



How sediment bacterial community shifts along the urban river located in mining city

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Abstract

Bacterial communities play critical roles in biogeochemical cycles and serve as sensitive indicators of environmental fluctuation. However, the influence of mineral resource exploitation on shaping the bacterial communities in the urban river is still ambiguous. In this study, high-throughput sequencing was used to determine the spatial distribution of the sediment bacterial communities along an urban river in the famous mining city Panzhihua of China. The results showed that mineral resource exploitation had a significant impact on the urban river bacterial community structure but not on the bacterial ecological functions. Distinct families of bacteria often associated with nutrients (i.e., *Comamonadaceae* and *Sphingomonadaceae*) and metal contaminants (i.e., *Rhodobacteraceae*) were more predominant in the residential and mining area, respectively. Relative to dispersal dynamics, environmentally induced species sorting may primarily influence bacterial community structure. Heavy metals and sediment physicochemical properties had both similar and significant influence on shaping bacterial community structure. Among heavy metals, essential metal elements explained more rates of bacterial variation than toxic metals at moderate contaminant levels. Moreover, the bacteria with multiple metal resistances identified in culture-dependent experiments were probably not suitable for indicating heavy metal contamination in field research. Thus, several sensitive bacterial genera such as *Rhodobacter*, *Hylemonella*, and *Dechloromonas* were identified as potential bioindicators to monitor metals (iron and titanium) and nutrients (phosphorus and organic carbon) in the river ecosystem of the Panzhihua region. Together, these results profiled the coupling effect of urbanization and mineral resource utilization on shaping sediment bacterial communities in urban rivers.

Keywords Bacterial community · Urban river · Heavy metals · Sediment physicochemical properties · Bioindicators

Introduction

Rivers and mineral resources are closely associated with the development of civilization (Jing et al. 1997; Rickard 1934). Many famous cities such as Pittsburgh in the USA, Dortmund in Germany, and Panzhihua in China are perched on riversides and have abundant mineral resources. The mineral resources are the basis of economic development whereas the rivers flowing through these cities are important sources of drinking water for citizens, but the rapid urbanization in mining cities

may cause environmental pollution and geological hazards, which in turn, make the urban river a sink for municipal sewage and mining wastewater. Bacterial communities are vital components of biogeochemical processes and food webs in the sediments of river ecosystems (Fernandez-Gonzalez et al. 2016). As the sink and source for chemicals and bacteria, sediments always contain most of contaminant and bacterial biomass in the urban river (Fischer and Pusch 1999). Previous studies indicated that sediment bacterial communities in urban rivers are subjected to anthropogenic stress during rapid urbanization (Gourvindu et al. 2015) because urban effluents like stormwater and sewage are continually discharged into the river (Paul and Meyer 2008). Urban effluents contaminate river water with nutrients, organic pollutants, suspended solids, and metals (Lewis et al. 2007; Wenger et al. 2009), and consequently alter sediment bacterial communities (Roberto et al. 2018). River water deterioration may be especially severe in mining city because the rivers receive not only urban discharges but also a large amount of metal inputs (Cao

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et al. 2017). However, there is no consensus as to how sediment bacterial communities respond and resilient to the multiple anthropogenic stressors in the urban rivers of mining cities (Sun et al. 2013).

Bacterial communities are extremely sensitive to environmental disturbances and spatial variations in freshwater sediments (Gibbons et al. 2014; Ibekwe et al. 2016). Geographic distance (Isabwe et al. 2018) and environmental factors such as hydrology (Roberto et al. 2018), nutrient levels (Wang et al. 2019), and heavy metals (Jroundi et al. 2020) are the major factors influencing bacterial communities. However, how bacterial communities are assembled under multiple disturbances in urban rivers still remains unclear (Roberto et al. 2018). Among the mentioned factors, heavy metals are always considered as toxic to microorganisms and can damage ecosystems, but some metals are essential for several cellular functions (Seiler and Berendonk 2012). In many cases, high concentrations of heavy metals may adversely affect bacterial communities (Li et al. 2021; Zhang et al. 2015). In contrast, some metals may promote microbial growth and increase bacterial diversity (Jingxin et al. 2013). Those contrasting conclusions shadowed the knowledge on effect of heavy metals on bacterial communities in river sediment. Therefore, an attempt to determine the how heavy metals influence sediment bacterial community has also been emphasized.

The variations of metal resistance bacterial are useful for monitoring heavy metal pollution in freshwater sediments because bacterial community function is a better indicator of environmental perturbation than bacterial community structure (Burke et al. 2011; Steffen et al. 2012). Since culture-dependent methods can more accurately represent physiological state of bacteria than culture-independent methods (Ellis et al. 2003), metal-resistant bacteria are usually identified by culture-dependent tests. However, it is unlikely that the metal-resistant bacteria found in laboratory studies could survive under field conditions. For this reason, metal resistance bacteria reported in the literature must be verified under field conditions. If these metal resistance bacteria fail to monitor heavy metal pollutants, some other sensitive candidate bacteria should be identified as potential indicators to monitor heavy metal contaminants.

Panzhihua is a famous mining city located along the Jinsha River in Sichuan Province, China. It has abundant mineral resources and accounts for 20% of the iron, 64% of the vanadium, and 53% of the titanium supply in China (Cao et al. 2017). However, its topography interlaces residential area with the mining industry (Teng et al. 2011). This configuration may increase potential human health risks by contaminating the drinking water with heavy metals discharged from the mining areas. Furthermore, the Jinsha River is the upper reach of the Yangtze River, a major source of fresh water in China (Wang et al. 2015). Heavy metal accumulation in the Jinsha River will probably deteriorate the water quality and alter the

bacterial community structure downstream of the Yangtze River, which might pose potential risk for both public health and ecosystems in China. Therefore, it is necessary to understand how the sediment bacterial community shifts along the urban river in Panzhihua and to identify sensitive bacteria which can monitor heavy metal contamination. It is hypothesized that high input of nutrient elements and heavy metals from various pollutant sources associated with mining city are important impetus for shifting sediment bacterial community. In this study, we investigated the following: (1) the alterations in the Jinsha River sediment bacterial community caused by inputs from Panzhihua city; (2) the major factors influencing dynamic changes in the sediment bacterial community, and (3) the feasibility of certain sensitive bacterial genera as bioindicators of heavy metals and nutrient contamination.

Materials and methods

Study area and sampling procedure

The Panzhihua region is located in Sichuan Province, China, along the middle stream of the Jinsha River. In this study, sediment samples were collected from eight sites in the Panzhihua region along the Jinsha River (Fig. 1). To minimize variance, three sediment samples were collected within meters of each other at each sampling site along the river. PZH-1 and PZH-3 are pristine sites. PZH-2, PZH-4, and PZH-5 represent residential areas. Within residential areas, PZH-2 is downstream from a village in the rural area. PZH-4 and PZH-5 are located in the urban area. PZH-6 is located in an area affected by both residential effluents and mining industry discharges (hereafter, the comprehensive area). PZH-7 and PZH-8 are influenced by V-Ti and Cu mining industry, respectively.

Each sediment sample was collected at 0 ~ 6 cm depth with an Ekman-Brige grab sampler (Hydro-Bios, Altenholz, Germany) then homogenized. For the sediment physicochemical characteristics and heavy metal analyses, 500 g of homogenized sediment was stored at -20°C before returned to the laboratory. Another 50 g of homogenized sediment was stored at -20°C in the field to acquire bacterial DNA. Samples were maintained at -80°C in the laboratory before DNA extraction.

Physicochemical characteristics and heavy metal analysis

Sampling location data were obtained with a GPS device (GPSMAP 62s; Garmin Ltd., Olathe, KS, USA) during field sampling. In the laboratory, all sediment samples were freeze-dried using a lyophilizer (Labconco, Kansas City, MO, USA) for environmental factor determinations. Electrical

conductivity (EC) and pH were measured for 1:5 sediment: water suspensions with a multiparameter water quality analyzer (HQ40d; Hach, Loveland, CO, USA). Total nitrogen (TN) and total phosphorus (TP) were determined by the Kjeldahl and sulfuric acid-perchloric acid oxidation methods, respectively (Ebina et al. 1983). Ammonium nitrogen (NH_4) was extracted with 2 M KCl and then measured with a flow injection autoanalyzer (Lachat QC8500; Lachat Instruments, Loveland, CO, USA). Available phosphorus (AP) was extracted with NaHCO_3 and measured by the molybdenum blue method in a spectrophotometer (Persee, Beijing, China). Total organic carbon (TOC) was measured by an Elementar Liqui TOCII analyzer (Frankfurt, Germany).

Heavy metals (Hg, Cu, Cd, Pb, V, Fe, and Ti) in the sediment were digested with concentrated hydrofluoric acid and determined by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500 series, USA) as described in the previous study (Gillan et al. 2015). Heavy metal pollution in the river sediment was defined using geoaccumulation and potential ecological risk indices in order to profile the pollution condition of heavy metals properly (Fu et al. 2009; Zhang et al. 2016). Heavy metal pollution in each area was represented by the average geoaccumulation and potential ecological risk indices of their sampling sites. The methods of these pollution assessments were presented in Text. S1 in the Supplementary material.

DNA extraction, high-throughput sequencing, and metal-resistant bacteria identification

DNA was extracted from 0.5 g samples using a PowerSoil DNA extraction kit (Mo Bio, Carlsbad, CA, USA) following the manufacturer's instructions. Agarose gel electrophoresis (1%) and spectrophotometry (NanoDrop ND 2000, Thermo

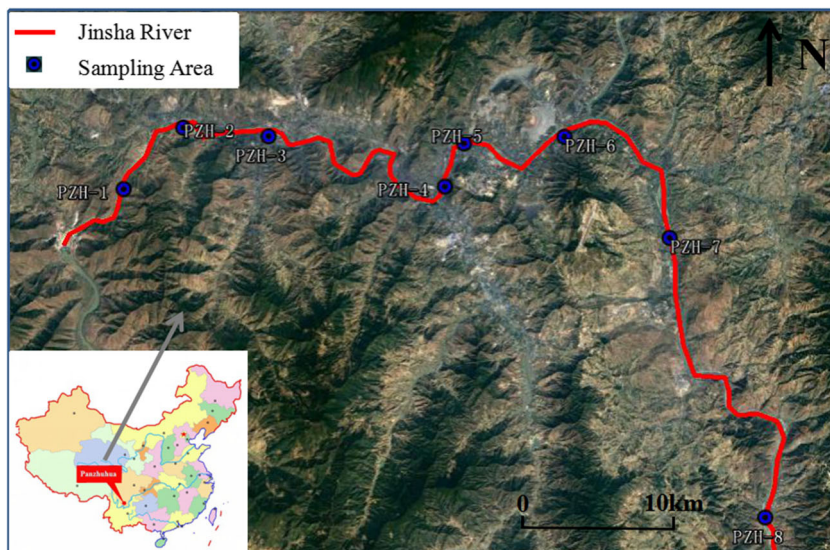
Scientific, DE, USA) were applied to verify the quality and quantity of extracted DNA.

The V4 region of 16S rRNA was amplified as follows: 94 °C for 5 min, 31 cycles at 94 °C for 30 s, 52 °C for 30 s, 72 °C for 45 s, and a final extension at 72 °C for 10 min. Universal primers were 515F (5'-GTGC CAGCMGCCGCGGTAA-3') and 806R (5'-GGAC TACHVGGGTWTCTAAT-3'). Triplicate PCRs were conducted for all samples to minimize potential bias and were combined equally for each sample. Amplicons were measured by the PicoGreen dsDNA assay kit (Invitrogen, Carlsbad, CA, USA).

The PCR product was sequenced on the Illumina Hiseq 2000 platform (Illumina, Inc., San Diego, CA, USA). Data processing was conducted according to a previous study (Li et al. 2017). Briefly, the raw sequences were trimmed to remove low-quality reads (Trimmomatic, version 0.33). Forward- and reverse-end sequences were attributed to their corresponding samples by MOTHUR (version 1.35.1) and were matched using FLASH (version 1.2.11). The matched reads containing an "N" or shorter than 200 bp were removed. Chimeras were discarded via the UCHIME algorithm in USEARCH. The filtered sequences were clustered into operational taxonomic units (OTUs) at 97% identification with the UPARSE algorithm in USEARCH (version 8.0.1517). The OTUs were then assigned taxonomically by aligning representative sequences to the Greengenes database (version 13.5) with the Ribosomal Database Project (RDP) Classifier (Cole et al. 2009).

Metal-resistant bacteria were identified from the literature based on the results of high-throughput sequencing (Mejias Carpio et al. 2018), which including 6 common genera of metal-resistant bacteria *Bacillus*, *Cupriavidus*, *Ochrobactrum*, *Paenibacillus*, *Pseudomonas*, and *Ralstonia* revealed by culture-independent methods in previous studies.

Fig. 1 Sampling areas in the Panzhuhua region of the Jinsha River. The satellite image was obtained from Google Earth



Bioinformatics analysis

The bioinformatics analysis was conducted based on the sequence data of bacterial community. Alpha-diversity of the bacterial community was evaluated by the Chao1 richness and Shannon diversity indices, which were calculated by QIIME (version 1.8.0) (Caporaso et al. 2010). The hierarchical clustering method UPGMA was used to present phylogenetic structure of the bacterial communities based on bacterial abundance-unweighted UniFrac distances. Analysis of similarities (ANOSIM) was performed to identify whether bacterial community structures significantly differed among areas. PICRUSt (Langille et al. 2013) was applied to predict bacterial community functions in the river sediment. It used the associations between the 16S rRNA markers found in the Greengenes database and the functional genes found in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database to reconstruct the potential functional genes in the samples (Roberto et al. 2018). Canonical correspondence analysis (CCA), variance partitioning analysis (VPA), and multivariate regression tree (MRT) were applied to reveal significant drivers influencing bacterial community. CCA was applied to calculate the influence of variation on the shift in bacterial community structure and tested by Monte Carlo permutation. VPA was conducted to compare the effect of geographic distance vs. local environmental condition as well as physicochemical characteristics vs. heavy metal based on CCA analysis. MRT was conducted to cluster bacterial community data by environmental condition and identify the crucial environmental factors that best impacted the bacterial community. A network was constructed with Cytoscape v. 3 (Saito et al. 2012) to visualize the correlations between bacterial taxa and environmental factors. The random matrix theory-based method was used to automatically define the threshold and Pearson correlation network were constructed based on bacterial genera level. (Ding et al. 2015; Xie et al. 2016). In addition, environmental parameters were tested for their associations and significances of the observed variations by Pearson correlation analysis and one-way ANOVA, respectively. All statistical analyses were conducted in the R packages *vegan* and *mvpart* (version 3.1.2, R Foundation for Statistical Computing, Vienna, Austria).

Results

Sediment physicochemical characteristics and heavy metal pollution

Sediment physicochemical properties (pH, EC, TOC, TN, NH₄, TP, and AP) are listed in Table S1 in the Supplemental Files. Both pH and EC were higher in the pristine site than in the other areas. TN was significantly lower in the mining area

than in the other areas ($P < 0.05$). Minimum NH₄ was measured for the pristine site and was relatively higher for the comprehensive area and the mining area. In contrast, TP was the highest for the mining area and significantly lower for the pristine site ($P < 0.05$). The pristine site had the highest AP of all. TOC was comparatively higher in the pristine site and ranged from 2.61–29.22 mg kg⁻¹.

Hg, Cu, Cd, Pb, V, Fe, and Ti were profiled by the geoaccumulation index (I_{geo}) shown in Fig. S1A. The geoaccumulation indices of all metals were < 1 in the pristine and residential areas. The highest accumulation level was measured for Ti in the Panzhuhua region especially in the comprehensive area and the mining area. According to the geoaccumulation indices, all areas ranged from uncontaminated to moderately contaminated with Hg, Cu, Cd, Pb, and Fe. Moreover, the levels of Ti, Fe, and V were significantly correlated along the river ($r > 0.7$; $P < 0.001$). The ecological risk indices of heavy metal along the Jinsha River around the Panzhuhua region were shown in Fig. S1B. The toxicities of Hg and Cd were the highest of all the metals analyzed because they are inherently highly toxic. Their potential ecological risk index was the highest in the comprehensive area, and even in this area, the ecological risks of Hg and Cd were moderate.

Bacterial community diversity, structure, and ecological function affected by the mining city

The high-quality sequences sampled from all sediments ranged from 55,286–76,925 (Table S2 in the Supplemental Files). The OTUs ranged from 1342–5287 in all samples and the average was 3120. PZH-5 had the maximum OTUs and was located in the residential quarters. The α -diversity was calculated based on the 55,286 reads randomly selected from each sample to minimize the effect of uneven sampling on the analysis. According to the ANOVA, both the Chao1 richness and Shannon diversity indices were significantly lower in the pristine site than in the other area ($P < 0.05$).

The ANOSIM indicated that the bacterial community structures in the pristine and residential areas were significantly different with other areas (Table S3). The bacterial community structure in the comprehensive area more nearly resembled that of the mining area than the other areas. UPGMA was applied to determine the phylogenetic structures of the bacterial communities (Fig. 2a). It clustered the sampling sites into three groups: PZH-1 and PZH-3 were clustered into the first group representing the pristine site; PZH-2, PZH-4, and PZH-5 were clustered into the second group and comprised the residential area; and PZH-6, PZH-7, and PZH-8 were clustered into the third group and constituted the comprehensive and mining areas.

The taxonomic distributions of all samples are illustrated in Fig. 2a. *Proteobacteria* was the dominant phylum in the bacterial community, which accounted for 28.51–57.55% of the

total bacteria (average: 43.36%). The second dominant phylum was *Firmicutes* (20.15%). *Bacteroidetes* (11.59%), *Actinobacteria* (8.67%), and *Verrucomicrobia* (8.48%) were the other major phyla in the community at relative abundances > 5%. The bacterial communities in pristine sites consisted mainly of *Proteobacteria* and *Firmicutes* (> 90%). In the mining area, the proportion of *Bacteroidetes* was higher than other areas. The relative abundance of *Actinobacteria* was the highest for the residential area, especially at PZH-2 (downstream from the village in the rural area). The relative abundance of *Verrucomicrobia* was the highest for PZH-6, which was affected both by residential effluents and mining industry discharges.

The most abundant bacterial family in all areas was the *Exiguobacteraceae* (*Firmicutes*) and followed by the *Moraxellaceae* (*Proteobacteria*). The *Exiguobacteraceae* and

the *Moraxellaceae* comprised 18.16% and 10.66% of the total bacterial community, respectively (Fig. 2c). Other bacterial families with relative abundances > 1% were the *Xanthomonadaceae*, *Pseudomonadaceae*, *Enterobacteriaceae*, *Oxalobacteraceae*, *Comamonadaceae*, *Sphingomonadaceae*, *Erythrobacteraceae*, *Rhodobacteraceae*, *Caulobacteraceae* (*Proteobacteria*), [*Weeksellaceae*], *Cytophagaceae*, *Chitinophagaceae* (*Bacteroidetes*), *Pirellulaceae* (*Planctomycetes*), *Nocardioideaceae*, *Micrococcaceae* (*Actinobacteria*), *Deinococcaceae* ([*Thermi*]), and *Verrucomicrobiaceae* (*Verrucomicrobia*). The proportion of *Proteobacteria* was high in the residential area and pristine site but it consisted of a greater number of distinct families and the *Xanthomonadaceae*, *Oxalobacteraceae*, *Comamonadaceae*, *Sphingomonadaceae*, *Erythrobacteraceae*, *Rhodobacteraceae*, and *Caulobacteraceae* were higher in residential area whereas

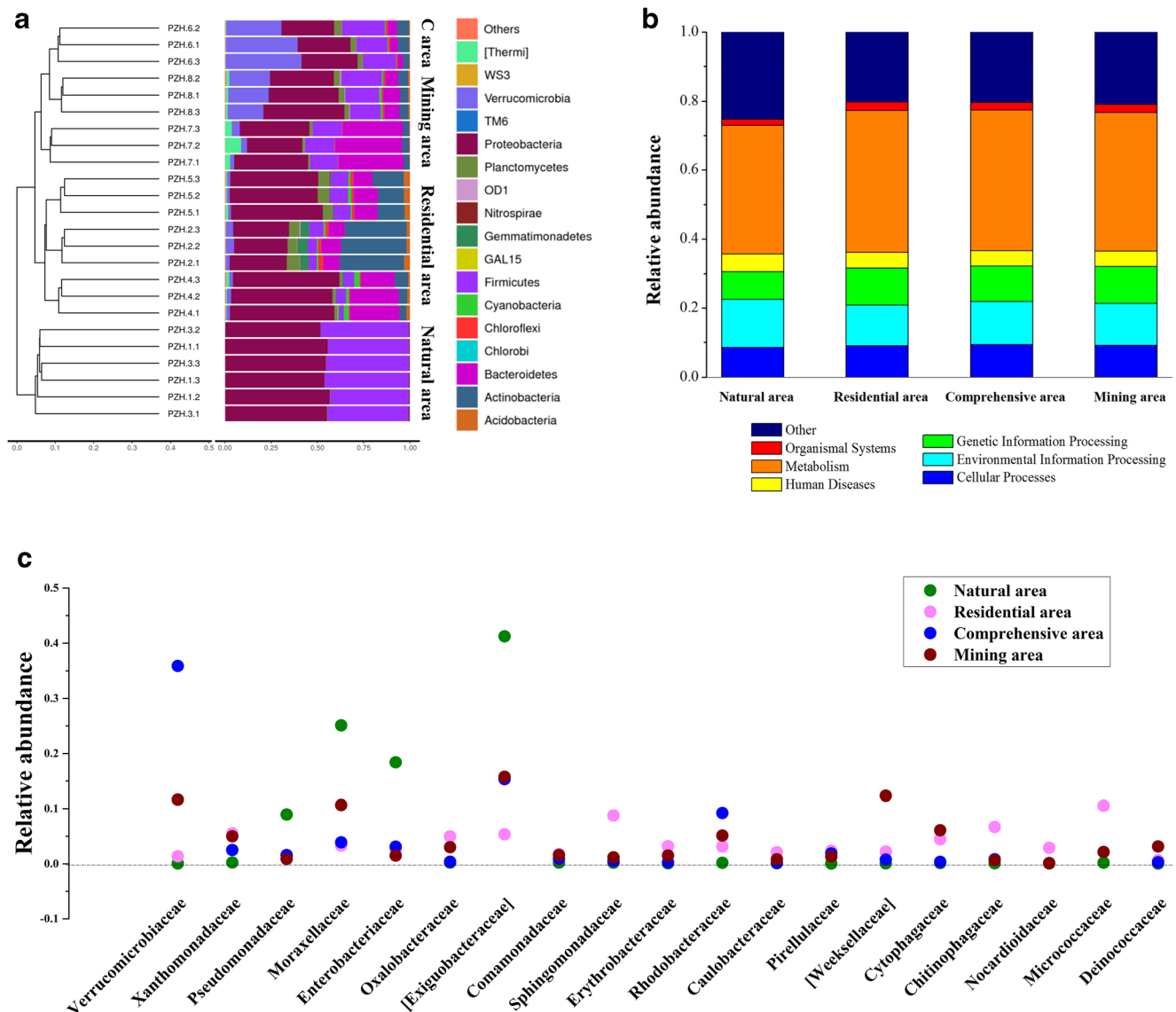


Fig. 2 UPGMA comparison of the bacterial communities based on the unweighted Unifrac metric and the bacterial community structures at the phylum level (a); bacterial functional groups (b); and relative bacterial family abundances (c) in different areas

Moraxellaceae, *Pseudomonadaceae*, and *Enterobacteriaceae* were peaked in pristine site. The *Pirellulaceae* (*Planctomycetes*), *Nocardiodaceae*, and *Micrococcaceae* (Actinobacteria) also had the highest relative abundances in the residential areas. The *Verrucomicrobiaceae* (of the *Verrucomicrobia*) were the most abundant in the comprehensive area. The *Deinococcaceae* ([*Thermi*]), *Cytophagaceae*, and *Chitinophagaceae* (*Bacteroidetes*) had the highest relative abundances in the mining area.

The major functional groups included related cellular processes, environmental information processing, genetic information processing, human diseases, metabolism, and organismal systems, and were investigated with a PICRUSt analysis (Fig. 2b). In all samples, the genes associated with metabolism were more abundant than those for all other genes. There were no significant differences among the areas in terms of functional groups.

Effect of environmental parameters on bacterial community

Bacterial community diversity was more highly correlated with physicochemical characteristics than with heavy metals. The Pearson correlation analysis indicated that both the Chao1 richness and Shannon diversity indices were significantly negatively correlated with TOC ($r = -0.672$, $P < 0.001$; $r = -0.733$, $P < 0.001$) and positively correlated with TP ($r = 0.682$, $P < 0.001$; $r = 0.694$, $P < 0.001$). No significant correlation was observed between bacterial diversity and heavy metals.

The influences of environmental parameters (including heavy metals and physicochemical characteristics) and spatial distance on the bacterial community structure were determined by CCA. The environmental parameters (89.21%) more strongly influenced the bacterial community than the geographic distance (19.63%) along the urban river (Fig. 4a). Among the environmental parameters, heavy metals and physicochemical characteristics had similar and strong influences on bacterial community composition and explained 69.31% and 70.69% of the variation, respectively (Fig. 4b). Fe and TOC significantly influenced changes in the bacterial community structure and accounted for 22.95% ($P = 0.001$) and 21.00% ($P = 0.001$) of the variation, respectively (Table 1). The MRT analysis was conducted to evaluate the impact of the environmental parameters on the bacterial community structure. As illustrated in Fig. 3b, the MRT was formed by three splits and four leaves according to the sediment environmental factors. The 24 samples were clustered by TOC into two groups. Then, the group with TOC < 15.51 g kg⁻¹ was further split by Fe concentration. Samples with Fe < 9.505 g kg⁻¹ were further separated by Ti concentration (2.055 g kg⁻¹). Therefore, Fe and TOC were the key factors influencing the bacterial community structure.

Moreover, the heavy metals with relatively greater potential ecological risk (Hg and Cd) explained a comparatively lower percentage of the variation in the bacterial communities than other metals. The relationship between the percentage explanation of bacterial community variation and heavy metal toxicity was also evaluated by a Pearson correlation analysis. The explanation percentage of bacterial community variation was significantly negatively correlated with potential ecological risk index ($r = -0.723$, $P < 0.05$).

Correlation network of sensitive bacteria to environmental variation

Seven metal-resistant bacterial genera reported in the literature were screened by Next Generation Sequencing. These included *Bacillus*, *Cupriavidus*, *Klebsiella*, *Ochrobactrum*, *Paenibacillus*, *Pseudomonas*, and *Ralstonia*. According to the results, 6 genera metal resistance bacteria were identified in the sediment of the Panzhihua region along the Jinsha River, and *Bacillus*, *Pseudomonas*, and *Ralstonia* occurred in every sample (Table S4 in the Supplemental Files). Metal-resistant bacteria constituted only 1% of the total bacterial community in all sediments, and the mining area harbored the lowest amount of these metal-resistant bacteria in the Panzhihua region. *Bacillus* was the dominant metal-resistant bacterial genus, accounting for 56.93% of the total population of metal-resistant bacteria. The relative abundance of *Bacillus* was higher in the pristine and comprehensive areas than the other areas. *Pseudomonas* and *Ralstonia* also comprised an important fraction of these heavy metal-resistant bacteria in the Panzhihua region. The relative abundance of *Pseudomonas* and *Ralstonia* was the highest in the pristine and residential areas, respectively. Moreover, no significant correlation was observed between the potential ecological risk index and metal-resistant bacterial population in the sediment along the Jinsha River.

Network analyses were conducted to determine the correlations between individual bacteria and environmental variables. It is suggested that metals (Fe and Ti) and nutrients (NH₄, TOC, and TP) were the main factors influencing bacterial community. Fe, Ti, NH₄, and TP were positively correlated with most of the bacteria in the sediment of the Panzhihua region, while TOC was negatively correlated with them (Fig. 4a). The abundances of *Rhodobacter*, *Hylemonella*, and *Dechloromonas* were directly correlated with these environmental variables. *Rhodobacter* was negatively correlated with TOC and positively correlated with Fe, Ti, and TP. *Hylemonella* was negatively correlated with TOC and positively correlated with Fe and TP. *Dechloromonas* was strongly negatively correlated with TOC and strongly positively correlated with Fe and Ti. Since that Fe had the strongest impacts on the bacterial community, it was solely used to run the network analysis with the bacterial community.

Table 1 The explanation percentages of environmental factors on bacterial community structures. The variation explains was calculated by CCA analysis and the *P* value was examined by Monte Carlo permutation (999 permutations)

| Parameters | | % Variation explains | <i>P</i> value | Parameters | | % Variation explains | <i>P</i> value |
|--------------|-----------|----------------------|----------------|---------------------------------|-----------------|----------------------|----------------|
| Heavy metals | Hg | 13.15 | 0.001 | Physicochemical characteristics | pH | 17.89 | 0.001 |
| | Cu | 12.25 | 0.005 | | EC | 9.99 | 0.021 |
| | Cd | 10.93 | 0.001 | | TOC | 21.00 | 0.001 |
| | Pb | 18.84 | 0.001 | | TN | 12.26 | 0.001 |
| | V | 16.38 | 0.001 | | NH ₄ | 15.95 | 0.001 |
| | Fe | 22.95 | 0.001 | | TP | 19.34 | 0.001 |
| | Ti | 21.41 | 0.001 | | AP | 9.01 | 0.002 |
| | Above all | 69.31 | 0.001 | | Above all | 70.69 | 0.001 |

Figure 4b shows that Fe was a hub node directly connected by 15 bacterial nodes. Fe and most of these bacterial nodes presented with the highest connectivity in the system. Therefore, Fe and its directly connected nodes played important roles in maintaining the network structure.

Discussion

Composition of the sediment bacterial community along the urban river in the mining area

The sediment bacterial community was detected to have significant differences in terms of diversity and taxonomic composition along the river (Ibekwe et al. 2012; Xia et al. 2013). However, it was largely unknown how bacterial communities shift along urban rivers in mining cities. In the present study,

relatively higher bacterial diversity was detected in the anthropogenically disturbed areas than the pristine areas. This might due to the oligotrophic bacteria are dominant in river sediment of pristine areas and these oligotrophic bacteria could not prevail in anthropogenically disturbed and eutrophic areas, while other bacterial taxa capable of using these resources, which leads to the prevalence of more diverse bacteria. This result is not in consistent with the previous studies reported that the lowest bacterial diversity was associated with the sediment in urban runoff (Wang et al. 2018). In our opinion, this observation may be explained by the fact that the region they investigated was located in a megalopolis with extremely high pollutant levels and only the bacteria with high tolerance of contamination can survive in these conditions (Chen et al. 2018). In contrast, the contaminant concentrations in Panzhihua City were comparatively moderate (Teng et al. 2011).

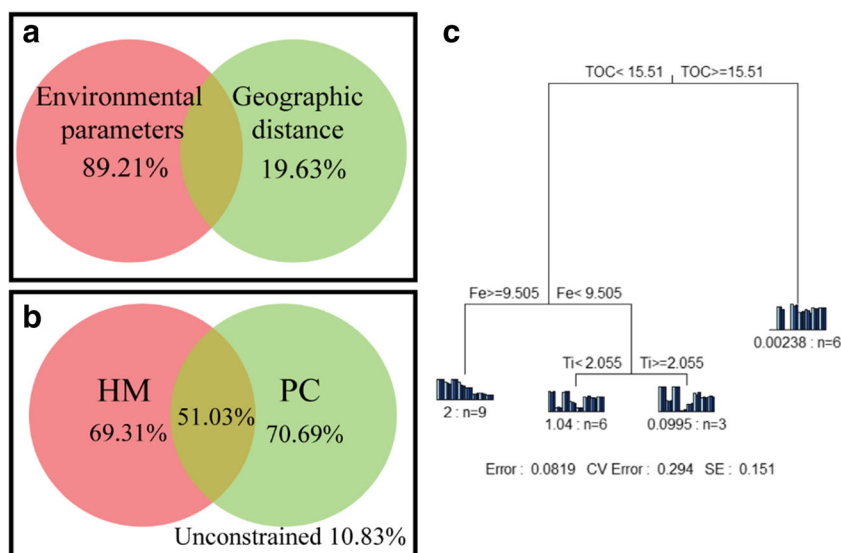


Fig. 3 Relative influences of determined factors on bacterial community structure according to VPA (a and b) and MRT (c). VPA is conducted to compare the effect of geographic distance vs. local environmental parameters (a) as well as physicochemical characteristics vs. heavy metals (b) based on CCA analysis. HM stands for heavy metals while

PC stands for physicochemical characteristics. Each split in MRT (c) is represented graphically as a branch in the tree. Bar plots show the multivariate means of different OTUs at each branch. CV Error stands for cross-validated relative error while SE stands for standard error

Moreover, the bacterial community structures in the various areas of the Panzhihua region were significantly different from each other based ANOSIM and UPGMA analysis, except that no significant difference was observed between comprehensive and mining areas. This result indicated that the sediment bacterial community structure in the urban river was significantly altered by the mining city. The sediment bacterial community in Panzhihua City is predominated by *Proteobacteria*, which agrees with previous studies conducted in several urban rivers (Gibbons et al. 2014; Xie et al. 2016). Both the pristine and residential areas harbored relatively high abundances of *Proteobacteria* but the compositions of their proteobacterial families were different. The proteobacterial families mostly known for dominating in oligotrophic environments (*Moraxellaceae* and *Enterobacteriaceae*) were found in high proportions in the pristine sites. On the other hand, the eutrophic proteobacterial families, including *Xanthomonadaceae*, *Oxalobacteraceae*, *Comamonadaceae*, *Sphingomonadaceae*, *Erythrobacteraceae*, *Rhodobacteraceae*, and *Caulobacteraceae* (Tang et al. 2017; Vetterli et al. 2015), were comparatively more abundant in the residential area. Therefore, these bacterial families may serve as urbanization bioindicators. With the increase of eutrophication, *Firmicutes* (mostly *Exiguobacteraceae*) could not thrive in other areas than pristine areas (Liu et al. 2018b). Consequently, other phyla could flourish in these regions along the river (Li et al. 2011). Families within the *Acidobacteria* prevail in a wide range of contaminated regions (Debarati et al. 2010; Sanchez-Peinado et al. 2010) whereas most *Bacteroidetes* families are resistant to organic pollutants and heavy metals (Chen et al. 2018; Obernosterer et al. 2011). Therefore, the dominance of *Acidobacteria* and *Bacteroidetes* in the residential and mining areas reflects the diversity of pollutant sources in residential areas and heavy metal contamination in mining areas, respectively. The higher abundance of *Verrucomicrobiaceae* (*Verrucomicrobia*) in the comprehensive areas may indicate that the *Verrucomicrobiaceae* remain competitive under multiple anthropogenic contaminants including nutrients and heavy metals. The fact that different bacterial families prevailed in each area may reflect distinct contaminant sources in each case. The residential area concentrated industrial contaminants and other chemicals from WWTP effluent and storm drain runoff from residential areas (Roberto et al. 2018). In contrast, the mining areas were impacted primarily by mining industry discharges. The comprehensive area was affected by all the mentioned pollution sources. Mining industry discharges have a relatively stronger impact on bacterial community structure in the comprehensive area because it clustered into the same group with the mining areas rather than the group including the residential areas.

Unlike bacterial diversity and community structure, bacterial community function was not significantly affected by the mining

city. Some bacterial groups may have served as seed banks in the environment and helped stabilize the ecosystem by facilitating functional redundancy (Loreau et al. 2001). As environmental conditions change, bacteria with relatively lower tolerance for the new condition revert to dormancy (Cardinale et al. 2012). Conversely, the functionally complementary groups proliferate and have minimal impact on ecological function (Jung et al. 2016). Therefore, the adaptation of local bacterial communities to alterations in environmental conditions may be independent of bacterial functional change (Bier et al. 2014; Fernandez-Gonzalez et al. 2016).

Vital factors influencing the sediment bacterial community along the urban river

There is no consensus on the vital factors influence sediment bacterial community composition. For this reason, the effects of multiple factors (physicochemical properties, heavy metals, and geographic location) were investigated on sediment bacterial communities. In the present study, the bacterial communities located at adjacent geographical positions were not clustered into the same group according to the UPGMA. For instance, the bacterial community impacted by resident in the rural area (PZH-2) had a structure more alike the distant sites in the residential quarter of the urban area (PZH-4 and PZH-5), rather than the adjacent pristine sites in the rural area (PZH-1 and PZH-3). On the other hand, environmental stressors accounted for a larger proportion of the bacterial community structure variation (89.21%) than the river length or the geographical position (21.06%). Therefore, the shift of the bacterial community structure along the river may follow environmental stressors rather than spatial distance in the local scale, which corroborates several previous studies (Logue and Lindström 2010; Staley et al. 2015; Winter et al. 2013).

Sediment bacterial communities may be directly or indirectly affected by environmental stressors (Chen et al. 2018; Li et al. 2017). In this study, the effects of metals and physicochemical characteristics on bacterial community structure were evaluated simultaneously. In general, both heavy metals and physicochemical characteristics explained high and nearly equal levels of the variation in bacterial community structure (69.31% and 70.69%, respectively). Metals with relatively low toxicity (Fe and Ti) and nutrients (TOC and TP) most strongly influenced bacterial community structure. Nutrients are essential environmental factors that significantly affect bacterial community structure because they provide necessary materials for the growth of river microorganisms (Davidson et al. 2007). In this study, TOC was the nutrient with the most powerful influence on bacterial community structure. Large amounts of carbon are discharged from terrestrial to aquatic ecosystems (Jones et al. 2010). In the sediment, carbon is the main energy source for heterotrophic bacteria (Kritzberg et al. 2004). Increases in TOC are often correlated with rises in cellulolytic and chitinolytic activities and may

further alter bacterial metabolism (Larson et al. 2002; Phillips et al. 2002). High inputs of TOC in the sediment may favor the proliferation of microorganisms which degrade carbon polymers (He et al. 2012). Under these conditions, the heterotrophic bacteria may effectively compete with autotrophs like *Chloroflexus* and *cyanobacteria* (Celine et al. 2010). The present study also confirmed that TP could also impact bacterial community structure. Phosphorus plays a major role in the riverine ecosystem (Miettinen et al. 1997), and serves as an essential nutrient for bacterial growth. The lack of phosphorus may be a principal factor limiting microbial productivity (Hu et al. 2014). Therefore, TOC and TP are essential nutrients influencing bacterial community structure.

Heavy metals are always considered to have a negative effect on the bacterial community since they are toxic and inactivate the sulfhydryl groups in proteins (Valls and De 2003). However, many metals are necessary for the bacterial community especially at the proper concentrations. In the present study, the percentage explanation of the variation in bacterial community structure was significantly negatively correlated with heavy metal toxicity. The metal contamination level is moderate in the Panzhihua region. At low concentrations, metals may function as essential nutrients rather than hazardous materials, which may have a more positive effect on bacterial communities (Jingxin et al. 2013; Liu et al. 2018a). It was determined that Fe was the most essential metal for the bacterial communities. Fe is widely distributed in mining cities because the mining industry discharges harbor very high Fe^{2+} and Fe^{3+} concentrations (Yang et al. 2007). Fe^{2+} is one of the key energy sources for bacteria and many bacterial species are capable of making use of ferrous iron (Yang et al. 2007). On the other hand, Fe^{3+} addition increases bacterial diversity and alters the bacterial community by changing the electric field. This effect enriches bacteria with Fe^{3+} -reducing capabilities, including the *Comamonadaceae* and the *Rhodocateraceae* (Jingxin et al. 2013). Low concentrations of Fe are also necessary for bacteria to catalyze enzymatic reactions and maintain protein structures, which may enhance the production of extracellular polymeric substances (EPS) (Stacy et al. 2016). Cytoactivity may also increase with Fe level (Jingxin et al. 2013). Therefore, the present study confirmed that Fe is a vital factor influencing bacterial community structure.

Networking analysis demonstrated that there are rules to bacterial community assembly which reflect ecological processes like cooperation and competition among different bacterial genera under environmental stress (Fuhrman 2009). Since Fe explained the highest percentage of bacterial community variation, it was selected as the main environmental stressor for the network analysis of bacterial species. Network analysis described a strong interaction between the bacteria and Fe stress. This association may be explained by certain deterministic processes like habitat filtering, reduction in trait dispersal, and common ecological tolerances (Cornwell et al. 2006). The high interaction indicated that the microbial

system is stable and resistant to environmental alterations, particularly at the functional level (Ding et al. 2015).

Response patterns of individual bacteria to contaminants

Metal-resistant bacteria in the sediment can indicate environmental perturbations (Xie et al. 2016). Bacteria with multiple metal resistances like *Bacillus*, *Cupriavidus*, *Paenibacillus*, and *Pseudomonas* were investigated in previous laboratory studies based on culture-dependent methods (Mejias Carpio et al. 2018). Nevertheless, their relative abundances were low in the present field study. Bacteria with multiple metal resistances only gain a competitive advantage for resource acquisition under extreme environmental conditions (Zhang et al. 2015). These metal-resistant bacteria in the river sediment may be considered alien species discharged from the mining industry, and the competition for vital resources and direct antagonism may lead to the suppression of these metal-resistant bacteria in the river sediment (Moynihan et al. 2015). Moreover, heavy metal contamination levels and the abundances of metal-resistant bacteria were not significantly correlated. Therefore, this type of bacteria derived from culture-dependent research may not be feasible as indicators of heavy metal contamination in the river. For this reason, effective river sediment contaminant indicators are still required further investigation.

Several bacterial genera were positively correlated with the contaminant levels according to the network analysis. Therefore, these bacteria could tolerate contaminants or actually utilize them as energy sources (Xie et al. 2016). These bacterial genera could be applied as contamination indicators. For instance, *Rhodobacter* has been reported to resist certain metals (Mishra and Malik 2013). In our study, *Rhodobacter* abundance was negatively correlated with Fe, Ti, and TP and positively correlated with TOC. For this reason, *Rhodobacter* may be a suitable candidate to monitor these substances. *Dechloromonas* are active denitrifiers in the river sediments (Price et al. 2017; Wang et al. 2017) and could also be contaminant indicators because their abundance was negatively correlated with Fe and Ti and positively correlated with TOC. *Hylemonella* can indicate the levels of Fe, Ti, and TOC, and its abundance was negatively correlated with TOC and positively correlated with Fe and TP.

There is a potential limitation of this study that needs further discussion. The quantitative relationships between sensitive bacteria and contaminant types and levels described in this study may also be influenced by other environmental factors unique to the Panzhihua region. Therefore, the indicator potential of these bacteria should also be investigated under other environmental conditions (Xie et al. 2016). Nevertheless, this study reveals the potential contaminant stressors affecting the river sediment bacterial community and identifies possible bacterial

indicators of environmental contamination, and our results show that ecogenomics could be effectively applied towards the improvement of river management (Gibson et al. 2015).

Conclusions

The present study showed that anthropogenic activities in both urban and mining areas had significant impacts on bacterial community structure and diversity but not on bacterial ecological function. Distinct bacterial families associated with municipal pollution, such as the *Comamonadaceae* and *Sphingomonadaceae*, and metal contamination, such as the *Rhodobacteraceae*, were more abundant in the urban and mining areas, respectively, than the other areas. Relative to dispersal dynamics, environmentally induced species sorting may be substantially more important in shaping bacterial community structure. Heavy metal concentrations and sedimentary physicochemical properties both significantly influenced bacterial community structure. In contrast, since the metal contamination levels were only moderate in the mining city, metals were more likely to be used by the bacteria as essential nutrients and less likely to be toxic to the bacterial community. Moreover, bacteria with multiple metal resistances identified by culture-dependent research may be unable to indicate heavy metals contamination in field studies. Therefore, several sensitive bacterial genera like *Rhodobacter*, *Hylemonella*, and *Dechloromonas* were identified as potential bioindicators for iron, titanium, phosphorus, and organic carbon in the riverine ecosystem. Overall, these results reveal that urbanization and mineral resource utilization jointly affect urban river sediment bacterial communities and provide a useful tool to monitor sediment contaminant levels.

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Data availability The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

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Consent for publication Not applicable

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