Environmental Pollution 212 (2016) 348–357

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Using *in situ* bacterial communities to monitor contaminants in river sediments☆

Yuwei Xie [a], Jizhong Wang [b], Yaketon Wu [b], Chen Ren [b], Chao Song [a], Jianghua Yang [a], Hongxia Yu [a], John P. Giesy [a, c, d, e], Xiaowei Zhang [a, *]

[a] State Key Laboratory of Pollution Control & Resource Reuse, School of the Environment, Nanjing University, Nanjing, 210023, China
[b] Laboratory for Nanomineralogy and Environmental Material, School of Resources and Environmental Engineering, Hefei University of Technology, Hefei, 230009, China
[c] Department of Veterinary Biomedical Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan, Canada
[d] School of Biological Sciences, University of Hong Kong, Hong Kong Special Administrative Region
[e] Department of Biology, Hong Kong Baptist University, Kowloon, Hong Kong Special Administrative Region

ARTICLE INFO

Article history:
Received 26 August 2015
Received in revised form
12 January 2016
Accepted 12 January 2016
Available online 8 February 2016

Keywords:
Metals
PAHs
Organophosphorous insecticides
Pyrethroid insecticides
Next generation sequencing

ABSTRACT

Bacterial communities in sediments of human-impacted rivers are exposed to multiple anthropogenic contaminants and eventually lead to biodiversity lost and ecological functions disable. Nanfei River of Anhui province has been contaminated by pollutants from industrial and/or agricultural sources. This study was conducted to investigate the structure of *in situ* sediment bacterial communities in Nanfei River and to examine the correlation between the different taxonomic components and contaminant concentrations. The bacterial communities were dominated by *Proteobacteria*, *Bacteroidetes* and *Chloroflexi*. Both the profiles of environmental predictors and the composition of microbial communities differed among agriculture, industrial and confluence regions. There were significant associations between bacterial community phylogenies and the measured contaminants in the sediments. Nutrients (TN and TP) and two metals (Cd and Zn) were negatively correlated with the essential "core" of the bacterial interaction network (*Betaproteobacteria* and *Deltaproteobacteria*). Metals (Fe, Ni and Zn) and nutrients (TN and TP) had higher impact on bacterial community compositions than PAHs, OPs and PRTs according to the correlation and network analyses. Furthermore, several sensitive candidate genera were identified as potential bioindicators to monitor key contaminants by species contaminant correlation analysis. Overall, *in situ* bacterial communities could provide a useful tool for monitoring and assessing ecological stressors in freshwater sediments.

1. Introduction

Microbes contribute the major biomass of river sediments (Fischer and Pusch, 2001). Microbial productions account for the major part of river ecosystem productions (Craft et al., 2002; Gibbons et al., 2014). Microbial communities are also crucial for ecosystem stability, resilience and services and play important roles in biogeochemical cycling (Ducklow, 2008; Reed and Martiny, 2013). Despite their importance, sediment microbes are always neglected (Torsvik et al., 2002).

Sediment microbial communities are under a range of natural and anthropogenic stressors, during rapid urbanization and land use changes especially in developing countries (Saxena et al., 2015). Contaminations absorbed by fine particles of sediments with organic matter could harm the associated biota and eventually lead to a loss of biodiversity (Burton and Johnston, 2010; Eggleton and Thomas, 2004; Lake et al., 2000). A decline in microbial diversity and activity could affect the geochemical cycling of elements in sediments and community formation of higher trophic levels (Yergeau et al., 2012). However, we are still at the very beginning of understanding bacterial community response and resilience to multiple anthropogenic drivers. There is also a lack of consensus between studies regarding bacterial community resilience to

☆ This paper has been recommended for acceptance by Elena Paoletti.

* Corresponding author. State Key Laboratory of Pollution Control & Resource Reuse, School of the Environment, Nanjing University, 163 Xianlin Great Rd, Nanjing, 210023, China.
E-mail addresses: howard50003250@yahoo.com, zhangxw@nju.edu.cn (X. Zhang).

http://dx.doi.org/10.1016/j.envpol.2016.01.031
0269-7491/

stressors (Sun et al., 2013).

Microbial communities are extremely sensitive to changes in the physicochemical state of freshwater sediments, according to previous sediment microbiome Investigations (Faris et al., 2009; Gibbons et al., 2014; Ramsey et al., 2005). Next generation sequencing, taxonomic reference databases and bioinformatics tools provide a great opportunity to characterize the complex relationship between microbial communities and contaminants in detail. However, only a few metagenomics studies have been conducted on freshwater sediments (Liu et al., 2014; Saxena et al., 2015). Relatively little is known about the interaction between sediment contaminants and microbiome and its role in the functioning and service of aquatic ecosystems (Nogales et al., 2011; Sun et al., 2013).

Here we hypothesize that the composition of *in situ* bacterial communities can be used to monitor key stressors among anthropogenic pollutants in freshwater sediments. Nanfei River of Anhui province in China provides a good study site to evaluate *in situ* bacterial communities and contaminant levels among industrial, agricultural and confluence regions (Fig. 1). Polycyclic aromatic hydrocarbons (PAHs) and metals are prevalent in urbanized and industrialized regions, while pesticides predominant in agricultural regions (Ren et al., 2015; Wu et al., 2014). The objectives of this study were to 1) to characterize the benthic bacterial communities of freshwater sediment collected from three different regions (industrial, agricultural and confluence regions) of Nanfei River, and 2) to examine the relationship between bacterial communities and concentrations of associated anthropogenic contaminants. In this study, the bacterial communities in sediments were assessed by semi-quantitative 16S rRNA gene amplicon next generation sequencing.

2. Materials and methods

2.1. Sampling sites

The Nanfei River is one of the largest rivers discharging into Lake Chao. It is 70 km long with a catchment area of 1446 km^2. The Shipu and Dianpu rivers are two tributaries of the Nanfei River. The Nanfei and Shipu Rivers pass through an urbanized region (population more than 3 million), and the Dianpu River passes through an agricultural region (population approximately 0.9 million). The sampling sites were grouped into three types (agriculture, industry and confluence) according to the hydrology and land-use characteristics (GlobeLand30 web tools) (Han et al., 2015) (Fig. 1, Table S1).

2.2. Sediment collection and processing

Sediments were collected on September 26, 2012, along the Nanfei, Shipu and Dianpu rivers. Three sediment grabs were collected at each site (approximately 2.5 m depth) using the Van Veen grab sampler. Surface sediment samples (<5 cm depth) were collected, pooled and homogenized. A 40 mL aliquot of homogenized surface sediments was stored in a sterile 50 mL Falcon tube. The remaining sediment and left surface sediment (approximately 2 L) were combined together and stored in a plastic bag. They were returned to the laboratory in the dark on ice within the same day and frozen at −20 °C. All sediment samples were freeze-dried and homogenized for predictor variable analyses, including pH, grain size, organophosphorous insecticides (OPs), pyrethroid pesticides

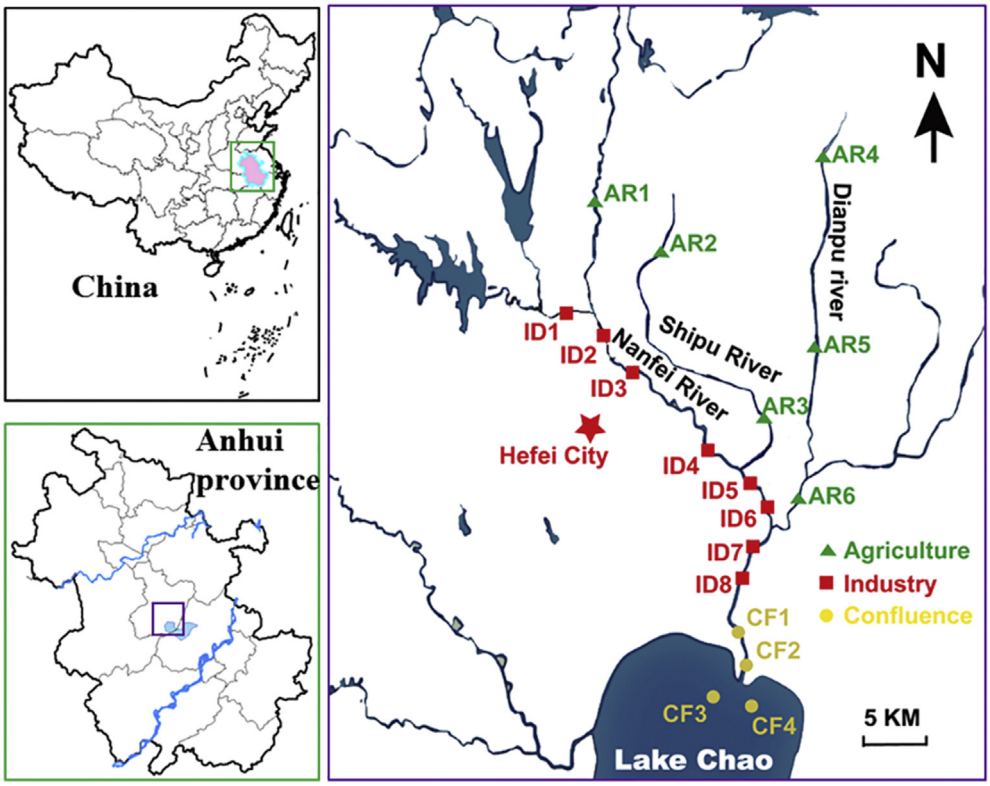

Fig. 1. Location of sampling sites of the Nanfei River of Anhui province in China. Sampling sites were grouped into three types according to land use and hydrology: green triangle, agriculture region; red square, industry region; yellow circle, confluence region. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(PRTs), PAHs and metals.

Concentration data for OPs (methidathion, dichlorvos, dyfonate, diazinon, chlorfenvinphos methyl, fenitrothion, malathion, parathion, chlorfenvinphos, ethion, carbophenothion and phosalone), PRTs (tefluthrin, bioallethrin, prallethrin, tetramethrin, permethrin, cyfluthrin, cypermethrin, fenvalerate, deltamethrin, cyhalothrin, esfenvalerate and ahethrin), and 16 EPA Criteria PAHs (naphthalene, acenaphtylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenzo[a,h]anthracene and benzo[g,h,i] perylene) were obtained from a co-author's lab (Liu and Zhang, 2014; Ren et al., 2015; Wu et al., 2014) and normalized to 1% TOC.

One gram of sediment was prepared according to the USEPA Method 3051A (2007) for metal analysis. Subsequently, the concentrations of Fe and Zn were measured with a flame atomic absorption spectrometer AF-610A (Varian, CA, USA), and Cd, Cr, Pb, Ni, Cu and Mn were analyzed using Atomic Absorption with a graphite furnace. Total nitrogen (TN) and total phosphorus (TP) were determined employing the Kjeldahl method and the sulfuric acid-perchloric acid oxidation method, respectively (Ebina et al., 1983).

DNA was extracted from a 0.25 g aliquot of a homogenized sample of surface sediment with the MoBio Power Soil DNA Kit (MoBio Laboratories Inc., CA, USA) following the manual. The extracted DNA was quantified and checked for purity at A260/280 nm in a Take3 microplate in the Synergy H4 Hybrid Multi-Mode Microplate reader (BioTek, VT, USA) prior to storage at −80 °C.

2.3. PCR amplification and next generation sequencing

Bacterial 16S rRNA genes were amplified by PCR from DNA using the V3 primers (modified primers 341F and 518R) (Klindworth et al., 2013). A 20 μL reaction system was used 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, 54 °C for 30 s and 68 °C for 30 s, and a final extension at 68 °C for 7 min. Triplicate PCR reactions were performed for each sample to minimize potential PCR bias. Ten ug of extracted DNA was used per 50 μL reaction mixture. PCR products were checked for size and specificity by electrophoresis on a 2% (w/v) agarose gel and were gel purified using the MinElute Gel Extraction Kit (Qiagen, CA, USA). The purified products were quantified with the Qubit™ dsDNA HS Assay Kits (Invitrogen, CA, USA) and adjusted to 10 ng/μL in molecular grade water. All purified PCR products were pooled equally for subsequent sequencing. Sequencing adapters were linked to the purified DNA fragments with the Ion Xpress™ Plus Region Library Kit (Life Technologies, CA, USA). Prior to next generation sequencing, all DNA products were assessed for length and concentration using the Bioanalyzer 2100 (Agilent Technologies, CA, USA). The samples were adjusted to a final concentration of 26 pM. Sequencing templates were prepared with the Ion One-Touch™ and sequenced in the Ion Torrent Personal Genome Machine (Life Technologies, CA, USA).

2.4. Bioinformatics analysis

2.4.1. Quality control

All PGM low quality sequence reads (quality < 20, scanning window = 50) and sequencing adapter sequences were filtered using the ION server (version 3.6.2). Reads containing ambiguous 'N', homopolymer and read lengths shorter than 150 bp or longer than 190 bp were discarded by the QIIME toolkit (Caporaso et al., 2010). *Denoising, chimera removal and operational taxonomic units (OTUs)-picking*: OTUs were selected with a sequence similarity cut-off of 97% following the UPARSE pipeline (Edgar, 2013). Chimeric and singleton OTUs (sizes less than 2) were removed. *Taxonomic assignment*: For each resulting OTU, a representative sequence was chosen. Taxonomy was assigned to the representative sequences using the RDP classifier (Wang et al., 2007) against the Greengenes database (DeSantis et al., 2006). A small fraction of unexpected archeal and chloroplast sequences was removed.

2.4.2. Alpha diversity

Alpha rarefaction was performed using the phylogenetic diversity, Chao 1 indices, observed species and Shannon index metrics. *Beta diversity and clustering*: All samples were rarefied at the lowest sequencing depth (16,726) to reduce biases resulting from differences in sequencing depth. Beta diversity was estimated by computing unweighted UniFrac distances between samples. *Interaction network*: To enhance the detection of diverse patterns among sediment communities under specific pressure, OTUs present in every sample were selected as core communities. Microbial co-occurrence relationships in the sediment were discovered using the ConNet app (Faust et al., 2012) in Cytoscape V3 (Saito et al., 2012). The correlation among taxa and anthropogenic contaminants was confirmed to be robust if the Spearman's correlation coefficient ($|r_{spearman}|$) was > 0.75 and the adjusted FDR was statistically significant ($P_{FDR} < 0.05$) (Yergeau et al., 2012).

2.5. Statistical analysis

Organic contaminants were first grouped or selected based on their chemical structure and concentration distribution to reduce the number of environmental variables. Environmental predictors (parameters and/or pollutants) with correlations $|r| > 0.75$ were grouped together to account for co-linearity between predictor variables (Sun et al., 2013). Environmental parameters were transformed ($\ln(x + 1)$) and normalized. The Mantel test was used to evaluate the association among community structures and the spatial and physicochemical attributes of the sediments (Diniz-Filho et al., 2013). The effects of anthropogenic activities (different land use groups) with the composition of bacterial communities were assessed by permutational multivariate analysis of variance test with unweighted UniFrac distance (PERMANOVA) with 9999 permutations with unweighted UniFrac distance matrices (Lozupone and Knight, 2005). The relative contribution of contaminant and environmental variables in explaining differences in community composition was determined with forward selection distance-based linear modelling (distLM). Principal component analysis (PCA) was conducted to cluster the sediments according to the environmental variables. We also performed a principal coordinate analysis (PCoA) to test and visualize the relationships between the environmental variables and community composition. Species response curves to each contaminate were generated using normalized abundance data for the top 150 genera in the form of generalized additive models (GAM) with Poisson distributions. The freedom of the GAM models was set as 2. P-values and smooth P-values for each curve were adjusted and filtered ($P_{FDR} > 0.05$) for further analysis. Statistical analyses were conducted in the "R" and Primer 7 environment.

2.6. Nucleotide sequence accession numbers

Sequence data were deposited into the NCBI Sequence Read Archive under accession number SRR1238092.

3. Results

3.1. Sediment chemical analysis

The contaminant profile varied in the sediments among agriculture, industry and confluence groups (Fig. 2). The concentrations of chemical parameters were summarized in Table S2. The concentrations of nutrient (TN, TP) and metals (Cd, Cr, Cu, Pb and Zn) in the industrial group were significantly greater than agriculture and confluence group, while the concentration of dichlorvos was less than agriculture group.

Highly correlated correlations were observed among contaminants (Table S3) which indicated the similar environmental sources and behaviors. PAH contaminants were summed as PAH_{EPA16} because all congeners behaved collinearly. PRTs were grouped into the TI-PRT and TII-PRT types based on a combination of toxicological and physical properties (HHS, 2003). The OPs were dominated by dichlorvos (approximately 87% of the total OPs). The dichlorvos concentration was correlated with the total OP concentration. Hence, we selected dichlorvos as the variable to represent the OPs. The dichlorvos concentration was significantly correlated with the TII-PRTs ($r = 0.84$, $P = 0.012$). According to the Pearson correlation, 14 environmental variables, or predictor variables were selected for further analysis (Table 1).

3.2. Diversity of bacterial communities

The structures of the bacterial communities varied between groups (Fig. 3). The sediment bacterial communities were diverse (11,470 non singleton OTUs) and colonized by 64 bacterial phyla across all samples. Bacterial communities were dominated by *Proteobacteria* (17.1–38.2%, mean 29%), *Bacteroidetes* (12.2–26.1%, mean 17.2%), *Chloroflexi* (2.3–11.8%, mean 6.2%), *Firmicutes* (0.7–7.9%, mean 4.3%), *Spirochaetes* (1.9–7.4%, mean 3.9%) and *Actinobacteria* (2.6–5.9%, mean 4%) (Figure S1-A). The sum of these groups accounted for 53.6–73.9% of all bacterial communities in the sediments from the Nanfei River. Following the dominant groups, there were a few other major phyla, including *Verrucomicrobia, Planctomycetes, Acidobacteria, Nitrospirae, OP1, OP3, OP8* and *Chlorobi* (mean abundance >1%). Within the *Proteobacteria*, *Betaproteobacteria*, *Deltaproteobacteria* and *Gammaproteobacteria* were most abundant. The proportion of *Acidobacteria*, *Gammaproteobacteria* and *Nitrospirae* was greatest in the confluence region, while the proportions of *Chloroflexi*, *Firmicutes*, *OP1*, *OP3*, and *OP8* in the industrial region were higher than in the other two types (Fig. 3B and C). The proportion of *Proteobacteria* in the agricultural and confluence region was greater than in the industrial region.

The patterns of both Chao1 and phylogenetic diversity were similar to the observed number of OTUs (Table S4). According to

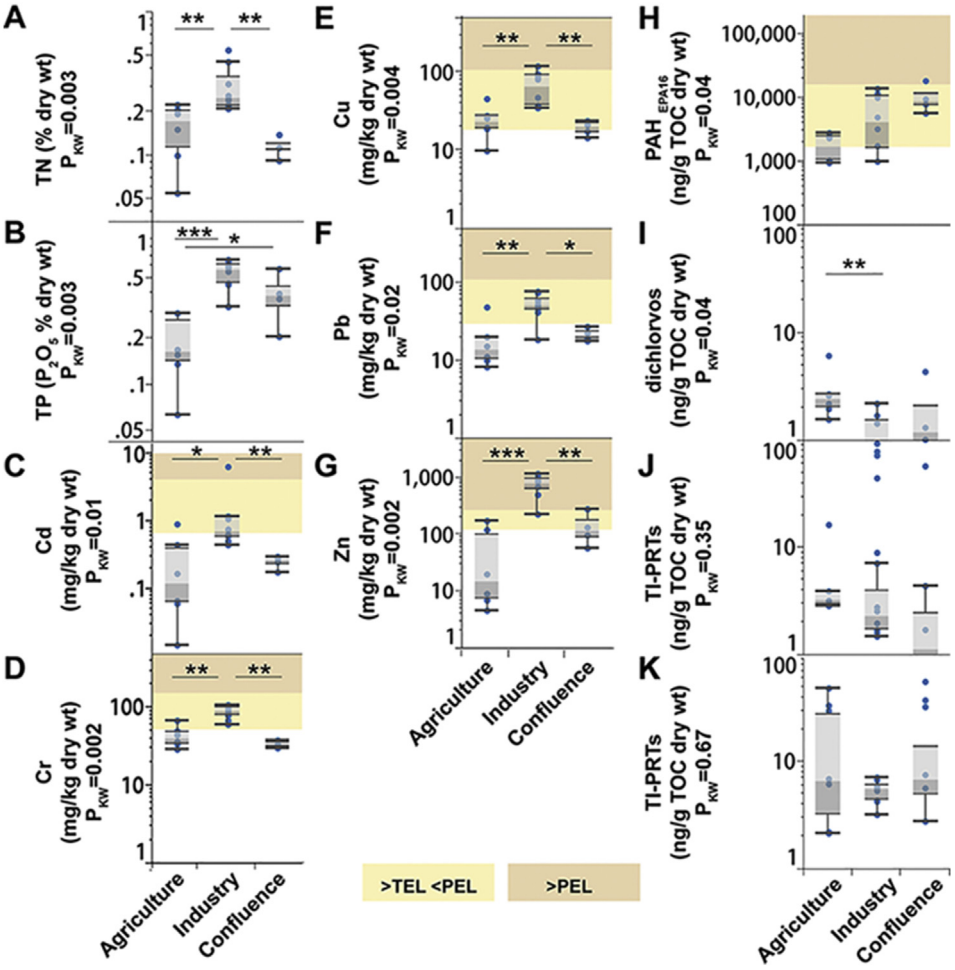

Fig. 2. Comparisons of important contaminant and environmental variables measured at each group. Concentrations were log-transformed. For metals and PAH_{EPA16}, threshold effects level (TEL) and probable effects level (PEL) of sediment quality guidelines (SQG) were colored in yellow and orange respectively. The Kruskal–Wallis test was performed, followed by post hoc Mann–Whitney U tests. P values for Kruskal–Wallis test (PKW) were present in column label. Significance of Mann–Whitney U tests was determined at P values < 0.001 (***), <0.01 (**), and <0.05 (*). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Variables included in the sediment microbial community multivariate analyses chosen by identifying collinear groups. Environmental variables with correlations |r| > 0.75 were grouped together to account for co-linearity between predictor variables. Correlations (r) were calculated with Pearson correlation analysis (Table S3).

Group name	Variable used in analysis	Correlated variables (|r| > 0.75)
PAH_s	$SUM(PAH_{EPA16})$	Acenaphthene, acenaphtylene, fluorene, naphthalene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenzo[a,h]anthracene, benzo[g,h,i]perylene
Dichlorvos (OPs/TII-PRTs)	dichlorvos	TII-PRTs, total Ops, dichlorvos
TI-PRTs	TI-PRTs	
Cu (TOC/TN)	Cu	TN, TOC, Cu
Zn (Pb/Cr)	Zn	Zn, Pb, Cr
PH	pH	
Silt	Silt	
TP	TP	
Cd	Cd	
Ni	Ni	
Fe	Fe	
Mn	Mn	
Latitude	Latitude	
Longitude	Longitude	

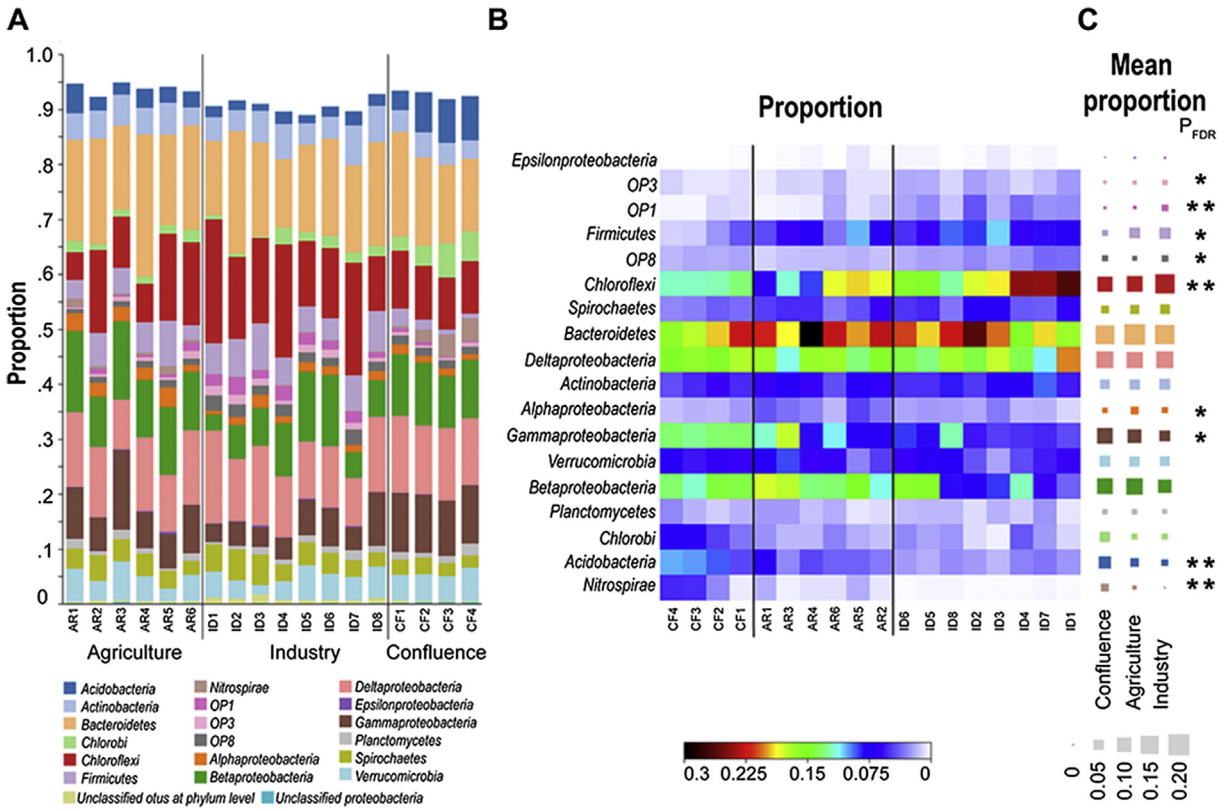

Fig. 3. The structures of the bacterial communities varied between groups. A, Proportion of different phyla and classes of *Proteobacteria* in sediments. Low abundance phyla (<1%) were not presented. A large fraction of denoised sequences could not be assigned to any taxa, indicating the extent of novel sequences in this study. B, heatmap representation of the abundant phyla and classes of *Proteobacteria* composition pattern across all samples. C, comparison of proportions across land-use groups. Mean proportion was tested with ANOVA, P < 0.05, followed by post hoc Tukey–Kramer tests between groups. P values were corrected using the Benjamin-Hochberg FDR method). Significance was determined at P values < 0.001 (***), <0.01 (**), and <0.05 (*).

rarefaction curves (Fig. S1), most of the abundant bacterial OTUs were captured by rarefaction at 16,726 sequences. Phylogenetic diversity of the bacterial communities in the agricultural group was significantly greater than in the industrial groups (Mann–Whitney U tests, P < 0.05).

There was significant covariance among the different taxonomic groups from the sediment microbial communities based on the relative abundance of each measured "core" OTU (384 OTUs were present in all sediments, Fig. S2). The resulting microbial network consisted of 231 nodes (OTUs) and 591 edges (average degree or node connectivity of 4.01). Only the 100 most abundant OTUs were presented. Several mutually exclusive nodes were identified between *Synergistia* and *Deltaproteobacteria*, *Acidobacteria-6* (Figure S2 C), *OP1* and *Proteobacteria* (Figure S2 D), and *Alphaproteobacteria* and *Thermobacula* (Figure S2 E). The complex bacterial network was dominated by highly connected classes (approximately 5 edges per node) such as *Acidobacteria-6*, *Leptospirae*, *Bacteroidia*, *Betaproteobacteria*, *Gammaproteobacteria*, *Anaerolineae*, *Clostridia*, and *Deltaproteobacteria* (Fig. 5A).

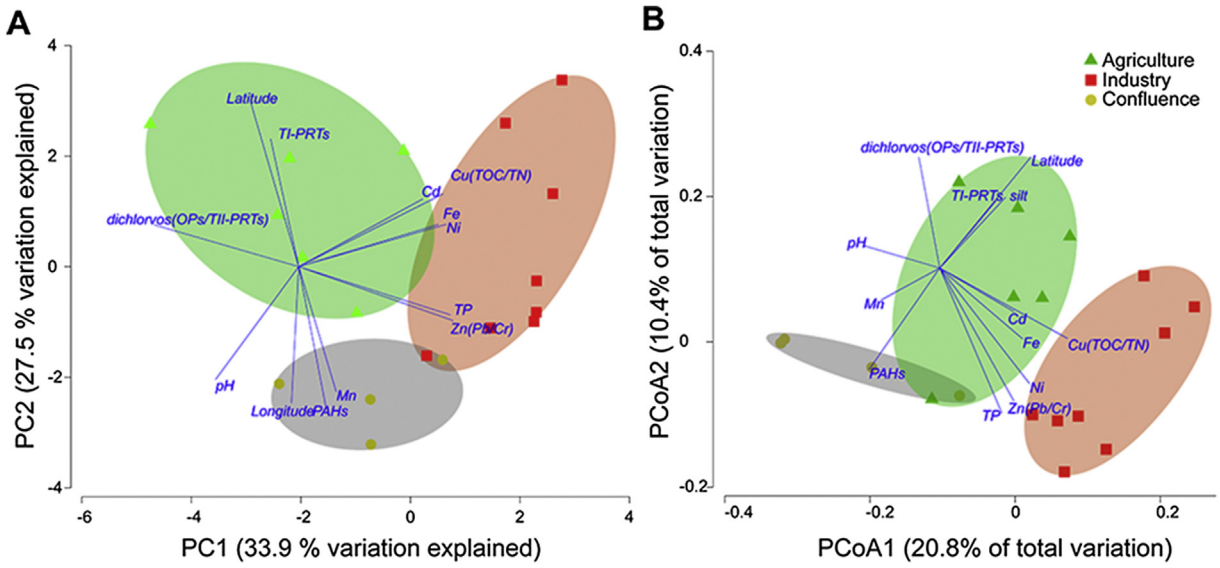

Fig. 4. Ordination of environmental variables (A: PCA with Euclidean distance) and bacterial community composition (B: PCoA with unweighted Unifrac distance matrices). Blue vectors (envfit $p < 0.05$, $|r| > 0.3$) point to the direction of the increase for a given variable so that sediments with similar environmental variable profiles or bacterial communities are localized in similar positions in the diagram.

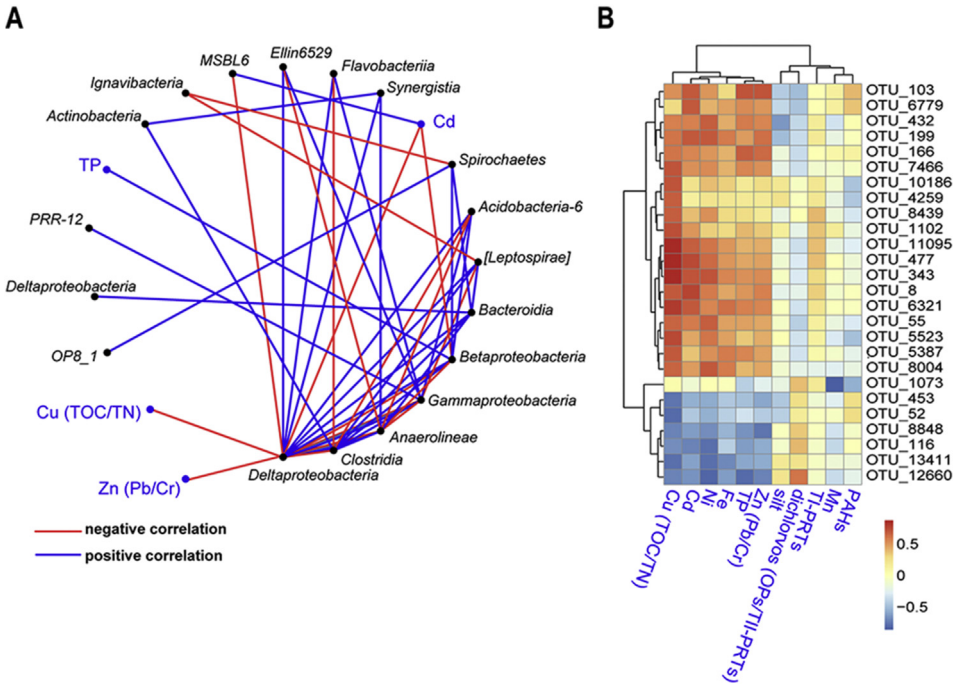

Fig. 5. Network (A) and correlation (B) between predictor variables and bacterial core OTUs (present in all sediments). A, core OTUs were collapsed at class level from core association network (Fig. S2). Predictor variables were in blue. Red line, negative correlation; green line, positive correlation. B, strongly correlated core OTUs ($|r_{speraman}| > 0.7$) with predictor variables were identified and presented. Predictor variables were in blue. Red, strong positive correlation; yellow, weak correlation; blue, strong negative correlation. OTU_8004 (*Leucobacter*); OTU_103 (*Actinobacteria*, At425_EubF1); OTU_8848 (*Actinobacteria*, OPB41); OTU_1102 and OTU_6779 (*Anaerolineae*); OTU_4259 (*Anaerolinaceae* WCHB1-05); OTU_5523 (*Anaerolineae* GCA004); OTU_1073 (*Chloroflexi* Ellin6529); OTU_55 and OTU_432 (*Clostridiales*); OTU_6321, OTU_343, OTU_11095, OTU_8 and OTU_477 (OP1 MSBL6); OTU_166 and OTU_199 (OP8_1); OTU_52 (*Methylocystaceae*); OTU_12660 and OTU_13411 (*Thiobacillus*); OTU_7466, OTU_5387 and OTU_10186 (*Syntrophaceae*); OTU_8439 (*Syntrophorhabdaceae*); OTU_116 (*Methylococcaceae*); OTU_453 (*Pedosphaerales*, auto67_4W). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.3. Impact of environmental predictors upon bacterial community structures

Both the profiles of environmental predictors and the composition of microbial communities differed among agriculture, industrial and confluence regions (Fig. 4). Industrial sediments were polluted with metals, while agriculture sediments absorbed higher concentration of OPs and PRTs (Figs. 2 and 4A). The concentration of PAHs and Mn were relatively higher (not significantly) than the other two regions. The PCoA (Fig. 4B) of the bacterial community composition in the sediments showed that an average 31.2% of the variability in the data were explained by the first two principle

Table 2
DistLM results of bacterial community composition against 14 predictor variables in the full analysis (9999 permutations). Bold = significantly correlated with community structure at $P < 0.05$.

Predictor variable	Marginal tests			Forward selection sequential tests			
	Pseudo-F	P	Percent variation explained	Pseudo-F	P	Percent variation explained	Cumulative variation explained
Cu (TOC/TN)	2.5891	**0.0005**	0.13928	2.5891	**0.0004**	0.13928	0.13928
Latitude	2.0085	**0.0061**	0.11153	1.9807	**0.0016**	0.1004	0.23968
Zn (Pb/Cr)	1.864	**0.0121**	0.10434	1.6149	**0.0069**	0.078632	0.31831
Silt	1.425	0.0637	0.081778	1.4865	**0.0139**	0.069951	0.38826
Mn	1.3192	0.1046	0.076169	1.1777	0.218	0.054671	0.44293
Cd	1.2928	0.1072	0.074757	1.1244	0.3035	0.051662	0.49459
Longitude	0.92428	0.539	0.054613	1.1078	0.3716	0.050407	0.545
TI-PRTs	1.2708	0.1261	0.073579	1.1212	0.3604	0.050404	0.59541
Fe	1.511	**0.048**	0.086288	1.1535	0.3449	0.050985	0.64639
Ni	1.9625	**0.008**	0.10925	1.0819	0.4116	0.047337	0.69373
Dichlorvos (OPs/TII-PRTs)	1.2295	0.1501	0.071359	0.98134	0.4966	0.043051	0.73678
PH	1.3519	0.0871	0.077911	0.99742	0.4682	0.043776	0.78055
TP	1.8358	0.0111	0.10293	1.0326	0.4468	0.045027	0.82558
PAHs	1.5316	**0.0486**	0.08736	1.0178	0.4597	0.044185	0.86977

components (PCoA1 and PCoA2). The variability in pH and Mn were mostly explained by PCoA1, whereas the other variables were mainly explained by PCoA2.

PERMANOVA tests (environmental predictors: Pseudo-F = 5.09, P_{PERM} = 0.0001; bacterial community composition: Pseudo-F = 2.47, P_{PERM} = 0.0001) were accepted as a reflection of true differences in the means, as distinct groups were observed (Fig. 4). About 89% of the variation in bacterial community dissimilarity was explained by all 14 predictor variables (DistLM test, Table 2). Cu(TOC/TN), Latitude, Zn(Pb/Cr), Fe, Ni and PAHs were significant variables in explaining changes in bacterial community composition across all sediments (Table 2; Marginal tests). In decreasing order of influence, Cu(TOC/TN), Latitude, Zn(Pb/Cr) and silt together explained 38.8% of community variation (Table 2; Forward selection sequential tests). Cu(TOC/TN), TI-PRTs, Mn, Geographic distance, Zn(Pb/Cr), pH and TP were significant positive correlations with microbial communities by the Mantel tests (Table 3). There was also a significant relationship between the bacterial community structure and spatial data.

3.4. Correlation and response of individual bacteria to predictor variables

Correlation between the relative abundance of core OTUs and the concentration/value of predictor variables facilitated the discovery of potential bioindicators of environmental contaminant (Fig. 5). The nutrients (TOC, TN and TP) and metals (Cu, Cd, Zn Pb and Cr) were negatively correlated with the *Betaproteobacteria* and *Deltaproteobacteria*, while Cd was positively correlated with *MSBL6* (Fig. 5A). Non-linear correlations between abundant genera and contaminant concentrations were identified with species response analysis. Abundant bacterial genera responded differently to predictor variables (Fig. S3, Table S5). We also identified that there are two OTU clusters of 27 core OTUs strongly correlated with environmental and contaminant concentrations (Fig. 5B). One cluster was positively correlated with metals and nutrients, the other was negatively correlated with metals and nutrients. Compared with metals and nutrients, fewer core OTUs exhibited negative correlation with organic contaminates.

Table 3
The association between bacterial community structures and each predictor variable 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 bacterial communities and predictor variables were constructed using unweighted UniFrac and Euclidean distances, respectively. Bold = significantly correlated with community structure at $P < 0.05$.

Predictor variable	Rho	P_{PERM}
Cu (TOC/TN)	0.35	0.003
TI-PRTs	0.328	**0.0091**
Mn	0.299	**0.0107**
Geographic distance (Latitude/Longitude)	0.28	**0.0063**
Zn (Pb/Cr)	0.219	**0.0299**
PH	0.189	**0.0452**
TP	0.181	**0.0386**
Ni	0.152	0.1006
Fe	0.118	0.1439
Silt	0.095	0.2097
PAHs	0.063	0.212
Cd	0.056	0.3063
Dichlorvos (OPs/TII-PRTs)	0.026	0.3971

4. Discussion

4.1. Bacterial communities significantly differed between land-use types

The bacterial community structure in the Nanfei River is largely in accordance with the results of several previous studies on sediments conducted at locations around the world (Gibbons et al., 2014; Liu et al., 2014; Saxena et al., 2015). These communities were dominated by *Proteobacteria*. The composition of bacterial communities was consistent with the profiles of environmental variables in sediments according to ordination analysis (Fig. 4). Strong, non-random associations were also found between phylogenetic traits and total measured predictors (Procrustes analysis, $M^2 = 51.19$, $P = 0.001$). The concentrations of both metals (except Mn, Fe and Ni) and PAH_{EPA16} exceeded threshold effects level (TEL) (ANZECC/ARMCANZ, 2000) in the industrial region, where possible adverse biological effects can be expected (Long, 1992; Sun et al., 2013). Metals in the environment are mainly derived from anthropogenic sources, including emissions from traffic and industries, domestic activities, weathering of buildings and pavement surfaces, and atmospheric deposition (Ordonez et al., 2003; Sindern et al., 2007). Compared to the relatively clean agriculture regions of the Nanfei River basin, traffic emissions in urban-industrial areas are the major source of PAHs. In contrast, pesticides enter the rivers mainly from nonpoint sources in the agricultural regions. Anthropogenic activities, such as transport, industry and agriculture, might shape the structures of bacterial communities in sediments through the deposition of metals and

pesticides into freshwater sediments (Halliday et al., 2014; Paerl et al., 2003).

4.2. Metals and nutrients are major pressures on bacterial communities

Bacterial communities dwelling in sediments have been shown to be influenced by direct or indirect anthropogenic activities, especially in industrial areas (Gibbons et al., 2014; Saxena et al., 2015; Yergeau et al., 2012). Network analysis based on correlations could help to identify the key contaminants. A network connection between two OTUs implies that both OTUs might respond to a common environmental parameter rather than interacting directly (Zhou et al., 2011). Concentrations of metals were negatively correlated with *Betaproteobacteria* and *Deltaproteobacteria*. Ubiquitous *Betaproteobacteria* can degrade hydrocarbons and related substituted molecules. Hydrocarbon-degrading *Alphaproteobacteria* have also been identified in polluted soil aquifers (Alfreider and Vogt, 2007). The *Deltaproteobacteria* can oxidize PAHs by using sulfate as the electron acceptor (Selesi et al., 2010). Thus, high concentrations of metals in industrial sediments might led to suppressed microbial activities and element cycling processes (Jose et al., 2011; Mackintosh et al., 2016). Phylogenetic diversity of bacterial communities dwelling in the industrial region had relatively lower than in the other two types. The impaired bacterial communities in industrial sediments might cause some degrees of loss of ecological functions (Jose et al., 2011).

Metal contaminants and nutrients had stronger influence on shaping the structure of sediment microbial communities than other measured variable. Although some metals are required in minute amounts to catalyze reactions or maintain protein structures, there is a subsidy-stress relationship that results in a window of optimal concentrations outside of which metals can cause adverse effects on bacterial communities. 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 to changes in concentrations of metals can include a decrease in microbial biomass (Gillan and Pernet, 2007), shifts in the dominant species (Gough and Stahl, 2011), changes in diversity (Sun et al., 2012), modulation of enzyme activities (Hoostal et al., 2008; Wainwright and Duddridge, 1982) and the introduction of genes resistant to metals (Ryan et al., 2005).

Besides metals and nutrients, PAHs can shape the structure of bacterial communities by increasing the proportions of bacteria that can degrade PAHs and use them as sources of carbon or energy (Tian et al., 2008). Responses of structures of bacterial populations to contamination with pesticides have not been well investigated to date. Pesticides could influence the biomass, community structure and enzymatic activity of sediment microbiome (Widenfalk et al., 2008). Influences of specific contaminants on the *in situ* bacterial communities might indicate the possible roles of bacteria in the element cycles (Vishnivetskaya et al., 2011; Yergeau et al., 2012).

4.3. Diverse response patterns of individuals to contaminants

The patterns of response to contaminants were diverse among bacterial species (OTUs) (Fig. 5B, Fig. S3 and Table S5). *Anaeromyxobacter* was sensitive to Ni and Fe but resistant to Cd, Zn and Mn, for the ability to reduce soluble and amorphous ferric iron and other oxidized metal species (Chao et al., 2010; Wu et al., 2006). Several of the measured contaminants were negatively correlated with core OTUs, most likely due to their toxicity (Nogales et al., 2011; Valls and de Lorenzo, 2002), despite inputs of nutrients. Core OTUs were positively correlated with metals, might for stimulating amino acid and carbohydrate metabolism. Several OTUs were positively correlated with the pesticides, which suggested that these taxa were tolerant or that they might be able to use some of these compounds as energy sources. It should also be noted that the quantitative relationship between bacteria/bacterial communities and contaminants described in this study may have been influenced by confounding factors, such as water depth, redox and organic carbon content, sulfide production, pH and grain size, but their effects can be difficult to differentiate from the toxic effects of the contaminants (Olson et al., 1990).

Here the relationships between environmental variables and bacterial communities were identified by correlation analysis, but not causal study. However, this analysis reveals the potential contaminant stressors from the microbial community aspect. Other approaches, such as toxicity identification evaluation (TIE) experiment and sediment mesocosm studies, could be conducted to confirm the actual causative agents (Yi et al., 2015). The result of this study highlighted the potential utilities of ecogenomics (both phylogenetic diversity and function characters) in the future development of river management frameworks (Gibson et al., 2015).

5. Conclusions

The present study showed that sediments from different land-use regions of Nanfei river contained distinct bacterial community structures, which were associated with the level of anthropogenic contaminants. Comparing to other two land use types, bacterial communities dwelling in the industrial region had relatively lower alpha diversity. There were significant associations between bacterial community phylogenies and the anthropogenic contaminants in the sediments. Components and structures of *in situ* sediment bacterial communities can be used to monitor the key stressors of freshwater river basin ecosystem. Metal contaminants and nutrients had stronger influence on shaping the structure of sediment microbial communities than the other measured variables. Furthermore, several sensitive candidate genera were identified as potential bio-indictors to monitor contaminants by species–contaminant correlation analysis. Nutrients (TN and TP) and two measured metals (Cd and Zn) were negatively correlated with the essential core of the bacterial interaction network (*Betaproteobacteria* and *Deltaproteobacteria*). While few OTUs were positively correlated with PAHs or pesticides. Future study should employ other diagnostic approaches like sediment TIE to confirm the key stressors to the ecosystem function. Overall, *in situ* bacterial communities could provide a useful tool for monitoring and assessing sediment quality.

Acknowledgments

This project was supported by the National Natural Science Foundation of China (Grant No. 21322704), Jiangsu Natural Science Foundation (SBK201310181) and Major Science and Technology Program for Water Pollution Control and Treatment (Grant# 2012ZX07506-003). Prof. Xiaowei Zhang was supported by Collaborative Innovation Center for Regional Environmental Quality. Prof. Giesy was supported by the program of “High Level Foreign Experts” (GDT20143200016) funded by the State Administration of Foreign Experts Affairs, the P.R. China to Nanjing University and the Einstein Professor Program of the Chinese Academy of Sciences. He was also supported by the Canada Research Chair program, a Visiting Distinguished Professorship in the Department of Biology and Chemistry and State Key Laboratory in Marine Pollution, City University of Hong Kong.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2016.01.031.

References

Alfreider, A., Vogt, C., 2007. Bacterial diversity and aerobic biodegradation potential in a BTEX-contaminated aquifer. Water Air Soil Poll. 183, 415–426.

ANZECC/ARMCANZ, 2000. Australian and New Zealand Guidelines for Fresh and Marine Water Quality. Australian and New Zealand Environment and Conservation Council, Canberra, Australia.

Burton, G.A., Johnston, E.L., 2010. Assessing contaminated sediments in the context of multiple stressors. Environ. Toxicol. Chem. 29, 2625–2643.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Tumbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336.

Chao, T.C., Kalinowski, J., Nyalwidhe, J., Hansmeier, N., 2010. Comprehensive proteome profiling of the Fe(III)-reducing myxobacterium anaeromyxobacter dehalogenans 2CP-C during growth with fumarate and ferric citrate. Proteomics 10, 1673–1684.

Craft, J.A., Stanford, J.A., Pusch, M., 2002. Microbial respiration within a floodplain aquifer of a large gravel-bed river. Freshw. Biol. 47, 251–261.

DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D., Hu, P., Andersen, G.L., 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl. Environ. Microbiol. 72, 5069–5072.

Diniz-Filho, J.A., Soares, T.N., Lima, J.S., Dobrovolski, R., Landeiro, V.L., de Campos Telles, M.P., Rangel, T.F., Bini, L.M., 2013. Mantel test in population genetics. Genet. Mol. Biol. 36, 475–485.

Ducklow, H., 2008. Microbial services: challenges for microbial ecologists in a changing world. Aquat. Microb. Ecol. 53, 13–19.

Ebina, J., Tsutsui, T., Shirai, T., 1983. Simultaneous determination of total nitrogen and total phosphorus in water using peroxodisulfate oxidation. Water Res. 17, 1721–1726.

Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods 10, 996–999.

Eggleton, J., Thomas, K.V., 2004. A review of factors affecting the release and bioavailability of contaminants during sediment disturbance events. Environ. Int. 30, 973–980.

Faris, K.P., Ramsey, P.W., Gibbons, S.M., Frazar, C., Rillig, M.C., Moore, J.N., Gannon, J.E., Holben, W.E., 2009. Hyporheic microbial community development is a sensitive indicator of metal contamination. Environ. Sci. Technol. 43, 6158–6163.

Faust, K., Sathirapongsasuti, J.F., Izard, J., Segata, N., Gevers, D., Raes, J., Huttenhower, C., 2012. Microbial co-occurrence relationships in the human microbiome. PLoS Comput. Biol. 8.

Fischer, H., Pusch, M., 2001. Comparison of bacterial production in sediments, epiphyton and the pelagic zone of a lowland river. Freshw. Biol. 46, 1335–1348.

Gibbons, S.M., Jones, E., Bearquiver, A., Blackwolf, F., Roundstone, W., Scott, N., Hooker, J., Madsen, R., Coleman, M.L., Gilbert, J.A., 2014. Human and environmental impacts on river sediment microbial communities. Plos One 9.

Gibson, J.F., Stein, E.D., Baird, D.J., Finlayson, C.M., Zhang, X., Hajibabaei, M., 2015. Wetland ecogenomics—the next generation of wetland biodiversity and functional assessment. Wetl. Sci. Pract. 32, 27–32.

Gillan, D.C., Pernet, P., 2007. Adherent bacteria in heavy metal contaminated marine sediments. Biofouling 23, 1–13.

Gough, H.L., Stahl, D.A., 2011. Microbial community structures in anoxic freshwater lake sediment along a metal contamination gradient. ISME J. 5, 543–558.

Halliday, E., McLellan, S.L., Amaral-Zettler, L.A., Sogin, M.L., Gast, R.J., 2014. Comparison of bacterial communities in sands and water at beaches with bacterial water quality violations. Plos One 9.

Han, G., Chen, J., He, C.Y., Li, S.N., Wu, H., Liao, A.P., Peng, S., 2015. A web-based system for supporting global land cover data production. Isprs J. Photogramm. 103, 66–80.

HHS, 2003. Toxicological Profile for Pyrethrins and Pyrethroids. U.S. Dept. of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Atlanta, Ga.

Hoostal, M.J., Bidart-Bouzat, M.G., Bouzat, J.L., 2008. Local adaptation of microbial communities to heavy metal stress in polluted sediments of Lake Erie. FEMS Microbiol. Ecol. 65, 156–168.

Jose, J., Giridhar, R., Anas, A., Loka Bharathi, P.A., Nair, S., 2011. Heavy metal pollution exerts reduction/adaptation in the diversity and enzyme expression profile of heterotrophic bacteria in Cochin estuary, India. Environ. Pollut. 159, 2775–2780.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Glöckner, F.O., 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acid. Res. 41, e1.

Lake, P.S., Palmer, M.A., Biro, P., Cole, J., Covich, A.P., Dahm, C., Gibert, J., Goedkoop, W., Martens, K., Verhoeven, J., 2000. Global change and the biodiversity of freshwater ecosystems: Impacts on linkages between above-sediment and sediment biota. Bioscience 50, 1099–1107.

Liu, F., Zhang, S., 2014. Ecological risk of organophosphorous and synthetic pyrethroid pesticides in water from Nanfeihe River. J. Herfei Univ. Technol. 37, 1499–1504.

Liu, J., Hua, Z.S., Chen, L.X., Kuang, J.L., Li, S.J., Shu, W.S., Huang, L.N., 2014. Correlating microbial diversity patterns with geochemistry in an extreme and heterogeneous environment of mine tailings. Appl. Environ. Microbiol. 80, 3677–3686.

Long, E.R., 1992. Ranges in chemical concentrations in sediments associated with adverse biological effects. Mar. Pollut. Bull. 24, 38–45.

Lozupone, C., Knight, R., 2005. UniFrac: a new phylogenetic method for comparing microbial communities. Appl. Environ. Microbiol. 71, 8228–8235.

Mackintosh, T.J., Davis, J.A., Thompson, R.M., 2016. Impacts of multiple stressors on ecosystem function: leaf decomposition in constructed urban wetlands. Environ. Pollut. 208, 221–232.

Nogales, B., Lanfranconi, M.P., Pina-Villalonga, J.M., Bosch, R., 2011. Anthropogenic perturbations in marine microbial communities. FEMS Microbiol. Rev. 35, 275–298.

Olson, R.J., Chisholm, S.W., Zettler, E.R., Armbrust, E.V., 1990. Pigments, size, and distribution of synechococcus in the North-Atlantic and Pacific oceans. Limnol. Oceanogr. 35, 45–58.

Ordonez, A., Loredo, J., De Miguel, E., Charlesworth, S., 2003. Distribution of heavy metals in the street dusts and soils of an industrial city in Northern Spain. Arch. Environ. Contam. Toxicol. 44, 160–170.

Paerl, H.W., Dyble, J., Moisander, P.H., Noble, R.T., Piehler, M.F., Pinckney, J.L., Steppe, T.F., Twomey, L., Valdes, L.M., 2003. Microbial indicators of aquatic ecosystem change: current applications to eutrophication studies. FEMS Microbiol. Ecol. 46, 233–246.

Ramsey, P.W., Rillig, M.C., Feris, K.P., Gordon, N.S., Moore, J.N., Holben, W.E., Gannon, J.E., 2005. Relationship between communities and processes; new insights from a field study of a contaminated ecosystem. Ecol. Lett. 8, 1201–1210.

Reed, H.E., Martiny, J.B.H., 2013. Microbial composition affects the functioning of estuarine sediments. ISME J. 7, 868–879.

Ren, C., Wu, Y., Zhang, S., Wu, L.-L., Liang, X.-G., Chen, T.-H., Zhu, C.-Z., Sojinu, S., Wang, J.-Z., 2015. PAHs in sediment cores at main river estuaries of Chaohu Lake: implication for the change of local anthropogenic activities. Environ. Sci. Pollut. Res. 22, 1687–1696.

Ryan, R.P., Ryan, D.J., Dowling, D.N., 2005. Multiple metal resistant transferable phenotypes in bacteria as indicators of soil contamination with heavy metals. J. Soils Sediments 5, 95–100.

Saito, R., Smoot, M.E., Ono, K., Ruscheinski, J., Wang, P.L., Lotia, S., Pico, A.R., Bader, G.D., Ideker, T., 2012. A travel guide to cytoscape plugins. Nat. Methods 9, 1069–1076.

Saxena, G., Marzinelli, E.M., Naing, N.N., He, Z.L., Liang, Y.T., Tom, L., Mitra, S., Ping, H., Joshi, U.M., Reuben, S., Mynampati, K.C., Mishra, S., Umashankar, S., Zhou, J.Z., Andersen, G.L., Kjelleberg, S., Swarup, S., 2015. Ecogenomics reveals metals and land-use pressures on microbial communities in the waterways of a megacity. Environ. Sci. Technol. 49, 1462–1471.

Selesi, D., Jehmlich, N., von Bergen, M., Schmidt, F., Rattei, T., Tischler, P., Lueders, T., Meckenstock, R.U., 2010. Combined genomic and proteomic approaches identify gene clusters involved in anaerobic 2-Methylnaphthalene degradation in the sulfate-reducing enrichment culture N47. J. Bacteriol. 192, 295–306.

Sindern, S., Lima, R.F.S., Schwarzbauer, J., Petta, R.A., 2007. Anthropogenic heavy metal signatures for the fast growing urban area of Natal (NE-Brazil). Environ. Geol. 52, 731–737.

Sun, M.Y., Dafforn, K.A., Brown, M.V., Johnston, E.L., 2012. Bacterial communities are sensitive indicators of contaminant stress. Mar. Pollut. Bull. 64, 1029–1038.

Sun, M.Y., Dafforn, K.A., Johnston, E.L., Brown, M.V., 2013. Core sediment bacteria drive community response to anthropogenic contamination over multiple environmental gradients. Environ. Microbiol. 15, 2517–2531.

Tian, Y., Liu, H.J., Zheng, T.L., Kwon, K.K., Kim, S.J., Yan, C.L., 2008. PAHs contamination and bacterial communities in mangrove surface sediments of the Jiulong River Estuary, China. Mar. Pollut. Bull. 57, 707–715.

Torsvik, V., Øvreås, L., Thingstad, T.F., 2002. Prokaryotic Diversity–Magnitude, dynamics, and controlling factors. Science 296, 1064–1066.

Valls, M., de Lorenzo, V., 2002. Exploiting the genetic and biochemical capacities of bacteria for the remediation of heavy metal pollution. FEMS Microbiol. Rev. 26, 327–338.

Vishnivetskaya, T.A., Mosher, J.J., Palumbo, A.V., Yang, Z.K., Podar, M., Brown, S.D., Brooks, S.C., Gu, B., Southworth, G.R., Drake, M.M., Brandt, C.C., Elias, D.A., 2011. Mercury and other heavy metals influence bacterial community structure in contaminated Tennessee streams. Appl. Environ. Microbiol. 77, 302–311.

Wainwright, M., Duddridge, J.E., 1982. Effects of heavy-metals on enzyme-synthesis in substrate-amended river sediments. Eur. J. Appl. Microbiol. Biotech. 15, 241–245.

Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73, 5261–5267.

Widenfalk, A., Bertilsson, S., Sundh, I., Goedkoop, W., 2008. Effects of pesticides on community composition and activity of sediment microbes - responses at various levels of microbial community organization. Environ. Pollut. 152, 576–584.

Wu, Q., Sanford, R.A., Loffler, F.E., 2006. Uranium(VI) reduction by anaeromyxobacter dehalogenans strain 2CP-C. Appl. Environ. Microbiol. 72, 3608–3614.
Wu, Y., Zhang, S., Ren, C., Xie, Y.-W., Zhang, X.-W., Sojinu, S.O., Chen, T.-H., Wang, J.-Z., 2014. Residues of organophosphorus insecticides in sediment around a highly eutrophic lake, Eastern China. J. Soils Sediments 15, 436–444.
Yergeau, E., Lawrence, J.R., Sanschagrin, S., Waiser, M.J., Korber, D.R., Greer, C.W., 2012. Next-generation sequencing of microbial communities in the Athabasca river and its tributaries in relation to oil sands mining activities. Appl. Environ. Microbiol. 78, 7626–7637.
Yi, X., Li, H., Ma, P., You, J., 2015. Identifying the causes of sediment-associated toxicity in urban waterways in South China: incorporating bioavailabillity-based measurements into whole-sediment toxicity identification evaluation. Environ. Toxicol. Chem. 9999, 1–7.
Zhou, J., Deng, Y., Luo, F., He, Z., Yang, Y., 2011. Phylogenetic molecular ecological network of soil microbial communities in response to elevated CO2. Mbio 2, e00122–11.

Using *in situ* bacterial communities to monitor contaminants in river sediments

Yuwei Xie[a], Jizhong Wang[b], Yaketon Wu[b], Chen Ren[b], Chao Song[a], Jianghua Yang[a], Hongxia Yu[a], John P. Giesy[a,c,d,e], Xiaowei Zhang[a*]

[a] State Key Laboratory of Pollution Control & Resource Reuse, School of the Environment, Nanjing University, Nanjing, 210023, China

[b] Laboratory for Nanomineralogy and Environmental Material, School of Resources and Environmental Engineering, Hefei University of Technology, Hefei, 230009, China

[c] Department of Veterinary Biomedical Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

[d] School of Biological Sciences, University of Hong Kong, Hong Kong, SAR, China

[e] Department of Biology, Hong Kong Baptist University, Kowloon, Hong Kong, SAR, China

* Corresponding author. State Key Laboratory of Pollution Control & Resource Reuse, School of the Environment, Nanjing University, 163 Xianlin Great Rd, Nanjing, 210023, China.

E-mail address: howard50003250@yahoo.com (XW Zhang)

Supporting Information

FIGURE LEGENDS

Figure S1. Phylogenetic diversity (A) and Shannon index (B) rarefaction curves of microbial communities in sediments. The patterns of these alpha diversity indices among groups were similar. Alpha rarefaction curves showed that 15,000-20,000 effective sequences captured most of the trends in the phylogenetic diversity richness indices.

Figure S2. Core microbial co-occurrence relationships in the sediments. Each node represents the top 100 abundant bacterial OTUs, and the size of nodes indicates the nodes' degree. Labels of nodes were set at the phylum level in A, D and E and at the class level in B and C.

Figure S3. Species response curves of the 5 most abundant bacterial genera to contaminate concentrations. Top 2nd genus was discarded for P_{FDR}>0.5. Genus 1, *Syntrophus*; genus 3, Dechloromonas; genus 4, *Geobacter*; genus 5, *Leptospirales SJA-88*.

TABLE LEGENDS

Table S1. Latitude/Longitude and group information for each sampling site.

Table S2. Means, std. errors, minimum and maximum values of environmental variables measured in the study sediments.

Table S3. Pearson correlation coefficients between environmental variables. Correlations ($|r| > 0.75$) were in red.

Table S4. Raw, denoised reads, plus numbers of alpha diversity of each samples.

Table S5. Predicted response curve of filtered top 150 abundant genera to contaminants.

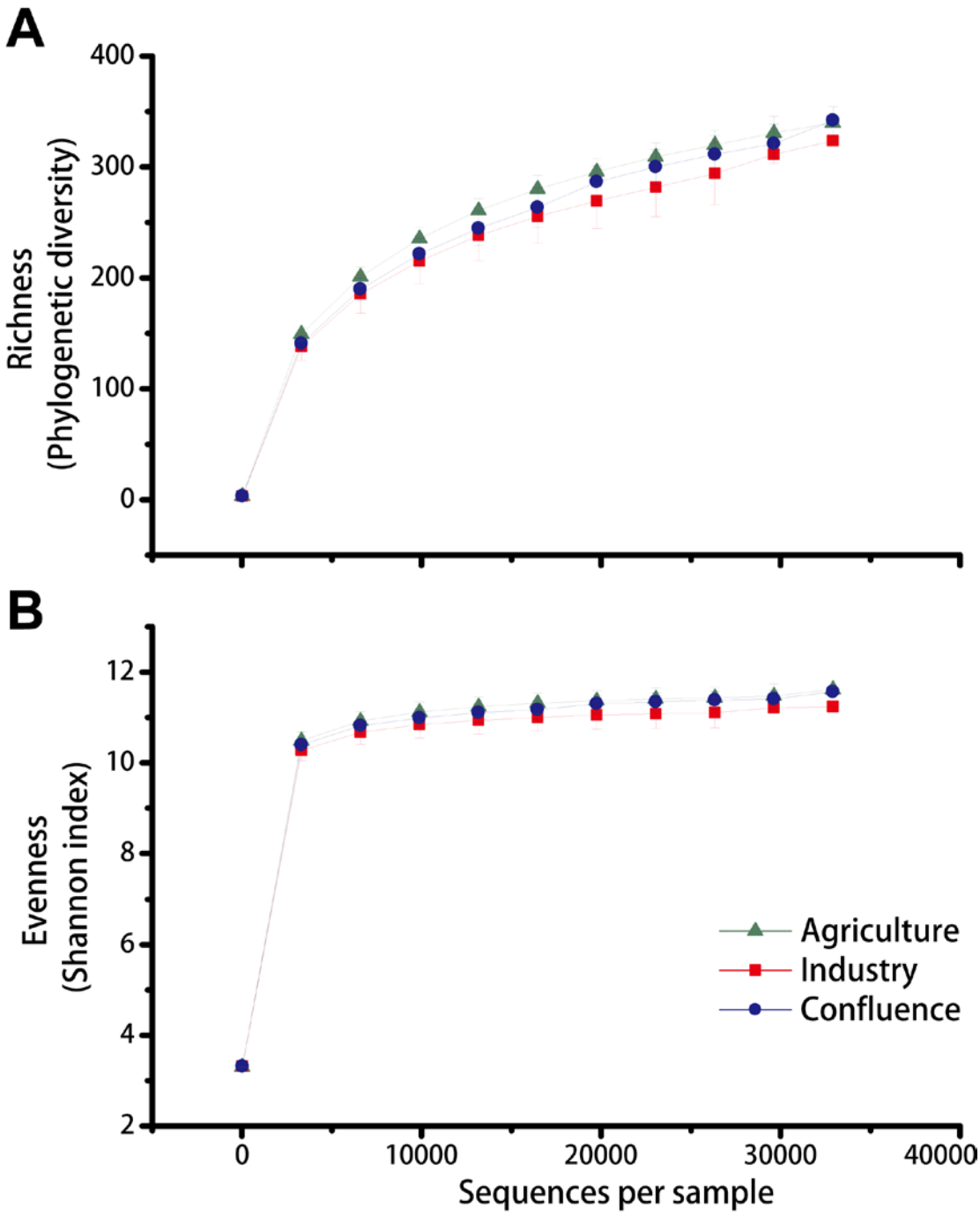
A
400
300
200
100
0
Richness
(Phylogenetic diversity)
B
12
10
8
6
4
2
Evenness
(Shannon index)
0
10000
20000
30000
40000
Sequences per sample
Agriculture
Industry
Confluence

Figure S1.

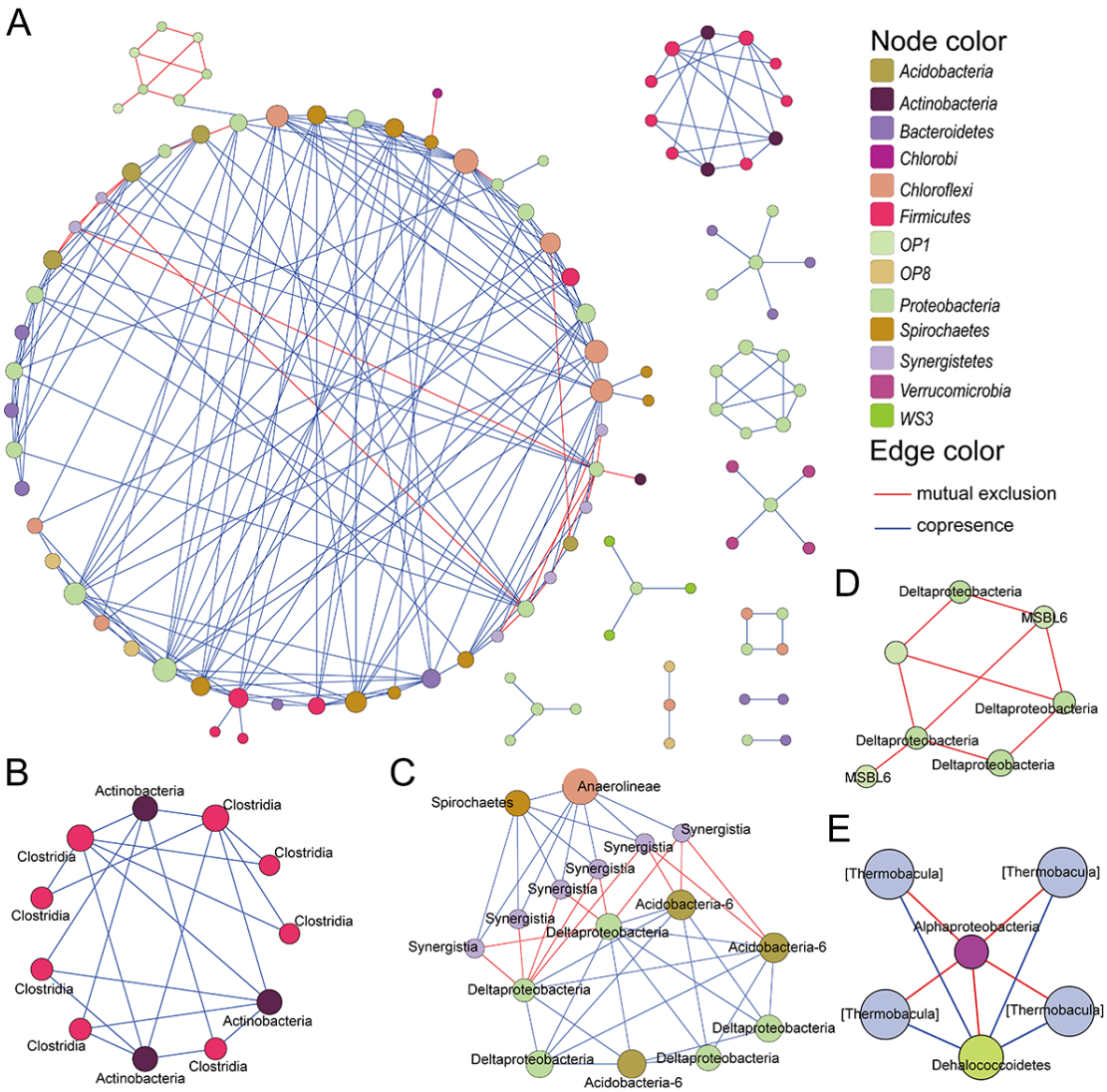
A
Node color
Acidobacteria
Actinobacteria
Bacteroidetes
Chlorobi
Chloroflexi
Firmicutes
OP1
OP8
Proteobacteria
Spirochaetes
Synergistetes
Verrucomicrobia
WS3
Edge color
mutual exclusion
copresence
B
Actinobacteria
Clostridia
Clostridia
Clostridia
Clostridia
Clostridia
Clostridia
Actinobacteria
Clostridia
Clostridia
Actinobacteria
C
Anaerolineae
Spirochaetes
Synergistia
Synergistia
Synergistia
Synergistia
Synergistia
Acidobacteria-6
Deltaproteobacteria
Acidobacteria-6
Deltaproteobacteria
Deltaproteobacteria
Deltaproteobacteria
Deltaproteobacteria
Acidobacteria-6
D
Deltaproteobacteria
MSBL6
Deltaproteobacteria
Deltaproteobacteria
Deltaproteobacteria
MSBL6
E
[Thermobacula]
[Thermobacula]
Alphaproteobacteria
[Thermobacula]
[Thermobacula]
Dehalococcoidetes

Figure S2.

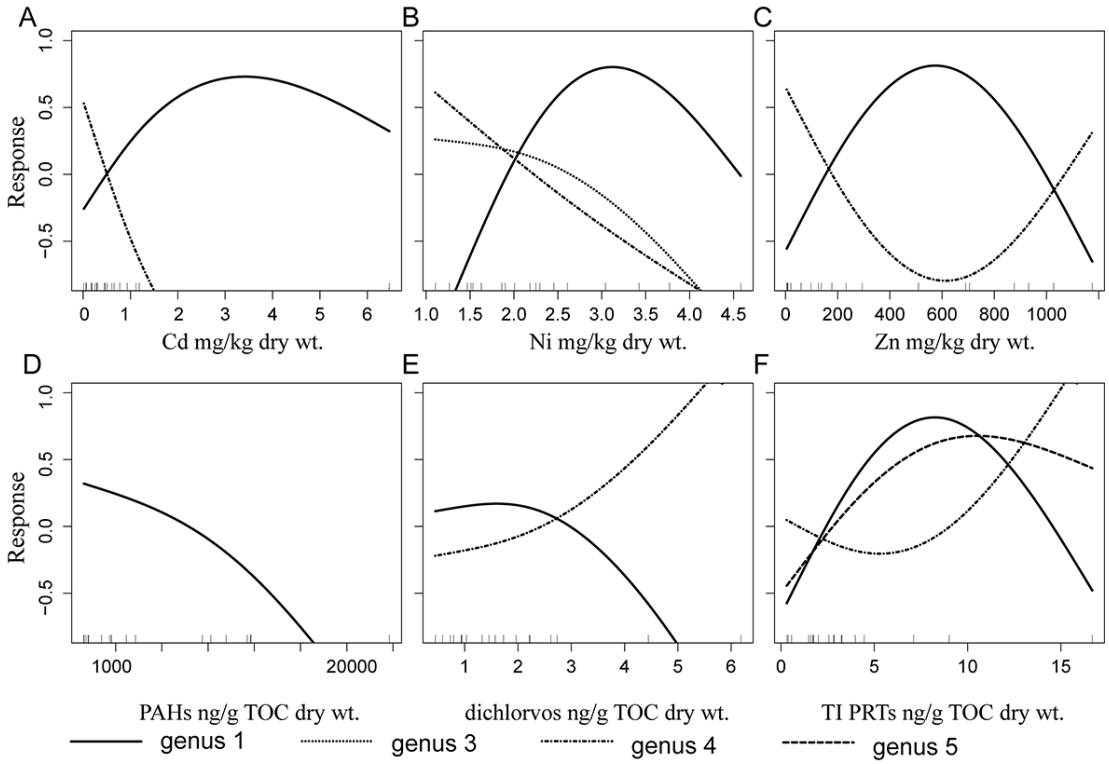
A
B
C
D
E
F
Response
Cd mg/kg dry wt.
Ni mg/kg dry wt.
Zn mg/kg dry wt.
PAHs ng/g TOC dry wt.
dichlorvos ng/g TOC dry wt.
TI PRTs ng/g TOC dry wt.
genus 1
genus 3
genus 4
genus 5

Figure S3.

Table S1.

Sampling Sites	Latitude	Longitude	Group
AR1	31.933333	117.28333	agriculture
AR2	31.9	117.31667	agriculture
AR3	31.833333	117.4	agriculture
AR4	31.883333	117.45	agriculture
AR5	31.816667	117.46667	agriculture
AR6	31.85	117.3	agriculture
CF1	31.7	117.4	confluence
CF2	31.683333	117.4	confluence
CF3	31.683333	117.4	confluence
CF4	31.683333	117.38333	confluence
ID1	31.9	117.23333	industry
ID2	31.866667	117.28333	industry
ID3	31.766667	117.41667	industry
ID4	31.8	117.36667	industry
ID5	31.783333	117.4	industry
ID6	31.766667	117.41667	industry
ID7	31.75	117.41667	industry
ID8	31.716667	117.38333	industry

Table S2.

Variable	Minimum	Maximum	Median	Means	Std. Deviation
TN (% dry wt)	0.055	0.546	0.211	0.215167	0.120873
TP (P2O5, % dry wt)	0.063505	0.659	0.380307	0.386264	0.185004
TOC (% dry wt)	0.160302	4.960946	1.370361	1.484325	1.066956
Cd (mg/kg dry wt)	0.015119	6.467233	0.459164	0.805707	1.415099
Cr (mg/kg dry wt)	29.91505	109.5725	56.29648	62.50796	27.95532
Pb (mg/kg dry wt)	8.4404	77.55762	25.25886	34.78047	21.63938
Ni (mg/kg dry wt)	1.104314	4.58302	2.09515	2.271942	0.900692
Cu (mg/kg dry wt)	9.890585	122.622	32.03916	43.15216	31.61006
Fe (mg/kg dry wt)	18244.84	48810.28	31902.89	31547.95	7788.115
Zn (mg/kg dry wt)	4.558189	1175.269	205.2049	393.2255	390.1714
Mn (mg/kg dry wt)	126.4011	368.2998	202.1128	215.1665	73.31803
naphthalene (ug/kg TOC dry wt)	194.79	36321.63	5660.32	10474.53	11710.54
acenaphtylene (ug/kg TOC dry wt)	518.55	7280.16	2533.13	3215.658	2219.546
acenaphthene (ug/kg TOC dry wt)	83.89	74424.28	1490.01	14273.89	20536.68
fluorene (ug/kg TOC dry wt)	827.63	104462.7	9514.53	22830.72	28978.44
phenanthrene (ug/kg TOC dry wt)	2989.38	173993.7	26366.82	47163.75	52307.06
anthracene (ug/kg TOC dry wt)	432.62	55903.95	3777.19	15006.21	17858.86
fluoranthene (ug/kg TOC dry wt)	8709.53	231412.4	42288.42	73447.05	72658.5
pyrene (ug/kg TOC dry wt)	3101.82	170701.8	31515.77	54962.96	58737.24
benzo[a]anthracene (ug/kg TOC dry wt)	6163.13	141781	30754.22	46888.67	42182.71
chrysene (ug/kg TOC dry wt)	9882.39	146554	38543.78	50236.54	41028.09
benzo[b]fluoranthene (ug/kg TOC dry wt)	1893.19	51016.95	12915.61	17767.94	15540.74
benzo[k]fluoranthene (ug/kg TOC dry wt)	5143.66	81684.91	17501.07	24685.65	21010.32
benzo[a]pyrene (ug/kg TOC dry wt)	6213.77	138447.8	24599.95	41004.63	36738.72
indeno[1,2,3-cd]pyrene (ug/kg TOC dry wt)	12684.56	251734.5	48982.18	63091.9	62994.13
dibenzo[a,h]anthracene (ug/kg TOC dry wt)	3645.64	83892.29	16855	22471.46	21948
benzo[g,h,i]perylene (ug/kg TOC dry wt)	14985.86	304854.2	51709.13	72623.35	73392.9
methidathion (ug/kg TOC dry wt)	0.5	12.87	0.92	3.149444	4.355776
dichlorvos (ug/kg TOC dry wt)	44.68	618.2	151.315	188.4233	141.3087
dyfonate (ug/kg TOC dry wt)	0.02	0.3	0.08	0.107778	0.070282
diazinon (ug/kg TOC dry wt)	0.46	47.06	6.98	14.69	16.26056
CFVM (ug/kg TOC dry wt)	0.35	8.93	1.785	2.878333	2.391676
fenitrothion (ug/kg TOC dry wt)	0.07	4.28	0.225	0.708333	1.034871
malathion (ug/kg TOC dry wt)	0.1	2.1	0.37	0.585556	0.603602
parathion (ug/kg TOC dry wt)	0.19	11.18	0.785	1.626667	2.484914
chlorfenvinphos (ug/kg TOC dry wt)	0.02	3.76	0.12	0.375556	0.839426
ethion (ug/kg TOC dry wt)	0.01	0.52	0.03	0.067778	0.115111
carbophenothion (ug/kg TOC dry wt)	0	0.1	0.01	0.018333	0.022423
phosalone (ug/kg TOC dry wt)	0.05	50.17	0.87	3.996111	11.27766
tefluthrin (ug/kg TOC dry wt)	0.01	3.26	0.15	0.664444	0.929685
bioallethrin (ug/kg TOC dry wt)	0.7	6.35	1.995	2.69	1.700212

Variable	Minimum	Maximum	Median	Means	Std. Deviation
prallethrin (ug/kg TOC dry wt)	0.07	1.93	0.285	0.472222	0.457828
tetramethrin (ug/kg TOC dry wt)	0.39	18.1	1.55	2.603333	3.907663
permethrin (ug/kg TOC dry wt)	24.79	1639.65	262.725	356.6189	378.5758
cyfluthrin (ug/kg TOC dry wt)	5.43	205.99	9.355	22.51444	45.18182
cypermethrin (ug/kg TOC dry wt)	76.94	1135.08	239.16	305.5556	282.4656
fenvalerate (ug/kg TOC dry wt)	39.46	2380.58	156.8	381.9911	584.3349
deltamethrin (ug/kg TOC dry wt)	11.59	113.93	23.475	33.11389	25.06824
Cyhalothrin (ug/kg TOC dry wt)	22.58	2580.55	81.795	230.8739	573.5289
esfenvalerate (ug/kg TOC dry wt)	12.02	545.97	36.75	96.72111	140.1759
allethrin (ug/kg TOC dry wt)	0.29	7.82	2.01	2.308333	1.726706

Table S3.
***Provided as another single csv file.**

Table S4.

Sample	Reads			Alpha diversity		
	Raw	Unique	Denoised	OTUs	Chao1	PD whole tree
AR1	47950	30540	39859	2305	2779	208
AR2	46269	29795	38565	2194	2639	202
AR3	42891	26319	33663	1996	2378	190
AR4	51525	35592	41060	2314	2747	212
AR5	34012	21944	27378	2071	2527	196
AR6	43654	28860	35132	2327	2777	218
CF1	40149	25018	33382	2381	2871	218
CF2	78396	44772	67137	2365	2901	217
CF3	20812	14240	16726	1609	2113	159
CF4	40239	27680	32392	1993	2384	189
ID1	35372	21849	28957	1676	2112	168
ID2	46144	29184	37330	2046	2471	197
ID3	32320	19670	26774	1604	2045	162
ID4	40264	25794	32463	2047	2468	198
ID5	35037	22593	27579	2224	2693	210
ID6	35102	23006	27514	2292	2802	216
ID7	28248	16206	23168	1903	2397	187
ID8	52722	33154	43165	2155	2638	200

Table S5.

phylum	family	genus	Cd	Ni	Fe	Zn	Mn	acenaphthene	benzo[a]pyrene	dichlorvos	TI PRTs
Bacteroidetes	*Flavobacteriaceae*	*Flavobacterium*	-	SR[a]	-	R[b]	-[c]	R	-	-	-
Bacteroidetes	*Rikenellaceae*	*Blvii28*	R	-	-	-	-	-	-	-	-
Bacteroidetes	*Cryomorphaceae*	*Fluviicola*	S[d]	S	S	S	SR	-	RS[e]	-	SR
Bacteroidetes	*Porphyromonadaceae*	*Paludibacter*	R	-	-	-	-	-	-	-	RS
Bacteroidetes	*Prevotellaceae*	*Prevotella*	-	S	S	-	R	S	S	S	S
Chloroflexi	*Anaerolinaceae*	*WCHB1-05*	RS	R	-	RS	RS	-	-	-	-
Cyanobacteria	*Microcystaceae*	*Microcystis*	SR	S	S	S	R	S	RS	SR	SR
Nitrospirae	*FW*	*4-29*	S	S	S	S	SR	-	R	-	SR
Nitrospirae	*Nitrospiraceae*	*Nitrospira*	S	S	S	S	SR	-	-	-	-
Nitrospirae	*Thermodesulfovibrionaceae*	*GOUTA19*	S	S	S	S	SR	-	R	-	SR
Proteobacteria	*Syntrophaceae*	*Syntrophus*	RS	RS	RS	RS	RS	-	S	S	RS
Proteobacteria	*Rhodocyclaceae*	*Dechloromonas*	-	S	-	-	-	-	-	-	-
Proteobacteria	*Geobacteraceae*	*Geobacter*	S	S	S	SR	SR	-	-	R	SR
Proteobacteria	*Hydrogenophilaceae*	*Thiobacillus*	SR	S	S	RS	SR	-	-	-	SR
Proteobacteria	*Myxococcaceae*	*Anaeromyxobacter*	SR	S	S	SR	SR	-	-	R	SR
Proteobacteria	*Crenotrichaceae*	*Crenothrix*	RS	SR	S	S	SR	-	-	-	-
Proteobacteria	*Methylococcaceae*	*Methylomonas*	-	-	-	-	R	-	R	-	-
Spirochaetes	*Sediment-4*	*SJA-88*	-	-	-	-	-	-	-	-	RS

a unimodal peak (U shape)

b resistant

c PFDR > 0.05

d sensitive

e unimodal peak (reversal U shape)