

Long-term effect of water diversion and CSOs on the remediation of heavy metals and microbial community in river sediments

Jun Wu, Zuxin Xu, Huaizheng Li, Peng Li, Mei Wang, Lijun Xiong and Jin Zhang

ABSTRACT

Untreated combined sewer overflows (CSOs) cause serious water pollution problems. In this study, the effects of CSO-induced heavy metals and the remediation practice of installation of a long-term water diversion (LTWD) on the microbial environment in river sediments were analyzed in an inland river. The Zn, Cd, Cr, and Cu contents in sediments and water were analyzed. DNA extraction and polymerase chain reaction analysis were conducted based on the Illumina MiSeq platform. The results showed that CSOs have a significant adverse impact on the diversity of microbial populations in river sediments. The LTWD is helpful in improving the richness of microorganisms and the proportion of Gram –ves, but it is challenging to reduce the accumulation of heavy metals in the sediment. The correlation analysis shows a strong relationship between some metabolic pathways and Zn and Cd accumulation in river sediments. Some detoxification compound metabolisms are also promoted at these sites. Thus, chronic exposure to environmental heavy metals from CSOs decreases the river microbial community, and further affects the ecological environment of the river. Therefore, without eliminating CSOs or reducing overflow frequency, it is difficult to alleviate the accumulation of heavy metals in river sediments and improve river ecology via water diversion alone.

Key words | bacterial community composition, combined sewer overflow, heavy metals, microbial

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INTRODUCTION

The discharge of a mixture of sewage and stormwater through combined sewer overflows (CSOs) into receiving waters is inevitable because of the capacity limitations of wastewater treatment plants during wet weather periods (Kaeseberg *et al.* 2018a, 2018b; Zhang *et al.* 2019). Therefore, untreated CSOs cause serious water pollution problems and are a major concern for the microbiological quality of the receiving waters (Xu *et al.* 2018).

According to many previous research studies (Houhou *et al.* 2009; Gasperi *et al.* 2010; Zgheib *et al.* 2012), the most prominent pollutants in CSOs are organic matter and heavy metals. Many studies set organic matter content or chemical oxygen demand as the reference substance for evaluating CSO pollution. In addition, some reports (Lau & Stenstrom 2005; Li *et al.* 2012) regarding heavy metals

have been limited to the content of heavy metals, while the source characteristics and their correlations between other factors were not analysed in detail. In fact, because of the relatively high stability of heavy metals, their accumulation in rivers is bound to have an impact on the rivers' ecological conditions (Wang *et al.* 2019). Ancion *et al.* (2013) have found that microbial communities in river channels are sensitive to heavy metals. In other studies, Song (2015) found that actinomycetes were the species most sensitive to heavy metals. Xu *et al.* (2016) found that scleroderma (including *Bacillus* and *Clostridium*) preferred areas with higher concentrations of heavy metals in estuarine sediments. Therefore, the microbial community of rivers is easily destroyed by the accumulation of heavy metals in river sediments. How to

mitigate or solve the impact of this adverse factor is a focus of researchers.

Urban environmental infrastructure construction (such as rainwater pipelines, sewage pipelines, urban sewage treatment plants, etc.) lags far behind the urbanisation process in most developing countries, resulting in deterioration of urban inland river water quality, which is mostly caused by overflow pollution. Because water diversion is a relatively effective ecological remediation practice for a CSO pollutant area, it is often favoured by decision makers, resulting in the acceptance of both diversions and CSOs in rivers (Yu *et al.* 2018).

Although ecological water diversion is capable of enhancing the self-purification capacity of river water, it is not clear whether it can play a significant role in the reduction of accumulated pollutants, such as heavy metals. In addition, to our knowledge, it has not been reported whether a water diversion can improve and promote the restoration of the microbial environment of river sediments under long-term CSO stress. In this study, the microbial community characteristics of river sediments were analysed based on the Illumina MiSeq sequencing platform to assess the impact of water diversion as a CSO remediation practice for the microbial biota in the adjacent aquatic environment.

Accordingly, this study investigates through field sampling (1) whether long-term water diversion (LTWD) can reduce the heavy metal pollution in river sediments under the influence of CSOs and (2) whether LTWD can improve the microbial community status of river sediments under the stress of CSOs.

MATERIALS AND METHODS

Study area

Chaohu City, adjacent to Chaohu Lake, is in Anhui Province, southeast China. The Huancheng River is a tributary of Chaohu Lake. Because the West Dam and East Dam were constructed to separate the inland water body from the external water system, the inland river is isolated, resulting in reduced self-purification and slow flow. Under normal conditions of dry days when the gates are open, the average retention time from upstream to downstream is 44.4 h on dry days without water diversion. This river is an inland river in the center of the city. There is no industrial wastewater discharged into the drainage system around the river. Domestic wastewater is centrally transported to the wastewater treatment plant (WWTP)

through three combined pumping stations, DG, CC and JK. The main sewage pipe for intercepting dry-day wastewater is constructed along the river, and the sewage is intercepted to the WWTP. The WWTP is located in the downstream area of the basin at a straight line distance of more than 10 km. Therefore, CSOs are the main contributors to the river pollution in our study area.

Water diversion program

According to the Clean Water Diversion Program (starting in June 2017), water is drawn from Chaohu Lake to the Huancheng River through the Xi'an Bridge Sluice to improve the inland water quality. This is a water supplement programme. The water is diverted from Xi'an Bridge Gate to Xihuancheng River. After flowing through Donghuancheng River, it enters the Xier pool through the box culvert of Chaohu Road, then enters Lujia River, and finally enters the lower reaches of the river. To guarantee the water level of the Huancheng River is lower than flood stage, the optimal water diversion is operated under a flow of 5 m³/s for 48 h/72 h according to the achievements of the Clean Water Diversion Programs.

Sampling method

The sampling collection was performed during June 2017 and June 2018, which is the beginning of the rainy season. The sampling sites for the river water and sediments are along the Huancheng River (Figure S1, available with the online version of this paper), the average water depth of the river at sampling points is 1.6 meters. Sediments were collected at a depth of 5 cm, and were collected using a hand dredge. This dredge is recommended for sampling sediments in calm, shallow streams and rivers. The dredge is equipped with perforated meshes on its sides to facilitate the removal of excess water. The water sampler was put into the river to 50 cm depth by taxiing the boat to each sampling point. When the river water filled the sampler, the sampler was lifted up and the collected river water was transferred to a polyethylene sampling bottle, then the polyethylene bottle was put into a storage box filled with ice. There were three parallel samples at each sampling point for the water and sediment. All the collected samples were stored in polypropylene containers with ice until delivery to the laboratory. The three parallel samples at each sampling point were mixed before analysis. A consolidation period in the laboratory was established to ensure that the overlying water did not influence the samples (Xu *et al.* 2017). The

overlying water was removed as the final step to prepare the sediments for the subsequent tests.

Chemical analysis

The chemical analyses were conducted by the State Key Laboratory of Pollution Control and Resource Reuse in Shanghai. The Cr, Cu, Zn, and Cd contents of 54 water and sediment samples were tested. All samples were analysed using inductively-coupled plasma optical emission spectroscopy. Sediment concentrations were expressed as the weight of dry sediment (mg/kg or $\mu\text{g}/\text{kg}$) according to the measured concentrations.

Samples were processed in triplicate throughout the analyses and the reported results were the averages of these values. Laboratory quality assurance/quality control was maintained according to the previous study (Xu *et al.* 2018). The recoveries of Cr, Cu, Zn, and Cd ranged from 65% to 89%, 75% to 118%, 89% to 125%, and 72% to 110%, respectively. The detection limit was 5.0 $\mu\text{g}/\text{L}$ for each metal.

Microbial diversity analysis

Microbial DNA was extracted from the sediment samples. The V4 to V5 region of the bacterial 16S ribosomal RNA gene was amplified by polymerase chain reaction. Illumina MiSeq sequencing was employed in this study. Raw fast files were demultiplexed and quality-filtered using QIIME (version 1.9.1) (Zhang *et al.* 2018; Chen *et al.* 2019). In addition, the Shannon and Simpson's indices were calculated for comparing species diversity and evenness as previously described (Schloss *et al.* 2009). The Phylogenetic Investigation of Communities by construction of Unobserved States (PICRUSt) software package was used to infer the metagenomic content of each sample (Langille *et al.* 2013). Detailed steps and instructions for the process have been previously published (Langille *et al.* 2013; Wang *et al.* 2018; Xu *et al.* 2018).

Data analysis

To generate a synthetic metagenome, the observed 16S rDNA sequences were clustered into a collection of OTUs using the pick closed reference otus.py script in QIIME. The resultant biom-formatted OTU table was first normalised with respect to inferred 16S rRNA gene copy numbers and then used to predict metagenomic functional content based on PICRUSt. This computational approach exploits the relationship between phylogeny and function

by combining 16S data with a database of reference genomes (Greengenes) to predict the presence of gene families. Functional predictions were exported as KEGG orthologs. Phylogenetic trees were generated using the neighbour-joining method in MEGA version 5.0 (Tamura *et al.* 2011).

RESULTS

Characterising heavy metal pollution

Samples were collected at nine locations from the upstream to the downstream of the river. As shown in Figure 1, the average Cr and Cu concentrations in the water samples are similar in the two inter-annual monitoring periods. The Cu concentration gradually increases from the upstream to the downstream. The Zn and Cd concentrations in the water samples are below the detection limit.

From the upstream to the downstream of the Huan-cheng River, three combined pumping stations, DG, CC, and JK, and two combined sewer outlets, JF and TJ, are sequentially distributed (Figure S1). The concentrations of the four heavy metals in sediment samples significantly increase after the DG pumping station along the river, indicating that external pollutants have entered the channel at least in the region from DG to TJ (Figure 1).

Since June 2017, the upstream lake (Chaohu Lake) has been continuously regulating its water supply to the Huan-cheng River. As shown in Figure 1, the difference in heavy metal content in the sediment is not significant between the two inter-annual monitoring periods, which demonstrates the rather stable yearly flux of heavy metals to the river. The sampling point C1 is at the lake and near the inlet of the river, and the heavy metal concentration of the water at this point is relatively low (Figure 1). After the LTWD, the sediment content at the bottom of the river does not obviously decrease (average concentrations before and after the LTWD, Zn: 213 mg/g and 207 mg/g, Cu: 59 mg/g and 64 mg/g, Cr: 132 mg/g and 150 mg/g, and Cd: 0.56 mg/g and 0.50 mg/g as shown in Figure 1). This indicates that heavy metals in river sediments have stable accumulation characteristics, and the impact of over-flow pollution can not be ignored.

Richness and diversity of the bacterial community

Illumina MiSeq sequencing of 16S rRNA genes at the whole community level provides a nearly unbiased profiling

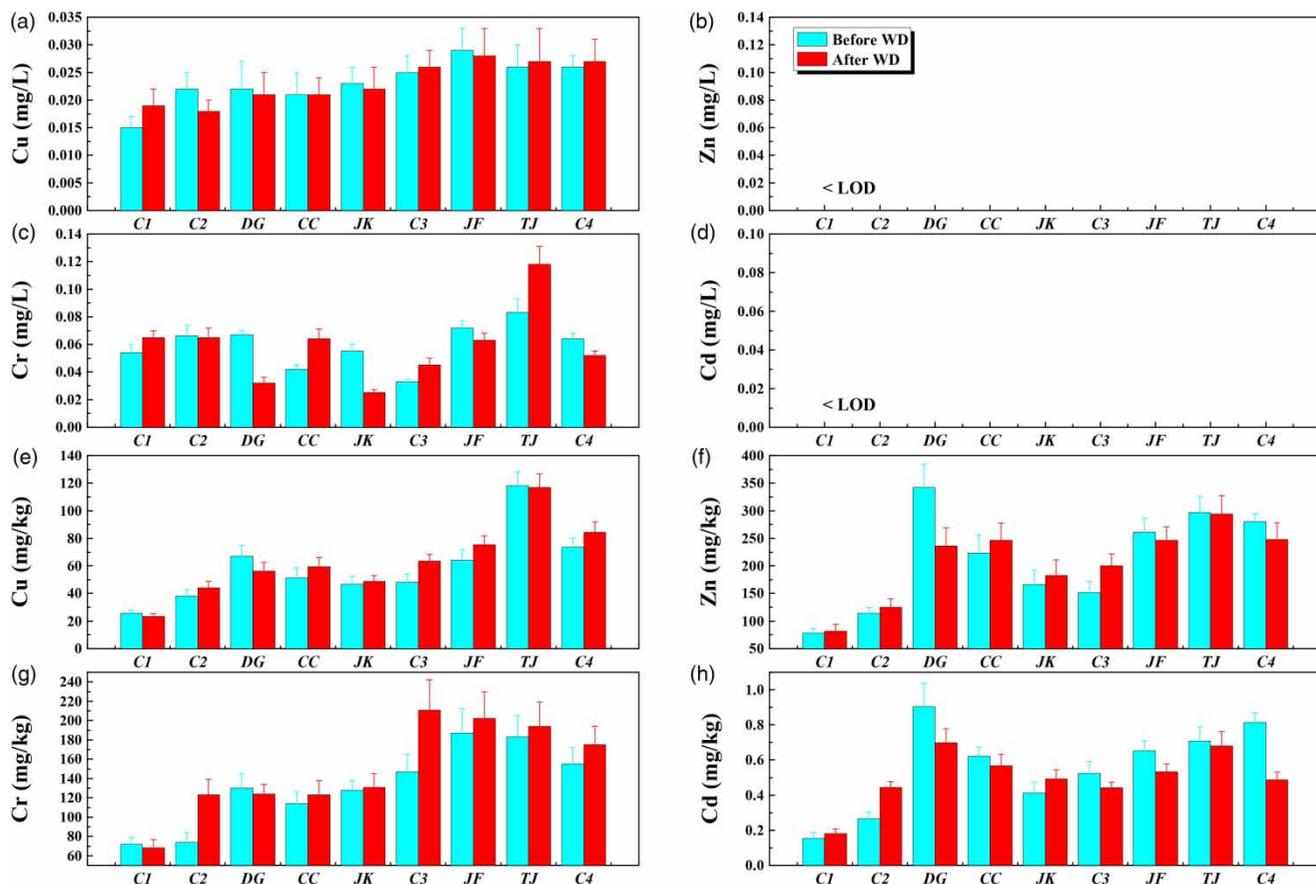


Figure 1 | The concentration of heavy metals in waters (a)–(d) and river sediments (e)–(h) at the nine sites before and after long-term water division. *LOD: limit of detection.

strategy for characterising the changes in diversity and relative proportions of microbial community in the flowing river. The Shannon diversity indexes suggest that the diversity varied along the flowing river from 6.22 to 7.09, with the lowest diversity at DG before the LTWD. The Shannon diversity index of the sediment bacterial communities after the LTWD is higher than that before the LTWD at each sampling site except TJ and JF (Figure S2, available with the online version of this paper).

Bacterial community structure and composition

Comparing the relative abundances of the specific families of bacteria across all sampling sites, the most abundant sequences are classified as a member of the *Cyanobacteria*, comprising ~24.0% of the sequencing reads, followed by *Anaerolineae* (~22.7%) (*Anaerolineaceae* in Figure S3(b)), *Betaproteobacteria* (~21.5%) (mainly *Rhodocyclaceae* (60.7%), *Hydrogenophilaceae*, *Comamonadaceae*, o_SC-I-84, and *Alcaligenaceae* as shown in Figure S5(a)), *Deltaproteobacteria* (~19.4%) (mainly *Syntrophaceae* (59.6%),

Bacteriovoracaceae, *Syntrophorhabdaceae*, *Syntrophobacteraceae*, o_Sva0485, *Archangiaceae*, *Desulfobacter*, and *Geobacteraceae* as shown in Figure S5(b)) and *Clostridia* (~10.8%) (mainly *Clostridiaceae_1* (35.6%), *Peptostreptococcaceae*, and *Ruminococcaceae* as shown in Figure S3(i)), etc.

Communities in the river sediments differ among the sites affected by CSOs (DG, CC, JK, TJ, and JF) where the higher prevalence of families are in the class *Acidobacteria*, *Bacilli*, *Bacteroidetes_vadinHA17*, *Bacteroidia*, *Caldisericia*, *Clostridia*, *Cyanobacteria*, *Dehalococcoidia*, *SB-5*, *Spirochaetes*, *Synergistia*, and *Deltaproteobacteria* (Figures S3–S5, available online). In contrast, the reference sites (C1, C2, C3, and C4) are dominated by families from the class *Belgica2005-10-ZG-3*, *Nitrospira*, *SBR2076*, *p_Chloroflexi*, and *Gammaproteobacteria* (Figures S3–S5).

The influence of the LTWD is also apparent at all taxonomic levels (Figure 2(a)). The plots represent a dbRDA ordination based upon the Bray-Curtis distance. Before and after the LTWD, the samples are distributed on the upper and lower sides of the X axis, and the DG site is the least affected by CAP2. According to Figure 2(b), the

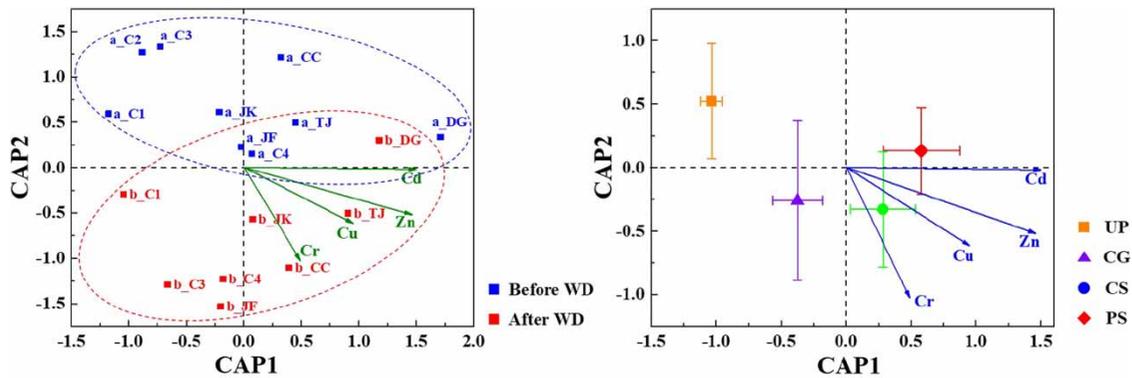


Figure 2 | Distance-based Redundancy Analysis (db-RDA) plot showing the influence of Zn, Cu, Cd and Cr to the microbial community structure. Each data point is the mean, with error bars representing the standard error. Arrows indicate the direction of the environmental gradient, and their lengths are proportional to their correlations with the ordination. (UP: Upstream sampling sites; CG: Control group sampling sites; CS: Combined sewer sampling sites; PS: Pumping station sampling sites).

CAP1 factor is significantly correlated with the Cd and Zn contents in the river sediments. The most significant impact is in the combined pumping station outlet area, followed by the combined sewer outlet area, the middle and lower reaches of the control section, and finally the upstream diversion area. The correlation between CAP1 and Cu and Cr content in the sediments is not significant. According to the above analysis, CAP1 and CAP2 may reflect the impact of CSOs and the difference between batches of sample collection, respectively.

Pearson correlations between the bacterial abundances of the family level and heavy metal contents were calculated, and significant correlations are apparent with specific family groups (Table 1). Zn and Cd have similar significant positive or negative effects on 20 groups of microorganisms at the family level. Among them, the significant positive effects appear on *Acidobacteria* (*norank_o_Gaiellales* and *Propionibacteriaceae*), *Bacilli*, *Bacteroidetes_vadinHA17*, *Caldisericia*, *Clostridia*, *Delta-proteobacteria*, *Spirochaetes*, *Synergistia*, and *unclassified_p_Chloroflexi*. However, significant adverse effects appear on *Acidobacteria* (*norank_c_Acidobacteria*), *Belgica2005-10-ZG-3*, *Betaproteobacteria*, *Gammaproteobacteria*, and *Nitrospira*. In addition, Cu and Cr both have a significant adverse effect on *Belgica2005-10-ZG-3* and *Nitrospira*.

In addition, the proportion of Gram-negative (–ves) bacteria before the LTWD at each site descends in the following order: C3 (87.4%) > C1 (86.5%) > JK (85.0%) > C4 (84.7%) > C2 (84.0%) > JF (82.9%) > CC (78.6%) > TJ (75.4%) > DG (67.3%). However, after the LTWD, it descends in the following order: C1 (88.9%) > C3 (86.0%) > JF (85.5%) > CC (83.6%) > C4 (82.1%) > JK (80.5%) > TJ (75.9%) > DG (71.5%) (Figure S6, available online). The proportion of Gram-positive (+ves) bacteria before the

LTWD at each site descends in the following order: DG (22.5%) > TJ (17.3%) > CC (13.9%) > JF (10.4%) > JK (9.9%) > C2 (9.2%) > C4 (7.9%) > C3 (7.4%) > C1 (7.4%). While after the LTWD, it descends in the following order: DG (17.2%) > TJ (16.3%) > JK (11.5%) > C4 (10.4%) > JF (10.3%) > CC (8.4%) > C3 (5.2%) > C1 (4.7%) as shown in Figure S6. Nasrazadani *et al.* (2011) reported that some Gram +ves bacteria with high contaminant resistance are commonly found in heavy-metal-polluted sediments, to a greater extent than Gram –ves. In our findings, an obvious difference is observed between the river sediments affected by the CSO discharge and the control samples; the proportion of Gram +ves surges in the river sediments affected by the CSO discharge. Furthermore, the proportion of Gram +ves is higher before the LTWD on average than that after the LTWD. The aforementioned results show a certain degree of ecological improvement facilitated by the LTWD of the river sediments.

Prediction of bacterial function

PICRUSt analysis indicates that the major functional gene families are related to cellular, metabolism, human diseases, environmental information processing, genetic information processing, and organismal systems (Figure 3). The annotations are classified into 310 functional KEGG or orthology pathways in nine samples at level 3, which include any type of biological reaction and regulation of gene expression. Although there are some differences in bacterial communities among different sampling sites, analysis of the community KEGG profiles show that there is no significant difference in functional groups regardless of the functional resolution (e.g. levels 1 and 2 in Figure 3) between sampling locations. However, the majority (~50.5%) of functional

Table 1 | Pearson correlations between bacterial abundances of family level and heavy metal contents by sampling location

Class	Family	Zn	Cu	Cr	Cd	
Acidobacteria	norank_c_Acidobacteria	– 0.523*	0.022	0.008	– 0.673**	
	Holophagaceae	0.315	0.269	0.312	0.227	
	norank_o_Gaiellales	0.522*	0.26	0.241	0.415	
	Mycobacteriaceae	0.07	0.252	0.093	0.046	
	Propionibacteriaceae	0.649**	0.021	0.121	0.671**	
	norank_o_PeM15	–0.299	–0.057	–0.175	–0.377	
Anaerolineae	Anaerolineaceae	–0.004	–0.173	–0.044	–0.077	
Bacilli	Carnobacteriaceae	0.527*	0.092	0.107	0.556*	
Bacteroidetes_vadinHA17	norank_c_Bacteroidetes_vadinHA17	0.654**	0.068	0.19	0.698**	
Bacteroidia	Draconibacteriaceae	0.094	0.3	0.311	–0.02	
Belgica2005-10-ZG-3	norank_c_Belgica2005-10-ZG-3	– 0.856**	– 0.501*	– 0.554*	– 0.861**	
Betaproteobacteria	Rhodocyclaceae	–0.256	0.265	0.213	–0.377	
	Hydrogenophilaceae	–0.4	–0.267	–0.171	– 0.521*	
	Comamonadaceae	– 0.519*	0.046	–0.128	–0.431	
	norank_o_SC-I-84	– 0.642**	–0.084	–0.085	– 0.762**	
	Alcaligenaceae	– 0.775**	–0.324	–0.312	– 0.821**	
	Caldisericia	Caldiseriaceae	0.706**	0.062	0.168	0.774**
Chloroflexia	unclassified_o_Chloroflexales	0.106	–0.178	–0.109	0.038	
Clostridia	Clostridiaceae_1	0.700**	0.088	0.196	0.710**	
	Peptostreptococcaceae	0.746**	0.186	0.281	0.738**	
	Ruminococcaceae	0.427	0.141	0.169	0.422	
	Cyanobacteria	norank_c_Cyanobacteria	–0.017	0.105	–0.107	0.149
Dehalococcoidia	norank_o_GIF9	–0.101	0.057	0.132	–0.168	
	norank_o_FS117-23B-02	0.302	0.129	0.143	0.34	
	Deltaproteobacteria	Syntrophaceae	0.561*	0.038	0.144	0.621**
	norank_o_Sva0485	–0.368	0.034	–0.006	–0.409	
	Geobacteraceae	–0.372	0.254	0.176	–0.412	
	Syntrophorhabdaceae	0.629**	0.357	0.449	0.568*	
	Syntrophobacteraceae	–0.118	–0.157	–0.132	–0.128	
	Desulfobacteraceae	–0.315	0.264	0.113	–0.363	
	Bacteriovoracaceae	0.533*	0.017	0.141	0.581*	
	Archangiaceae	–0.317	0.085	0.081	–0.407	
	Flavobacteriia	Flavobacteriaceae	–0.411	0.032	–0.147	–0.266
	Gammaproteobacteria	Xanthomonadales_Incertae_Sedis	–0.413	–0.069	–0.051	– 0.595*
norank_o_SZB30		–0.433	–0.282	–0.242	– 0.565*	
Xanthomonadaceae		– 0.6*	–0.111	–0.249	–0.464	
Nitrospira	norank_c_Nitrospira	– 0.805**	– 0.565**	– 0.532*	– 0.821**	
norank_p_Aminicenantes	norank_p_Aminicenantes	0.078	–0.28	–0.229	0.171	
norank_p_Latescibacteria	norank_p_Latescibacteria	–0.143	–0.408	–0.214	–0.231	
OPB35_soil_group	norank_c_OPB35_soil_group	–0.255	–0.244	–0.353	–0.15	
SB-5	norank_c_SB-5	0.321	0.232	0.334	0.245	
SBR2076	norank_c_SBR2076	–0.436	–0.081	–0.006	–0.479	
SJA-15	norank_c_SJA-15	–0.032	–0.077	–0.058	–0.081	
Sphingobacteriia	Lentimicrobiaceae	–0.374	0.054	–0.048	–0.341	
Spirochaetes	Leptospiraceae	0.606**	0.357	0.438	0.509*	
	Spirochaetaceae	0.029	0.068	0.139	–0.047	
Synergistia	Synergistaceae	0.581*	–0.109	-	0.633**	
unclassified_k_norank	unclassified_k_norank	0.443	–0.165	0.002	0.437	
unclassified_p_Chloroflexi	unclassified_p_Chloroflexi	– 0.494*	–0.247	–0.156	– 0.570*	

Bold representation of significance correlation, * indicates that the *p* value is less than 0.05, ** indicates that the *p* value is less than 0.01.

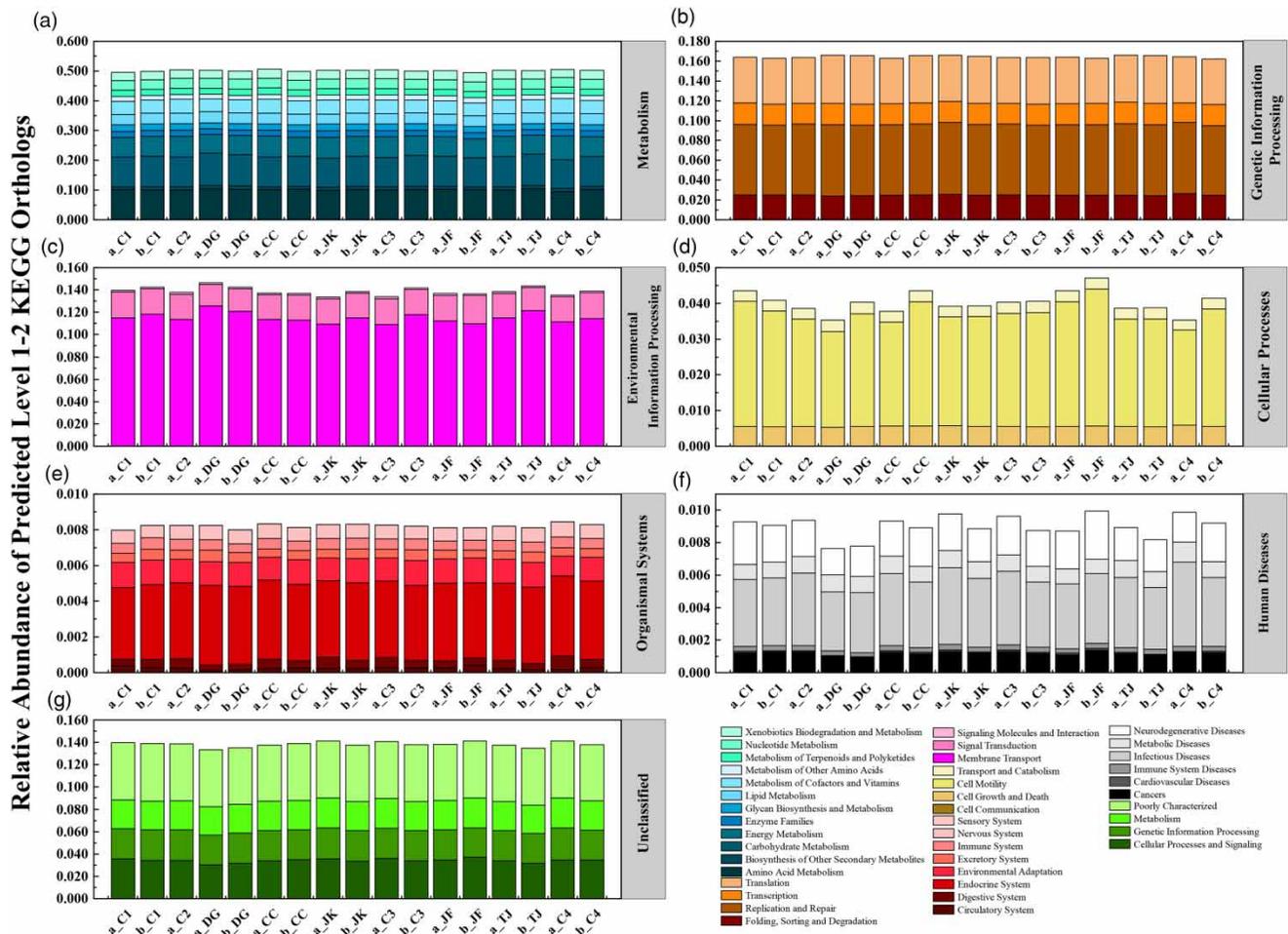


Figure 3 | Sampling location and water division effects on predicted functional groups of OTUs in river sediment bacteria based on KEGG database.

genes are related to metabolic functions, followed by genetic information processing (~16.6%). The most abundant pathway observed in the nine samples is ko02010, which can be assigned to ABC transporters. The ABC transporters are able to transport inorganic ions such as metal ions outside of cells (ter Beek *et al.* 2014).

In addition, we have defined the dominant pathways as those with a relative abundance greater than 1.00% of the total observed pathways. Then, we obtained 27 dominant pathways in all samples (Table S3, available online), where some detoxification compound metabolisms (Purine metabolism; Arginine, and proline metabolism) can combine with Cd and decrease its biological toxicity (Feng *et al.* 2018). Pearson correlations between the predicted functional groups of the OTUs based on the KEGG database and heavy metal content showed that Cd has a positive effect on the two detoxification compound metabolisms (Table S3).

The relative abundance ratio of the selected samples (DG, CC, JK, JF, and TJ) to the control samples (C1, C2,

C3, and C4) in 27 dominant pathways were calculated with the average value of each sample group (the S/C ratio). An S/C ratio >1 means that the relative abundance of a dominant pathway in the selected sample is higher than that of the control sample; in contrast, the relative abundance of the control sample is higher. Among the 27 dominant pathways, the values of 17 pathways are greater than 1, as shown in Figure S7 (available online). This result reflects the difference between the samples affected by the CSOs and the control sites. In addition, among the 17 pathways, Cd and Zn have significant positive effects on 'Pyrimidine metabolism' and 'Ribosomes'. Zn has a significant positive effect on 'DNA repair and recombination proteins'. Cd has a significant negative effect on 'Methane metabolism' (Table S3). Cd and Zn have significant negative effects on 'Chaperones and folding catalysts' and 'Oxidative phosphorylation' with a S/C ratio less than 1. Cd also has a significant negative impact on the 'Secretion system' and 'Two-component system' (Table S3). The aforementioned

conclusions indicate that Cd and Zn in the CSOs have significant positive and negative effects on the functions of group A ('Pyrimidine metabolism', 'Ribosomes', and 'DNA repair and recombination proteins') and group B ('Chaperones and folding catalysts', 'Oxidative phosphorylation', 'Secretion system', and 'Two-component system'), respectively. 'Methane metabolism' is inevitably influenced by other more sensitive factors than Cd, and it subsequently increased at the sampling sites affected by CSOs (Figure S7). The anaerobic conditions, with a high quantity of organic matter in CSOs influencing the area, are the key factors facilitating the function of 'Methane metabolism' (Sonoki *et al.* 2012; Guo *et al.* 2019).

DISCUSSION

CSOs are one of the main contributors to river pollution. The main pollutants in CSOs include organic matter, heavy metals, polycyclic aromatic hydrocarbons, etc. (Launay *et al.* 2016; Xu *et al.* 2018). In developing countries, overflow pollution has existed for a long time in urban inland rivers on account of the imbalance between urban development and drainage system construction, resulting in difficult elimination along rivers. 'Water diversion' has been widely used as a quick-acting and easy-to-manage measure to remove black and odorous material (Chen *et al.* 2014). Although conventional water quality indicators of most regulated rivers have been significantly improved after water diversion (Fan *et al.* 2012; Deng *et al.* 2018), there are few reports on the improvement of the microbial environment in river sediments. As an indispensable part of the river ecosystem, river sediment is an important benthic habitat, and has a great impact on the aquatic and terrestrial ecological balance and water quality (Su *et al.* 2002; Haimann *et al.* 2018). In this study, water quality and sediment microbial characteristics under the influences of the long-term water diversion and CSOs were assessed. It was found that CSOs have a significant impact on the diversity of microbial populations in river sediments. An LTWD is helpful in improving the diversity of the microbial environment, but had difficulty reducing the accumulation of heavy metals in sediment within the cycle of this research.

Heavy metals in the river sediments affected by CSOs along the river

The influence of CSOs on the accumulation of heavy metals in the Huancheng River sediment was investigated in our

previous study (Xu *et al.* 2018). Along the river, the contents of heavy metals in the river sediments affected by CSOs are significantly higher than those at the control sites in this study. As shown in Figure 1, the Cu, Cd, and Zn concentrations (Cu: <0.033 mg/L, Cr <0.131, and Cd and Zn below the limit of detection) in the water diversion source are all lower than the Grade II surface water standard, which is mainly suitable for the first-class protection area of surface water source areas for centralised drinking water and so on (China 2002) (only the concentration of Cr⁶⁺ is within this standard, which is less than 0.5 mg/L in Grade II), but the long-term clean water diversion has not reduced the accumulation of heavy metals in river sediments. Although hydraulic conditions are important factors affecting pollutant migration in river sediments, the long-term impact of overflow pollution cannot be ignored in this area. The aforementioned results also indicate the long-term and stable characteristics of heavy metals in river sediments affected by CSOs. Heavy metals are considered to have good stability and are easily enriched in farmland, soil, sludge, and other environments (Sainz *et al.* 2004; Fu & Wang 2011; Jing *et al.* 2018). Therefore, without eliminating CSOs or reducing overflow frequency, it is difficult to alleviate the accumulation of heavy metals in river sediments by water diversion alone.

The richness of the microorganisms has been improved after installation of the LTWD

The DG area has been affected by CSOs for a long time, and the overflow frequency at this point is the highest of the five outlets according to our long-term field investigation. In our study, the Shannon index at site DG is the lowest among all the sampling sites (Figure S6), which means that the microbial richness in the river sediments was significantly affected by CSOs. Under high heavy metal contamination, the Shannon diversity can remain at a low level (Qu *et al.* 2017). Chang *et al.* (2018) also found that the Shannon index in a sewer deposit discharging area was the lowest of all the selected sites. However, after installation of the LTWD, the richness of the microorganisms has been improved, indicating a certain ecological flow can promote the improvement of the microbial environment in river sediments.

The dominant microbial populations in river sediments affected by CSOs

The dominant microbial populations in river sediments affected by CSOs are different from those of the control

points. In the sediments affected by CSOs, the main classes include *Acidobacteria*, *Bacilli*, *Bacteroidetes_vadinHA17*, *Bacteroidia*, *Caldisericia*, *Clostridia*, *Cyanobacteria*, *Dehalococcoidia*, *SB-5*, *Spirochaetes*, *Synergistia*, and *Deltaproteobacteria*. Among them, *Acidobacteria*, *Bacteroidetes*, *Deltaproteobacteria*, and *Spirochaetes* were found harbouring heavy-metal resistance/reduction genes in the river sediments (Chen et al. 2018). The proportion of Gram +ves in the sediments affected by CSOs is also significantly higher than that in the control areas. The cell wall surface of Gram +ves contains an extremely resistant compound that can withstand long-term contaminants (Lăzăroaie 2010), which favours them dominating in heavy-metal-polluted sediments and historically contaminated soils (Nasrazadani et al. 2011; Kuppusamy et al. 2016). Following installation of the LTWD, the proportion of Gram +ves decreased at nearly all of the sampling sites, which also indicates that the LTWD is gradually improving the microbial environment in the river sediment.

Overflow pollution contains a variety of pollutants closely related to the types of land use along the river and the coverage of the drainage system. The families *Ropionibacteriaceae*, *Bacilli*, *Bacteroidetes_vadinHA17*, *Caldisericia*, *Clostridia*, *Deltaproteobacteria*, *Spirochaetes*, and *Synergistia* were positively correlated with Zn and Cd at the sampling sites ($P < 0.05$, Table 1). Members of these families are commonly found in contaminated environments (Onunga et al. 2015; Wang et al. 2015; Li et al. 2016) and reactors for removing organic, nutrient, and heavy metal pollutants (Sundar et al. 2011; Shen et al. 2013; Zhu et al. 2017). In addition to the aforementioned taxa, *Bacteroidia*, *Cyanobacteria*, and *Dehalococcoidia* do not significantly ($P > 0.05$, Table 1) correlate with Zn and Cd, but are also dominant in these contaminated areas. Because of the continuous water diversion from upstream lakes, where *Microcystis* is the main cyanobacterial species (Cai et al. 2012; Cai & Kong 2013; Yu et al. 2014), a large number of *Cyanobacteria* enter the urban river and grow vigorously in the water column, where nitrogen is more abundant in CSO-affected sites (Figure S8, available online). *Dehalococcoidia* is well known for its potential to remediate aromatics and halogenated ethenes (Maphosa et al. 2012), which may indicate some persistent organic pollutants are discharged through these outlets. This will be the focus of the next stage of this research. *Bacteroidia* is also found in some reactors for removing pollutants (Cho et al. 2013; Narciso-da-Rocha & Manaia 2017), but some other environmental factors may alter its growth at these sampling sites. In general, the complex characteristics of the overflow pollution

have direct impacts on the microbial environment of the river sediments. The bacterial communities are distributed according to the heavy metal content along the first axis of the dbrDA. The significant impacts of Cd and Zn in the study area are closely related to the type of pollutants locally discharged and their accumulation in surface sediments. The dbrDA plot also shows that the bacterial communities are ordinated before and after the LTWD, along with its second axis. Water diversion projects are usually used to replenish water-scarce rivers, lakes, and wetlands (Dadaser-Celik et al. 2009). During recent years, water diversion measures (Zhu et al. 2008; Song et al. 2018; Yan et al. 2018) have often been used to dilute polluted water, improve a water's self-purification capacity, and rapidly improve a water's ecological environment because of the worsening situation of river black odour and lake eutrophication in urban China (Song et al. 2018). However, these studies are often based on hydrodynamic and water quality models. Therefore, the analysis is usually limited by the selected parameters of these models (Song et al. 2018; Žganec 2012). In addition, some researchers (Yan et al. 2018) have analysed the migration of organic micro-pollutants in water diversion projects. Although the organic micro-pollutant pollution is not optimistic in the aforementioned study, these analyses often are of a large spatial scale and affected by complex factors. Therefore, it is difficult to determine the key factors of pollutant migration, and it is also impossible to comprehensively and qualitatively discriminate the changes in the water conditions. Similarly, the variation in heavy metal concentration in the sediments before and after the LTWD installation is not obvious in this study. However, the increase in biodiversity and the proportion of Gram –ves bacteria shows that although the pollution concentration has not been significantly reduced, the microbial environment of the urban water body has been improved by the LTWD. This is partly because of the better water quality and continuous fluidity brought by the water diversion, which can not only dilute the pollution caused by CSOs, but also improve the self-purification ability of the water body (Dadaser-Celik et al. 2009; Song et al. 2018).

The bacterial function in river sediments affected by CSOs

The microbial population richness was improved after installation of the LTWD, but the change in gene functions predicted by the PICRUSt analysis is not significant. However, the correlation analysis shows that there is a significant relationship (Table S3) between some metabolic

pathways and the Zn and Cd accumulation in the river sediments. At the selected sampling sites, the discharged Zn and Cd is as serious as the organic pollutants, resulting in mostly anaerobic or anoxic environments in the sediment. Oxidative phosphorylation is the process yielding an anaerobic condition and making ATP. When collecting water samples, dissolved oxygen (DO) was detected by a rapid determinator. The average DO of water samples collected near the outlet was only 2.95 mg/L (collecting depth was 0.5 m). Although the redox conditions in sediment samples have not been analyzed, the DO content in sediments with an average depth of 1.6 m, in theory, must be significantly less than 2.95 mg/L. The boundary value of DO under anoxic and aerobic conditions in microbial treatment technology is 2 mg/L (Meng *et al.* 2019). This may be the reason why oxidative phosphorylation is inhibited. Two-component systems are signaling pathways that regulate many bacterial characteristics, such as virulence, pathogenicity, metabolic regulation, etc. Under Cd stress, two-component systems are suppressed, affecting their transmission of toxic signals. Under the stress of heavy metals, DNA repair and recombination protein pathways have been promoted, indicating long-term exposure to heavy metals is associated with a significant increase in DNA repair genes (Al Bakheet *et al.* 2013). Some detoxification compound metabolisms (Purine metabolism; Arginine and proline metabolism) are also promoted under this condition. Therefore, chronic exposure to environmental heavy metals differentially alters the expression of genes involved in detoxification and the DNA repair process (Al Bakheet *et al.* 2013), and hence decreases the survival of river microorganisms, in turn affecting the ecological environment of the river.

CONCLUSION

In this study, we found that the LTWD cannot significantly reduce the pollution of heavy metals in the river sediments, but the Shannon diversity of the sediment bacterial communities after the LTWD was higher than that before. Furthermore, the proportion of Gram +ves was higher before the LTWD on average than that after. In addition, an obvious difference was observed between the river sediments affected by the CSO discharge and the control samples; the proportion of Gram +ves surged in the river sediments affected by the CSO discharge. We also found that some heavy metals in sediments showed a positive effect on the two detoxification compound metabolisms.

This work demonstrates that CSOs have a significant adverse impact on the diversity of microbial populations in river sediments, but an LTWD is capable of improving this unfavourable ecological environment. Therefore, eliminating CSOs is crucial to river ecological improvement.

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