Bangladesh J. Bot. **55**(1): 21-30, 2026 (March) DOI: https://doi.org/10.3329/bjb.v55i1.88626

REVEALING THE DYNAMICS OF ULTRAPHYTOPLANKTON DIVERSITY IN CIHU LAKE BY INTEGRATED HPLC AND MOLECULAR SEQUENCING ANALYSES

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Keywords: Ultraphytoplankton, HPLC-CHEMTAX, Single-molecule real-time sequencing, Biodiversity, Cihu Lake

Abstract

Eutrophication is a major ecological issue in freshwater lakes. Ultraphytoplankton, with small cell sizes and high sensitivity, are effective indicators for water trophic status assessment. In this study, a two-year ecological survey was conducted in Cihu Lake, a shallow urban lake in the middle Yangtze River, using HPLC-CHEMTAX and 16S rDNA high-throughput sequencing to explore ultraphytoplankton community diversity and its environmental drivers. Results showed Cihu Lake shifted from lightly eutrophic (2022) to moderately eutrophic (2023), with its trophic state significantly affected by Dissolved Oxygen, Potassium Permanganate Index, Total Phosphorus, Ammonium Nitrogen (N-NH_4^+), Secchi Depth, and Chlorophyll a. Nitrogen limitation occurred in the first three seasons of 2022 and throughout 2023, while nitrogen-phosphorus co-limitation occurred in winter 2022. Fucoxanthin, Alloxanthin, Zeaxanthin, and Chlorophyll b were major photosynthetic pigments while Euglenophyta, Cryptophyta, Cyanophyta, and Chrysophyta were dominant phytoplankton, indicating distinct seasonal succession regulated by Total Phosphorus, Secchi Depth, and Water Temperature. Meanwhile, dominant bacterial phyla included Proteobacteria, Bacteroidota, Cyanobacteria, Actinobacteriota, and Verrucomicrobiota, with Phosphate (PO_4^{3-}), Potassium Permanganate Index, and Chlorophyll a as key influencing factors, and most phytoplankton taxa were negatively correlated with bacterial communities.

Introduction

Freshwater lakes are vital ecological and socioeconomic resources, but some of them suffer eutrophication and water quality degradation due to human activities and climate change (O'Farrell *et al.* 2021). More than half of China's freshwater lakes are located in the middle and lower reaches of the Yangtze River, many of which are currently in the eutrophic state (Shi *et al.* 2020). Eutrophication of water bodies leads to the disruption or degradation of aquatic ecosystem structure and function, directly affecting the sustainability of socioeconomic development and human livelihood needs (Shi *et al.* 2020), and is prone to trigger algal blooms, which emit unpleasant odors and result in decreased dissolved oxygen levels and mass mortality of aquatic organisms (Feng *et al.* 2012). Therefore, timely monitoring of the dynamic changes in lake eutrophication is essential to provide data support for the scientific prevention and control of algal blooms, safeguarding lake ecosystems and ensuring water supply security. Cihu Lake, a core urban lake of Huangshi City in the middle Yangtze River, has been affected by historical mining activities and urbanization, resulting in long-term fluctuations in water quality between Class III and Class V, with prominent eutrophication issues (He *et al.* 2022). Cihu Lake has currently

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evolved into a closed inland water body with limited water storage capacity and a fragile ecosystem that is highly sensitive to water quality fluctuations, leading to significant impacts on aquatic communities and a declining trend in biodiversity (Lili *et al.* 2008). Ultraphytoplankton (0.7-5.0 μm), due to their small cell sizes and high sensitivity to environmental changes, serve as ideal indicators for assessing trophic status (Sieburth *et al.* 1978, Davis *et al.*1985). High-performance liquid chromatography-chemotaxonomy (HPLC-CHEMTAX) enables quantitative assessment of phytoplankton community composition through photosynthetic pigment analysis (Agirbas *et al.* 2015), while single-molecule real-time sequencing can reveal diversity of planktonic bacteria (Lucie *et al.* 2020). In this study, we monitored the eutrophication status of Cihu Lake for eight seasons in 2022 and 2023, integrating HPLC-CHEMTAX and PacBio sequencing to analyze the community structure, seasonal succession, and environmental drivers of ultraphytoplankton. The findings will support ecological monitoring and management of Cihu Lake and other freshwater lakes under the risk of eutrophication.

Materials and Methods

Cihu Lake (62.19 km^2, average depth 2.70 m), located in Huangshi, Hubei Province, China (30°11'14"N–30°13'00"N, 115°01'59"E–115°03'45"E), was monitored at five sites (CH1-CH5) across four seasons from 2022 to 2023, yielding 80 surface water samples (Fig. 1). Water samples were collected at 0.5 m depth, stored in pre-cleaned bottles wrapped in aluminum foil to prevent light exposure, and processed within 24 hrs. The <5 μm fraction was collected on 0.7μm GF/F filters and stored at -80 °C for pigment and molecular analyses. In situ measurements of water temperature (WT), dissolved oxygen (DO), specific conductance (SpCond), salinity (Sal), total dissolved solids (TDS), and Secchi depth (SD) were conducted using a YSI EXO3 multiparameter probe and Secchi disk. Concentrations of total nitrogen (TN), total phosphorus (TP), chemical oxygen demand (COD_{Mn}), phosphate (PO_4^{3-}), ammonium nitrogen ($N\text{-}NH_4^+$) , and chlorophyll a (Chl *a*) were determined according to GB3838-2002 (Agirbas *et al.* 2015). Photosynthetic pigments were extracted using a hot solvent method, analyzed by HPLC (Agilent 1260, Eclipse XDB-C8 column; Mobile phase A consisted of methanol (HPLC grade) and 1 mol/l

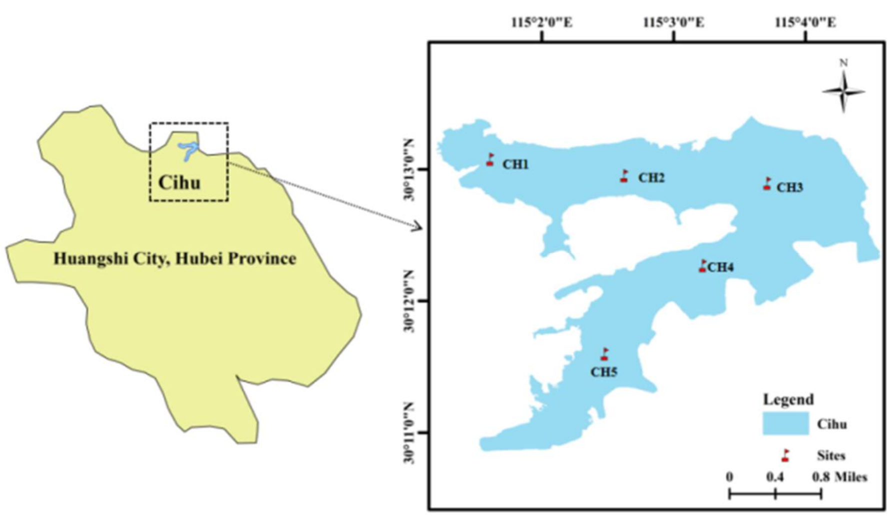

Fig. 1. Sampling and monitoring stations in Cihu Lake.

ammonium acetate solution (4:1, v/v), with the pH adjusted to 7.2. Mobile phase B was methanol (HPLC grade), and mobile phase C was ultrapure water. The gradient elution program is presented in Table 1, and quantified against external standards. Genomic DNA was extracted using a modified CTAB protocol (Lucie *et al.* 2020), checked for purity and integrity, and amplified for full-length 16S rDNA gene sequencing on the PacBio Sequel II platform. Sequence data were processed using QIIME2 (v2020.6) and taxonomically assigned against the SILVA database (v138) (Quast *et al.* 2013). Alpha diversity (Chao1, Shannon), beta diversity (Bray-Curtis), NMDS, PCoA, RDA (R v4.1.0, Canoco 5.0), and Spearman correlation analysis (SPSS v25.0) were performed. Trophic state was evaluated using the TLI, and phytoplankton composition was inferred using CHEMTAX.

Table 1. HPLC gradient elution program settings.

Time (min	Mobile phase A (%)	Mobile phase b (%)	Mobile phase C (%)
0	100	0	0
2	100	0	0
16	55	45	0
27	0	100	0
32	0	100	0
36	100	0	0

Results and Discussion

Cihu Lake exhibited a clear trend of deteriorating water quality from 2022 to 2023 (Fig. 2). Total nitrogen (TN) showed no significant interannual difference (mean: 1.17 mg/l in 2022 vs. 1.29 mg/l in 2023; $p > 0.05$), although a slight increase was observed, potentially masked by high spatiotemporal variability. In contrast, total phosphorus (TP) increased significantly in 2023 ($p < 0.001$), indicating intensified phosphorus loading. Chemical oxygen demand (COD_{Mn}) rose from 5.01 mg/l in 2022 to 6.70 mg/l in 2023 ($p < 0.01$), while chlorophyll a (Chl *a*) peaked at 68.61 μg/l in summer 2023 (maximum: 103.93 μg/l), far exceeding the 10 μg/l algal bloom threshold; values in 2022 ranged between 9.42 and 25.52 μg/l. Mean dissolved oxygen (DO) remained high (>9 mg/l), suggesting strong reoxygenation capacity despite increasing eutrophication pressure.

The TN:TP ratio declined significantly from 2022 (4.25-37.65) to 2023 (2.2-12.22, $p < 0.01$), suggesting a shift from potential nitrogen-phosphorus co-limitation in winter 2022 toward increasing nitrogen limitation throughout 2023 (Table 2). TN and TP were predominantly classified as Class IV-V, and COD_{Mn} as Class III-IV according to GB3838-2002, reflecting a typical " nitrogen-phosphorus-driven " pollution pattern. The lake transitioned from lightly eutrophic (TLI: 48.26 – 58.64) to moderately eutrophic (59.36-64.39) in 2023 (Fig. 3A). TLI was positively correlated with Chl *a* ($r = 0.67$, $p < 0.001$), TP ($r = 0.73$, $p < 0.001$), and COD_{Mn} ($r = 0.71$, $p < 0.001$), and negatively with Secchi depth (SD; $r = -0.74$, $p < 0.001$) and DO ($r = -0.53$, $p < 0.001$) (Fig. 3B), confirming that nutrient enrichment drives algal proliferation and degrades light and oxygen conditions (Filazzola *et al.* 2020, Qin *et al.* 2020) .

HPLC analysis revealed key diagnostic pigments: fucoxanthin (Fuco), alloxanthin (Allo), zeaxanthin (Zea), lutein (Lut), chlorophyll b (Chl *b*), and chlorophyll a (Chl *a*). Fuco (avg: 63.63 ng/l) peaked in spring (Fig. 4), indicating diatom dominance. Allo (avg: 247.11 ng/l) surged in autumn-winter, with a peak of 1461.90 ng/l at site CH5 in winter 2023, over three times higher than in 2022, suggesting cryptophyte proliferation during cold periods (Huang *et al.* 2007). Zea peaked in summer (120.57 ng/l), indicative of cyanobacterial activity. All pigment maxima occurred at site CH5, implying localized nutrient enrichment or hydrological stagnation.

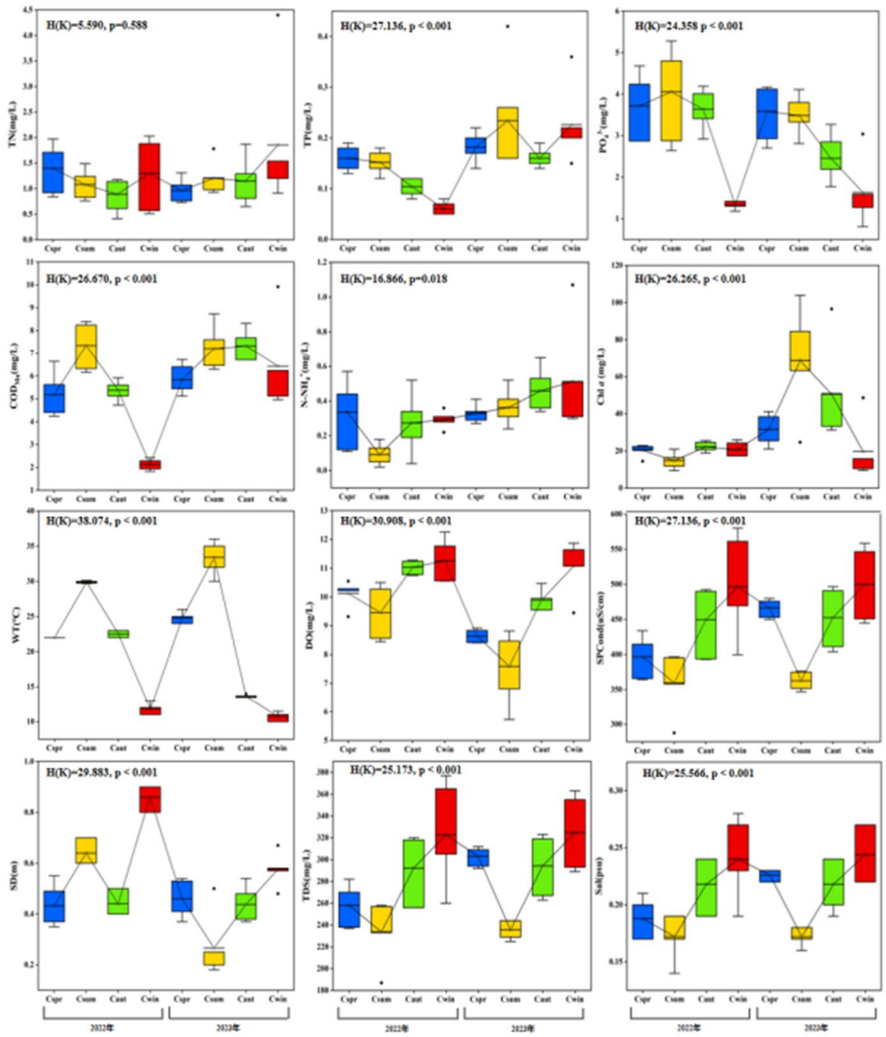

Fig. 2. Analysis of physicochemical parameters in Cihu Lake across four seasons in 2022 and 2023.

Table 2. TN/TP ratio of Cihu Lake.

Site	TN/TP	Annual average
22Cspr	8.59±2.30	11.47
22Csum	7.25±2.34	
22Caut	8.54±3.87	
22Cwin	21.49±11.53	
23Cspr	5.36±1.24	6.48
23Csum	5.92±2.15	
23Caut	7.09±2.31	
23Cwin	7.53±2.98	

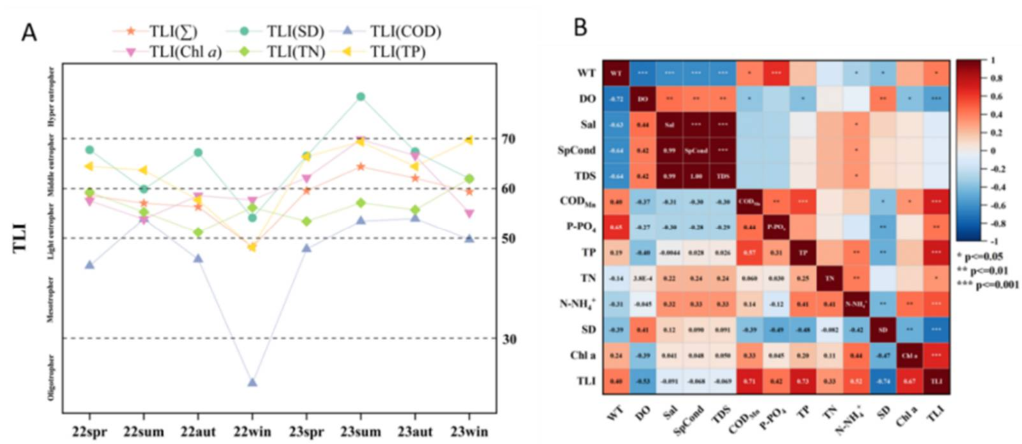

Fig. 3. Trophic State Assessment and Correlations with Physicochemical Parameters in Cihu Lake. A. Comprehensive assessment of trophic state in Cihu Lake. B. Heatmap of correlations between physicochemical parameters and the comprehensive trophic state index in Cihu Lake.

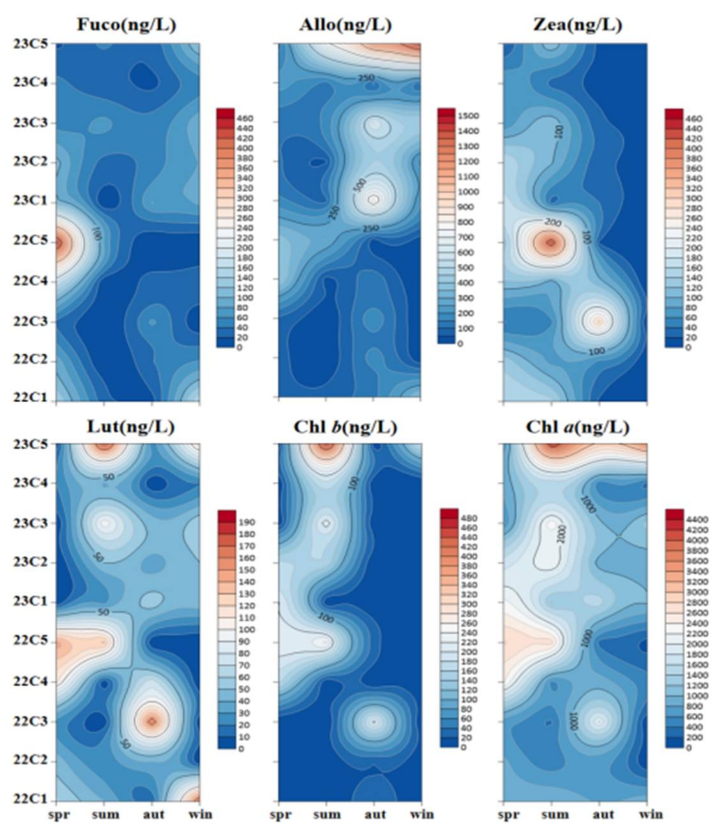

Fig. 4. The spatiotemporal distribution characteristics of characteristic photosynthetic pigments of ultra - microphytoplankton in Cihu Lake.

CHEMTAX identified six phytoplankton groups: Cryptophyta, Bacillariophyta, Chrysophyta, Dinophyta, Euglenophyta, and Chlorophyta (Fig. 5). In 2022, Euglenophyta dominated spring-summer (up to 99.99%), replaced by Cryptophyta in autumn-winter. In 2023, Chrysophyta (18.70 – 84.93%) and Cryptophyta co-dominated year-round. NMDS showed (Fig. 6) high reliability (Stress < 0.08, R^2 > 0.97), with reduced seasonal clustering in 2023, suggesting diminished seasonality and potential community adaptation to sustained nutrient stress (Olefeld *et al.* 2020).

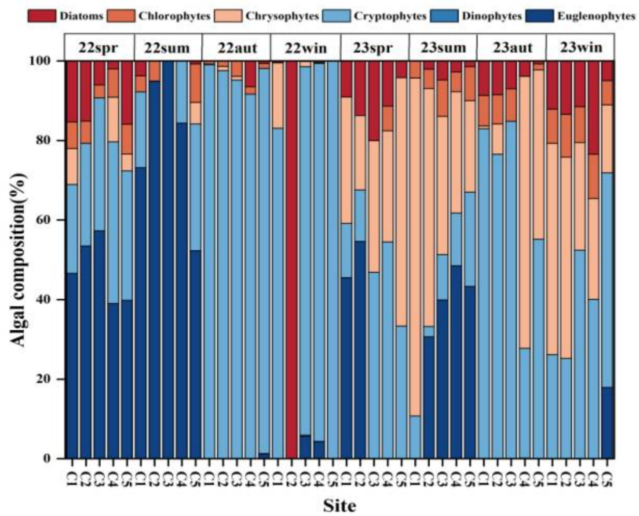

Fig. 5. Community structure composition of ultra - microeukaryotic phytoplankton in Cihu Lake based on the HPLC - CHEMTAX method.

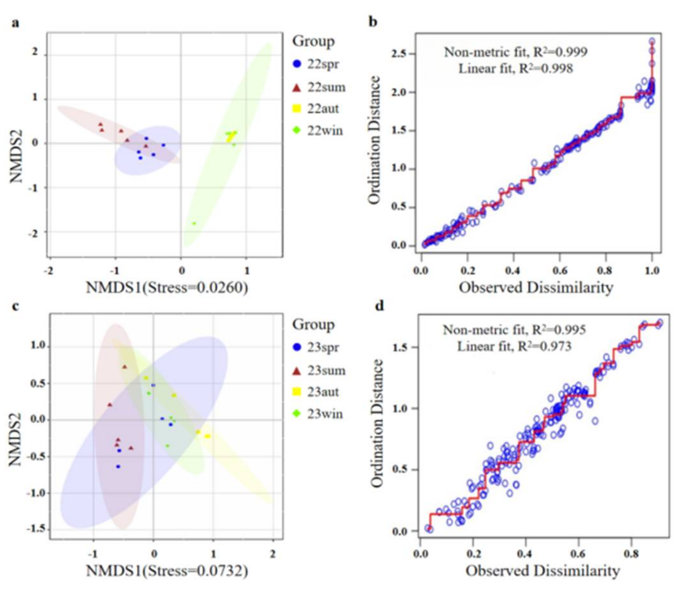

Fig. 6. The NMDS figure and Shepard figure of Cihu Lake. 2022 year ：a. NMDS figure b. Shepard figure ；2023 year ：c. NMDS figure ；d. Shepard figure.

A total of 31 bacterial phyla were detected, dominated by Proteobacteria (29-42%) and Bacteroidota (12-30%) (Fig. 7A), consistent with typical freshwater lakes. At genus level (Fig. 7B), *Candidatus Fonsibacter ubiquis* increased in relative abundance (7-13%) in 2023, suggesting potential adaptation under eutrophic conditions (Jun *et al*. 2021). Cyanobacteria abundance rose, particularly in autumn 2023, signaling elevated harmful algal bloom risks (Kim *et al*. 2020, King *et al*. 2022). Alpha diversity peaked in summer 2023 (Fig. 8), contrasting with a spring peak in 2022, reflecting shifts in microbial assembly processes. PCoA explained 55% of variance (Fig. 9A), showing temporal separation along PCoA2, with stations clustered, indicating seasonality outweighs spatial heterogeneity. Winter communities diverged distinctly, supported by hierarchical clustering (Fig. 9B).

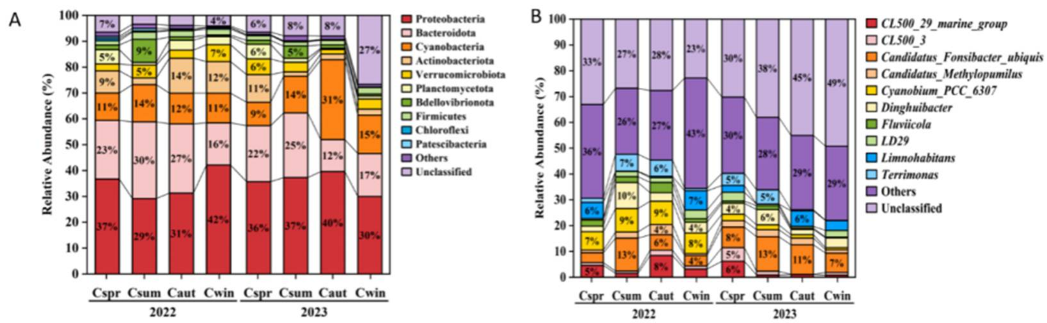

Fig. 7. The composition of relative abundances of ultra-microplanktonic bacteria in Cihu Lake A. phylum level B. genus level.

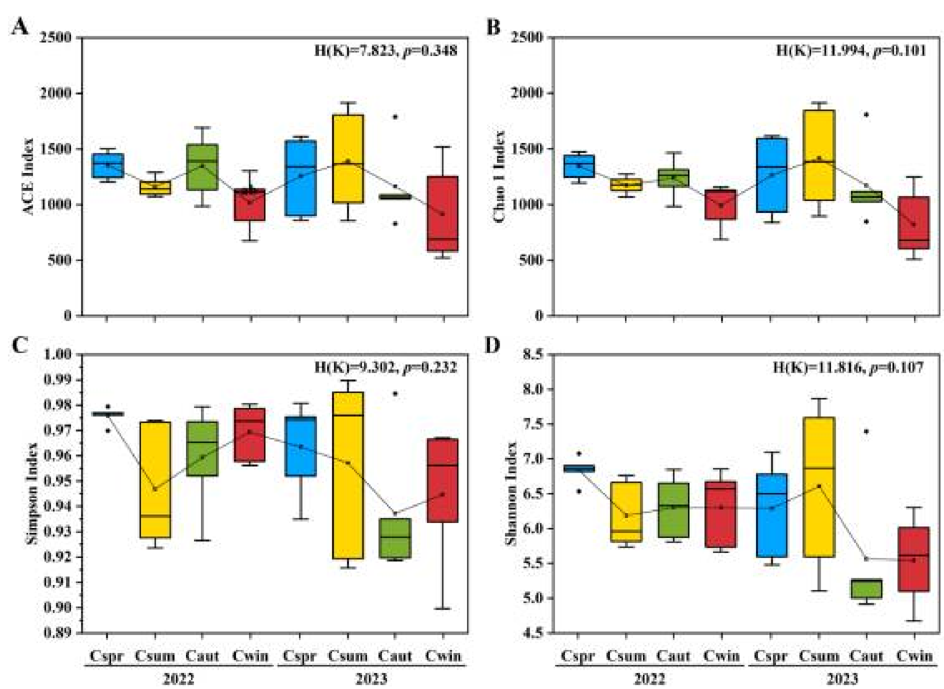

Fig. 8. α diversity indices of ultra - bacterioplankton community in Cihu Lake. (A. ACE index; B. Chao1 index; C. Simpson index; D. Shannon index).

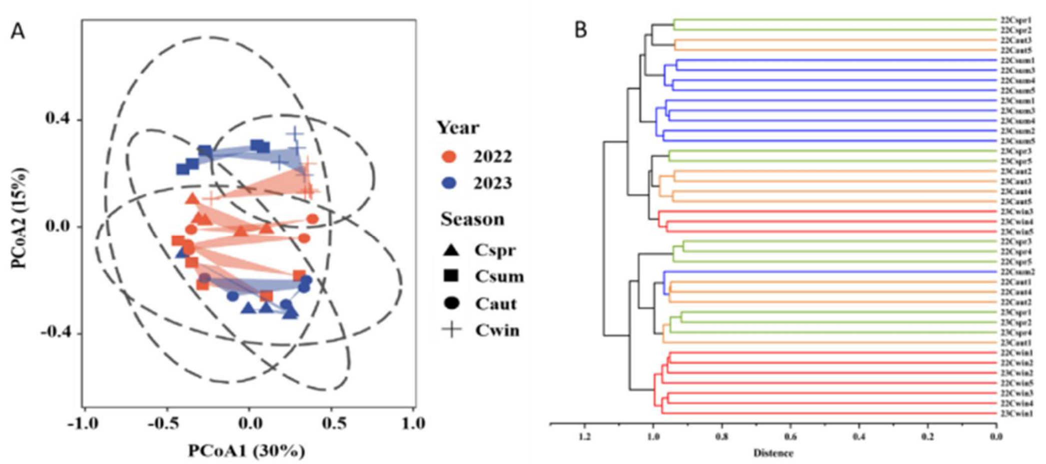

Fig. 9. Community Structure of Ultrabacterioplankton in Cihu Lake Revealed by Hierarchical Clustering and Principal Coordinate Analysis. A. Principal Coordinate Analysis. B. Hierarchical Clustering.

RDA identified TP ($p = 0.050$), SD ($p = 0.026$), and temperature ($p = 0.002$) as key drivers of eukaryotic communities (Fig. 10A, 53.16% explained), with temperature favoring Euglenophyta and TP promoting most algae except Dinophyta. For bacteria, phosphate (PO_4^{3-}; $p = 0.022$), COD_{Mn} ($p = 0.020$), and Chl *a* ($p = 0.046$) were significant (Fig. 10B, 85.84%), linking bacterial structure to organic pollution and algal-derived organics (Xu *et al.* 2020) . Heatmap analysis (Fig. 11) revealed Bacteroidota positively correlated with Euglenophyta ($p < 0.05$) but negatively with Bacillariophyta ($p < 0.01$); Firmicutes negatively with Euglenophyta ($p < 0.05$); Chloroflexi negatively with Chrysophyta ($p < 0.05$), suggesting complex interactions including competition and facilitation (Sanguo *et al.* 2023).

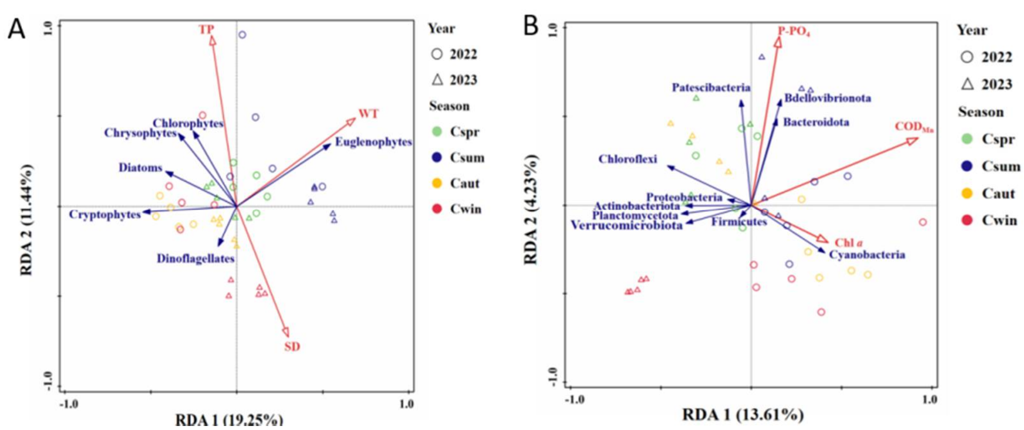

Fig. 10. Redundancy Analysis Revealing Relationships Between Environmental Factors and Ultra-Microbial Communities in Cihu Lake. A.Eukaryotic phytoplankton community. B. Bacterioplankton community.

These findings demonstrate that Cihu Lake is undergoing rapid eutrophication driven by external inputs and internal phosphorus cycling (Qin *et al.* 2020). The shift toward nitrogen limitation implies that management should prioritize nitrogen control while monitoring sediment P flux. Seasonal succession in both eukaryotic and bacterial communities is shaped by environmental filtering and biological interactions, highlighting the need for integrated strategies, including source control, internal load reduction, and ecological restoration, to prevent recurrent algal blooms and facilitate ecosystem recovery (Kai *et al.* 2022).

This study elucidates the community structure, succession patterns, and potential environmental response mechanisms of ultraphytoplankton in Cihu Lake, providing a scientific basis for lake ecological monitoring and management. The use of integrating HPLC-CHEMTAX and single-molecule real-time sequencing reveals facilitates continuous monitoring of lake eutrophication status and timely management of environment. Future efforts should extend monitoring duration, expand investigations across particle size fractions, and further explore algal-bacterial interaction mechanisms to optimize eutrophication mitigation strategies.

Acknowledgement

National Natural Science Foundation of China (41171045), Open Project Funding of Key Laboratory of Intelligent Health Perception and Ecological Restoration of Rivers and Lakes, Ministry of Education, Hubei University of Technology.

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(Manuscript received on 30 September, 2025; revised on 16 March, 2026)