

Original Research

# Aquatic Microbial Community Characteristics and Influencing Factors in Urban Landscape Rivers

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## Abstract

Urban rivers play an important role in ecological landscapes, but the influencing factors of microbial community structure and diversity have not been further studied. This study took three urban landscape river water bodies in Wuhu, Anhui Province, as examples. Determination of the physical and chemical properties of the water samples collected was performed. The Illumina platform was used for double-terminal sequencing of community DNA fragments. The specific composition of samples at different taxonomic levels was obtained to determine the microbial community structure. The influence of environmental differences on river water quality, bacterial community structure and diversity of urban rivers was discussed. The results showed that (1) a maximum of 35 phyla were detected in urban landscape water bodies, among which *Proteobacteria* were the most dominant phyla, accounting for 63.62% on average. Then, *Actinobacteria* and *Bacteroidetes* were observed. The content of cyanobacteria in the streams near the residential area is higher than that in the other two streams. (2) Due to the different functional areas nearby, there were spatial differences among microorganisms in urban river water. The abundance and diversity of bacteria in streams near residential areas decreased significantly. The abundance and diversity of bacteria in river water samples with a more complex surrounding environment were the highest. (3) DO, pH, NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N and EC were key factors affecting bacterial communities. *Proteobacteria* was positively correlated with DO. *Actinobacteria* were positively correlated with NO<sub>3</sub><sup>-</sup>-N. At the genus classification level, *Azospirillum* was highly correlated with DO and EC.

**Keywords:** urban river, microbial community structure, environmental factors, redundancy analysis

## Introduction

Rivers, lakes and other landscape water bodies that flow through the city have many functions, such as providing water resources, exerting ecological effects and carrying urban life [1, 2]. They are often distributed in areas where people gather and have the characteristics of poor water environment capacity and poor water self-purification capacity. Water is vulnerable to domestic sewage, rain and garbage [3, 4]. Urban river pollution is an increasingly serious problem. For example, previous reports have described seriously polluted urban waterbodies becoming black and odorous in diverse regions, including the Cheonggye Stream in Seoul (South Korea), the Seine River in Paris (France), the Emscher River in Nordrhein-Westfalen (Germany) and the Danube River in Vienna (Austria) [5]. Urban black and odorous water remediation is an important part of improving the urban living environment. With the attention of the Chinese government and people to environmental protection, ecological restoration has been implemented, and urban landscape rivers has been improved to some extent [6].

By the end of 2020, the elimination rate of 2914 black and odorous water bodies in prefecture-level and above cities in China reached 98.2%. The treatment of black and odorous water bodies has made positive progress. However, due to the lack of long-term measures in some cities, some rivers return to black and odorous again. In December 2018, the first round of the Central Environmental Protection Inspectorate found 46 black and odorous water bodies that had been completed in Wuhu, Anhui Province, and nearly 1/3 of them returned to black and odorous. The main reason may be that the urban sewage pipe network is not complete, there are a large number of human activities near the shore, and rainwater scouring leads to pollutants entering the river [7]. Second, some water bodies have endogenous pollution problems, and the heavily polluted sediment has not been effectively removed. Once a large amount of rainfall occurs, there is a large area of mud dumping in the completed rivers, which inevitably leads to serious seasonal or local pollution of urban inland rivers.

Microbial communities are an important part of aquatic ecosystems and play an important role in pollutant degradation and nutrient cycling [8, 9]. Previous assessments of the impact of human activities on urban rivers have focused on the ecological risk of typical pollutants (e.g., heavy metals), the distribution of hydrocarbons, nitrogen and phosphorus and the characteristics of algae [10]. There is still a lack of in-depth understanding of the characteristics and diversity of microbial communities in urban landscape rivers. However, there is a mutual influence and interaction between microorganisms and the ecological environment of rivers. Microorganisms decompose pollutants to purify the water quality of rivers. In turn, changes in the ecological environment of rivers

will affect the distribution and diversity of microbial populations. Therefore, the dynamic changes in the distribution of microorganisms in rivers can be used as an important indicator to indicate whether the ecological environment of rivers is healthy. Analysing and understanding the microbial community structure of rivers plays an irreplaceable role in the ecological restoration of rivers [11, 12]. A large number of studies at home and abroad in recent years have shown that microorganisms in freshwater bodies mainly belong to *Proteobacteria*, *Bacteroides*, *Actinobacteria*, *Cyanobacteria*, *Firmicutes* and *Verrucomicrobia* [13].

Microorganisms are sensitive to environmental fluctuations in rivers and are easily affected by spatial changes in physical, chemical and biological factors [14-16]. Their community structure and diversity change with environmental conditions. Urban rivers are quite uneven ecosystems, and human activities directly or indirectly affect bacterial communities [17]. Pollutants from urban environments, for example, reduce microbial composition [18, 19]. For polluted rivers, the pollution levels in different regions of rivers may be different, thus hiding different bacterial communities. Significant changes have taken place in river community composition due to eutrophication [20]. Studies have shown that the bacterial communities in rivers under human disturbance vary from place to place, but the seasonal effect is not obvious [21]. Therefore, it is necessary to study the composition and diversity of microorganisms in rivers with different spatial locations. Biological and abiotic factors are reported to contribute to bacterial community composition [22]. Among them, heavy metals and water quality parameters significantly affect the diversity and composition of bacterial communities [23-26]. Some researchers suggested that organic pollutants from human activities affect river bacterial communities [27]. It has been reported that bacterial diversity is highly correlated with phosphate, and ammonia nitrogen is the main driving factor for bacterial community structure [28]. Temperature, nutrients and dissolved oxygen are the most important factors affecting microbial communities in polluted rivers [17, 29]. It was found that bacterial communities were affected by nutrition, hydrology and environmental factors. The abundance of *Armatimonas*, *Roseomonas*, *Limonhabitans* and *Flavobacterium* was positively correlated with pH, flow rate and DO [9]. These studies help to understand the response of microbial communities to environmental changes.

Many scholars have conducted a large number of studies on the spatial distribution, community structure, diversity and environmental factors of microorganisms in different types of rivers, lakes and marine water bodies. However, there are relatively few studies on the microbial community of urban landscape water in the period of easy return to black and odorous [9, 13, 15, 20-23]. In addition, the exploration of microbial communities in urban landscape rivers not only helps

to understand the degree of river pollution but also plays an important role in predicting the changes in river ecosystems and judging the potential risks of returning black to smell [30, 31]. Therefore, in this study, the urban river in Wuhu city, Anhui Province, was selected as the research object. By collecting water samples near different pollution sources, Illumina MiSeq high-throughput sequencing technology was used to comprehensively analyse the bacterial community structure and diversity changes in urban rivers affected by different pollution sources. The purpose of this study was (1) to present the taxonomic composition of microbial communities in urban landscape rivers (2) to explore the differences of Aquatic Microorganisms in urban landscape rivers in different functional areas; and (3) to analyse the relationship between microbial communities and environmental factors. This study is expected to provide an important basis for understanding the microbial diversity of urban rivers, exploring the relationship between microbial community characteristics and river ecosystem functions, and laying a foundation for further bioremediation of urban rivers.

resources for many years are 3.165 billion cubic metres, and the average shallow groundwater resources for many years are 703 million cubic metres.

Urban landscape rivers have always been an important gathering place for urban waste, domestic sewage and storm runoff. The study area is three urbanized rivers in Wuhu city: the Huicheng River (HC), Zhongyangcheng River (ZCY) and Zhongshan South Road River (ZSNL). The three rivers flow through different functional areas such as residential areas, commercial areas and schools, and receive the pollution of rainfall surface runoff and sewage pipe network leakage. The HC River is located east of Wuhu Hospital of Traditional Chinese Medicine, Jiuhua South Road. The ZCY River is located in the Wuhu City Planning Center and the core area of University City, adjacent to the international convention and exhibition centre and five-star hotels. The ZSNL River is located southwest of the intersection of the Qingyi River and the Yangtze River. It is surrounded by residential areas. The locations of the sampling points are shown in Fig. 1.

### Material and Methods

#### Area of Study

Wuhu city is located in southeastern Anhui Province and the lower reaches of the Yangtze River. The central geographical coordinates are 118°21'E and 31°20'N. The total area of the city is 6026 square kilometres, and the Yangtze River flows through the city. In addition to the Yangtze River water resources, Wuhu city is rich in local surface water resources. The average water

#### Sample Collection

Around the above river waters, 18 sampling points were selected, and water samples were collected in accordance with the water sample collection specifications (HJ 495-2009). Sampling at intervals of 100 to 200 metres. Three surface water samples were collected manually from each sampling point at a depth of approximately 50 cm in the river centre. Sampling was conducted on October 31, 2020, and the temperature in the study area was suitable for bacterial growth. In addition, selected 10 samples (ZCY1-3, HC1-4,

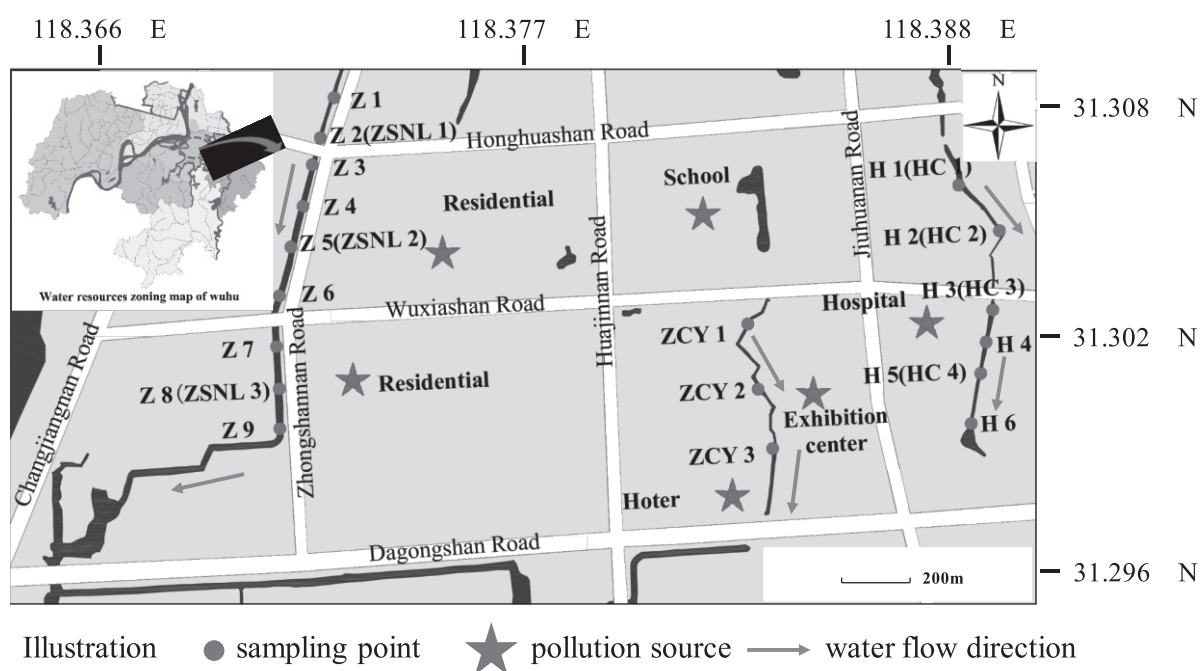


Fig. 1. This figure shows the locations of the sampling points in Wuhu city, Anhui Province.

ZSNL1-3) for microbiological examination. Water samples were collected for cryopreservation to detect microorganisms in the samples.

### Determination of Physicochemical Property

River water temperature (WT) and depth (WD) were measured on site. The transparency (SD) measurement was measured using secchi disk. The pH, dissolved oxygen (DO), redox point (ORP), conductivity (EC) and total dissolved solids (TDS) of the water samples were measured using a portable water quality detector (T3WS-GP). The chemical oxygen demand ( $COD_{Cr}$ ) was determined by the dichromate method (HJ 828-2017) in the laboratory,  $BOD_5$  was determined by dilution and inoculation (HJ 505-2009), total nitrogen (TN) was determined by alkaline potassium persulfate digestion UV spectrophotometry (HJ 636-2012), total phosphorus (TP) was determined by the ammonium molybdate spectrophotometric method (GB 11893-89), chlorophyll a (chl a) was determined by spectrophotometry (HJ 897-2017), and ammonia nitrogen ( $NH_4^+-N$ ) was determined by Nessler reagent spectrophotometry (HJ 535-2009). The nitrate ( $NO_3^- -N$ ,  $NO_2^- -N$ ) and phosphate ( $PO_4^{3-} -P$ ) were determined by Ion Chromatography (DIONEX ICS-5000).

### Microbial DNA Sequencing

Microbial sampling in strict accordance with the test requirements, refrigerated conditions sent to the laboratory stored at  $-20^{\circ}C$  and complete bacterial genomic DNA extraction as soon as possible. Genomic DNA was extracted with an E.Z.N.A.® Soil DNA Kit (Omega Bio-Tek, Norcross, GA, U.S.), operation steps in accordance with the kit instructions. After genomic DNA extraction, 1% agarose gel electrophoresis was used to detect the extracted genomic DNA. Primers F: ACTCCTACGGGAGGCAGCA and R: GGACTACHVGGGTWTCTAAT were used for PCR amplification to amplify the V3-V4 region of the 16S rRNA gene in urban river water. The reaction conditions were as follows:  $95^{\circ}C$  for 2 min; denaturation at  $95^{\circ}C$  for 30 s, annealing at  $55^{\circ}C$  for 30 s, and extension at  $72^{\circ}C$  for 30 s for 15 cycles. After the cycle,  $72^{\circ}C$  was finally extended for 5 min. Each sample was mixed and repeated three times to reduce the error caused by the experimental operation in the DNA extraction process. After the amplification product was detected by agarose gel electrophoresis, DNA was purified and recovered by a nucleic acid purification kit. High-throughput sequencing was carried out based on the Illumina MiSeq platform at Shanghai Parsono Biotechnology Co., Ltd.

### Statistics and Software Analysis

Repeated detection of water samples at each sampling point for three times using Excel to calculate

the average value of water index parameters and microbial datas. The one-way analysis of variance (ANOVA) with Fisher's least significant difference (LSD) post-hoc test was performed using the SPSS Statistics software. The One-way ANOVA was used to test the difference of environmental factors among different groups. The DADA2 method was used to remove primers, quality filtering, denoising, splicing and chimeraism [32]. Using the R language script, the length distribution of the high-quality sequences contained in all samples was counted. The classification-sklearn algorithm of QIIME2 was used to annotate the feature sequences of each ASV using the default parameters and naive Bayes classifier. Based on ASV dilution curve analysis, the coverage, Chao1 richness index and Shannon-Weiner diversity index were calculated. Nonmetric multidimensional scaling (NMDS) analysis of sampling points based on Bray-Curtis distance was performed using R language to determine the differences in bacterial community structure among different sampling points. The relationship between bacterial community structure and environmental factors was determined by redundancy analysis (RDA). The functional annotation of bacterial composition was performed by using the database FAPROTAX on the normalized ASV table. Each taxonomically annotated ASV was automatically compared with the FAPROTAX 1.1 database (including 7820 annotations, covering 4724 taxa) using a web-based platform [33].

## Results

### Physicochemical Properties of Water Bodies

Table 1 lists the physicochemical properties of different sampling points. The One-way ANOVA results showed that the pH, COD, TN,  $NH_4^+ -N$ ,  $NO_2^- -N$ , TP of the three river samples showed little difference ( $P > 0.05$ ). Significant differences in the SD, DO,  $BOD_5$ ,  $NO_3^- -N$ ,  $PO_4^{3-} -P$  between different rivers ( $P < 0.01$ ). The surface water temperature in urban rivers in autumn was between  $18.0$  and  $19.9^{\circ}C$ . The pH ranged from 8.43 in ZYC to 7.65 in ZSNL, showing weak alkalinity. In general, the transparency of HC river waters was significantly lower than that of ZYC river waters and ZSNL river waters. The dissolved oxygen content in the ZYC water sample was the lowest and that in the ZSNL water sample was the highest. The chlorophyll a content decreased with the flow direction in all three rivers. The TP content was the lowest in ZYC river waters and the highest in HC river waters. The content of  $NH_4^+ -N$  was the highest in the ZSNL sample and the lowest in HC river waters. The content of  $NH_4^+ -N$  in ZSNL river waters exceeded the V class water standard value in the *Environmental Quality Standard for Surface Water* (GB3838-2002).

Table 1. Results of physicochemical indexes of different water samples (\* P < 0.05, \*\* P < 0.01).

Sample	WD (m)	WT* (°C)	SD** (cm)	pH	DO** (mg/L)	ORP* (mV)	EC* (ms/m)	TDS (mg/L)	BOD <sub>5</sub> ** (mg/L)	CODcr (mg/L)	TN (mg/L)	NH <sub>4</sub> <sup>+</sup> -N (mg/L)	NO <sub>3</sub> <sup>-</sup> -N** (mg/L)	NO <sub>2</sub> <sup>-</sup> -N (mg/L)	TP (mg/L)	PO <sub>4</sub> <sup>-</sup> -P** (mg/L)	Chla* (ug/L)
ZYC1	2.09	18.2	81	8.43	5.3	180	392	197	4.3	4.72	4.08	2.344	0.5619	0.072	0.152	0.046	10
ZYC2	1.99	18.0	75	8.10	3.3	95	413	206	2.0	2.74	4.97	3.44	0.3785	0.038	0.238	0.118	11
ZYC3	1.82	19.0	97	7.82	4.5	136	424	213	3.9	4.67	5.36	3.724	0.4689	0.042	0.476	0.186	7
H1 (HC1)	1.05	18.4	40	7.91	7.6	157	397	197	5.6	5.73	4.89	3.23	0.3165	0.041	0.196	0.073	23
H2 (HC2)	1.11	19.3	52	7.99	6.8	182	392	195	7.1	4.94	4.60	3.07	0.3880	0.046	0.483	0.013	39
H3 (HC3)	1.37	19.2	48	8.10	7.2	197	376	185	7.5	4.26	5.73	3.08	0.3475	0.039	0.218	0.076	57
H4	1.90	19.9	50	7.84	5.7	160	402	202	4.8	10.7	4.08	2.464	0.0855	0.012	0.799	0.142	32
H5(HC4)	2.53	18.7	53	7.91	7.1	158	408	202	6.1	6.97	4.33	2.132	0.2784	0.043	0.395	0.076	22
H6	3.34	19.5	56	7.91	7.1	195	398	240	5.6	7.36	5.71	2.832	0.2808	0.038	0.365	0.016	21
Z1	1.37	18.7	105	7.87	8.8	188	408	201	6.9	6.05	3.93	3.244	0.0736	0.032	0.255	0.256	26
Z2 (ZSNL1)	1.45	18.4	74	7.65	7.6	186	405	205	5.3	4.61	4.39	3.048	0.1689	0.023	0.270	0.188	30
Z3	1.59	18.3	92	7.74	8.3	186	402	200	6.6	6.44	2.22	2.008	0.1927	0.042	0.233	0.200	47
Z4	1.73	18.3	80	7.92	7.2	176	419	210	5.9	4.87	4.17	3.18	0.1451	0.029	0.351	0.220	53
Z5 (ZSNL2)	1.60	18.3	81	8.07	9.8	172	433	193	8.9	5.15	5.49	3.512	0.1284	0.024	0.292	0.092	71
Z6	1.10	18.8	85	7.81	8.4	181	416	209	6.6	7.80	6.88	4.16	0.1260	0.040	0.285	0.296	37
Z7	1.13	18.7	79	7.80	8.2	182	428	214	5.9	10.70	5.13	4.00	0.1379	0.121	0.299	0.308	36
Z8 (ZSNL3)	1.35	18.8	90	8.41	7.7	167	436	218	6.2	7.81	5.48	4.56	0.1784	0.172	0.336	0.244	27
Z9	1.42	19.1	84	7.84	7.6	173	461	229	5.2	2.2	5.9	4.44	0.1736	0.094	0.476	0.128	13

Table 2. Bacterial community abundance and diversity index of different water samples.

Sample	ASVs	Chao1	Observed species	Shannon	Simpson	Goods coverage
ZYC1	1511	1531.48	1478.5	6.74073	0.950309	0.997719
ZYC2	1865	1893.77	1829.7	8.43357	0.990225	0.997651
ZYC3	1429	1452.40	1368.2	8.11368	0.987824	0.997915
HC1	1372	1387.68	1351.2	8.46390	0.991333	0.998778
HC2	1496	1529.50	1487.6	7.68224	0.976259	0.998065
HC3	1909	1955.46	1830.7	8.22451	0.987945	0.996159
HC4	1617	1645.41	1602.3	6.95963	0.947310	0.997773
ZSNL1	1266	1290.59	1250.8	7.41887	0.976035	0.99846
ZSNL2	1262	1300.67	1214.5	6.12043	0.943752	0.997403
ZSNL3	1043	1061.97	1029.6	6.16163	0.954304	0.998473

### Alpha and Beta Diversity of the Bacterial Community

A total of 868827 high-quality gene sequences were obtained by high-throughput sequencing of 10 water samples from three rivers, and 8999 different ASVs. The number of ASVs in a single water sample was between 1043 and 1909, with an average of 1477. Sequence length was mainly distributed in 404-432 bp, accounting for 99.67% of the total sequence, and the average sequence length was 419.02 bp. The overall number of ASVs decreased in the order ZYC>HC>ZSNL.

High-throughput sequencing technology was used to screen and remove the chimeric sequences, and the related data of 10 surface water samples in the Wuhu urban river were analysed. To comprehensively evaluate the alpha diversity of the microbial communities, the Chao1 and observed species indices were used to characterize richness, the Shannon and Simpson indices were used to characterize diversity, and charts (Table 2) were obtained. The maximum value of the

Chao1 index appeared at sampling point 3 of the HC River (1955.46), and the minimum value appears at sampling point ZSNL3 (1061.97). Table 2 shows that the Goods coverage values were all greater than 0.996, indicating that the probability of gene sequence detection in the sample was high, and the sequencing results could represent the real situation of the bacterial community in water samples. The Chao1 index and Shannon index were HC > ZYC > ZSNL, and the order of the Simpson index was ZYC > HC > ZSNL. These results indicated that there was spatial variability in bacterial diversity and abundance among the three rivers. The abundance and species diversity of the microbial communities in the ZYC and HC water samples were higher than those in the ZSNL water samples.

Sparse curves were drawn using QIIME software. All the bacterial sparse curves based on the  $\alpha$  diversity index reached or approached a steady state (Fig. 2a), which also reflected that the sequencing depth met the requirements and had high reliability.

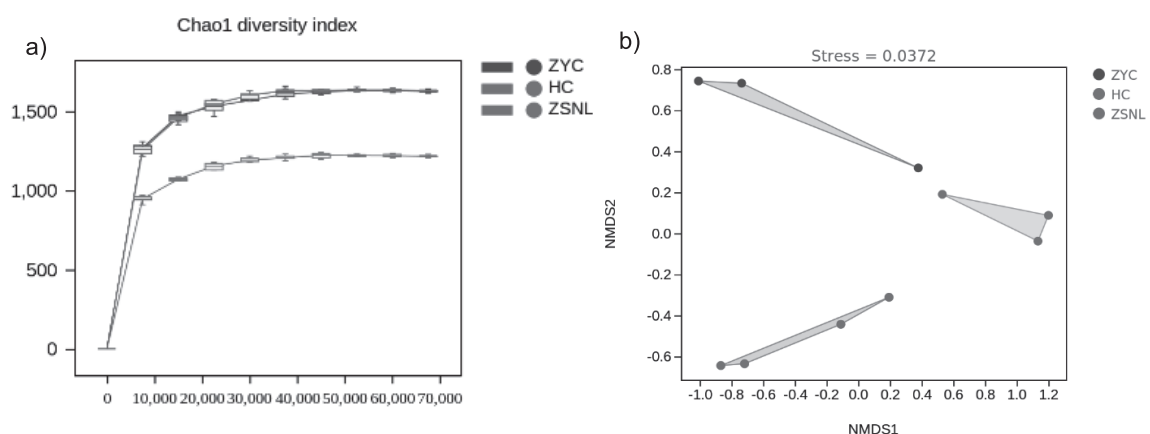


Fig. 2. a) Sparse curve of the microbial diversity index. b) Nonmetric multidimensional scale (NMDS) analysis diagram of sampling points based on Bray-Curtis distance.

The NMDS analysis was performed on the distance matrix information. NMDS analysis (Fig. 2b) showed that there were significant differences in the distribution of different samples on the NMDS axis. On the NMDS1 axis, HC and ZYC water samples were distributed in the negative direction, and ZSNL samples were distributed in the positive direction. On the NMDS2 axis, the HC water samples were distributed in the negative direction, and the HC water samples were distributed in the positive direction. The water samples from the three rivers were far away from each other, and there was no overlapping phenomenon, indicating that the sampling points of the same river had similar bacterial community structures and that the bacterial communities at the different river sampling points were significantly different.

### Composition of the Bacterial Community at the Phylum and Genus Levels

As shown in Fig. 3, the bacterial sequences in the three river water bodies were mainly distributed in 10 phyla, of which the sum of *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Firmicutes* and *Verrucomicrobia* reached more than 95% in each sample. *Proteobacteria* at each sampling point was the first dominant class, accounting for 46.87%-80.88% of all bacterial communities, and its abundance was ZSNL>HC>ZYC. *Actinobacteria* was

the second dominant phylum, with an abundance of 4.19%-27.00%. *Cyanobacteria* in ZSNL river waters was the third dominant bacteria with an abundance of 6.75%, but *Bacteroidetes* was the third dominant bacteria in HC and ZYC river waters with an abundance of 9.8% and 10.54%, respectively, while *Cyanobacteria* had a low content. The content of *Verrucomicrobia* was relatively high at the HC2 and HC3 sampling points, and the abundances were 3.27% and 4.49%, respectively, compared with those at each sampling point. Other dominant phyla detected were *Deinococcus-Thermus*, *Patescibacteria*, *Acidobacteria* and *Chloroflexi*. *Proteobacteria* are usually dominant phyla in aquatic ecosystems. The microbial population structures of the three rivers were similar at the gate level, but there were certain differences in the abundance of each gate. The content of *Cyanobacteria* in ZSNL samples was higher than that in the other two river waters, and the abundance of *Proteobacteria* in the three river waters was ZSNL>HC>ZYC.

At the genus level, the bacteria detected at river water sampling points were mainly *Aquabacterium*, and the abundances were quite different. The abundances of other genera in the three river water samples were quite different. *Chloroplast*, *Sporichthyaceae*, *hgcI\_clade* and *Limnohabitans* were the remaining bacteria with high abundance in HC river waters, and the average proportions were 4.38%, 4.25%, 3.93% and 3.59%, respectively. *Aquabacterium*, *hgcI\_clade*

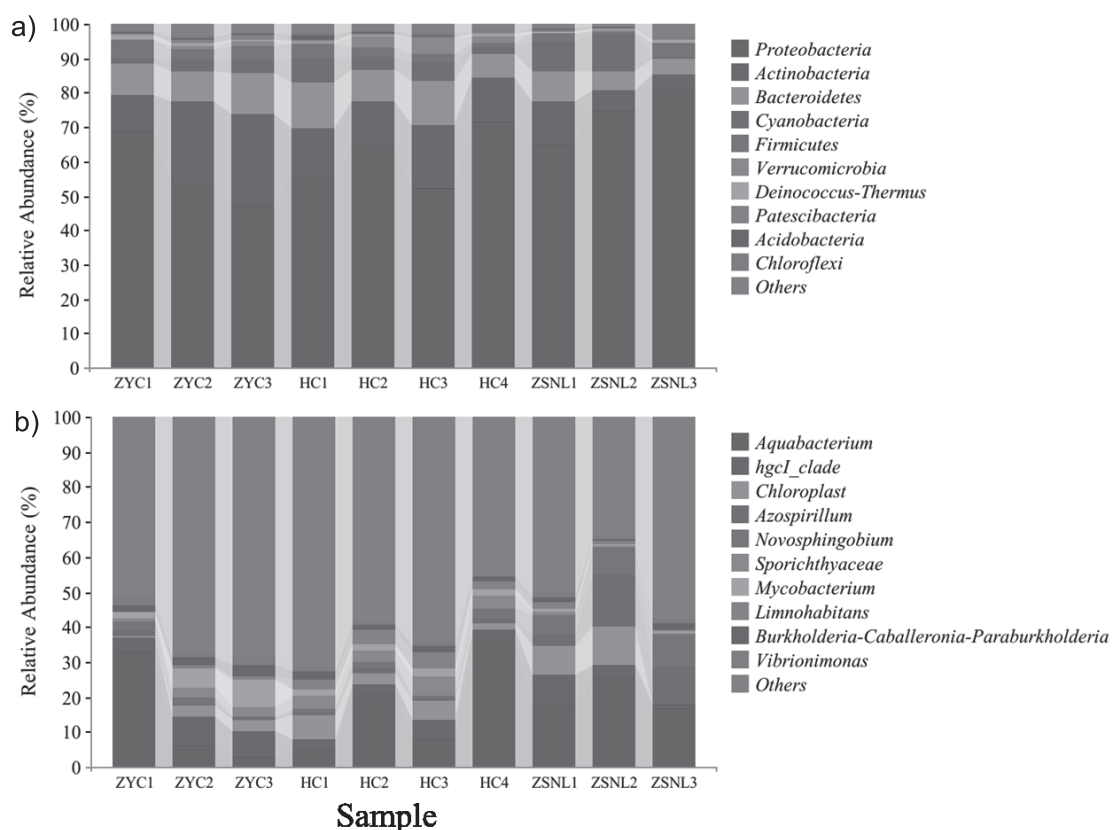


Fig. 3. Relative abundance of bacterial communities at the phylum a) and genus b) levels in the water samples.

and *Mycobacterium* were the dominant genera in ZYC and ZYC river waters, and the average proportions were 13.96%, 6.49% and 4.92%, respectively. *Aquabacterium*, *Azospirillum*, *Novosphingobium*, *Chloroplast* and *hgcl* clade were the dominant genera in ZSNL river waters, accounting for 20.10%, 9.49%, 7.49%, 6.52% and 4.33%, respectively.

#### Analysis of the Relationship between Microbial Community Structure and Environmental Factors

RDA was performed to determine the correlation between the physicochemical properties of water (pH, EC,  $\text{COD}_{\text{Cr}}$ , TN, TP,  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ , ORP and DO) and the composition of different aquatic bacterial communities. Fig. 4 shows the redundancy analysis of the bacterial community and water environmental factors at the phylum and genus levels. At the phylum level, RDA1 and RDA2 explained 44.83% and 31.12% of the total variation, respectively, and they explained 74.95% of the variance change, indicating that the relationship between bacterial colonies and environmental variables was reliable. The RDA data showed that the environmental variables that had important effects on bacterial community composition were DO, pH,  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$  and EC. Other environmental factors also had a certain degree of influence on bacterial community structure but failed to reach a significant level. In addition, *Proteobacteria* was positively correlated with pH,  $\text{NH}_4^+\text{-N}$ , DO and EC. *Actinobacteria* and *Bacteroidetes* were negatively correlated with DO and EC and positively correlated with  $\text{NO}_3^-\text{-N}$ .

At the genus level, RDA1 and RDA2 explained 52.82% and 20.02% of the total variation, respectively, and they explained 72.84% of the variance change,

indicating that the relationship between bacterial colonies and environmental factors at the genus level was reliable. The RDA data showed that the environmental variables that had important effects on bacterial community composition were DO, pH,  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$  and EC. In addition, *Azospirillum* was positively correlated with DO and EC. *Sporichthyaceae* was not highly correlated with all factors.

#### Functional Predictions of Bacterial Communities

FAPROTAX was used to predict the functional profiles of microbial communities at different sampling points (Fig. 5). The functional annotation of ASVs revealed a rich repertoire of metabolic functional types. Forty-five functional groups were obtained to annotate the function of the microbial community. Bacterial functional abundance related to energy source (chemoheterotrophy and aerobic chemoheterotrophy), carbon cycle (fermentation and hydrocarbon degradation), and nitrogen cycle (nitrate reduction and nitrogen fixation) were identified in these samples. Although with relatively low abundances, some bacterial functional abundances associated with the sulfur cycle (dark oxidation of sulfur compounds and sulfate respiration) were also found.

In addition to the sample ZSNL1, there was no significant difference in the functional map clustering analysis of the three rivers. This may be due to the differences in nutrients, bacterial communities and functional components between the sample ZSNL1 and other river samples. The potential biogeochemical activities of these sites are further demonstrated. The results showed that the ZSNL community had a higher percentage of ASVs and oxidative heterotrophic activity. Among the cycle-related functional types,

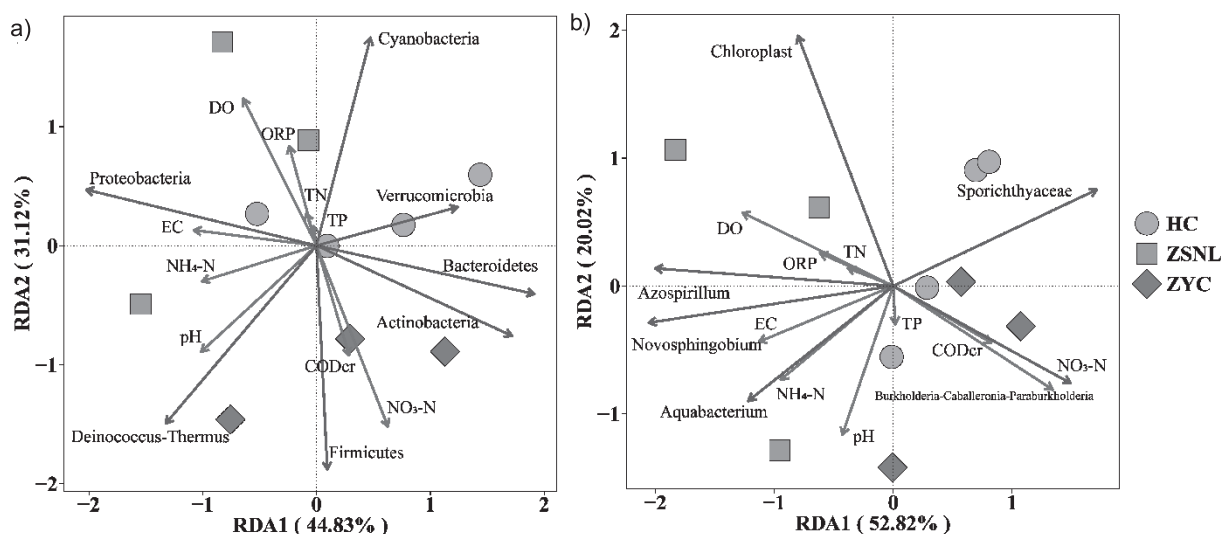


Fig. 4. RDA based on community composition and environmental factors of dominant species at the phylum a) and genus b) levels in urban river water samples



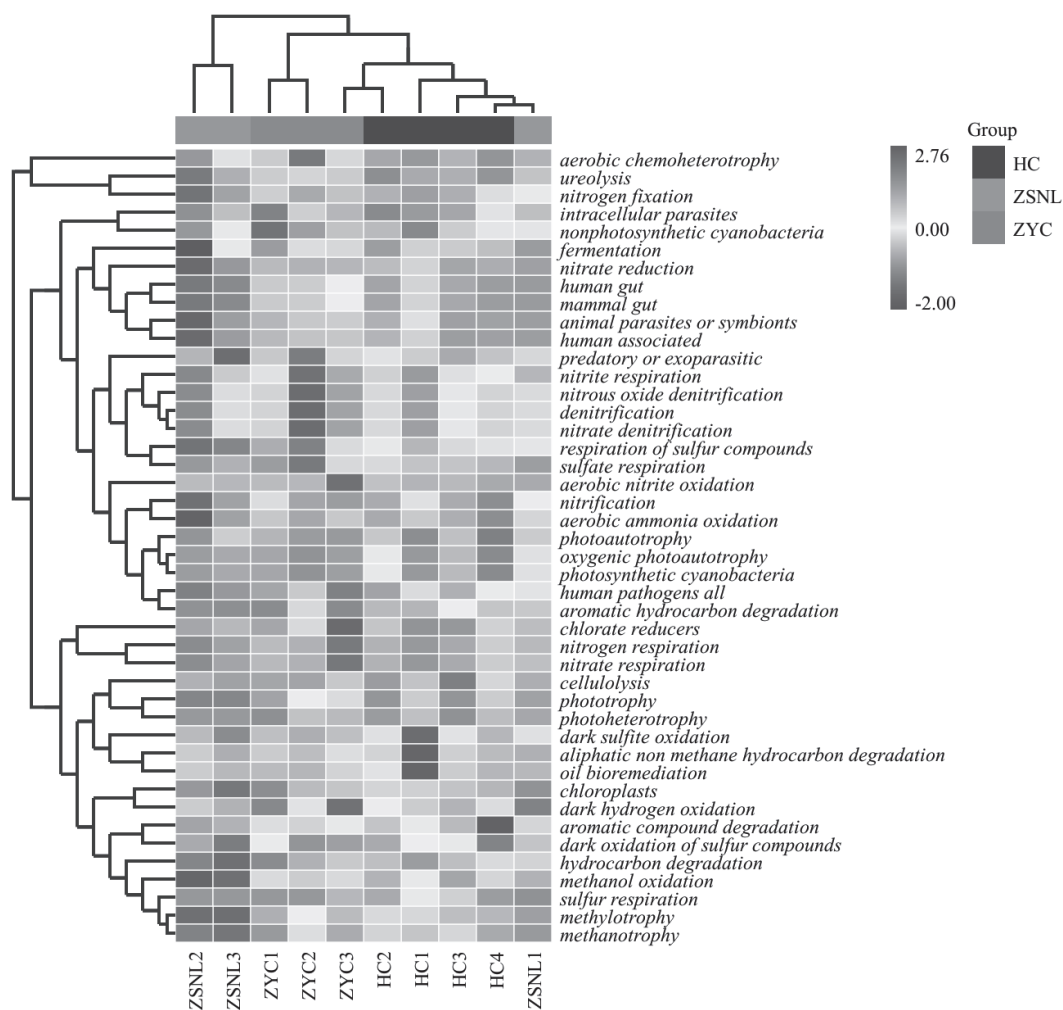


Fig 5. Heatmap representing major differences in predicted functions among different sampling points based on FAPROTAX.

Carbon cycle-related functions (fermentation, hydrocarbon degradation) and sulfur cycle-related functions (sulfate respiration, respiration of sulfur compounds) are abundant in ZYC water. Nitrogen fixation was significantly enriched in ZSNL water, and methanol oxidation was significantly enriched in ZYC water. Overall, there was no significant spatial difference in bacterial function prediction in urban rivers.

### Discussion

Urban rivers are unique ecosystems in which pollution occurs frequently, resulting in significant changes in the chemical and biological characteristics of water [24]. However, the effects of urbanization on the diversity and structure of river microbial communities have not been fully studied [18]. In this study, Illumina high-throughput sequencing technology based on 16S rRNA genes was used to detect the distribution of microbial communities in three urban rivers affected by different pollution sources, and a comprehensive

analysis was conducted to analyse the important influence of the main pollution factors on the structure of bacterial communities in urban rivers.

### Changes in the $\alpha$ and $\beta$ Diversity of Bacterial Communities

Studies have shown that the richness and diversity of microbial communities in freshwater rivers have obvious spatial heterogeneity, and bacterial diversity is greatly affected by temperature. The higher bacterial diversity in surface water is related to the outbreak of algae in summer [34]. In urban areas, due to urbanization and industrial and domestic sewage into rivers, microorganisms deteriorate due to sewage pollution conditions. The results of diversity analysis showed that the abundance and species diversity of microbial communities in ZYC and HC water samples were higher than those in ZSNL water samples, probably due to the adaptation of *Proteobacteria* to the living conditions in sewage and its accumulation, resulting in a decline in microbial community diversity [3]. This indicated that

the presence of pollutants near urban rivers had a great influence on the  $\alpha$  diversity of microbial communities. Differences in microbial diversity among rivers are the result of interactions between water quality characteristics and human disturbance factors [35]. NMDS analysis showed that the sampling points in the same river channel had similar bacterial community structures, but the bacteria at different river sampling points were significantly different. This indicated that the potential pollution sources near urban rivers affect the microbial community structure of urban river water. The research and analysis showed that the environmental driving factors affecting bacterial diversity were the combination of environmental variables (TN, TP, DO, pH, etc.) [28]. At HC sampling points near the hospital, the growth of bacteria may be affected by hospital sewage leakage, resulting in a decline in bacterial diversity. Due to the complex surrounding environment, the bacterial abundance and diversity of the ZYC sampling sites were high, and the bacterial community structure changed. Studies have shown that human disturbance significantly alters bacterial communities and functions [25, 36].

### Bacterial Community Composition

Bacterial community structure plays an important role in urban landscape river ecosystems, including participating in energy flow and the cycle of carbon and related major elements [35]. In this study, up to 35 phyla were detected in urban river water ecosystems. The highest relative abundance was *Proteobacteria* (63.62%), followed by *Actinobacteria* (14.25%) and *Bacteroidetes* (8.84%). They are common in aquatic microorganism - induced black and odorous waters and sediments [5]. The results of domestic and foreign studies on river bacterial community composition and influencing factors show that *Proteobacteria*, *Actinobacteria*, *Bacteroidetes* and *Firmicutes* are the dominant microbial flora in urban river water [37]. These dominant freshwater phyla were essentially consistent with the dominant phyla found in this study. Zhang et al. found in a study of the Qingliu River that bacterial communities in the Qingliu River under the influence of different domestic sewage had similar dominant phyla. The relative abundance of *Bacteroidetes* at different sampling points was significantly different [17]. Wang et al. studied the bacterial community structure in the water body of the Le'an River from upstream to downstream and showed that the dominant bacterial groups in the water body were *Betaproteobacteria*, *Actinobacteria* and *Bacteroidetes*. Downstream affected by agricultural and domestic wastewater, bacterial community richness and diversity increased [38]. It was found that the diversity of ZSNL water samples near the residential area was significantly lower than that of HC and ZYC water samples with a complex surrounding environment, and domestic sewage could cause a change in river microbial community diversity.

The microbial community structure of water samples can accurately predict changes in environmental conditions [24]. The microbial population structure of the three rivers was similar at the phylum level. The most dominant population in the water was *Proteobacteria* [39], which is involved in the biotransformation and migration of nutrients [40]. However, its abundance was significantly different in the three rivers, ZYC (56.40%)<HC (61.01%)<ZSNL (73.44%). ZSNL waters are surrounded by a large number of residential areas, and sewage may be combined. Increased nutrients in water and *Proteobacteria* abundance increased. This result indicated that *Proteus* had an obvious response to pollution, showing spatial differences. *Actinobacterias* are the second main gate and play an important role in removing complex organic matter, nitrogen and phosphorus [41]. *Bacteroidota* is the third major bacterial phylum of ZYC and HC and is a representative organic degradation product. The results of this study also found that the relative abundance of *Cyanobacteria* was high in the sampling points of ZSNL river waters. *Cyanobacteria* could play a positive role in water self-purification and could absorb nutrients such as nitrogen and phosphorus in water. *Cyanobacteria* could be used as an indicator of water eutrophication and could also reflect the high nutrient content in the river waters of ZSNL [42, 43]. *Firmicutes* are abundant in HC waters and are usually involved in the decomposition of organic pollutants and microbial nitrogen fixation [44]. *Firmicutes* are also the most abundant microorganisms in the black and odorous reaches of Jinchuan, Nanjing, China, and are proposed as an effective indicator of faecal pollution [45]. Bacterial function prediction showed that the highest relative abundance of microorganisms related to the functional metabolism of energy, carbon and nitrogen cycles was observed at ZSNL2. However, there is no significant difference in microbial function between urban rivers.

### Environmental Impact Factors

River bacterial communities are affected by climate, hydrology, nutrients, heavy metal pollution and many other environmental factors [46, 47]. The differences in the surrounding environment of different rivers, regional conditions, and sampling time conditions make different studies obtain different environmental impact factors [24, 48]. There are few studies on the response of river bacterial communities to environmental factors in urban landscape rivers. Wang et al. analysed the impact of urban landscapes on bacterial communities based on SourceTracker data, and the results showed that the impact of urban landscapes on bacterial communities was between 17% and 34% [49]. In the process of the development and change of urban rivers, they are greatly affected by the surrounding environment and pollutant emissions, and the bacterial community structure will change differently with the influence of environmental factors [50]. Through the study

of the microbial community in urban landscape river water, redundancy analysis showed that microbial community structure was affected by many environmental factors. The environmental variables that had important effects on bacterial community composition were DO, pH,  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ , EC, ORP and  $\text{COD}_{\text{Cr}}$ , which were consistent with the results of previous studies. In this study, TN and TP had little effect on microorganisms, which may be due to the small changes in TN and TP contents between the three rivers. When the change is small, the effect of environmental factors on bacterial community composition is not obvious [17]. By comparing the relationship between planktonic bacteria and environmental factors, DO, pH,  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ , EC were found to be the key factors affecting the bacterial community in this study. *Proteobacteria* was positively correlated with DO, and *Actinobacteria* was positively correlated with  $\text{NO}_3^-\text{-N}$ . The other chemical indicators of the three rivers monitored in this study included ORP and  $\text{COD}_{\text{Cr}}$ , which had less influence on microbial community structure than DO and pH. These results show that due to the combined influence of the relevant environmental variables caused by the surrounding environment of urban rivers and sewage discharge [37], the river microbial community shows spatial variability in urban areas.

Through the study of the sampling points of different bacterial diversity in urban rivers, it was found that urban rivers are greatly affected by human life [25]. The pollution around rivers in different cities is different, and the sampling points will have more complex environmental factors than natural rivers and lakes. Therefore, to reveal the correlation between bacterial population structure and environmental factors in urban rivers, we need to further investigate the bacterial diversity in urban rivers and ultimately provide a more favourable basis for the bioremediation of urban rivers.

## Conclusions

Urban rivers are vulnerable to human disturbance and receive different types of emissions, which is beneficial to the enrichment of microorganisms related to sewage and affects the biogeochemical cycle of local microorganisms involved in freshwater ecosystems. *Proteobacteria* was the dominant microbial species in urban landscape water, followed by *Actinobacteria* and *Bacteroidetes*. Compared with the other two rivers, the content of cyanobacteria in ZSNL water samples was higher. The genus with the highest relative abundance was *Aquabacterium*. At the level of species classification, there are a large number of unknown bacteria in urban landscape river water. There was no significant difference in the structure of microorganisms in urban landscape water near different function areas, but the abundance of bacterial communities was

significantly different. DO, pH,  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$  and EC were the key factors affecting the bacterial community. *Proteobacteria* was positively correlated with DO. *Actinobacteria* was positively correlated with  $\text{NO}_3^-\text{-N}$ . The results for prediction of bacterial function showed that the ZSNL community had a higher percentage of ASVs and oxidative heterotrophic activity. Carbon cycle-related functions are abundant in ZYC water. Our study will aid in understanding the relationship between river pollution and microbial population dynamics and provide a theoretical basis for the management and restoration of urban water ecosystems.

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## Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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