbon (TOC), total phosphorus (TP), total nitrogen (TN),  $\text{SO}_4^{2-}$ ,  $\text{NH}_4^+$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$  and heavy metals (Cr, Cu, Zn and Pb), are listed in Table 1. Salinity and pH of sediments were determined with a portable instrument (HQ 40d, HACH, USA), after fresh sediments were mixed with  $\text{CO}_2$ -free Milli-Q water at a ratio (sediment/water) of 1:2.5 (Y.L. Zheng et al., 2014; B.H. Zheng et al., 2014). The LS 13 320 Laser grain sizer (Beckman Coulter, USA) and the TOC automatic analyzer (SSM-5000A, Shimadzu, Japan) were used to determine the sediment grain size and TOC concentration, respectively. TP was analyzed using the Mo-Sb colorimetric method with the UV-4802 UV/Vis spectrophotometer (Unico, China), after digestion with  $\text{H}_2\text{SO}_4$  and  $\text{HClO}_4$  (Hou et al., 2013). TN was measured using a CN thermal combustion furnace analyzer (Elementar analyzer vario Max CN, Germany), after samples were leached with a 1 M HCl solution (Li et al., 2014).  $\text{SO}_4^{2-}$ ,  $\text{NH}_4^+$ ,  $\text{NO}_2^-$  and  $\text{NO}_3^-$  levels were determined using an auto discrete analyzer (EasyChem Plus, Systea, Italy) with methods of Method EASY-SO4-01 rev 0, EASY-NH3-02 rev 0, Method EASY-Nitrate-354.1-01 rev 1 and Method EASY-Nitrate-353.2 rev 0, respectively (Hou et al., 2007). Heavy metals (Cr, Cu, Zn and Pb) were extracted according to the sediment environmental quality standard method in China (GB15618-1995) and measured using an inductively coupled plasma mass spectrometer (ICP-MS, PerkinElmer NexION 350D, USA).

**Table 1**  
Physicochemical properties of the sediments taken along the Yangtze Estuary and its coastal area.

Sample sites	Salinity (‰)	pH	Moisture content (%)	Clay content (%)	Silt content (%)	Sand content (%)	TOC (mg/kg)	TN (mg/kg)	TP (mg/kg)	SO <sub>4</sub> <sup>2-</sup> (mg/kg)	NO <sub>2</sub> <sup>-</sup> (mg/kg)	NO <sub>3</sub> <sup>-</sup> (mg/kg)	NH <sub>4</sub> <sup>+</sup> (mg/kg)	Cr (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Pb (mg/kg)
XP	0.18	7.73	33.09	24.46	74.32	1.21	14.42	487.34	911.68	66.35	0.06	4.05	27.12	48.50	65.29	256.74	46.74
QYK	0.27	7.44	52.48	23.89	74.70	1.41	27.96	673.03	996.25	77.93	0.07	3.83	28.13	36.39	67.36	261.02	47.72
LHK	0.26	7.52	42.71	23.97	74.44	1.59	18.90	596.87	942.06	188.74	0.08	3.64	37.20	42.73	79.55	339.12	56.15
SDK	0.16	7.67	42.62	27.50	72.21	0.29	70.46	1328.17	1747.41	211.93	0.13	3.95	98.97	76.50	178.76	1049.93	77.88
WSK	0.28	7.76	44.24	26.59	72.38	1.03	32.74	699.39	1238.77	245.54	0.10	4.17	62.28	41.25	76.09	335.57	57.86
SCK	0.23	7.76	31.11	20.47	78.61	0.92	15.16	481.63	861.50	203.15	0.08	4.94	24.70	32.73	43.56	193.34	42.67
CYNC	0.47	7.82	41.06	19.65	79.32	1.03	15.25	670.84	865.57	156.84	0.10	5.93	28.67	24.80	50.02	192.83	41.02
DHNC	1.11	7.97	58.92	33.56	66.44	0.00	22.88	643.03	881.79	222.73	0.07	5.14	33.95	31.70	82.96	284.11	84.54

Notes: the grain size of Clay < 4 μm, the grain size of Silt 4–63 μm, the grain size of Silt > 63 μm, Cr chromium, Cu copper, Zn zinc, Pb lead. Notes: Significant differences are indicated by the different letters in each row ( $P < 0.05$ ).

### 2.3. Illumina MiSeq sequencing analysis of microbial communities

The DNA in the sediment and sludge samples were extracted using the OMEGA Mag-Bind Soil DNA Kit (Omega Bio-Tek, Norcross, GA, USA) according to the manufacturer's protocols. The V4–V5 region of the 16S rRNA gene was amplified using previously described primers and conditions (Guo et al., 2018b). After purification using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantification using the QuantiFluor™-ST system (Promega, USA), the amplicons were pooled in equimolar amounts and paired-end sequenced ( $2 \times 250$ ) on an Illumina MiSeq platform at Personal Biotechnology Co., Ltd., Shanghai, China, according to the standard protocols. The original paired Illumina MiSeq reads were deposited in the NCBI sequence read archive (accession number: SRP136966).

### 2.4. PCR amplification and real-time qPCR

The functional gene of methanogens and methanotrophs were amplified with the primer sets 1106F (5'-TTWAGTCAGGCAACGAGC-3')/1378R (5'-TGTGCAAGGAGCAGGGAC-3') (Watanabe et al., 2006) and A189F (5'-GGNACTGGGACTTCTGG-3')/Mb661R (5'-ACRTAGTGTTAA CCTTG YAA-3') (Kolb et al., 2003), respectively. The PCR procedure, plasmid and standard curve construction are presented in the Supplemental Information (SI). Samples and standard reactions were conducted using an ABI 7500 Sequence Detection System (Applied Biosystems, Canada), and the procedure of qPCR are listed in the SI. Two strong linear relationships between the threshold cycles ( $C_T$ ) and the natural log<sub>10</sub>-transformed values of gene copy numbers ( $r^2 = 0.994$  and  $r^2 = 0.994$ , respectively) were obtained, and their corresponding amplification efficiencies were 97.1% and 98.2%, respectively. The abundances of the target genes were calculated based on the standard curve, and then converted into copies per gram of dry sediment. Three replicates were conducted for each sample.

### 2.5. Bioinformatics and statistical analyses

Raw data were processed using the USEARCH (version 5.2.236, <http://www.drive5.com/usearch/>) in QIIME software (version 1.8.0; Caporaso et al., 2010). Sequences were binned into operational taxonomic units (OTUs) by UCLUST, using a 97% identity threshold (Edgar, 2010). Alpha diversity indices, including those for the abundance based coverage estimator (Ace; Chao and Yang, 1993) and Shannon diversity (Shannon, 1948), were calculated using the MOTHUR 1.15.0 (Schloss et al., 2009). The cluster analysis was calculated using Primer 6 software (Primer-E Ltd., UK). A one-way analysis of similarity (ANOSIM) was performed with the sampling sites as the factor (Clarke and Warwick, 1994), and the value of ANOSIM statistic R ranged from -1 to 1. Redundancy discriminant analysis (RDA) with 9999 Monte Carlo permutation tests was conducted to reveal the relationship between bacterial assemblages and environmental factors using the R base packages (Liu et al., 2015). The metabolic functions of microbial communities were predicted in KEGG using the PICRUSt software (Langille et al., 2013). A network analysis was performed to indicate the co-occurrence and co-exclusion patterns among different microbial species (Faust and Raes, 2012), using the software of MOTHUR 1.15.0 and Cytoscape (<http://www.cytoscape.org/>) (Shannon et al., 2003; Schloss et al., 2009).

Spearman's rank correlation coefficient was used to evaluate the relationship between environmental factors and the diversity or abundance of microbial community using the SPSS.19.0 software (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test was performed to measure the difference of microbial diversity indices, the abundance of specific microbial taxon and tested genes. Differences were considered significant at  $P < 0.05$ .



### 3. Results and discussion

#### 3.1. Physicochemical properties of sediments

The physicochemical characteristics of the sediments taken from the estuarine and coastal areas are shown in Table 1. The salinity ranged from 0.16‰ to 1.11‰, which did not show a gradual increase from the inside river to the outside estuary in the Yangtze Estuary as presented in winter (Guo et al., 2018b). This was attributed to the abundant freshwater from the Yangtze River during the flood season. Additionally, the pH values ranged from 7.44 to 7.97, showing no significant difference between each other ( $P > 0.05$ ). The water contents of the sediments ranged from 31.11% to 58.92%. The sediments were mainly comprised of clay and silt (>98%), which was conducive to the adsorption of pollutants (Weber et al., 2001; Zhou et al., 2004). TOC concentrations changed distinctly among the eight sampling sites, with the highest concentration at SDK (70.46 mg/kg, dry weight). The average concentrations of TP in the sediments were 1055.63 mg/kg, and these concentrations at SDK and WSK were significantly higher than those at other sampling sites ( $P < 0.05$ ). The concentrations of sulphate ( $\text{SO}_4^{2-}$ ) ranged from 66.35 mg/kg to 245.54 mg/kg. The TN,  $\text{NO}_2^-$  and  $\text{NH}_4^+$  contents showed the high values at SDK, with concentrations of 1328.17 mg/kg, 0.13 mg/kg and 98.97 mg/kg, respectively. However, no significant differences were found in the concentrations of  $\text{NO}_3^-$  among the eight sites ( $P > 0.05$ ).

The average concentrations of Cr, Cu, Zn and Pb were 41.28 mg/kg, 80.54 mg/kg, 364.08 mg/kg and 56.82 mg/kg, respectively. All of these heavy metals had highest concentrations at SDK (76.50 mg/kg for Cr, 178.76 mg/kg for Cu, 1049.93 mg/kg for Zn and 77.88 mg/kg for Pb), except for Pb at DH (84.54 mg/kg). Moreover, all of the concentrations of target heavy metals in this study were higher compared to those in a previous study (28 mg/kg for Cr, 17 mg/kg for Cu, 49 mg/kg for Zn and 20 mg/kg for Pb) in the sediments taken along the Yangtze Estuary (Xu et al., 1997). These results indicated the heavy metal contamination could be more severe, which may be caused by the urbanization and industrial development in the recent two decades. Nevertheless, according to the soil environment guidelines (GB15618-1995; Cr  $\leq$  350 mg/kg, Cu  $\leq$  100 mg/kg, Zn  $\leq$  300 mg/kg, Pb  $\leq$  350 mg/kg), most of the heavy metal levels were within the prescribed values, except for Cu at SDK, Zn at LHK, SDK and WSK. Overall, elevated concentrations of nutrients and heavy metals including TOC, TN, TP,  $\text{SO}_4^{2-}$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$  and Zn, were found at SDK and WSK, where sediments were seriously affected by human activities, such as the WWTP effluent and urban domestic waste.

#### 3.2. Abundance and diversity of microbial community

A total of 791,695 valid sequences were generated from the eight sediment samples and 251,776 OTUs were obtained to calculate the rarefaction curves, rank-abundance curves, abundance and diversity of the microbial communities at a sequence divergence of 3%. The rarefaction curves for all samples tended to approach the saturation plateau, and new phylotypes continued to emerge even after 10,000 reads sampling with pyrosequencing (Fig. S2A). The rank-abundance curves revealed that a minority of sequences belonged to the highly abundant bacteria, whereas a majority of the sequences belonged to rare microorganisms (Fig. S2B).

The abundance and diversity of the microbial communities in the sediments are shown in Table S1. The average abundance (Ace index) in this study was 2177.5, which was nearly half of a previous determination (an average Ace index of 4236) in winter (Guo et al., 2018b). In addition, the diversity (Shannon index) was 9.01, which was comparable to the result in winter (an average Shannon index of 8.82) (Guo et al., 2018b). These seasonal variations of the abundance and diversity in the same sampling area could be mainly determined by temperature (Sun et al., 2014; Gao et al., 2016).

Spatially, the abundance of the microbial communities at SDK, WSK and DH were significantly lower than those at the other sampling sites ( $P < 0.05$ ), with an average Ace index of 1685.74, 1676.25 and 1656.61, respectively. Two environmental factors including the sand contents and  $\text{SO}_4^{2-}$  concentrations were significantly correlated with the abundances of microbial communities in this study (Table 2). As for the diversity of microbial communities, the lower values were found at SDK and WSK ( $P < 0.05$ ), with an average Shannon index of 7.85 and 8.25, respectively. These results may be attributed to the fact that WWTP effluent and urban runoff containing toxicants could potentially inhibit bacterial growth, and thus affect the microbial abundance and diversity in sediments (Drury et al., 2013; Wang et al., 2016). Furthermore, several environmental factors showed significant and negative correlations to the microbial diversity in the present study, such as TOC ( $r = -0.814$ ,  $P = 0.014$ ),  $\text{SO}_4^{2-}$  ( $r = -0.802$ ,  $P = 0.017$ ),  $\text{NH}_4^+$  ( $r = -0.790$ ,  $P = 0.020$ ), Cu ( $r = -0.826$ ,  $P = 0.011$ ), Zn ( $r = -0.886$ ,  $P = 0.003$ ) and Pb ( $r = -0.886$ ,  $P = 0.003$ ) (Table 2). Overall, these results might indicate that anthropogenic activities could cause the change of microbial abundance and diversity in sedimentary environments (Lu and Lu, 2014).

#### 3.3. Microbial taxonomic assignment and community composition

In total, 66 different phyla were determined in the eight sampling sites, where a total of 61, 64, 58, 55, 59, 64, 61 and 56 phyla were identified in the sediments collected from XP, QYK, LHK, SDK, WSK, SCK, CY and DH sites, respectively (Fig. 1 and Table S2). As compared to the results in winter (Guo et al., 2018b), seven new phyla appeared, including BHI80-139, FCPU426, H-178, Kazan-3B-28, MVS-104, OC31 and OP9, whereas ZB3 disappeared in summer (Table S2). The main dominant phyla and their relative abundance showed a similar variation trend among the eight sediment samples. *Proteobacteria* was the first dominant phylum for both summer (39.21%) and winter (47.4%) (Guo et al., 2018b), which was in line with previous studies on the bacterial compositions in the Yangtze Estuary (Feng et al., 2009; Sun et al., 2014; Liu et al., 2015). Besides of *Proteobacteria*, the three other dominant bacterial phyla were *Bacteroidetes* (13.68%), *Chloroflexi* (11.52%) and *Acidobacteria* (8.02%) at eight sampling sites. In addition, several studies have demonstrated that *Proteobacteria*, *Bacteroidetes* and *Acidobacteria* were generally the dominant microorganisms in sedimentary environments and are always important contributors to the biogeochemical processes, such as anaerobic ammonium oxidation and sulphate reduction (Xia et al., 2013; Guo et al., 2018b). Therefore, these results suggested that the biogeochemical processes may occur properly due to the relatively stable dominant bacterial community in the sediments along the Yangtze Estuary in summer. Noticeably, the relative abundances of *Euryarchaeota* at WSK (11.43%) and SDK (12.51%) were significantly higher than those at other sampling sites ( $P < 0.05$ ). Other two archaea phyla including *Crenarchaeota* and *Parvarchaeota* were also found among those eight sites. However, *Parvarchaeota* was not found only at SDK (Fig. 1 and Table S2).

The abundances of nitrite-oxidizing *Nitrospira* ranged from 0.02% to 2.43%, and the highest abundance of this genus was found at CY, indicating the microbial-related nitrogen cycle may occur frequently in this area (Fig. S3). *Dechloromonas*, WCHB1-05 and *Methanosaeta*, associated with the anaerobic organic matter degradation (Oakley et al., 2012; Yan et al., 2016), were enriched at WSK (6.86%, 2.58% and 8.10%, respectively) and SDK (5.98%, 2.12% and 9.01%, respectively) with elevated TOC contents (Table 1), suggesting that these bacteria may largely participate in the metabolism of organic matter (Fig. S3 and Table S3).

The dendrogram based on the Bray-Curtis dissimilarity method showed that the microbial communities of the eight sampling sites were divided into two clusters (Fig. 2). The communities in the sediments at WSK and SDK were clustered together, with a similarity of 89.28%, indicating a more similar community structure between these two sites. In addition, the communities at XP and QYK were clustered

**Table 2**  
Correlation analysis between the environmental factors and the diversity and abundance of microbial community in sediments.

	Salinity (%)	pH	Moisture content (%)	Clay content (%)	Silt content (%)	Sand content (%)	TOC (mg/kg)	TN (mg/kg)	TP (mg/kg)	SO <sub>4</sub> <sup>2-</sup> (mg/kg)	NO <sub>2</sub> <sup>-</sup> (mg/kg)	NO <sub>3</sub> <sup>-</sup> (mg/kg)	NH <sub>4</sub> <sup>+</sup> (mg/kg)	Cr (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Pb (mg/kg)
Abundance	<i>r</i>	-0.429	-0.695	-0.262	0.595	0.826*	-0.405	-0.262	-0.024	-0.929**	-0.436	-0.524	-0.500	0.190	-0.429	-0.310	-0.595
	<i>P</i>	0.289	0.056	0.531	0.120	0.011	0.320	0.531	0.955	0.001	0.280	0.183	0.207	0.651	0.289	0.456	0.120
Diversity	<i>r</i>	0.240	0.090	-0.479	0.778*	0.410	-0.814*	-0.563	-0.683	-0.802*	-0.439	0.287	-0.790*	-0.443	-0.826*	-0.886**	-0.886**
	<i>P</i>	0.568	0.831	0.230	0.023	0.314	0.014	0.146	0.062	0.017	0.276	0.490	0.020	0.272	0.011	0.003	0.003

Notes: *r*: Spearman's rank order correlation analysis, *P*: *P*-value, \*, significant at  $P < 0.05$ , \*\*, significant at  $P < 0.01$ .

together, with the most similarity of 94.16%. These two samples were then clustered with other sediments taken from LHK, CY, SCK and DH. The *R* value was 0.922, indicating that there was a significant difference in the microbial communities at the phylum level among the eight sediment samples ( $P < 0.05$ ).

#### 3.4. Abundances of the methane metabolism genes, methanogens and methanotrophs

The microbial communities at SDK and WSK were clustered together, due to the strong impact of human activities at these areas. Moreover, there was significantly elevated abundance of *Euryarchaeota* found at SDK and WSK (Fig. 1). Therefore, *Euryarchaeota* at the genera level were analyzed in all samples to further investigate the impact of anthropogenic activities on the composition of microbial community in estuarine sediments. In total, 336 different genera were detected (Table S3), and significant differences among the communities at the different sampling sites were observed, with an *R* value of 0.957 ( $P < 0.05$ ). Interestingly, most of genera belonging to *Euryarchaeota* at SDK and WSK were related to the methane metabolism, such as *Methanobacterium*, *Methanobrevibacter*, *Methanocorpusculum*, *Methanoregula*, *Methanospirillum*, *Methanosaeta*, *Methanomethylovorans* and *Methanomassiliococcus* (Table S3).

The relative abundance of the KEGG orthologs related to methane metabolism was analyzed in all sediment samples taken from the Yangtze Estuary and its coastal area. As shown in Fig. 3A, the relative abundance of methane metabolic pathways ranged from 1.10% to 1.77%. The high values of these metabolic pathways were found at SDK and WSK, which were consistent with the distribution pattern of *Euryarchaeota*. In general, methanogens and methanotrophs were involved in the metabolism of methane. The abundance of these two types of microbes was further quantified in the sediments along the Yangtze Estuary. As shown in Fig. 3B and C, compared with the abundance of methanotrophs (ranging from  $6.30 \times 10^3$  to  $8.03 \times 10^4$  copies/g), methanogens abundances were dominant in the sediments at all sampling sites, with abundances ranging from  $1.55 \times 10^6$  to  $4.99 \times 10^7$  copies/g. In addition, the higher abundances of methanogens were found at WSK and SDK ( $P < 0.05$ ), whereas the abundances of methanotrophs were lower at WSK and SDK, compared to other samples ( $P < 0.05$ ). These results suggested that the WWTP effluent and urban domestic outfall could affect the functional microbe of methane metabolism.

Elevated relative abundances of *Euryarchaeota* were found in the sediments at SDK and WSK, especially for the methanogens (Figs. 1 and 3). Therefore, considering these two samples sites were strongly impacted by human activities, including WWTP and urban runoff, two hypotheses were proposed to explain the high abundance of these archaea: (1) these archaea could be exogenous, transported by WWTP and urban runoff; (2) these archaea could be indigenous, whose growth could be promoted by extensive nutrition input from WWTP and urban runoff. In this respect, a activated sludge sample was taken from a nearby WWTP, which is located at the upstream of the sampling site SDK. The relative abundances and composition of *Euryarchaeota* were analyzed in the sludge samples. By comparing the abundances and composition of *Euryarchaeota* in WWTP sludge and SDK samples, we were able to get an insight of the potential reasons for the elevated abundance of these ecologic functional microbes in this area. The results showed that the relative abundance of *Euryarchaeota* in the activated sludge was only 1.35% (Fig. S4 and Table S5), which was comparable to previous studies that reported the relative abundance of *Euryarchaeota* were <5% in municipal WWTP sludge (Ju et al., 2014; Yang et al., 2014). Moreover, the absolute abundance of methanogens and methanotrophs, for the genera level, in the sludge was  $8.39 \times 10^6$  copies/g and  $2.00 \times 10^4$  copies/g, respectively (Fig. S5), and the sum of the abundance of these two groups abundance was lower than those in sediments taken from SDK ( $2.10 \times 10^7$  copies/g). Therefore,

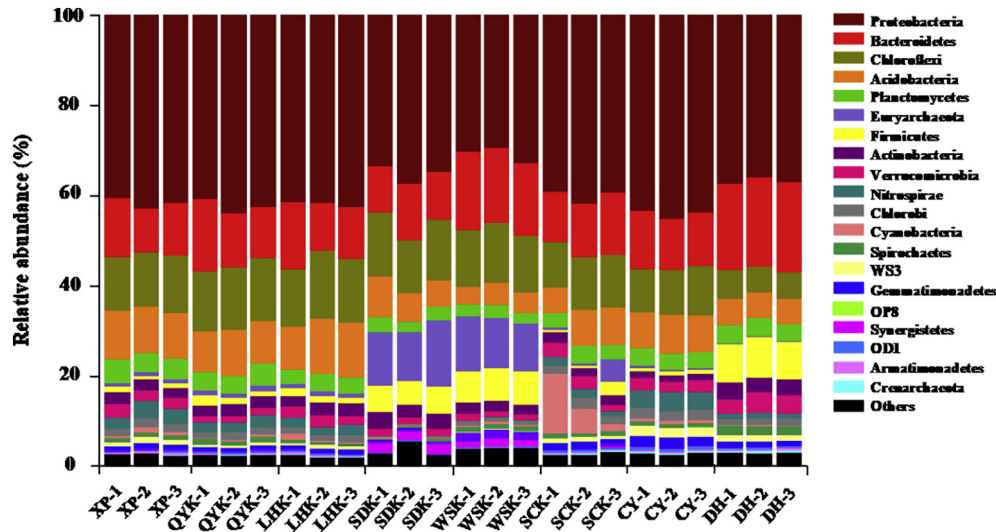


Fig. 1. Relative abundance of the top 20 phylum in the sediments taken along the Yangtze Estuary and its coastal area. Three replicates for each sample are labelled with the numbers 1, 2 and 3.

both the relative abundance of *Euryarchaeota* and the sum of the methanogens and methanotrophs abundance in sludges were significantly lower than those in the sediments at SDK, affected by WWPT effluent. Moreover, nine genera related to the metabolism of methane were detected in the sludge (Table S6), including *Methanobacterium* (0.24%), *Methanobrevibacter* (0.01%), *Methanomicrobiales* (0.01%), *Methanosphaerula* (0.04%), *Methanospirillum* (0.01%), *Methanosaeta* (0.52%), *Methanomethylovorans* (0.01%), *Methanosarcina* (0.40%) and *Methanomassiliicoccus* (0.003%). Six of them covered these genera in the sediments and the three others (*Methanomicrobiales*, *Methanosphaerula* and *Methanosarcina*) were not detected in the sediments at SDK. Additionally, the abundance of *Methanosaeta* in sludge was significantly lower than those in the sediments at SDK, with an abundance of 9.01% ( $P < 0.05$ ). Moreover, *Methanoregula*, *Methanocorpusculum* and *Methanolobus* were not found in WWPT sludge, but detected in the sediments at SDK (Table S3). Overall, these results suggested that the high relative abundance of *Euryarchaeota* at SDK may mainly be caused by the massive growth of indigenous *Euryarchaeota*, promoted by the extensive nutrient input by WWTP effluent, and the exogenous bacteria sources from the WWPT discharge cannot be excluded in the current study.

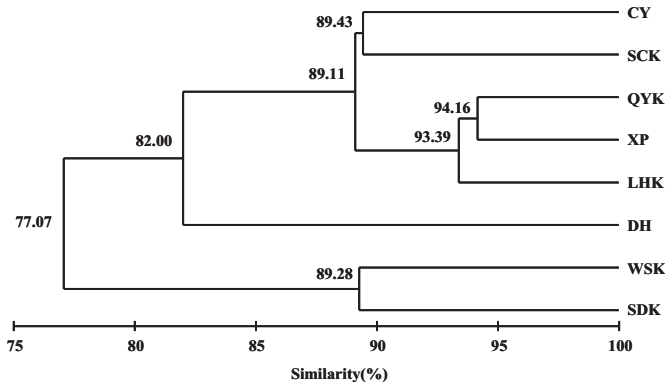


Fig. 2. Cluster analysis of microbial communities of the eight sediments taken along the Yangtze Estuary and its coastal area based on the Bay-Curtis dissimilarity.

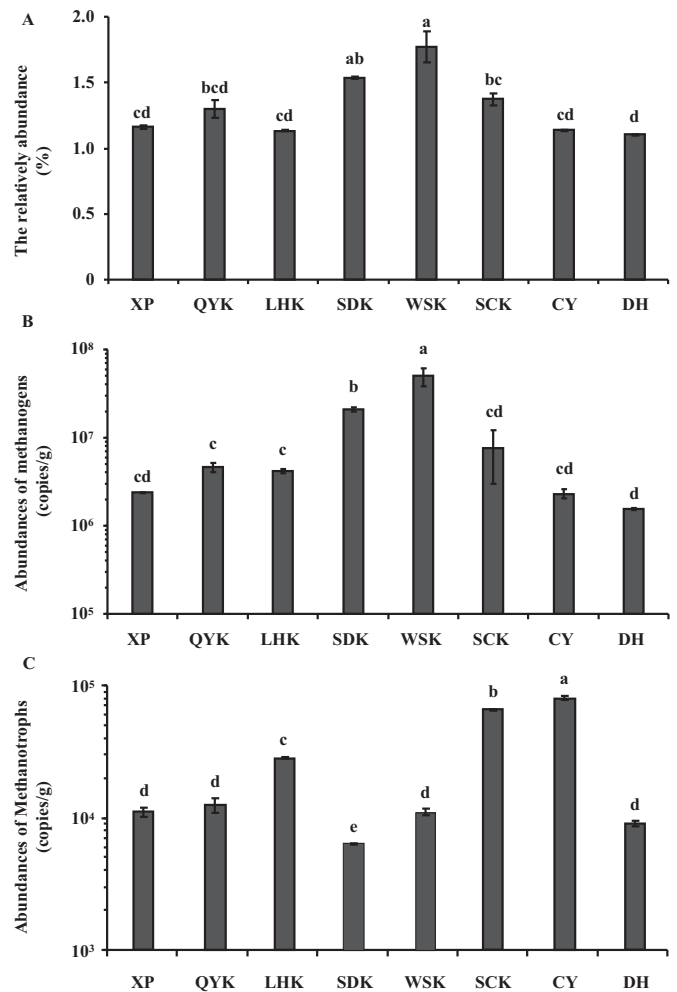


Fig. 3. Abundance of the methane metabolism genes, methanogens and methanotrophs in sediments along the Yangtze Estuary and its coastal area. A, methane metabolism genes, B, methanogens, C, methanotrophs.



### 3.5. Network analyses of the microbial community

The interrelationships among different microbial communities are vital to the structure, function and stability of microbial ecosystems (Fuhrman, 2009). However, the relationships among different microbial communities were generally neglected in previous studies on the sedimentary microbial communities in the Yangtze Estuary (Guo et al., 2018b). In this study, the interaction among microbial communities at the genera level was preliminarily explored to evaluate the role of a certain genera in the whole community in the sediments (Fig. 4). The top ten putative keystone genera that related to other bacteria were *Desulfobulbus*, *Methanosaeta*, *Nitrospira*, *Syntrophomonas*, *Syntrophus*, *Dechloromonas*, *Longilinea*, *Chitinophaga*, *Robiginitalea* and *Fluviicola*, with the number of associated genera not less than fifteen. These results

may be typically interpreted by the fact that other genera respond more strongly to the metabolites produced by these ten genera (Chaffron et al., 2010). For the top ten most abundant genera (Fig. S3), only six of them (*Dechloromonas*, *Methanosaeta*, *Nitrospira*, *Planctomyces*, *Luteolibacter* and *Geobacter*) were found in the network, among which three genera (*Dechloromonas*, *Methanosaeta* and *Nitrospira*) were ranked as the top ten genera that related to other bacteria. Moreover, although the relative abundance of *Desulfobulbus* was low (0.21%, Table S3), it directly linked to other nineteen genera, and six of them were co-excluded and thirteen of them were co-occurred in the study area (Fig. 4). Therefore, more attention should be given for such genera, as a key node in the microbial community, although its abundance may not be high (Chaffron et al., 2010; Faust and Raes, 2012).

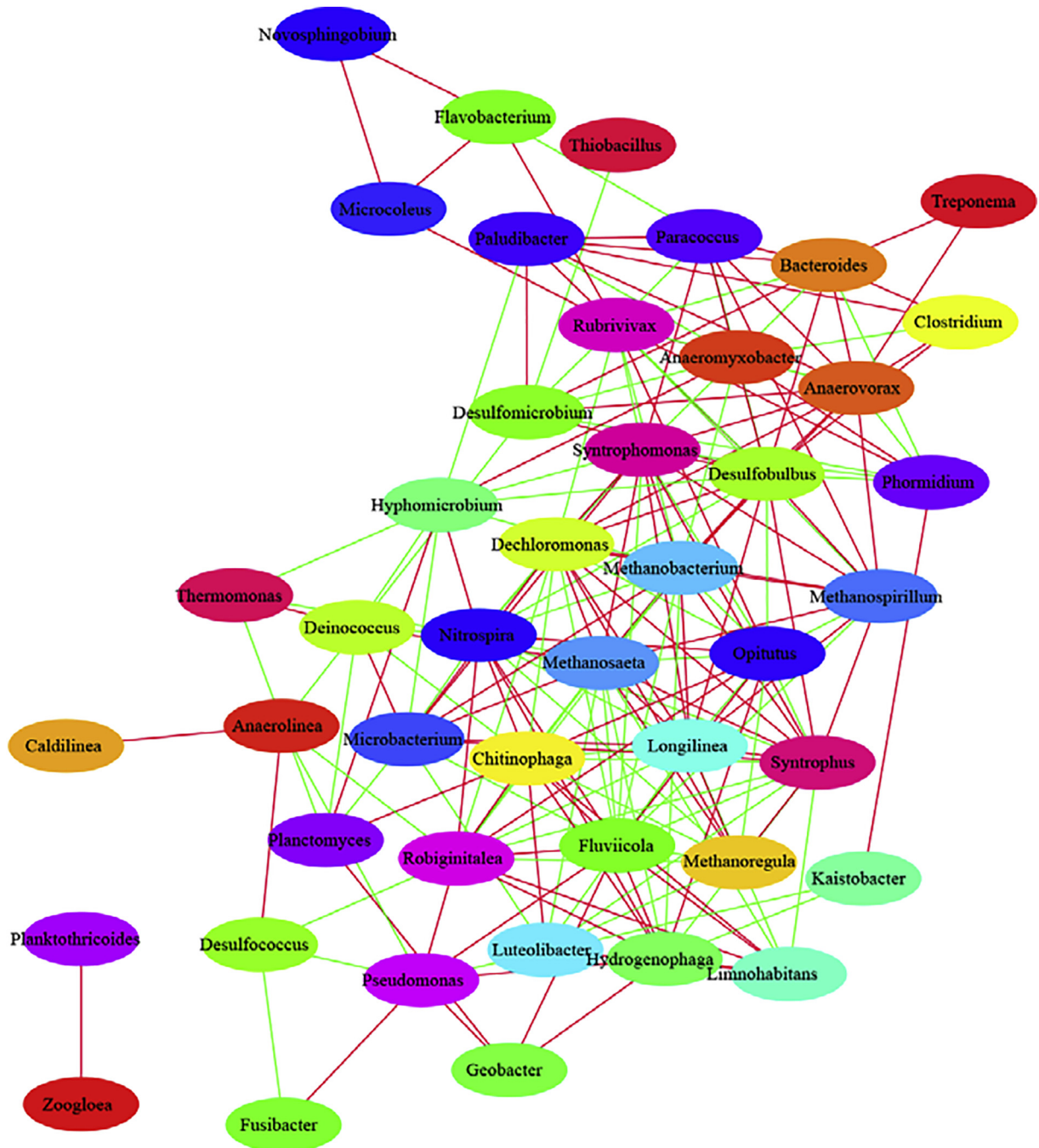


Fig. 4. Network analysis depicting co-occurrence and co-exclusion patterns among the top 50 abundant genera in sediments along the Yangtze Estuary and its coastal area. Red line means: co-occurrence, green line means: co-exclusion.

In addition, to investigate the effect of the high abundance and diversity of genera in *Euryarchaeota* on the whole bacterial community, the network of microbial communities at the genera level was reconstructed without SDK and WSK samples. SDK and WSK samples were excluded because this two sampling sites were seriously impacted by WWTP effluent and urban river runoff with relatively high level of contaminations (Table 1), and their microbial communities in these two samples were more similar to each other compared to those in the other six samples (Figs. 1, 2). The results showed that the relationships among microbes in the reconstructed network were changed greatly, and only *Methanoregula* and *Methanosaeta* were involved in the reconstructed network, which were different from the previous results that *Methanobacterium*, *Methanospirillum*, *Methanosaeta* and *Methanoregula* showed close correlation to other microbial communities (Fig. S6). These results suggested that the high abundance of *Euryarchaeota*, especially for the microbe related to the metabolism of methane, may play an important role on the interassociation and interaction among different species in the whole microbial community.

### 3.6. Correlation between the microbial communities and environmental factor

To explore the contribution of the environmental factors on microbial abundance and community composition, RDA was used to reveal the correlation between the microbial community and environment factors. As shown in Fig. 5, six of seven nutritional factors, including TOC, TN, TP,  $\text{SO}_4^{2-}$ ,  $\text{NO}_2^-$  and  $\text{NH}_4^+$  were more correlated to the microbial community at SDK and WSK, except for the  $\text{NO}_3^-$ , as the angle between the lines of  $\text{NO}_3^-$  and SDK or WSK was  $>90^\circ$ . Moreover, the relationships between the environmental factors and the abundances of the methane metabolic pathways, methanogens and methanotrophs were also analyzed, and the results showed that several nutritional factors were positively and significantly correlated to the abundance of methanogens, including TN ( $r = 0.54$ ,  $P = 0.01$ ), TP ( $r = 0.54$ ,  $P = 0.01$ ),  $\text{NO}_2^-$  ( $r = 0.59$ ,  $P < 0.01$ ) and  $\text{NH}_4^+$  ( $r = 0.50$ ,  $P = 0.01$ ). These results suggested that nutrients may promote the growth of methanogens in the sediments along the Yangtze Estuary, which was especially true for the samples taken at WSK and SDK. In addition, we found metal concentrations also have significant correlations to the abundance of methanogens

according to the RDA and relationship analysis (Table S4 and Fig. 5), and these may be explained by the fact that metals can work as stimulating agents for increasing methanogens growth and methane production in a certain dosage (Demirel and Scherer, 2011; Mudhoo and Kumar, 2013).

Several studies have demonstrated that salinity was vital to the distribution patterns of microbial communities in estuarine environments (Bouvier and Giorgio, 2002; Gao et al., 2016; Zheng et al., 2016; Guo et al., 2018b). However, in this study, salinity did not show a significant effect on both the abundance and composition of the microbial community (Table 2 and Fig. 5). This may be attributed to the small range of salinity during the flood season in the study area. Additionally, pH was also not significantly correlated with the abundance, diversity and community structure in this study (Table 2 and Fig. 5), and this result is in line with the finding in our previous research, as the pH values remained relative constant at different sampling sites (Guo et al., 2018b). Moreover, all three grain size fractions of sediments were correlated to the microbial community structure in the sediments (Pr values were all 0.001, Fig. 5), which is in accordance with previous researches (Wang et al., 2013; Y.L. Zheng et al., 2014; B.H. Zheng et al., 2014; Wang et al., 2017).

## 4. Conclusion

This study provides detailed information regarding the composition and structure of microbial communities and its influencing factors in the sediments taken along the Yangtze Estuary and its coastal area. *Proteobacteria* dominated the bacterial communities and their relative abundances had low variation at all sampling sites, whereas *Euryarchaeota* varied greatly among all of the samples, especially at WSK and SDK. At the genera level, *Euryarchaeota* were mainly comprised of methanogens, related to the methane metabolism. The abundances of methanogens and methanotrophs varied among different sampling sites, and they were significantly correlated to the concentrations of anthropogenic nutrients and metals. As compared to the *Euryarchaeota* in sludge, the high relative abundance of *Euryarchaeota* in sediment could be largely caused by the massive growth of indigenous species, promoted by extensive nutrients input by WWTP effluent. Network analyses showed that microbial communities related to the metabolism of methane may play an important role in the relationships of the whole microbial community. Environmental factors including grain size, metals and nutritional factors (TOC, TN, TP,  $\text{SO}_4^{2-}$ ,  $\text{NO}_2^-$  and  $\text{NH}_4^+$ ) influenced the composition and structure of microbial communities. Overall, methanogens, methanotrophs and their communities were suggested as potential bio-indicator species and biomarker communities to indicate anthropogenic activities in the sediments along the Yangtze Estuary and its coastal area. In addition, although certain microbial communities, such as *Desulfobulbus* in this study had a low relative abundance, it could also play a significant role as a key node in the whole community network.

## Acknowledgement

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2018.08.162>.

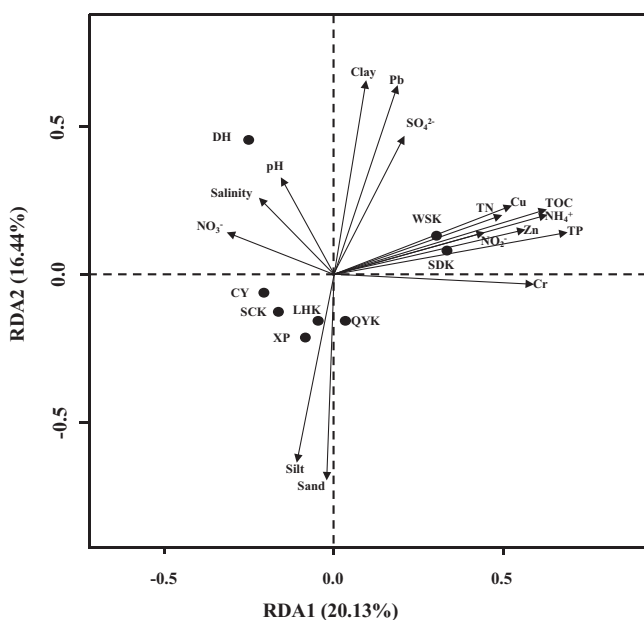


Fig. 5. Redundancy discriminant analysis (RDA) biplot of the distribution of microbial communities with environmental factors along the Yangtze Estuary and its coastal area.



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