Ecogenomic responses of benthic communities under multiple stressors along the marine and adjacent riverine areas of northern Bohai Sea, China

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HIGHLIGHTS

- Bacterial, protistan and metazoan communities were characterized with ecogenomics.
- The influences of salinity on benthic communities overwhelmed these of pollutants.
- Variations of community structures also associated with DDTs, PAHs or metals.

ARTICLE INFO

ABSTRACT

Benthic communities in the aquatic ecosystem are influenced by both natural and anthropogenic stressors. To understand the ecogenomic responses of sediment communities to the multiple stressors of polluted environments, the bacteria, protistan and metazoan communities in sediments from marine and adjacent riverine areas of North Bohai Sea were characterized by environmental DNA meta-systematics, and their associations with environmental variables were assessed by multiple statistical approaches. The bacterial communities were dominated by *Firmicutes* (mean 22.4%), *Proteobacteria* (mean 21.6%) and *Actinobacteria* (mean 21.5%). The protistan communities were dominated by *Ochrophyta* (33.7%), *Cercozoa* (18.9%) and *Ciliophora* (17.9%). *Arthropoda* (71.1%) dominated the metazoan communities in sediments. The structures of communities in sediments were shaped by both natural variables (spatial variability and/or salinity (presented as Na and Ca)) and anthropogenic contaminants, including DDTs, PAHs or metals (Cu, Al, Co, Cr, Cu, Fe, K, Mg, Mn, Ni and Zn). Particularly, the correlation network of multiple communities was modulated by the concentrations of Na and DDTs at the family level. Overall, environmental DNA meta-systematics can provide a powerful tool for biomonitoring, sediment quality assessment, and key stressors identification.

1. Introduction

The increasing contamination of sediment is a severe environmental problem throughout the world as it affects aquatic life and human health (Schwarzenbach et al., 2006; Zhao et al., 2016). The contaminants can cause systematic community-level effects in an ecosystem and eventually, lead to losses of biodiversity and ecological functions (Lake et al., 2000). Popular sediment quality assessment approach includes sediment chemistry, sediment toxicity tests, and benthic macroinvertebrates monitoring (Gerbersdorf et al., 2011). However, the ecological effects of multiple anthropogenic contaminants on multiple communities are largely unknown. Particularly, sediment microbiota is always neglected, despite their central role in the biogeochemical cycling (Fischer and Pusch, 2001), contaminant decomposition and other

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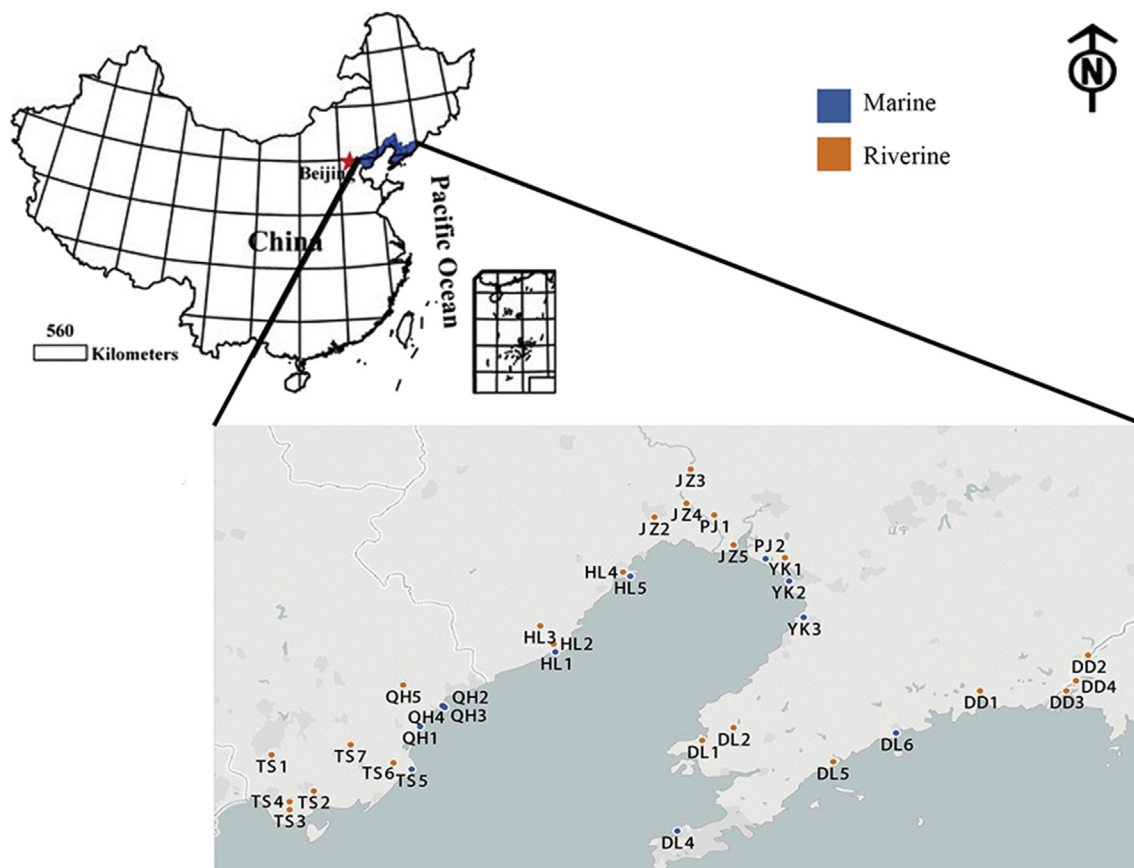[1] Equal contributors.

Fig. 1. Location of sampling sites in the marine and adjacent riverine areas of North Bohai Sea.

functions of aquatic ecosystem (Ducklow, 2008; Reed and Martiny, 2013).

The Bohai Sea, along with nearby rivers/estuaries and coast, are under rapid industrialization and urbanization (Hu et al., 2010). The Bohai Sea is threatened by numerous synthetic chemicals, and geogenic compounds discharged from agricultural, industrial and domestic activities. According to the results of previous environmental monitoring projects, we found ecotoxicological risks were posed by concentrations of organochlorine pesticides (OCPs) (Hu et al., 2010), heavy metals (chromium, lead, cadmium, zinc and mercury), metalloids (arsenic) (Luo et al., 2010), polychlorinated-dibenzo-p-dioxins and -dibenzofurans (PCDD/Fs) (Naile et al., 2011), and polycyclic aromatic hydrocarbon (PAHs) (Jiao et al., 2012). However, due to lack of sufficient biodiversity data in sediments from the Bohai Sea, the ecological association of concentrations of contaminants with multiple communities (bacterial, protistan and metazoan communities) were largely unknown.

Benthic communities, including bacterial, protistan and metazoan communities, can be affected by multiple anthropogenic contaminants (Saxena et al., 2015; Schwarzenbach et al., 2006; Xie et al., 2016). Comprehensive effects of anthropogenic contaminants on multiple communities are very labor-intensive, time-consuming and difficult to achieve if we chose the morphology-based approaches. The taxa/OTU matrix generated by environmental DNA (eDNA) meta-systematics could be used to identify the major stressors from the multiple ones (Saxena et al., 2015; Xie et al., 2016). eDNA meta-systematics provide a powerful tool for monitoring multiple communities in aquatic ecosystems (Gibson et al., 2014). Bulk sediment DNA records the phylogenetic information of sediment communities. eDNA meta-systematics can identify and

enumerate individual taxa that allow fine-scale analyses of ecosystems (Thomsen and Willerslev, 2015).

Here, we hypothesize that the effects of anthropogenic variables (chemical contaminants) on *in situ* bacterial, protistan and metazoan communities could be different from that of natural environmental variables (salinity). The objectives of this study were 1) to characterize the bacterial, protistan and metazoan communities in sediments from the Bohai Sea; and 2) to examine the relationships between the diversities and structures of bacterial, protistan and metazoan communities and concentrations of anthropogenic contaminants. In this study, the bacterial, protistan and metazoan communities were characterized from the bulk sediment DNA using the eDNA meta-systematics approach.

2. Materials and methods

2.1. Sediments and concentrations of anthropogenic contaminants

Ten marine and twenty-five riverine sediment samples were collected along the marine and adjacent riverine/estuarine areas of the northern Bohai Sea, China, in May 2008 (Fig. 1). The top 10-cm layer of sediments was collected from the sedimentation basin of the bed close to the bank using a trowel. Five subsamples from an area of about 5 m^2 at each sampling site (~1 kg) were mixed for a representative sample (Hu et al., 2010). The sediments were wrapped in polyethylene boxes and transported to the laboratory on ice. The sediments were then homogenized and freeze-dried before analysis. According to USEPA Method 3051, an aliquot of sediments were digested with HNO$_3$ and H$_2$O$_2$ before quantifying arsenic (As), cadmium (Cd), copper (Cu), chromium (Cr), lead (Pb),

and zinc (Zn). According to USEPA Method 7471 A, to determinate the concentration of mercury (Hg), an aliquot of sediments was digested with a combination of H_2SO_4 and HNO_3. Quantification of As and metals in sampling solutions were conducted on an inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7500) (Luo et al., 2010). Ten g dried sediments were Soxhlet extracted with 400 ml of 1:1 hexane/dichloromethane (Omni-Solv grade, EMD Chemicals Gibbstown, NJ, USA) and concentrations of PCDD/Fs were characterized by isotope-dilution following USEPA Method 1613 (Naile et al., 2011). Five g sediment was mixed with 2 g of anhydrous sodium sulfate and 1 g of activated Cu, and then extracted with 210 ml of methylene chloride in a Soxhlet apparatus for 48 h. Concentrations of PAHs in extracted solutions were determined by an Agilent 6890 gas chromatograph (GC) equipped with a 5973 mass selective detector (MSD) in selective ion monitoring (SIM) mode (Jiao et al., 2012). OCPs were quantified by solid−liquid extraction followed by quantification through gas chromatography (GC) equipped with electron capture detector (ECD) following the previously described procedures (Hu et al., 2010). Total organic carbon contents (TOC) in sediments were measured using an elemental analyzer (EA, Elementar, Hanau, Hesse, Germany) after removing inorganic carbon using 1M hydrochloric acid (HCl, Sigma Aldrich). The concentrations of sedimentary organic compounds were normalized to TOC (1%).

2.2. DNA isolation, PCR amplification, and next generation sequencing

DNA was extracted from a 0.25 g aliquot of a homogenized sample with the MoBio Power Soil DNA Kit (MoBio Laboratories Inc., CA, USA). Bacterial 16s rRNA genes were amplified following the previously published protocol (Xie et al., 2016). Protistan 18S rRNA genes were amplified by PCR from DNA using the V9 primers (Amaral-Zettler et al., 2009). A 20 μL reaction system was set up for each PCR amplification with Platinum® Taq polymerase (Life Technologies, CA, USA). The amplification was conducted in a SureCycler 8800 Thermal Cycler (Agilent Technologies, CA, USA) under the following conditions: initial denaturation at 94 °C for 2 min, 28 cycles at 94 °C for 15 s, 50 °C for 30 s and 68 °C for 30 s, and a final extension at 68 °C for 7 min. The metazoan mitochondrial Cytochrome Oxidase subunit I gene (COI) were amplified using mlCOIintF and dgHCO2198 primers (Leray et al., 2013). Triplicate PCR reactions were performed for each sample to minimize potential PCR bias. Ten ng of extracted DNA was used per 50 μL reaction mixture. PCR products were checked, purified and quantified. All purified PCR products were pooled equally for subsequent sequencing. Sequencing templates were prepared with the Ion OneTouch V2™ and sequenced in the Ion Proton or PGM sequencer (Life Technologies, CA, USA).

2.3. Bioinformatics analyses

Low quality raw reads (mean quality < 20, scanning window = 50) and sequences which contained ambiguous 'N', homopolymer and were shorter than 100 bp in length were discarded by the QIIME toolkit (Caporaso et al., 2010). Chimera removal and operational taxonomic units (OTUs) clustering were conducted following the UPARSE pipeline (Edgar, 2013). Taxonomy of bacterial, protistan and metazoan OTUs was assigned against the Greengenes database (DeSantis et al., 2006), the Protist Ribosomal Reference database (Guillou et al., 2013) and self-constructed metazoan COI database, respectively. OTU table was rarefied at equal sequencing depth to reduce biases resulting from differences in sequencing depth. Richness (Observed OTUs #) and alpha-diversity (Shannon entropy) were calculated on all twenty equal-depth rarefactions and then averaged. Beta-diversity was estimated by computing unweighted unifrac distances between samples. Sequence data were deposited in the NCBI Sequence Read Archive under accession number SRP095859.

2.4. Statistical analyses

Statistical analyses of communities in sediment were performed using R Statistical Language (https://cran.r-project.org/) and PRIMER7 with PERMANOVA + add-on software (PRIMER-E Ltd, Ivybridge, UK) (Lozupone and Knight, 2005). The statistical significance was set at P < 0.05. Environmental variables were firstly grouped based on the concentration distribution. Environmental variables were transformed ($Log_e(x + 1)$) and normalized. The nonparametric Kruskal-Wallis (KW) test was conducted to detect significant differential features between the marine and riverine sediments. Unweighted unifrac distance matrices were compared using permutational multivariate analysis of variance test (PERMANOVA, 9999 permutations) (Lozupone and Knight, 2005) to determine whether community structures were different between the marine and riverine sediments. The relative contributions of environmental variables in explaining differences in community structure were determined with forward selection distance-based linear modeling (distLM) (Lozupone and Knight, 2005). The Mantel test was used to evaluate the association among community structures and the environmental variables (Diniz-Filho et al., 2013). The principal coordinate analysis (PCoA) was performed to visualize the relationships between the environmental variables and community structures (Lozupone and Knight, 2005). A correlation network among families was generated by SparCC with 100 bootstraps to assign P-values (Friedman and Alm, 2012). The network was filtered to include only correlations with a correlation $\rho > 0.65$ and a 'two-tailed' P value < 0.01. The correlations between families and environmental variables were confirmed to be robust if the Spearman's correlation coefficient ($|r_{spearman}|$) was > 0.6 and the adjusted FDR was statistically significant ($P_{FDR} < 0.05$). The network was displayed and analyzed with Cytoscape V3 (Shannon et al., 2003).

3. Results and discussion

3.1. Summary of sedimentary chemistry

The concentrations of inorganic (As, metals) and organic contaminants were varied across samples (Fig. 2; detail information is available in supporting information Table S1). The concentrations of Cu were significantly correlated with that of aluminum (Al), cobalt (Co), Cr, Cu, iron (Fe), kalium (K), magnesium (Mg), manganese (Mn), nickel (Ni) and Zn (supporting information Fig. S1). These correlations indicated the similar environmental sources and behaviors of metals in the area of Bohai Sea. Cu was selected for further analyses to represent those correlated metals (Al, Co, Cr, Cu, Fe, K, Mg, Mn, Ni and Zn).

The sediments collected from the marine and adjacent riverine area of north Bohai Sea were under the stress of multiple stressors based on the ecotoxicological evaluation. The concentrations of As, Cd, Cr, Cu, Pb, Ni, PAHs and DDTs of some sediments from both freshwater ecosystem and marine ecosystem in the area of Bohai Sea exceeded the corresponding threshold effects levels (TEL) (Fig. 2) (ANZECC/ARMCANZ, 2000), where possible adverse biological effects can be expected (Long, 1992; Sun et al., 2013). Furthermore, the concentrations of PAHs of both YK3 and TS1 exceeded the probable effects level (PEL), and 40% brine sediments contained a higher concentration of DDTs than PEL (Fig. 2) (ANZECC/ARMCANZ, 2000). Two brine sediments (DL4 and QH1)
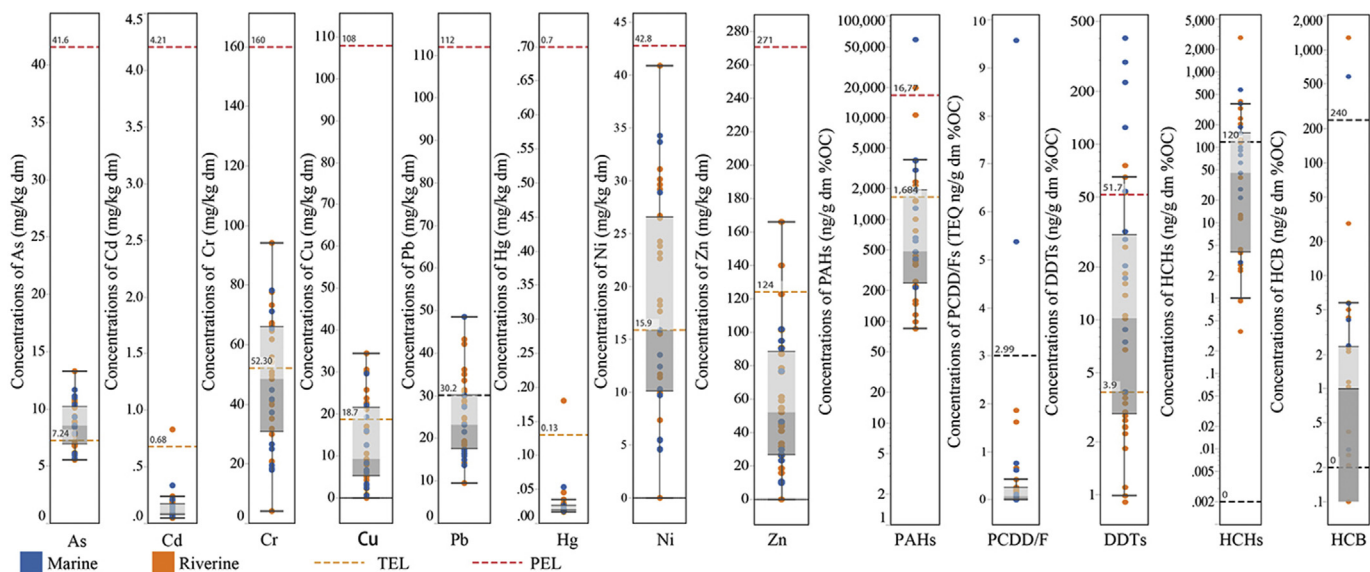
Fig. 2. Potential ecological risk caused by multiple contaminants in sediments from the north Bohai Sea. The threshold effects level (TEL) and probable effects level (PEL) of sediment quality guidelines were colored in yellow and red respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

contained a higher concentration of PCDD/Fs than the equilibrium partitioning sediment quality benchmark (Friedman and Lohmann, 2014). The concentrations of HCHs of about 34% sediments were more than the Severe Effect Level, and the concentrations of HCB of 46% sediment were more than the Lowest Effect Level of sediment quality guidelines in Ontario (Fig. 2) (Persaud et al., 1969). Sediments contaminated by multiple contaminants might cause

systematic adverse effects at all levels of the biological organization from molecule, individual to population and community in the aquatic ecosystems (Fent, 2003).

3.2. Next generation sequencing data

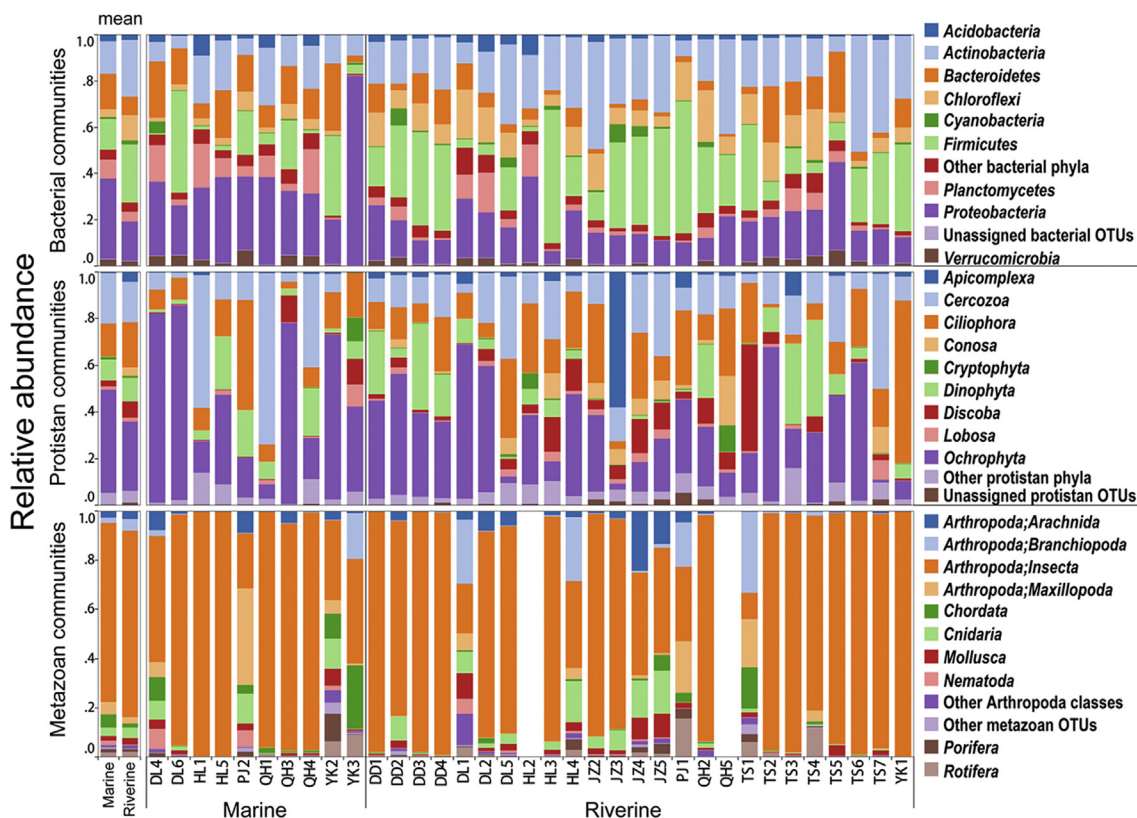After the quality check of raw reads, there were totally 4,487,830

Fig. 3. Composition of the bacterial, protistan and metazoan communities in sediment samples from the Bohai Sea. (A) Mean of the relative abundance of different taxa in both brine and freshwater sediments. (B) Relative abundances of different taxa at phylum level in each sample. Low abundance phyla (<1%) were not presented.

bacterial 16S V3 sequences, 2,179,742 protistan 18S V9 sequences, and 856,220 metazoan COI sequences across all sediment samples from the Bohai Sea. Reads due to the poor quality of sequencing, short length, PCR bias, lack of annotated references or lineage filtering were discarded (Bragg et al., 2013). Totally 37,621 bacterial OTUs, 12,416 protistan OTUs, and 5662 metazoan OTUs were clustered from the filtered sequences, representing 75 bacterial phyla, 25 protistan phyla and 13 metazoan phyla. The rarefaction curves of bacterial, protistan and metazoan communities started saturated from more than 160,000, 60,000 and 25,000 sequences per sample respectively. The OTU tables for bacterial, protistan and metazoan communities were randomly sampled at 12,399, 8653 and 2940 sequences per sample respectively, capturing most of the trends in the diversity of all sediments (Supporting information Fig. S2). Due to low sequencing depth, HL2 and QH5 were discarded from the metazoan community analyses.

3.3. Compositions of multiple communities in sediments from the Bohai Sea

The compositions of benthic communities varied across all sediment samples, as revealed by eDNA meta-systematics (Fig. 3).

Diversities of communities in sediment were dominated by a few abundant phyla. *Firmicutes* (0.5–57.5%, mean 22.4± 16.6%), *Proteobacteria* (5.8–81.8%, mean 21.6± 13.6%), *Actinobacteria* (5.1–50.2%, mean 21.5± 11.3%) and *Bacteroidetes* (1.2–28.9%, 10.2± 7.9%) dominated the bacterial communities. *Ochrophyta* (1.1–83.0%, 33.7± 23.9%), *Cercozoa* (2.3–73.7%, 18.9± 15.9%) and *Ciliophora* (1.6–68.8%, 17.9± 14%) dominated the protistan communities. The metazoan communities were dominated by *Arthropoda* (7.3–99.7%, 71.1± 27.9%). The observation that bacterial communities in the sediment samples from the Bohai Sea were dominated by *Proteobacteria*, *Actinobacteria* and *Bacteroidetes* is consistent with the results of previous studies on freshwater and brine sediments around the world (Costa et al., 2014; Saxena et al., 2015; Xie et al., 2016). The dominant role of *Ochrophyta* and *Ciliophora* in protistan communities dwelling in sediments was also observed in other aquatic ecosystems (Lei et al., 2014; Massana et al., 2015; Simon et al., 2015). Additionally, the protistan communities were more significantly correlated with the bacterial communities (Mantel test: Rho = 0.726, p < 0.001) than the metazoan communities (Mantel test: Rho = 0.331, p < 0.001), revealing intimate connections between bacteria and protists in the food web.
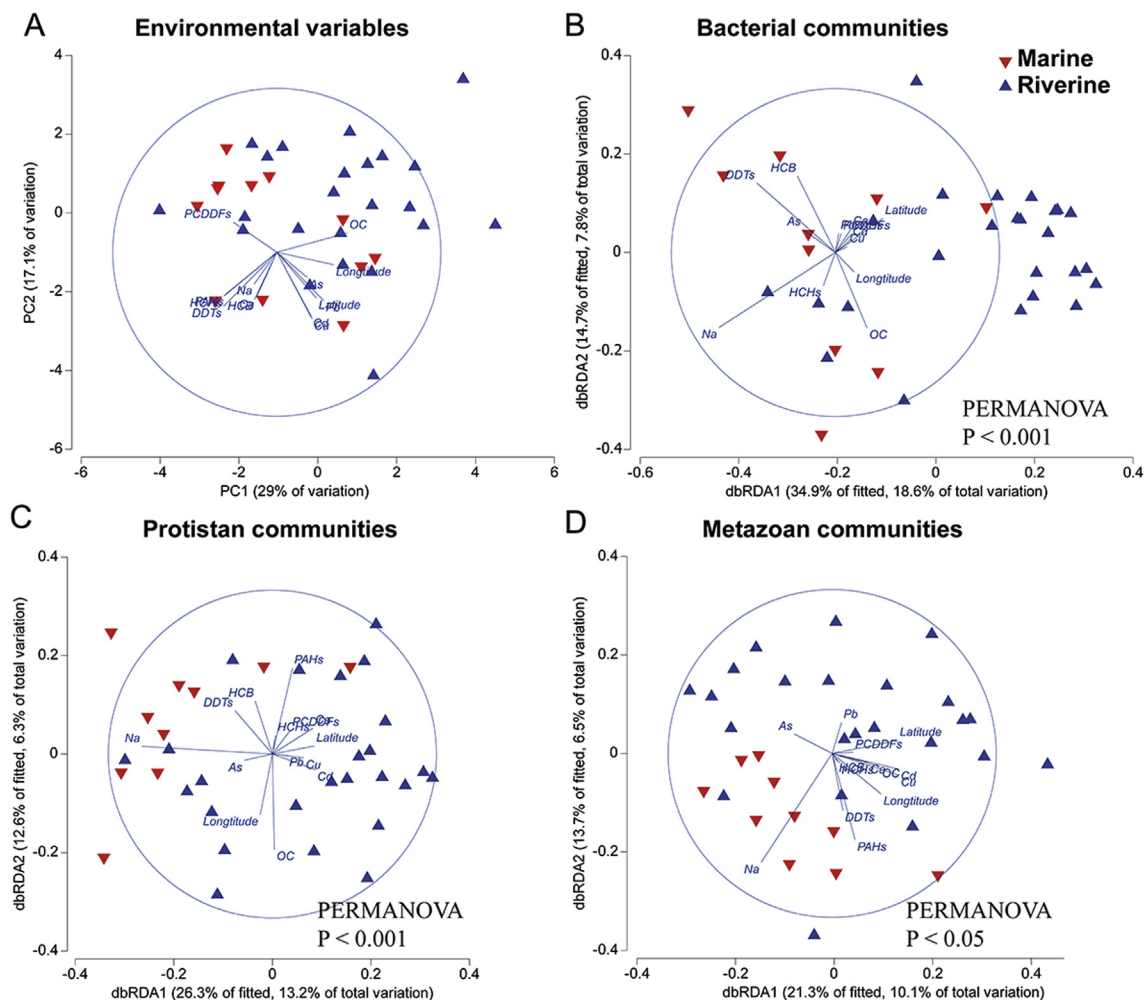
Fig. 4. Ordination of measured environmental variables (A), bacterial communities (B), protistan communities (C) and metazoan communities (D). (A) PCA ordination plot. (B–D) Ordination plot of PCoA with unweighted Unifrac distance matrices. The structures of communities in marine sediments were compared with communities in riverine sediments using PERMANOVA (9999 permutations). Blue vectors point to the direction of the increase for a given variable. Sediments with similar environmental variable profiles or eukaryotic communities are localized in similar positions in the diagram. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
DistLM results of multiple community structures against 14 predictor variables in the full analysis (9999 permutations). Significance was determined at P values < 0.001 (***), <0.01 (**), <0.05 (*) and non-significant (ns). **Bold** = significantly correlated with community structure at a = 0.05. Prop. = Proportion of variation explained; Cumul. = Cumulative proportion of variation explained.

Community	Marginal tests			Sequential tests		
	Variable	F_{Pseudo}	Prop.	F_{Pseudo}	Prop.	Cumul.
Bacteria	Na	4.94	**13.0%, ***	4.94	**13.0%, ***	13.0%
	DDTs	3.53	**9.7%, ***	2.52	**6.4%, ***	19.4%
	Ca	0.67	2.0%, ns	1.67	**4.1%, *	23.5%
	Latitude	2.03	**5.8%, *	1.42	**3.4%, *	27.0%
	HCB	1.73	**5.0%, *	1.38	3.3%, ns	30.3%
	PAHs	1.61	**4.7%, *	1.32	3.1%, ns	33.4%
	Longitude	1.42	4.1%, ns	1.10	2.6%, ns	36.0%
	As	0.99	2.9%, ns	1.16	2.7%, ns	38.8%
	HCHs	1.44	4.2%, ns	1.14	2.7%, ns	41.4%
	OC	2.00	**5.7%, *	1.29	3.0%, ns	44.4%
	Cd	0.88	2.6%, ns	1.20	2.8%, ns	47.2%
	PCDDFs	0.76	2.2%, ns	0.93	2.1%, ns	49.3%
	Pb	0.74	2.2%, ns	0.91	2.1%, ns	51.4%
	Cu	0.82	2.4%, ns	0.81	1.9%, ns	53.3%
Protista	Na	3.05	**8.5%, ***	3.05	**8.5%, ***	8.5%
	Ca	0.99	2.9%, ns	2.14	**5.7%, ***	14.2%
	PAHs	1.79	**5.1%, **	1.94	**5.0%, ***	19.2%
	Longitude	1.51	**4.4%, *	1.45	**3.7%, *	23.0%
	DDTs	1.92	**5.5%, **	1.25	3.2%, ns	26.2%
	Latitude	1.57	**4.5%, *	1.21	3.1%, ns	29.2%
	HCB	1.19	3.5%, ns	1.17	3.0%, ns	32.2%
	Cd	1.18	3.4%, ns	1.14	2.8%, ns	35.0%
	PCDDFs	1.02	3.0%, ns	1.02	2.5%, ns	37.6%
	Cu	1.05	3.1%, ns	1.02	2.6%, ns	40.1%
	HCHs	1.19	3.5%, ns	0.98	2.5%, ns	42.6%
	OC	1.89	**5.4%, **	0.98	2.5%, ns	45.0%
	Pb	1.07	3.1%, ns	0.99	2.5%, ns	47.5%
	As	1.09	3.2%, ns	0.98	2.4%, ns	49.9%
Metazoan	Latitude	1.83	**5.6%, **	1.83	**5.6%, **	5.6%
	Na	1.77	**5.4%, **	1.64	**4.9%, *	10.5%
	PAHs	1.37	4.2%, ns	1.47	**4.3%, *	14.8%
	Cu	1.63	**5.0%, *	1.51	**4.3%, *	19.2%
	Cd	1.50	**4.6%, *	1.12	3.2%, ns	22.4%
	PCDDFs	0.86	2.7%, ns	1.12	3.2%, ns	25.6%
	Longitude	1.21	3.8%, ns	1.07	3.1%, ns	28.6%
	As	0.82	2.6%, ns	1.15	3.3%, ns	31.9%
	OC	1.31	4.1%, ns	1.07	3.0%, ns	34.9%
	DDTs	1.40	4.3%, ns	1.03	2.9%, ns	37.8%
	Pb	1.03	3.2%, ns	0.95	2.7%, ns	40.5%
	HCHs	0.96	3.0%, ns	0.88	2.5%, ns	43.0%
	Ca	0.82	2.6%, ns	0.79	2.3%, ns	45.3%
	HCB	0.74	2.3%, ns	0.70	2.0%, ns	47.3%

Table 2
Association between multiple community structures and environmental variables of the sediments with Mantel test (9999 permutations). Geographic distances between every two sampling locations were calculated using the "Vincenty" formula (WGS-84) from the latitudes and longitudes. Dissimilarity matrices for the eukaryotic communities and environmental variables were constructed using unweighted unifrac and Euclidean distances, respectively. Significance was determined at P values < 0.001 (***), <0.01 (**), <0.05 (*) and non-significant (ns). **Bold** = significantly correlated with community structure at a = 0.05.

Variable	Community		
	Bacteria	Protista	Metazoan
Geo	−0.019, ns	0.054, ns	0.104, ns
OC	−0.005, ns	0.085, ns	−0.024, ns
Na	**0.405, ***	0.367, ***	0.112, ns
Ca	0.053, ns	0.047, ns	−0.074, ns
(Na, Ca)	**0.406, ***	0.374, ***	0.101, ns
As	−0.023, ns	0.003, ns	−0.021, ns
Cd	−0.038, ns	−0.017, ns	0.148, ns
Cu	−0.036, ns	0.005, ns	−0.065, ns
Pb	−0.068, ns	−0.018, ns	−0.037, ns
(As, Cd, Cu, Pb)	−0.122, ns	−0.068, ns	−0.01, ns
Inorganic	0.002, ns	0.074, ns	0.021, ns
DDTs	**0.284, ***	0.229, ***	0.202, *
HCB	0.044, ns	−0.052, ns	0.287, *
HCHs	0.024, ns	0.02, ns	0.113, ns
(DDTs, HCB, HCHs)	**0.249, ***	0.153, *	0.249, **
PAHs	0.086, ns	0.167, *	0.062, ns
PCDD/F	0.015, ns	0.014, ns	0.056, ns
(PCDD/F, PAH)	0.092, ns	0.158, *	0.115, ns
Organic	**0.233, ***	0.179, **	0.214, *
All-measured	0.097, ns	0.14, *	0.113, ns

Fortunato et al., 2012; Herlemann et al., 2011; Rousk et al., 2010; Telesh et al., 2013).

Despite the important roles of spatial variability and/or salinity (presented as the concentrations of Na and Ca), the structures of sedimentary communities from the Bohai Sea were also influenced by anthropogenic pollutants, including DDTs, PAHs or metals (Cu, Al, Co, Cr, Cu, Fe, K, Mg, Mn, Ni and Zn) (Tables 1 and 2 and Fig. 4). The change of bacterial community structures was associated with both inorganic (Na and Ca) and organic compounds (DDTs, HCB, and PAHs). The changes of protistan community structures were associated with salinity, PAHs, and DDTs, while the shifts of metazoan communities were correlated with metals (Cd, Cu, Al, Co, Cr, Cu, Fe, K, Mg, Mn, Ni and Zn) and organochlorine pesticide (DDTs and HCB). According to the results of Mantel test, among these three communities, only the structures of protistan communities were significantly associated with the distance matrix of all environmental variables (Table 2). About half of the variations of communities cannot be explained by the measured environmental variables. Environmental variables other than those examined in this study could also influence the communities in sediments. Additionally, the alpha-diversity (Shannon entropy) of protistan communities was negatively correlated with the concentration of HCHs (linear regression, P < 0.05), which suggested that the overall diversity of protistan communities might be disturbed by HCHs.

The correlation network revealed the significant ecological effects of Na and DDTs on communities in sediment samples from the Bohai Sea (Fig. 5), which is in consistence with the results of the multiple statistical approaches (PCoA, distLM, and Mantel tests). Strong correlations among relative abundances of taxa at the family level and concentrations of environmental variables were explored to get deep insight into a vast array of complex and previously poorly understood phenomena. Among the inorganic variables, Na was the dominant variable correlated with 241 nodes (accounting for 39.4% of all families) in the correlation network. Among the organic variables investigated, DDT was the dominant variable that connected with 46 nodes (accounting for 7.7% of all families).

3.4. Natural variables and anthropogenic contaminants shaped the structures of communities in sediments from the Bohai Sea

Spatial variability and salinity played dominant roles in shaping the structures of bacterial, protistan and metazoan communities in sediments from Bohai Sea (Fig. 4). Despite the spatial effects, the concentration of Na was the key driver for the changes of bacterial, protistan and metazoan communities in sediments according to the results of distLM and Mantel tests (Table 1 and 2). The alpha-diversities or structures of bacterial, protistan and metazoan communities shifted along a river to sea salinity gradient (Campbell and Kirchman, 2013). The alpha-diversity (Shannon entropy) of protistan communities in marine sediments were significantly lesser than that of riverine sediments (Kruskal-Wallis test). As pelagic and edaphic communities, the influences of natural variables (for example, spatial variability, salinity and pH) overwhelmed anthropogenic contaminations in both prokaryotic and eukaryotic communities in aquatic sediments across a salinity gradient (presented as the concentration of Na and Ca) (Fierer and Jackson, 2006;
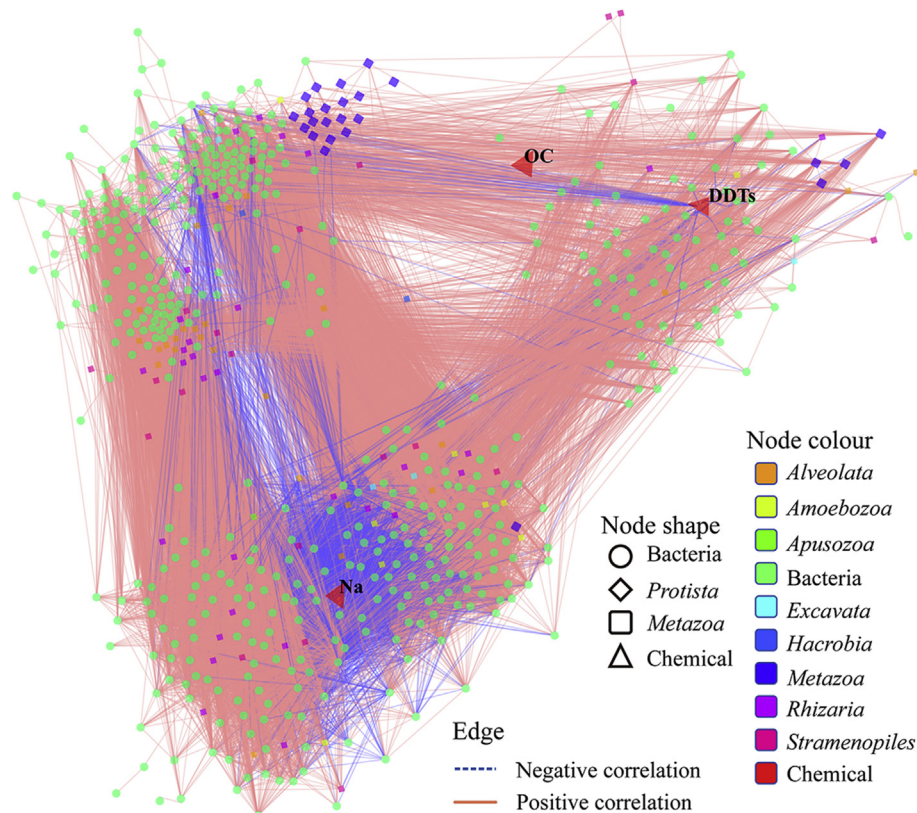
Fig. 5. Correlation network between bacterial, protistan and metazoan communities and environmental variables in sediment samples from the Bohai Sea. The correlations between families were generated by SparCC with 100 bootstraps to assign P-values. The associations were filtered to include only correlations with a correlation $\rho > 0.65$ and a 'two-tailed' P value < 0.01. The correlations between eukaryotic classes and environmental variables were confirmed to be robust if the Spearman's correlation coefficient (|rspearman|) was >0.50 and the adjusted FDR was statistically significant (PFDR < 0.05). Correlation coefficients between two nodes were labeled, positive coefficient in red, while negative coefficient in blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Within these 46 nodes, 45 nodes were negatively correlated with DDTs except *Pelagibacteraceae*. Although the associations were correlation-based, it highlighted significant roles of DDTs or other DDTs associated factors in succession of sedimentary communities in the Bohai Sea area. The results confirmed that the sediment communities can be affected by both natural variables and anthropogenic activities. However, the complex interaction pattern between natural factors and anthropogenic contaminants should be investigated in further studies.

Anthropogenic contaminants (metals, PAHs and pesticides) can influence multiple communities in complex ways. Although some metals (for instance, Zn, Mn) have been classified as a micro-nutrient required by living organisms and are involved as cofactors in enzymes (Andreini et al., 2008; Hansch and Mendel, 2009), high concentration metals are toxic to ciliated protists (Madoni and Romeo, 2006), algae (Duong et al., 2010; Peterson et al., 1984; Wong et al., 1978), and benthic macroinvertebrates (Faupel et al., 2012; Leung et al., 2016; Monteiro et al., 2014). The adverse effects of high concentration of some metals are due to their abilities to block and inactivate the sulfhydryl groups of proteins (Valls and de Lorenzo, 2002). Responses of sediment communities to changes of metals' concentration can include a decrease in biomass (Gillan and Pernet, 2007), changes in alpha-diversity (Xie et al., 2016) and structures and modulation of enzyme activities (Hoostal et al., 2008; Wainwright and Duddridge, 1982). Additionally, organic contaminants (for example, DDTs, PAHs) jeopardize the health of freshwater ecosystems and can contribute to diversity losses of freshwater and marine ecosystems (Malaj et al., 2014). Organic pollutants can cause adverse ecotoxicological effects on multiple

communities in sediments through indirect effects (such as via food webs) as well (Almeda et al., 2014; Cerezo and Agusti, 2015; Enrique et al., 2007).

4. Conclusion

Our study characterized the diversity and structures of bacterial, protistan and metazoan communities and assessed their association with environmental variables in sediment samples collected from the marine and adjacent areas of northern Bohai Sea. The communities of freshwater sediments were significantly different from the communities in brine sediments. The communities in sediments were influenced by both natural salinity gradient and anthropogenic contaminants. However, there were the other non-measured environmental variables which could affect the stability of communities. Since this analysis reveals the potential contaminant stressors (for example, DDTs) from the community aspect, toxicity identification evaluation experiment, and sediment mesocosm studies could be conducted to confirm the actual causative agents.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2016.12.121.

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Supporting information for

Ecogenomic Responses of Benthic Communities under Multiple Stressors along the Marine and Adjacent Riverine Areas of Northern Bohai Sea, China

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31   Supplementary figures

32   Fig. S1. Heatmap of Pearson correlation coefficient between concentrations of analytes.

33   Blue block, positive correlation; red block, negative correlation; size of the block,

34   degree of the correlation.

35   Fig. S2. Rarefaction curves of observed OTU numbers of bacterial (A), protistan (B)

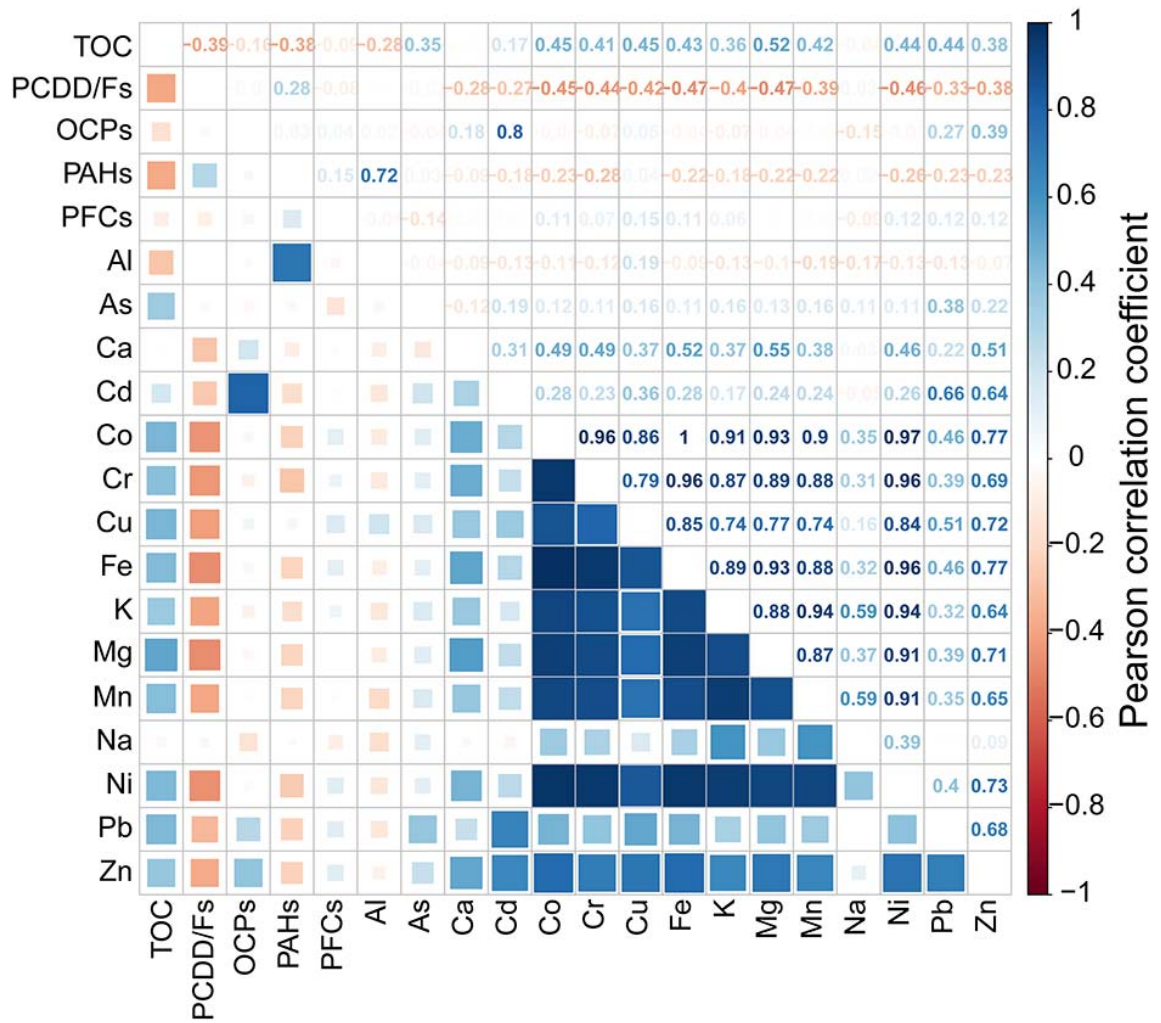36   and metazoan (C) communities.

37

38   Supplementary tables

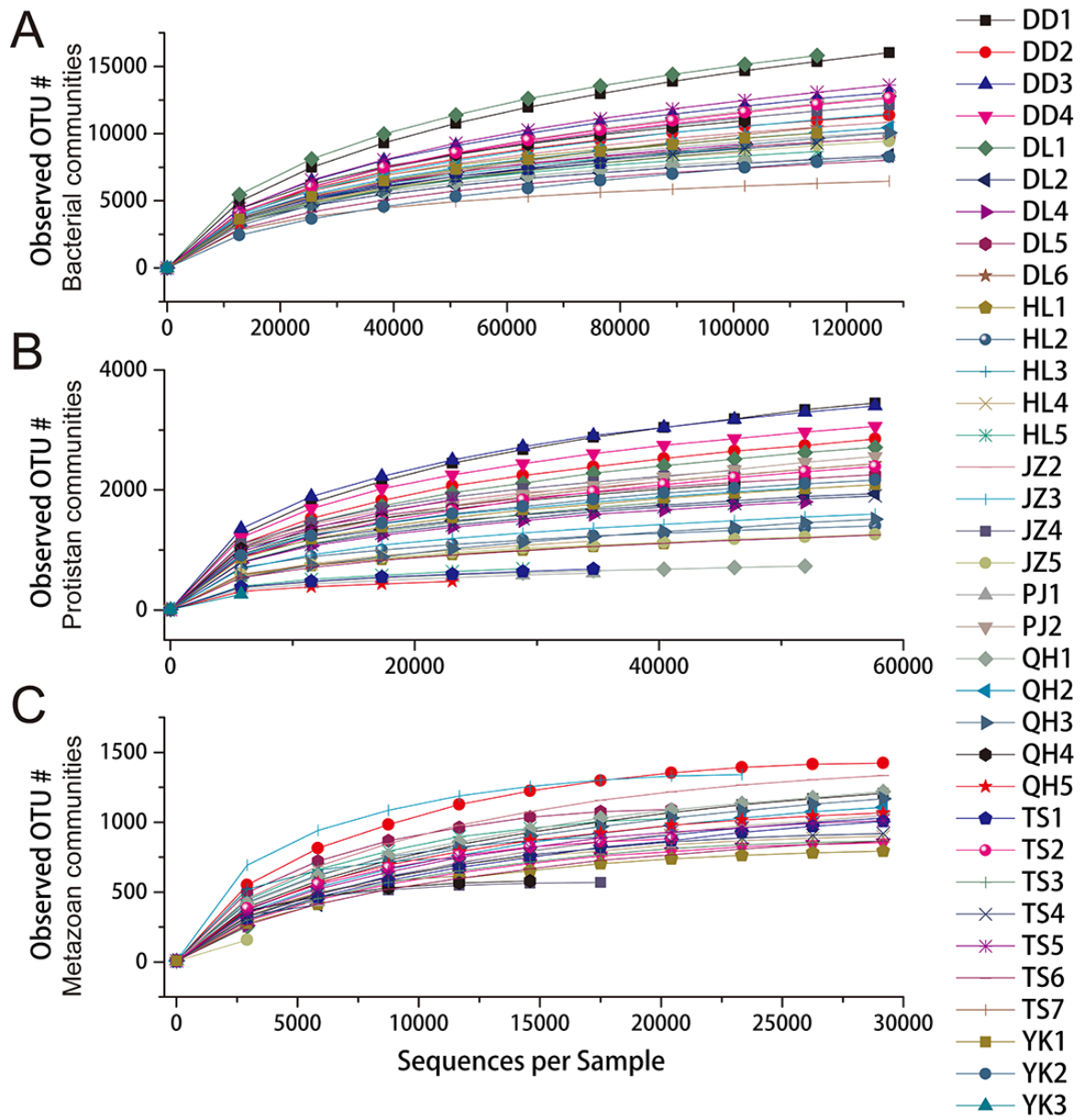39   Table S1. Sampling site, location and concentrations of chemical analyte for each

40   sediment (Hu et al., 2010; Jiao et al., 2012; Luo et al., 2010; Luo et al., 2013; Luo et

41   al., 2012; Naile et al., 2011; Wang et al., 2011).

42

Fig .S1.

44
45   Fig. S2.

46   Table S1 was represented as a separated excel table.

47

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Table S1.

ID	Sampling Site		Location		Organic chemicals					As	Al	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Pb	Zn
	Province	Region	Latitude °	Longtitude °	TOC %	PCDD/Fs # pg/g, dw	OCPs $ ng/g, dw	PAHs * ng/g, dw	PFCs ‡ ng/g, dw															
TS1	Tangshan	Dou River	39.512	118.199	0.09	8.69	89.9	1870.6	0.21	5.56	7165	9765	0.06	34.3	30.02	4.99	10049	1498	2302	676	660	9.92	14.15	78.7
TS2		Qing Long River	39.268	118.524	1.46	25.00	125.0	147.8	0.32	9.38	40061	8410	0.11	135.9	94.15	30.48	33302	11161	10339	4638	9664	40.89	24.87	101.7
TS3		Shuang Long River	39.144	118.339	0.71	29.23	361.7	157.0	<LD※	8.90	5775	7066	0.10	21.2	20.86	0.78	6102	1923	3965	695	2993	7.34	18.56	15.5
TS4			39.199	118.339	1.10	13.25	348.5	129.9	0.26	6.80	12051	10875	0.09	45.5	37.34	4.30	12979	3567	7159	1676	4157	15.55	18.53	33.3
TS5		Luanhe River	39.418	119.276	0.18	<LD	28.7	124.0	0.21	6.07	10444	8668	0.07	51.2	44.91	6.15	14759	2744	5137	1494	4910	13.55	15.02	29.6
TS6			39.461	119.134	0.07	5.57	2.3	160.2	0.24	7.93	6042	9268	0.09	50.7	48.63	2.16	14544	1430	3738	1290	767	11.36	27.69	26.0
TS7			39.582	118.807	1.27	8.89	4.7	192.4	0.21	6.98	10709	8307	0.15	71.2	61.70	7.89	20319	2390	4738	1414	535	15.53	18.90	40.7
QH1	Qinhuangdao	Bohai Sea	39.702	119.336	0.02	0.96	5.5	55.4	0.66	10.98	3687	4537	0.07	19.5	17.95	2.30	5911	1316	1346	1261	4198	5.42	13.78	10.7
QH2			39.841	119.511	0.20	7.99	232.7	257.0	4.45	5.70	5085	7801	0.10	21.6	18.16	0.53	6455	1188	1665	677	524	4.58	16.34	26.2
QH3			39.840	119.515	0.44	30.14	28.9	274.1	2.21	5.96	4660	3318	0.13	30.9	26.60	8.24	8069	1280	1778	1678	8769	10.26	17.06	10.2
QH4			39.831	119.527	0.08	15.55	22.3	144.7	<LD	8.57	4946	6324	0.06	25.6	19.54	2.93	7662	1307	2317	871	4952	4.62	16.52	23.3
QH5		Tian Ma Lake	39.980	119.210	0.05	2.41	13.0	74.3	<LD	6.81	10186	7084	0.09	40.4	31.75	5.44	11867	2373	3777	779	3660	11.64	28.67	43.6
HL1	Huludao	Bohai Sea	40.197	120.374	0.03	<LD	60.2	118.5	0.42	8.57	6568	10005	0.07	34.8	24.97	6.59	10247	1922	2894	1222	4500	9.67	17.36	26.9
HL2		Liugu River	40.253	120.360	0.76	13.40	1312.9	369.0	0.22	6.91	34556	6950	0.19	95.4	67.30	25.67	25018	5715	6060	1863	845	30.15	35.10	101.2
HL3			40.370	120.258	0.84	11.35	227.0	336.4	0.24	6.86	33693	9639	0.18	100.9	66.55	29.99	26377	5663	6794	2406	867	29.62	31.10	86.7
HL4		Wu Li River	40.727	120.894	0.68	<LD	3959.2	541.8	0.60	8.92	24330	10409	0.83	75.3	54.24	21.09	19978	4244	6219	2312	1283	23.11	43.36	165.9
HL5		Bohai Sea	40.700	120.948	0.07	<LD	14.1	253.2	0.26	11.67	5705	11074	0.34	54.7	37.18	12.48	15739	2588	3373	1199	4205	12.42	48.49	101.8
JZ2	Jinzhou	Xiaoling River	41.085	121.133	1.61	15.47	124.5	1657.5	0.10	7.81	24142	11981	0.22	78.6	64.66	23.70	21073	4446	7129	2775	1613	26.44	36.22	90.5
JZ3		Daling River	41.399	121.411	0.91	7.05	14.1	329.8	<LD	7.20	17604	9955	0.15	79.6	67.12	16.19	21790	3596	5997	1847	1061	23.83	19.24	51.9
JZ4			41.177	121.378	2.44	<LD	36.8	549.5	0.30	7.73	19883	10577	0.21	66.2	54.25	22.22	17790	4036	6241	1773	834	24.21	23.51	54.5
JZ5			40.904	121.739	0.30	26.38	208.3	729.4	2.90	10.57	8031	8954	0.11	36.1	35.28	5.19	10630	1671	3627	798	694	9.86	24.62	31.5
PJ1	Panjin	Shuangtaizi River	41.100	121.591	0.91	5.19	<LD	204.1	<LD	6.16	21753	9367	0.08	64.1	49.85	10.46	17724	4508	5895	1471	1331	22.66	18.52	45.2
PJ2		Bohai Sea	40.813	121.986	1.04	8.21	49.3	434.8	1.09	10.35	33372	9422	0.21	99.4	71.27	19.29	25808	8616	8620	3253	12175	34.26	27.48	94.8
YK1	Yingkou	Daliao River	40.821	122.133	1.03	<LD	10.2	380.6	0.74	10.08	14281	7926	0.05	51.7	39.79	9.18	14420	3172	4829	1455	882	15.61	9.50	61.3
YK2		Bohai Sea	40.665	122.164	0.88	<LD	127.5	425.1	0.64	11.12	28257	9064	0.16	88.1	65.60	15.92	23274	7002	7511	2383	9572	28.86	23.11	76.9
YK3			40.427	122.277	0.02	<LD	20.4	1097.2	0.15	8.43	14422	6544	0.08	60.3	41.78	29.58	17062	3290	5633	1195	807	15.89	21.62	46.6
DL1	Dalian	Fuzhou River	39.611	121.499	1.13	<LD	179.4	1782.2	<LD	8.82	36103	9940	0.20	105.7	77.75	22.66	27888	9479	8242	3868	11293	31.09	28.81	76.2
DL2			39.694	121.737	0.07	3.82	25.9	759.8	0.18	10.79	9070	5939	0.07	35.2	55.89	8.56	10217	3065	2001	1346	1019	18.23	17.53	18.7
DL4		Bohai Sea	39.000	121.309	0.05	12.75	41.2	98.3	<LD	9.29	3402	5705	0.07	19.0	18.60	3.39	5495	1289	1655	543	4452	5.54	15.89	9.8
DL5		Bilia River	39.465	122.500	0.28	<LD	4.7	127.7	0.45	6.87	16194	7378	0.09	56.5	41.24	15.71	15904	3554	3861	1477	549	17.67	19.39	54.9
DL6		Bohai Sea	39.657	122.986	1.63	37.76	354.2	353.5	<LD	7.90	36217	7721	0.07	105.6	78.22	22.05	27542	8286	8818	3535	13011	33.67	30.20	90.3
DD1	Dandong	Dayang River	39.942	123.628	1.50	2.45	10.5	128.9	<LD	9.31	17864	6276	0.09	65.5	50.95	6.20	17975	3996	6298	1891	1431	18.67	29.94	58.8
DD2		Yalu River	40.176	124.455	3.90	28.32	12.6	634.6	<LD	13.29	29678	8891	0.24	110.6	73.25	34.47	29022	4969	9581	2641	718	29.17	42.22	122.9
DD3			39.941	124.288	2.78	<LD	26.4	1138.5	0.24	10.54	55	<LD	0.15	0.2	4.21	<LD	<LD	235	<LD	171		62	<LD	33.60	<LD
DD4			40.006	124.363	1.97	42.43	64.9	492.9	<LD	10.23	26295	8027	0.14	90.6	66.97	17.01	24591	5559	7568	2656	956	26.67	31.41	139.9

: Sum of concentrations of 12378-PeCDF, 1234678-HpCDD, OCDD, 2378-TCDF, 12378-PeCDF, 23478-PeCDF, 123478-HxCDF, 123678-HxCDF, 234678-HxCDF, 1234678-HpCDF and OCDF

$: Sum of concentrations of α-HCH, β-HCH, γ-HCH, δ-HCH, p, p′-DDE, p, p′-DDD, o, p′-DDT, p, p′-DDT and HCB

*: Sum of concentrations of 16 EPA priority PAHs

‡ : Sum of concentrations of PFOS, PFOA, PFHxA, PFHpA, PFNA, PFDA and PFUnA

※: less than limits of detection to be equal to zero for statistical purpose