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Water temperature and organic carbon control spatio-temporal dynamics of particle-attached and free-living bacterial communities in a hypereutrophic urban river network

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Abstract

1. Bacteria are an important component of the aquatic ecosystem playing a key role in the transformation and mineralisation of allochthonous organic matter, nutrients and pollutants. However, there is no clear understanding of spatio-temporal patterns of particle-attached versus free-living bacterial abundances, diversities and communities, which limits our in-depth understanding of hypereutrophic urban river ecosystems and the mechanisms regulating nutrient cycling and biogeochemical transformations.

2. In order to investigate spatio-temporal patterns and associated factors for aquatic particle-attached versus free-living bacterial communities, we conducted a 2-year study of four contrasting habitats in a hypereutrophic urban river network of eastern China. Bacterial abundance and community structure in relation to various environmental variables were assessed by epifluorescence microscopy and high-throughput sequencing techniques.

3. Results revealed that particle-attached bacterial abundance depended mainly on suspended particle concentrations, whereas free-living bacterial abundance was correlated with nutrient availability and water temperature. Organic carbon was related to a decrease of free-living and particle-attached bacterial richness, and particulate matter input increased free-living bacterial diversity. Seasonal changes of water temperature were linked to the temporal dynamics of bacterial community composition, and the differences in total organic carbon concentrations contributed to spatial differences in bacterial community composition. There were significant differences in bacterial community composition between particle-attached and free-living assemblages in low-turbidity/low-nutrient rivers found in land areas comprising mainly natural vegetation. These differences were especially prominent for the relative abundances of Cyanobacteria and Actinobacteria, which were higher in the particle-attached and free-living fractions, respectively. However, no significant differences were found in turbid/nutrient-rich rivers. Total organic carbon was the most significant factor shaping the composition similarity between particle-attached and free-living bacterial communities.

4. Our findings indicate that spatio-temporal variations in water temperature, suspended particle and resource availability, especially for organic carbon, appreciably contribute to changes in the abundance, diversity and composition of aquatic particle-attached versus free-living bacterial communities in the hypereutrophic urban river network.

5. Considering that biodiversity is important for supporting ecosystem functioning, this study informs spatio-temporal patterns of particle-attached and free-living bacterial abundance, diversity and composition of communities, and their underlying control mechanisms, thereby providing fundamental knowledge of microbial ecological functions for remediation and sustainable management of hypereutrophic urban river ecosystems.

KEYWORDS
aquatic ecosystems, diversity, free-living bacteria, particle-attached bacteria, water quality

1 | INTRODUCTION

Urban population has been rapidly increasing in recent decades with more than half of the global population now living in urban areas (Grimm et al., 2008). Urban rivers, as an important component of urban ecological systems, provide numerous ecosystem services for urban residents, such as recreation, water transportation, aquaculture and water supply (Araya et al., 2003; Vörösmarty et al., 2010). Hence, urban rivers are of great significance to the health and well-being of urban residents and environments. However, numerous anthropogenic activities, such as wastewater/industrial discharge, atmospheric deposition, stormwater runoff, water diversions/consumption, and channelisation/bank reinforcement, negatively impact the natural ecological characteristics of urban rivers. Frequent studies report that organic, metal, sediment and nutrient pollutants impair the water quality and ecological health of urban river networks (Dong & Xu, 2019; Schliemann et al., 2021; Ye et al., 2021; Zhang et al., 2015).

In urban river networks, bacteria are an important component of the aquatic ecosystem playing a key role in the transformation and mineralisation of allochthonous organic matter, nutrients and other pollutants (Azam, 1998; Fuhrman, 2009; Hui et al., 2022). Bacterial communities are highly dynamic, experience fast growth rates and are highly responsive to environmental fluctuations making them a potentially sensitive bioindicator of aquatic ecosystem health and function (Araya et al., 2003; Paerl et al., 2003). For example, cyanobacteria blooms may be a symptom of advanced eutrophication, and levels of coliform bacterial indicators (e.g., *Escherichia coli*) provide information about faecal contamination (Paerl et al., 2003; Reinl et al., 2021). Hence, studying bacterial abundance and community structure is fundamentally important for gaining a better understanding of the ecological characteristics and health of urban river systems. In the context of urban river environmental alterations, several studies have examined the effects of urbanisation and changes in water quality on bacterial community composition, focusing mainly on the bacteria in sediments. These results showed

that urbanisation had significant impacts on the bacterial community composition (Hosen et al., 2017; Li et al., 2020; Simonin et al., 2019; Wang et al., 2011). However, there has been far less effort to understand how different lifestyles affect bacterial abundance, diversity and community structure of urban rivers in response to urban stressors (e.g., nutrient concentrations, organic pollutants, impervious surfaces) (Simonin et al., 2019; Wang et al., 2011; Wenger et al., 2009).

The Wen-Rui Tang River is a typical coastal plain urban river network in eastern China that is representative of many degraded urban rivers in coastal areas of eastern China and elsewhere (Lu et al., 2011; Mei et al., 2014). According to the concentration of ammonia under the guidance of National Water Quality Guidelines for Surface Water (State Environment Protection Bureau of China, 2002), this river network is hypereutrophic with high mean ammonia concentrations ($>2\,mg\,N\,L^{-1}$) that exceed Type V waters – the lowest water-quality classification that supports aquatic ecosystem health. Insufficient water flows are commonly encountered in the Wen-Rui Tang River. The direction and velocity of flow are regulated by the operation of sluice gates (i.e., flood control structures) in the lower catchment. For most of the year, the sluice gates are closed and water bodies are relatively stagnant. The Wen-Rui Tang River and its numerous urban tributaries often contain steep gradients in environmental conditions (e.g., total organic carbon [TOC], ammonium [NH_4^+], dissolved oxygen [DO], turbidity) and impaired habitats resulting from a multitude of human impacts. Hence, the Wen-Rui Tang River was selected as a representative research area to investigate bacterial communities under sharply different environmental conditions within the hypereutrophic urban river network.

In order to study bacterial communities more mechanistically, aquatic bacteria are often classified into two major types based on their different lifestyles: particle-attached and free-living bacteria (Grossart, 2010). Considering that particle association provides bacteria with several benefits (e.g., increased nutrient availability, enhanced environmental resistance) (Dang & Lovell, 2016; Hu et al., 2020), we hypothesised that particle-attached and free-living bacterial communities would differ in their diversity and

composition, and that their spatio-temporal dynamics would be associated with different environmental factors in hypereutrophic urban river networks. To avoid artefacts associated with cultivation-dependent methods, we employed cultivation-independent molecular techniques to provide a more comprehensive understanding of real-world bacterial communities, such as high-throughput sequencing technologies (Lemos et al., 2011; Logares et al., 2012). Therefore, we used high-throughput sequencing to explore particle-attached and free-living bacterial community structure in the Wen-Rui Tang River. The primary objectives of this 2-year study were to: (1) examine the abundance, diversity and composition of particle-attached versus free-living bacterial communities; (2) investigate the relationships and spatio-temporal patterns of particle-attached and free-living bacterial communities relative to environmental conditions; and (3) identify the variables showing the strongest correlation with the abundance, diversity and composition of particle-attached and free-living bacterial communities in a hypereutrophic urban river network.

2 | MATERIALS AND METHODS

2.1 | Study sites and sampling protocols

As a representative example of a hypereutrophic urban river network in the coastal plain, the Wen-Rui Tang River was selected as the research area to take advantage of its many contrasting aquatic environments. This area's climate is subtropical monsoon with a mean annual precipitation of ~1800 mm and mean annual temperature of ~18°C. Rainfall occurs mainly in May–September with a typhoon season in July–September. Water samples were collected from four rivers in the Wen-Rui Tang River network based on their distinctly different water-quality conditions and surrounding land use types (Figure 1). River A (27.97°N, 120.67°E) is located in a downtown area surrounded by commercial and residential land use, and is prone to receiving untreated municipal wastewater. River B (27.96°N, 120.74°E) is close to an industrial area and River C (27.94°N, 120.68°E) is surrounded by agricultural land. River D (27.87°N, 120.68°E) was considered a reference river owing to its location in a natural area with limited human impact. At each river, three sampling sites separated by a 100-m to 1-km distance were established to provide within-site replication. A total of eight samples at each sampling site were collected seasonally over a 2-year time period: 18 July 2017 (summer), 11 November 2017 (autumn), 13 January 2018 (winter), 21 April 2018 (spring), 7 August 2018 (summer), 10 November 2018 (autumn), 12 January 2019 (winter) and 30 March 2019 (spring). Surface waters (top 50 cm) were collected with a 3-L Schindler sampler, placed in sterilised polyethylene bottles, and transported to the laboratory in a cooler with ice packs for immediate processing.

Bacteria in water samples were divided into particle-attached and free-living assemblages. Particle-attached bacteria were retained by filtering 100–600 mL of sample through a 5.0-μm polycarbonate membrane (47-mm diameter; Millipore); and free-living bacteria were collected by filtering 100–300 ml of the 5.0-μm filtrate through a 0.2-μm polycarbonate membrane (47-mm diameter; Millipore) (Kellogg & Deming, 2009; Xie et al., 2020). After filtration, the membrane was cut into pieces, placed in a 2-ml sterile centrifuge tube, and then stored at −80°C for subsequent bacterial genomic DNA extraction. Additionally, ~10 ml aliquots of the unfiltered and filtered (<5.0-μm pore size) water samples were immediately fixed with 2% formaldehyde (pre-filtered through 0.2-μm pore size; final concentration) and stored at 4°C for later direct counting of total bacterial abundance and free-living bacterial abundance, respectively. Particle-attached bacterial abundance was calculated as total bacterial abundance per unit volume minus free-living bacterial abundance per unit volume. All laboratory equipment and operating procedures followed strict aseptic protocols to prevent post-collection contamination.

### 2.2 | Measurement of water-quality variables and enumeration of bacteria

Water temperature (T), specific conductivity (EC), pH, dissolved oxygen (DO) and turbidity (TU) were measured in the field using a multi-parameter water quality sonde (YSI 650MDS/6920, Xylem). The other physicochemical variables were quantified immediately upon return to the laboratory. Ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-) and orthophosphate (PO_4^{3-}) were determined on a filtered subsample (0.2-μm pore size) using Nesslerisation colorimetric, N-(1-naphthyl)-ethylenediamine colorimetric, ultraviolet spectrophotometric and ammonium molybdate spectrophotometric methods, whereas total organic carbon (TOC), Chlorophyll-a (Chl-a) and suspended solids (SS) were analysed on non-filtered subsamples using a TOC analyser (TOC-L CPH; Shimadzu), spectrophotometric method following hot-ethanol extraction and gravimetric methods, respectively (Jin & Tu, 1990). All analytes were quantified at concentrations well above analytical detection limits.

Bacterial abundance was determined by epifluorescence microscopy after staining with DAPI (4′, 6′-diamidino-2-phenylindole; at a final concentration of 1 μg/ml) for 10 min in the dark (Cai et al., 2018; Porter & Feig, 1980). Then, the sample was filtered onto a black polycarbonate filter (0.2-μm pore size, 25 mm diameter; Millipore) with a vacuum of <10-mm Hg. At least 20 randomly selected fields of view with a range of >400 bacterial cells were counted per slide using a Leica fluorescent microscope (DM4000B).

2.3 | DNA extraction, 16S rDNA amplification, sequencing and data processing

Samples were divided into two annual groups: first year (July 2017 to April 2018) and second year (August 2018 to March 2019). Bacterial genomic DNA was extracted for all samples using the FastDNA® SPIN Kit for Soil (MP Biomedicals) according to the manufacturer's
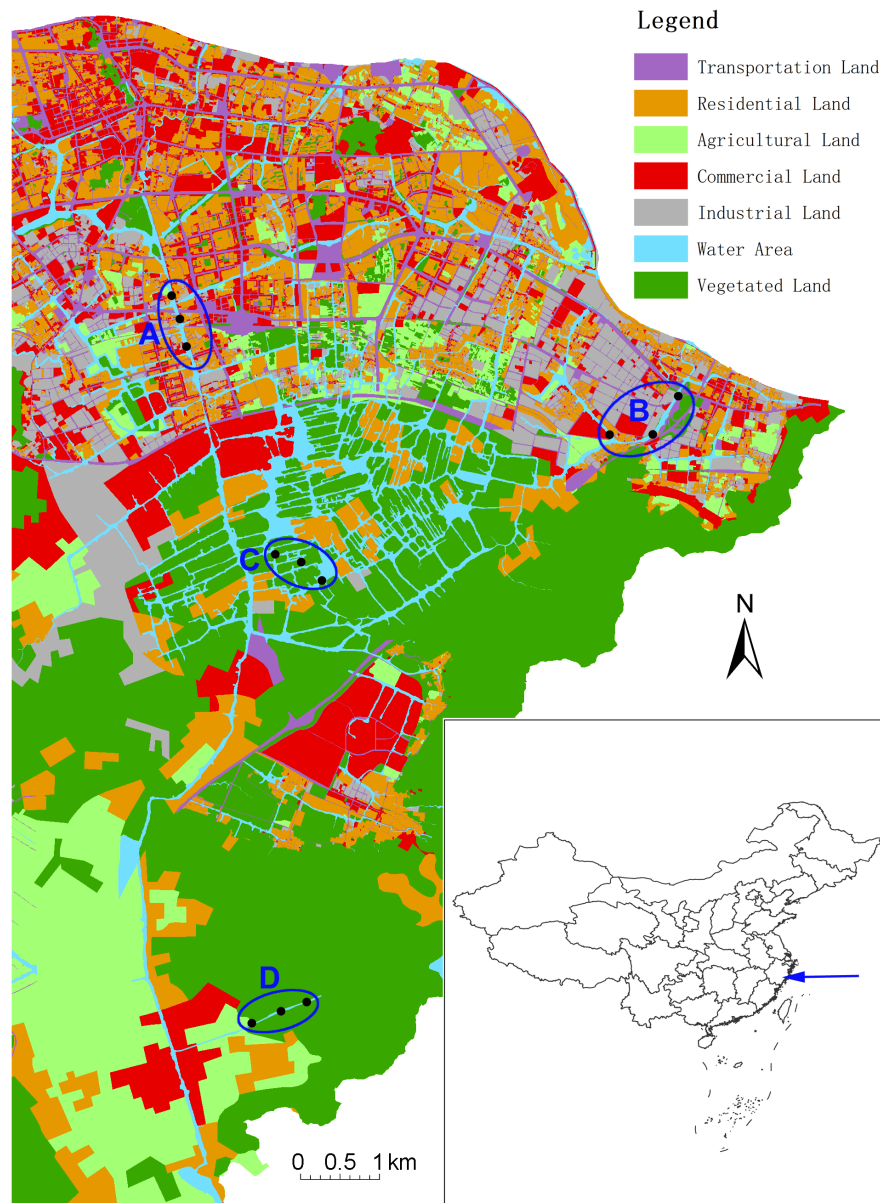
FIGURE 1 Map of Wen-Rui Tang River (Wenzhou, China) showing the location of river sampling sites (rivers A, B, C and D) and land use/land cover.

protocols. The DNA concentration and quality were determined by NanoDrop Spectrophotometer (Nano-200; Hangzhou Allsheng Instruments) and agarose gel electrophoresis. All qualifying samples were amplified with the universal primer pair S-D-Bact-0341-b-S-17, 5′-CCTACGGGNGGCWGCAG-3′ and S-D-Bact-0785-a-A-21, 5′-GACTACHVGGGTATCTAATCC-3′ (Klindworth et al., 2013). For the first year samples, the PCR reactions were performed using a 2×KAPA HiFi HotStart ReadyMix PCR Kit (Kapa Biosystems). A 25-µl reaction volume was subjected to an initial denaturation step at 95°C for 3 min, followed by 25 cycles each of denaturation at 95°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 30 s, followed by a final extension at 72°C for 5 min. The purified, barcoded amplicons were sequenced using an Illumina HiSeq platform at Xiangyin Biotechnology with 2×250 paired-end chemistry. For the second year samples, the PCR reactions were performed using a TranGen Kit (TransGen AP221-02: TransStart Fastpfu DNA Polymerase; TransGen Biotech). A 20-µl reaction volume underwent

an initial denaturation step at 95°C for 3 min, followed by 27 cycles each of denaturation at 95°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 45 s, followed by a final extension at 72°C for 10 min. Sequencing was performed on an Illumina MiSeq platform at Majorbio Bio-pharm Technology using 2×300 paired-end chemistry. Although the HiSeq and MiSeq platforms differ markedly in scale, α- and β-diversity of microbial communities generated by the two platforms are similar (Caporaso et al., 2012; Na et al., 2020).

The raw paired-end sequencing reads were assigned to samples based on their unique barcode and truncated by cutting off the barcode and primer sequence. Merging of overlapping paired-end reads and quality control of reads were performed using FLASH (Tanja & Salzberg, 2011) and Fastp (Chen et al., 2018). Chimeric sequences were filtered using USEARCH (Edgar, 2010) and the denoised sequences were clustered to operational taxonomic units (OTUs) at a 97% similarity level. A representative sequence for each OTU was assigned a taxonomic identity using the RDP classifier (Cole

et al., 2014) based on the RDP release 11.5 database. OTUs assigned to mitochondria and chloroplasts were removed. OTU abundances were normalised using rarefaction corresponding to the sample with the least sequences. The lowest sequence amounts for the first and second year samples that were used in the standardisation were 65,362 and 22,424, respectively. OTU data were assessed by Good's coverage, Chao1 richness estimator and Shannon index. All α-diversity indices were calculated with QIIME (Caporaso et al., 2010). One sample (1 of 96 for second year) failed to adequately sequence; however, this failure had little effect on the overall statistical analyses. Mean Good's coverage for the first and second year samples was >98.5% and 97.5%, respectively, indicating that most phylotypes were detected.

2.4 | Data analysis

Univariate data were presented as mean±SE. Water-quality variables for each year were analysed by one-way repeated measures ANOVA, with river site as the main factor and sampling date as the repeated measure. Bacterial abundance and α-diversity indices of each year were subjected to two-way repeated measures ANOVA, with river site and assemblage type (particle-attached vs. free-living; nested within river site) as the factors and sampling date as the repeated measure. Subsequently, multiple comparisons were made using the Duncan's multiple range test with a $p < 0.05$ significance level. The differences between two assemblage types of each river in each year were tested by one-way repeated measures ANOVA. Statistical comparisons between means were performed with SPSS 22.0 (IBM-SPSS Statistics). To correct for violations of sphericity, the Greenhouse–Geisser adjustment was used. Relationships between water-quality variables and bacterial abundances/α-diversity indices were examined using Pearson's correlation (two-tailed).

Principal coordinates analysis (PCoA) on a Bray–Curtis dissimilarity matrix based on OTU relative abundance was applied to investigate how community composition varied between particle-attached and free-living fractions as a function of river sites and sampling dates. One-way permutational multivariate ANOVA (PERMANOVA) was used to assess the effects of sampling date, river site and assemblage type (particle-attached vs. free-living) on bacterial community composition in each year. ADONIS is a PERMANOVA using a distance matrix as response variable. We used a Bray–Curtis dissimilarity matrix and 999 permutations. Additionally, the Whittaker index between particle-attached and free-living fractions from each river at each sampling date was performed using PAST (PAleontological STatistics version 3.14) (Hammer et al., 2001). The relationships between Whittaker index and water-quality variables were explored using linear regression models. Redundancy analysis (RDA) was used to examine correlations between bacterial communities and water-quality variables, and assess which variables were significantly associated with the bacterial data. In the RDA plot, only the 10 OTUs that explained the most variance were shown. The significance of the RDA analysis was tested by Monte Carlo permutation tests with 999 permutations. PCoA and RDA were conducted using Canoco for Windows 5.0 (Ter Braak & Šmilauer, 2012).

3 | RESULTS

3.1 | Water-quality characterisation

Many water-quality variables (e.g., DO, TU, NH_4^+, PO_4^{3-}, SS) showed steep gradients among the four rivers in the Wen-Rui Tang River network (Figure 2). For example, DO concentrations showed an increase from river A (commercial and residential) to river D (natural). There were significant differences in the DO concentrations among the rivers in the second year ($p < 0.001$). Turbidity, NH_4^+, NO_2^-, NO_3^-, PO_4^{3-}, TOC and SS displayed gradient changes among rivers, with the lowest values recorded at river D (natural). Compared to the other rivers, turbidity (first year: $p < 0.001$; second year: $p < 0.001$), PO_4^{3-} (first year: $p < 0.001$; second year: $p < 0.001$) and SS (first year: $p < 0.001$; second year: $p < 0.001$) were significantly higher in river A (commercial and residential) in both years. The NO_2^- concentration was progressively lower in the order of river B (industrial), C (agricultural), A (commercial and residential) and D (natural) for both years (first year: $p < 0.001$; second year: $p < 0.001$). For river A (commercial and residential), several variables, including EC, NH_4^+ and TOC decreased markedly, and the DO concentration increased from the first to the second year. For river C (agricultural), although the EC decreased markedly, the PO_4^{3-}, Chl-a and SS increased from the first year to second year. Notably, PO_4^{3-} concentration increased from the first year to second year in all four rivers. Mineral N concentrations followed $NH_4^+ > NO_3^- >> NO_2^-$ for the rivers strongly impacted by human activities (rivers A, B and C), whereas NO_3^- was the dominant form at the reference river (river D). Although changes in water quality were observed between the 2 years, the spatial pattern for overall water-quality conditions did not change appreciably.

Water temperature (T) displayed a similar seasonal variation among the different rivers with maxima in summer and minima in winter. Chl-a showed a similar pattern to T owing to its growth dependence on incident solar radiation and temperature. In the more nutrient-rich rivers (A, B and C vs. D), NO_3^- concentrations displayed significant seasonal changes with minima in spring–summer and maxima in autumn-winter. The other water-quality variables showed no distinct seasonal variability.
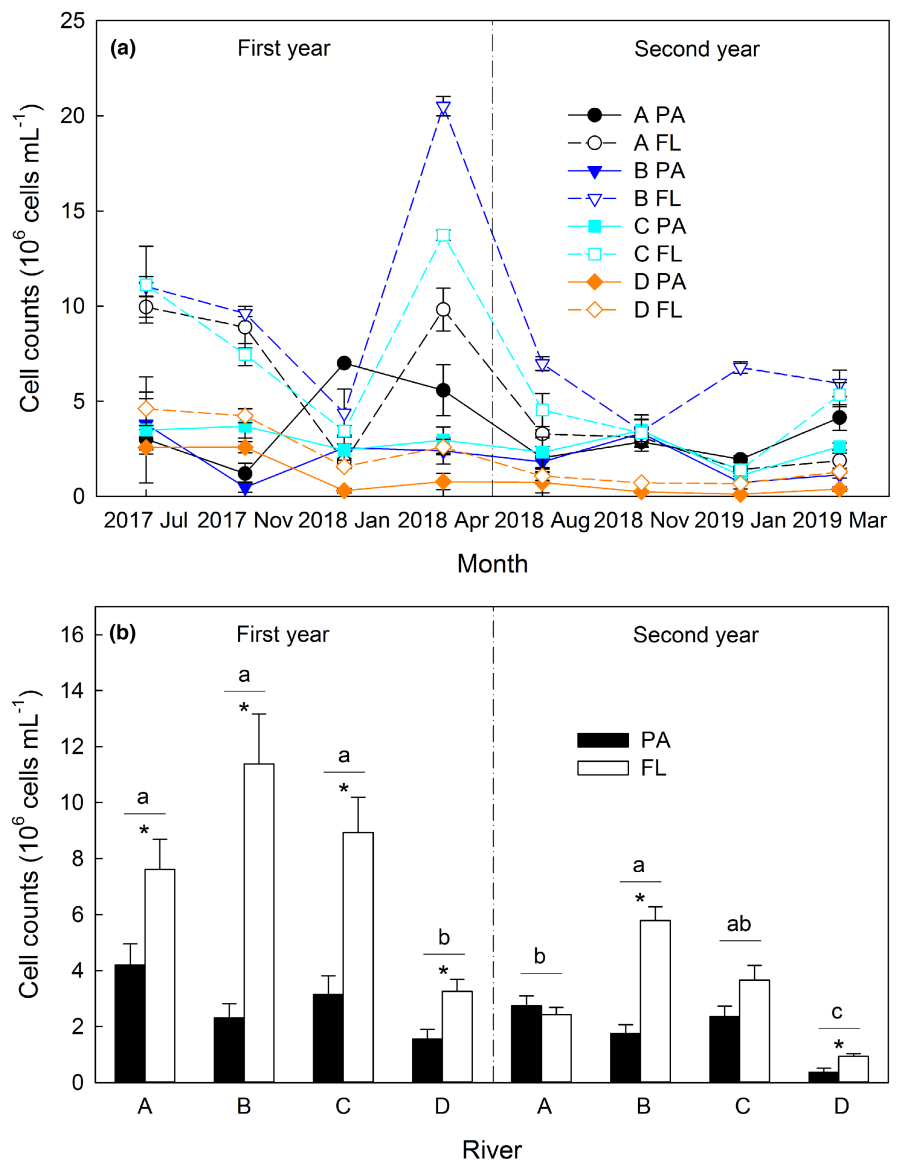
3.2 | Bacterial abundance

Free-living and particle-attached bacterial abundance in the different rivers displayed large seasonal variations, and in general bacterial abundance was lower in winter (Figure 3a). There were similar variations in the spatial pattern between the 2 years, with the bacterial abundance being significantly lower in river D (natural) compared to the other rivers (first year: $p < 0.001$; second year: $p < 0.001$). In the first year, there were no significant differences
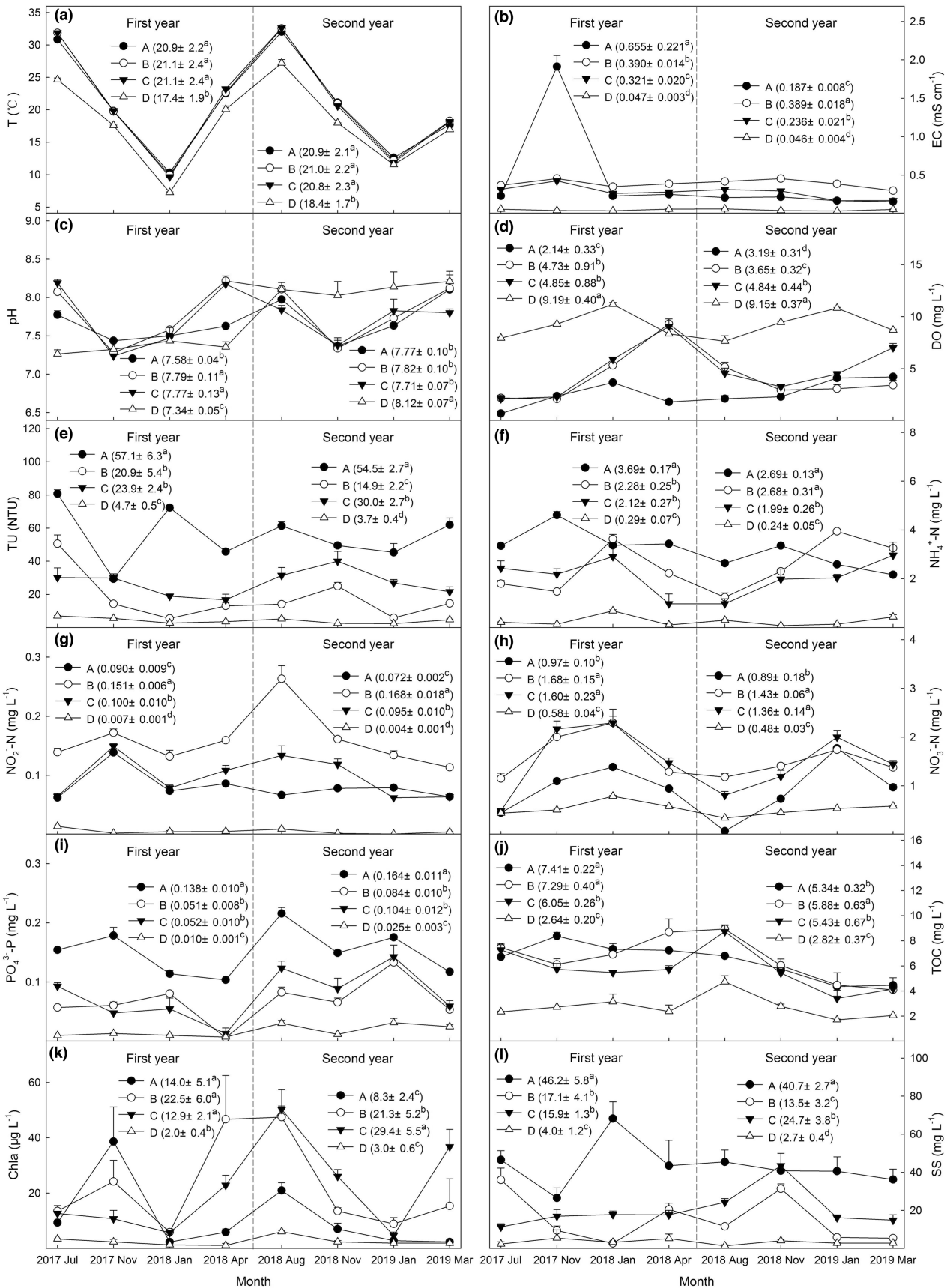
FIGURE 2 Temporal variations (mean±*SE*, *n*=3) for water-quality variables at the four rivers (A, B, C and D) in the Wen-Rui Tang River network. Numbers in parentheses represent the mean±*SE* (*n*=12) for each year at each river. Different letters indicate significant differences among rivers at *p*<0.05 by the Duncan's multiple range test.

FIGURE 3 Temporal (a) and spatial (b) changes of bacterial abundances for particle-attached and free-living assemblages (mean $\pm$ *SE*; *n* = 3 and 12 per river for (a, b), respectively) for four different rivers (A, B, C and D). Different letters indicate significant differences at *p* < 0.05 among rivers for each year. Asterisk indicates a significant difference at *p* < 0.05 between particle-attached and free-living assemblages. FL, free-living; PA, particle-attached.

among the more nutrient-rich rivers (A, B and C vs. D). However, in the second year, the total bacterial abundance was significantly higher in river B (industrial) than in river A (commercial and residential) (*p* < 0.001). The highest free-living bacterial abundance was found at river B (industrial), whereas the highest particle-attached bacterial abundance was found at river A (commercial and residential) (Figure 3b). Free-living bacterial abundances were all significantly higher than particle-attached bacterial abundances in the different rivers during the first year (river A: *p* = 0.004; river B: *p* < 0.001; river C: *p* = 0.003; river D: *p* = 0.014), but no significant difference was found between free-living and particle-attached bacterial abundance at rivers A (commercial and residential) and C (agricultural) in the second year. Free-living bacterial abundance showed a significant positive relationship with TOC, NO_2^-, Chl-*a*, *T*, EC and NH_4^+. Particle-attached bacterial abundance displayed a significantly positive relationship with SS, TU, TOC, NH_4^+ and PO_4^{3-}, whereas it showed a significant negative relationship with DO (Table 1).

3.3 | Bacterial α-diversity

Across all samples, particle-attached bacterial communities had significantly higher species richness and diversity than free-living bacterial communities for both years (Table 2). Observed OTU richness and Chao1 for bacterial communities generally displayed seasonal changes with maxima in autumn–winter and minima in spring–summer (Figure 4a,e). The value of Chao1 was significantly higher at rivers A (commercial and residential) and D (natural) compared to the other rivers for both years (first year: *p* < 0.001; second year: *p* = 0.001; Figure 4f). River D, having relatively low nutrient contents, had significantly higher observed OTU richness than the other rivers for both years (first year: *p* < 0.001; second year: *p* < 0.001; Figure 4b). For particle-attached and free-living bacteria in different rivers, the differences between the two assemblages varied in time and space (Figure 4). For example, observed OTU richness showed a similar trend among different rivers in the first year, being significantly higher in the particle-attached than in the

free-living assemblage (river A: $p = 0.004$; river B: $p = 0.007$; river C: $p < 0.001$; river D: $p = 0.022$). Conversely, in the second year, significant differences between the two assemblages appeared only in river B (industrial) ($p = 0.001$; Figure 4b). The changing patterns for Shannon index (Figure 4d) were similar to those of observed OTU richness.

In correlation analysis of water-quality variables and α-diversity indices, we focused on the similar results between the 2 years. The observed OTU richness of free-living bacteria showed a significant negative relationship with NO_2^-, TOC and Chl-a. Shannon index of free-living bacteria showed a significant positive relationship with TU, NH_4^+, PO_4^{3-} and SS, whereas it showed a significant negative relationship with DO. Chao1 of free-living bacteria showed a significant negative relationship with T, NO_2^-, TOC and Chl-a. For

TABLE 1 Pearson's correlation coefficients for different water-quality variables and bacterial abundance.

| Variables	Free-living bacterial abundance	Particle-attached bacterial abundance
T	0.416**	0.140
EC	0.360**	0.009
pH	0.193	−0.135
DO	−0.186	−0.344**
TU	0.073	0.592**
NH_4^+	0.252*	0.353**
NO_2^-	0.505**	0.176
NO_3^-	0.155	0.100
PO_4^{3-}	−0.096	0.202*
TOC	0.553**	0.418**
Chl-a	0.484**	0.003
SS	0.058	0.610**

Abbreviations: Chl-a, Chlorophyll-a; DO, dissolved oxygen; EC, specific conductivity; NH_4^+, ammonium; NO_2^-, nitrite; NO_3^-, nitrate; PO_4^{3-}, orthophosphate; SS, suspended solids; T, water temperature; TOC, total organic carbon; TU, turbidity.

*$p < 0.05$; **$p < 0.01$.

particle-attached bacteria, the observed OTU richness showed a significant negative relationship with NH_4^+, NO_2^-, TOC and Chl-a for both years (Table 3).

3.4 | Community composition

Across all seasons and all rivers, the four predominant phyla appearing in both the particle-attached and free-living bacterial communities were Proteobacteria, Bacteroidetes, Actinobacteria and Cyanobacteria (Figure 5). At the phylum level, particle-attached and free-living bacterial communities in river A (commercial and residential) displayed a similar composition (Figure 5a,b). By contrast, there were marked differences in bacterial communities between particle-attached and free-living bacterial communities in river D (natural) (Figure 5g,h). For example, in river D (natural), the relative abundance of Cyanobacteria was higher in the particle-attached than free-living fraction in both years, whereas the relative abundance of Actinobacteria was the opposite.

Sampling date, river site and assemblage type (particle-attached vs. free-living) were significantly associated with the composition of the bacterial communities (Figure 6). ADONIS analysis further indicated that sampling date (first year, $R^2 = 0.284$, $p = 0.001$; second year, $R^2 = 0.307$, $p = 0.001$) and river site (first year, $R^2 = 0.228$, $p = 0.001$; second year, $R^2 = 0.146$, $p = 0.001$) were more strongly correlated with community variations than assemblage type (first year, $R^2 = 0.052$, $p = 0.001$; second year, $R^2 = 0.022$, $p = 0.022$), regardless of year. PCoA analysis showed that bacterial communities were clearly separated between river D (natural) and the other nutrient-rich rivers (A, B and C). Meanwhile, PCoA analysis demonstrated that the major separations for river A (commercial and residential), B (industrial) and C (agricultural) were caused by sampling date rather than assemblage type, regardless of year. By contrast, in river D (natural), assemblage type was significantly correlated with the separation of bacterial communities in both the first (ADONIS, $r^2 = 0.270$, $p = 0.001$) and second (ADONIS, $r^2 = 0.104$, $p = 0.009$) years (Table 4).

TABLE 2 Estimates (mean ± SE) of richness and diversity for bacterial communities between particle-attached and free-living fractions in the Wen-Rui Tang River.

	Good's coverage (%)	OTUs	Shannon	Chao1
First year				
Particle-attached	98.5 ± 0.05	2592 ± 93	5.45 ± 0.06	3818 ± 114
Free-living	98.5 ± 0.05	2248 ± 64	5.14 ± 0.05	3591 ± 104
F-value	–	18.66	100.54	3.40
p-value	–	<0.001***	<0.001***	0.034*
Second year				
Particle-attached	97.5 ± 0.15	1395 ± 79	5.04 ± 0.12	2192 ± 112
Free-living	97.7 ± 0.14	1167 ± 53	4.62 ± 0.11	1945 ± 100
F-value	–	8.23	4.70	3.53
p-value	–	0.001**	0.012*	0.032*

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.
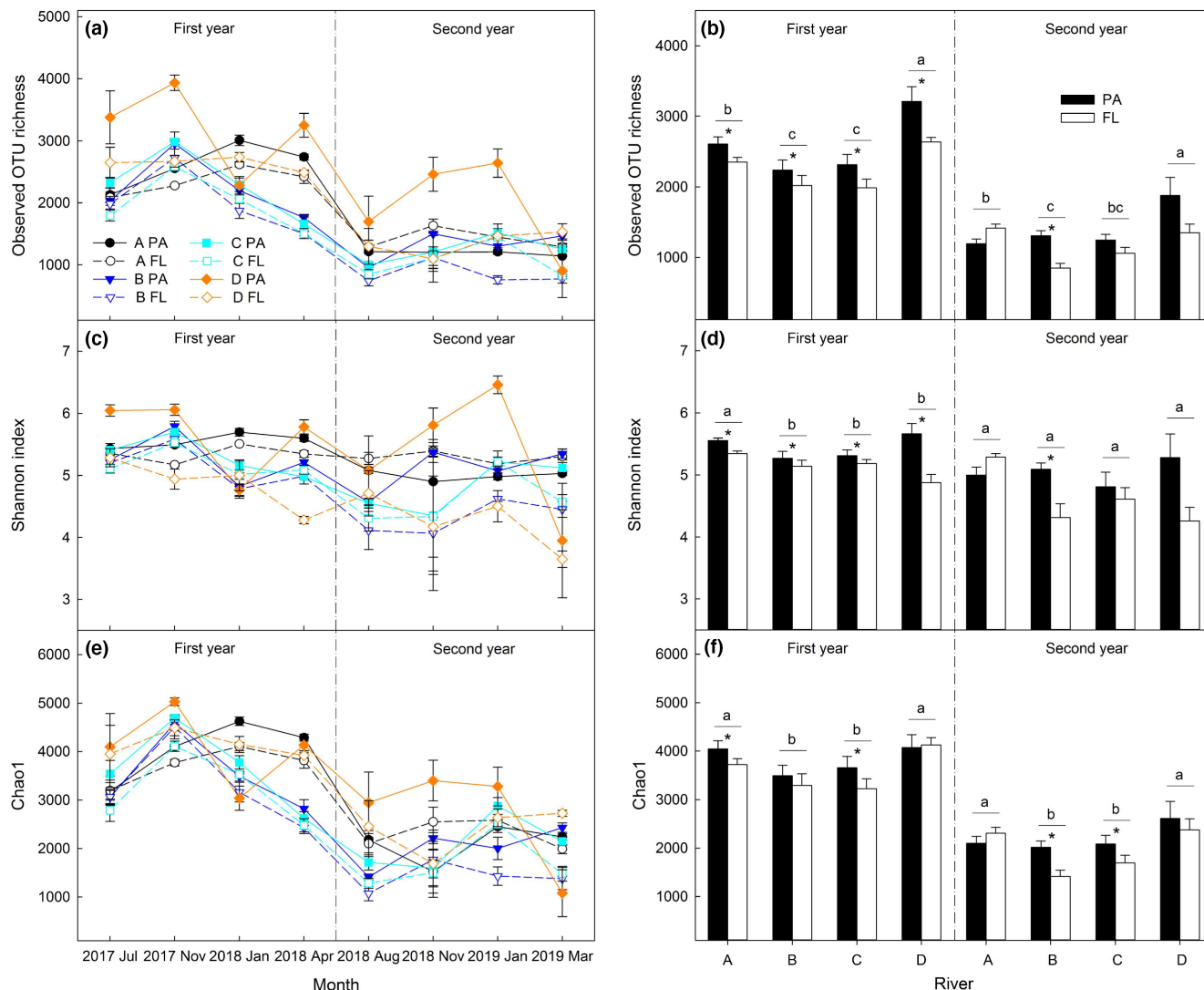
FIGURE 4 Changes of bacterial richness and diversity indices for particle-attached and free-living assemblages (mean ± SE; $n = 3$ and 12 per river for (a, c, e) and (b, d, f), respectively) for different rivers (A, B, C and D). Different letters indicate significant differences at $p < 0.05$ among rivers for each year. Asterisk indicates a significant difference at $p < 0.05$ between particle-attached and free-living assemblages. FL, free-living; PA, particle-attached.

The Whittaker index was used to evaluate the difference between the community compositions; a higher index indicates a greater difference. Therefore, the Whittaker index can be used to reflect the differences in bacterial community structure between particle-attached and free-living assemblages under different environmental conditions. The mean differences in bacterial community structure between particle-attached and free-living assemblages in the different rivers based on the Whittaker index followed river D > river B > river C > river A in the first year and river D > river B > river A > river C in the second year (Table 4). Additionally, we assessed factors shaping differences between particle-attached and free-living bacterial communities. We used the Whittaker index and different water-quality variables in a linear regression analysis (Figure 7). Regression results for both years revealed a significant positive correlation between the Whittaker index and DO, and significant negative correlations for Whittaker index with T, EC, TU, NH_4^+, NO_2^-, PO_4^{3-}, TOC, Chl-a and SS. Among them, TOC had the largest R^2 value.

Redundancy analysis (RDA) indicated that 12 environmental variables were significantly associated with changes in bacterial communities (Figure 8a,b; Table 5). Temperature and TOC were the top two factors, each explaining more than 10% of the total variance in both years. A few OTUs stood out in the RDA analysis in both years, belonging to *Terrimonas*, *Ilumatobacter* and unclassified Burkholderiales were more closely associated with water temperature.

4 | DISCUSSION

4.1 | Bacterial abundance

Bacterial abundance is a fundamental metric for understanding the population dynamics of bacteria (Khalili et al., 2019). Based on our 2-year data record, the relative importance of water-quality variables on the free-living and particle-attached bacterial abundances were

TABLE 3 Pearson's correlation analysis for different water-quality variables and α-diversity indices.

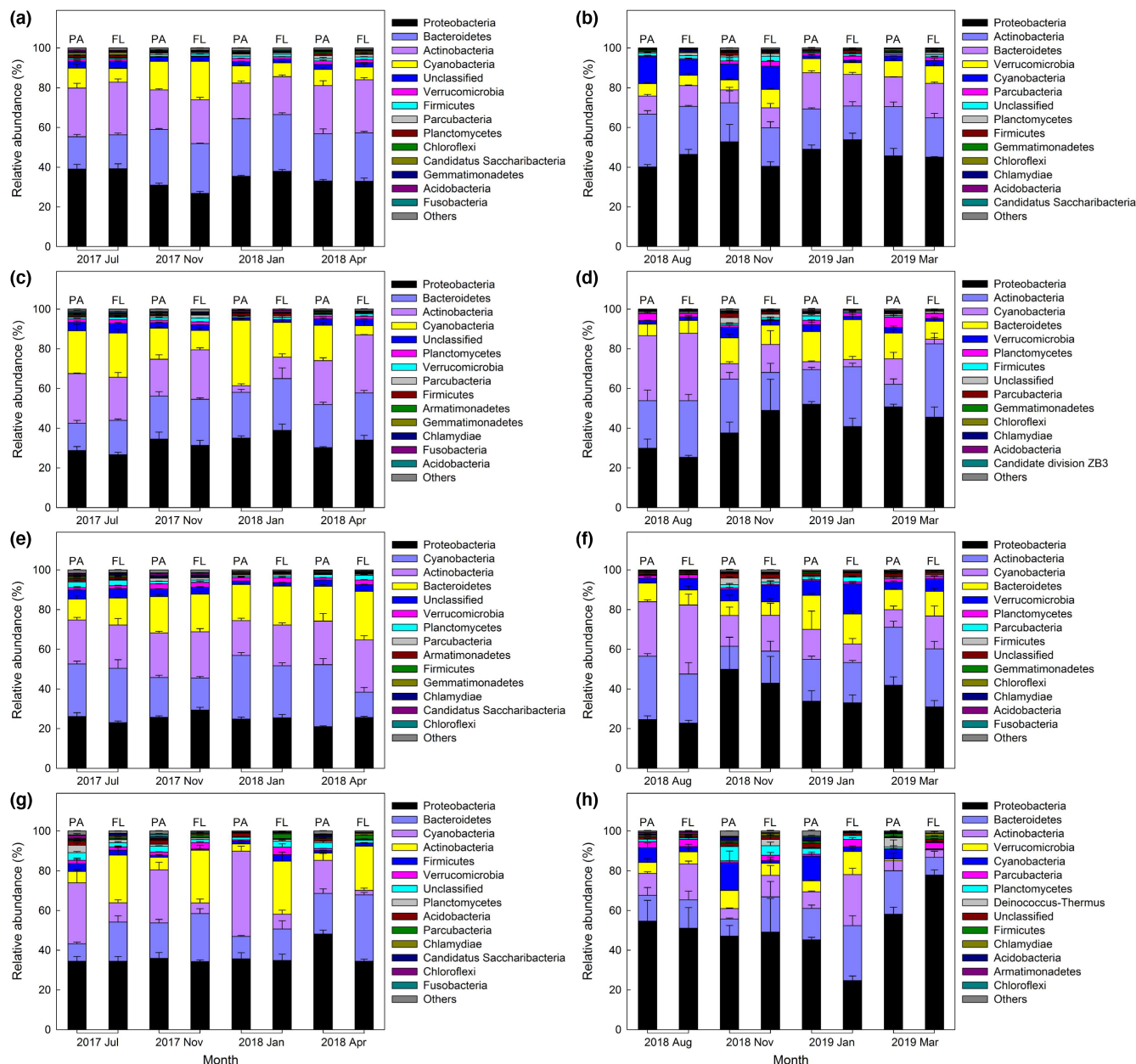|	First year			Second year		
	OTUs	Shannon	Chao1	OTUs	Shannon	Chao1
T						
Particle-attached	−0.163	0.244	−0.221	−0.333*	−0.272	−0.312*
Free-living	−0.335*	0.160	−0.395**	−0.254	−0.089	−0.329*
EC						
Particle-attached	−0.156	−0.046	0.048	−0.386**	−0.176	−0.375**
Free-living	−0.113	0.150	−0.039	−0.489**	−0.091	−0.537**
pH						
Particle-attached	−0.694**	−0.428**	−0.661**	0.167	0.148	0.180
Free-living	−0.823**	−0.082	−0.843**	−0.043	−0.108	0.094
DO						
Particle-attached	0.047	−0.179	−0.219	0.475**	0.205	0.304*
Free-living	−0.027	−0.523**	−0.030	0.107	−0.335*	0.202
TU						
Particle-attached	−0.174	0.072	−0.009	−0.352*	−0.118	−0.175
Free-living	−0.052	0.492**	−0.105	0.210	0.444**	0.063
NH_4^+						
Particle-attached	−0.348*	−0.255	−0.036	−0.340*	−0.051	−0.218
Free-living	−0.249	0.299*	−0.199	−0.184	0.306*	−0.183
NO_2^-						
Particle-attached	−0.449**	−0.244	−0.134	−0.417**	−0.208	−0.403**
Free-living	−0.382**	0.361*	−0.290*	−0.510**	−0.134	−0.564**
NO_3^-						
Particle-attached	−0.243	−0.321*	0.048	−0.181	−0.026	−0.016
Free-living	−0.150	0.173	−0.022	−0.235	0.067	−0.184
PO_4^{3-}						
Particle-attached	−0.122	0.011	0.113	−0.354*	−0.091	−0.143
Free-living	−0.018	0.369**	−0.004	0.116	0.467**	0.063
TOC						
Particle-attached	−0.564**	−0.323*	−0.250	−0.427**	−0.258	−0.342*
Free-living	−0.515**	0.347*	−0.458**	−0.349*	0.020	−0.431**
Chl-*a*						
Particle-attached	−0.360*	−0.150	−0.209	−0.350*	−0.227	−0.371*
Free-living	−0.395**	0.087	−0.321*	−0.538**	−0.210	−0.582**
SS						
Particle-attached	−0.149	0.054	0.038	−0.350*	−0.184	−0.257
Free-living	−0.033	0.421**	−0.064	0.213	0.348*	0.048

Abbreviations: Chl-*a*, Chlorophyll-*a*; DO, dissolved oxygen; EC, specific conductivity; NH_4^+, ammonium; NO_2^-, nitrite; NO_3^-, nitrate; PO_4^{3-}, orthophosphate; SS, suspended solids; *T*, water temperature; TOC, total organic carbon; TU, turbidity.
*$p < 0.05$; **$p < 0.01$.

somewhat different across the urban river network. TOC, NO_2^-, Chl-*a*, *T*, EC and NH_4^+ were identified as important environmental factors correlated with increases in the free-living bacterial abundance. By contrast, SS, TU, TOC, NH_4^+, PO_4^{3-} and DO were identified as important environmental factors correlated with variation in particle-attached bacterial abundance.

Organic matter, both allochthonous and autochthonous sources, can increase the biomass and productivity of bacteria (Gao et al., 2007; Morán et al., 2020). TOC, as a possible energy source for heterotrophic bacteria, is a measure of the aquatic carbon content in dissolved and particulate organic compounds (Cai et al., 2018; Siepak, 1999). Additionally, in this study, TOC was

FIGURE 5 Dominant bacterial phyla for particle-attached and free-living assemblages: (a, b) first and second year at river A; (c, d) first and second year at river B; (e, f) first and second year at river C; (g, h) first and second year at river D (respectively).The sum of relative abundances for the dominant phyla exceeded 97%. FL, free-living; PA, particle-attached.

measured from non-filtered samples meaning that these TOC values were not independent from organic carbon contained in the bacterial cells. This is likely to be one reason why TOC is related to both free-living and particle-attached bacterial abundance. The concentration of Chl-*a* is used as a proxy for algal biomass. Algal-derived dissolved organic matter is considered highly labile and is easily degraded by bacteria. For example, heterotrophic bacterial production increased by 25-fold after algal lysate addition (Pérez & Sommaruga, 2006). The NO_2^-, NH_4^+ and NO_3^- forms of mineral N are generated during the biodegradation–mineralisation–nitrification of domestic/industrial nitrogenous wastes as well as some fertilisers. Hence, the presence of NO_2^- and NH_4^+ is an

indication of organic pollutants (e.g., municipal domestic sewage) which contribute to bacterial proliferation (Aydin et al., 2005).

The EC is directly correlated with the ionic strength of a solution and is therefore correlated with dissolved ionic nutrients in river water. For example, in this study, EC showed a significant positive relationship with NH_4^+ ($R = 0.526$, $p < 0.01$), NO_2^- ($R = 0.513$, $p < 0.01$), NO_3^- ($R = 0.228$, $p < 0.05$) and PO_4^{3-} ($R = 0.365$, $p < 0.01$). Free-living bacteria need to absorb nutrients from water to satisfy their growth requirements, therefore the Chl-*a*, NO_2^-, NH_4^+ and EC variables all reflect the potential availability of nutrients to support free-living bacteria.

Compared to free-living bacteria, particle-attached bacteria not only absorb nutrients from water, but also can benefit from the
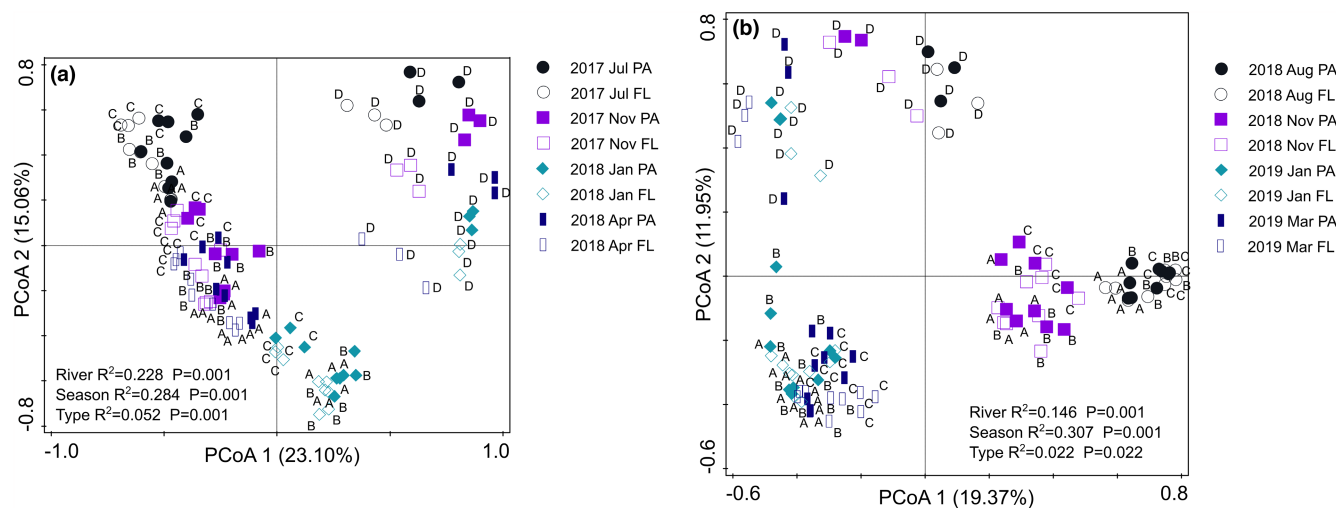
FIGURE 6 Principal coordinates analysis (PCoA) showing differences between particle-attached and free-living bacterial communities in different rivers (A, B, C and D) with time: (a) first year, (b) second year.. FL, free-living; PA, particle-attached. The R^2-values and p-values from the ADONIS analysis are reported.

TABLE 4 ADONIS and Whittaker index assessing differences between particle-attached and free-living bacterial communities in different rivers.

|	Adonis		Whittaker index
	R^2-value	p-value	
First year			
River A	0.024	0.620	0.351 ± 0.003
River B	0.085	0.060	0.384 ± 0.011
River C	0.052	0.267	0.376 ± 0.005
River D	0.270	0.001**	0.499 ± 0.009
Second year			
River A	0.023	0.759	0.464 ± 0.022
River B	0.064	0.168	0.472 ± 0.024
River C	0.027	0.725	0.461 ± 0.021
River D	0.104	0.009**	0.643 ± 0.042

**$p < 0.01$.

organic compounds and nutrients released from the particles (Cai et al., 2022; Gerba & McLeod, 1976; Tang et al., 2017). Particulate algal biomass can serve as habitat for particle-attached bacteria (e.g., biofilm communities), as well as a readily available carbon source for bacterial metabolism, often leading to high rates of oxygen consumption (Volkmar & Dahlgren, 2006). Nutrients released from particulate matter supplement dissolved nutrient availability supporting the nutritional needs of particle-attached bacteria. The particulate fraction was a key factor influencing particle-attached bacterial abundance. For example, the SS, TU and PO_4^{3-} variables were all significantly positively related to particle-attached bacterial abundance. TU measurements are often used as a proxy for SS in the water column (Rügner et al., 2013). Likewise, SS can serve as a vector for phosphorus through sorption/desorption buffering reactions (Bilotta & Brazier, 2008). In the present work, there were similar

spatial patterns between PO_4^{3-} and SS concentrations with the highest concentrations occurring in river A over the 2-year study periods. Therefore, the TU and PO_4^{3-} variables appear to be strongly associated with the amount of particles in the water column. Furthermore, the increases of SS, TU and PO_4^{3-} imply an increase in access to nutrients and habitats for particle-attached bacteria.

Water temperature is an important environmental factor that affects a myriad of bacterial physiological and biochemical processes. Bacterial activity is strongly a function of temperature with bacterial growth rates often having Q_{10} value of 2–3 (Cai et al., 2013; Li & Dickie, 1987). Notably, in the present work, T was significantly positively related to free-living bacterial abundance, but not to particle-attached bacterial abundance. Bacterial abundance was generally lower in the winter and was mainly a consequence of free-living bacteria accounting for the majority of the total bacteria. Aquatic particulate content of urban rivers is dependent on anthropogenic activities, as well as seasonal weather patterns (Komínková, 2012; Sun et al., 2016; Zhao et al., 2022). The autumn–winter dry season results in less urban storm runoff, thereby decreasing the amount of SS (Komínková, 2012; Sun et al., 2016) and, consequently, the abundance of particle-attached bacteria. These results clearly demonstrate the important influence of aquatic particulate matter on particle-attached bacterial abundance.

Dissolved oxygen was identified as a significant factor associated with particle-attached bacterial abundance. DO is certainly a very important factor affecting many geochemical and microbiological processes in aquatic environments (He et al., 2011). Biochemical processes related to organic pollution and nutrient concentrations may result in an effect of particle-attached bacterial abundance on DO (Banerjee et al., 2019; Zhang et al., 2022). For example, considering the bacterial biological oxygen demand, inputs of large amounts of particulate materials containing organic substrates/nutrients and associated bacteria were possibly responsible for contributing to a decreased DO concentration. Thus, the particulate matter input appears to be the variable most strongly associated
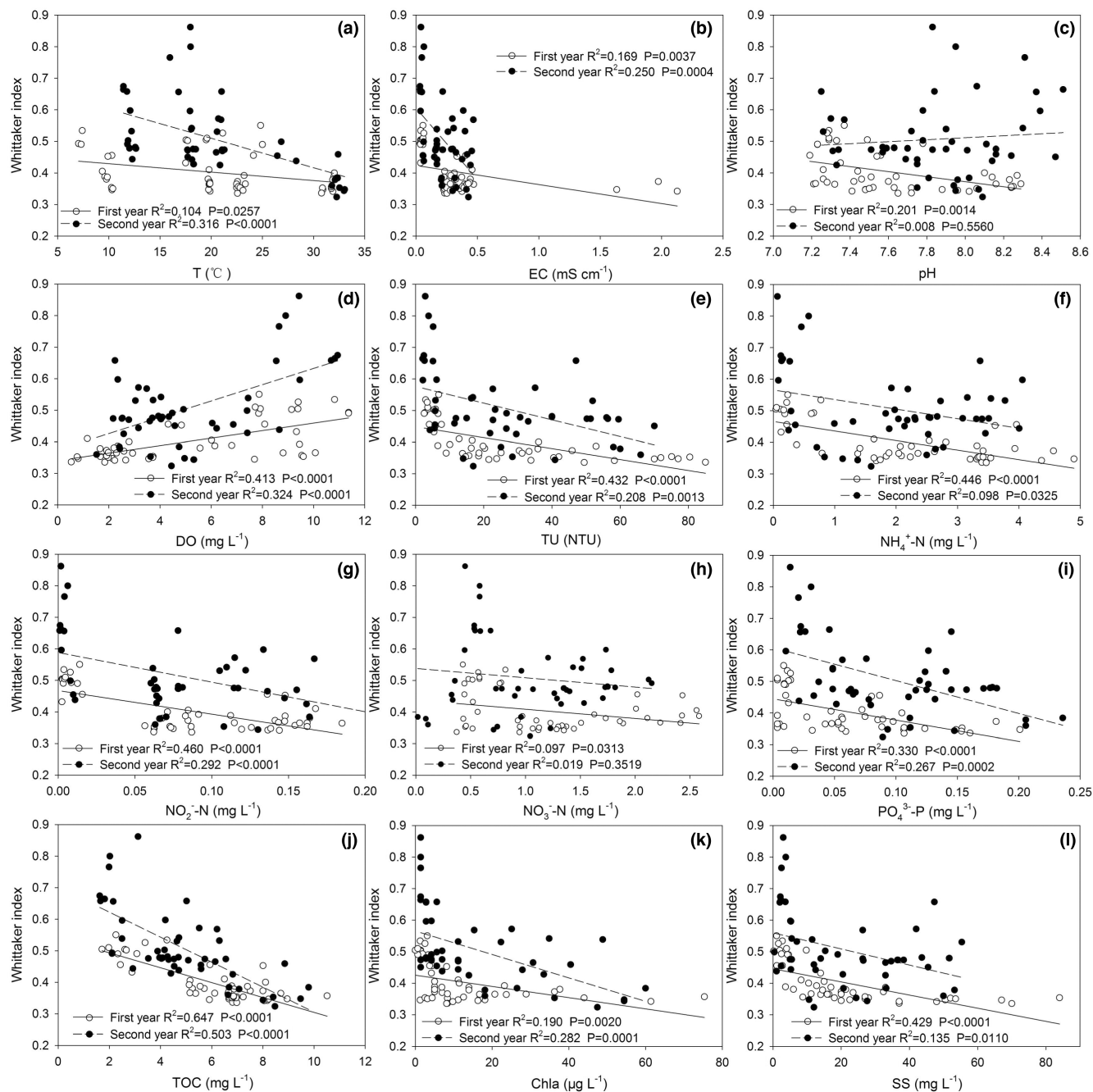
FIGURE 7 Linear relationships between the similarity for particle-attached and free-living bacterial communities with key water-quality variables.

with both particle-attached bacterial abundance and DO concentrations. Finally, considering that bacterial abundance estimated by DAPI does not differentiate between live and dead cells, additional studies are warranted to elucidate the difference in the proportions of live and dead cells between particle-attached and free-living assemblages, and their relationship with environmental factors.

4.2 | Bacterial α-diversity

High-throughput sequencing enables exploration of complex microbial communities, allowing researchers to establish links

between the microbial communities and their environment (Dean et al., 2021; Logares et al., 2012). MiSeq and HiSeq from Illumina are among the most widely used high-throughput sequencing platforms to study microbial communities. Although the HiSeq and MiSeq platforms differ markedly in scale, the α- and β-diversities of microbial communities generated by the two platforms are similar (Caporaso et al., 2012; Na et al., 2020). In fact, our results are consistent with this conclusion. For a specific river, we found some significant differences in bacterial α-diversity between particle-attached and free-living bacterial communities, but not for all cases. Across all samples, regardless of the high-throughput sequencing platform, significantly higher α-diversity was found
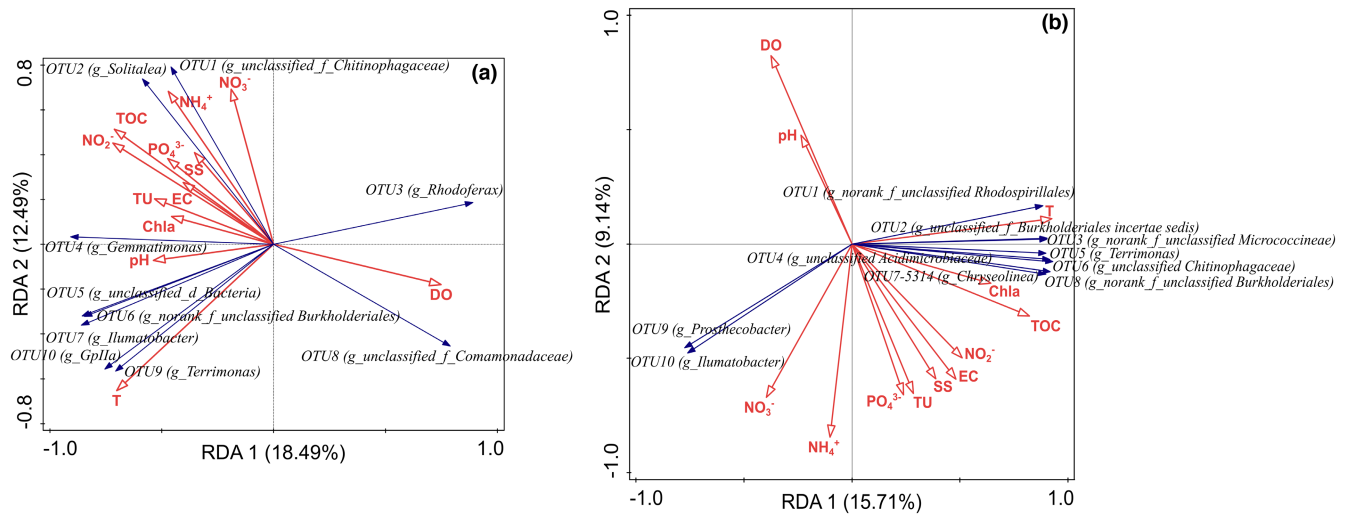
FIGURE 8 Redundancy analysis (RDA) showing the effects of environmental factors on bacterial communities: (a) first year, (b) second year. For the graph, the 10 OTUs that explain the greatest variance are presented.

TABLE 5 Percentage of the total variance for bacterial communities explained by environmental factors as determined by redundancy analysis (RDA).

	Explanatory variables	Explained variance %	*F*-value	*p*-value
First year	T	14.6	16.1	0.001
	TOC	13.0	14.1	0.001
	NO_2^-	12.9	13.9	0.001
	DO	12.3	13.2	0.001
	NH_4^+	10.6	11.2	0.001
	pH	8.3	8.5	0.001
	PO_4^{3-}	8.2	8.4	0.001
	NO_3^-	7.9	8.0	0.001
	TU	7.6	7.7	0.001
	EC	6.5	6.5	0.001
	SS	5.8	5.8	0.001
	Chl-*a*	5.7	5.6	0.001
Second year	T	14.0	15.1	0.001
	TOC	11.9	12.5	0.001
	DO	8.7	8.8	0.001
	Chl-*a*	8.2	8.3	0.001
	EC	7.7	7.7	0.001
	NO_2^-	7.3	7.3	0.001
	NO_3^-	7.2	7.2	0.001
	NH_4^+	7.1	7.1	0.001
	SS	6.7	6.7	0.001
	TU	6.2	6.2	0.001
	PO_4^{3-}	5.9	5.9	0.001
	pH	4.8	4.7	0.001

Abbreviations: Chl-*a*, Chlorophyll-*a*; DO, dissolved oxygen; EC, specific conductivity; NH_4^+, ammonia; NO_2^-, nitrite; NO_3^-, nitrate; PO_4^{3-}, orthophosphate; SS, suspended solids; *T*, water temperature; TOC, total organic carbon; TU, turbidity.

in the particle-attached versus free-living bacterial communities, consistent with the findings of several previous reports (Liu et al., 2019; Ortega-Retuerta et al., 2013; Tang et al., 2017). This may result from particle association providing bacteria with several benefits, such as access to growth resources (e.g., organic substrates and nutrients), a favourable habitat (e.g., biofilm communities) and protection from predators and environmental stress (Dang & Lovell, 2016; Hu et al., 2020).

Most previous studies concluded that inorganic nitrogen and phosphorus enrichment led to a decrease in bacterial α-diversity of aquatic ecosystems (Hu et al., 2020; Kiersztyn et al., 2019; Tang et al., 2021). However, our previous study indicated that the species richness and diversity of ammonia-oxidising bacteria increased with increasing NH_4^+ (Cai et al., 2022). In addition to nitrogen and phosphorus, organic carbon is an additional important factor that shapes bacterial α-diversity (Deng et al., 2018; Jiang et al., 2020). However, the effects of organic carbon on bacterial α-diversity are variable. For example, Deng et al. (2018) indicated that the addition of organic carbon sources to biofloc systems improved the overall bacterial α-diversity. However, this is contradictory with the findings of Jiang et al. (2020), who reported that diversity of the bacterial community did not change much with the addition of carbon sources. In this study, we observed an initial decrease followed by an increase in bacterial α-diversity as nutrient concentrations increased (from river D to river A). However, when contrasted with river C (agricultural), river A (commercial and residential) had a significant higher richness and diversity in bacterial communities throughout the 2-year study period. This may reflect the integrated results of multi-factor effects (e.g., nutrient and organic carbon sources). For river B (industrial), the appreciably lower bacterial richness and diversity metrics are possibly associated with inputs of untreated wastewater (i.e., toxicity potential) from the surrounding industrial area (Subrahmanyam et al., 2014; Subrahmanyam et al., 2016). Thus, care must be taken to consider both bacterial growth promoters (e.g., organic carbon and nutrient sources) and potential toxicity factors (e.g., metals and organic pollutants) when interpreting aquatic bacterial communities.

The effects of water-quality variables on bacterial α-diversity may be more complex than previously thought. In our 2-year study, no consistent relationship was found between water-quality variables and the Shannon index and Chao1 of particle-attached bacteria. NH_4^+, NO_2^-, TOC and Chl-*a*, which reflected organic pollution and organic matter (Aydin et al., 2005), were identified as important factors associated with reductions in the observed OTU richness of particle-attached bacteria. It is interesting that NO_2^-, TOC and Chl-*a*, which also reflected organic pollution and organic matter, were identified as important factors associated with reductions in the observed OTU richness of free-living bacteria. Additionally, the factors (*T*, NO_2^-, TOC and Chl-*a*) were correlated with reductions in the Chao1 of free-living bacteria were basically the same factors (NO_2^-, TOC and Chl-*a*) that affected the observed OTU richness of free-living bacteria. In the present study, *T* showed a significant positive relationship with NO_2^- ($R=0.283$, $p<0.01$), TOC ($R=0.481$, $p<0.01$)

and Chl-*a* ($R=0.447$, $p<0.01$). Therefore, these results indicated that organic carbon was related to decreased free-living bacterial richness and observed OTU richness of particle-attached bacteria. These results were different from those of Li et al. (2020), who found that organic matter content correlated positively with bacterial richness in sediments along the Yangtze River that were subject to different levels of urbanisation. Furthermore, Hosen et al. (2017) suggested that processes driving changes in the bacterial community of urbanised water column versus sediment environments are distinct. Hence, a possible reason for the difference between our results and those of Li et al. (2020) may result from the sample media (i.e., water column vs. sediment) used for studying the bacterial community.

In addition, it is notable to find that TU, NH_4^+, PO_4^{3-}, SS and DO were identified as environmental factors strongly associated with variations in the Shannon index of free-living bacteria. The SS, TU and PO_4^{3-} variables appeared to be strongly associated with the amount of particles in the water column. NH_4^+ is an indication of organic pollutants (Aydin et al., 2005). Considering that particulate matter inputs containing organic substrates/nutrients appear to be strongly associated with DO concentrations, these results reflected that particulate matter input increased free-living bacterial diversity. This implies that many bacteria may alternate between particle-attached and free-living forms.

4.3 | Bacterial community composition

Aquatic microorganisms can be classified into particle-attached and free-living assemblages according to their different lifestyles (Grossart, 2010), and exploring the relationships between the two assemblages has been a long-standing interest to microbiologists (Hu et al., 2020). Previous research examining the community structure of particle-attached and free-living bacteria in aquatic ecosystems resulted in several conflicting observations. While some studies revealed distinct differences between particle-attached and free-living bacterial community compositions (Liu et al., 2019; Salazar et al., 2015; Zhao et al., 2017), others showed similar bacterial compositions between the two assemblages (Tang et al., 2015; Tang et al., 2017). One reason for this discrepancy may be that there are few bacterial species that are exclusively particle-attached or truly free-living bacteria (Tang et al., 2017). Hence, many bacteria may alternate frequently between particle-attached and free-living forms depending on the influence of environmental factors.

In the present study, 10 water variables (*T*, EC, TU, NH_4^+, NO_2^-, PO_4^{3-}, TOC, Chl-*a*, SS and DO) were correlated with community similarity between particle-attached and free-living fractions. TOC (first year: $R^2=0.647$, $p<0.0001$; second year: $R^2=0.503$, $p<0.0001$) was identified as the most important environmental factor associated with community composition similarity between particle-attached and free-living assemblages. Suspended particles are an important nutritional source (e.g., of carbon) for aquatic organisms (Gloeckler et al., 2018). Although free-living bacteria and particle-attached bacteria are living in the same river, they colonise

different niches with distinct nutrient conditions. This provides a similar environment on-and-off particles as TOC concentrations increase. This may be one reason for the similar bacterial communities between free-living and particle-attached fractions in the high-TOC river water. Additionally, the higher the concentration of suspended particles, the higher the possibility of bacteria exchange between particle-attached and free-living forms. Moreover, considering the lower oxygen concentration in the interior of suspended particles, the surrounding water with lower DO concentration has a more similar environment to the inside of suspended particles. Thus, the oxygen gradient within particles supports a similar community composition between particle-attached and free-living assemblages.

A primary objective of this work was to investigate which factors are associated with changes in bacterial community composition in hypereutrophic urban river networks. Based on our 2-year data record, 12 water variables (T, TOC, NO_2^-, DO, NH_4^+, pH, PO_4^{3-}, NO_3^-, TU, EC, SS and Chl-a) were correlated with influencing the variation in bacterial community composition. Clearly, multiple physicochemical variables interact to influence the bacterial community composition in this urban river network. Additionally, RDA analysis demonstrated that T and TOC were the factors most strongly correlated with bacterial community composition in both years. Li et al. (2020) reported that water temperature was significantly correlated with the composition of the bacterial community in sediments of the Yangtze River exposed to different levels of urbanisation. Water temperature is one of the most important factors controlling aquatic microbial community activity, with each bacterial species having its own optimal temperature range for growth. Among all assessed water-quality variables, the most distinct seasonal variation occurred for T. Hence, seasonal variations in bacterial community composition were linked to the seasonal changes of T.

Organic carbon is a critical resource for the proliferation of heterotrophic bacteria. Aquatic bacterial communities are strongly influenced by the bottom-up effects of resource supply (i.e., organic carbon substrates, nutrients; Muscarella et al., 2019). Many freshwater systems receive substantial inputs of terrestrial organic matter (Brett et al., 2017), which is especially true for urban rivers. For example, the genera *Solitalea* and *Chryseolinea* associated with TOC were isolated from soil, implying that the study area received inputs of terrestrial organic matter. The amount and characteristics of organic matter in urban rivers are related to the surrounding land-use type (Tang et al., 2019). In the present work, the selected rivers had different surrounding land-use types, such as commercial and residential (river A), industrial (river B), agricultural (river C) and natural wildlands (river D). Although we had no data on the composition of organic matter, significant differences were observed in TOC concentrations among rivers. Notably, TOC concentrations showed no regular seasonal patterns resulting from wet–dry season alternation. Combined with the results of RDA analysis, we posit that differences in TOC concentrations were an important factor contributing to spatial differences in bacterial community composition in the hypereutrophic urban river network.

The OTUs identified as influential taxa via the RDA were different than those previously identified in an urban stream ecosystem studied by Hosen et al. (2017) (e.g., *Polynucleobacter*, *Candidatus Planktophila limnetica*, *Paucibacter*). An OTU from the genus *Rhodoferax*, which is frequently found in stagnant aquatic environments exposed to light (Hiraishi et al., 1991; Jin et al., 2020), was found under similar hydrodynamic conditions in our study area. We also found the genus *Ilumatobacter*, which is of marine origin (Khan et al., 2012; Matsumoto et al., 2009), suggesting that the study area receives some atmospheric inputs from the nearby ocean. The occurrence of the genus *Chryseolinea* may further suggest a tolerance to organic contaminants as it has been found previously in environments containing phenanthrene, pyrene and 4-chlorophenol (He et al., 2016; Zhao et al., 2018). Likewise, the genus *Terrimonas* was linked to the degradation of anthracene or benzo-a-pyrene (Song et al., 2015; Zhang et al., 2011), a possible industrial pollutant in our study area. Considering that these bacteria are strongly related to their environments, the presence of these specific taxa might serve as a bioindicator for a potential threat of toxic organic pollutants to human health in our hypereutrophic urban river network. Accordingly, these results indicate that the bacterial composition may be useful as an indicator for impaired water quality and aquatic ecosystem health.

5 | CONCLUSIONS

Spatio-temporal dynamics of bacterial abundance, diversity and community composition in both particle-attached and free-living assemblages were investigated within a hypereutrophic urban river network of eastern China. Our results revealed that particle-attached bacterial abundance depended mainly on the amount of suspended particles, whereas free-living bacterial abundance correlated with the nutrient availability and water temperature. Across all samples, significantly higher bacterial α-diversity was found in particle-attached assemblages compared to free-living assemblages. However, spatio-temporal dynamics of bacterial α-diversity were more complex. Our results indicated that organic carbon reduced the free-living and particle-attached bacterial richness, and particulate matter input increased free-living bacterial diversity. Further analysis of taxonomic composition revealed four predominant phyla appearing in both the particle-attached and free-living assemblages: Proteobacteria, Bacteroidetes, Actinobacteria and Cyanobacteria. Relative to a low-nutrient/low-turbidity river, there were no significant differences in bacterial community composition between particle-attached and free-living assemblages in nutrient-rich urban rivers. Our results suggest that multiple physicochemical variables interact to influence the composition similarity between particle-attached and free-living bacterial communities, and among them TOC was the most significant factor. Spatio-temporal variations of water quality were accompanied by changes in the composition of particle-attached and free-living bacterial communities. Although multiple physicochemical variables interact to influence the bacterial community

composition, RDA analysis demonstrated that T and TOC were the factors most strongly correlated with the seasonal and spatial dynamics of bacterial community compositions. Considering the importance of organic matter to bacterial communities in hypereutrophic urban river networks, additional studies are warranted to further elucidate the role of organic matter (quality and quantity) and its underlying mechanisms.

AUTHOR CONTRIBUTIONS

Conceptualisation: Xianlei Cai, Randy A. Dahlgren. Developing methods: Xianlei Cai, Ling Yao. Data analysis: Xianlei Cai, Ling Yao, Yuanyuan Hu. Preparation of figures and tables: Xianlei Cai, Ling Yao, Zhenfeng Wang. Conducting the research, data interpretation: Xianlei Cai, Ling Yao, Yuanyuan Hu, Siyu Wang. Writing: Xianlei Cai, Ling Yao, Yuanyuan Hu, Siyu Wang, Zhenfeng Wang, Randy A. Dahlgren.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interests.

DATA AVAILABILITY STATEMENT

The raw sequence data in this study were submitted to the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under accession number PRJNA847357 (first year) and PRJNA847387 (second year). The data supporting the conclusions of this article are included within the article.

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