



OPEN Similar but different assembly processes of bacterial and micro-eukaryotic communities in an urban river

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Bacteria and micro-eukaryotes play important roles in river ecological systems. The processes that govern bacterial and micro-eukaryotic communities in urban rivers are still uncertain. The spatiotemporal characteristics and assembly processes of bacterial and micro-eukaryotic communities in the Xiangjianghe River were explored using 16 S and 18 S rRNA gene amplicon sequencing in the present study. The results indicate that the bacterial and micro-eukaryotic community composition exhibited distinct temporal and spatial variation. The topological characteristics of co-occurrence networks demonstrate that the bacterial and micro-eukaryotic community coexistence patterns vary significantly between the four seasons. Water temperature (WT) and oxidation-reduction potential (ORP) were detected as the most critical factors affecting bacterial and micro-eukaryotic community structure. The stochastic process (dispersal limitation) was the dominant assembly process for bacteria and micro-eukaryotes in all seasons. Deterministic and stochastic processes influenced the bacteria and micro-eukaryotes differently. Compared to bacteria, the values of niche breadth were relatively lower, and the proportion of deterministic processes was relatively higher in micro-eukaryotes. These results expand our understanding of spatiotemporal patterns, assembly mechanisms, and influencing factors of bacterial and micro-eukaryotic communities in urban rivers.

Keywords Bacteria, Micro-eukaryotes, Seasonal variation, Community assembly, Stochastic process

Bacteria and micro-eukaryotes are crucial components in river ecosystems and play important roles in the degradation of pollutants, biogeochemical processes, enhancing self-purification ability of water environments, and the restoration of riverine ecosystems¹. Therefore, understanding the diversity and distribution of bacterial and micro-eukaryotic communities is vital to improve river environments and exerting ecological benefits.

The mechanisms of microbial community assembly have always been a core issue in research on aquatic ecology². It is crucial to the diversity, distribution, function, biogeographical patterns, and succession processes of microbial community^{3–5}. Based on niche theory, deterministic processes such as environmental filtering (pH, temperature, dissolved oxygen, organic matter, salinity, etc.) and interspecific interactions (competition, cooperation, or predation) dominate the composition and distribution of microbial community^{6,7}. In contrast, neutral theory postulates that the stochastic processes such as birth, death, migration, speciation, and dispersal limitation shape the structure of microbial community^{8,9}. Actually, deterministic and stochastic processes can coexist within the same system or context, as they are not mutually exclusive concepts. It is generally acknowledged that microbial community assembly is jointly driven by deterministic and stochastic processes¹⁰. In recent years, the mechanisms underlying microbial community assembly in multiple environments have been studied. However, these studies have primarily focused on low-mobility ecosystems such as oceans¹¹, soils¹², wetlands¹³, lakes¹⁴, and activated sludges¹⁵. In contrast to these low-mobility ecosystems, river environments and their microbial community exhibit more complex and dynamic characteristics¹⁶. Furthermore, studies have revealed that seasonality is a crucial driving factor of microbial community diversity and assembly processes within riverine ecosystems^{17–19}. Environmental factors including dissolved oxygen concentration, water temperature, and light will exhibit significant synergistic changes with seasonal variations, directly or indirectly affecting the structure, diversity, functions, and assembly processes of microbial community in the river^{5,8}. Nevertheless, research on seasonal succession and assembly mechanisms of microorganisms in river

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ecosystems remains limited, especially for micro-eukaryotes¹. Therefore, understanding the seasonal succession and assembly processes of bacteria and micro-eukaryotes in rivers will facilitate a deeper comprehension of the mechanisms for maintaining microbial diversity and ecological functions in river ecosystems.

Unlike natural river channels, urban rivers possess not only ecological functions such as flood control, water storage, and local climate regulation but also economic functions such as sewage reception and irrigation, as well as humanistic values like landscaping and entertainment²⁰. On the one hand, most of the water sources for urban rivers come from relatively stable effluent discharged from sewage treatment plants, resulting in small seasonality in water quality and quantity, and higher water temperature²¹. On the other hand, urban river channelization may cause habitat homogenization, resulting in overall decline in biodiversity²². Whether these differences compared to natural rivers lead to spatial and seasonal variation of microbial communities in urban rivers need to be further studied.

The Xiangjianghe River (XJH) is a tributary of the Wujiang River and is located in the northern part of China's Guizhou Province, and belongs to the Yangtze River basin. It flows through the urban area of Zunyi City with more than one million residents. Human activities may affect the ecological stability of XJH. Besides, the ecological health of XJH is vital to the sustainable development in this area. In the present study, microbial diversity patterns, community networks, and community assembly processes of bacteria and micro-eukaryotes in the XJH under four seasons were performed by 16 S and 18 S rRNA amplicon sequencing to address the following questions: (1) Do the bacterial and micro-eukaryotic communities exhibit spatiotemporal variability across different seasons? (2) What are the main influencing factors that affect the bacterial and micro-eukaryotic communities? (3) Do deterministic or stochastic processes dominate community construction of bacteria and micro-eukaryotes? The present study will provide an important and comprehensive insight for better understanding the bacterial and micro-eukaryotic community patterns in urban river environments.

Materials and methods

Sampling and environmental factors measurement

A total of 84 surface water samples were collected along the Xiangjianghe River (XJH) from seven sites (3 replicates) in May, July, and September 2022, and January 2023, which present spring, summer, autumn, and winter, respectively (Fig. 1). Sites 3 and 5 are located in the tributary, and sites 1, 2, 4, 6, and 7 are located in the mainstream of the XJH. Water samples were collected in sterile bottles and transported to the laboratory at 4 °C within 6 h for subsequent analysis. Water temperature (WT), pH, oxidation-reducing potential (ORP), dissolved oxygen (DO), and electrical conductivity (EC) were measured in situ using a YSI Professional Plus meter. Total nitrogen (TN) was determined by a UV spectrophotometric method with alkaline potassium persulfate elimination. Total phosphorus (TP) was determined by the ammonium molybdate spectrophotometric method. Chemical oxygen demand (COD_{Mn}) was measured using the potassium permanganate titration method.

DNA extraction, PCR amplification, and illumina miseq sequencing

For microbial community DNA extraction, 1000 ml water samples were filtered through 0.22 μ m filters within 6 h after collection and the membranes were stored at - 80 °C ultra-low temperature freezer before further

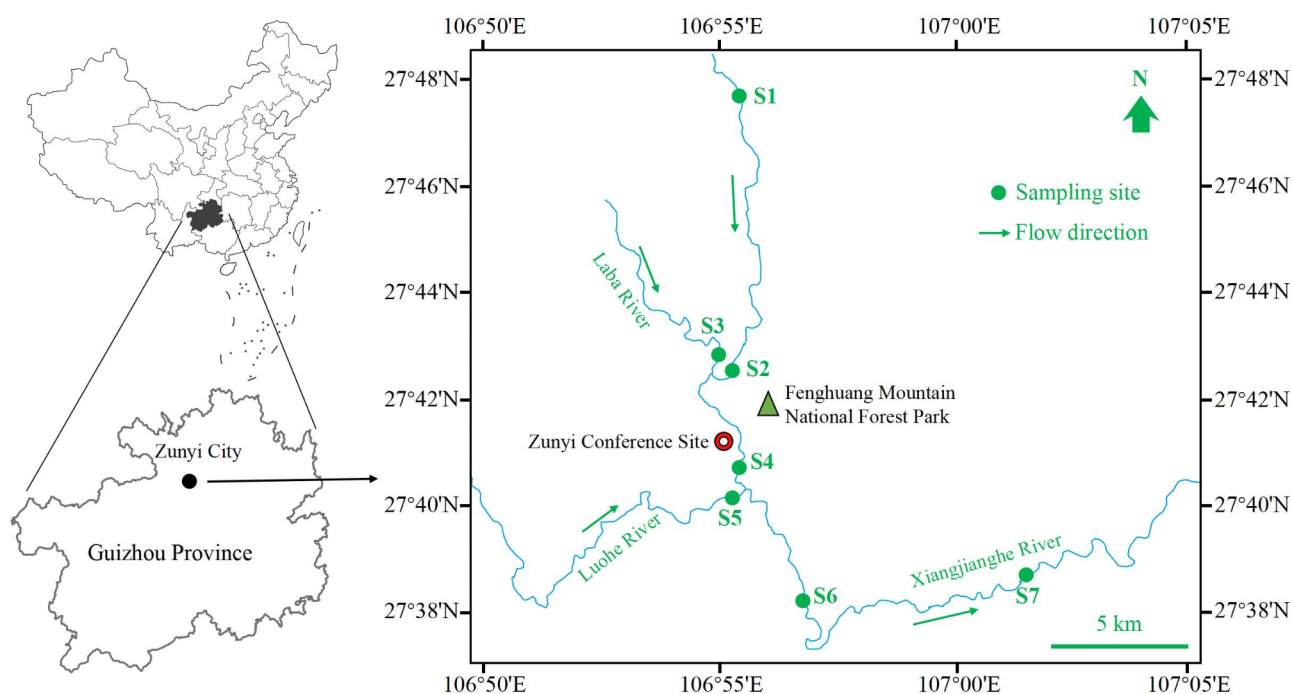


Fig. 1. Location of the sampling sites in the Xiangjianghe River.

treatment. Microbial DNA was extracted using the E.Z.N.A.[®] soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to the manufacturer's instructions. The V3-V4 region of the 16 S rRNA gene and the V4 region of the 18S rRNA gene were amplified by using the primer pairs 338/806R and 528 F/706R, respectively. The PCR reactions were performed in triplicate, with each 20 μ L mixture containing 4 μ L of 5 \times FastPfu Buffer, 2 μ L of 2.5 mM dNTPs, 0.8 μ L of 5 μ M primer, 0.4 μ L of FastPfu Polymerase, and 10 ng of template DNA. Subsequently, the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and QuantiFluor[™]-ST (Promega, USA) were used for the purification and quantization of PCR products. The purified products were pooled in equimolar and paired-end sequenced (2 \times 300) on an Illumina MiSeq platform (Illumina, San Diego, USA) in Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

Statistical analysis

All bacterial and micro-eukaryotic sequence analyses were performed on the i-sanger cloud platform of Majorbio BioTech Co., Ltd (<http://www.i-sanger.com/>). Operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using UPARSE (version 7.1 <http://drive5.com/uparse/>) with a novel “greedy” algorithm that performs chimera filtering and OTU clustering simultaneously. All chloroplasts, mitochondria, and OTUs with total sequences of less than 20 were removed. The data was normalized by subsampling to the least number of reads in order to synchronize the sequencing depth across all samples, following which the distinctive sequences were selected for subsequent analysis. The bacterial and micro-eukaryotic taxonomies were analyzed by the RDP Classifier algorithm against the silva138/16s_bacteria and silva138.1/18s_eukaryota database using a confidence threshold of 70%, respectively. Kruskal-Wallis H test was used to determine the differences in Chao1 and Shannon indices between different seasons and sampling sites. The β NTI (β nearest-taxon index) and RC_{Bray} (modified Raup-Crick index) were calculated to evaluate the impact of stochastic and deterministic processes on the aggregation of microbial communities across different spatial and temporal scales. The neutral community model (NCM) was also performed to quantify the influence of stochastic processes in shaping microbial community. Co-occurrence characteristics of the microbial community in four seasons were revealed by network analysis according to the Spearman correlation coefficients ($|r| > 0.8$, $p < 0.05$). The OTUs with relative abundance greater than 1% were retained for better visualization. The visualization and topological analysis of network was conducted in Gephi (version 0.9.2). In order to identify the keystone taxa, the nodes were classified into four topological roles (Module hubs, Connectors, Network hubs, and Peripherals) according to the values of within-module connectivity (Z_i) and among-module connectivity (P_i). In this study, the analysis of Co-occurrence pattern, NCM, and niche breadth were performed in R software (version 4.3.1), while others such as the α -diversity index (Chao1 and Shannon), non-metric multidimensional scaling (NMDS), microbial spatiotemporal distribution characteristics, β NTI/ RC_{Bray} and Z_i/P_i analysis were performed by the online tools of Majorbio Cloud Platform (<https://cloud.majorbio.com/page/tools/>)²³.

Results

Diversity of microbial community

In total, 4,064,995 high-quality 16 S rRNA sequences and 5,537,316 high-quality 18 S rRNA sequences were obtained. The sequencing coverage rates ranged from 98.4 to 99.8% and the rarefaction curves of all samples reached a plateau (Fig. S1), which indicates the current sequencing depth is sufficient. The Chao1 and Shannon indices were calculated to assess the richness and diversity of microbial community (Fig. 2). For bacteria, no significant difference was found in the Chao1 index between the four seasons ($p > 0.05$). The Shannon index in winter was higher than that in other seasons, and significantly higher than that in summer ($p < 0.05$). For micro-eukaryotes, however, the Chao1 index in spring was significantly higher than that in autumn and winter ($p < 0.001$), and the Shannon index in spring was significantly higher than that in autumn ($p < 0.01$). In addition, the Chao1 and Shannon indices of bacteria and micro-eukaryotes also displayed significant differences across sampling sites. The maximum Chao1 index of bacteria was found in S6 for spring, summer, and winter, while in S5 for autumn. The minimum value of Chao1 and Shannon index of bacteria was found in S4 for all four seasons, except for the Shannon index in autumn. The maximum Chao1 index of micro-eukaryotes was found in S6 for spring, S5 for summer and autumn, and S3 for winter. The minimum Chao1 index of micro-eukaryotes was found in S2 for spring and summer, and S4 for autumn and winter. Similar trends were also found in the Shannon index of micro-eukaryotes for spring and winter.

Microbial community composition

The community composition of bacteria and micro-eukaryotes exhibited distinct temporal and spatial variations. For bacteria, the OTUs number in each sample ranged from 510 to 1425, with an average of 957. A total of 1741 core OTUs which present in all four seasons was found, and samples from spring, summer, autumn, and winter had 4, 1, 32, and 6 unique OTUs, respectively (Fig. 3a). The results of NMDS revealed that the bacterial community was grouped by seasons (Fig. 3c). Although there were overlaps between spring and winter, as well as between summer and autumn, the clustering still manifested more obvious seasonal patterns. Notably, the spring and winter samples were separated from the summer and autumn samples. The samples of S4 in summer, autumn, and winter were separated far from other samples (Fig. S3a). The mean bacterial relative abundance at phylum level is present in Fig. 3e. Proteobacteria, Bacteroidota, and Actinobacteriota were predominant (with a mean relative abundance range from 57.6 to 96.0%) in most samples. In particularly, the relative abundance of Cyanobacteria (23.6%) at S4 in summer was higher than that of Bacteroidota (12.0%) and Actinobacteriota (10.2%). The mean relative abundance of Proteobacteria in spring (46.9%) was higher than that in other seasons (41.7% in summer, 37.0% in autumn, and 43.2% in winter, respectively), while the mean relative abundance of Bacteroidota (28.6%) and Cyanobacteria (5.5%) in summer, Actinobacteriota (24.6%) and Verrucomicrobiota (3.7%) in autumn, and Campylobacterota (7.3%) and Firmicutes (6.9%) in winter was higher than that in other

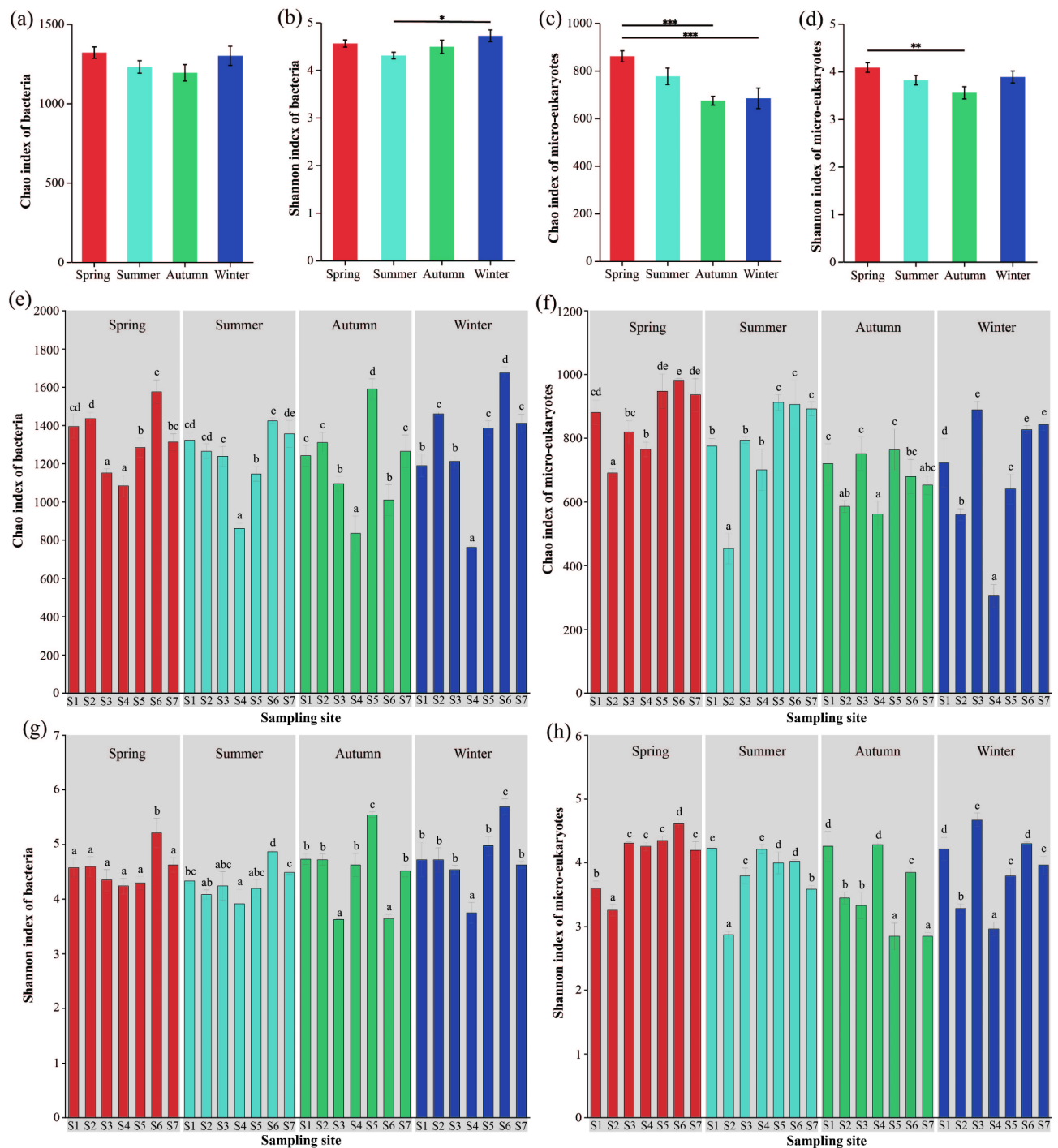


Fig. 2. Alpha diversity of bacterial and micro-eukaryotic communities. *, **, and *** represent $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Different letters above the bar chart indicate significant differences between sampling sites ($p < 0.05$).

seasons. Kruskal-Wallis test results also showed that there were significant differences in the mean relative abundance of abundant bacterial taxa among the different seasons (Fig. 3g). At the genus level, *Limnohabitans* (13.3%), *Flavobacterium* (11.2%), and *hgcI*_clade (5.5%) were the dominant bacteria (Fig. S2a). The abundance of *Flavobacterium* in spring (11.3%) and winter (13.8%) was much higher than that in summer (9.0%) and autumn (10.7%), while the abundance of *hgcI*_clade in spring (4.8%) and winter (2.4%) was much lower than that in summer (6.8%) and autumn (8.1%).

For micro-eukaryotes, the average number of OTUs was 584. A total of 951 core OTUs was found in four seasons, and 40, 20, 101, and 56 unique OTUs were found in spring, summer, autumn, and winter, respectively (Fig. 3b). Compared to bacteria, micro-eukaryotic community composition exhibited more intense

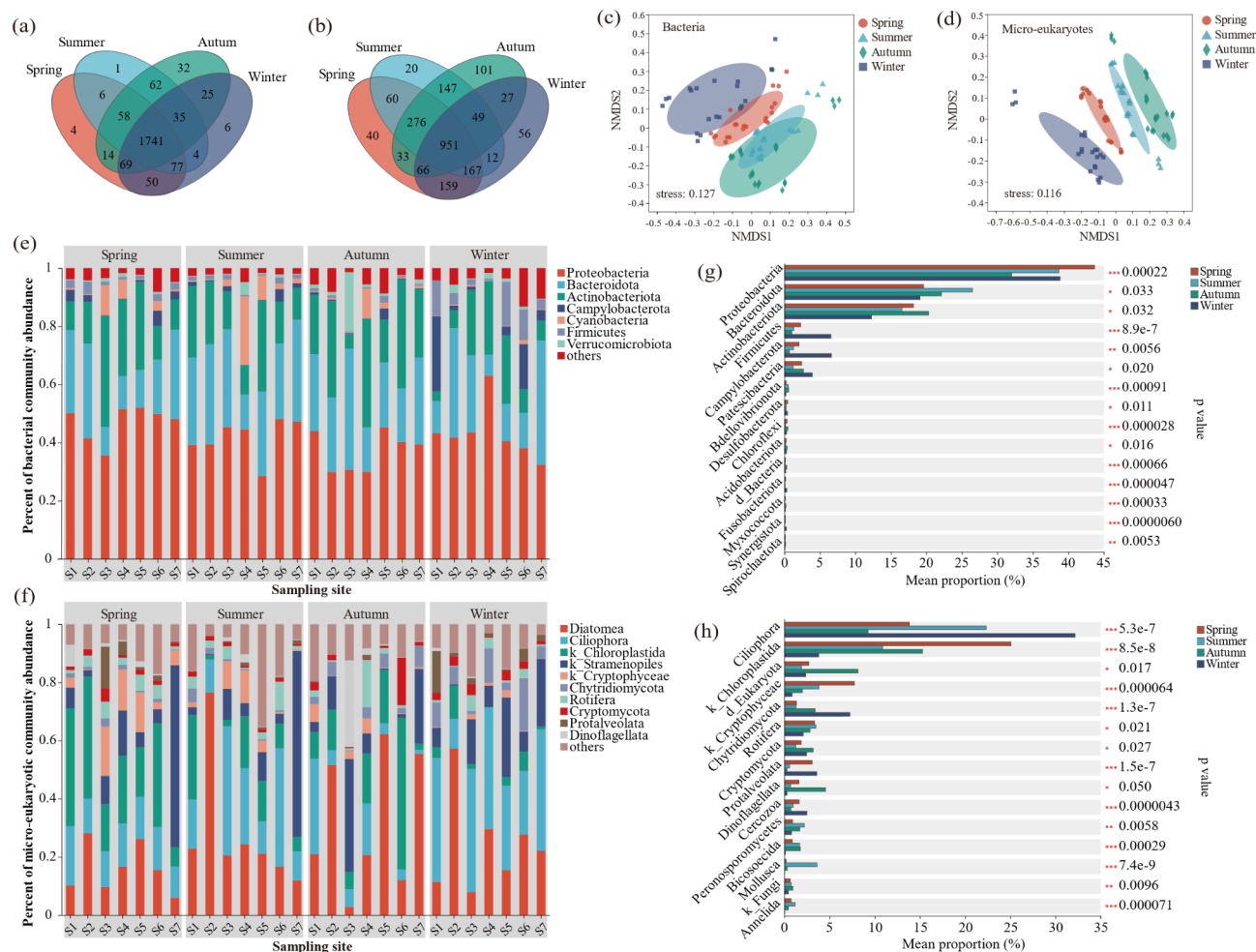


Fig. 3. Seasonal variation of bacterial and micro-eukaryotic community composition in the XJH. Venn diagram displaying the OTUs richness distribution of bacteria (a) and micro-eukaryotes (b) in different seasons. Nonmetric multidimensional scaling analysis (NMDS) of bacterial (c) and micro-eukaryotic (d) community at OTU level based on Bray-Curtis distance in different seasons. Mean relative abundance of different phyla at different sampling sites in four seasons for bacteria (e) and micro-eukaryotes (f). Significance test of the difference in the relative bacterial (g) and micro-eukaryotic (h) abundance at the phylum level among four seasons using the Kruskal-Wallis H test.

spatiotemporal differences. Four clusters of micro-eukaryotes community composition of the samples were distinguished clearly from each other (Fig. 3d), indicating significant differences between the four seasons. In addition, the samples at S2 in summer, and at S4 in autumn and winter were separated far from other samples (Fig. S3b). The difference for each site was also significant. The top 10 taxa in the mean relative abundance of micro-eukaryotes at phylum level are shown in Fig. 3f. Diatomea, Ciliophora, unclassified_k__Chloroplastida, and unclassified_k__Stramenopiles were dominant in all samples. Unclassified_k__Chloroplastida was the most predominant taxa in spring (25.9%), Diatomea was the most abundant taxa in summer (27.7%) and autumn (32.2%), and Ciliophora was the most abundant taxa in winter (32.9%). The relative abundance of Diatomea at S2 in spring (28.1%), summer (76.5%), and winter (51.6%) was higher than that at other sites. In addition, 8 of the 10 top abundant taxa had significant differences among the four seasons, while other two abundant taxa Diatomea and unclassified_k__Stramenopiles showed no significant differences among the different seasons (Fig. 3h). At genus level, Cyclotella (11.1%), Ochromonas (6.3%), and Cladophora (4.7%) were the dominant micro-eukaryotes (Fig. S2b). The abundance of Cyclotella in spring (2.7%) was much lower than that in summer (14.9%), autumn (16.5%), and winter (10.3%), while Cladophora abundance in spring (14.3%) was much higher than that in summer (0.5%), autumn (1.7%), and winter (2.2%). The unclassified_f__Choreotrichia abundance at S4 in four seasons was much higher than that at other sampling sites.

Co-occurrence patterns of microbial community

Network analysis was conducted to assess the microbial community complexity affected by seasonality (Fig. 4a). The results of topological properties and network correlations were summarized in Table S1. The topological characteristics demonstrate that the bacterial and micro-eukaryotic community coexistence patterns vary

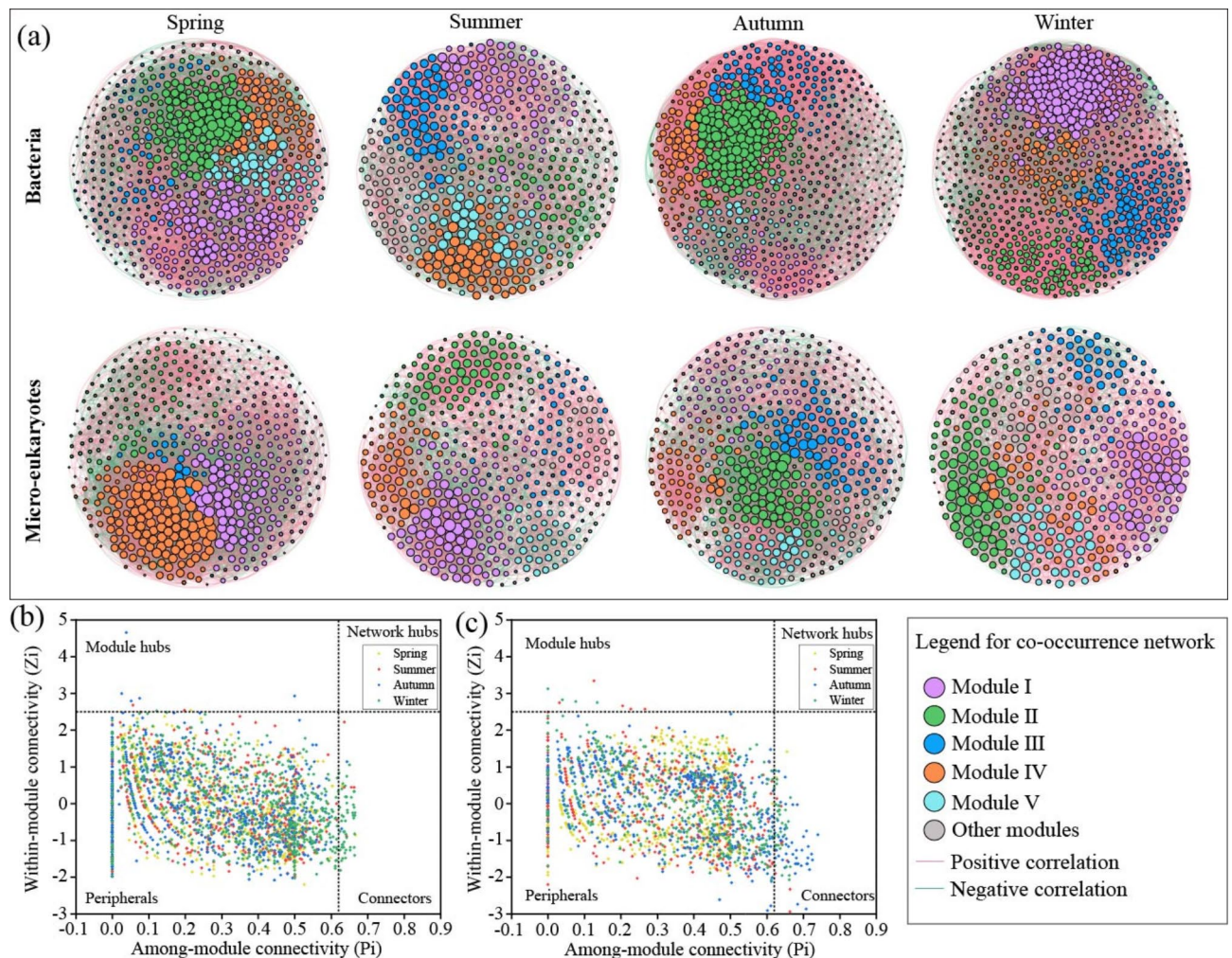


Fig. 4. Co-occurrence networks of bacterial and micro-eukaryotic communities in different seasons (a), and keystone species analysis based on Zi-Pi plots for bacteria (b) and micro-eukaryotes (c).

significantly between the four seasons. The top 5 major modules in networks of bacteria and micro-eukaryotes account for 81.64–87.88% and 85.69–91.12% of the nodes in four seasons, respectively. The number of nodes and edges, average degree, and average clustering coefficient of the bacterial network in winter were higher than those in the other three seasons, indicating that the most complex bacterial network was manifested in winter, followed by autumn, spring, and summer. The lowest value of graph density and the highest values of modularity and average path length were also found in summer. The network of micro-eukaryotes in spring was much more complex and compact, with significantly higher nodes, edges, graph density, average degree, and average clustering coefficient, and significantly lower modularity and average path length than other seasons. Obviously, most of the edges in these networks were positive, suggesting that positive correlation accounted for most of bacterial and micro-eukaryotic communities. The proportions of positive correlation for bacteria and micro-eukaryotes were ordered as autumn (86.26%) > winter (85.24%) > spring (67.44%) > summer (67.26%), and winter (80.61%) > summer (74.03%) > autumn (69.58%) > spring (66.31%), respectively.

According to the values of within-module connectivity (Z_i) and among-module connectivity (P_i), the positioning of nodes (OTUs) in the network was classified into the following four categories: module hubs ($Z_i > 2.5$, $P_i < 0.62$), network hubs ($Z_i > 2.5$, $P_i > 0.62$), peripherals ($Z_i < 2.5$, $P_i < 0.62$), and connectors ($Z_i < 2.5$, $P_i > 0.62$). The module hubs, network hubs, and connectors are commonly defined as keystone species. No node in the network hubs and more than 92% of the nodes in the peripherals were observed in the network of both bacteria (Fig. 4b) and micro-eukaryotes (Fig. 4c). For bacteria, there were 1, 2, 5, and 1 module hubs, and 2, 5, 2, and 49 connectors in spring, summer, autumn, and winter, respectively (Fig. 4b, Table S2). The nodes of module hubs belonged to the dominant phyla, such as Bacteroidota, Proteobacteria, Actinobacteriota, Patescibacteria, and Acidobacteriota. For micro-eukaryotes, no module hub was found in spring and autumn, while 5 and 4 module hubs were found in summer and winter, respectively. They belonged to Cryptomycota, Labyrinthulomycetes, Ciliophora, Protalveolata, unclassified_k__Chloroplastida, and Cercozoa. In addition, 2, 10, 48, and 34 connectors were found in spring, summer, autumn, and winter, respectively (Fig. 4c, Table S3).

Correlations between microbial communities and environmental factors

The relationship between microbial communities and environmental factors was conducted to discern significant linkages. Among the selected environmental factors in the present study, TN, WT, pH, ORP, and DO were significantly varied in different seasons (Table S4), and TN, TP, COD, and EC were significantly varied in different sampling sites (Table S5). The results of mantel test revealed that WT and ORP were significantly correlated with community composition of both bacteria and micro-eukaryotes ($r > 0.2$, $p < 0.01$), and the strongest relationship was found between WT and bacterial community ($r > 0.4$, $p < 0.01$) (Fig. 5a). In addition, in order to identify the main factors affecting the bacterial and micro-eukaryotic communities, the correlation analysis was conducted between the top 10 abundant phyla of microbial communities and the environmental factors. TP was significantly positively correlated with Campylobacterota, Firmicutes, and Patescibacteria ($p < 0.05$). WT was significantly positively correlated with Cyanobacteria, while significantly negatively correlated with Firmicutes ($p < 0.01$). ORP was significantly positively correlated with Bdellovibrionota ($p < 0.05$), while significantly negatively correlated with Proteobacteria ($p < 0.01$), Campylobacterota ($p < 0.05$), and Firmicutes ($p < 0.001$). Environmental factors were

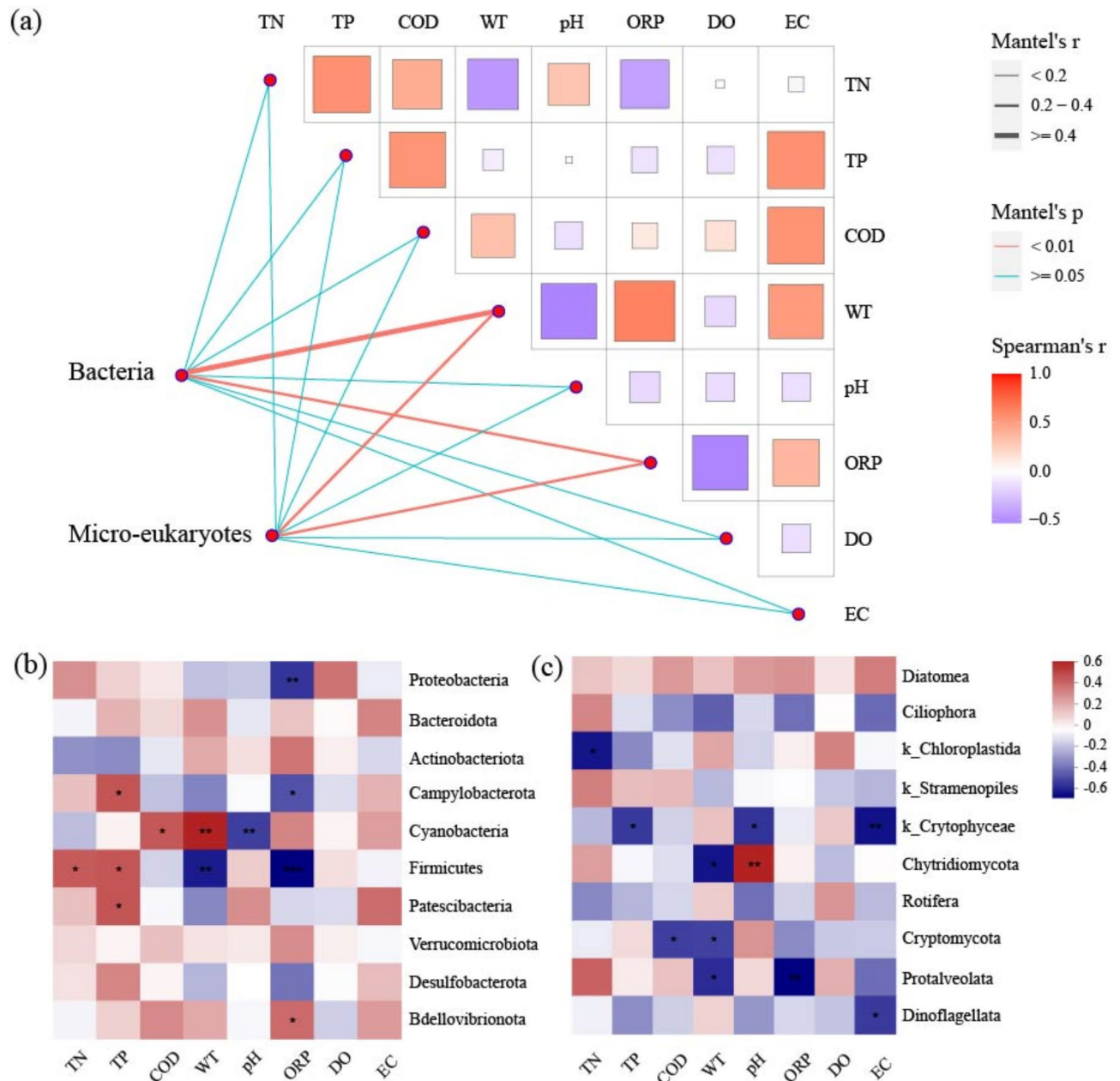


Fig. 5. Environmental factors affecting microbial community. (a) Environmental drivers of the bacterial and micro-eukaryotic communities by Mantel test; (b) Correlation of top 10 abundant phyla to environmental factors in bacteria; (c) Correlation of top 10 abundant phyla to environmental factors in micro-eukaryotes. *, **, and *** represent $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

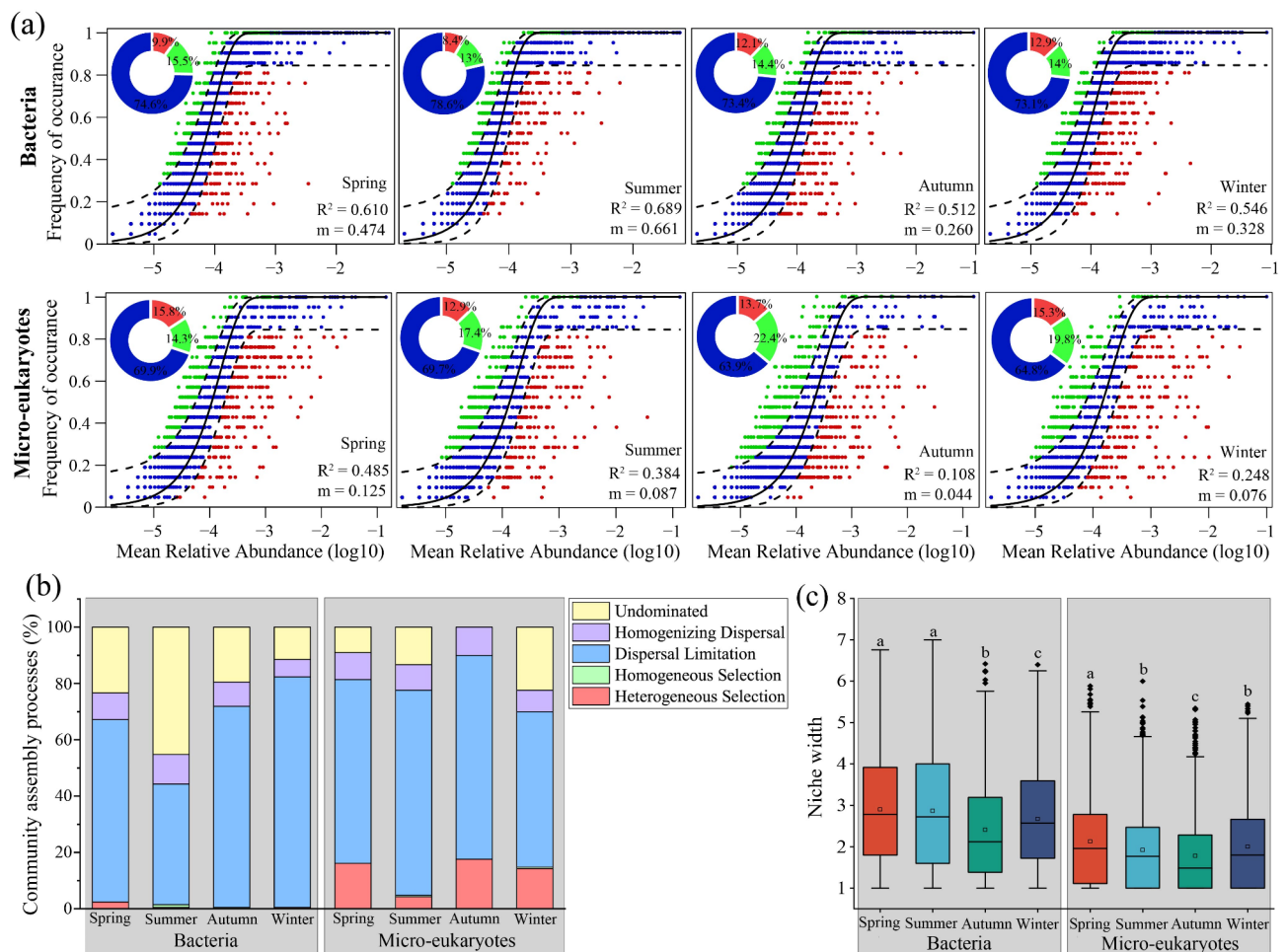


Fig. 6. Assembly process of the microbial community in different seasons. **(a)** Fit of the neutral community model (NCM) of bacteria and micro-eukaryotes in four seasons. Green and red dots represent the OTUs occurred more and less frequently than predicted, respectively. The black solid line represents the best fit to the NCM and the black dashed lines indicate the 95% confidence interval. **(b)** Relative contributions of different ecological processes in assembling the bacterial and micro-eukaryotic community. **(c)** Levins' niche breadth of bacterial and micro-eukaryotic in four seasons. Different letters represent significant differences among the four seasons ($p < 0.01$, multiple comparisons after the Kruskal-Wallis test).

mainly negatively correlated with the micro-eukaryotic community (Fig. 5c). WT was significantly negatively correlated with Chytridiomycota, Cryptomycota, and Protalveolata ($p < 0.05$). ORP was significantly negatively correlated with Protalveolata ($p < 0.01$). EC was significantly negatively correlated with unclassified_k_Cryptophyceae ($p < 0.01$) and Dinoflagellata ($p < 0.05$).

Assembly process of microbial communities

The neutral community model (NCM) was employed to assess the potential contribution of stochastic processes to the community assembly of bacteria and micro-eukaryotes (Fig. 6a). Most proportions of taxa for bacteria (73.1%–78.6%) and micro-eukaryotes (63.9%–69.9%) were within the dashed line. In addition, the NCM was strong in explaining the bacterial community with the R^2 were 0.610, 0.689, 0.512, and 0.546 in spring, summer, autumn, and winter, respectively, indicating the important roles of stochastic processes on bacterial community assembly. The migration rate (m) was highest in summer (0.661), followed by spring (0.474), winter (0.328), and autumn (0.260). The values of R^2 (0.108–0.485) and migration rate (0.044–0.125) in four seasons for micro-eukaryotes were lower than bacteria, suggesting that less stochastic processes in micro-eukaryotes compared to bacteria. Furthermore, the tendency of niche breadth in different seasons for the bacterial and micro-eukaryotic communities was similar to the migration rate (Fig. 6c). Bacterial communities exhibited relatively wider niche breadth than micro-eukaryotic communities.

The null model based on the β NTI and RC_{Bray} was applied to further determine the relative contributions of stochastic and deterministic processes (Fig. 6b). The results showed that stochastic process was the dominant process in all seasons for both bacteria and micro-eukaryotes. The deterministic process accounted for a negligible proportion of variation in bacterial communities (0–0.5% for heterogeneous selection and 0–1.0% for homogeneous selection). Dispersal limitation was the most important process affecting bacterial community

assembly in spring (64.8%), autumn (71.4%), and winter (81.9%), while undominated was the most important process in summer (45.2%). Dispersal limitation was dominant for micro-eukaryotic community assembly, with proportions of 65.2%, 72.9%, 72.4%, and 55.2% in spring, summer, autumn, and winter, respectively. The proportion of stochastic process for micro-eukaryotic communities in summer was higher than in other seasons. In the deterministic process, the proportions of heterogeneous selection (4.3–17.6%) were significantly higher than homogeneous selection (0–0.5%) in four seasons. Compared to bacteria, the deterministic process in micro-eukaryotes was relatively higher.

Discussion

Spatiotemporal distribution characteristics of microbial community structure

The α -diversity and taxonomic composition of bacteria and micro-eukaryotes in the XJH were all affected by seasonal variations. Similar results were also found in other urban rivers^{24,25}. However, it is inconsistent with the result that the α -diversity of microbial communities was not affected by seasonal variation in the Yarlung Zangbo River²⁶. Several possible reasons might explain this phenomenon. First, differences in environmental conditions may result in differences of microbial community composition and diversity. The distribution of bacteria and micro-eukaryotes in the XJH could be influenced by human activities such as sewage discharge, urban runoff, and river canalization²⁷. While the Yarlung Zangbo River is a typical natural river and less disturbed by human activities. Second, nutrients (nitrogen, phosphorus, etc.) and organic matter are considered as crucial factors affecting the microbial abundance and diversity²⁸. Usually, natural rivers lack nutrient sources compared to urban rivers, and nutrients varied greatly across different seasons in urban rivers than natural rivers. Third, natural rivers possess greater self-purification abilities²⁹, whereas urban rivers may have diminished self-purification capacity due to high pollution loads, channelization, and human interference, then resulting in significant variations in microbial community structure. Some other studies have manifested that microbial diversity in summer was higher than in other seasons^{17,19}. However, in the present study, the highest α -diversity of bacteria and micro-eukaryotes was found in spring and winter. Similar results were also manifested in other studies^{30,31}. It could be attributed to the precipitation. The XJH belongs to the subtropical monsoon humid climate zone. Heavily precipitation usually occurs in summer in this region. Although precipitation could wash nutrients and microorganisms from urban surface into surrounding rivers which leads to higher microbial diversity³², higher water flow and water volume are likely to strongly dilute the microorganisms and nutrients. Indeed, the TN and TP concentrations in summer were much lower than those in winter (Table S4), suggesting that nutrients might be diluted by heavy precipitation in summer. The effect of exogenous input is smaller than dilution due to precipitation. Besides, in summer, numerous aquatic macrophytes emerged in this river. It could compete with microorganisms for nutrients, thereby reducing the diversity of bacteria and micro-eukaryotes. Usually, the spatial variation is not obvious on a small spatial scale because of the river connectivity³³. However, significant spatial variation of α -diversity was found in this study area. In particular, the Chao1 index of bacteria and micro-eukaryotes in S4 was relatively lower than in other sites. It is probably due to the effects of human activities. High intensity of human activity could reduce microbial diversity³⁴. The S4 is located upstream of a dam, which is used for flood control. Most microorganisms are attached to suspended particles in rivers. As the sedimentation of suspended particles is due to dam construction, the quantity of microbes in the surface water will be decreased³⁵. In addition, the S4 is located in the central urban area of Zunyi. Exogenous pollution through surface runoff, untreated domestic wastewater, and water sports like swimming and bamboo drifting could reduce bacterial and micro-eukaryotic diversity.

The abundance of top three dominant bacterial phyla (Proteobacteria, Bacteroidota, and Actinobacteriota) showed significant differences between different seasons ($p < 0.05$). It is consistent with several other studies^{30,35,36}. These phyla are common and typical freshwater bacteria in rivers. Temperature variation in different seasons has been reported to influence the temporal variability of bacterial assemblages³⁷. Diatomea is the largest group of micro-eukaryotes and widely exists in flowing rivers. Interestingly, although the abundance of Diatomea showed obvious difference between different sampling sites, no significant difference was found between different seasons. It indicates that seasonal variation might be not the main factor affecting the distribution of Diatomea. It has been revealed that the bacterial and micro-eukaryotic community composition all exhibited seasonal variation according to the NMDS results. For bacteria, the samples from summer and autumn clustered together and deviated from spring and winter. It might be attributed to the relatively lower temperature difference between summer and autumn. For micro-eukaryotes, however, there were no overlap samples from different seasons, demonstrating that micro-eukaryotes could have different adaptation and assembly mechanisms compared to bacteria.

Co-occurrence networks of bacteria and micro-eukaryotes in different seasons

The interactions between microbial communities contribute more to ecological process than the relative abundance and diversity of species in water ecosystems. It will affect the microbial community composition to a certain extent and play important roles in maintaining the stability of ecosystem functions and structures⁸. Co-occurrence network analysis can be effectively used to characterize the interaction between microbial groups^{38,39}. Positive and negative correlations between edges of two nodes in co-occurrence networks represent the reciprocal and competitive relationships, respectively. The proportions of positive correlations among bacteria and micro-eukaryotes in all seasons were significantly higher than the negative correlations, indicating the importance of synergistic cooperation among microorganisms in this urban river habitat. Most bacteria and micro-eukaryotes resist the interference of external environmental variations through cooperative relationships with other species. This is consistent with other studies^{26,30,40}. The number of nodes, edges, and average degree of bacteria were higher in winter than in other seasons, indicating a high degree of complexity in winter. However, the complexity of micro-eukaryotes in winter was lower than in other seasons. It might be due to the competitive

relationship between bacteria and micro-eukaryotes. Usually, the increased complexity of co-occurrence network could lead to higher community stability³⁴. However, the modularity of bacteria and micro-eukaryotes was higher in summer than in other seasons, which was not consistent with the complexity. It indicates that network complexity does not necessarily represent network stability¹⁸. The network modularity can enhance the network stability under human activity interference⁴¹. Furthermore, the different co-occurrence characteristics between different seasons might be affected by other environmental factors such as WT and nutrients.

The keystone species play an essential role in the assembly and ecological functions of microorganisms in co-occurrence networks. Although most of the keystone species belong to the dominant phyla, some keystone species belong to Dependistia, Synergistota, Gastrotricha, Vertebrata, Hyphochytriomycetes, Labyrinthulomycetes, Mucoromycota, and Cnidaria with low abundance were also found (Table S2). It suggests that some of the low abundance species could play non-negligible roles in maintaining microbial community stability. Previous studies have shown that more keystone species may promote network stability to some extent^{18,42}. The number of keystone species in winter for bacteria and in autumn and winter for micro-eukaryotes was significantly higher than in other seasons. However, it cannot be concluded that the microbial networks are most stable in winter according to the topological properties of co-occurrence network. In addition, the keystone species varied significantly between different seasons in this study. Only one keystone species of micro-eukaryotes (*Paraphysomonas*) was found in both autumn and winter (Table S3). Furthermore, no consistent keystone species was observed between the four seasons of bacteria. Therefore, the stability of microbial networks could be determined by multiple factors such as the complexity of networks, interactions of species, keystone species, as well as external environmental interference.

Environment factors and assembly mechanisms of the microbial communities

The spatial variations of WT, pH, ORP, and DO were not obvious, probably because of the water connectivity in this studied urban river with a small spatial scale. However, the concentrations of TN, TP, COD, and EC downstream were relatively higher than those upstream. It might be due to the domestic sewage and surface runoff which bring abundant nutrients in the city area. Increased human activities along the river could increase the nutrient contents and change the proportion and forms of nutrients⁴³. The Environmental variations determine the temporal and spatial distribution of microbial community in riverine ecosystems. Nutrients, DO, pH, WT, and metals were determined to be the main environmental factors that could influence the microbial community¹⁹. WT and ORP were detected as the most critical factors affecting bacterial and micro-eukaryotic communities in the XJH. WT promotes the natural succession of microbial communities by influencing the growth, reproduction, and metabolic capacity of microorganisms directly^{44–46}. The seasonal variation in ORP is also influenced by its temperature-dependency⁴⁷. Furthermore, other detected environmental factors such as TN, TP, COD, pH, and EC also appear to have significant impacts on bacterial and micro-eukaryotic communities. TN, TP, and COD are the foundation of microbial metabolism. They play pivotal roles in modifying the trophic state of aquatic environments, thereby influencing the composition and dynamics of the microbial community^{25,48}. pH is considered to mediate the availability of ions and influence cellular osmotic pressure and enzyme synthesis of microorganisms⁴⁹. EC provides an indication of the overall concentration of dissolved ions present in aquatic environments and has play an important role in the community structure of micro-eukaryotes²⁶. The significant negative relationship between EC and most micro-eukaryotes in the present study might suggest that EC is an inhibitory factor of micro-eukaryotes.

Previous studies have shown that environmental factors cannot completely explain the microbial variations due to the influence of diffusion and drift processes⁵⁰. The NCM and null model were then applied to reveal the assembly process of bacterial and micro-eukaryotic communities in the XJH. The fitted values of NCM suggest that stochastic processes play important roles in assembly of bacterial and micro-eukaryotic communities. Other studies have confirmed that the stochastic process dominates the construction of microbial communities in river ecosystems, which is consistent with our findings^{8,38,51}. Besides, channelization of river could accelerate the water velocity⁵². The Zunyi urban section of the XJH has been channelized for landscape and flood control. Therefore, the results of stochastic process in this study area may be attributed to relatively high flow velocity which could be conducive to the microbial dispersal process. The migration rates (m) of bacteria and micro-eukaryotes in spring and summer were higher compared with that in autumn and winter, indicating the dispersal rates of microbial community in spring and summer were higher than in autumn and winter. It might be attributed to the higher river connectivity in spring and summer. Frequent rainfall events in these seasons can cause high connectivity in rivers and then enhance the migration and movement of organisms across the river⁸. In addition, the migration rates of bacteria in all four seasons were significantly higher than those of micro-eukaryotes. It indicates that bacterial cell dispersal might happen more frequently as a compensate mechanism for the random loss of an individual bacteria compared to the micro-eukaryotic species⁵³.

The results of contribution ratio between stochastic and deterministic processes further confirmed that the stochastic process was dominated in bacterial and micro-eukaryotic communities in the XJH. It may be mainly caused by dispersal limitation and undominated process such as drift. Besides, the deterministic process could not easily be detected during this limited sampling period, which may not able to clearly observe the evolutionary dynamics of bacterial and micro-eukaryotic communities⁵⁴. The contribution ratio of deterministic process during the four seasons for bacteria was 0.5–2.4%, which can be ignored. The community variation explained by deterministic process for micro-eukaryotes was 4.3–17.6%, which was significantly higher than that for bacteria. It suggests that deterministic and stochastic processes affect the bacteria and micro-eukaryotes differently. Different microorganisms have differences in body size, metabolic capacity, and dispersal ability. It will affect the relative contributions of stochastic and deterministic processes. Smaller organisms such as bacteria are less environment filtered than micro-eukaryotes, which are relatively larger organisms. Because the former is more likely to have plasticity in metabolic abilities and have greater environmental tolerance than the latter⁵⁵.

Liu et al. also found that bacteria had higher adaptability to environmental changes than micro-eukaryotes¹. Micro-eukaryotes have more complex cellular structures and longer lifespans compared to bacteria. They usually need more time for evolution and speciation, and then be more susceptible to deterministic factors⁵⁶. Therefore, deterministic process may have a greater influence on the assembly of micro-eukaryotes than bacteria. Furthermore, the microbial community structure has changed due to the heterogeneity of environmental factors in the XJH. So, the heterogeneous selection dominated the assembly of micro-eukaryotes in deterministic process. The wider niche width in bacterial community also proves that bacterial community was less affected by deterministic process⁵⁵. The results also manifest that although stochastic process significantly influenced the community assembly of bacteria and micro-eukaryotes, the NCM did not fully fit the bacterial and micro-eukaryotic communities. It indicates that there could be a coexistence of other community assembly processes and mechanisms such as species interactions and environmental filtering⁸. Undetected variables might lead to unexplained variations in microbial communities⁵⁷. The variables may include some abiotic factors such as metals and organic compounds^{58,59}, and some biological factors such as lytic bacterial viruses, protists, zooplankton, aquatic plants, etc^{60–62}.

Conclusions

This study demonstrates that diversity and taxonomic composition of bacterial and micro-eukaryotic communities in the XJH were all affected by seasonal and spatial variations. Compared to bacteria, micro-eukaryotic community composition exhibited more intense spatiotemporal differences. There are significant differences in the coexistence patterns of bacterial and micro-eukaryotic communities among the four seasons. The proportion of positive correlations among bacteria and micro-eukaryotes in all seasons was significantly higher than the negative correlations, indicating the importance of synergistic cooperation among microorganisms in this urban river habitat. WT and ORP showed significantly correlated with the composition of both bacteria and micro-eukaryotes, and they were the primary environmental factors influencing the bacterial and micro-eukaryotic community construction process. The results of NCM and null model indicate important roles of stochastic processes on community assembly of bacteria and micro-eukaryotes. In addition, dispersal limitation was the most important process affecting the community assembly of both bacteria and micro-eukaryotes. It also indicates that deterministic process has a greater influence on the assembly of micro-eukaryotes than bacteria. Furthermore, relatively wider niche breadth was found in micro-eukaryotes compared to bacteria. Overall, the assembly processes of bacterial and micro-eukaryotic communities in this urban river were similar but exhibited different characteristics. These observations provide scientific references for further research on the spatiotemporal variation and assembly mechanisms of microorganisms in urban rivers. However, more biological and environmental factors that could influence microbial assembly processes in a larger study area with more sampling sites and the mechanisms of difference in bacteria and micro-eukaryotes still need to be revealed.

Data availability

All the data generated or analyzed during this study are included in this manuscript. The raw sequence data supporting the findings of this study have been deposited at the Sequence Read Archive (SRA) in of National Center for Biotechnology Information (NCBI) under accession numbers PRJNA1175016 and PRJNA1175020 (<https://www.ncbi.nlm.nih.gov/sra/>).

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References

1. Liu, L., Wang, S. & Chen, J. Transformations from specialists to generalists cause bacterial communities are more stable than micro-eukaryotic communities under anthropogenic activity disturbance. *Sci. Total Environ.* **790**, 148141 (2021).
2. Zhou, J. Z. & Ning, D. L. Stochastic community assembly: Does it matter in microbial ecology? *Microbiol. Mol. Biol. R.* **81**, 00002–17 (2017).
3. Nemergut, D. R. et al. Patterns and processes of microbial community assembly. *Microbiol. Mol. Biol. R.* **77**, 342–356 (2013).
4. Obieze, C. C. et al. Anthropogenic activities and geographic locations regulate microbial diversity, community assembly and species sorting in Canadian and Indian freshwater lakes. *Sci. Total Environ.* **826**, 154292 (2022).
5. Zhang, T. et al. Similar geographic patterns but distinct assembly processes of abundant and rare bacterioplankton communities in river networks of the Taihu basin. *Water Res.* **211**, 118057 (2022).
6. Lin, L. et al. Microbial interactions strengthen deterministic processes during community assembly in a subtropical estuary. *Sci. Total Environ.* **906**, 167499 (2024).
7. Yu, J., Tang, S. N. & Lee, P. K. H. Microbial communities in full-scale wastewater treatment systems exhibit deterministic assembly processes and functional dependency over time. *Environ. Sci. Technol.* **55**, 5312–5323 (2021).
8. Chen, W. et al. Stochastic processes shape microeukaryotic community assembly in a subtropical river across wet and dry seasons. *Microbiome* **7**, 138 (2019).
9. Zhou, L. et al. Stochastic determination of the Spatial variation of potentially pathogenic bacteria communities in a large subtropical river. *Environ. Pollut.* **264**, 114683 (2020).
10. Stegen, J. C., Lin, X., Konopka, A. E. & Fredrickson, J. K. Stochastic and deterministic assembly processes in subsurface microbial communities. *ISME J.* **6**, 1653–1664 (2012).
11. Chen, Z., Gu, T. & Sun, J. Community assembly and network stability of picoeukaryotic plankton communities in the Northeast Indian ocean. *Prog. Oceanogr.* **219**, 103157 (2023).
12. Barbour, K. M. & Martiny, J. B. H. Investigating eco-evolutionary processes of microbial community assembly in the wild using a model leaf litter system. *ISME J.* **18**, wrac043 (2024).
13. Huang, L. B. et al. Different stochastic processes regulate bacterial and fungal community assembly in estuarine wetland soils. *Soil. Biol. Biochem.* **167**, 108586 (2022).

14. Bai, C. R. et al. Contrasting diversity patterns and community assembly mechanisms of bacterioplankton among different aquatic habitats in lake Taihu, a large eutrophic shallow lake in China. *Environ. Pollut.* **315**, 120342 (2022).
15. Xia, Y., Wen, X. H., Zhang, B. & Yang, Y. F. Diversity and assembly patterns of activated sludge microbial communities: A review. *Biotechnol. Adv.* **36**, 1038–1047 (2018).
16. Hui, C. et al. Modelling structure and dynamics of microbial community in aquatic ecosystems: The importance of hydrodynamic processes. *J. Hydrol.* **605**, 127351 (2022).
17. Liu, B. et al. Temporal and Spatial dynamic changes of planktonic bacteria community structure in Li river, China: A seasonal survey. *Environ. Sci. Pollut. R.* **30**, 111244–111255 (2023).
18. Fang, W. et al. Seasonal changes driving shifts in microbial community assembly and species coexistence in an urban river. *Sci. Total Environ.* **905**, 167027 (2023).
19. Ouyang, L. et al. Characteristics of Spatial and seasonal bacterial community structures in a river under anthropogenic disturbances. *Environ. Pollut.* **264**, 114818 (2020).
20. Bedla, D. & Halecki, W. The value of river valleys for restoring landscape features and the continuity of urban ecosystem functions—A review. *Ecol. Indic.* **129**, 107871 (2021).
21. Yang, S. et al. A novel assessment considering spatial and temporal variations of water quality to identify pollution sources in urban rivers. *Sci. Rep.* **11**, 8714 (2021).
22. Dutta, V. et al. Impact of river channelization and riverfront development on fluvial habitat: Evidence from Gomti river, a tributary of Ganges, India. *Environ. Sustain.* **1**, 167–184 (2018).
23. Ren, Y. et al. Majorbio cloud: A one-stop, comprehensive bioinformatic platform for multiomics analyses. *IMeta* **1**, e12 (2022).
24. Shu, W., Wang, P., Zhang, H., Ding, M. & Wu, B. Seasonal and spatial distribution and assembly processes of bacterioplankton communities in a subtropical urban river. *FEMS Microbiol. Ecol.* **96**, faa154 (2020).
25. Wang, H. et al. Spatial and Temporal dynamics of microbial community composition and factors influencing the surface water and sediments of urban rivers. *J. Environ. Sci.* **124**, 187–197 (2023).
26. Yang, Q. et al. Distribution patterns and community assembly processes of eukaryotic microorganisms along an altitudinal gradient in the middle reaches of the Yarlung Zangbo river. *Water Res.* **239**, 120047 (2023).
27. Wang, L. et al. Shift in the microbial community composition of surface water and sediment along an urban river. *Sci. Total Environ.* **627**, 600–612 (2018).
28. Zeglin, L. H. Stream microbial diversity in response to environmental changes: Review and synthesis of existing research. *Front. Microbiol.* **6**, 00454 (2015).
29. Nascimento, J. R., dos Santos, J. F., Pinha, G. D., Cabral, J. B. & Simões, N. R. Influence of aquatic biodiversity on the self-purification of tropical rivers. *Ecol. Eng.* **212**, 107540 (2025).
30. Bian, R. et al. Spatial and Temporal distribution of the microbial community structure in the receiving rivers of the middle and lower reaches of the Yangtze river under the influence of different wastewater types. *J. Hazard. Mater.* **462**, 132835 (2024).
31. Lv, J., Yuan, R. & Wang, S. Water diversion induces more changes in bacterial and archaeal communities of river sediments than seasonality. *J. Environ. Manag.* **293**, 112876 (2021).
32. Nyirabuhoro, P. et al. Responses of abundant and rare bacterioplankton to temporal change in a subtropical urban reservoir. *FEMS Microbiol. Ecol.* **97**, fiab036 (2021).
33. Yang, Z. et al. Spatial and temporal changes in bacterial community structure in adjacent waters of Daguer river estuary of Jiaozhou Bay (China) revealed by high-throughput sequencing. *Reg. Stud. Mar. Sci.* **52**, 102302 (2022).
34. Wu, B. et al. Spatial and temporal distribution of bacterioplankton molecular ecological networks in the Yuan river under different human activity intensity. *Microorganisms* **9**, 1532 (2021).
35. Chen, J. et al. Distinct assembly mechanisms underlie similar biogeographic patterns of rare and abundant bacterioplankton in cascade reservoirs of a large river. *Front. Microbiol.* **11**, 158 (2020).
36. Fortunato, C. S. et al. Determining indicator taxa across Spatial and seasonal gradients in the Columbia river coastal margin. *ISME J.* **7**, 1899–1911 (2013).
37. Liu, T. et al. Integrated biogeography of planktonic and sedimentary bacterial communities in the Yangtze river. *Microbiome* **6**, 16 (2018).
38. Sun, H. et al. Assembly processes and co-occurrence relationships in the bacterioplankton communities of a large river system. *Ecol. Indic.* **126**, 107643 (2021).
39. Coyte, K. Z., Schluter, J. & Foster, K. R. The ecology of the microbiome: Networks, competition, and stability. *Science* **350**, 663–666 (2015).
40. Zhao, J. et al. Co-occurrence patterns and assembly processes of abundant and rare bacterioplankton in plain river network areas of Eastern China. *Ecol. Indic.* **150**, 110204 (2023).
41. Banerjee et al. Determinants of bacterial communities in Canadian agroforestry systems. *Environ. Microbiol.* **18**, 1805–1816 (2016).
42. Liu, S. et al. Ecological stability of microbial communities in lake Donghu regulated by keystone taxa. *Ecol. Indic.* **136**, 108695 (2022).
43. Read, D. S. et al. Catchment-scale biogeography of riverine bacterioplankton. *ISME J.* **9**, 516–526 (2015).
44. Chiriac, C. M. et al. Differences in temperature and water chemistry shape distinct diversity patterns in thermophilic microbial communities. *Appl. Environ. Microbiol.* **83**, e01363–e01317 (2017).
45. Shang, Y. et al. Factors affecting seasonal variation of microbial community structure in Hulun lake, China. *Sci. Total Environ.* **805**, 150294 (2022).
46. Yu, B., Xie, G., Shen, Z., Shao, K. & Tang, X. Spatiotemporal variations, assembly processes, and co-occurrence patterns of particle-attached and free-living bacteria in a large drinking water reservoir in China. *Front. Microbiol.* **13**, 1056147 (2023).
47. Guo, L., Wang, G., Sheng, Y., Shi, Z. & Sun, X. Groundwater microbial communities and their connection to hydrochemical environment in Golmud, Northwest China. *Sci. Total Environ.* **695**, 133848 (2019).
48. Monchamp, M. E., Spaak, P. & Domaizon, D. P. Francesco. Homogenization of lake cyanobacterial communities over a century of climate change and eutrophication. *Nat. Ecol. Evol.* **2**, 317–324 (2018).
49. Santini, T. C., Gramenz, L., Southam, G. & Zammit, C. Microbial community structure is most strongly associated with geographical distance and pH in salt lake sediments. *Front. Microbiol.* **13**, 920056 (2022).
50. Zhao, J., Peng, W., Ding, M., Nie, M. & Huang, G. Effect of water chemistry, land use patterns, and geographic distances on the spatial distribution of bacterioplankton communities in an anthropogenically disturbed riverine ecosystem. *Front. Microbiol.* **12**, 633993 (2021).
51. Ren, K. et al. Dynamics and determinants of amoeba community, occurrence and abundance in subtropical reservoirs and rivers. *Water Res.* **146**, 177–186 (2018).
52. Bukaveckas, P. A. Effects of channel restoration on water velocity, transient storage, and nutrient uptake in a channelized stream. *Environ. Sci. Technol.* **41**, 1570–1576 (2007).
53. Burns, A. R. et al. Contribution of neutral processes to the assembly of gut microbial communities in the zebrafish over host development. *ISME J.* **10**, 655–664 (2016).
54. Siriarchawatana, P. et al. Elucidating potential bioindicators from insights in the diversity and assembly processes of prokaryotic and eukaryotic communities in the Mekong river. *Environ. Res.* **243**, 117800 (2024).

55. Wu, W. et al. Contrasting the relative importance of species sorting and dispersal limitation in shaping marine bacterial versus protist communities. *ISME J.* **12**, 485–494 (2018).
56. Logares, R. et al. Contrasting prevalence of selection and drift in the community structuring of bacteria and microbial eukaryotes. *Environ. Microbiol.* **20**, 2231–2240 (2018).
57. Mo, Y. et al. Low shifts in salinity determined assembly processes and network stability of microeukaryotic plankton communities in a subtropical urban reservoir. *Microbiome* **9**, 128 (2021).
58. Zhang, F. et al. Different response of bacterial community to the changes of nutrients and pollutants in sediments from an urban river network. *Front. Environ. Sci. Eng.* **14**, 28 (2020).
59. Yan, Z. et al. Co-occurrence patterns of the microbial community in polycyclic aromatic hydrocarbon-contaminated riverine sediments. *J. Hazard. Mater.* **367**, 99–108 (2019).
60. Souffreau, C. et al. Environmental rather than Spatial factors structure bacterioplankton communities in shallow lakes along a >6000 Km latitudinal gradient in South America. *Environ. Microbiol.* **17**, 2336–2351 (2015).
61. Wu, H. et al. Bacterial community composition and function shift with the aggravation of water quality in a heavily polluted river. *J. Environ. Manage.* **237**, 433–441 (2019).
62. Ren, Y. M., Shi, W., Chen, J. W. & Li, J. J. Water quality drives the reconfiguration of riverine planktonic microbial food webs. *Environ. Res.* **249**, 118379 (2024).

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Author contributions

S. C. conceived the idea of the study and drafted the original manuscript. J. Z., E. S., and L. F. performed the experiments. Z. S. analyzed the data. Y. L. revised and edited the manuscript. All authors read and approved the final manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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