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Bacterial communities along a 4,500-meter elevation gradient in the sediment of the Yangtze River: what are the driving factors?

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ABSTRACT

Microorganisms are important transporters of mass and energy under the influence of external disturbances. However, the bacterial communities in the sediment of the Yangtze River and the influence of the synergistic effect of geographical characteristics and anthropogenic activities remain poorly understood. To analyze the bacterial community compositions and determine the driving factors that cause the niche segregation of the bacterial assembly, samples located along the Yangtze River were analyzed using Illumina Miseq sequencing. Based on the results, Proteobacteria, Bacteroidetes, Actinobacteria, Acidobacteria, and Chloroflexi were identified as dominant phyla, and Alphaproteobacteria was the most dominant subdivision. The bacterial diversity was most significantly correlated with elevation ($p < 0.01$), followed by urbanization rate ($p < 0.01$), and organic matter ($p < 0.05$). A decreasing trend of bacterial diversity was found along the elevation and a threshold of 400 masl was observed with regard to the bacterial richness and community compositions in response to elevation gradient. Above 400 masl, the bacterial richness was closely associated with elevation gradient, followed by total phosphorus and total nitrogen, while below 400 masl elevation, urbanization rate and Cu were dominant variables. Redundancy analysis indicated that bacterial community compositions were mostly related to the input of organic matter (20.0%, $p = 0.001$), followed by elevation (10.4%, $p = 0.003$), urbanization rate (9.0%, $p = 0.017$) and Pb (9.5%, $p = 0.026$). Distance-decay correlation analysis also showed that the variations of bacterial community structures were significantly positively correlated with elevation and organic matter. Based on the results of the meta-community, in general, the altitudinal gradient exerted a more notable influence than anthropogenic disturbance on bacterial communities. However, increased concentrated anthropogenic exploitation and interference might contribute more to deterministic processes driven by competition and niche differentiation of bacterial community structures in the sediment of the Yangtze River along the elevation gradient.

Keywords: Bacterial community; Deterministic processes; Anthropogenic effects; Elevation; Organic matter; Yangtze River

1. Introduction

Streams and rivers are essential links between terrestrial ecosystems and the global marine continuum, and are essential sources for hydropower, industrial, agricultural, and domestic water [1,2]. The ecological security of rivers is related to global water environmental security and human health. The streams system is subjected to significant changes in response to anthropologic activities, and the Yangtze River, which is the longest river in Asia, was particularly influenced by urbanization and hydraulic engineering [3,4]. It is important to understand the role of the river system

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within the interaction of biogeochemical nutrients and the transformation and temporary storage of terrestrially-derived organic matter (OM) [2,5]. These are based on the function of the microbial community in riverbed sediment [6,7]. Microbial communities have been suggested to play central roles in aquatic ecosystems and biogeochemistry cycles [8,9]. Moreover, bacterial assemblages quickly respond to abrupt environmental perturbation and are sensitive to anthropogenic disturbances [1].

Recently, two types of processes (deterministic processes and stochastic processes) have been applied to explain the response of microbial community variations to environmental disturbance [10]. Although stochastic processes, related to neutral theory, based on the diffusion and migration of bacterial communities as a result of the water flow, had been established, a multitude of microbial communities were structured by their local environment [11]. Considering special geographical characteristics, such as elevation and rapid development of the Yangtze basin, deterministic processes, driven by species competition, selection, and niche differentiation, were considered to determine the mechanisms of bacterial distribution and succession. Previous studies indicated that microbial communities could be largely structured by deterministic effects, such as niche differentiation [12]. Thus, deterministic processes, that assess the interaction between natural selection and anthropogenic selection effects, may cause variations in response to altitude.

With regard to the effects of natural selection, altitudinal patterns of diversity and bacterial community functional traits had been proved in previous studies [12,13]. However, the bacterial diversity and community functional traits along large elevation gradients in river sediment have not been studied in depth, particularly in the Yangtze river. Elevation gradient, as a typical geographical factor, was suggested to cause strong variances in climate and biotic features over short geographic distances [14]. Bryant et al. [15] reported that altitudinal gradients correlate with the taxonomic changes of microorganisms and macroorganisms. Previous investigations considered mountainous systems [14,16,17] and glacial ecosystems [18] to analyze the effects of altitudinal patterns of microbial communities. Geographical variation trends were observed in bacterial diversity along elevation gradients based on various ecosystems. Wilhelm et al. [12] documented that the microbial community compositions of the biofilm in alpine streams changed along the elevation gradient. Hence, whether the altitudinal gradient is one of the key factors affecting microbial diversity and community compositions in stream and river ecosystems should be investigated. It is of great importance to further determine how altitudinal gradients affect bacterial communities in the sediment of the Yangtze River continuum.

The Yangtze River closely link Eurasia with the marine ecosystem, and is the third longest river in the world and the longest among all rivers in Asia [19]. Its water quality is significantly correlated to global water environmental safety. Except for the characteristic altitude gradients of the Yangtze River, anthropogenic selection effects should also be considered. The Yangtze River watershed is one of the most economically developed basins in China. Its urban agglomeration has become a remarkable phenomenon, and undoubtedly caused underlying ecological destruction driven by human activities [20]. Anthropogenic inputs (e.g., OM) affect bacterial communities and their function in the sediment of the Yangtze River and have inevitably become a key problem that disturbs the stability and security of this ecosystem. Yang et al. [21] reported that the Yangtze River faces unprecedented ecological risk caused by allochthonous inputs. However, previous studies mainly focused on functional bacteria and the microbial distribution in small specific areas or overlying water bodies [22–24]. The bacterial communities in the sediment of the Yangtze River under the influence of elevation gradients, as well as the anthropogenic interferences have not been investigated so far. Hence, knowledge about the driving factors, elevation gradients, or anthropogenic inputs, that result in the variance of microbial community compositions in the sediment of the Yangtze River is of great importance.

In this study, high-throughput sequencing technology was applied to explore the phylogenetic diversity patterns of bacteria and relative environmental variables along the elevation gradient in the sediment of the Yangtze River. The objectives of the study were (1) to explore the biogeographical patterns and driving factors for the bacterial composition of the sediment of the Yangtze River; (2) to identify the most significant driving factors, elevation gradients, or anthropogenic inputs (e.g., OM) that cause the deterministic process related to niche differentiation; (3) to further determine the key variables among anthropogenic selection processes if the dominant drivers were anthropogenic inputs only. To do so, statistical methods were used to detect the distribution pattern based on operational taxonomic units (OTUs) and environmental factors, that is, non-metric multidimensional scaling analysis (NMDS), canonical correspondence analysis (CCA), and redundancy analysis (RDA). Accordingly, the study provides theoretical support for the initiation of a water environmental evaluation index system toward the repair of the water quality of the Yangtze River.

2. Materials and methods

2.1. Study site and sample collection

Surveys were carried out at eight state-controlled sections along the Yangtze River (Fig. 1). Each section set three sampling sites, located in the upstream of city, downstream of city and inner city, respectively, so that there were 24 sampling sites in total. All sediment samples were collected using mud bucket in July 2014 and January 2015, respectively. Triplicate sediments were collected at 20 m intervals. Each sample was made by mixing three triplicates randomly and saved in dry-ice box immediately after removing visible plant particles and transported to the laboratory as soon as possible. Each sample was split into two parts, with one stored at –4°C for physicochemical properties analysis and the other was stored at –80°C in the laboratory until bacterial molecular genomic determination.

2.2. Environmental physicochemical properties analysis

Water temperature was measured using a multiparameter water quality analyzer (HQ30d, Hach, USA) in situ. Soil pH (water:soil ratio of 2.5:1) was determined by a

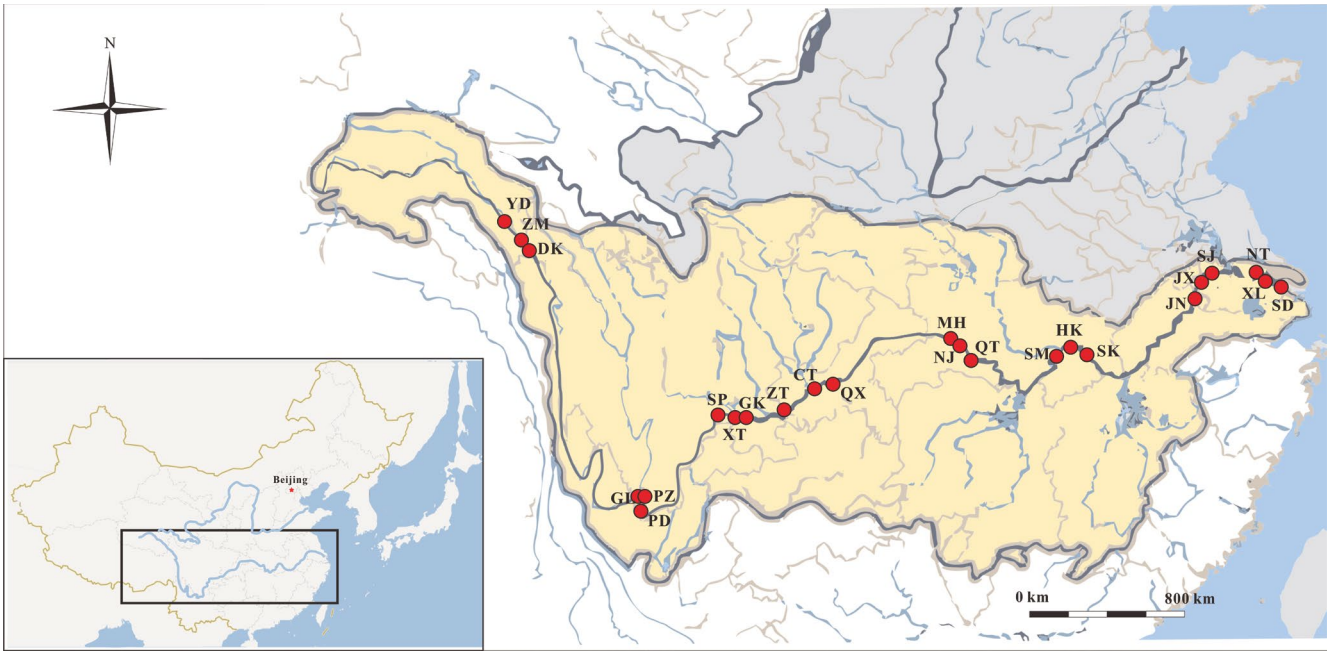

Fig. 1. Map showing the 24 sampling sites located along the Yangtze River.

digital pH meter. Total nitrogen (TN) was determined by an element analyzer (VarioEL cube, Elementar, Germany) and total phosphorus (TP) was measured by spectrophotometry after digestion. The sediment organic matter (OM) content was analyzed using the method provided in previous study [25]. Copper (Cu), lead (Pb), cadmium (Cd) [5] and arsenic (As) were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) as previous study introduced [26] and hydrargyrum (Hg) was analyzed by Mercury Analyzer (MA-800, Taiwan). The physicochemical properties of the aquatic environment and sediment collected are summarized (Table 1), as well as the geographical characteristics and urban development level of typical cities (Table 2).

2.3. DNA extraction and high-throughput sequencing

Bacterial DNA was extracted from sediments using the FastDNA spin kit (MP Biomedicals, USA) according to the manufacturer's instructions. The extracted DNA was examined with 3 μL in 1% agarose gels by electrophoresis. Other extracted DNA were sent to Shanghai Majorbio for high-throughput sequencing on an Illumina MiSeq instrument (San Diego CA, USA) using a paired-end 150 bp sequence read run. A set of primers, 338F (5′-ACTCCTRCGGGAGGCAGCAG-3′) and 806R (5′-GGACT ACCVGGGTATCTAAT-3′), was applied to amplify the V3-V4 hypervariable region of bacterial gene. A sample-specific barcode was added to reverse primer for identifying individual samples in a mixture within a single pyrosequencing [27]. A 20 μL reaction system was applied to amplify the bacterial DNA using the following protocol: 95°C for 3 min, 27 cycles of 30 s at 95°C, 30 s at 55°C, and 45 s at 72°C, and a final extension at 72°C for 10 min.

2.4. Sequence analysis

The 16S rRNA-based Illumina Miseq sequencing data were analyzed using Mothur software package and SILVA database [27,28]. Raw sequences were removed if any sequences length was <50 bp or if they had even a single base bias. PCR chimeras were checked and removed using the chimera.uchime command implemented in mothur. The remaining sequences were aligned to the RDP Classifier database to determine their phylogenetic assignments, with a confidence threshold of 80%. A clustering threshold of 97% similarity could be adopted to establish an OTU table in Mothur [27].

2.5. Statistical analyses

The α-diversity indices (e.g., OTU numbers, Shannon index) for each sample were calculated using vegan package in R (v. 3.12; http://www.r-project.org/). Community analysis was conducted based on phylum and class levels, and comparisons of relative abundance of bacterial community genus among samples were performed using pheatmap package and package gplots. NMDS was carried out employing the software Past, which is based on Bray–Curtis distances, to illuminate the similarity between different samples. A Pearson correlation analysis using SPSS 20.0 to understand the linkage between bacterial α-diversity and environmental variables, and an RDA was applied to study the correlations between the microbial community compositions and environmental variables with 999 permutations in R.

2.6. Nucleotide sequence accession number

The raw data obtained from the 48 samples were submitted separately to the NCBI Sequence Read Archive database under the accession number SRR6012654.

Table 1
Physiochemical properties of water and sediments of the Yangtze River

Name	Location	*T* (°C)	pH	Cu (mg/kg)	Pb (mg/kg)	As (mg/kg)	Hg (mg/kg)	Cd (mg/kg)	TP (mg/kg)	TN (mg/kg)	N/P	C/N	OM (%)
YD	Yedulong	4.5 ± 7.5	7.65 ± 0.15	2.44 ± 0.57	ND	ND	ND	ND	229.5 ± 8.5	1,630 ± 260	7.0 ± 0.9	0.20 ± 0.07	0.053 ± 0.012
ZM	Zhimenda	7.5 ± 8.5	7.75 ± 0.05	2.97 ± 0.43	ND	ND	ND	ND	168.0 ± 23.0	1,130 ± 240	6.6 ± 0.5	0.37 ± 0.03	0.072 ± 0.010
DK	Dengke	7.1 ± 8.0	7.75 ± 0.05	3.56 ± 0.77	ND	ND	ND	ND	210.5 ± 21.5	1,820 ± 570	9.0 ± 3.6	0.33 ± 0.07	0.097 ± 0.010
GL	Geliping	16.34 ± 6.1	8.20 ± 0.08	28.34 ± 5.30	22.26 ± 2.21	6.82 ± 1.76	0.071 ± 0.059	0.426 ± 0.012	752.8 ± 36.9	6,480 ± 1,590	8.5 ± 1.7	0.46 ± 0.15	0.470 ± 0.040
PZ	Panzhihua	15.7 ± 6.9	8.40 ± 0.11	39.27 ± 4.10	24.69 ± 0.76	7.96 ± 0.99	0.109 ± 0.002	0.667 ± 0.047	523.7 ± 68.4	8,250 ± 2,510	9.8 ± 2.2	0.88 ± 0.53	1.027 ± 0.366
PD	Pingdizhen	15.8 ± 5.8	8.10 ± 0.10	17.20 ± 3.00	18.62 ± 1.39	4.37 ± 0.28	0.051 ± 0.003	0.441 ± 0.062	782.0 ± 42.0	6,600 ± 1,500	8.4 ± 1.5	0.61 ± 0.30	0.621 ± 0.187
SP	Shaping	15.1 ± 8.0	8.03 ± 0.05	57.70 ± 1.40	32.68 ± 6.67	4.19 ± 1.67	0.078 ± 0.017	0.319 ± 0.012	598.3 ± 130.2	5,010 ± 2,510	7.2 ± 2.0	0.57 ± 0.14	0.533 ± 0.106
XT	Xintan	15.2 ± 7.6	8.12 ± 0.08	48.34 ± 5.30	20.57 ± 3.72	6.28 ± 0.37	0.108 ± 0.002	0.628 ± 0.003	672.8 ± 89.3	6,820 ± 1,830	10.0 ± 1.4	0.44 ± 0.04	0.532 ± 0.181
GK	Guankou	14.3 ± 8.2	8.07 ± 0.12	40.23 ± 3.10	18.78 ± 2.72	4.85 ± 1.82	0.081 ± 0.007	0.211 ± 0.017	537.9 ± 56.3	5,790 ± 2,680	9.1 ± 2.6	0.29 ± 0.08	0.350 ± 0.020
ZT	Zhutuo	17.5 ± 5.5	8.20 ± 0	60.51 ± 3.50	43.50 ± 1.50	6.28 ± 1.57	0.137 ± 0.049	0.467 ± 0.021	760.5 ± 101.5	8,770 ± 3,310	11.1 ± 2.9	0.68 ± 0.25	0.885 ± 0.015
CT	Cuntan	17.0 ± 7.1	8.20 ± 0	61.00 ± 9.01	87.01 ± 8.00	9.78 ± 2.60	0.387 ± 0.071	0.763 ± 0.033	1,004.5 ± 163.5	6,490 ± 3,130	6.1 ± 2.1	1.33 ± 0.66	1.130 ± 0.320
QX	Qingxichang	13.0 ± 5.0	8.20 ± 0	79.50 ± 36.50	62.00 ± 36.00	6.88 ± 0.25	0.148 ± 0.064	0.519 ± 0.015	850.5 ± 270.5	5,780 ± 3,400	6.1 ± 2.0	1.01 ± 0.25	0.860 ± 0.340
MH	Miaohe	15.1 ± 6.2	8.08 ± 0.05	67.04 ± 26.00	64.50 ± 38.50	9.19 ± 1.37	0.198 ± 0.006	0.517 ± 0.010	948.5 ± 274.5	10,830 ± 2,370	11.7 ± 0.9	0.52 ± 0.07	0.935 ± 0.085
NJ	Nanjinguan	14.3 ± 6.2	8.08 ± 0.08	61.00 ± 27.00	41.10 ± 21.02	11.36 ± 5.08	0.171 ± 0.039	0.682 ± 0.021	732.0 ± 176.0	8,450 ± 4,250	10.8 ± 3.2	0.89 ± 0.16	1.170 ± 0.410
QT	Quantong	14.6 ± 6.1	8.08 ± 0.08	97.50 ± 11.50	38.50 ± 6.50	7.41 ± 0.12	0.289 ± 0.040	0.309 ± 0.009	539.5 ± 112.5	6,690 ± 80	12.6 ± 1.2	0.78 ± 0.13	0.885 ± 0.045
SM	Shamao	16.1 ± 8.8	8.12 ± 0.10	23.50 ± 2.50	19.50 ± 5.50	3.98 ± 0.23	0.072 ± 0.01	0.198 ± 0.004	718.0 ± 64.0	10,520 ± 690	14.7 ± 0.3	0.46 ± 0.03	0.825 ± 0.005
HK	Hankou	16.6 ± 9.8	8.00 ± 0	9.00 ± 1.02	17.01 ± 1.00	4.56 ± 1.27	0.063 ± 0.036	0.438 ± 0.011	468.0 ± 75.0	10,100 ± 4,970	20.4 ± 7.3	0.65 ± 0.57	0.645 ± 0.435
SK	Shakouhe	14.7 ± 8.5	8.06 ± 0.06	13.01 ± 2.01	21.06 ± 8.00	3.92 ± 0.09	0.083 ± 0.020	0.287 ± 0.007	634.0 ± 113.0	9,300 ± 1,410	14.7 ± 0.4	0.56 ± 0.02	0.910 ± 0.170
JN	Jiangninghe	16.0 ± 11.6	9.05 ± 0.05	23.65 ± 4.75	18.05 ± 2.05	9.50 ± 0.70	0.077 ± 0.015	0.749 ± 0.032	848.0 ± 107.0	11,020 ± 2,810	13.6 ± 5.0	0.81 ± 0.21	1.440 ± 0.510
JX	Jiuxianghe	17.6 ± 11.4	8.70 ± 0	74.00 ± 11.80	57.40 ± 14.90	17.40 ± 1.10	0.150 ± 0.027	0.656 ± 0.008	1,781.5 ± 203.5	11,710 ± 6,480	7.1 ± 4.4	1.01 ± 0.52	1.160 ± 0.080
SJ	Sanjianghe	16.4 ± 11.6	9.35 ± 0.05	12.60 ± 1.90	14.40 ± 0.80	10.04 ± 3.17	0.050 ± 0.001	0.414 ± 0.024	695.5 ± 45.5	8,190 ± 3,560	11.5 ± 4.4	0.93 ± 0.22	1.185 ± 0.235
NT	Nantong	17.2 ± 11.5	8.25 ± 0.05	19.15 ± 0.95	20.90 ± 0.80	11.00 ± 1.20	0.092 ± 0.009	0.572 ± 0.018	676 ± 102.3	9,470 ± 1,850	13.9 ± 0.5	0.96 ± 0.15	1.225 ± 0.055
XL	Xuliujing	17.9 ± 11.7	8.05 ± 0.05	19.45 ± 0.25	18.95 ± 0.25	9.30 ± 0.90	0.067 ± 0.019	0.669 ± 0.037	665.2 ± 131.0	11,280 ± 1,930	17.0 ± 0.6	0.82 ± 0.08	1.565 ± 0.125
SD	Shidongkou	18.4 ± 10.5	7.65 ± 0.05	18.50 ± 1.50	20.50 ± 3.50	9.40 ± 0.80	0.199 ± 0.067	0.424 ± 0.029	677.0 ± 5.0	7,480 ± 4,680	11.0 ± 6.8	1.25 ± 0.86	0.925 ± 0.095

Table 2
Geographical characteristics and urban development level of the typical cities along the Yangtze River

Location	Yushu			Panzhihua			Yibin			Chongqing			Yichang			Wuhan			Nanjing			Shanghai		
	YD	ZM	DK	GL	PZ	PD	SP	XT	GK	ZT	CT	QX	MH	NJ	QT	SM	HK	SK	JN	JX	SJ	NT	XL	SD
Geographic feature																								
Longitude	97.2	97.3	101.8	101.5	101.7	101.8	104.7	104.5	105	105.9	106.6	108.9	110.9	111.3	111.4	114.9	114.3	114.5	118.5	118.8	119.1	120.9	121.3	121.4
Latitude	33	33	36.6	26.6	26.6	26.2	28.8	28.5	28.8	29	29.6	28.4	30.9	30.8	30.5	30.3	30.6	30.7	31.8	32.1	32.2	32	31.3	31.5
Elevation	3,675	3,635	2,257	1,029	1,156	1,827	342	507	372	191	221	378	78	117	50	21	27	26	6	13	3	2	3	1
Urban development																								
Urbanization rate (%)	50.3	50.3	50.3	64.74	64.74	64.74	45.1	45.1	45.1	60.94	60.94	60.94	55.65	55.65	55.65	79.3	79.3	79.3	80.5	80.5	80.5	62.76	73.95	88.02
Urban people (10^4)	295.98			79.79			202.5			1,838.41			225.92			775.98			661.4		2,135.08			
Rural people (10^4)	292.45			43.46			246.5			1,178.14			180.05			202.56			160.21		290.6			
Total people (10^4)	588.43			123.25			449			3,016.55			405.97			978.54			821.61		2,425.68			
Population density (people/km^2)	3	3	5	163	163	115	695	378	582	952	3,339	205	159	153	387	449	25,415	404	496	1,099	1,099	852	3,374	7,469

3. Results and discussion

3.1. Diversity of bacterial communities

A total of 83,857 bacterial OTUs were generated in the study, and the number of OTUs varied from 446 (YDwi: sampling at YD site in winter) to 3,076 (XLsu: sampling at XL site in summer) per sample (Table S1). In the present study, the low OTU numbers (only 446–751 diversity in winter and 551–694 in summer) were observed in Qinghai, a city located at the source of the Yangtze River. Considering the lower contents of nutrients and carbon resources, which resulted from lower urbanization levels, lower population density, and higher elevation, the lower bacterial richness could be acceptable. Diversity patterns of species have been reported to be shaped by energy availability [29–31]. Increasing trends of OTU richness and evenness along the decreasing elevation gradient were observed although the bacterial evenness was generally low (Table S1). Moreover, the varied range of OTU richness and evenness in summer exceeded the range of bacterial diversity variations in winter.

3.2. Bacterial community composition and relative abundance

The bacterial community compositions were analyzed at the phylum level and Proteobacteria was determined at the class level based on normalized library size. All sampling sites were analyzed based on average values of bacterial abundance in both summer and winter. Fig. 2a shows that Proteobacteria (averaging 42.23%), Bacteroidetes (averaging 12.78%), Actinobacteria (averaging 9.10%), Acidobacteria (averaging 8.33%), and Chloroflexi (averaging 7.94%) occupied dominant positions among all identified phyla. Only four main subdivisions affiliated with Proteobacteria are summarized in Fig. 2a, since Epsilonproteobacteria accounted for only a very low percentage and was only clustered into "others". Alphaproteobacteria was the most abundant subdivision (averaging 15.85%), followed by Beta-, Gamma-, and Deltaproteobacteria (11.16%, 9.28%, and 5.62%, respectively). Alphaproteobacteria had an advantage at high elevation when Proteobacteria was the most dominant position among all phyla. At the genus level, a heatmap (Fig. 2b) showed that *Subgroup 6* (Acidobacteria), *Flavobacterium* (Bacteroidetes), *Anaerolineaceae_uncultured* (Chloroflexi), and *Nitrosomonadaceae_uncultured* (Betaproteobacteria) were commonly found at all sampling sites and accounted for relatively high abundance.

To the best of our knowledge, Proteobacteria was the most abundant phylum, which was in accordance with the results of previous studies where Proteobacteria was the most abundant phylum in diverse ecosystems and was more common in freshwater environments [32–34]. Sekiguchi et al. [19] reported that Alpha- and Betaproteobacteria were dominant bacterial groups in the Yangtze River prior to the construction of the Three Gorges Dam, which was in line with the present study. However, the result of a previous study, which reported that Gamma- and Deltaproteobacteria were minor groups differed from what has been reported in this study. However, Wilhelm et al. [12] reported that abundant classes were Alpha-, Beta-, Gamma-, and Deltaproteobacteria when investigating variations of microorganism in three headwater streams (Obertalbach, Steinriesenbach, and Reisachbach)

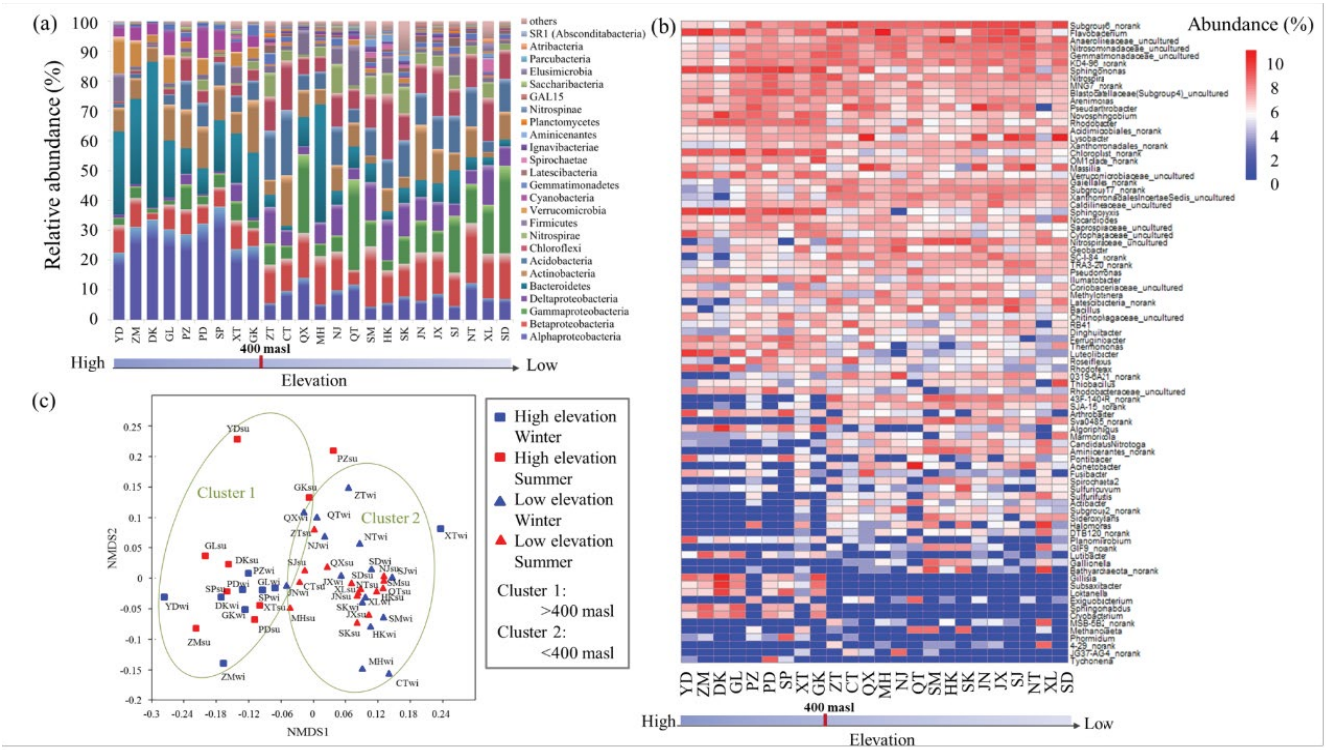

Fig. 2. (a) Relative abundances of bacterial phyla and classes of Proteobacteria in the sediment of the Yangtze River based on 24 sampling sites. The top 10 phyla of bacteria were summarized, and remaining bacterial phyla were included in "others"; (b) Heatmap based on the abundance of top 10 bacterial genera in the sediment of the Yangtze River. The value of each sampling sites was based on the average value of bacterial abundance in summer and in winter; (c) Non-metric multidimensional scaling analysis of the bacterial community compositions in the sediment of the Yangtze River.

in the Schladminger-Tauern, Austria, along the altitudinal gradient. Thus, considering the different molecular technologies, urban development, and the construction of hydraulic engineering, the reported changes of dominant subphyla of Proteobacteria were reasonable.

With regard to Bacteroidetes, previous investigations indicated that Bacteroidetes accounted for 11%–37% of bacteria detected in a tributary of the Yangtze River [24,35]. This is in accordance with that the result of this study, where Bacteroidetes comprised 12.78% on average. The lower abundance of bacteria in the Yangtze River may be related to the high OM or humus acid in the sediment of a tributary where Bacteroidetes could degrade high-molecular-weight OM to some extent [35]. With regard to the bacteria at the highest elevation (>2,000 m), the high abundance of Bacteroidetes may be related to the high altitude which results in a complicated autochthonal environment. Dorador et al. [36] reported that Bacteroidetes (together with Proteobacteria) are the most abundant groups in high-altitude rivers when studying the diversity of Bacteroidetes in high-altitude basins. In addition, the abundance of nitrogen cycle bacteria along the Yangtze River was generally high. With the exception of Nitrospira, Betaproteobacteria was assumed the clade that performs ammonia oxidation [37]. Considering the changes of bacterial composition, although the water quality in the Yangtze River is relatively high, the deterioration of water quality influenced by human activities should receive more attention.

3.3. Changes of bacterial community along the elevation gradient

In this study, NMDS was applied and a clear separation of the bacterial community responding to high (>400 masl) and low (<400 masl) elevation could be documented (Fig. 2c). In general, heterogeneity exists in the bacterial communities along the altitudinal gradient and an obvious threshold was also reported in previous studies [12,30]. To the best of our knowledge, heterogene community compositions are correlated with niche segregation as a result of habitat heterogeneity affected by human activities. Similar results from a previous study indicate that niche segregation influenced by allochthonous and autochthonous contents contributing to material resources was correlated with microbial diversity, specialization, and functional traits [12,38]. In addition, the deterministic processes were considered as a key theoretical process to explain the response of the microbial community to environmental disturbance. Hence, niche segregation affected by different dominant environmental factors should be considered to cause deterministic processes along altitudinal gradients.

With regard to bacterial richness, relatively lower OTU numbers were observed (<1,400) above 400 masl, while higher numbers (>1,400) were observed below 400 masl. The bacterial richness and evenness showed increasing tendencies with decreasing elevation from upstream to downstream, while the number of OTUs decreased slightly near

the estuary of the Yangtze River (Table S1). Linear regression analysis (Fig. 3) also indicated that the OTU numbers of bacteria in this study also decreased with increasing elevation, while a stable trend was obtained at high elevation and a threshold of 400 masl could be identified. Furthermore, the bacterial evenness generally decreased with increasing elevation and a linear decreasing trend above 400 masl in winter (R^2 = 0.787, p < 0.001) was observed. Among proteobacterial classes, significant variation trends were observed along the altitudinal gradient. Fig. 2a shows that the abundance of Alphaproteobacteria was significantly higher above 400 masl, while increasing trends of the abundances of Beta-, Gamma-, and Deltaproteobacteria along decreased elevation were observed. Siles and Margesin [14] reported the significant variations of Alpha-, Gamma-, and Betaproteobacteria over the altitudinal gradient, which matched the results found in this study. Moreover, Bacteroidetes also had a relatively high abundance at high elevation, which corresponds to the results reported by Dorador et al. [36]. Niu et al. [30] also indicated that Bacteroidetes on the Tibetan grassland could adapt to this extreme environment by accumulating a large number of endemic stress genes. Above 400 masl, the majority of the top 10 bacterial phyla occupied more than 97%, while at low elevation, the bacterial abundance was more diverse and the community compositions were more heterogeneous. Obvious shifts of bacterial genera between different samples and distribution variations along the elevation gradient could be observed in Fig. 2b. Markedly different relative abundances of bacterial communities at different elevations were found. For example, *Sphingomonas* (Alphaproteobacteria), *Chloroplast_norank* (Cyanobacteria), and *Sphingopyxis* (Alphaproteobacteria) showed significantly higher relative abundance above 400 masl and significant decreasing trends along decreasing elevation were observed. Several genera, for example *43F-1404R_norank* (Deltaproteobacteria) and *Aminicenantes_norank* (Aminicenantes), followed opposite trends.

Clear increasing trends of bacterial diversity were found along the Yangtze River except for the estuary. As previous studies showed, high salinity possibly inhibits the growth of a number of bacteria that cannot adapt to the saline environment and result in decreased biodiversity of the estuary sediment [27,39]. In addition to diversity, the bacterial community structure also showed significant variations along altitudinal gradients. On the one hand, the highly dynamic nature of the Yangtze River, which resulted in variations of autochthonous materials, changed the composition of main bacterial phyla along the elevation gradient. On the other

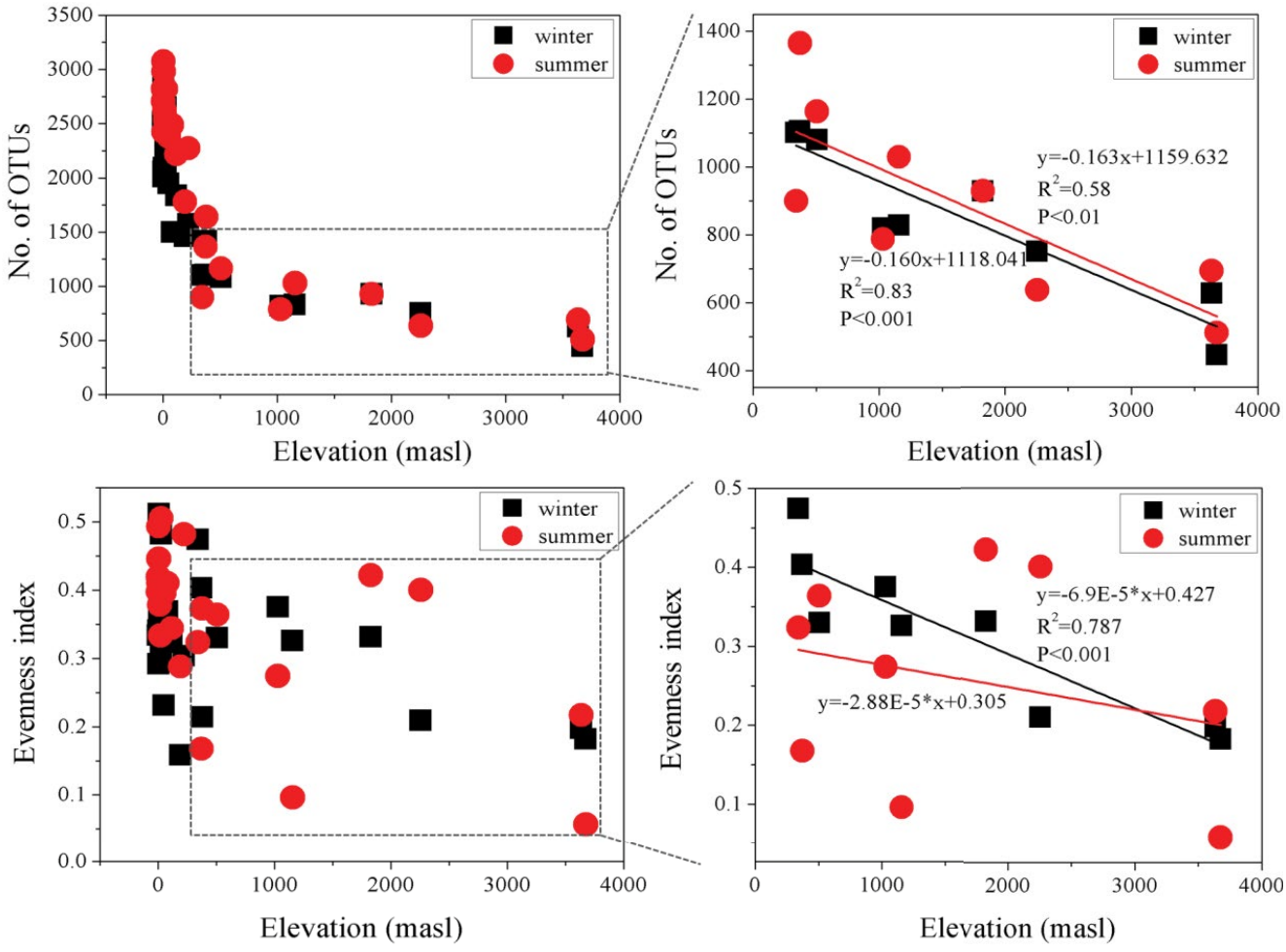

Fig. 3. Variations of bacterial richness and evenness along the elevation gradient.

hand, allochthonous resources structured different community compositions via matter transformation, diffusion, and energy flow [12]. In the previous study, Betaproteobacteria was positively related to the contents of humus and nutrients [14], while Zhang et al. [32] reported that the higher ratios of Gammaproteobacteria indicate low water quality, which is in accordance with the result of this investigation. In this study, Betaproteobacteria and Gammaproteobacteria were dominant below 400 masl, which may be correlated to the environmental complexity and high denaturation of the sediment of the Yangtze River at low elevation.

As a result, the changes of bacterial communities along the altitude may be affected by two deterministic processes. On the one hand, significant geographical environmental differentiation associated with altitudinal gradient may influence the autochthonous bacterial constituents and form diverse microbial compositions and functional traits [12]. On the other hand, flexible environmental factors have been associated with changeable bacterial communities, and were affected by niche partitioning, competition, and selection. Except for the effect of natural selection, anthropogenic selection interacts with environmental stochasticity and further develops the generalists and specialists of microbial function. Hence, determining the role of natural selection and anthropogenic selection in structuring ecological communities is of great importance.

3.4. Relationships between bacterial community diversity and environmental factors

To better understand the linkages between bacterial community diversity and environmental factors, the OTU numbers, Shannon index, Simpson index, and Evenness index were investigated among samples at the OTU level (Table S1). Along altitudinal gradients, decreasing trends were observed for most physicochemical properties (Fig. S1). Moreover, neutral pH and relatively lower nutrient concentrations (TN and TP) were found at higher altitudes (Tables 1 and 2). This represents the higher water quality and lower contents of available biomaterials.

To determine the environmental variables that drove the variations of bacterial community richness along the elevation, this study applied a Pearson analysis. Fig. 4 shows that, based on all bacterial diversity indices, elevation (all correlation coefficients of <–0.54, $p < 0.01$), urbanization rate (all correlation coefficients of >0.40, $p < 0.01$), and OM (all correlation coefficients of >0.39, $p < 0.05$) were most significantly correlated with both OTU richness and evenness. Above 400 masl, the bacterial diversity was closely associated with the elevation gradient ($p < 0.05$), followed by TP ($p < 0.05$) and TN ($p < 0.05$). However, below 400 masl elevation, altitude ($p < 0.05$), urbanization rate ($p < 0.05$), and Cu ($p < 0.05$) were dominant variables (Table S2). Among all determined variables (Fig. 4), both OTU numbers and evenness index were most associated with altitude and urbanization rate. The correlations between physicochemical properties, elevation, and urbanization rate were analyzed (Table S3). The results showed that both elevation and urbanization rate were most correlated with OM. Population density was significantly related to N/P values.

As previously reported, nutrients increased with decreasing altitude and accordingly changed the relative abundance of bacterial communities [14]. This study also showed that nutrients (e.g., TN, N/P) could influence bacterial diversity. Wilhelm et al. [12] reported that microbial diversity was negatively correlated with altitude at higher elevation, which was in accordance with the findings of the present study. Previous investigations showed that a significant effect of organic matter was observed on microbial diversity [14,27]. The large input of allochthonous matters results from quick urban development and increases the bacterial diversity in diversification of energy sources would develop the otherness of bacteria. Fagervold et al. [40] reported that OM with different quality and source was strongly correlated with microbial community diversity.

Based on these results, bacterial diversity correlated most with elevation, urbanization rate, and OM and the variations of OTU richness were significantly influenced by altitude above 400 masl. As previous studies reported, bacterial community richness decreased with increasing elevation in the

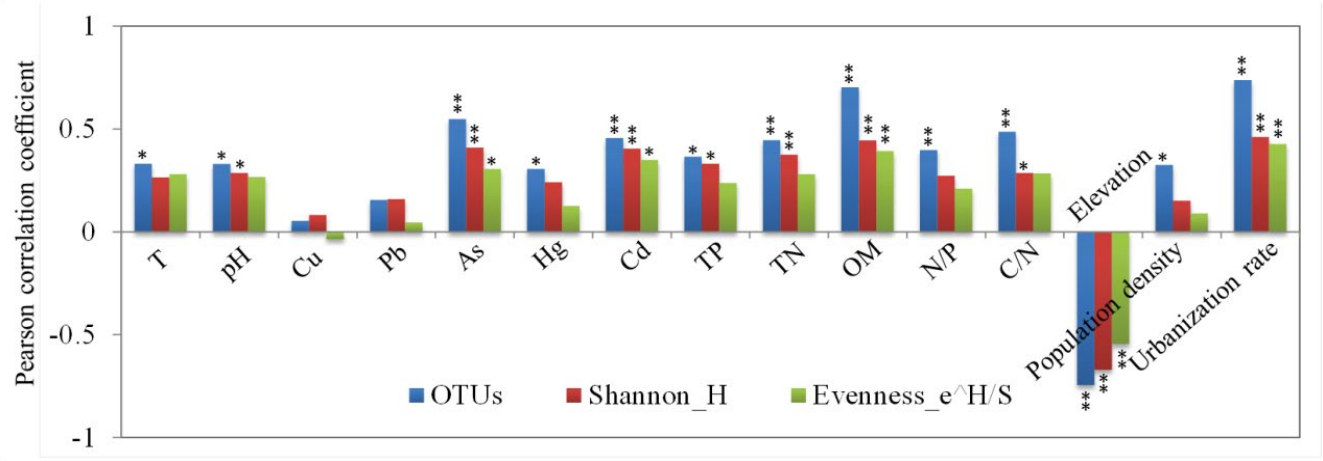

Fig. 4. Pearson correlation coefficients for relationships between bacterial diversity indices and environmental variables in the sediment of the Yangtze River. Two asterisks indicate that the variable is significantly correlated to the alpha diversity index ($p < 0.01$) and an asterisk also represents the relevance between the variable and the diversity index ($p < 0.05$).

natural ecosystem, such as mountain soil [16]. On the Tibetan Plateau grassland, for the extreme environmental conditions caused by high altitude, endemic stress genes (σ^{24} and *obg*E) were reported to be enriched in the microbial community [41]. Therefore, the observed significant correlation between high elevation and low bacterial richness was reasonable.

3.5. Linkages between microbial communities and environmental factors

Based on the results about bacterial community richness, both elevation and physicochemical properties, such as OM and urbanization rate, were significantly associated with bacterial diversity. In addition to bacterial diversity, bacterial community composition was also an important determining factor that influences the function of the microbial ecosystem in the sediment of the Yangtze River. In general, bacterial community compositions were correlated to environmental factors. However, among all factors, the determinant of the community compositions in the sediment of the Yangtze River requires further investigation. Thus, RDA was performed to analyze the potential relationships between bacterial community compositions and elevation, as well as physicochemical properties, using 999 Monte Carlo permutations (Fig. 5).

Only significant factors are shown in Fig. 5. The first axis explained 47.9% and the second axis explained 18.6%. OM (20.0%, p = 0.001) was the most significant physicochemical property that influenced the bacterial community structures, followed by elevation (10.4%, p = 0.003), urbanization rate (9.0%, p = 0.017), and Pb (9.5%, p = 0.026). The results indicate that the variance of community composition correlated significantly with elevation above 400 masl, while OM, urbanization rate, and Pb (mainly caused by anthropogenic activities) structured the heterogeneity of bacterial community below 400 masl. The bacterial communities located between 50 and 400 masl were most correlated with Pb, while the bacterial community located below 50 masl showed a significant relationship to the urbanization rate. The content of OM correlated with overall bacterial assemblies below 400 masl. The higher urbanization development level of the Yangtze River Delta compared with the cities located in the middle reaches of the Yangtze River, where industry developed quickly, may be influences of the bacterial distribution.

To determine linkages between specific bacteria and significantly relevant variables, a Pearson analysis was conducted (Table S4) based on the top 10 genera. *Sphingopyxis* (Alphaproteobacteria; correlation coefficient of 0.592, p < 0.01) and *Xanthomonadales_norank* (Gammaproteobacteria; correlation coefficient of 0.590, p < 0.01) were most associated with elevation. Furthermore, *Sphingopyxis* (Alphaproteobacteria; correlation coefficient of 0.540, p < 0.01) and *SC-I-84_norank* (Betaproteobacteria; correlation coefficient of 0.535, p < 0.01) were most correlated with OM. *Subgroup18_norank* (Acidobacteria; correlation coefficient of 0.643, p < 0.01) and *KD4-96_norank* (Chloroflexi; correlation coefficient of 0.575,

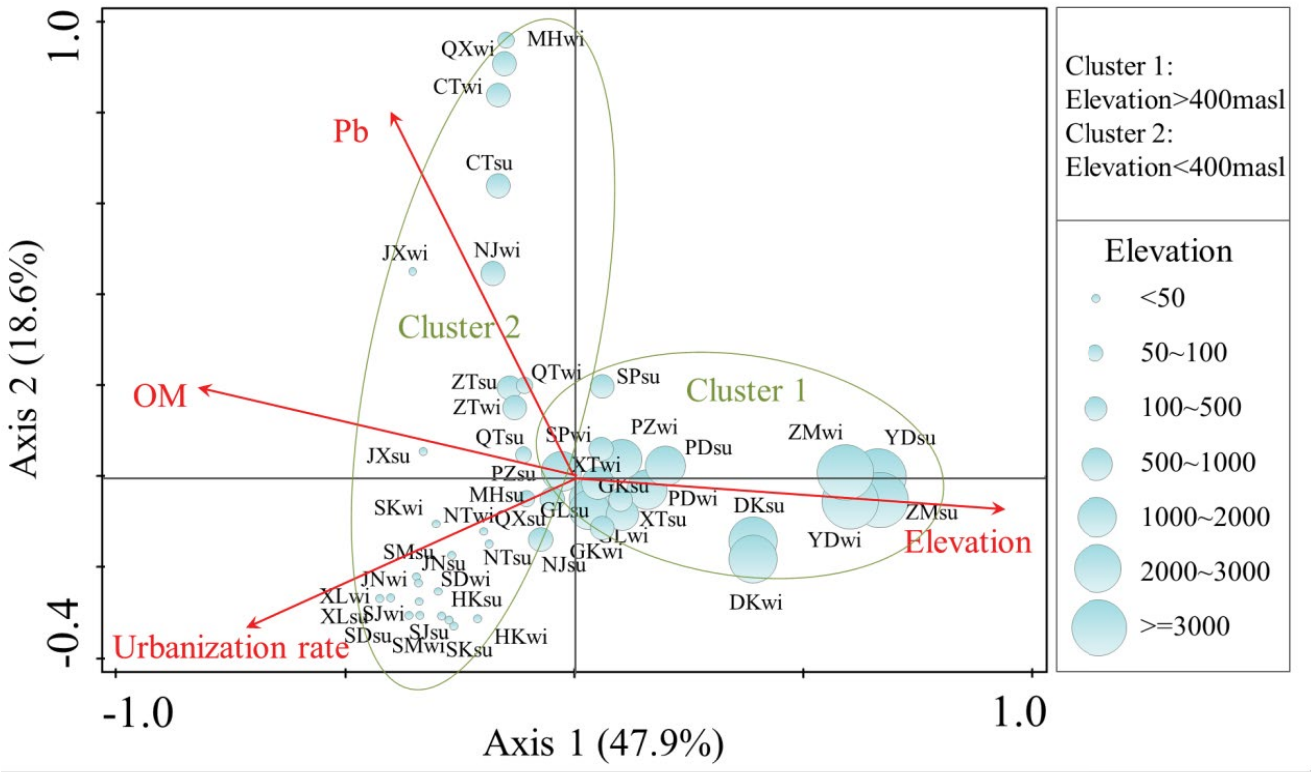

Fig. 5. RDA ordination plots of the relationships between bacterial community structure and environmental factors in the sediment of the Yangtze River. Elevation of each sampling site is represented by the size of symbol. Only significantly correlated variables (p < 0.05) are represented in the figure.

$p < 0.01$) showed significant relationships to urbanization rates.

Based on the top 10 phyla, Alphaproteobacteria, Deltaproteobacteria, Bacteroidetes, and Chloroflexi were significantly correlated with elevation, OM, and urbanization rate (Table S5). Except for the evidence of different habitat environment of different subdivision of Proteobacteria, Bacteroidetes were also reported to be related to degraded complex organic macromolecules [34]. This corresponds to the present result where the negative correlations between Bacteroidetes and OM, as well as urbanization rate were generated. It is worth noting that the influence of anthropogenic activities caused by the developed economy could change the bacterial community compositions and functional traits via effects of resource and energy flow [12]. The important influence of allochthonous inputs on the bacterial community composition was also reported previously [1].

The linkages between variations in bacterial community structure and three significantly dominant environmental variables selected above were further analyzed based on distance-decay correlation analysis (Fig. 6). The Euclidean distance was considered to calculate and analyze dissimilarities of the bacterial community structure in the sediment of the Yangtze River. The results showed that most significant and positive correlations of variations between bacterial community structure and elevation were observed ($R^2 = 0.277$, $p < 0.01$), followed by OM ($R^2 = 0.07$, $p < 0.01$). However, no obvious correlation of variations in bacterial community structure and urbanization rate was found in Fig. S2. Larger variations of elevation gradient or OM content indicated larger dissimilarities of bacterial communities.

Among all significantly correlated variations, geographical parameter elevation and OM derived from human activity, were always dominant variables. Both are related to bacterial richness and community compositions in the sediment of the Yangtze River. As a geographical parameter, elevation was significantly correlated with bacterial community variations in previous studies [30,42]. The niche segregation related to elevation might be more attributed to natural selection based on deterministic process rather than the influence of environmental stochasticity. Siles and Margesin [14] indicated that dramatic changes in climate and biotic characteristics over short geographic distance were found along altitudinal gradients and thus affected microbial habitats. Considering the significant correlation between elevation and OM (Table S3), elucidating the significant effects of organic matter on bacterial communities is very important.

The vital role of OM in structuring the bacterial community provides further evidence for the hypothesis that deterministic processes, driven by species competition for more material and energy sources, could cause niche segregation. Focusing on the deterministic process, anthropogenic selection may be the most significant factor to cause the niche differentiation of bacterial communities. Previous studies suggested that allochthonous resources more likely formed the bacterial functional specialization that is correlated with niche differentiation. OM is an important allochthonous resource and was found to exert a dominant role in the formation of bacterial communities.

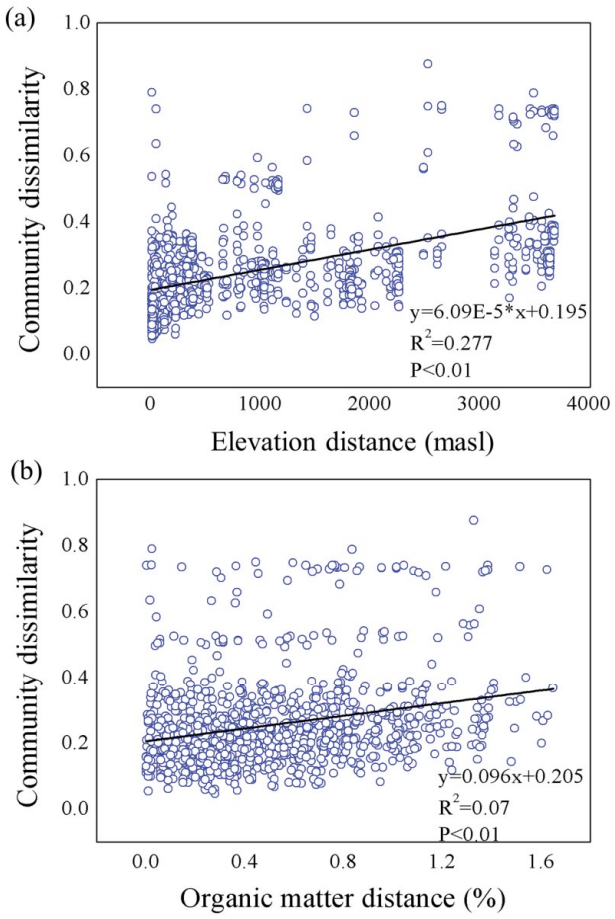

Fig. 6. Distance-decay relationships for bacterial communities in the sediment of the Yangtze River. Scatter plots indicate community dissimilarity represented by Euclidean distance (vertical axes) versus (a) elevation and (b) organic matter along the Yangtze River (horizontal axes). Regression analysis was adopted in that significant and positive correlations of variances in bacterial communities were achieved for elevation and organic matter.

Fagervold et al. [40] confirmed that river OM shapes the microbial communities, and the degradation of microbial-driven organic matter is considered fundamental to microbial function. Fagervold et al. [40] also reported that the variance of the microbial community composition was most closely related to $\delta^{13}C$ values and the proportion of saturated or polyunsaturated fatty acids, which were associated with both the source and lability of OM, respectively. Moreover, Cotano and Villate [43] reported that OM distribution was influenced by anthropogenic activities, such as the discharge of sewage. Allochthonous OM as an important factor structuring the resources of bacterial activities may shed new light on the relationship of anthropogenic interference and niche segregation. The present study identified OM as central for shaping the bacterial community composition. However, based on the various sources and instability of sediment OM, how OM influences the Yangtze River sedimentary bacterial community should be further investigated.

4. Conclusion

This study determined the bacterial community composition in the sediment of the Yangtze River as well as the linkages between bacterial communities and elevation and anthropogenic disturbance. A threshold at approximately 400 masl of elevation was found in response to variations of bacterial diversity and community composition. Bacterial diversity was most correlated with elevation, followed by urbanization rate ($p < 0.01$), and OM ($p < 0.05$) along the Yangtze River. The variations of bacterial community structure also indicated significantly positive relevance with OM (20.0%), elevation (10.4%), and urbanization rate (9.0%). Despite the dominance of natural selection of elevation, anthropogenic effects (especially OM input) were important in structuring the diversity and composition of bacterial communities along the altitudinal gradients. Niche differentiation and competition between species as modulated by deterministic process should be considered and may be decisive for the stabilization of the ecosystem. Therefore, the study provides theoretical support for the formation of a water environmental evaluation index system and for the repair of the water quality of the Yangtze River.

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Supplementary information

Table S1
Bacterial diversity indices of collected samples at 97% similarity level in summer and in winter, respectively

Taxon	No. of OTUs	Simpson_ 1-D	Shannon_ H	Evenness_ e^H/S
YDwi	446	0.8603	2.483	0.1816
ZMwi	627	0.8981	2.759	0.1972
DKwi	751	0.9006	2.841	0.2090
GLwi	820	0.9525	3.473	0.3749
PZwi	829	0.9304	3.285	0.3259
PDwi	929	0.9395	3.313	0.3310
SPwi	1,101	0.9582	3.660	0.4741
XTwi	1,081	0.9229	3.034	0.3300
GKwi	1,107	0.9568	3.591	0.4032
ZTwi	1,458	0.7617	2.622	0.1583
CTwi	1,575	0.9236	3.261	0.3032
QXwi	1,416	0.8914	2.970	0.2142
MHwi	1,500	0.9520	3.535	0.3689
NJwi	1,836	0.9302	3.413	0.3230
QTwi	1,947	0.8911	3.180	0.2313
SMwi	2,296	0.9573	3.590	0.3856
HKwi	2,131	0.9292	3.395	0.3043
SKwi	2,649	0.9631	3.834	0.4819
JNwi	2,094	0.9709	3.894	0.5116
JXwi	2,482	0.9550	3.594	0.3714
SJwi	2,009	0.9479	3.368	0.3414
NTwi	2,057	0.9359	3.301	0.2917
XLwi	2,968	0.9634	3.689	0.4042
SDwi	2,514	0.9437	3.436	0.3340

Table S1 (*Continued*)

Taxon	No. of OTUs	Simpson_ 1-D	Shannon_ H	Evenness_ e^H/S
YDsu	511	0.4280	1.322	0.0568
ZMsu	694	0.9030	2.803	0.2170
DKsu	637	0.9442	3.347	0.4003
GLsu	788	0.9235	3.112	0.2740
PZsu	1,030	0.6637	2.065	0.0962
PDsu	929	0.9579	3.556	0.4219
SPsu	900	0.9363	3.266	0.3237
XTsu	1,164	0.9486	3.522	0.3640
GKsu	1,365	0.8576	2.757	0.1675
ZTsu	1,779	0.9205	3.331	0.2882
CTsu	2,274	0.9686	3.844	0.4818
QXsu	1,640	0.9455	3.456	0.3729
MHsu	2,487	0.9652	3.792	0.4104
NJsu	2,219	0.9419	3.387	0.3441
QTsu	2,381	0.9596	3.594	0.3955
SMsu	2,538	0.9436	3.434	0.3335
HKsu	2,820	0.9587	3.626	0.3955
SKsu	2,445	0.9732	3.932	0.5050
JNsu	2,981	0.9687	3.836	0.4457
JXsu	2,622	0.9467	3.582	0.3783
SJsu	2,425	0.9539	3.665	0.4110
NTsu	2,821	0.9618	3.716	0.4192
XLsu	3,076	0.9698	3.918	0.4932
SDsu	2,708	0.9535	3.682	0.3974

Table S2
Pearson correlation coefficients for relationships between bacterial diversity indices and environmental variables in the sediment of the Yangtze River (a) above 400 masl and (b) below 400 masl, respectively

(a) Above 400 masl

	T	pH	Cu	Pb	As	Hg	Cd	TP	TN	OM	N/P	C/N	Elevation	Population density	Urbanization rate
	(°C)					(mg/kg)				(%)			masl	(people/km^2)	(%)
OTUs	0.495*	0.619**	0.777**	0.656**	0.688**	0.753**	0.586*	0.580*	0.500*	0.524*	0.233	0.275	–0.849**	0.766**	–0.183
Shannon_H	0.16	0.254	0.405	0.415	0.244	0.323	0.286	0.509*	0.486*	0.109	0.302	–0.11	–0.570*	0.427	0.017
Evenness_e^H/S	0.135	0.197	0.411	0.382	0.188	0.263	0.263	0.508*	0.503*	0.136	0.268	–0.103	–0.529*	0.418	–0.014

(b) Below 400 masl

	T	pH	Cu	Pb	As	Hg	Cd	TP	TN	OM	N/P	C/N	Elevation	Population density	Urbanization rate
	(°C)					(mg/kg)				(%)			masl	(people/km^2)	(%)
OTUs	0.468**	0.011	–0.576**	–0.588**	0.051	–0.344	–0.04	–0.167	–0.114	0.244	0.13	0.263	–0.696**	0.202	0.561**
Shannon_H	0.373*	0.122	–0.444*	–0.359	0.14	–0.165	0.117	–0.141	–0.146	0.215	0.026	0.277	–0.487**	0.016	0.386*
Evenness_e^H/S	0.356	0.162	–0.461*	–0.349	0.076	–0.198	0.147	–0.156	–0.158	0.235	0.012	0.288	–0.409*	–0.035	0.383*

Table S3
Relationships between physicochemical parameters and geographical properties, along with urban development level characteristics described using Pearson correlation analysis

	T	pH	Cu	Pb	As	Hg	Cd	TP	TN	OM	N/P	C/N
	(°C)					(mg/kg)				(%)		
Elevation (masl)	–0.285*	–0.434**	–0.420**	–0.432**	–0.646**	–0.475**	–0.628**	–0.527**	–0.592**	–0.680**	–0.366*	–0.368**
Population density (people/km^2)	0.067	–0.135	–0.21	–0.054	–0.001	0.013	0.074	–0.084	0.172	0.023	0.433**	0.106
Urbanization rate (%)	0.176	0.412**	–0.23	–0.005	0.356*	0.033	0.304*	0.371*	0.480**	0.537**	0.390**	0.340*

Significance levels are shown at *$p < 0.05$ and **$p < 0.01$.

Table S4
Relationships between bacterial community compositions considering the top 10 most abundant bacterial genera and environmental variables using Pearson correlation analysis

Phylum/class/genus	*T*	pH	Cu	Pb	As	Hg	Cd	TP	TN	OM	N/P	C/N	Elevation	Population density	Urbanization rate
	(°C)					(mg/kg)				(%)			(m)	(people/km^2)	(%)
Proteobacteria/Gammaproteobacteria/*Acinetobacter*	–0.04	0.013	0.18	0.123	–0.009	0.041	0.056	0.07	0.118	0.027	0.035	–0.024	–0.085	–0.051	–0.06
Firmicutes/Bacilli/*Exiguobacterium*	–0.019	0.097	0.134	0.1	–0.034	–0.028	0.039	0.077	0.149	0.052	0.077	–0.037	–0.095	–0.039	0.002
Firmicutes/Bacilli/*Tumebacillus*	–0.038	0.017	0.16	0.121	–0.046	–0.012	0.017	0.087	0.164	0.035	0.087	–0.083	–0.081	–0.035	–0.044
Proteobacteria/Gammaproteobacteria/*XanthomonadalesIncertaeSedis_uncultured*	0.205	0.216	0.179	0.185	0.569**	0.257	0.358*	0.352*	0.297*	0.512**	0.107	0.385**	–0.495**	0.074	0.451**
Chloroflexi/Caldilineae/*Caldilineaceae_uncultured*	0.03	0.391**	0.238	0.272	0.550**	0.053	0.339*	0.412**	0.389**	0.426**	0.088	0.187	–0.314*	0.043	0.365*
Firmicutes/Clostridia/*Clostridium sensu stricto12*	–0.11	–0.018	0.229	0.078	–0.032	0.064	0.094	0.08	0.171	0	0.124	–0.1	–0.116	0.001	–0.144
Actinobacteria/Actinobacteria/*Pseudarthrobacter*	–0.161	0.135	0.395**	0.424**	0.225	–0.01	0.185	0.238	0.147	0.189	–0.044	0.05	–0.095	–0.044	–0.005
Chloroflexi/KD4-96/*KD4-96_norank*	0.171	0.098	–0.031	–0.005	0.435**	0.217	0.288*	0.127	0.26	0.403**	0.238	0.363*	–0.497**	0.381**	0.575**
Acidobacteria/Subgroup 6/*Subgroup 6_norank*	0.154	0.123	–0.044	0.017	0.399**	0.191	0.163	0.118	0.253	0.443**	0.257	0.369**	–0.486**	0.198	0.549**
Proteobacteria/Alphaproteobacteria/*Sphingomonas*	0.014	0.056	–0.005	–0.093	–0.11	–0.17	–0.102	–0.181	–0.219	–0.322*	–0.249	–0.184	0.195	–0.209	–0.273
Chloroflexi/SJA-15/*SJA-15_norank*	0.029	0.158	0.067	0.072	0.272	0.084	0.268	0.196	0.423**	0.307*	0.459**	0.13	–0.387**	0.582**	0.461**
Firmicutes/Bacilli/*Planomicrobium*	0.107	0.022	–0.042	–0.031	0.033	–0.064	–0.029	–0.001	–0.101	–0.127	–0.128	–0.062	0.085	–0.069	–0.022
Proteobacteria/Alphaproteobacteria/*MNG7_norank*	0.106	0.034	0.066	–0.04	0.069	0.007	0.126	0.064	–0.08	–0.108	–0.194	0.089	–0.091	–0.093	–0.07
Proteobacteria/Alphaproteobacteria/*Brevundimonas*	–0.061	0.063	0.127	0.078	0.064	–0.019	0.144	–0.067	0.042	–0.092	–0.075	–0.139	0.153	–0.19	–0.19
Proteobacteria/Alphaproteobacteria/*Rhodobacter*	–0.07	–0.067	–0.116	–0.2	–0.271	–0.159	–0.123	–0.254	–0.244	–0.382**	–0.163	–0.175	0.309*	–0.188	–0.361*
Proteobacteria/Betaproteobacteria/*Massilia*	–0.051	0.025	0.462**	0.351*	0.24	0.171	0.114	0.074	0.024	0.196	–0.084	0.055	–0.023	–0.123	–0.171
Actinobacteria/Acidimicrobiia/*Ilumatobacter*	0.043	–0.115	–0.137	–0.198	–0.301*	–0.269	–0.18	–0.101	–0.23	–0.367*	–0.249	–0.193	0.324*	–0.173	–0.275
Chloroflexi/Anaerolineae/*Anaerolineaceae_uncultured*	0.061	0.096	–0.04	0.019	0.228	0.006	0.213	0.067	0.400**	0.255	0.566**	0.067	–0.396**	0.712**	0.452**
Actinobacteria/*Coriobacteriia*/*Coriobacteriaceae_uncultured*	0.147	0.084	0.046	–0.039	0.184	0.222	0.196	0.004	0.169	0.242	0.303*	0.253	–0.376**	0.388**	0.346*
Firmicutes/Bacilli/*Bacillus*	0.17	0.003	0.055	–0.003	0.032	0.027	–0.058	–0.104	–0.185	–0.122	–0.148	0	–0.111	0.045	–0.17
Proteobacteria/Betaproteobacteria/*SC-I-84_norank*	0.076	0.115	0.152	0.154	0.571**	0.256	0.327*	0.312*	0.341*	0.535**	0.18	0.327*	–0.466**	0.183	0.466**

Table S4 (*Continued*)

Phylum/class/genus	*T*	pH	Cu	Pb	As	Hg	Cd	TP	TN	OM	N/P	C/N	Elevation	Population density	Urbanization rate
	(°C)		(mg/kg)							(%)			(m)	(people/km^2)	(%)
Proteobacteria/Betaproteobacteria/*Nitrosomonadaceae_uncultured*	0.209	0.124	–0.061	–0.017	0.28	0.21	0.342*	0.131	0.2	0.449**	0.227	0.333*	–0.453**	0.148	0.380**
Acidobacteria/Blastocatellia/*Blastocatellaceae (Subgroup 4)_uncultured*	0.108	0.119	–0.049	–0.119	0.026	–0.139	0.063	–0.065	–0.039	–0.152	–0.132	–0.07	–0.003	–0.16	–0.115
Actinobacteria/Acidimicrobiia/*Acidimicrobiales_norank*	0.001	0.108	0.096	0.01	0.292*	0.073	0.310*	0.137	0.097	0.217	–0.026	0.202	–0.17	–0.049	–0.055
Nitrospirae/Nitrospira/*Nitrospiraceae_uncultured*	0.212	0.099	–0.071	0.066	0.176	0.106	0.233	0.416**	0.303*	0.301*	0.27	0.258	–0.373**	0.476**	0.530**
Actinobacteria/Actinobacteria/*Nocardioides*	–0.144	–0.028	0.052	0.095	0.198	0.219	0.061	0.018	0.134	0.086	0.114	–0.02	–0.084	0.088	0.212
Verrucomicrobia/Verrucomicrobiae/*Verrucomicrobiaceae_uncultured*	–0.208	–0.107	–0.146	–0.245	–0.330*	–0.226	–0.274	–0.225	–0.201	–0.351*	–0.108	–0.229	0.314*	–0.153	–0.329*
Chloroflexi/Chloroflexia/*Roseiflexus*	–0.082	0.166	–0.111	–0.045	0.263	–0.031	0.117	0.052	0.108	0.172	0.067	0.12	0.268	0.097	0.068
Proteobacteria/Alphaproteobacteria/*Variibacter*	–0.03	0.057	0.189	0.071	0.267	0.174	0.281	0.122	0.161	0.199	0.105	0.136	0.173	–0.084	–0.029
Aminicenantes/norank/*Aminicenantes_norank*	0.054	–0.017	–0.048	0.133	–0.01	–0.012	0.006	0.207	0.353*	0.149	0.406**	0.005	0.362*	0.069	0.459**
Bacteroidetes/Bacteroidetes vadinHA17/*Bacteroidetes vadinHA17_norank*	0.175	0.064	–0.053	–0.103	0.095	0.149	0.192	0.015	0.082	0.193	0.168	0.224	0.376**	0.029	0.266
Acidobacteria/Subgroup 17/*Subgroup 17_norank*	0.221	0.082	–0.049	0.038	0.247	0.12	0.081	0.154	0.271	0.360*	0.278	0.248	0.577**	0.168	0.526**
Actinobacteria/Thermoleophilia/*Gaiellales_norank*	–0.108	0.029	0.072	0.083	0.404**	0.121	0.244	0.174	0.297*	0.344*	0.226	0.148	0.418**	0.113	0.102
Proteobacteria/Gammaproteobacteria/*Halomonas*	–0.023	0.063	0.146	0.496**	0.096	0.600**	0.257	0.348*	0.096	0.155	–0.129	0.106	–0.002	–0.031	0.022
Gemmatimonadetes/Gemmatimonadetes/*Gemmatimonadaceae_uncultured*	0.073	0.104	–0.069	–0.138	0.11	–0.02	0.149	0.062	0.193	0.235	0.191	0.063	0.292*	–0.122	0.141
Cyanobacteria/Chloroplast/*Chloroplast_norank*	–0.068	–0.18	–0.196	–0.285*	–0.365*	–0.273	–0.188	–0.22	–0.254	–0.480**	–0.154	–0.325*	–0.459**	–0.142	–0.378**
Proteobacteria/Gammaproteobacteria/*Lysobacter*	0.095	0.078	0.302*	0.134	0.14	0.145	0.112	–0.039	–0.093	0.154	–0.143	0.159	–0.164	–0.289*	–0.127
Actinobacteria/MB-A2-108/*MB-A2-108_norank*	0.128	0.099	0.092	0.107	0.443**	0.116	0.288*	0.196	0.195	0.397**	0.05	0.329*	0.459**	–0.019	0.340*
Firmicutes/Clostridia/*Clostridium sensustricto 1*	–0.129	–0.029	0.032	–0.033	–0.002	–0.017	0.167	0.029	0.228	–0.066	0.328*	–0.12	0.042	–0.1	–0.016
Proteobacteria/Gammaproteobacteria/*Pseudomonas*	0.087	0.084	0.071	0.001	0.036	0.184	0.099	–0.066	–0.143	–0.013	–0.13	0.184	0.049	–0.129	–0.023
Actinobacteria/Thermoleophilia/*Gaiella*	0.075	0.182	0.182	0.078	0.394**	0.224	0.211	0.07	0.133	0.431**	0.111	0.365*	0.400**	0.027	0.171

Table S4 (*Continued*)

Phylum/class/genus	*T*	pH	Cu	Pb	As	Hg	Cd	TP	TN	OM	N/P	C/N	Elevation	Population density	Urbanization rate
	(°C)					(mg/kg)				(%)			(m)	(people/km^2)	(%)
Proteobacteria/Alphaproteobacteria/*Rhodobacteraceae_uncultured*	–0.13	–0.132	–0.163	–0.209	–0.295*	–0.253	–0.237	–0.139	–0.202	–0.362*	–0.17	–0.231	–0.410**	–0.203	–0.226
Bacteroidetes/Sphingobacteriia/*Dinghuibacter*	0.064	0.125	0.390**	0.229	0.251	0.252	0.093	0.123	0.057	0.179	–0.015	0.073	0.122	–0.099	–0.015
Bathyarchaeota/norank/*Bathyarchaeota_norank*	–0.154	–0.038	0.151	0.404**	0.016	0.261	–0.009	0.345*	0.289*	0.122	0.059	–0.095	0.117	0.018	0.137
Nitrospirae/Nitrospira/*Nitrospira*	0.116	0.141	–0.061	–0.004	0.033	–0.075	0.033	0.119	0.207	0.167	0.169	–0.022	0.194	–0.132	0.225
Actinobacteria/Acidimicrobiia/*CL500-29marinegroup*	–0.049	0.127	0.017	–0.084	0.008	–0.156	0.061	0.059	0.016	–0.138	–0.076	–0.173	–0.177	–0.237	–0.135
Proteobacteria/Deltaproteobacteria/*H16*	0.066	0.226	0.045	–0.004	0.290*	0.123	0.308*	0.221	0.272	0.410**	0.223	0.187	0.465**	0.089	0.24
Proteobacteria/Gammaproteobacteria/*Xanthomonadales_norank*	0.314*	0.254	0.028	0.005	0.298*	0.15	0.292*	0.292*	0.163	0.462**	0.116	0.410**	0.590**	0.154	0.408**
Proteobacteria/Alphaproteobacteria/*Novosphingobium*	–0.169	–0.205	–0.134	–0.158	–0.225	–0.15	–0.23	–0.258	–0.304*	–0.278	–0.194	–0.107	–0.370**	0.112	–0.275
Bacteroidetes/Cytophagia/*Pontibacter*	0.102	–0.008	–0.033	–0.033	0.004	–0.073	–0.076	–0.071	–0.154	–0.166	–0.181	–0.08	–0.246	–0.094	–0.087
Actinobacteria/*Acidimicrobiia/OM1 clade_norank*	0.089	0.033	–0.061	–0.084	–0.086	–0.126	–0.058	–0.007	–0.068	–0.018	–0.119	0.036	–0.162	–0.306*	–0.057
Proteobacteria/Alphaproteobacteria/*A0839_norank*	0.225	0.224	0.187	0.337*	0.366*	0.306*	0.440**	0.340*	0.065	0.335*	–0.111	0.494**	0.233	0.037	0.165
Proteobacteria/Gammaproteobacteria/*Arenimonas*	–0.188	–0.046	0.1	–0.013	0.014	0.088	–0.121	–0.125	–0.228	–0.159	–0.119	0	–0.122	0.294*	–0.246
Bacteroidetes/Sphingobacteriia/*Pedobacter*	–0.012	–0.08	0.094	0.062	–0.139	–0.204	–0.046	–0.034	–0.121	–0.288*	–0.203	–0.274	–0.451**	–0.445**	–0.241
Latescibacteria/norank/Latescibacteria_norank	0.087	0.243	–0.101	–0.103	0.118	0.05	–0.017	0.053	0.267	0.367*	0.376**	0.142	0.542**	0.192	0.472**
Proteobacteria/Deltaproteobacteria/*Geobacter*	0.09	–0.049	0.158	–0.049	0.08	0.127	0.163	–0.018	0.037	0.073	0.101	0.056	0.06	–0.074	–0.155
Proteobacteria/Betaproteobacteria/*Comamonadaceae_uncultured*	–0.063	–0.1	–0.233	–0.159	–0.232	–0.04	–0.127	–0.152	–0.169	–0.235	0.02	–0.093	–0.097	0.282	–0.104
Acidobacteria/Subgroup 22/*Subgroup22_norank*	0.121	0.231	–0.093	–0.086	0.132	0.057	0.03	0.113	0.28	0.372**	0.334*	0.142	0.554**	0.19	0.492**
Bacteroidetes/Sphingobacteriia/*Saprospiraceae_uncultured*	0.099	0.202	0.02	–0.044	0.138	–0.016	0.231	0.017	0.077	0.123	0.082	0.074	0.04	–0.139	–0.037
Proteobacteria/Alphaproteobacteria/*Sphingopyxis*	–0.134	–0.22	–0.193	–0.253	–0.386**	–0.306*	–0.283	–0.300*	–0.314*	–0.540**	–0.211	–0.368**	–0.592**	–0.161	–0.396**
Bacteroidetes/Flavobacteriia/*Actibacter*	0.05	0.061	0.084	0.027	0.088	0.192	0.166	–0.008	–0.14	–0.022	–0.178	0.22	0.02	–0.123	0
Bacteroidetes/Cytophagia/*Cytophagaceae_uncultured*	–0.185	–0.137	–0.179	–0.1	–0.235	–0.111	–0.193	–0.14	–0.18	–0.201	–0.096	–0.105	–0.234	0.094	–0.165
Proteobacteria/Deltaproteobacteria/*Sva0485_norank*	–0.011	0.05	–0.138	0.067	0.068	0.07	0.204	0.185	0.426**	0.227	0.535**	–0.005	0.415**	0.081	0.471**

Table S4 (*Continued*)

Phylum/class/genus	*T*	pH	Cu	Pb	As	Hg	Cd	TP	TN	OM	N/P	C/N	Elevation	Population density	Urbanization rate
	(°C)					(mg/kg)				(%)			(m)	(people/km^2)	(%)
Nitrospinae/Belgica2005-10-ZG-3/*Belgica2005-10-ZG-3_norank*	0.461**	–0.055	–0.03	–0.06	0.233	0.168	0.137	0.053	–0.038	0.268	0.049	0.554**	0.519**	0.122	0.436**
Proteobacteria/Alphaproteobacteria/*Altererythrobacter*	0.144	0.241	–0.004	0.016	0.12	–0.003	0.156	–0.112	–0.134	0.063	–0.175	0.227	–0.109	0.052	–0.014
Bacteroidetes/Sphingobacteriia/*Chitinophagaceae_uncultured*	0.068	0.013	–0.114	–0.144	–0.122	–0.144	–0.159	–0.165	–0.221	–0.201	–0.213	–0.047	–0.171	0.232	–0.133
Acidobacteria/Subgroup 18/*Subgroup18_norank*	0.138	0.029	–0.181	–0.097	0.129	0.037	0.068	0.107	0.301*	0.307*	0.440**	0.182	0.572**	0.156	0.643**
Actinobacteria/Actinobacteria/*Arthrobacter*	0.012	–0.113	–0.155	–0.151	–0.163	–0.204	–0.228	–0.161	–0.254	–0.311*	–0.23	–0.169	–0.318*	0.051	–0.154
Verrucomicrobia/Verrucomicrobiae/Luteolibacter	–0.312*	–0.233	–0.212	–0.279	–0.410**	–0.278	–0.404**	–0.327*	–0.314*	–0.435**	–0.107	–0.271	–0.375**	0.231	–0.358*
Chloroflexi/norank/*Chloroflexi_norank*	–0.087	–0.004	0.087	0.436**	0.017	0.525**	0.177	0.235	0.167	0.094	0.026	–0.053	–0.015	–0.048	0.02
Proteobacteria/Betaproteobacteria/*TRA3-20_norank*	0.059	0.224	–0.043	–0.1	0.068	0.036	0.122	–0.018	0.126	0.290*	0.236	0.132	0.334*	0.111	0.185
Bacteroidetes/Flavobacteriia/*Flavobacterium*	–0.125	–0.202	–0.166	–0.195	–0.333*	–0.244	–0.393**	–0.267	–0.329*	–0.397**	–0.209	–0.247	–0.360*	0.196	–0.308*
Acidobacteria/Blastocatellia/*Blastocatella*	0.116	–0.025	–0.174	–0.212	–0.223	–0.255	–0.224	–0.163	–0.166	–0.262	–0.139	–0.146	–0.219	0.017	–0.104
Bacteroidetes/Sphingobacteriia/*Ferruginibacter*	–0.105	–0.178	–0.14	–0.229	–0.375**	–0.291*	–0.264	–0.257	–0.255	–0.508**	–0.206	–0.352*	–0.516**	–0.118	–0.381**
Proteobacteria/Alphaproteobacteria/*Sphingomonadaceae_uncultured*	0.012	0.156	0.404**	0.346*	0.440**	0.275	0.321*	0.146	0.137	0.372**	0.002	0.2	0.037	–0.038	–0.05
Firmicutes/Clostridia/*Fusibacter*	–0.093	0.015	0.352*	0.091	0.02	0.333*	–0.098	–0.024	–0.023	–0.015	–0.001	–0.028	0.009	–0.003	–0.115
Proteobacteria/Gammaproteobacteria/*Thermomonas*	–0.009	0.077	0.21	0.069	–0.008	0.054	0.019	–0.158	–0.135	–0.047	–0.193	0.031	–0.288*	–0.094	–0.142
Proteobacteria/Epsilonproteobacteria/*Sulfuricurvum*	0.241	0.194	0.131	0.092	0.136	0.024	0.153	0.576**	0	0.093	–0.242	0.279	0.173	0.098	0.185
Proteobacteria/Betaproteobacteria/*Ramlibacter*	0.128	–0.038	0.111	–0.041	–0.134	–0.087	–0.108	–0.209	–0.350*	–0.175	–0.345*	0.011	–0.330*	–0.099	–0.330*
Actinobacteria/Actinobacteria/*Marmoricola*	–0.169	–0.146	0.124	0.15	0.186	0.287*	0.071	0.052	0.169	0.114	0.158	–0.029	0.193	0.019	0.198
Bacteroidetes/Sphingobacteriia/*Flavihumibacter*	0.075	0.129	0.306*	0.113	0.163	0.18	0.13	–0.036	–0.045	0.203	–0.082	0.183	–0.109	–0.242	–0.067
Proteobacteria/Betaproteobacteria/*Paucimonas*	0.164	0.117	0.245	0.081	0.212	0.18	0.136	–0.045	–0.099	0.173	–0.139	0.199	–0.172	–0.311*	–0.091
Proteobacteria/Deltaproteobacteria/*43F-1404R_norank*	0.138	0.09	–0.163	–0.066	0.119	0.059	0.178	0.144	0.323*	0.415**	0.423**	0.157	0.566**	0.156	0.555**
Proteobacteria/Betaproteobacteria/*Methylotenera*	0.202	–0.095	–0.251	–0.249	–0.146	–0.19	0.002	–0.187	–0.002	0.065	0.198	–0.102	0.084	0.067	–0.047

Table S4 (*Continued*)

Phylum/class/genus	*T*	pH	Cu	Pb	As	Hg	Cd	TP	TN	OM	N/P	C/N	Elevation	Population density	Urbanization rate
	(°C)					(mg/kg)				(%)			(m)	(people/km²)	(%)
Proteobacteria/Betaproteobacteria/*Acidovorax*	0.078	0.11	–0.093	–0.14	–0.038	–0.019	–0.004	–0.173	–0.041	–0.026	–0.028	–0.005	–0.04	0.06	0.058
Proteobacteria/Deltaproteobacteria/*Bacteriovorax*	–0.034	–0.144	–0.146	–0.162	–0.242	–0.134	–0.285*	–0.21	–0.194	–0.227	–0.082	–0.143	–0.191	0.172	–0.116
Firmicutes/Erysipelotrichia/*Erysipelothrix*	–0.005	0.075	–0.037	–0.078	0.02	0.065	0.071	–0.05	0.054	0.032	0.107	–0.062	0.04	0.1	–0.056
Chloroflexi/MSB-5B2/*MSB-5B2_norank*	–0.103	–0.05	0.286*	0.539**	0.144	0.257	0.087	0.289*	0.245	0.096	0.011	–0.095	0.025	0.018	–0.072
Chloroflexi/Dehalococcoidia/*GIF9_norank*	–0.067	–0.048	0.118	0.377**	0.039	0.142	0.04	0.309*	0.332*	0.08	0.218	–0.107	0.172	0.056	0.177
Spirochaetae/Spirochaetes/*Spirochaetaceae_uncultured*	–0.082	–0.05	0.264	0.437**	0.142	0.153	0.047	0.245	0.218	0.079	0.021	–0.079	0.051	0.057	–0.074
Atribacteri/*norank*/*Atribacteria_norank*	–0.104	–0.053	0.282	0.477**	0.145	0.172	0.062	0.254	0.224	0.075	0.008	–0.091	0.022	0.027	–0.087
Proteobacteria/Gammaproteobacteria/*pLW-20_norank*	–0.095	–0.052	0.252	0.547**	0.119	0.327*	0.098	0.285*	0.233	0.111	0.011	–0.086	0.029	0.016	–0.046
Elusimicrobia/Elusimicrobia/*4-29_norank*	–0.114	–0.028	0.236	0.594**	0.107	0.492**	0.165	0.319*	0.209	0.125	–0.043	–0.07	–0.019	–0.019	–0.067
Proteobacteria/Betaproteobacteria/*Candidatus* Nitrotoga	0.044	0.085	–0.003	0.289*	0.133	0.269	0.24	0.261	0.324*	0.362*	0.219	0.012	0.335*	0.093	0.282
Proteobacteria/Betaproteobacteria/*Gallionellaceae_uncultured*	0.012	–0.106	–0.076	0.091	–0.071	–0.008	–0.019	0.029	0.304*	0.012	0.463**	–0.131	0.244	0.057	0.284
Chloroflexi/*S085*/*S085_norank*	–0.1	0.259	0.139	0.178	0.317*	0.185	0.173	0.282	0.404**	0.463**	0.321*	0.141	0.559**	0.178	0.441**
Proteobacteria/Gammaproteobacteria/*Sulfurifustis*	–0.003	–0.002	0.113	0.476**	0.096	0.538**	0.227	0.343*	0.191	0.213	–0.002	0.081	0.154	0.031	0.122
Proteobacteria/Deltaproteobacteria/*DTB120_norank*	–0.059	0.018	0.134	0.484**	0.068	0.551**	0.188	0.263	0.166	0.172	–0.005	–0.005	0.031	–0.015	0.01
Proteobacteria/Betaproteobacteria/*Sideroxydans*	–0.13	–0.137	–0.104	0.025	–0.085	0.188	–0.119	0.075	0.308*	0.052	0.356*	–0.146	0.286*	0.037	0.393**
Proteobacteria/Deltaproteobacteria/*Polyangiaceae_uncultured*	–0.07	0.01	0.118	0.462**	0.06	0.559**	0.207	0.247	0.112	0.127	–0.069	–0.005	–0.032	–0.036	–0.042
Proteobacteria/Betaproteobacteria/*Gallionella*	–0.095	–0.062	–0.179	–0.099	–0.189	–0.077	–0.16	0.009	0.266	–0.031	0.392**	–0.171	0.205	0.021	0.346*
GAL15/*norank*/*GAL15_norank*	0.037	0.252	0.034	–0.045	0.075	0.036	–0.07	–0.006	0.14	0.251	0.245	0.078	0.291*	0.147	0.217
Spirochaetae/Spirochaetes/*Spirochaeta2*	0.092	0.031	–0.258	–0.118	–0.065	–0.089	0.055	0.07	0.308*	0.134	0.507**	–0.012	0.403**	0.112	0.499**
Acidobacteria/Acidobacteria/*Acidobacteriaceae(Subgroup1)_uncultured*	–0.11	–0.037	0.045	–0.051	0.074	0	0.141	0.052	0.095	0.053	0.074	–0.024	0.032	–0.073	–0.153
Proteobacteria/Betaproteobacteria/*Rhodoferax*	–0.041	–0.21	–0.078	–0.155	–0.402**	–0.224	–0.309*	–0.191	–0.298*	–0.416**	–0.238	–0.213	–0.393**	–0.119	–0.398**
Acidobacteria/*Subgroup 2*/*Subgroup 2_norank*	–0.07	0.021	0.072	–0.055	0.027	0.003	0.136	0.036	0.083	0.055	0.087	–0.033	0.009	–0.072	–0.149
Proteobacteria/Betaproteobacteria/*Polaromonas*	–0.028	–0.186	0.127	–0.111	–0.332*	–0.109	–0.253	–0.174	–0.299*	–0.454**	–0.26	–0.303*	–0.420**	–0.17	–0.555**

Table S4 (*Continued*)

Phylum/class/genus	*T*	pH	Cu	Pb	As	Hg	Cd	TP	TN	OM	N/P	C/N	Elevation	Population density	Urbanization rate
	(°C)					(mg/kg)				(%)			(m)	(people/km^2)	(%)
Proteobacteria/Betaproteobacteria/*Dechloromonas*	0.377**	–0.099	–0.132	–0.069	0.238	0.026	0.12	0.012	–0.1	0.215	–0.112	0.534**	0.397**	0.147	0.346*
Proteobacteria/Betaproteobacteria/*Thiobacillus*	0.153	0.06	0.028	–0.042	0.099	0.017	0.169	–0.075	–0.072	0.187	–0.088	0.247	–0.12	–0.246	0.027
Bacteroidetes/Bacteroidia/*Paludibacter*	–0.246	–0.158	–0.102	–0.176	–0.264	–0.167	–0.228	–0.198	–0.192	–0.269	–0.099	–0.173	–0.276	0.073	–0.296*
Bacteroidetes/Cytophagia/*Hymenobacter*	–0.235	–0.265	–0.188	–0.203	–0.317*	–0.268	–0.353*	–0.258	–0.321*	–0.395**	–0.205	–0.256	–0.394**	0.156	–0.256
Bacteroidetes/Cytophagia/*Algoriphagus*	–0.106	–0.295*	–0.261	–0.281	–0.415**	–0.300*	–0.423**	–0.348*	–0.357*	–0.421**	–0.221	–0.233	–0.449**	0.101	–0.290*
Actinobacteria/Actinobacteria/*Frankiales_norank*	0.014	–0.161	–0.179	–0.204	–0.273	–0.229	–0.289*	–0.22	–0.272	–0.328*	–0.238	–0.147	–0.306*	0.191	–0.226
Nitrospirae/Nitrospira/*0319-6A21_norank*	–0.089	0.007	0.061	–0.051	0.081	0.028	0.161	0.035	0.1	0.086	0.102	–0.012	0.054	–0.054	–0.138
Cyanobacteria/Cyanobacteria/*Phormidium*	–0.005	0.089	–0.009	–0.018	0.015	–0.003	0.118	–0.086	0.119	–0.05	0.04	–0.115	–0.154	–0.239	0.01
Acidobacteria/Blastocatellia/*RB41*	–0.156	0.112	–0.043	–0.076	0.219	–0.006	0.086	–0.008	0.168	0.206	0.158	0.052	0.221	0.026	0
Bacteroidetes/Flavobacteriia/*Lutibacter*	0.085	–0.097	0.063	–0.003	–0.24	–0.088	–0.15	–0.049	–0.164	–0.229	–0.175	–0.093	–0.191	–0.155	–0.304*
Proteobacteria/Gammaproteobacteria/*Alkanindiges*	–0.073	–0.139	–0.139	–0.166	–0.212	–0.152	–0.211	–0.177	–0.186	–0.233	–0.121	–0.158	–0.245	0.13	–0.206
Proteobacteria/Alphaproteobacteria/*Sphingorhabdus*	0.057	–0.112	–0.035	–0.052	–0.232	–0.153	–0.08	–0.026	–0.147	–0.275	–0.175	–0.157	–0.281	–0.277	–0.215
Bacteroidetes/*Flavobacteriia*/*Gillisia*	–0.202	–0.296*	–0.238	–0.24	–0.345*	–0.266	–0.370**	–0.26	–0.321*	–0.358*	–0.199	–0.216	–0.396**	0.067	–0.23
Proteobacteria/Alphaproteobacteria/*Loktanella*	–0.165	–0.348*	–0.302*	–0.316*	–0.461**	–0.331*	–0.503**	–0.381**	–0.414**	–0.465**	–0.255	–0.252	–0.421**	0.362*	–0.303*
Actinobacteria/Actinobacteria/*Cryobacterium*	0.002	–0.191	–0.117	–0.159	–0.355*	–0.253	–0.214	–0.109	–0.228	–0.382**	–0.193	–0.227	–0.401**	–0.258	–0.313*
Bacteroidetes/Flavobacteriia/*Subsaxibacter*	–0.114	–0.292*	–0.236	–0.251	–0.371**	–0.266	–0.400**	–0.311*	–0.332*	–0.371**	–0.197	–0.198	–0.378**	0.156	–0.25
Actinobacteria/Actinobacteria/*Knoellia*	0.118	–0.026	–0.097	–0.098	–0.048	–0.121	–0.099	–0.061	–0.178	–0.234	–0.186	–0.117	–0.271	–0.193	–0.09
Firmicutes/Bacilli/*Planococcus*	0.122	0.015	–0.07	–0.056	0.028	–0.076	–0.032	–0.002	–0.116	–0.154	–0.142	–0.075	–0.226	–0.292*	–0.016
Bacteroidetes/Cytophagia/*Rhodonellum*	–0.268	–0.274	–0.189	–0.193	–0.285*	–0.204	–0.310*	–0.222	–0.24	–0.292*	–0.118	–0.208	–0.289*	0.144	–0.186
Cyanobacteria/Cyanobacteria/*Tychonema*	–0.019	–0.019	–0.04	–0.05	–0.057	–0.094	–0.014	0.045	0.022	–0.126	–0.016	–0.133	–0.16	–0.244	0.009
Chloroflexi/JG37-AG-4/*JG37-AG-4_norank*	–0.093	–0.049	0.08	–0.027	0.018	–0.001	0.13	0.036	0.054	–0.021	0.028	–0.073	–0.084	–0.113	–0.212
Chloroflexi/Ktedonobacteria/*HSB OF53-F07_norank*	–0.176	0.011	–0.092	–0.04	0.208	–0.017	0.075	0.04	0.14	0.154	0.117	0.036	0.214	0.101	–0.013

Significance levels are displayed at *$p < 0.05$ and **$p < 0.01$.

Table S5
Relationships between top 10 bacterial phyla and elevation, organic matter, and urbanization rate using Pearson correlation analysis

Phylum	Elevation (masl)	OM (%)	Urbanization rate (%)
Alphaproteobacteria	0.653**	–0.696**	–0.648**
Betaproteobacteria	–0.442*	0.616**	0.497*
Gammaproteobacteria	–0.395	0.440*	0.372
Deltaproteobacteria	–0.592**	0.596**	0.571**
Bacteroidetes	0.596**	–0.684**	–0.592**
Actinobacteria	–0.011	0.038	–0.12
Acidobacteria	–0.561**	0.587**	0.362
Chloroflexi	–0.609**	0.668**	0.623**
Nitrospirae	–0.505*	0.505*	0.442*
Firmicutes	0.057	–0.06	–0.16
Verrucomicrobia	0.833**	–0.717**	–0.416*
Cyanobacteria	0.516**	–0.608**	–0.428*
Gemmatimonadetes	–0.334	0.17	–0.032
Latescibacteria	–0.476*	0.525**	0.593**
Spirochaetae	–0.266	0.371	0.338
Ignavibacteriae	–0.604**	0.522**	0.528**
Aminicenantes	–0.371	0.226	0.576**
Planctomycetes	–0.377	0.487*	0.334
Nitrospinae	–0.499*	0.519**	0.338
GAL15	–0.366	0.495*	0.460*
Saccharibacteria	–0.1	0.143	–0.252
Elusimicrobia	–0.18	0.424*	0.173
Parcubacteria	0.157	–0.488*	–0.14
Atribacteria	–0.134	0.389	0.169
SR1 (Absconditabacteria)	0.503*	–0.425*	–0.356
Others	–0.396	0.38	0.433*

Significance levels are shown at *$p < 0.05$ and **$p < 0.01$.

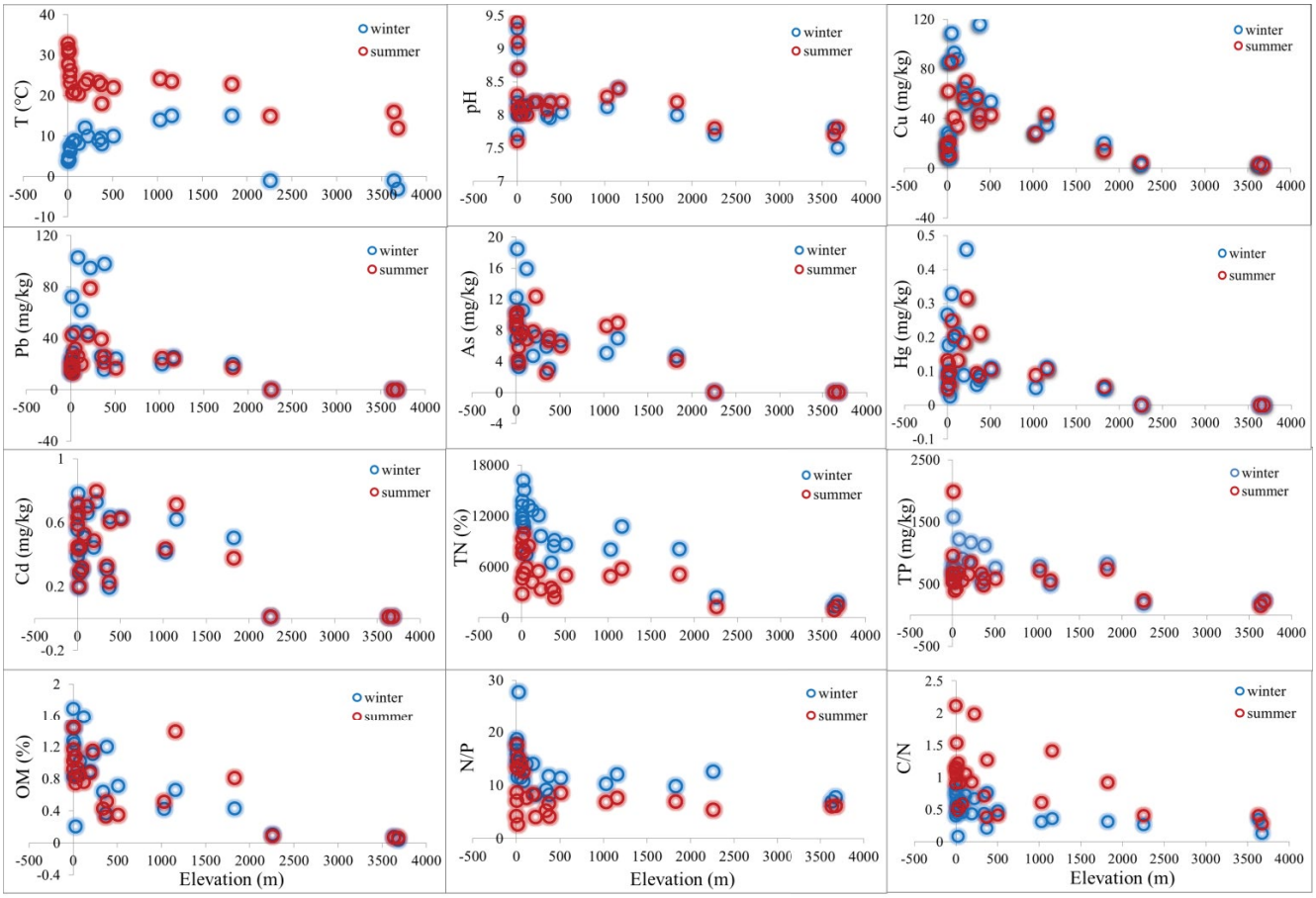

Fig. S1. Variations of physicochemical properties and OTU numbers along elevation gradient. Similar decreased trends of all parameters were observed except temperature and pH.

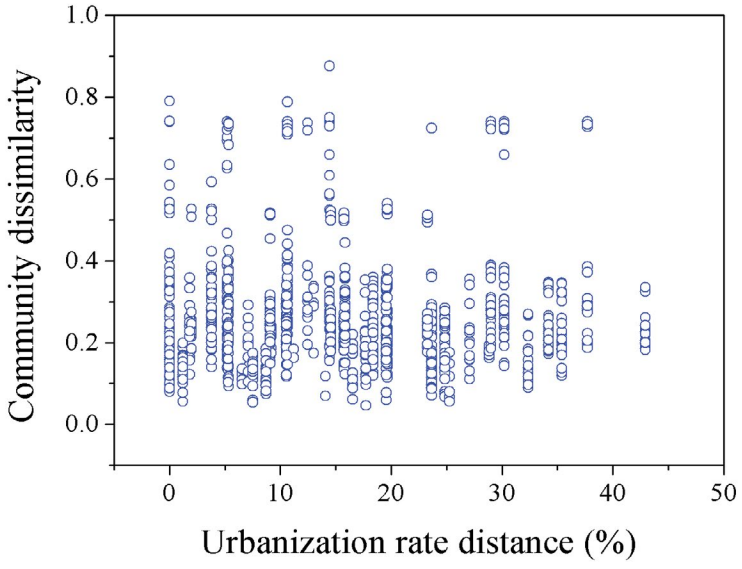

Fig. S2. Distance-decay relationships for bacterial communities in the sediment of the Yangtze River. Scatter plots indicate community dissimilarity represented by Euclidean distance (vertical axes) vs. urbanization rate along the Yangtze River (horizontal axes).