Spatiotemporal Correlations Between Water Quality And Microbial Community of Typical Inflow River Into Taihu Lake, China

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Abstract

Changxing River, which is a typical inflow river into the Taihu Lake and occurs severe algae invasion, is selected to study the effect of different pollution sources on the water quality and ecological system. Four types of pollution sources, including the estuary of Taihu Lake, discharge outlets of urban wastewater treatment plants, storm water outlets, and non-point source agricultural drainage areas are chosen, and next-generation sequencing and multi-variate statistical analyses are used to characterize the microbial communities and reveal their relationship with water physicochemical properties. Results showed that ammonia nitrogen ($\text{NH}_4^+\text{-N}$), total nitrogen (TN), and total phosphorus (TP) are the main pollutant in Changxing River, especially at storm water outlets. At the same time, the diversity of microbial communities was the highest in the summer, and dominant phyla included *Proteobacteria* (40.9%), *Bacteroidetes* (21.0%) and *Euryarchaeota* (6.1%) under the condition of algal bloom. Water temperature (T), air pressure (P), concentrations of TP and COD_{Mn} were the important variables for the succession of microbial community. From the perspective of different pollution types, relative abundances of *Microcystis* and *Nostocaceae* at the estuary of Taihu Lake were correlated positively with dissolved oxygen (DO) and pH, and *Pseudomonas* and *Arcobacter* were correlated positively with concentrations of TN and nitrate nitrogen ($\text{NO}_3^-\text{-N}$) at storm water outlets. The results provide a reference for the impact of pollution types on river microbial ecosystem under complex hydrological condition and a guidance for the selection of restoration techniques for polluted rivers entering an important lake.

1 Introduction

Over the past decades, rapid industrialization and urbanization around the Taihu Lake Basin in China have caused severe water pollution issues, attracting the considerable attention (Yang et al., 2013; Zhang et al., 2019c). According to the Health Status Report of Taihu Lake in 2018, the average comprehensive trophic level index (TLI) was 60.3, indicating moderate eutrophication (The higher the index value, the more nutritious it is). The annual total nitrogen in the most polluted area in Taihu Lake was above 2.0 mg/L, and the average concentration of chlorophyll a was 31.5 mg/m³. The input and retention of high-load pollutants have caused the eutrophication and frequent algae outbreaks in Taihu Lake.

Based on the statistics of Taihu Lake Basin and Southeast Rivers Water Resources Bulletin in 2018, 10 of 22 major inflow rivers around the Taihu Lake were contaminated, with COD above 20 mg/L and TN above 1.0 mg/L. Previous studies (Wang et al., 2007; Rodriguez-Mozaz et al., 2015; Zhou et al., 2016a; Du et al., 2017; Zhang et al., 2019c) showed that inflow rivers were the main sources that contribute to eutrophication, algae blooms, and water quality deterioration in downstream receiving lakes (reservoirs), especially for Taihu Lake. Wang et al. (2011) calculated that more than 90% of the total nutrient load of COD, ammonia nitrogen, total nitrogen, and total phosphorus in Taihu Lake were attributed to the rivers in the Changxing County and Huzhou Urban Area. The highest concentration of $\text{NO}_3^-\text{-N}$ in Changxing River reached 14 mg/L (Yang et al., 2013). Since the water diversion from Yangtze River to Taihu Lake was implemented in 2002, the water level of Taihu Lake has risen each year, which is worsened by the over-

exploitation and utilization of water resources and meteorological factors, such as precipitation, wind velocity, and wind direction (Liu et al., 2013). Frequent backwatering of the lake and serious accumulation of cyanobacterial blooms in the river flowing into the lake also weaken the natural restoration. Over the past years, water quality of the section of Changxing River remain unstable without meeting the environmental quality standard for surface water in China. Therefore, the development of efficient polluted river restoration technology is needed to prevent and control eutrophication of Taihu Lake.

Microorganisms exist in river ecosystems either in the suspended phase or attached to biofilms (Battin et al., 2016). The composition and structure of microorganisms in river ecosystems are highly dynamic. They can reflect different ecological functions and show a high degree of sensitivity to changes of environmental condition (Lundgard et al., 2017), and are recognized as one of the important indicators for the stability of aquatic ecosystems (Liao et al., 2018; Sharuddin et al., 2018; Santos et al., 2019). A number of studies noted that the effect of seasons on microbial community in rivers were greater than locations (Zhang et al., 2019a, b; Zhu et al., 2019). Microbial community changes generally follow a pattern of periodic recurrence and exhibit a predictable temporal pattern in a single habitat (Gilbert et al., 2012; Portillo et al., 2012). Zhang et al. (2012) found that there was a significant difference in microbial community compositions between samples collected in the lower temperature months (December to February of the following year) and the higher temperature months (June to October) ($R = 0.102$, $p < 0.005$). Further, the relative abundance of cyanobacteria in Fast River during the rainy season was about 10 times higher than that in dry season, and the abundance of actinomycetes in the dry season was much higher than that in rainy season by contrast (Sun et al., 2017). However, other studies have found that the differences in the spatial pattern of microbial communities may be greater than the seasonal differences (Ouyang et al., 2020). For instance, a significant positive correlation was reported between the *Gammaproteobacteria* and TN in Chaohu Lake, China, which suggested their roles in nitrogen transformation and cycling in aquatic ecosystems (Zhang et al., 2020). However, studies have also shown that small phytoplankton with low nutrient requirements for nitrogen and phosphorus could become dominant algal species in oligotrophic waters (Jiang et al., 2015).

Therefore, correlating physiochemical characteristics with changes in the microbial communities provides hypotheses as to the ecological function of rivers and likely successful remediation strategies. In this study, Changxing River was selected as a major inflow river located at South Taihu Lake Basin. We collected four types of impacted sites, including the estuary of Taihu Lake, discharge outlets of urban wastewater treatment plants, storm water outlets, and non-point source agricultural drainage areas over four seasons. The microbial communities, water physicochemical properties, and their spatiotemporal correlation was investigated. The results provide a reference for the impact of pollution types on river microbial ecosystem under complex hydrological condition and a guidance for the selection of restoration techniques for polluted rivers entering an important lake.

2 Materials And Methods

2.1 Study area and sampling sites

Changxing River is one of the main inflow rivers in south Taihu Basin. It plays a vital role in water supply, irrigation, tourism and fishery, and is one of the major navigable rivers in Zhejiang Province (Liu et al., 2011; Wu et al., 2018). However, there are many sources of pollution along the river, such as farmland, residences, catering, industry and urban wastewater treatment plants. Continuous discharge of irrigation water, urban sewage, and rainwater runoff is conducive to complex and serious water pollution in Changxing River.

Based on pollution sources along the Changxing River, the pollution types were divided into four categories: the estuary of Taihu Lake (I), discharge outlets of urban wastewater treatment plants (II), storm water outlets (III), and non-point source agricultural drainage areas as (IV). For each type, we selected 2-3 sites for water sample collection in the summer (August 2018), autumn (October 2018), winter (January 2019), and spring (March 2019), respectively. Geographic locations of the sampling sites are described in Table 1 and depicted in Fig. 1.

Table 1
Location of sampling sites along the Changxing River

Type	No.	Name of sites	Geographic locations	
			East longitude	North latitude
I	1	XinTang section	119.992641	31.032865
	2	Hexixingang	119.986131	31.054735
II	3	Shenchang sewage treatment plant	119.944546	31.007868
	4	Xingchang sewage treatment plant	119.930341	31.002718
III	5	Cangqian sluice	119.911909	30.999738
	6	FengHuang Community	119.901406	31.000428
	7	ShuiMuHuaDu Community	119.888209	30.999821
IV	8	Xizhuang	119.856806	30.986991
	9	Changqiao	119.865614	30.984250

Water samples were collected using a plexiglass sampler. At each sampling site, three water samples were taken in 500-mL bottles. After acidification to pH < 2 with H₂SO₄, samples were stored at 4°C prior to physicochemical analysis. 1 L of surface water at each sampling site was also collected into glass bottles for algae examination by microscopy. Another 1 L of surface water at each sampling site was sampled and filtered through 0.22-µm glass fiber filters (50 mm diameter, Shanghai) by vacuum filtration to collect the microorganisms. The filters were immediately stored at -80°C in the laboratory prior to DNA extraction (Liao et al., 2018).

2.2 Physicochemical analysis methods

The water temperature (T), air pressure (P), dissolved oxygen (DO), electrical conductivity (EC), total dissolved solids (TDS), salinity (SAL), pH, and oxidation reduction potential (ORP) were measured *in situ* at each site by using a multi-parameter water quality monitor (YSI Professional Plus, USA). Total Organic Carbon (TOC) and total nitrogen (TN) were measured by TOC analyzer (TOC-VCPH, SHIMADZU). Nessler's reagent spectrophotometry, N-(1-naphthyl) ethylene diamine dihydrochloride spectrophotometric, ultraviolet spectrophotometry and ammonium molybdate spectrophotometric methods were used to determine the concentrations of ammonia nitrogen ($\text{NH}_4^+\text{-N}$), nitrate ($\text{NO}_3^-\text{-N}$), nitrite ($\text{NO}_2^-\text{-N}$), and total phosphorus (TP) in surface water, respectively (State Environmental Protection Administration of China., 2002). All physicochemical parameters were calculated as the average of triplicate measurements.

2.3 Analysis of microbial community

The PowerSoil DNA extraction kit (MoBio Laboratories, CA, USA) was used to extract the DNA of microorganisms filtered on water filtration membranes. The V3-V4 region of 16S rRNA was amplified using universal primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') (Liao et al., 2018). All PCR reactions were performed using Phusion® high-fidelity PCR Master Mix (New England Biolabs). The PCR products were then purified using GeneJET™ Gel extraction kit (Thermo Scientific), and finally sequenced using the Ion S5™ XL platform.

Sequence analysis was performed by the Uparse software (Version 7.0.1001), which assigned sequences with $\geq 97\%$ similarity to OTUs for species annotation. Finally, the diversity indexes were estimated using QIIME (Version 1.7.0).

2.4 Statistical analysis

Principal Component Analysis (PCA) was analyzed using the IBM SPSS Statistics 20 software to pre-process the data. Spearman's rank correlation after Shapiro-Wilk test was used to analyze the correlation of water quality physicochemical indicators. One-way ANOVA, Kruskal Wallis test, and ANOSIM were used to determine the significance of differences in physicochemical indicators of water quality and relative microbial abundance in different seasons and pollution types with SPSS, GraphPad Prism, and R (Version 3.5.2). Canoco 4.5 redundancy analysis (RDA) was used to determine the relationship between the environment variables and microbial community structures. The ward.D was used to perform clustering analysis (CA) based on the relative abundance of microbial communities. BIOENV correlation analysis was used to study the relationship between microbial community structure and the environment variables in different seasonal and identify the best combination of environmental variables.

3 Results And Discussion

3.1 Spatial and temporal variations of water quality characteristics

As shown in Fig. 2a, the change trend of the average water temperature (T) in Changxing River was summer (31.3 °C) > autumn (22.6 °C) > spring (14 °C) > winter (7.6 °C). pH was neutral to slightly alkaline (7.0-7.9), which may be related to the nature of the upstream soil (Guo, 2017), or affected by the surface runoff of irrigation from farmland (Feng et al., 2017). The concentrations of DO in summer and autumn were 3.98 mg/L and 3.49 mg/L, respectively. They were both lower than the Class III standard limit (≥ 5 mg/L) of the surface water environmental quality standard (GB3838-2002), and significantly ($p < 0.0001$) lower than those in winter and spring (9.50, 7.77 mg/L). There was a significant negative correlation between DO and T ($r = -0.790$, $p < 0.01$), indicating that DO was generally higher in the dry season due to the influence of rainfall and temperature (Shi et al., 2017).

The concentration of TN in autumn was the highest (4.99 mg/L), followed by spring (4.50 mg/L), winter (4.31 mg/L) and summer (3.22 mg/L). The concentration of $\text{NH}_4^+\text{-N}$ in autumn was also the highest (1.92 mg/L). In other seasons, $\text{NH}_4^+\text{-N}$ concentrations also exceeded the limit of Class III of the environmental quality standard for surface water (≤ 1 mg/L). $\text{NO}_3^-\text{-N}$ was the main form of inorganic nitrogen in the water body of Changxing River (58%). The concentrations of $\text{NO}_3^-\text{-N}$ in summer and autumn (1.65 and 1.96 mg/L, respectively) were significantly lower than those in winter and spring (2.74 and 3.06 mg/L, respectively). The concentrations of TP in summer and autumn (0.39 and 0.44 mg/L, respectively) were higher than those in winter and spring (0.16 and 0.18 mg/L, respectively).

ANOVA analysis in Fig. 2b found that T, P, pH, ORP, $\text{NO}_2^-\text{-N}$, and $\text{NO}_3^-\text{-N}$ didn't change significantly among the four pollution types ($p > 0.05$). The concentrations of TDS, EC, and SAL in water samples from the discharge outlets of urban wastewater treatment plants (II) were 307 mg/L, 422 $\mu\text{S/cm}$ and 0.24 ng/L, respectively, which were all higher than other pollution source types. It was mainly related to the effluent composition of the sewage treatment plant. The concentrations of TN, $\text{NH}_4^+\text{-N}$, TP, TOC, and COD_{Mn} were the highest at the monitoring points of the storm water outlets (III), indicating that the pollution of the storm water outlets (III) was relatively serious. The average concentrations of TN, $\text{NH}_4^+\text{-N}$, and TP in water samples from the storm water outlets (III) were 5.30, 2.19, and 0.55 mg/L, respectively, which were 1.2-1.7, 2.4-5.2 and 3.0-3.9 times higher than those for other pollution types ($p < 0.01$). According to the statistical analysis, the $\text{NH}_4^+\text{-N}$ emissions of domestic sewage in Changxing County (597 tons) accounted for 89.6% of the total $\text{NH}_4^+\text{-N}$ emissions in 2018, indicating that the rain sewage discharge at the storm water outlets (III) had an impact on the concentration increase of TN, $\text{NH}_4^+\text{-N}$ and TP in Changxing River sections.

In addition, the lowest amount of DO was found at the estuary of Taihu Lake (I) during the autumn cyanobacteria bloom (2.20 mg/L) in Fig. 2c. Simultaneously, comparing with other seasons and pollution types, the concentrations of $\text{NH}_4^+\text{-N}$ and TP were particularly prominent at the storm water outlets (III) in autumn (3.62 and 0.99 mg/L, respectively). The issue of commingling discharge of rain precipitation and municipal sewage at the storm water outlets (III) in autumn had a particularly significant impact on the nutrient level in Changxing River.

In general, the average concentrations of $\text{NH}_4^+\text{-N}$, TN, and TP in Changxing River in autumn were 1.92, 4.99, and 0.44 mg/L, respectively, which were the highest. The sites with the highest concentrations of nutrient pollution occurred in the storm water outlets (III) with $\text{NH}_4^+\text{-N}$, TN, and TP concentrations of 2.19, 5.30 and 0.55 mg/L, respectively.

3.2 Analysis of microbial community in Changxing river

The Shannon index characterizes the diversity of microbial community, whereas the Chao1 index represents the species richness in microbial communities (Ren et al., 2018). The results showed that seasonal variation was the main factor affecting the diversity of microbial community (Fig. 3). The diversity of aquatic microbial communities in summer was the highest, with Shannon and Chao1 indexes of 9.4 and 3,278, respectively. In summer, high temperature can enhance the activity of microorganisms, and surface runoff from precipitation can introduce microorganisms and nutrients from the upstream (Feng et al., 2016), contributing to the highest diversity.

The estuary of Taihu Lake (I) had the highest Shannon index (8.2), while the non-point source agricultural drainage areas (IV) had the lowest Shannon index (7.1). The Chao1 index of the discharge outlets of urban wastewater treatment plants (II) was the highest (1,974), while the Chao1 index of the non-point source agricultural drainage areas (IV) was the lowest (1502). According to the analysis, the geographic location of the estuary of Taihu Lake (I) and the effluent sludge of the urban wastewater treatment plants (II) both affected the diversity of the microbial population of the sewage body (Lu et al., 2016; Tang et al., 2016). In general, the diversity of the microbial community at the monitoring points of different pollution types was not significant. The functional redundancy between microbial groups and the hydraulic distribution of rivers may stabilize microbial diversity against environmental fluctuations (Zeglin, 2015).

Proteobacteria was the dominant phylum across all monitoring sites (accounting for 45.8% on average), followed by *Bacteroidetes* (18.9%), *Cyanobacteria* (12.8%), *Firmicutes* (4.6%), *Actinobacteria* (4.0%), *Euryarchaeota* (1.7%), *Chloroflexi* (1.6%), *Acidobacteria* (1.5%), *Verrucomicrobia* (1.0%), and *Nitrospirae* (0.9%). According to the Kruskal-Wallis Test, the dominant phyla in summer included *Proteobacteria* (40.9%), *Bacteroidetes* (21.0%), *Euryarchaeota* (6.1%). Further, the relative abundance of *Euryarchaeota* (6.12%, $p < 0.001$) and *Nitrospirae* (3.13%, $p < 0.001$) in summer were significantly higher than other seasons. Analysis of similarities (ANOSIM) revealed significant differences in the relative abundance of dominant microbial community ($R = 0.62$, $p = 0.001$) between different seasons.

Note that the relative abundance of *Cyanobacteria* was the highest at the non-point source agricultural drainage areas (IV, 32.3%) (Fig. 4) due to the influence of agricultural non-point source nitrogen pollution load. This phenomenon was especially prominent in autumn. At the estuary of Taihu Lake (I), *Cyanobacteria* were highly enriched, in autumn and spring, at the highest of 41.9% in spring. This was in good agreement with previous studies reporting the highest algae bloom in March at the estuary of Taihu Lake (I) (Hampel et al., 2018). At the same time, the backflow and retention of inflow-rivers are more frequent during normal and dry seasons (i.e., spring). Therefore, *Cyanobacteria* in Changxing River are

attributed to the eutrophication of the non-point source agricultural drainage areas in autumn and backflow of algae in Taihu Lake in spring.

However, *Proteobacteria* was relatively abundant in water samples collected at the discharge outlets of urban wastewater treatment plants (II, 53.8%) and the storm water outlets (III, 55.2%), which may be related to their roles in nitrification, denitrification, and other functions.

At the genus level, more taxa showed distinctive differences in their relative abundances among different seasons or pollution types. The heatmap in Fig. 6 showed the genera with the top 80 relative abundances in Changxing River. Dominant genera included *Microcystaceae* (19.3%), *Flavobacterium* (9.6%), and *Pseudomonas* (8.9%).

Microcystis in the genera of *Microcystaceae* is one of the most dominant cyanobacteria found in the algae blooms at Taihu Lake, which has attracted great attention since it is culprit that secretes highly toxic and persistent microcystin (Shi et al., 2015). The microbial community analysis results showed that the relative abundance of *Microcystaceae* in spring and autumn (19.8% and 14.3%, respectively) was higher than that in summer and winter (2.4% and 9.7%, respectively). The two highest relative abundances were observed at the non-point source agricultural drainage areas (IV, 47.7%) in autumn and the estuary of Taihu Lake (I, 41.9%) in spring. Although *Microcystaceae* was relatively high in general at the non-point source agricultural drainage areas (IV) due to the input of agricultural non-point source nitrogen load (Paerl et al., 2016), only in the spring, the *Microcystis* at the estuary of Taihu Lake (I) had the highest relative abundance (41.9%). Previous investigations have shown March as the month with the heaviest cyanobacterial blooms in Taihu Lake (Hampel et al., 2018). During the dry season or the flat water period, backflow stagnation occurs frequently at the river channels around the lake. This likely explains why the *Microcystaceae* was highly enriched in the Taihu Lake in the spring. The optimal TN/TP ratio in water to promote algae blooms is between 13-35. When this ratio is below 13, cyanobacteria bloom can rarely occur. According to our water quality monitoring data, the TN/TP ratio at the estuary of Taihu Lake (I) was 8 in summer, and the other seasonal ratios were between 20 and 35, but there was no cyanobacteria bloom at the estuary of Taihu Lake (I) in winter. It indicated that the relatively high abundance of *Microcystis* at the estuary of Taihu Lake (I) in spring and autumn was mainly caused by algae backflow.

The relative abundance of *Flavobacterium* belonging to *Bacteroidetes* was relatively high in winter (11.0%), which may be related to the psychrophilic nature of several species in *Flavobacterium* (Lopes et al., 2016). In addition, *Flavobacterium* exhibited the highest relative abundances (15.6%-19.0%) at the discharge outlets of urban wastewater treatment plants (II) and the storm water outlets (III). Some *Flavobacterium* species can participate in denitrification, although this genus also contains fish pathogens (Liu et al., 2017b).

The relative abundances of *Pseudomonas* in winter and spring (7.5%-8.3%) were higher than in summer and autumn (0.5%-1.6%). Bacteria belonging to *Pseudomonas* were highly enriched at the discharge outlets of urban wastewater treatment plants (II, 12.6%-17.6%) and the storm water outlets (III,

12.0%-12.4%). *Pseudomonas* contains a diversity of heterotrophic bacteria and some of them are known to perform denitrification at low temperature (Yang et al., 2018b).

In addition to *Flavobacterium* and *Pseudomonas*, there were other denitrifying bacteria prevalent in Changxing River *Acinetobacter*, *Rhodobacter*, *Dechloromonas*, *Thiobacillus*, *Hydrogenophaga*, *Aeromonas*, *Stenotrophomonas*, *Zoogloea*, *Comamonas*, etc. (Zhang et al., 2011; Zhou et al., 2016b; Kim et al., 2018; Li et al., 2018b; Martinez-Santos et al., 2018; Zhou et al., 2019) showed high relative abundances in autumn and significant enrichment at the storm water outlets (III). Microorganisms can participate in ammonia oxidation alone (Fitzgerald et al., 2015), such as *Sphingomonas* and *Nitrospirae*, showed relatively low abundance (0.1% and 0.9%).

3.3 Correlation between microbial communities and environmental variables

Redundancy analysis (RDA) was conducted to evaluate relationships between relative abundances of dominant genera and environmental variables detected in the water samples. As shown in Fig. 7a, T, COD_{Mn}, DO, P, and NO₂⁻-N had a relatively large impact on the seasonal distribution differences of the microbial community in Changxing River. Due to the large number of environmental variables with influence effects, the BIOENV analysis was used to identify the best combination of environmental variables. Therefore, T, P, TP, and COD_{Mn} were the highest combination and the interpretation quantity of the BIOENV analysis is 78.3%. Although the environmental variables had little influence on the distribution of microbial community in different pollution types in Changxing River. The microbial community structure at the storm water outlets (III) was still significantly affected by TP, T and COD_{Mn} in summer, and affected by NH₄⁺-N in autumn (Fig. 7b).

As shown in Fig. 8a, *Proteobacteria*, *Bacteroidetes* and *Firmicutes* were positively correlated with EC, TP, TN, TDS, and SAL. *Actinobacteria* and *Cyanobacteria* were positively correlated with DO, NO₃⁻-N, and P. *Acidobacteria*, *Euryarchaeota*, *Chloroflexi*, *Acidobacteria*, *Verrucomicrobia* and *Nitrospirae* were positively correlated with COD_{Mn}, T, ORP, and pH.

At Taihu Lake, *Microcystis* and *Nostocaceae* were highly abundant and their relative abundances were positively correlated with pH. Previous studies revealed that higher pH of the water body can promote the growth and reproduction of *cyanobacteria* (Unrein et al., 2010; Wood et al., 2015). *Microcystis* was also positively correlated to DO (Fig. 8b). In addition, *Microcystis* is more competitive for NH₄⁺-N than most nitrifying bacteria (Paerl et al., 2014; Hampel et al., 2018), and serious accumulation of *Microcystis* may inhibit the nitrogen cycle of aquatic ecosystems.

3.4 Suggestion for remediation of Changxing River

According to the above results, restoration of the estuary of Taihu Lake (I) should focused on addressing the accumulation problem of cyanobacterial blooms caused by the backflow of cyanobacteria from Taihu Lake and strengthening the colonization of nitrifiers and denitrifiers. In the spring, the DO is

sufficient and the pH is high. The pH of the water body can be lowered by increasing the hydraulic circulation in the detention area (Cерco et al., 2013), such as connecting water systems, ecological water replenishment and installing aeration push flow (Lilindenschmidt, 1999). Simultaneously, the cyanobacteria can be removed *in situ* by arranging enclosures, floating dam interceptions. In the autumn, the DO content is low and the $\text{NH}_4^+\text{-N}$ content is high due to the eutrophication at the non-point source agricultural drainage areas (IV). Reoxygenation and capping zeolite or clay materials can be used to enhance the colonization of ammonia-oxidizing bacteria (Arnon et al., 2007; Chen et al., 2018). Moreover, the external load into the lake can be reduced by increasing rural sewage treatment and controlling agricultural non-point source pollution. Precise ecological dredging of sediments can also be carried out to effectively reduce the internal load and curb the release of nutrients from sediments.

This study found that the DO content at the storm water outlets (III) was low, especially in summer and autumn. The lowest DO was only 1.7 mg/L, impairing nitrifying bacteria. Current common rainwater restoration technologies include artificial aeration (Tang et al., 2009; He et al., 2012), aquatic plant restoration (Zeng et al., 2017; Liu et al., 2019), etc., but their effects on nitrification and denitrification is still limited (Lu et al., 2014). Effective nitrification requires high DO levels (e.g., >8 mg/L). At low DO levels (3 mg/L), ammonia nitrogen removal efficiency can be greatly hindered by 50% or more. Nitrobacteria generally turn to anaerobic respiration when the oxygen concentration is lower than 6.4 mg/L (Wang et al., 2015). Therefore, intermittent aeration may be needed to maintain the DO concentration between 5-6 mg/L at the storm water outlets (III). Biological processes can be integrated to bolster nitrifying bacteria populations and two successful cases include a novel water biofilm pretreatment process with reed addition (Feng et al., 2013) and bioactive thin layer coverage that enhances *in situ* microbial regeneration (Zhou et al., 2016b).

4 Conclusions

For the Changxing River as an important inflow river into the Taihu Lake, human activities, such as the discharge of domestic sewage and agricultural sewage, have affected its microbial ecology. Thereinto, T, P, concentrations of TP and COD_{Mn} were the main factors that govern the overall microbial community and distribution of dominant bacteria in the algae-polluted river. The results provided a reference for the impact of pollution types on river microbial ecosystems under complex hydrological condition and a guidance for the recovery of natural water ecological function in algae-polluted rivers.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

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Lecheng Wei: Methodology, Formal analysis, Investigation, Visualization;

Mengyan Li: Methodology, Investigation, Data Curation, Visualization;

Weitang Zhu: Formal analysis, Investigation, Data Curation;

Liang Zhu: Resources, Supervision, Funding acquisition

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Figures

Figure 1

Locations of sampling sites along Changxing River

Figure 2

Physicochemical characteristics in water samples collected in Changxing River in response to (a) seasons, (b) pollution types and (c) sites (I: Estuary of Taihu Lake; II: Discharge outlets of urban wastewater treatment plants; III: Storm water outlets; IV: Non-point source agricultural drainage areas)

Figure 3

Alpha-diversity of microbial community in water samples collected in Changxing River (I: Estuary of Taihu Lake; II: Discharge outlets of urban wastewater treatment plants; III: Storm water outlets; IV: Non-point source agricultural drainage areas)

Figure 4

Microbial community compositions water samples collected in Changxing River (at phylum level) (I: Estuary of Taihu Lake; II: Discharge outlets of urban wastewater treatment plants; III: Storm water outlets; IV: Non-point source agricultural drainage areas)

Figure 5

PCA of aquatic microbial communities in water samples collected in the Changxing River at the genus level

Figure 6

Relative abundance of the 80 most abundant genera in aquatic microbial populations in Changxing River

Figure 7

Redundancy analysis of dominant microorganisms and environmental variables in response to (a) seasons and (b) pollution types

Figure 8

RDA of key species and environmental variables at the (a) phylum level and (b) the genus level