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Wan, Wenjie; Gadd, Geoffrey Michael; He, Donglan; Liu, Wenzhi; Xiong, Xiang; Ye, Luping

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Abundance and diversity of eukaryotic rather than bacterial community relate closely to the trophic level of urban lakes

Running title: Biotic effect on water trophic level

Wenjie Wan\textsuperscript{a, e}, Geoffrey Michael Gadd\textsuperscript{c, d}, Donglan He\textsuperscript{b}, Wenzhi Liu\textsuperscript{a, e}, Xiang Xiong\textsuperscript{a, e}, Luping Ye\textsuperscript{a, e}, Yarui Cheng\textsuperscript{f}, Yuyi Yang\textsuperscript{a, e} *

\textsuperscript{a}Key Laboratory of Aquatic Botany and Watershed Ecology Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan 430074, PR China
\textsuperscript{b}College of Life Science, South-Central University for Nationalities, Wuhan 430074, PR China
\textsuperscript{c}Geomicrobiology Group, School of Life Sciences, University of Dundee, Dundee, Scotland DD1 5EH, UK
\textsuperscript{d}State Key Laboratory of Heavy Oil Processing, State Key Laboratory of Petroleum Pollution Control, China University of Petroleum, Beijing 102249, PR China
\textsuperscript{e}Danjiangkou Wetland Ecosystem Field Scientific Observation and Research Station, Chinese Academy of Sciences & Hubei Province, Wuhan 430074, PR China
\textsuperscript{f}College of Chemistry and Environmental Engineering, Hanjiang Normal University, Shiyan 442000, PR China

*Corresponding Author

E-mail: yangyy@wbgcas.cn (Yuyi Yang)

ORCID: Yuyi Yang: https://orcid.org/0000-0001-9807-6844
Wenjie Wan: https://orcid.org/0000-0001-7150-6138


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Originality-Significance Statement

Plankton play crucial roles in biogeochemical cycles in aquatic ecosystems and affect algal blooms. Yet, it remains unclear whether planktonic bacteria or eukaryotes contribute greatly to water trophic level of urban lakes. Our DNA-based datasets and statistical analyses reveal different distribution patterns of planktonic bacteria and eukaryotes and their differentiation strategies in response to environmental factors. We point out a potential interconnection between strong relationship of planktonic diversity-multinutrient cycling and close relationship of planktonic diversity-water trophic level. Therefore, abundance and diversity of eukaryotic rather than bacterial community relate closely to water trophic state despite significantly higher bacterial abundance and diversity. Our findings enrich the knowledge of diversity maintenance mechanisms of planktonic bacteria and eukaryotes underlying algal blooms and highlight ecological roles of plankton in shaping water trophic state of urban lakes.

Summary

Scientific understanding of biotic effects on the water trophic level is lacking for urban lakes during algal bloom development stage. Based on the Illumina MiSeq sequencing, quantitative PCR, and multiple statistical analyses, we estimated distribution patterns and ecological roles of planktonic bacteria and eukaryotes in urban lakes during algal bloom development stage (i.e., April, May, and June). *Cyanobacteria* and *Chlorophyta* mainly dominated algal blooms. Bacteria exhibited significantly higher absolute abundance and community diversity than eukaryotes, whereas abundance and diversity of eukaryotic rather than bacterial community relate closely to the water trophic level.
Multinutrient cycling (MNC) index was significantly correlated with eukaryotic diversity rather than bacterial diversity. Stronger species replacement, broader environmental breadth, and stronger phylogenetic signal were found for eukaryotic community than for bacterial community. In contrast, bacterial community displayed stronger community stability and environmental constraint than eukaryotic community. Stochastic and differentiating processes contributed more to community assemblies of bacteria and eukaryotes. Our results emphasized that a strong linkage between planktonic diversity and MNC ensured a close relationship between planktonic diversity and the water trophic level of urban lakes. Our findings could be useful to guide the formulation and implementation of environmental lake protection measures.

**Keywords:** multinutrient cycling, environmental breadth, phylogenetic signal, species replacement, environmental constraint, community stability

**Introduction**

Waterbodies in the Anthropocene, particularly in the rapid urbanization period, are subject to massive algal especially cyanobacterial blooms due to water eutrophication (Griffin, 2017; Sha *et al*., 2021). Many approaches (e.g., dredging) have been employed to mitigate bloom development and purify eutrophic waterbodies (Waajen *et al*., 2016; Wan *et al*., 2021a). However, algal blooms, including those of potential cyanobacteria, emerge seasonally and periodically in inland aquatic ecosystems despite strict control of external nutrient loading (Reinl *et al*., 2021). Therefore, it is essential to disentangle factors that affect the trophic state of freshwater bodies.
The content of chlorophyll-α (Chl-α), regarded as one of key parameters of water trophic level, depends on the biomass of Chl-α-containing organisms (e.g., prokaryotic cyanobacteria and eukaryotic algae) (Zhang et al., 2011). The biomass and community composition of such Chl-α-containing organisms (e.g., cyanobacteria and diatom) are significantly affected by abiotic factors, e.g., total nitrogen and total phosphorus (Te et al., 2017; Wan et al., 2021a). Additionally, biotic factors (e.g., Chl-α free organism) also affect the biomass of Chl-α-containing organisms (Bai et al., 2011; Zhang et al., 2018; Cook et al., 2020). For instance, a marine actinomycete display algicidal effect on *Alexandrium tamarense* (Bai et al., 2011). Flagellates can degrade toxic *Microcystis* sp. via regulating transcription level to produce functional compounds (e.g., peroxiredoxin and phosphatase) (Zhang et al., 2018). Therefore, the trophic state of waterbodies is a comprehensive consequence of nutrient availability and biotic factors (e.g., interaction patterns and population levels of plankton).

Microorganisms mediate the transformations of key nutrients, e.g., carbon, nitrogen, and phosphorus, in water-sediment system (Wörner and Pester, 2019; Yuan et al., 2020). For instance, microorganisms participate in denitrification in Keurusselkä Lake sediments (Aalto et al., 2018) and organic phosphorus mineralization in Lake Taihu (Yuan et al., 2020). Additionally, microorganism-mediated nutrient transformations are also subject to specific environmental conditions (e.g., oxygen level, temperature, and pH) in water-sediment systems (Brigolin et al., 2021; Huo et al., 2022). Consequently, the abundance, diversity, and community composition of bacterioplankton and eukaryotes are controlled by nutritional (e.g., total nitrogen and phosphorus) and abiotic (e.g., temperature and pH) factors.
total phosphorus) and non-nutritional (e.g., temperature and pH) factors (Te et al., 2017; Mo et al., 2021; Wan et al., 2021a). Microbial taxonomic and phylogenetic α-diversities are closely linked with MNC in terrestrial and aquatic environments (Wan et al., 2021b; Zhang et al., 2021). Therefore, it is of great importance to decipher planktonic diversity and diversity-driven MNC.

Many ecological theories have attempted to explain diversity maintenance of plankton at temporal and spatial scales, including species replacement and richness difference, coexistence patterns, environmental adaptability, and ecological assembly processes (Legendre, 2014; LeBrun et al., 2018; Huber et al., 2020; Wan et al., 2021a). Compositional dissimilarity can be disassembled by species replacement and richness difference (Legendre, 2014). Coexistence patterns can reveal mutualistic or antagonistic co-occurrence patterns among interactive taxa (Huber et al., 2020). Environmental adaptability can reflect the strength of species resistance to environmental change (Goberna and Verdú, 2016; LeBrun et al., 2018). Ecological assembly processes include stochastic and deterministic (i.e., sorting) processes, with the former arising from random events and the latter imposed by environmental filtering (Stegen et al., 2013; Jiao et al., 2020). A prior study reports that bacterioplankton display stronger environmental adaptability and a more complex coexistence pattern before dredging disturbance in Lake Nanhu (Wan et al., 2021a). Bacterioplankton community assembly is determinism-dominated in Lake Donghu across the four seasons (Yan et al., 2017). However, species replacement and richness difference, coexistence patterns, environmental adaptability, and ecological assembly processes of
plankton during algal bloom development stage has seldom been reported. We selected 12 urban lakes located in Wuhan City, China (Table S1; Fig. S1), and collected water samples during algal bloom development stage (i.e., April, May, and June) (Wuhan Water Authority 2014, http://en.0430.com/cn/web113017/). In this study, we aimed to (i) investigate linkage between water trophic level and planktonic abundance and diversity (i.e., bacterioplankton and microeukaryotes), (ii) decipher relationship between MNC and planktonic diversity, and (iii) reveal the formation and maintenance mechanisms of planktonic diversity underlying algal blooms. Because aquatic bacteria and eukaryotes are two distinctly different plankton and have different trophic grades in aquatic food web (McMeans et al., 2016), we hypothesized that abundances and diversities of bacteria and eukaryotes would contribute differently to water trophic level.

**Experimental procedures**

**Water sampling, physicochemical property determination, and water trophic level estimation**

In each selected month (i.e., April, May, and June) in 2021, we collected 27 water samples from 27 representative sites in 12 lakes located in Wuhan, China (Table S1; Fig. S1). These lakes were reported with different water quality and were selected as research area. Surface (0–0.3 m), middle, and bottom (1–1.2 m below the surface) waters at each site were collected by using a Wanzun Water Sampler, and waters from three layers were mixed evenly to form one water sample. A total of 81 water samples
were collected and stored in sterile water bags. Once waters were collected, samples were immediately placed in a portable refrigerator with a temperature setting of 4°C.

Collected water samples were filtered through sieve with 5-mm mesh to remove visible objects (e.g., leaves, plastics, and small animals). One part of the filtered water sample was used to measure physicochemical properties, and the remaining part was filtered through polycarbonate membranes with 0.22-μm pore diameter (Millipore Corporation, Billerica, MA, USA) for subsequent analyses (i.e., chlorophyll-α content determination, gene quantitation, and Illumina MiSeq Sequencing).

Water pH, temperature (Tem), DO, EC, and Tur were measured in situ using a portable YSI Pro1020 Water Quality Tester (Visal, USA). We determined contents of water TP, SRP, total nitrogen (TN), NH₄⁺–N, NO₃⁻–N, COD, Ca, Mg, Fe, and Chl-α according to standard approaches and protocols (APAH, 1998). Technical details of the determination of water physicochemical properties are summarized in supporting information (Supplementary Method 1).

We used Chl-α, TN, TP, and COD as evaluation factors to calculate the trophic level index (TLI) of lakes, and detailed protocol descriptions and algorithms have been reported previously (Zhang et al., 2011) and also summarized in supplementary materials (Supplementary Method 1). Mg is an important element for chlorophyll molecular structure, and Ca and Fe are deactivator for free phosphorus (e.g., PO₄³⁻). Therefore, we used these physicochemical factors (i.e., TP, SRP, TN, NH₄⁺–N, NO₃⁻–N, COD, Ca, Mg, and Fe) to calculate the MNC index. The physicochemical factors were transformed to a 0 to 1 range using the following equation: 

\[ SV_i = \frac{(E_i - E_{imin})}{(E_{imax} - E_{imin})} \]
\[ E_{\text{imin}} \] \ (Jiao et al., 2021b), where \( SV \) is the standardized variable of the physicochemical factor \( i \), \( E_i \) is the physicochemical factor \( i \), and \( E_{\text{imin}} \) and \( E_{\text{imax}} \) are the minimum and maximum values of physicochemical factor \( i \) across all samples, respectively. The MNC index is the average of \( SV \) values of nine selected physicochemical factors.

**Molecular analysis of bacterial and eukaryotic communities**

DNA was extracted from the filter by applying a PowerWater DNA Isolation Kit (MOBIO, USA) according to the manufacturer’s instructions. Universal primers 338F (5’-ACT CCT ACG GGA GGC AGC A-3’) and 806R (5’-GGA CTA CHV GGG TWT CTA AT-3’) were applied to partially amplify the bacterial 16S rRNA gene targeting the V3-V4 region (Mori et al., 2014). Universal primers Eu565F (5’-CCA GCA SCY GCG GTA ATT CC-3’) and Eu981R (5’-ACT TTC GTT CTT GAT YRA TGA-3’) were used to partially amplify the eukaryotic 18S rRNA gene targeting the V4 region (Stoeck et al., 2010).

The absolute abundances of 16S rRNA gene and 18S rRNA gene were determined using qPCR. Quantitation was conducted in triplicates applying an ABI VIITA 7 Cycle Real-time PCR System (Applied Biosystems, Foster City, CA, USA) in a 10-μL reaction system. The reaction conditions were as follows: 95°C for 5 min, followed by 40 cycles of 95°C for 15 s, 56°C for 30 s, and 72°C for 40 s.

Gene amplification for Illumina MiSeq sequencing was conducted in a 20-μL reaction system. The PCR conditions were as follows: 5 min at 95°C, followed by 30 cycles at 95°C for 40 s, 56°C for 40 s, and 72°C for 40 s, and a final extension at 72°C
for 10 min. High throughput sequencing was undertaken at Personal Biotechnology Co., Ltd (Shanghai, China). The raw sequences were run through the QIIME2 pipeline to gain denoised, chimera-free, non-singleton ASVs (Bolyen et al., 2019). For subsequent analyses, we removed all ASVs that contained < 20 reads. The phylogenetic trees of bacteria and eukaryotes were constructed by applying the FastTree tool (Price et al., 2009).

**Data analyses**

Distance-decay relationships between geographical distance and community similarity were estimated at both taxonomic (1-Bray-Curtis dissimilarity) and phylogenetic (1 - beta mean nearest taxon distance [βMNTD]) (Wan et al., 2021c). The β-diversities of bacterial and eukaryotic communities were disassembled by species replacement and richness difference (Legendre, 2014). Co-occurrence networks of bacteria and eukaryotes were built based on Spearman’s correlations between species relative abundances and visualized by Gephi software. We set correlation coefficients > 0.6 and significance level < 0.01, and extracted topological parameters (e.g., links and nodes) of the network and subnetwork.

Environmental adaptability of bacteria and eukaryotes was estimated based on environmental breadths at the taxonomic level and phylogenetic signals at the phylogenetic level (Baker and King, 2010; Goberna and Verdú, 2016). Threshold indicator taxa analysis (TITAN) was used to compute environmental breadths, reflecting species threshold ranges along specific environmental gradients (Baker and King, 2010; Wan et al., 2021a). The Fritz-Purvis D-test was employed to calculate
phylogenetic signals to reveal phylogenetic conservation for functional traits (Goberna and Verdú, 2016; Wan et al., 2021b). A relatively large $-D + 1$ value demonstrates a relatively strong phylogenetic signal.

Mantel correlograms were built to reflect whether phylogenetic signals of bacteria and eukaryotes occurred at short phylogenetic distances along environmental gradients (Wang et al., 2013). The Bray-Curtis-based Raup-Crick ($RC_{bray}$) and β-nearest taxon index ($βNTI$) were computed based on the null model (Stegen et al., 2013). The ranges of $RC_{bray}$ and $βNTI$ values reflect ecological assembly processes, including variable selection ($βNTI > 2$), homogeneous selection ($βNTI < -2$), homogenizing dispersal ($|βNTI| < 2$ and $RC_{bray} < -0.95$), dispersal limitation ($|βNTI| < 2$ and $RC_{bray} > 0.95$), “undominated” processes ($|βNTI| < 2$ and $|RC_{bray} < 0.95$), deterministic processes ($|βNTI| > 2$), stochastic processes ($|βNTI| < 2$), homogenizing processes (homogenizing dispersal plus homogeneous selection), and differentiating processes (variable selection plus dispersal limitation). Detailed descriptions of these ecological assembly processes have been reported previously (Stegen et al., 2013; Feng et al., 2018). Neutral model analysis was used to further reveal the contribution of stochasticity to community assembly by considering migration rate ($m$) (Sloan et al., 2006).

Results

Relationships between physicochemical factors and Chl-α, TLI, and MNC

The Chl-α content (2.21–564.27 μg/L) varied in different sites and sampling months (Fig. S2), and was significantly correlated with turbidity (Tur) \((r = 0.546, p < 0.001)\), dissolved oxygen (DO) \((r = 0.468, p < 0.001)\), total phosphorus (TP) \((r = 0.257, p < 0.05)\), chemical oxygen demand (COD) \((r = 0.286, p < 0.05)\), calcium (Ca) \((r = 0.315, p < 0.01)\), magnesium (Mg) \((r = 0.251, p < 0.05)\), and iron (Fe) \((r = 0.292, p < 0.01)\). TLI (37.11–100.16) differed in different samples (Fig. S2), and was notably correlated with Tur \((r = 0.478, p < 0.001)\), electrical conductivity (EC) \((r = 0.279, p < 0.05)\), soluble reactive phosphorus (SRP) \((r = 0.425, p < 0.001)\), ammonia nitrogen \((\text{NH}_4^+–\text{N})\) \((r = 0.521, p < 0.001)\), nitrate nitrogen \((\text{NO}_3^-–\text{N})\) \((r = 0.359, p < 0.001)\), Ca \((r = 0.628, p < 0.001)\), Mg \((r = 0.556, p < 0.001)\), and Fe \((r = 0.660, p < 0.001)\). MNC (0.005–0.726) was noticeably correlated with dissolved oxygen \((r = -0.277, p < 0.05)\) and electrical conductivity \((r = 0.452, p < 0.001)\). These results indicate that abiotic factors potentially affected water trophic state and MNC.

General distribution patterns of bacteria and eukaryotes

The absolute abundances of bacteria \((1.10 \times 10^5–8.51 \times 10^8 \text{ copies/L water})\) and eukaryotes \((7.24 \times 10^2–9.62 \times 10^6 \text{ copies/L water})\) varied between different samples (Fig. S2). Pearson’s correlations showed that bacterial abundance was significantly positively correlated with temperature \((r = 0.250, p < 0.05)\), turbidity \((r = 0.237, p < 0.05)\), and DO \((r = 0.539, p < 0.001)\), but notably negatively correlated with pH \((r = -0.242, p < 0.05)\), SRP \((r = -0.234, p < 0.05)\), and \(\text{NO}_3^-–\text{N}\) \((r = -0.305, p < 0.01)\) (Table
1). In contrast, eukaryotic abundance was significantly positively correlated with temperature \((r = 0.271, p < 0.05)\), Tur \((r = 0.541, p < 0.001)\), DO \((r = 0.325, p < 0.01)\), Ca \((r = 0.332, p < 0.01)\), and Fe \((r = 0.329, p < 0.01)\), but dramatically negatively correlated with pH \((r = -0.273, p < 0.05)\). Bacterial abundance was significantly higher than eukaryotic abundance \((p < 0.001; \text{Wilcoxon rank-sum test})\), but linear regression showed that eukaryotic \((R^2 = 0.225, p < 0.001)\) rather than bacterial \((R^2 = 0.030, p > 0.05)\) abundance was notably correlated with TLI (Fig. 1). These results indicate that environmental factors potentially influenced planktonic abundance, and eukaryotic rather than bacterial abundance contributed more to the water trophic level.

Taxonomic α-diversity represented by the Shannon-Wiener index was significantly higher for bacteria \((2.43–6.04)\) than for eukaryotes \((0.03–5.35)\) \((p < 0.001)\). Temperature, rather than other physicochemical factors, was notably correlated with taxonomic α-diversity of both bacteria and eukaryotes (Table 1). Linear regression revealed that the taxonomic α-diversity of eukaryotes \((R^2 = 0.053, p < 0.05)\) rather than that of bacteria \((R^2 = -0.012, p > 0.05)\) was correlated significantly with TLI (Fig. 1). Additionally, significant correlation between community diversity and the MNC index was found for eukaryotes \((R^2 = 0.049, p < 0.05)\) rather than bacteria \((R^2 < 0.001, p > 0.05)\) based on linear regression (Fig. 1). These results indicated that eukaryotic diversity closely linked with MNC contributed more to the water trophic level.

A total of 10,511 and 4,487 amplicon sequence variants (ASVs) were found for bacteria and eukaryotes, respectively. Bacterial ASVs were mainly identified as Proteobacteria \((10.11–87.02\%)\), Actinobacteria \((0.35–75.49\%)\), Bacteroidetes \((0.06–\)
38.62%), *Cyanobacteria* (0–76.39%), *Firmicutes* (0.04–24.10%), *Deinococcus-Thermus* (0–31.10%), *Verrucomicrobia* (0–8.35%), *Epsilonbacteraeota* (0–17.40%), *Chloroflexi* (0–2.86%), and *Patescibacteria* (0–5.46%) (Fig. S3). Similarly, eukaryotic ASVs were mainly identified as *Chlorophyta* (0–91.34%), *Rotifera* (0.01–86.91%), *Arthropoda* (0.01–99.83%), *Bacillariophyta* (0–59.29%), *Dinophyceae* (0–47.62%), *Chrysophyceae* (0–96.87%), *Chytridiomycota* (0–61.58%), *Synurophyceae* (0–29.12%), *Streptophyta* (0–26.12%), and unclassified (0.05–95.10%). The relative abundances of bacteria and eukaryotes were differently correlated with Chl-α, TLI, and MNC index (Table S2). For example, *Cyanobacteria* and *Chlorophyta* were significantly positively correlated with Chl-α and TLI (*p* < 0.05 or *p* < 0.001), suggesting that *Cyanobacteria* and *Chlorophyta* mainly dominated algae blooms in these urban lakes. Taxonomic and phylogenetic similarities of bacterial and eukaryotic communities decayed with geographical distance (Fig. 2a). Although the distance decay relationships (DDRs) were significant (*p* < 0.01 or *p* < 0.001), the fitness values were quite low (R² < 0.1), suggesting weak DDRs. Additionally, taxonomic distance was higher for bacteria than for eukaryotes, but the opposite occurred for phylogenetic distance (Fig. 2a). Mantel’s tests showed that temperature showed stronger effects on community structure of bacteria and eukaryotes in comparison with other physicochemical factors (*p* < 0.001; Table 1), suggesting temperature mainly shaping planktonic community composition.

**Species replacement and coexistence pattern of plankton**
Species replacement (for bacteria, 0.4064; for eukaryotes, 0.4172) was stronger than richness difference (for bacteria, 0.0241; for eukaryotes, 0.0160) for both bacteria and eukaryotes (Fig. 3a). The ratio of species replacement/dissimilarity of eukaryotic community (ratio = 0.9631) was larger than that of bacterial community (ratio = 0.9440). Subsequent result using redundancy analysis showed tested physicochemical factors explained the higher compositional variation in the bacterial community than in the eukaryotic community (Fig. 3b). These results indicate that species replacement was stronger for eukaryotic community than that of bacterial community.

Bacteria displayed more complex coexistence patterns than eukaryotes according to co-occurrence networks, showing comparably more links and nodes (Fig. 4a; Table S3). The bacterial rather than eukaryotic co-occurrence network showed lower graph density and higher average degree, diameter, modularity, average clustering coefficient, and average path length (Table S3). The results for network stability were opposite to those of species replacement. Additionally, topological parameters (i.e., node, link, and degree) of both bacterial and eukaryotic subnetworks were significantly correlated with temperature in comparison with other physicochemical factors ($p < 0.05$ or $p < 0.001$; Fig. 4b). Relative abundances of top 10 core nodes (those with the highest betweenness centrality) identified from bacterial and eukaryotic networks exhibited different correlations with water physicochemical factors (Table S4, S5). For instance, ASV_216647 belonging to *Actinobacteria* was significantly positively correlated with temperature. These results indicate temperature-dependent coexistence patterns of
bacteria and eukaryotes, and bacteria rather than eukaryotes exhibited stronger community stability.

**Phylogenetic signals and ecological assembly processes of plankton**

Subsequently, we estimated the environmental adaptability of bacteria and eukaryotes using environmental breadth and phylogenetic signal analyses (Fig. 5). By considering changing points of species abundance along specific environmental gradients (Fig. S4–S6), we found eukaryotes displayed wider environmental breadths for 78.6% of the tested physicochemical variables (Fig. 5a). Furthermore, eukaryotes exhibited stronger phylogenetic signals in response to 71.4% of the tested physicochemical parameters (Fig. 5b). These results indicated eukaryotes harbored stronger environmental adaptation than bacteria.

Significantly positive phylogenetic signals of bacteria and eukaryotes were found at short phylogenetic distances along the same environmental gradient (Fig. 6a). The β-nearest taxon index (βNTI) values of bacterial and eukaryotic communities mainly fell between -2 and 2, with medians of 1.16 and 0.43 for bacterial and eukaryotic communities, respectively (Fig. 6b). Subsequently, we found dispersal limitation (for bacteria, 55.90%; for eukaryotes, 43.89%) showed main effects on community assemblies of bacteria and eukaryotes, followed by variable selection (for bacteria, 34.23%; for eukaryotes, 17.44%), “undominated” processes (for bacteria, 8.09%; for eukaryotes, 35.34%) (Fig. 6c). Homogeneous selection (for bacteria, 0.93%; for eukaryotes, 2.59%) and homogenizing dispersal (for bacteria, 0.86%; for eukaryotes, 0.74%) displayed limited influences. Consequently, stochastic processes (for bacteria,
64.85%; for eukaryotes, 79.97%) and differentiating processes (for bacteria, 90.12%; for eukaryotes, 61.32%) mainly affected community assemblies of bacteria and eukaryotes. The βNTIs of bacterial and eukaryotic communities were notably correlated with changes in temperature, pH, and SRP ($p < 0.05$ or $p < 0.01$ or $p < 0.001$; Table 1). Additionally, larger ratio of sorting to dispersal limitation was found for bacterial community than eukaryotic community based on the null model (Fig. 6d). The Sloan neutral model displayed a relatively small migration rate for bacteria ($m = 0.0044$; $R^2 = 0.029$) than for eukaryotes ($m = 0.0075$; $R^2 = 0.181$) (Fig. 6e). These results indicate that bacteria rather than eukaryotes were more environmentally restricted.

**Discussion**

**Bacterial and eukaryotic communities respond notably to water temperature**

Significant DDRs of bacterial and eukaryotic communities were found in urban lakes during bloom development stage, which is similar to previous findings for bacterioplankton in Xiangshan Bay (Xiong et al., 2016) and microeukaryotes in subtropical Tingjiang River (Chen et al., 2019). The DDRs were relatively weak for bacteria, which might be mainly from strong differentiating processes. An earlier study reports that differentiating processes leads to compositional dissimilarity (Feng et al., 2018). Water temperature showed positive effects on abundance, diversity, structure, and assemblies of bacterial and eukaryotic communities, which are similar to findings for eutrophic Lake Donghu (Yan et al., 2017), Tibetan Plateau lake (Liu et al., 2017a), and Nanjing freshwater lakes (Jiao et al., 2021a). This phenomenon might be primarily
due to distinct shifts in water temperature (17.8–34.9°C) during bloom development stage. Temperature affects sediment microbial activity for nutrient (e.g., nitrogen and phosphorus) turnover (Zhou et al., 2016), and in turn affects exchanges in nutrients and microorganisms between sediments and water (Wan et al., 2020). Some plankton (e.g., members of the Alteromonadaceae, Comamonadaceae, Halomonadaceae, Methylophilaceae, and Rhodospirillaceae) are temperature-sensitive (Xiong et al., 2016), and such plankton are closely connected in important ecological processes (Chase et al., 2017; Huber et al., 2020). Consequently, abundance changes in some species and nutrient fluctuations can lead to changes in abundance, diversity, and structure of entire planktonic communities (Chase et al., 2017; Liu et al., 2017b). Additionally, temperature can directly affect cell metabolism and growth, which in turn affect species dispersal potential (Vass et al., 2021).

**Distinct divergence in diversity maintenance between bacteria and eukaryotes**

Species replacement rather than richness difference displayed stronger effects on β-diversities of bacterial and eukaryotic communities, which is similar to prior findings for diatom community in Paraná River (Ruwer and Rodrigues, 2022) and microorganisms (i.e., bacteria and fungi) in a mountain system (Shen et al., 2020). Temperature affected network complexities of bacterial and eukaryotic communities, which is similar to an earlier study describing that co-occurrence network of planktonic bacteria is temperature-dependent in Lake Nanhu (Wu et al., 2021). We estimated environmental breadths and phylogenetic signals of planktonic bacteria and eukaryotes responding to 14 physicochemical factors, which is unlike studies reporting
environmental breadths and phylogenetic signals are mainly used for specific environmental factor and microbial sub-communities (e.g., rare vs. abundant and specialist vs. generalist) (LeBrun et al., 2018; Wan et al., 2021b; Yan et al., 2022). Stochastic processes dominated community assemblies of bacteria and eukaryotes, which agrees with bacterial community assembly in the Han River (Sun et al., 2021) and eukaryotic community assembly in the Tingjiang River (Chen et al., 2019). However, deterministic processes dominate bacterial community assembly in the Paraná River (Huber et al., 2020). Whether stochasticity and/or determinism dominating planktonic community assembly seems to be influenced by geospatial differences and environmental heterogeneity (Huber et al., 2020; Pearman et al., 2022).

Stronger species replacement, broader environmental breadths, and stronger phylogenetic signals were found for the eukaryotic community rather than the bacterial community. In contrast, the bacterial community exhibited stronger community stability and environmental constraint in comparison with the eukaryotic community. To our knowledge, this is the first report of a distinct divergence in diversity maintenance between bacteria and eukaryotes during algal bloom development stage. Typically, the cell wall and cell membrane of bacteria is relatively simpler than that of eukaryotes, and bacteria are relatively slow to adapt to sudden environmental changes (e.g., high temperature, acidification, and salinization) (Pennisi, 2019). For instance, Chlamydomonas reinhardtii CHL13 belonging to the Chlorophyta display a higher growth rate than that of cyanobacterial species (e.g., Anabaena sp. PCC7122, Cylindrospermopsis raciborskii CIRF-01, Planktothrix agardhii CYA116, and
Microcystis aeruginosa PCC7941) under the same temperature conditions (Lürling et al., 2013). Therefore, environmental bacteria are usually embedded in biofilms, which are an omnipresent and successful form of lifestyle, which, in turn, limits bacterial dispersal (Flemming et al., 2016). Additionally, many eukaryotes (e.g., protozoa and algal flagellates) possess high dispersal potential by using pseudopodia and flagella (Finlay, 2002; Bahram et al., 2016), which enable relatively easy escape from unfavorable conditions (e.g., high temperature, high salinity, and low pH) and predators (e.g., small animals). Stochastic processes contributed more to the community assembly of eukaryotes rather than bacteria, which might imply that stochasticity-dominated assembly results from high dispersal and ecological drift (Bahram et al., 2016). Consequently, the divergences in cellular structure, planktonic living style, and dispersal potential might result in distinct divergences in diversity maintenance and ecological functions between bacteria and eukaryotes. Evaluating species replacement, coexistence pattern, environmental adaptability, and ecological community assembly of bacteria and eukaryotes in aquatic environments is important to estimate and predict diversity maintenance and diversity-driven ecosystem functions (Hector and Bagchi, 2007; Jiao et al., 2021b; Wan et al., 2021a). Species replacement can decipher the generation of taxonomic β-diversity (Carvalho et al., 2012; Legendre, 2014), which in turn can reveal coexistence pattern, environmental adaptability, and environmental constraint. Future work will be conducted to confirm these findings in different aquatic ecosystems.

Planktonic diversity drives multinutrient cycling
Bacteria and eukaryotes participate in key nutrient cycles (e.g., carbon decomposition, denitrification, and phosphorus cycling) in aquatic systems (Wörner and Pester, 2019; Yuan et al., 2020), which might relate differently to multinutrient cycling. Numerous studies have reported a strong linkage between microbial diversity and multinutrient cycling in terrestrial ecosystems (e.g., grassland, farmland, and forest soils) (Jiao et al., 2021b; Wan et al., 2021b), whereas this has been reported poorly for aquatic ecosystems. Taxonomic $\alpha$-diversity of bacterioplankton and phytoplankton significantly relate to ecosystem multifunctionality in a eutrophic lake (Zhang et al., 2021). In this study, significant linkage between multinutrient cycling index and taxonomic $\alpha$-diversity was found for eukaryotes rather than for bacteria during algal bloom development stage. This phenomenon is similar to prior findings (Wan et al., 2021b, 2021d), showing microbial diversity contributing more to multinutrient cycling when the microorganisms displayed relatively strong environmental adaptability. This is expected because strong biodiversity maintenance ensures efficient ecological processes and functions in various habitats (Hector and Bagchi, 2007; Lohbeck et al., 2016). Considering the coupling of key nutrient (e.g., carbon, nitrogen, phosphorus, and sulfur) cycles mediated by microorganisms in sediment-water systems (Zhao et al., 2020; Pelsma et al., 2022), more abiotic variables (e.g., nutrients and greenhouse gas) will be considered to estimate multinutrient cycling in future studies.

*Abundance and diversity of the eukaryotic community rather than the bacterial community reflect water trophic level*
The abundance or population of Chl-α-containing organisms (e.g., cyanobacteria and eukaryotic algae) directly determine the water trophic level according to calculated trophic level indices (Zhang et al., 2011). Here, significant linkage between trophic level index and gene abundance were found for eukaryotic community rather than bacterial community during algal bloom development stage. Many Chl-α-containing eukaryotic organisms (e.g., diatoms, chlorophytes, and algal flagellates) can be found in algal blooms (Nygaard and Tobiesen, 1993; Lin et al., 2016), and abundances of these plankton contribute to the water trophic level in a direct manner. Cyanobacteria are also bloom organisms and their abundance is also closely linked with water trophic level (Te et al., 2017; Wan et al., 2021a). However, cyanobacteria were not the first-class dominant organisms in most samples, and the abundance of most Chl-α-free bacteria (e.g., Proteobacteria) contributed less to the water trophic level in a direct way.

Water trophic state affect phytoplankton diversity in the Iranian Caspian Sea (Nasrollahzadeh et al., 2008), whereas potential effect of planktonic diversity on water trophic state is rarely investigated. A stronger linkage between the water trophic level and community diversity was found for eukaryotes than for bacteria despite significantly higher bacterial diversity. This might be primarily attributable to significant linkage between eukaryotic community diversity and multinutrient cycling. This is reasonable because key nutrient availability (e.g., soluble reactive nitrogen and phosphorus fractions) satisfy the growth and reproduction demands of bloom-related organisms (Nygaard and Tobiesen, 1993; Huo et al., 2022). Seven out of ten key species identified from co-occurrence network analysis were Chl-α-containing eukaryotic
organisms (i.e., Chlorophyta, Bacillariophyta, and Dinophyceae), and were correlated positively with Chl-α content. Changes in key species potentially affects community stability and ecosystem processes and functions (Huber et al., 2020; Wan et al., 2021a). For instance, an earlier study reports that an increase in Chlorophyta alter nitrogen cycling and metabolic functions (Oleksy et al., 2021). From a practical water restoration viewpoint, rational approaches (e.g., algicide usage and fish introduction) could be implemented to reduce the abundance and diversity of eukaryotes to mitigate or even prevent outbreak of algal blooms. Future work will evaluate effects of diversity change (e.g., dilution experiments) and key species (e.g., inoculation of reported important organisms) on the water trophic level in a controllable way in the laboratory.

**Conclusions**

Collectively, this work demonstrates that abundance and diversity of eukaryotic community rather than bacterial community relate closely to the trophic level of urban lakes during algal bloom development stage. A strong linkage between the multinutrient cycling index and community diversity was also found for eukaryotes than for bacteria. The eukaryotic community displayed stronger species replacement and environmental adaptability as well as weaker community stability and environmental constraints than the bacterial community. Our findings enrich understanding of biotic effects on the water trophic level during algal bloom development stage, and might be helpful to inform restoration of eutrophic lakes. Future work is needed to disentangle abiotic and biotic influences on the water trophic level at cellular and molecular levels.
Conflict of interest

The authors declare no competing financial or non-financial interests.

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Author contributions

All authors contributed intellectual input and assistance to this study and manuscript. Y.Y., W. W., and D.H. provided experimental design. W.W., D.H., and X.X. collected experimental materials and conducted experiments. W.W analyzed the data and wrote the manuscript. G.M.G., W.L., L.Y., Y.C., and Y.Y revised the manuscript, and Y.Y submitted the manuscript.

Data availability statement

The sequencing datasets were deposited in the NCBI Short Read Archive database under accession numbers SRR18822101–SRR18822181 for bacteria and SRR18822020–SRR18822100 for eukaryotes.

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Huber, P., Metz, S., Unrein, F., Mayora, G., Sarmento, H., and Devercelli, M. (2020) Environmental heterogeneity determines the ecological processes that govern


microeukaryotic plankton communities in a subtropical urban reservoir.  


Figure 1. Linear regressions between trophic level index and gene abundance and community diversity (Shannon-Wiener index), between multinutrient cycling index and community diversities of bacteria and eukaryotes. Asterisks represent significance (*, $p < 0.05$; ***, $p < 0.001$).
Figure 2. Taxonomic and phylogenetic β-diversities of bacteria and eukaryotes. (a) Distance-decay curves of community similarity at taxonomic and phylogenetic levels. (b) Differences in taxonomic and phylogenetic distances between bacteria and eukaryotes. Asterisks represent significance (**, $p < 0.01$; ***, $p < 0.001$).
Figure 3. Species replacement and responses to environmental factors. (a) Triangular plots (simplices) show community ecological processes (i.e., species replacement and richness difference) of bacteria and eukaryotes among the 6480 pairs of samples. Each point (yellow and blue dots) represents a pair of samples. Its position is determined by a triplet of values from the Similarity ($S = 1 - D$; dissimilarity), Repl (replacement), RichDiff (richness difference) matrices; each triplet sums to 1. (b) Redundancy analysis display effects of environmental factors on community composition of bacteria and eukaryotes.
Figure 4. Coexistence patterns of plankton and correlations between environmental factors and network topological parameters. (a) Co-occurrence networks of bacteria and eukaryotes. The nodes (circles) in the networks represent ASVs, and the size of the nodes reflect the value of betweenness centrality. (b) Pearson’s correlations between topological parameters (i.e., nodes, links, and degrees) of sub-networks and physicochemical factors. Asterisks denote significance (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).
Figure 5. Environmental adaptability of bacteria and eukaryotes in urban lakes. (a) Environmental breadths of bacteria and eukaryotes in response to environmental variables. The threshold values in the figures were standardized using log_{10} (original threshold value +1). (b) Phylogenetic signals reflecting the trait conservatism for environmental preferences of bacteria and eukaryotes.
Figure 6. Ecological community assembly processes of bacteria and eukaryotes in urban lakes. (a) Mantel correlograms reflect whether significant phylogenetic signals of bacterial and eukaryotic communities occur at short phylogenetic distances along environmental gradients. Each point denotes the Mantel correlation coefficient of the given range in phylogenetic distances. Red, blue, and grey symbols represent highly significant ($p < 0.01$), significant ($p < 0.05$) and insignificant ($p > 0.05$) correlations, respectively. (b) Kernel density estimates for distributions of abundances of βNTI for bacterial and eukaryotic communities. (c) Stacking diagram shows percentages of ecological processes. (d) The ratio of sorting to dispersal limitation based on null model. (e) Fit of the neutral community model displaying community assemblies of bacteria and eukaryotes. The solid black lines reflect the best fit to the neutral model, and the dashed black lines represent 95% confidence intervals around the model prediction.
Table 1  Pearson’s correlation between physicochemical variables and gene abundance and community diversity (Shannon-Wiener index) of bacteria and eukaryote, Permutational multivariate analysis of variance showing physicochemical variables on community composition of bacteria and eukaryote, and Mantel’s correlations between changes in physicochemical factors and phylogenetic turnover represented by βNTI. Asterisks denote significance (*, \(p < 0.05\); **, \(p < 0.01\); ***, \(p < 0.001\)).

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