

Characterization of the bacterioplankton community and the influencing factors in the upper reaches of the Han River basin

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Abstract

The upper reaches of the Han River are the source region of water for the Middle Route of China's South-to-North Water Diversion Project, mainly for household, industrial, and irrigation purposes. Planktonic bacteria are more sensitive than macroorganisms to water physical and chemical properties and play a critical role in biogeochemical processes in river ecosystems. In November 2017 and April 2018, a systematic and methodical survey was carried out to evaluate the water quality and bacterial communities, on the mainstem of the Han River and its five main tributaries. In this study, high-throughput sequencing technology has been employed to investigate the bacterioplankton community composition. The results indicated that: 1) Diversity increased downstream, especially in the upper reaches of the Han River. 2) The relative abundance of *Actinobacteria* increased with the increase of river length, while that of *Bacteroidetes* decreased slightly. 3) Five tributaries were found to be importance sources of taxa to the Han River, however in both months a large proportion of OTUs (37.84% and 36.34%, respectively) had unknown sources. 4) Finally, redundancy analysis (RDA) and Bioenv analysis showed that environmental parameters (pH, TN, Cond, $\text{NH}_4^+\text{-N}$, DO, $\text{NO}_2^-\text{-N}$, Chl-*a* and T) had a great influence ($p \leq 0.05$) on the bacterioplankton community. These research results are beneficial for the managing the ecological system, protecting the tributary biodiversity, and conserving the mainstem and tributaries of the Han River basin.

1. Introduction

Rivers are the main source of industrial, agricultural, and domestic water for humans, linking the water cycle and the recycling of carbon, nitrogen and phosphorus (Schultz et al. 2013, Adhikari et al. 2019). Among the critical components of river ecosystems, microbes play an important role in degrading the multi-resourced pollutants (Llirós et al. 2014, Sorokin et al. 2014). As an important producer and decomposer of aquatic ecosystems, bacterioplankton can remineralize elements making them available for primary producers (Wu et al. 2015). Moreover, the diversity of microorganisms is an important indicator in water quality evaluations, which could reflect the degree of water pollution (Findlay 2010).

Recently, the spatiotemporal variation of bacterioplankton composition has been demonstrated, which tends to have repeatable and predictable patterns in river ecosystems (Chen et al. 2019). The phyla *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, and *Firmicutes* dominantly constituted the bacterioplankton communities in rivers, as evidenced by 16S rRNA gene-based high-throughput sequencing studies (Tamames et al. 2010, Jani et al. 2018). Further investigations continually showed that environmental parameters (e.g., temperature, pH, and nutrient levels) regulated complex microbial communities (Hu et al. 2014, Staley et al. 2015). Temperature changes the abundance of various bacteria in the river by affecting the growth rate of the bacteria (Sieburth 1967) in the river and their motility (McCaulou et al. 1995). Nutrients could affect the bacterial communities in water by changing its composition and distribution via the "bottom-up effect" (Pérez & Sommaruga 2006, Bouvy et al. 2011). Reversely, bacteria can also be influenced by the composition and abundance of predators (e.g., ciliates) (Nakano et al. 1998).

As the largest tributary of the Yangtze River, the upper reaches of the Han River are the major component of the Middle Route of China's South-to-North Water Diversion Project. With the rapid development of the urban economy in the related area, the industrial wastewater and domestic sewage discharged from the upper reaches of the Han River may damage the health of people along the river. Therefore, it is highly desirable for us to understand the biodiversity of the upper reaches of the Han River. The unique geographical location and complex natural environment make the area rich in biological resources, such as macroinvertebrate (Cao et al. 2016, Wang et al. 2019). As a tributary of the Han River, the headwaters originate in the Qinling Mountains. Because of its limited water volume, it is particularly sensitive to changes in the surrounding environment. Therefore, the streams-related research has attracted intensive attentions (Adhikari et al. 2019). Recently, the Han River basin has undergone significant anthropogenic interference, ranging from agricultural, industrial, and urban aspects, that presents remarkable environmental impact on the lotic ecosystems (Li et al., 2009). As part of the ecosystem, studying the ecological distribution of microorganisms has important implications for the ecological balance of the Qinling Mountains and the sustainable development and utilization of bacterial resources.

Currently, research on the upstream reaches of the Han River and streams from the southern Qinling Mountains is mainly concentrated on the following two aspects: (1) most of the previous studies focused on larger organisms, such as macroinvertebrate (Wang & Tan 2017, Li et al. 2019); (2) only bacteria in the sediments were studied, and only denitrifying bacteria respond to the environment (Chen 2017), not the entire bacterial community. Moreover, the existing research has mainly focused on streams, while there have been few studies on both rivers and streams. Based on the important role of planktonic bacteria in river ecosystems, it is necessary to investigate bacterioplankton community in the main tributaries of the upper reaches of the Han River.

In November 2017 and April 2018, water quality and bacterioplankton along the Han River mainstem and its five main tributaries (Xushui River, Jinshui River, Yue River, Xun River and Jinqian River) were systematically investigated from the southern foot of the Qinling Mountains. The major objectives of the study were to (1) compare the diversity and structure of planktonic bacterial community along the Han River and its main tributaries, and (2) identify the dominant environmental variables influencing planktonic bacterial community. Besides, a more thorough understanding on the water environmental quality in Han River is much appreciated as it provides a reasonable basis for environmental monitoring and pollution prevention.

2. Materials And Methods

2.1 Study area, water sampling and water chemistry

As the largest tributary of the Yangtze River, the Han River originates from the Bozhong Mountains and flows through Hanzhong city and Ankang city. It enters Hubei Province in Baihe County, with an upstream length of 956 km and a catchment area of 95,220 km². The basin is situated in a subtropical climate, the respective average annual air temperature and precipitation is 12-16°C, and 700-1800 mm (Liu 2014). The wet season lasts from May to October, during which 80% of the annual precipitation falls. There are many tributaries in the upper reaches of the Han River. There are 68 rivers with a length of more than 50 km and 18 rivers with a length of more than 100 km. According to the climatic characteristics, habitat types and human disturbance of different rivers, we chose five (Xushui River, Jinshui River, Yue River, Xun River and Jinqian River) of the main tributaries.

Water samples were randomly obtained from 7 sections along the Han River and 25 sections from the tributaries at two separate sampling occasions: in October-November 2017 and April-May 2018 (Fig. 1). There are 7 sections in the upper reaches of the Han River, 5 sections in each tributary (Xushui River, Jinshui River, Yue River, Xun River and Jinqian River) and 3 sample points in each section. The water samples of 3 sample points of each section are evenly mixed into one sample. So, 64 samples (3L) were collected at a depth of 50 cm in the center of the river using organic glass hydrophores with good lighting. All samples were amplified and sequenced to determine bacterial community composition.

The conductivity (Cond), water temperature (T), pH and dissolved oxygen (DO) were measured with a Hach HQ40D portable multiparameter analyzer; the turbidity (Turd) was measured with a Hach 2100Q portable turbidimeter; According to the Water and Wastewater Detection and Analysis Method (4th edition), the total phosphorus (TP), total nitrogen (TN), ammonia nitrogen (NH₄⁺-N), nitrate nitrogen (NO₃⁻-N) and nitrite nitrogen (NO₂⁻-N) were determined. To prevent the dissolution of pigments caused by acidification, the content of chlorophyll-a (Chl-a) was determined in the water according to the Code for Investigation of Lake Eutrophication (2nd edition). Please refer to this article (zhao et al. 2020) for the determination of physical and chemical factors. For bacterioplankton community analyses, water samples (3 L) were transported to the laboratory in polyethylene bottles at 0-4 °C and then filtered through a 0.22-µm Durapore membrane filter (diameter 25 mm; Xinya, China) within 24 h to collect microbial cells. Filters were stored at -80°C for later analysis.

2.2 DNA extraction, amplification and sequencing

Filters with the total DNA of the water samples were cut into small fragments. The extraction of the total DNA from filters was performed using the Bioteke Water DNA Kit (Beijing, China) according to the manufacturer's protocols. The V4-V5 regions of the 16S rRNA genes used for construction were amplified by PCR (95 °C for 2 min, followed by 25 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s and a final extension at 72 °C for 5 min) using the primers 515F (5'-GTGCCAGCMGCCGCGG-3') and 907R (5'-CCGTCAATTCMTTTRAGTTT-3') (Shan et al. 2015). PCR was performed in triplicate in 20 µL mixtures containing 4 µL of 5 × FastPfuBuffer, 2 µL of 2.5 mM dNTPs, 0.8 µL of each primer (5 µM), 0.4 µL of FastPfu polymerase, and 10 ng of template DNA. Amplicons were extracted from 2% agarose gels and purified with the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, USA) according to the manufacturer's instructions and quantified using QuantiFluor™-ST (Promega, USA). Purified amplicons were pooled in equimolar amounts and sequenced using the Illumina MiSeq platform.

2.3 Statistical and bioinformatics analysis

Raw FASTQ files were demultiplexed and then quality-filtered using QIIME1 according to the reported procedures (Liu et al. 2018). Subsequent analysis was proceeded using UPARSE (version 7.1) and RDP Classifier (Wang et al. 2007) against the Greengenes 16S rRNA database (McDonald et al. 2012). Since uneven sampling may affect statistical analyses, the sequence data was in each sample randomly resampled to the lowest number of reads (9544).

2.4 Statistical analysis

Chao 1 richness, the Shannon-Wiener index and Pielou's evenness based on the identified OTUs were estimated. The Mann-Whitney *U* test was used to compare the alpha diversity between sampling months (Mo et al. 2018). Beta-diversity was represented by Bray-Curtis distance matrices generated based on the OTU table (Bray & Curtis 1957). Principal component analysis (PCA) was used to analyze the difference in the environmental gradient between the sampling sections of the mainstream and tributaries of the Han River in different samples. Because the data is normally distributed, Pearson correlations were calculated between dendritic distance and relative abundance of bacterial phyla in the mainstream. The dendritic distance (km), which is a measure of the cumulative length of the branching river network (river length) of two sampling sites, was calculated using the ArcGIS V10.6 software (Chen et al. 2019).

SourceTracker, a Bayesian approach (Knights et al. 2011), was performed to infer the extent to which the bacterial communities in the water samples from the five tributaries (source) could explain that in the main stream (sink). The tool was able to identify the proportion of the microbial community in the mainstream that came from its tributaries. Therefore, the source referred to the five tributaries, while the sink referred to the mainstream.

Redundancy analysis (RDA) was performed to explore the relationship between environmental parameters and the bacterioplankton community composition (OTU). Before analysis, detrended correspondence analysis (DCA) was employed, and the longest gradients generated from DCA were

< 2, which indicates that RDA is suitable in the present study (Li et al. 2019). Before RDA, all environmental data (pH, Cond, T, DO, Turd, TP, TN, NH_4^+ -N, NO_3^- -N, NO_2^- -N and Chl-*a*) needed to be standardized, while biological data also needed Hellinger transformed. Environmental variables with variance inflation factors (VIFs) > 20 were removed to avoid collinearity among factors. Then, the function “envfit” was run with 999 permutations to select the significant parameters (pH, TN, Cond, NH_4^+ -N, DO, NO_2^- -N, Chl-*a* and T) (Liu et al. 2018). Finally, the significance testing was assessed by the “permutest” function based on 999 permutations. Bioenv analysis calculates spearman's rank correlation between Bray-Curtis distance matrices of bacterial community and Euclidian distance matrices of the environmental factors, and selects the best combination of environmental factors. Function ‘Bioenv’ in the R package ‘Vegan’ was also performed to identify the subsets of environmental factors that best predicted the bacterial community composition. With the exception of RDA, Pearson correlation and Bioenv analysis, all other analyses were performed with seasons separate. All statistical analyses were performed using R programming (version 3.6.2).

3. Results

3.1 Water chemical analysis

The basic physical and chemical data of the different water samples, obtained from upstream sites of the Han River and from its tributaries, were listed in Table 1. According to PCA results, samples were separated in different seasons (Fig. 2a), indicating that the environmental background values of rivers in two seasons had obvious difference, but the difference between rivers was not obvious. In the PCA results, the two extracted components accounted for 58.8% of the total variance.

3.2 Bacterioplankton diversity

A total of 610816 high quality reads and 14105 operational taxonomic units (OTUs) were collected by analyzing these 64 samples. The rarefaction curves illustrated that the OTUs recovered by the current sequencing depth were sufficient to represent the bacterioplankton communities (Fig. 2b). In addition, the Good's coverage in each sample was ranged from 96.96 to 99.99%, indicating the characteristic representation of microbial communities by the current bacterial profiles (Table 2). The total number of OTUs in November was 8,515, much higher than the 4,402 in April. And the OTUs shared by the two seasons accounted for 17.5% of all sample OTUs (Fig. 2c).

According to the Mann-Whitney *U* tests, the Pielou's evenness in April notably exceeded that in November ($p = 0.001$) (Table 2). In contrast, there is no significant difference on the Shannon index ($p = 0.2$) and the Chao1 richness index ($p = 0.07$). On the other hand, the Shannon index and Pielou's evenness increased slightly from headwaters towards river mouths in the upper reaches of the Han River, but Shannon index fluctuated widely among its tributaries.

3.3 Bacterial community at the phylum and genus level

The percentage of reads within different taxonomic groups are shown in Fig. 3. The most abundant genera were *Comamonadaceae_unclassified* (22.6%), *Flavobacterium* (13.4%), *Planktophila* (4.6%) and *Siphonobacter* (3.8%). The relative abundance of *Actinobacteria* increased significantly with the increase of river length in the mainstem of the Han River ($r = 0.96$, $p < 0.01$), while that of *Bacteroidetes* decreased slightly based on Pearson correlation analysis ($r = -0.65$, $p = 0.11$) (Fig. 4).

SourceTracker analysis based on the OTU table revealed that proportion of the microbial community in the mainstream came from its tributaries. Five tributaries were found to be importance sources of taxa to the Han River, however in both months a large proportion of OTUs (37.84% and 36.34%, respectively) had unknown sources (Fig. 5a, c). At the same time, Fig. 5 (b, d) shows that the proportion of OTUs of unknown origin generally increased in downstream stations.

3.6 Influence of environmental parameters on the bacterioplankton community

RDA was employed to analyze the correlation between OTU relative abundance and driving parameters ($p \leq 0.050$) (Fig. 6). Among the main factors (Table 3), pH ($p = 0.001$), TN ($p = 0.001$), Cond ($p = 0.001$), NH_4^+ -N ($p = 0.002$), DO ($p = 0.002$), NO_2^- -N ($p = 0.003$), Chl-*a* ($p = 0.027$) and T ($p = 0.039$) exhibited significant impact on the bacterioplankton community. Bioenv analysis in Table 4 showed that pH was the optimal single-parameter to explain the variance of OTU relative abundance (Bioenv correlation= 0.4629).

4. Discussion And Conclusions

It is reported that *Proteobacteria*, *Bacteroidetes* and *Actinobacteria* are three of the most abundant phyla in rivers (Wang et al. 2017, Liu et al. 2018). However, the relative abundance of *Actinobacteria* increased gradually from headwaters to lower reaches, while that of *Bacteroidetes* decreased gradually in the present study. However, there was no similar pattern of *Actinobacteria* abundance in the tributaries. At the same time, the rapidly growing *Bacteroidetes* based on the r-strategist lifestyles, utilized available resources in the ‘new’ environment of upper reaches of the river (Newton et al. 2011, Read et al. 2015). However, competition may become more intense in lower reaches, favoring species that were more competitive and had lower growth rates (Read et al. 2015). For example, the growth rate of Freshwater *Actinobacteria* was reported to be slower than other phyla

(Šimek et al. 2006). Furthermore, the size of *Actinobacteria* was small, increasing the difficulty for them to graze (Wang et al. 2019). All these factors were beneficial to relative abundance of *Actinobacteria* increased with the increase of river length. Water residence time was correlated with the length of the river network, in general, it took more time for surface water to travel throughout longer networks (Stewart et al. 2009). We speculated that this was why community succession occurred in the mainstream rather than in the tributaries.

Our results indicated that the diversity in the Han River increased slightly towards river mouths, with the lowest diversity observed from headwaters, which was consistent with Read et al. (2015). As a river flowed downstream, the bacterial communities were better adjusted to the 'natives' environment, further to develop and outcompete the transient 'vagabond' or 'tourist' species (Newton et al. 2011, Crump et al. 2012). This conclusion was confirmed by the slightly increased Pielou's evenness from headwaters towards river mouths, as well as the succession in bacterial composition of the planktonic community. However, this result was different from that reported by Savio et al. (2015), which was ascribed to the spatial scale of research activities. For example, a small-spatial scale (spanning from 2°8'W to 43°E) of taxa was studied by Read et al. (2015), whereas Savio's study focused on a large-scale analysis (ranging from 10 to 30°E). Along the 2,600-kilometer Danube River, which was bordered by many large cities, the disturbance to the bacterioplankton community had been greatly intensified. Our result was also inconsistent with previous studies of lakes proposing that bacterioplankton richness decreased with the direction of water flow (Ruiz-Gonzalez et al. 2015, Logue et al. 2012). The reason may be that bacterioplankton richness in the lake was affected by the nutrient availability (Cardinale et al. 2009), lake chain number (Kratz et al. 1997), lake area and catchment area (MacArthur & Wilson, 1967). For example, Kratz et al. (1997) hypothesized that lake's landscape location can determine the hydrology, geomorphology and biological characteristics of the lake, which may have an impact on bacterioplankton richness. Therefore, with shifts of hydrological channels, geomorphology and biological characteristics, bacterioplankton richness may change in dependence of lake chain number (Logue et al. 2012).

SourceTracker, a Bayesian approach has been applied to predict the source proportions (Knights et al. 2011), to identify microbial source of cesarean-born infants (Dominguez-Bello et al. 2016), and to evaluate water source contributions of microbial contamination (Henry et al. 2016). In our study, the tributaries were demonstrated to be the original microbial part of the main stream. The proportion of OTUs of unknown origin (according to the SourceTracker analysis) generally increased in the main stream from headwaters to lower reaches. The reason may be the increase of downstream tributaries and the enhancement of human activities (Li et al. 2009). First, as the number of tributaries from downstream into the mainstream increases, the bacteria from the tributaries will accumulate in the mainstream. And we only studied five of the many tributaries, and there were still many tributaries that had not been studied (Finn et al. 2011). Second, previous studies showed that human disturbance and farmland activities in the downstream were increasing, which would lead to the import of foreign bacteria (Li et al. 2009). Genera-*Acinetobacter* appeared in the downstream of the mainstream, and this kind of bacteria is generally more in urban wastewater (Newton et al. 2013). As a result, the proportion of unknown bacteria was accordingly increased.

The previous studies indicated that the surrounding conditions and available nutrient sources exhibited direct impact on the composition and biodiversity of microbial community in the river (Huang et al. 2011, Cole et al. 2012). RDA and Bioenv analysis showed that environmental parameters were correlated with the changes in the bacterioplankton community. pH can directly affect the growth of microorganisms and affect the community structure and diversity of microorganisms by changing the physical and chemical properties of water (Yannarell et al. 2005, Langenheder et al. 2006). Chl-*a* was a good indicator of the stock of phytoplankton (Bai et al. 2009), and studies found that planktonic bacteria were closely related to the community structure of phytoplankton (Kirchman et al. 1991, Teeling et al. 2012). Bacteria not only absorb the organic matter produced by phytoplankton but also provide organic nutrients and growth factors for phytoplankton. They may be mutually beneficial and symbiotic relationship (Zhu et al. 2018). Previous studies in freshwater environments found that nitrogen nutrients and phosphorus were key factors affecting the composition of microorganisms (Liu et al. 2012); however, our study demonstrated that phosphorus had little effect on the composition of microorganisms. This difference may be due to the use of phosphorus-containing detergents and chemical fertilizers, resulting in higher phosphorus concentrations, and nitrogen nutrients became a limiting factor affecting the distribution of microorganisms (Lü et al. 2011).

As the main source of water for the Middle Route of China's South-to-North Water Diversion Project, the upper reaches of the Han River are the water supply area of the Danjiangkou Reservoir. Hence, it is necessary to strengthen the monitoring of water quality in near cities, as well as to strictly control the inflow of domestic sewage and industrial wastewater. At the same time, rivers also require the strengthening of control over activities such as dredging. Some rivers are disturbed by activities such as dredging and agricultural production, which destroy the original habitat conditions and cause fluctuations in species diversity. The study also found that the river length affected the growth and survival of certain planktonic bacteria. Therefore, ecological assessment should be performed before building a dam to reduce the impact of the dam on the environment. The diversity and spatial distribution have been highlighted to evaluate the bacterioplankton communities in the main tributaries of the Han River. On the other hand, there results in this study provide an insightful reference to understand the bacterioplankton diversity, to clarify the environmental factors affecting the distribution of bacterioplankton diversity in the region, and to provide data support for further improvement of the water quality in the region. In addition, a large number of bacterioplankton have not been identified, and subsequent development of functional bacterioplankton resources in the region can be enhanced by combining new technologies such as metagenomics.

Declarations

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Authors' contributions

All authors contributed to the study conception and design. **He Sun:** Investigation, Writing - original draft, Writing - review & editing, Visualization. **Baozhu Pan:** Investigation, Writing - original draft, Writing - review & editing, Visualization. **Haoran He:** Investigation, Writing - review & editing, Visualization. **Gengnan Zhao:** Writing - review & editing, Supervision. **Xiaoming Jiang:** Investigation, Writing - review & editing. **Xu Han, Hao Wang:** Conceptualization, Writing - review & editing, Supervision, Project administration. All the authors read and approved the manuscript.

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Data availability

Data that support the findings of this study have been deposited in the public National Center for Biotechnology Information (NCBI) with the accession codes: SRP142494.

Compliance with ethical standards

Competing interests

The authors declare that they have no competing interests

Ethical approval and consent to participate

Not applicable

Consent to publish

Not applicable

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Tables

Table 1 Water environmental parameters (mean ± standard deviation) of the survey sample in mainstem and tributaries of the Han River

Notes: temperature=T; conductivity=Cond; dissolved oxygen=DO; turbidity=Turd; total nitrogen=TN; ammonia nitrogen= $\text{NH}_4^+\text{-N}$; nitrate nitrogen= $\text{NO}_3^-\text{-N}$; nitrite nitrogen= $\text{NO}_2^-\text{-N}$; total phosphorus=TP; and chlorophyll *a*= Chl *a*.

Sampling time	Rivers	T	pH	Cond	DO	Turd	TN	$\text{NH}_4^+\text{-N}$	$\text{NO}_3^-\text{-N}$	$\text{NO}_2^-\text{-N}$	TP	Chl <i>a</i>
		°C		$\mu\text{S cm}^{-1}$	mg L^{-1}	NTU	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg m^{-3}
In November 2017	Upper reaches of the Han River	14.56±	8.37±	382±112	9.56±	31.28±	3.53±	0.53±	2.45±	0.03±	0.050±	1.75±
		1.51	0.14		0.79	15.12	0.23	0.14	0.19	0.02	0.015	1.16
	Xushui River	12.75±	8.49±	192±54	10.37±	2.75±	1.65±	0.49±	1.12±	0.03±	0.048±	3.39±
		0.61	0.17		0.25	1.10	0.75	0.01	0.74	0.01	0.007	1.63
	Jinshui River	11.02±	8.57±	187±88	10.58±	2.77±	0.98±	0.49±	0.47±	0.02±	0.036±	1.48±
		1.35	0.03		0.19	2.49	0.19	0.02	0.18	0.01	0.004	1.09
	Yue River	15.36±	8.39±	384±33	9.83±	33.90±	1.97±	0.50±	1.33±	0.03±	0.083±	4.09±
		1.17	0.18		0.48	32.20	0.10	0.01	0.09	0.02	0.011	1.57
	Xun River	14.06±	8.63±	356±140	9.82±	8.21±	1.92±	0.33±	1.45±	0.02±	0.055±	2.67±
		2.65	0.14		0.42	3.83	0.26	0.19	0.25	0.01	0.005	1.22
	Jinqian River	14.69±	8.76±	347±58	9.62±	7.88±	1.96±	0.39±	1.42±	0.02±	0.050±	3.86±
		1.58	0.29		0.56	6.93	0.34	0.18	0.31	0.01	0.006	2.71
In April 2018	Upper reaches of the Han River	15.40±	7.38±	221±22	8.58±	37.29±	2.17±	0.70±	1.10±	0.11±	0.071±	3.08±
		2.30	0.14		0.62	16.88	0.30	0.16	0.48	0.09	0.008	1.45
	Xushui River	12.52±	7.49±	137±38	9.23±	23.93±	1.99±	0.58±	1.39±	0.05±	0.068±	2.46±
		0.84	0.17		0.42	9.95	0.27	0.15	0.61	0.04	0.005	0.41
	Jinshui River	15.32±	7.56±	145±64	9.82±	7.80±	1.79±	0.76±	0.70±	0.05±	0.042±	1.45±
		3.50	0.03		1.04	5.86	0.28	0.23	0.36	0.03	0.008	0.33
	Yue River	22.36±	7.40±	198±29	8.78±	85.46±	2.49±	1.44±	0.84±	0.06±	0.086±	2.37±
		2.49	0.18		1.09	64.83	0.43	0.08	0.37	0.05	0.014	0.72
	Xun River	17.84±	7.66±	196±67	9.21±	20.60±	1.91±	0.95±	0.58±	0.09±	0.055±	3.76±
		2.96	0.11		0.46	7.91	0.28	0.31	0.28	0.06	0.004	1.65
	Jinqian River	21.02±	7.78±	260±45	8.30±	19.80±	2.29±	0.58±	0.68±	0.14±	0.062±	3.31±
		2.89	0.27		0.31	5.40	0.22	0.12	0.22	0.10	0.008	1.27

Table 2 Summary of alpha diversity indices, including Chao1, Shannon, Pielou and Good's coverage

Sample ID	Month	No. of OTU	Chao1	Shannon	Pielou	Good's coverage
HJ1	November	634	676	7.01	0.75	99.08%
HJ2	November	691	716	8.13	0.86	99.44%
HJ3	November	1230	1420	8.45	0.81	96.96%
HJ4	November	843	891	8.16	0.83	98.93%
HJ5	November	1150	1205	9.33	0.92	98.71%
HJ6	November	648	653	8.27	0.89	99.77%
HJ7	November	869	937	8.37	0.85	98.71%
XS1	November	340	341	7.33	0.87	99.94%
XS2	November	448	458	7.32	0.83	99.71%
XS3	November	401	406	7.31	0.84	99.84%
XS4	November	426	450	6.29	0.70	99.39%
XS5	November	373	390	6.48	0.76	99.68%
JS1	November	1199	1348	8.06	0.78	97.37%
JS2	November	1225	1324	9.13	0.89	97.96%
JS3	November	1252	1305	9.64	0.94	98.68%
JS4	November	319	319	7.46	0.90	99.99%
JS5	November	418	426	7.01	0.81	99.74%
Y1	November	190	190	6.61	0.87	99.99%
Y2	November	496	525	6.43	0.71	99.33%
Y3	November	544	552	7.88	0.86	99.72%
Y4	November	582	602	7.50	0.81	99.56%
Y5	November	638	743	6.76	0.70	98.31%
X1	November	477	479	8.17	0.92	99.91%
X2	November	524	537	7.86	0.87	99.75%
X3	November	255	256	6.68	0.83	99.94%
X4	November	459	474	6.98	0.78	99.63%
X5	November	495	510	7.49	0.83	99.62%
JQ1	November	935	990	8.94	0.90	98.89%
JQ2	November	546	555	7.91	0.87	99.69%
JQ3	November	800	881	7.41	0.76	98.60%
JQ4	November	459	486	6.28	0.70	99.48%
JQ5	November	1094	1186	8.74	0.86	98.21%
HJ1	April	530	538	7.24	0.80	99.69%
HJ2	April	687	706	8.70	0.92	99.63%
HJ3	April	647	669	8.16	0.87	99.47%
HJ4	April	722	728	8.73	0.92	99.75%
HJ5	April	594	608	8.58	0.93	99.70%
HJ6	April	583	597	8.61	0.94	99.73%
HJ7	April	482	486	8.08	0.90	99.83%
XS1	April	782	794	9.00	0.94	99.66%

XS2	April	390	393	7.62	0.89	99.88%
XS3	April	499	509	8.36	0.93	99.79%
XS4	April	552	568	7.40	0.81	99.59%
XS5	April	728	744	8.75	0.92	99.57%
JS1	April	694	706	8.54	0.90	99.66%
JS2	April	500	504	8.51	0.95	99.90%
JS3	April	958	976	9.38	0.95	99.46%
JS4	April	388	390	7.35	0.85	99.90%
JS5	April	380	385	7.18	0.83	99.81%
Y1	April	603	615	8.44	0.91	99.69%
Y2	April	333	339	6.79	0.81	99.84%
Y3	April	415	422	7.93	0.91	99.86%
Y4	April	644	674	8.75	0.94	99.57%
Y5	April	601	604	8.67	0.94	99.84%
X1	April	470	471	8.27	0.93	99.94%
X2	April	504	506	8.17	0.91	99.90%
X3	April	373	379	7.23	0.84	99.80%
X4	April	281	286	7.53	0.93	99.94%
X5	April	341	343	7.35	0.87	99.90%
JQ1	April	578	597	8.33	0.91	99.71%
JQ2	April	379	383	6.40	0.75	99.85%
JQ3	April	292	303	5.42	0.66	99.79%
JQ4	April	311	318	6.74	0.81	99.86%
JQ5	April	314	325	6.84	0.82	99.81%

Table 3 Effects of geographical and environmental factors on bacterial community composition

Variables	r ²	Pr(>r)
pH	0.2063	0.001
TN	0.1149	0.001
Cond	0.1163	0.001
DO	0.0956	0.002
NH ₄ ⁺ -N	0.0834	0.002
NO ₂ ⁻ -N	0.0579	0.003
Chl- <i>a</i>	0.0294	0.027
T	0.0137	0.039

Table 4 Correlations between environmental variables and OTU relative abundance

Environmental variables	Correlation
pH	0.4629
pH+Cond	0.5654
pH+Cond+DO	0.5468
pH+Cond+NH ₄ ⁺ -N+Tur	0.5477
pH+ Cond+NH ₄ ⁺ -N+ Do+ TN	0.5502
pH+ Cond+ NH ₄ ⁺ -N + Do+ TN+Tur	0.5569
pH+ Cond+ NH ₄ ⁺ -N + Do+ TN+Tur+TN	0.5444
pH+ Cond+ NH ₄ ⁺ -N + Do+ TN+Tur+TP+ NO ₃ ⁻ -N	0.5310

Figures

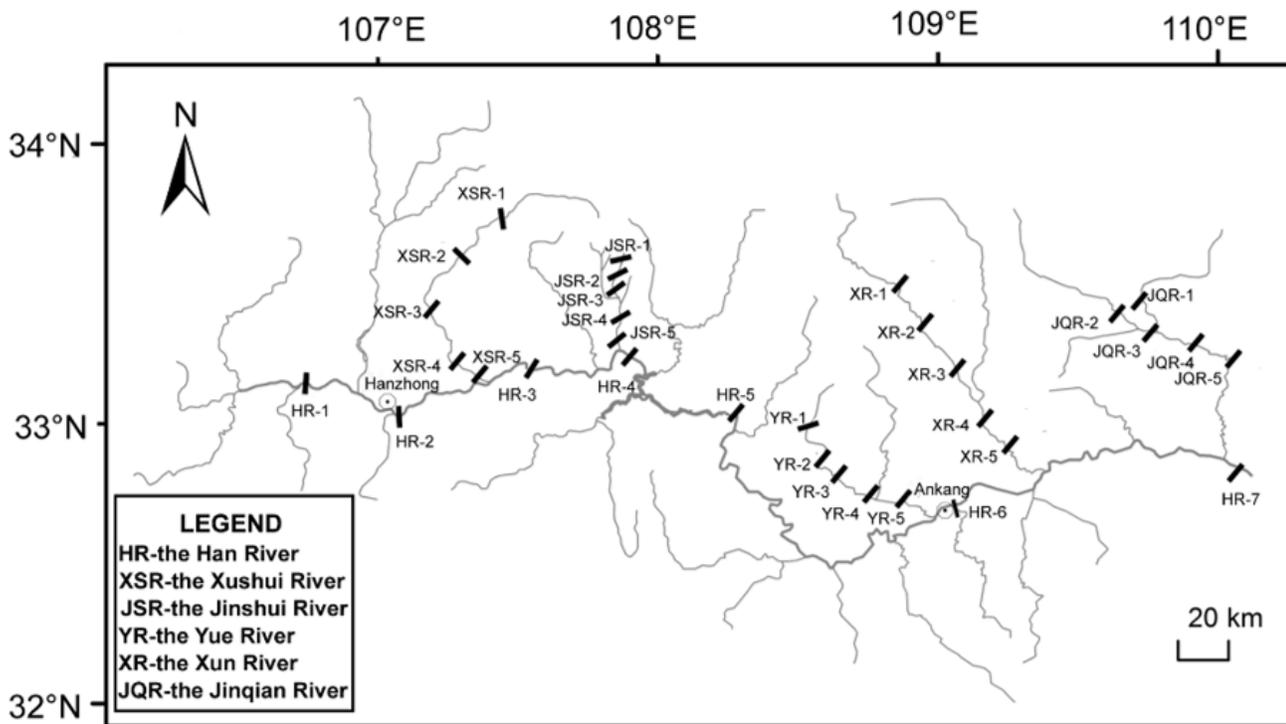


Figure 1

Distribution of sampling sections in the mainstem and tributaries of the Han River. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

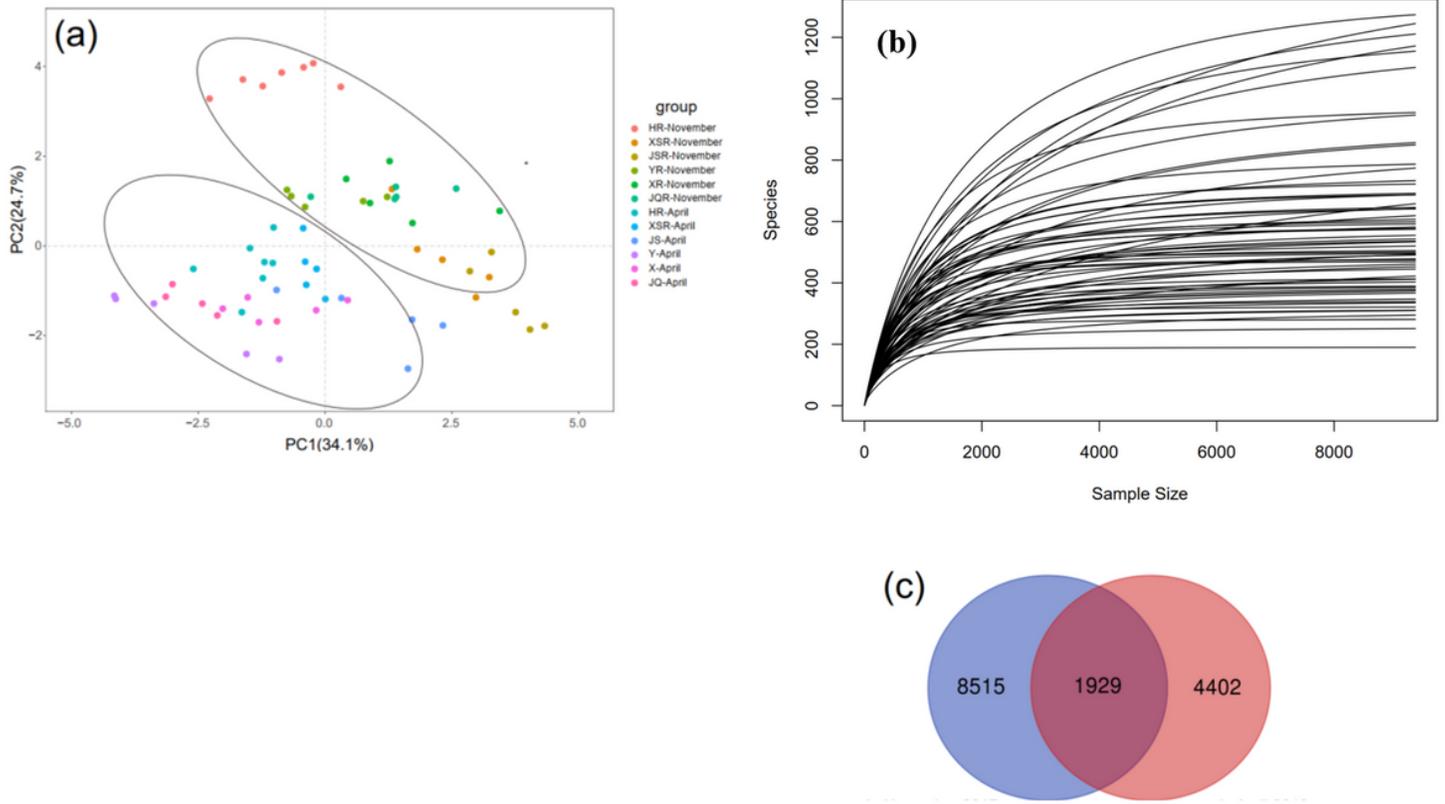


Figure 2

(a) PCA ordination of the physico-chemical parameters in the Han River and its tributaries. (b) Rarefaction curves of bacterial richness of each sample (c) Venn diagram showing the number of OTUs that are unique and shared between two sampled months.

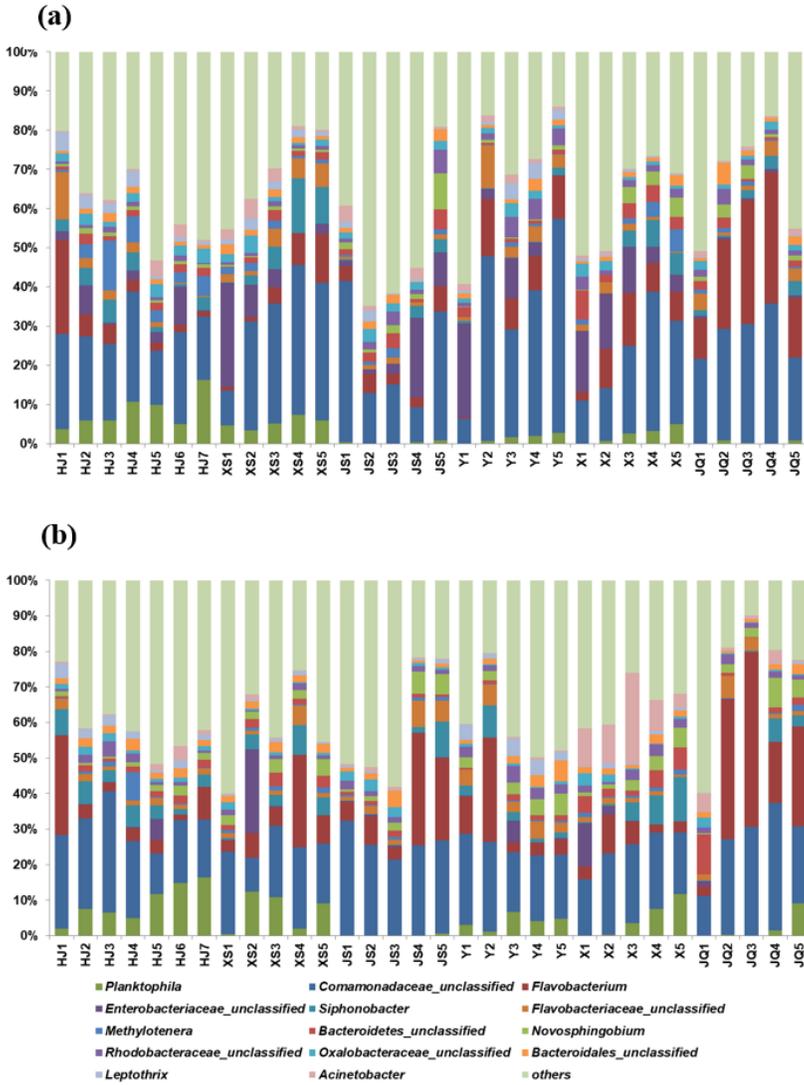


Figure 3

Genus-level taxonomic composition of the bacterioplankton communities in the mainstem and tributaries of the Han River: (a) in November 2017, (b) in April 2018 (Bacterial taxa with less than 1% of mean relative abundance are classified as others)

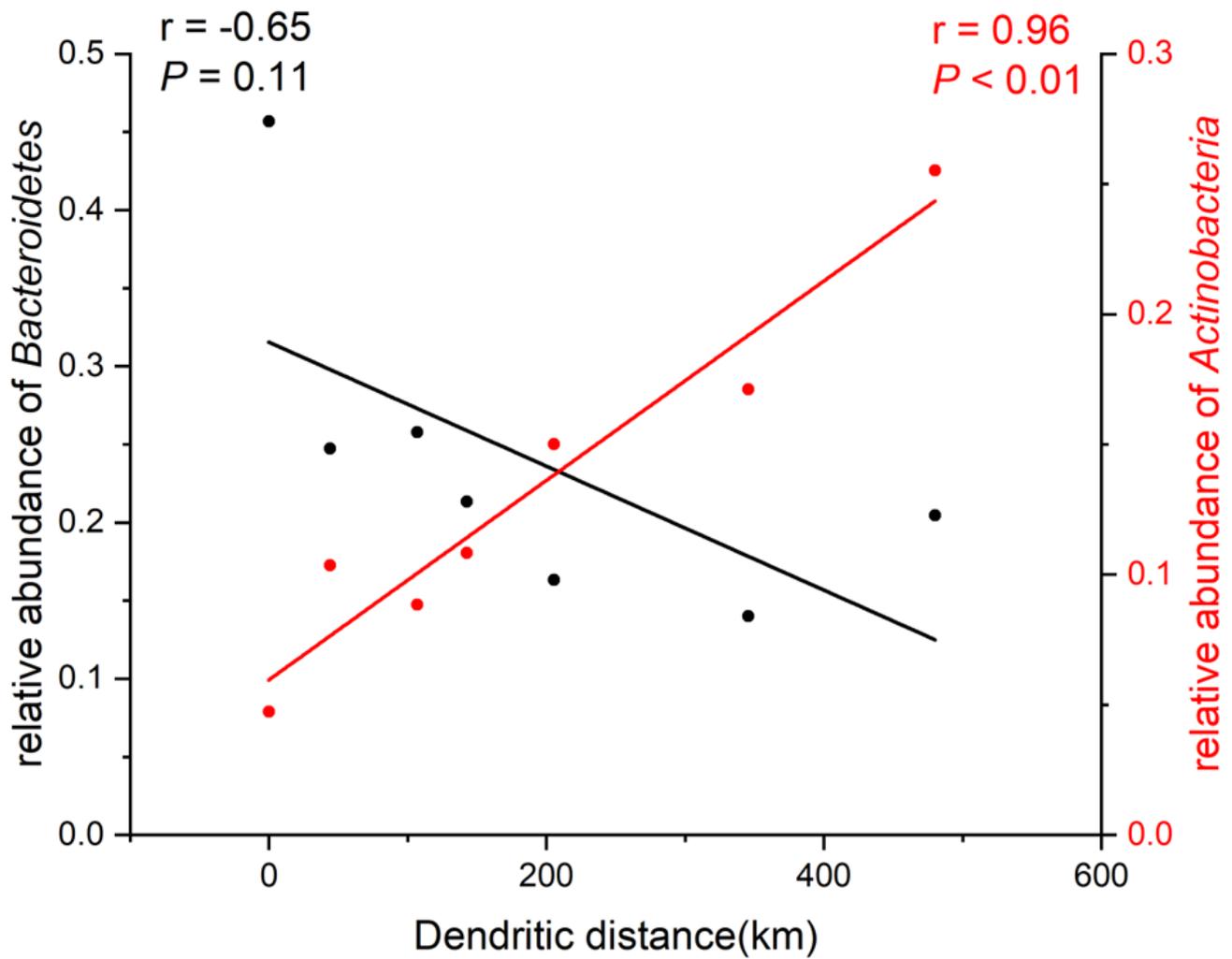


Figure 4
 Pearson correlation between dendritic distance (river length) and relative abundance of Bacteroidetes and Actinobacteria in the mainstream

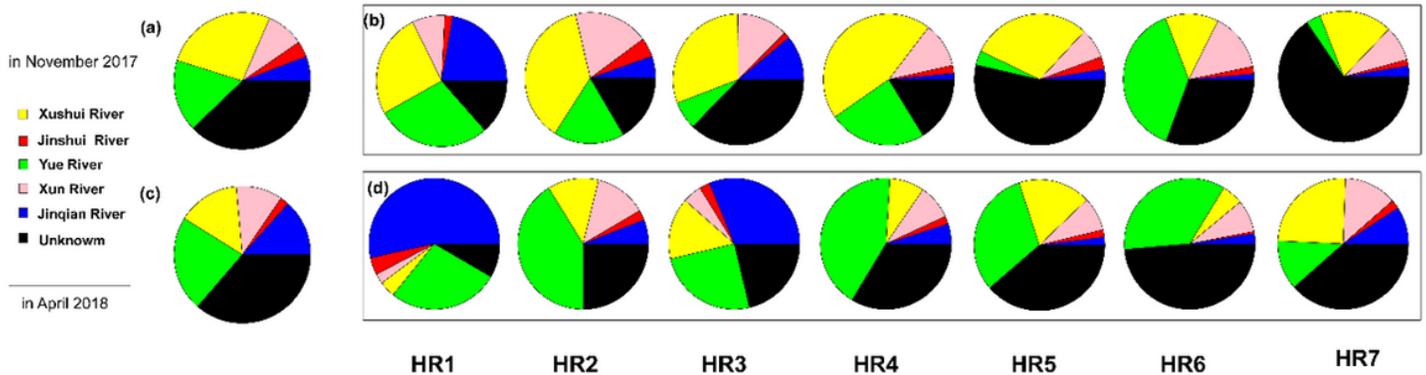


Figure 5
 SourceTracker proportion estimates for the microbial community in the mainstream that came from its tributaries (a, c) Han River, (b, d) different sections

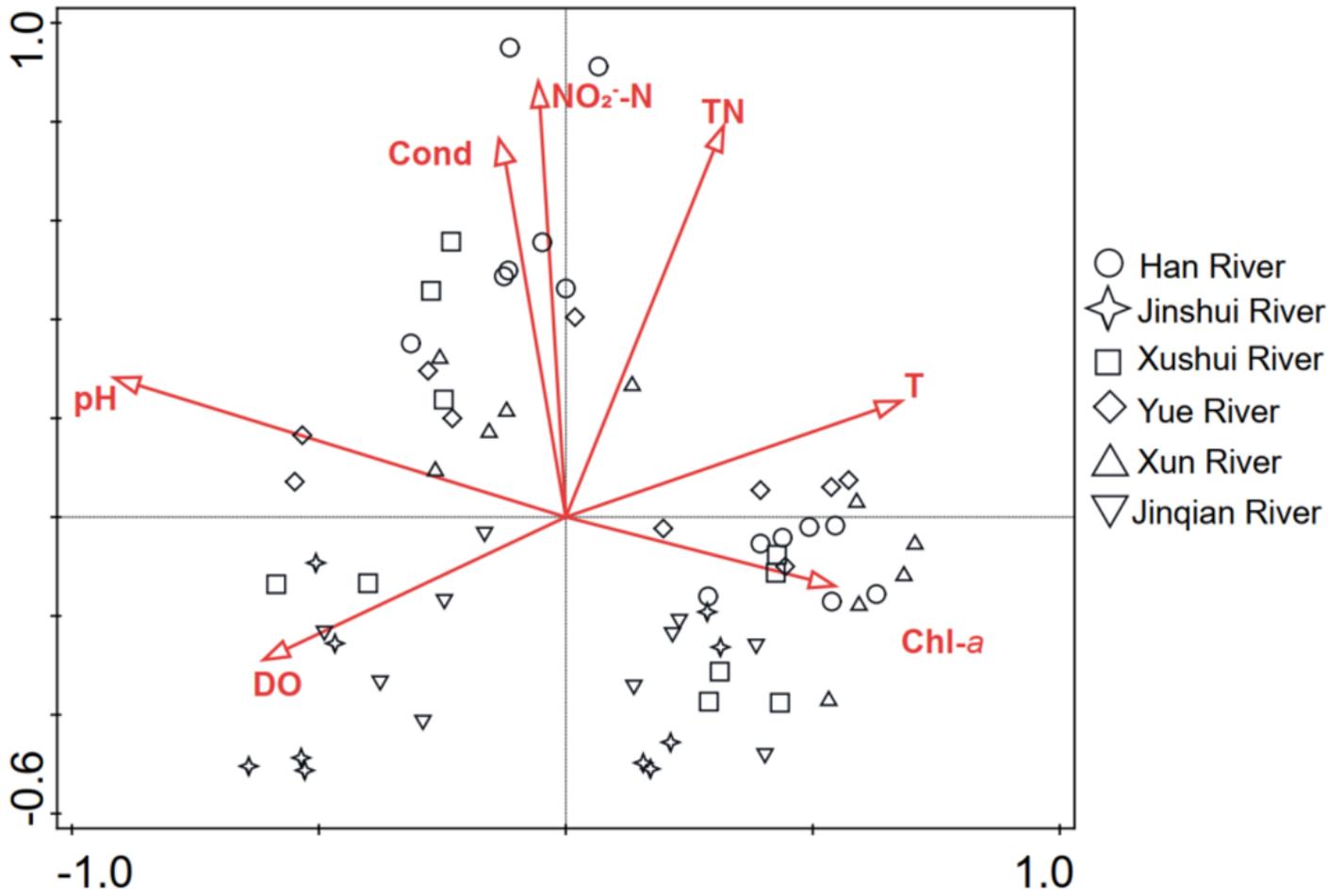


Figure 6

Redundancy analysis (RDA) of bacterioplankton (OTU) and environmental parameters