

Article

A Preliminary Assessment of Phytoplankton Community Characteristics and Environmental Associations in an Extremely High-Altitude River–Lake Confluence Zone

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Abstract

Phytoplankton are sensitive indicators of environmental change in high-altitude aquatic ecosystems, but their community responses across river–lake confluence zones remain insufficiently understood. We investigated phytoplankton community characteristics and environmental associations in the Siling Co river–lake continuum. This endorheic system lies above 4500 m, where river inflows, lake–water mixing, and evaporative concentration generate marked hydrochemical gradients. Genus-level cell density and estimated biomass were assessed with 22 environmental variables from 14 sampling events at 10 lake and river sites. We recorded 54 phytoplankton genera, with Bacillariophyta dominant. Lake and River samples overlapped in composition, whereas sampling period accounted for more community variation than water-body type. Major-ion and salinity-related gradients were associated with cell-density community dissimilarity after accounting for water-body type. Cell-density and biomass composition also showed partly distinct associations with pH and SiO₂, and with Cl[−], respectively. These observations indicate that phytoplankton communities in the Siling Co river–lake confluence zone are more closely associated with sampling-period variation and hydrochemical gradients than with Lake/River habitat classification. These findings provide an initial basis for integrated biological and hydrochemical monitoring.

Keywords: plateau lakes; river–lake confluence zones; phytoplankton; ionic composition; total dissolved solids; principal coordinates analysis (PCoA); permutational multivariate analysis of variance (PERMANOVA); distance-based redundancy analysis (db-RDA); mantel tests



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1. Introduction

Phytoplankton are key components of primary production, material cycling, and energy transfer through aquatic food webs. Their community composition, abundance, and diversity often respond rapidly to changes in water temperature, light availability, nutrient

concentrations, hydrodynamic conditions, and water chemistry. They therefore serve both as indicators of ecosystem status and as sensitive biological signals of environmental filtering processes [1,2].

River–lake confluence zones formed where rivers enter lakes are not simply juxtaposed riverine and lacustrine water bodies. Within a limited spatial extent, runoff inputs, evaporative concentration, ion exchange, water mixing, and variation in water residence time can collectively generate continuous gradients in salinity, dissolved substances, nutrients, and suspended particulate matter. Hydrodynamic processes can also alter the transport pathways and availability of inflowing water and associated nutrients within the euphotic zone [3,4]. Consequently, phytoplankton responses to such gradients are often taxon-specific, and interactions among physicochemical variables may explain community variation better than any single factor alone.

In waters with relatively high salinity, osmoregulatory costs, ionic ratios, and nutrient availability can jointly alter competitive relationships among algal groups. Recent field and experimental studies have shown that even modest changes in salinity can restructure planktonic communities, alter community assembly processes, and affect the relative dominance of groups such as cyanobacteria through responses of competitors or grazers [5–10]. In addition, cell density and biomass do not provide equivalent descriptions of community change. Small-sized or filamentous taxa may contribute high cell densities, whereas large taxa or taxa with high per-cell biomass may dominate biomass accumulation. Joint analyses of cell density, biomass, α -diversity, and genus-level composition can therefore reduce the risk of interpreting a single quantitative metric as a complete proxy for community ecological function.

The main basin of Siling Co, its littoral sites, and the confluence zones of its inflowing rivers together form a high-altitude river–lake transitional system with pronounced hydrochemical heterogeneity. Plateau lakes are highly sensitive to hydroclimatic change, salinity differentiation, and nutrient redistribution. Recent studies from the Tibetan Plateau have further linked hydroclimatic processes, gradients in conductivity or salinity, and nutrient stoichiometry to the structure and assembly of aquatic communities [11–15]. Available data indicate numerical differences between lake and river sites in salinity, ionic composition, nutrients, and silicate. However, owing to limited sample size and multiple-testing constraints, between-group differences for individual variables are not consistently detected. Thus, rather than focusing solely on whether lake and river sites support completely distinct communities, it is necessary to identify continuous environmental gradients spanning both site types and examine their relationships with community turnover.

Based on genus-level cell-density and biomass matrices from 14 sampling events, this study first characterized environmental conditions and the abundance, diversity, and taxonomic composition of phytoplankton communities. We then used Bray–Curtis distances, principal coordinates analysis (PCoA), non-metric multidimensional scaling (NMDS), and permutational multivariate analysis of variance (PERMANOVA) to assess community differences associated with water-body type and sampling period. Spearman correlation, distance-based redundancy analysis (db-RDA), Mantel tests, and partial Mantel tests were further combined to identify environmental associations at the levels of individual variables, constrained ordination, and distance matrices. Finally, candidate associations were evaluated according to convergent evidence from multiple analytical approaches. This framework was designed to distinguish environment–community associations supported by the reported analyses from exploratory signals and to provide a reproducible analytical pathway for phytoplankton monitoring in high-altitude river–lake confluence zones.

2. Materials and Methods

2.1. Study Area and Sampling Design

The study area comprised Siling Co and adjacent river–lake confluence zones at elevations above 4500 m. Sampling sites encompassed the main basin and littoral areas of Siling Co, as well as confluence zones associated with the Zagya Zangbo, Zhagen Zangbo, Ngari Zangbo, and Dara Zangbo rivers. In total, 10 spatial sampling sites (S1–S10) were established, spanning 88.522939–89.530935° E and 31.519086–32.042631° N (Figure 1).

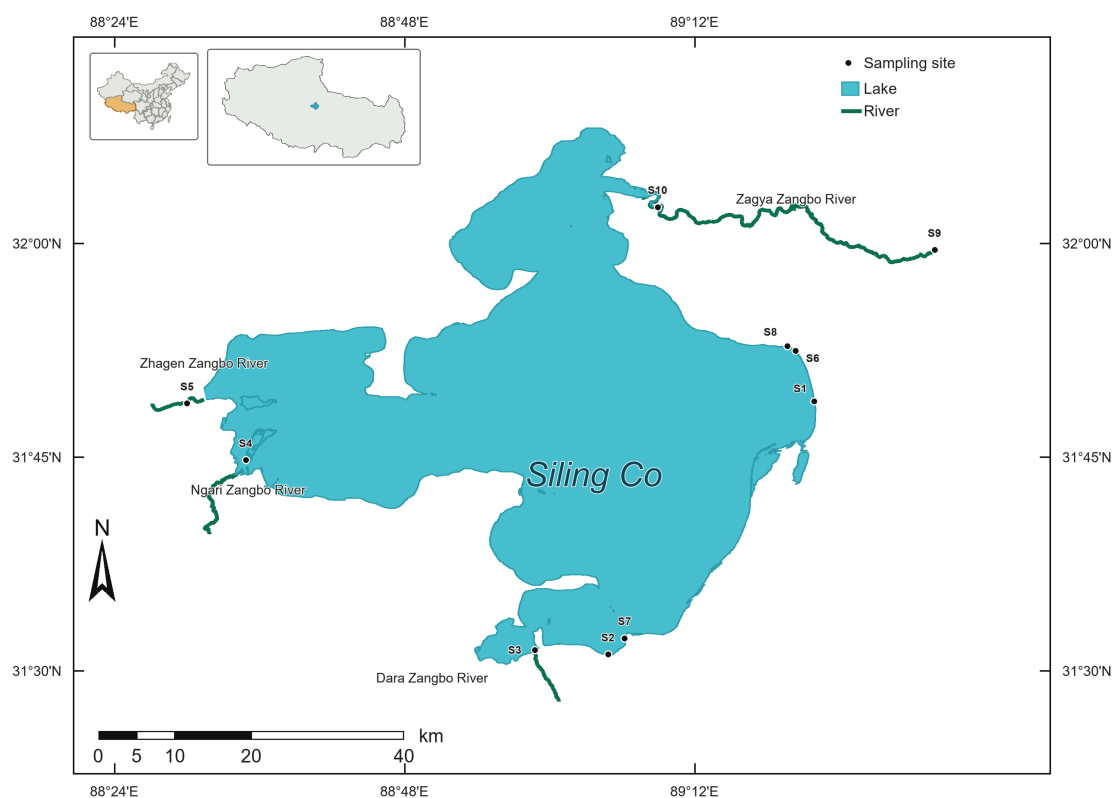


Figure 1. Distribution of sampling sites in Siling Co and adjacent river–lake confluence zones. Black circles indicate sampling sites, blue areas indicate lakes, and green lines indicate the major rivers associated with the sampling sites. S1–S10 denote sampling-site identifiers.

A total of 14 sampling events were included in this study, comprising seven lake samples (Lake) and seven river samples (River). Sampling was conducted in June, August, and October 2023, and in September 2024. Sites S1, S2, S3, and S5 were repeatedly sampled across sampling periods. Because months and years were not fully crossed in the sampling design, all time-related inferences are described as differences among sampling periods and are not interpreted as strictly independent seasonal or interannual effects.

2.2. Field Sampling, Environmental Measurements, and Phytoplankton Identification

Each sampling event was assigned a unique identifier (sample_uid) and linked to the corresponding sample_id, sampling-site name, geographic coordinates, water-body type, sampling year, and sampling month. Environmental data comprised 22 variables, including water temperature (WT), dissolved oxygen (DO), pH, turbidity (Tur), conductivity (CON), total dissolved solids (TDS), salinity (SAL), nitrogen and phosphorus nutrients, major ions, total hardness (TH), iron (Fe), sulfide (S^{2-}), and silica (SiO_2).

2.2.1. Sampling for Physicochemical Water-Quality Variables

Six variables, namely pH, water temperature, dissolved oxygen, total dissolved solids, conductivity, and salinity, were measured in situ using an AZ86031 multiparameter water-quality meter (AZ Instrument Corp, Taichung, Taiwan). Water samples for the remaining variables were collected from the surface layer, 10 cm below the water surface, using clean 500 mL plastic bottles. Before sampling, each bottle was rinsed with the water to be sampled. The rinse water was discarded, after which the bottle was filled with sample water while avoiding air bubbles and immediately sealed tightly. Samples were stored in a vehicle-mounted refrigerator at 4 °C and transported to the laboratory within 48 h for physicochemical analyses (Table 1).

Table 1. Physicochemical water-quality variables, units, analytical methods, and instruments.

Physicochemical Variable	Code	Unit	Analytical Method	Instrument
pH	pH	—	In situ measurement	AZ86031 multiparameter water-quality meter
Water temperature	WT	°C	In situ measurement	AZ86031 multiparameter water-quality meter
Dissolved oxygen	DO	mg/L	In situ measurement	AZ86031 multiparameter water-quality meter
Total dissolved solids	TDS	Ppm	In situ measurement	AZ86031 multiparameter water-quality meter
Conductivity	CON	µS/cm	In situ measurement	AZ86031 multiparameter water-quality meter
Salinity	SAL	Ppt	In situ measurement	AZ86031 multiparameter water-quality meter
Turbidity	Tur	TU	—	Merck Prove 600 laboratory multiparameter water-quality analyzer
Potassium	K ⁺	mg/L	GB/T 11904-1989 [16]	Merck Prove 600 laboratory multiparameter water-quality analyzer
Sodium	Na ⁺	mg/L	GB/T 11904-1989	Merck Prove 600 laboratory multiparameter water-quality analyzer
Calcium	Ca ²⁺	mg/L	GB/T 11904-1989	Merck Prove 600 laboratory multiparameter water-quality analyzer
Silicon	SiO ₂	mg/L	GB/T 13196-1991 [17]	Merck Prove 600 laboratory multiparameter water-quality analyzer
Iron	Fe	mg/L	HJ/T 345-2007 [18]	Merck Prove 600 laboratory multiparameter water-quality analyzer
Chloride	Cl [−]	mg/L	HJ/T 84-2001 [19]	Merck Prove 600 laboratory multiparameter water-quality analyzer
Sulfate	SO ₄ ^{2−}	mg/L	GB/T 13196-1991	Merck Prove 600 laboratory multiparameter water-quality analyzer
Sulfite	SO ₃ ^{2−}	mg/L	GB/T 13196-1991	Merck Prove 600 laboratory multiparameter water-quality analyzer
Nitrate	NO ₃ [−]	mg/L	GB/T 7480-1987 [20]	Merck Prove 600 laboratory multiparameter water-quality analyzer
Nitrite	NO ₂ [−]	mg/L	GB/T 8538-2008 [21]	Merck Prove 600 laboratory multiparameter water-quality analyzer
Ammonium nitrogen	NH ₄ ⁺	mg/L	GB/T 7479-1987 [22]	Merck Prove 600 laboratory multiparameter water-quality analyzer
Total phosphorus	TP	mg/L	GB/T 11894-1989 [23]	Merck Prove 600 laboratory multiparameter water-quality analyzer
Total nitrogen	TN	mg/L	GB/T 11894-1989	Merck Prove 600 laboratory multiparameter water-quality analyzer
Sulfide	S ^{2−}	mg/L	GB/T 16489-1996 [24]	Merck Prove 600 laboratory multiparameter water-quality analyzer

2.2.2. Phytoplankton Sampling and Identification

A 10 L water sample was filtered through a No. 25 plankton net with a mesh size of 64 µm. The retained material was fixed with Lugol's solution, and the final concentrated sample volume was 50 mL. After thorough homogenization, a 0.1 mL aliquot was transferred to a 0.1 mL counting chamber containing 100 grid squares. Typically, 50 randomly selected squares were examined at 400× magnification (10× ocular and 40× objective). Phytoplankton were identified to genus level using the taxonomic keys and illustrations in Atlas of Common Algae in China's Inland Waters [25]. Individual cells were counted, and counts were recorded by genus. Each sample was counted twice, and the mean estimate was used. When either count differed from the mean by 10% or more, a third count was performed.

Cell density was calculated as

$$D_i = \frac{n_i \times \left(\frac{100}{k}\right) \times \left(\frac{50}{0.1}\right)}{10} = 5000 \frac{n_i}{k} \text{ cells/L}$$

where D_i is the cell density of genus i (cells/L), n_i is the number of cells counted, and k is the number of grid squares examined. The calculation accounts for the counted chamber fraction, the concentrate volume, and the original water volume. For counts covering 50 squares, the expression simplifies to $D_i = 100n_i$.

Biomass was estimated using reference mean wet weights from Table 6-1-3 in Hydrobiology, second edition [26]. The intermediate size-class value was selected where available; otherwise, the single listed value was used. This rule standardized coefficient selection in the absence of direct cell-size measurements. For reference coefficients expressed per cell, biomass was calculated as

$$B_i = D_i \times a_i \times 10^{-4}$$

$$B = \sum B_i$$

where a_i is the numerical coefficient listed in the textbook, in units of 10^{-4} mg per individual. The factor 10^{-4} converts the coefficient to mg/cell. Genus-level biomass B_i and summed biomass B were expressed as estimated wet weight in mg/L.

The density estimates represent cells recovered by the net per litre of original water, without correction for filtration losses. Comparisons of phytoplankton sampling methods show that method selection can affect density estimates and community composition [27]. Both cell density and estimated biomass therefore describe the net-collected assemblage. No separate whole-water samples were collected to quantify the contribution of organisms passing through the mesh.

2.3. Environmental Data Quality Control and Preprocessing

For environmental characterization and between-group comparisons, missing values were first quantified and potential outliers were identified using the interquartile range method. In descriptive statistics and Mann–Whitney U tests, missing records were automatically excluded on a variable-wise basis. Given the small sample size and the skewed distributions of some variables, differences between Lake and River samples were assessed using two-sided Mann–Whitney U tests, whereas differences among sampling periods were evaluated using Kruskal–Wallis tests. All p values were adjusted for the false discovery rate (FDR) using the Benjamini–Hochberg procedure [28].

For correlation analyses and multivariate ordination, missing environmental values were imputed using variable medians. Non-negative variables with an absolute skewness > 1 and deemed suitable for transformation were $\log_{10}(x + 1)$ -transformed and subsequently standardized as z scores. Principal component analysis (PCA) was performed on the standardized environmental variables, equivalent to PCA of their correlation matrix.

The biplot was used to summarize environmental gradients and visualize sample scores together with variable loadings. To reduce redundancy among explanatory variables, collinearity was assessed using Spearman rank correlations and variance inflation factors (VIFs). Highly correlated variables, including pH, TDS, and CON, were not simultaneously retained in the same constrained ordination model. Analysis-specific environmental-variable selection criteria are described in Section 2.7 and the Supplementary Materials. For the Lake–River comparison of total cell density, total biomass, and α -diversity, site-level sensitivity analyses used the median value of each metric within each sample_id.

2.4. Phytoplankton Community Metrics and Taxonomic Composition

Phytoplankton communities were characterized using genus-level cell-density and biomass matrices comprising 14 samples and 54 genera. Total cell density (N) and total biomass (B) for each sample were calculated as the sums of the cell densities and biomasses, respectively, of all detected genera. Based on cell density, the Margalef richness index (d) and Shannon–Wiener diversity index (H) were calculated following Magurran [29]. Pielou evenness (J) [30] and Simpson dominance (S) [31] were also calculated. Here, p_i denotes the relative cell density of genus i in a sample, and S_r denotes the number of recorded genera: $d = \frac{S_r - 1}{\ln N}$, $H = -\sum p_i \times \ln p_i$, $J = \frac{H}{\ln S_r}$, $S = \sum p_i^2$.

Genus-level identifications followed the taxonomic reference described in Section 2.2.2 [25]. Phylum-level composition was summarized using the genus-to-phylum taxonomic mapping table. Relative cell density and relative biomass were calculated separately [32]. The relative contribution of phylum i was calculated as $R_{Ai} = \frac{A_i}{\sum A_i} \times 100\%$, where A_i represents its cell density or biomass. Dominant phyla were identified by integrating mean relative cell density, mean relative biomass, and frequency of occurrence. For genus-level visualization and correlation analyses, genera were selected from the union of the eight highest-ranked genera by mean relative cell density, the eight highest-ranked genera by mean relative biomass, and genera contributing at least 20% of either measure in any individual sample. Means were calculated across all 14 sampling events, including zeros. When this union exceeded 14 genera, the 14 highest-scoring genera were retained using $S = \max(\bar{D}, \bar{B}) + 0.20 \times \max(D_{max}, B_{max}) + 0.02 \times F$, where $\bar{D}$ and $\bar{B}$ are mean relative cell density and biomass, D_{max} and B_{max} are their respective maxima across samples, and F is occurrence frequency. All inputs were expressed as percentages. This study-specific heuristic rule incorporated average contribution, occasional high abundance, and occurrence frequency. As cell density and biomass represent numerical structure and material contribution, respectively, they were analyzed independently and cross-checked during interpretation.

2.5. Community β -Diversity, Ordination, and Permutation Tests

Genus-level abundances were first converted to within-sample relative abundances and then Hellinger-transformed to reduce the disproportionate influence of dominant genera on distance calculations and linear ordination [33]. Bray–Curtis dissimilarities were calculated from the transformed matrices [34], and principal coordinates analysis (PCoA) was conducted separately for cell-density and biomass communities [35]. Two-dimensional non-metric multidimensional scaling (NMDS) was used as a complementary ordination, with ordination quality evaluated using stress values [36]. In ordination plots, colours distinguished Lake and River samples, point shapes indicated sampling periods, and 95% confidence ellipses were displayed.

PERMANOVA with 4999 permutations was used to evaluate variation in community composition associated with water-body type (Type), sampling period (Period: June 2023, August 2023, October 2023, and September 2024), the additive Type + Period model, and

environmental PCA axes [37]. The standalone Year model was excluded from the reported results because the sampling design did not allow interannual differences to be distinguished from differences among sampling months. The event-level PERMANOVA analyses used unrestricted permutations among sampling events. Within-site dependence was assessed separately through the site-level sensitivity analysis of water-body type, rather than through site-restricted permutations in the main models. The proportion of explained variation was expressed as R^2 . PERMDISP with 999 permutations was additionally performed to test for differences in within-group dispersion [38]. As an additional rank-based test of habitat-level community differentiation, analysis of similarities (ANOSIM) was performed separately for the genus-level cell-density and biomass datasets using the same Bray–Curtis dissimilarity matrices [36]. Statistical significance was assessed using 9999 permutations. The ANOSIM R statistic was used to quantify the relative magnitude of between-group versus within-group dissimilarity, with larger positive R values indicating stronger separation between Lake and River communities.

For the site-level sensitivity analysis of water-body type, relative genus compositions were averaged across sampling events within each sample_id. This produced one community profile per spatial site, comprising five Lake and five River sites. The averaged profiles were Hellinger-transformed, and Bray–Curtis dissimilarities were recalculated before testing the Type effect. The Type effect was then retested using these 10 site-level profiles, preventing repeatedly sampled sites from contributing multiple observations to this comparison. This analysis addressed the between-site Type comparison and did not constitute a correction for repeated observations in the sampling-period analyses.

The association between cell-density and biomass Bray–Curtis distance matrices was assessed using a permutation-based distance-matrix correlation analysis.

2.6. Community–Environment Association Analyses

Spearman rank correlations were used to examine associations between environmental variables and total cell density, total biomass, α -diversity metrics, phylum-level relative abundance, and relative abundance of dominant genera. Multiple-testing correction was performed separately within five analytical families: overall community metrics, phylum-level cell density, phylum-level biomass, cell density of dominant genera, and biomass of dominant genera. Associations with $|\rho| \geq 0.60$ and an FDR-adjusted $p < 0.05$ were defined as statistically supported strong correlations. Associations with $|\rho| \geq 0.60$ and an unadjusted $p < 0.05$, but a non-significant FDR-adjusted p value, were defined as exploratory strong correlations. Site-level sensitivity analyses were conducted by taking within-site medians of the preprocessed environmental variables and community response metrics, yielding one observation per sample_id. Spearman correlations were then recalculated across the 10 sites, with the corresponding within-family FDR corrections reapplied. These analyses covered overall community metrics, phylum-level relative cell density and biomass, and the relative contributions of dominant genera.

2.7. Constrained Ordination and Environmental Distance-Matrix Analyses

Constrained ordination analyses used genus-level cell-density and biomass community matrices as response variables. Hellinger-transformed redundancy analysis (Hellinger-RDA) [33], canonical correspondence analysis (CCA) [39], and distance-based redundancy analysis (db-RDA) based on Bray–Curtis distances were compared according to gradient lengths from correspondence analysis, the proportion of zero values, and model suitability. db-RDA was selected as the primary model [40]. The initial candidate pool for constrained ordination comprised SAL, TN, TP, NH_4^+ , NO_3^- , Cl^- , SO_4^{2-} , Ca^{2+} , Mg^{2+} , Na^+ , WT, DO, pH, and SiO_2 , representing salinity, nutrient availability, major-ion composition, and basic

physicochemical conditions. Other measured variables were not included in this restricted pool to limit model complexity. SAL was retained to represent salinity-related conditions in the candidate pool, whereas CON and TDS were excluded. Variables with more than 20% missing observations were excluded, which removed Na^+ (35.7% missing). Mg^{2+} was excluded because it was strongly correlated with SO_4^{2-} (Spearman $\rho = 0.837$, exceeding the $|\rho| \geq 0.70$ screening threshold), with SO_4^{2-} retained to represent this shared gradient. Forward selection then evaluated the remaining 12 candidates. At each step, the variable with the lowest conditional permutation p value was considered, with ties resolved by higher adjusted R^2 and then greater incremental R^2 . Addition required $p < 0.05$, a maximum VIF ≤ 5 , and an increase in adjusted R^2 after the first selected variable. Selection stopped when these conditions were not met or three variables had been retained. Forward selection used 4999 permutations, and final models were evaluated using 9999 permutations.

Mantel tests compared Bray–Curtis dissimilarities calculated from Hellinger-transformed community matrices with Euclidean distances calculated from standardized environmental variables, using Spearman correlations and 9999 two-sided permutations [41]. Six environmental distance matrices were constructed, comprising one matrix containing all 22 measured environmental variables and five thematic subsets representing basic physicochemical conditions, salinity-related variables (CON, TDS, and SAL), nutrients, major ions, and other variables (Table S6). The “other variables” subset comprised additional chemical descriptors rather than a single ecological mechanism. Turbidity and SO_3^{2-} were included only in the full environmental matrix. Thus, the five subsets were not an exhaustive partition of all measured variables. Missing environmental values were imputed using the median of the corresponding variable, followed by Z-score standardization without logarithmic transformation before calculating Euclidean distances. FDR correction was applied separately to the six Mantel tests for cell density and biomass. A binary type distance matrix was further constructed for partial Mantel tests to assess whether the observed associations persisted after accounting for Lake/River classification [42]. As geographic distance was not controlled, these results were interpreted as sensitivity evidence rather than causal tests.

2.8. Supplementary Exploratory Analyses

Random forest models and heuristic multi-method evidence integration were conducted as supplementary exploratory analyses [43]. Given the limited dataset of 14 sampling events from 10 sites, random forest variable importance was not used to establish the main environmental associations. Site-grouped resampling was used for model evaluation, but it does not overcome the limited number of independent sites or establish the stability of variable-importance estimates. Detailed methods and results are provided in the Supplementary Information.

2.9. Statistical Software

Data processing, statistical analyses, and figure generation were conducted primarily in Python 3.14 using pandas (version 2.3.3), NumPy (version 2.3.5), SciPy (version 1.17.1), statsmodels (version 0.14.6), scikit-learn (version 1.9.0), Matplotlib (version 3.10.7), openpyxl (version 3.1.5), and related packages. The number of permutations used for each permutation-based test and the FDR-correction procedure are specified in the corresponding analyses.

3. Results

3.1. Environmental Gradients and Lake–River Sample Distribution

A total of 14 sampling events were included in the environmental analysis, comprising seven Lake and seven River samples. Missing values were recorded for Na^+ (five values),

SO_3^{2-} (four values), Tur (two values), K^+ (two values), and S^{2-} (one value); all other environmental variables had complete data. In the environmental principal component analysis, PC1 and PC2 explained 27.56% and 22.80% of the environmental variation, respectively, accounting for 50.36% cumulatively (Figure 2). Strong correlations among environmental variables after FDR correction are shown in Table 2.

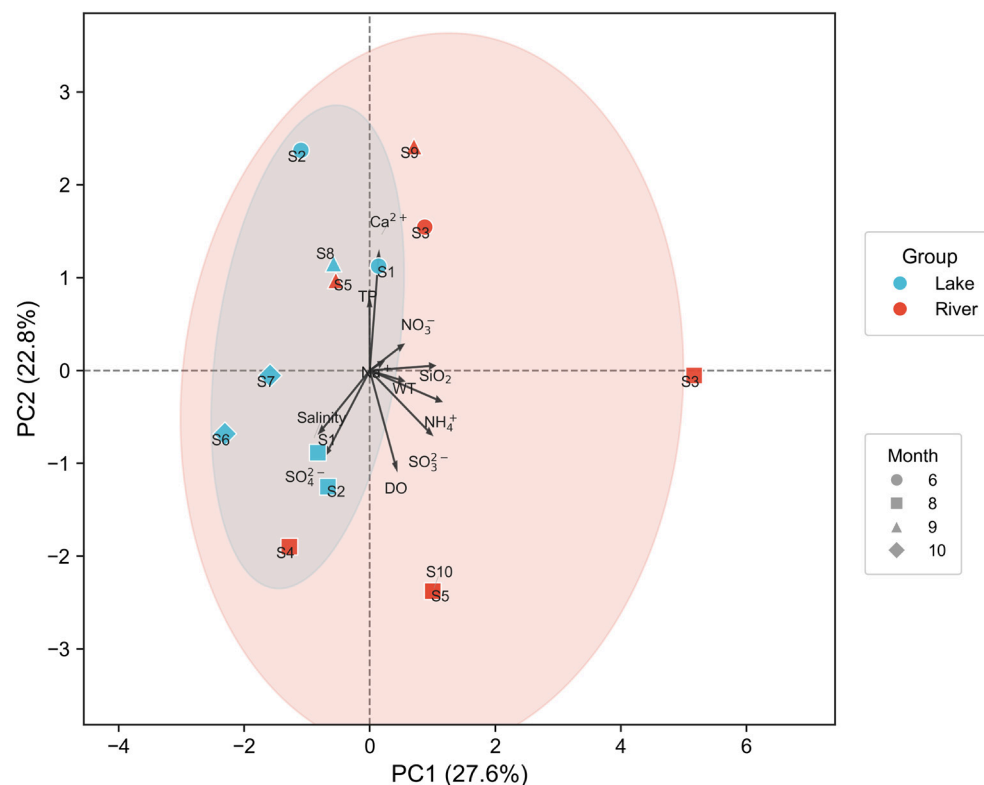


Figure 2. PCA biplot of environmental variables in the Siling Co lake–river confluence zone. Points represent samples, colours indicate water-body type, and point shapes denote sampling periods. Arrows indicate the loading directions and relative contributions of environmental variables to PC1 and PC2. Lake samples appeared more tightly clustered than River samples in the PC1–PC2 plane, which accounted for 50.36% of the total variance. This pattern describes dispersion in the displayed ordination space and does not establish temporal stability.

The largest positive loadings on PC1 were NH_4^+ (0.494), SiO_2 (0.449), and SO_3^{2-} (0.429), whereas the largest negative loadings were SAL (−0.349) and SO_4^{2-} (−0.303). For PC2, Ca^{2+} (0.552) and TP (0.333) showed the largest positive loadings, while DO (−0.462), SO_4^{2-} (−0.394), SO_3^{2-} (−0.301), and SAL (−0.292) showed the largest negative loadings. The mean PC1 scores were −0.99 for Lake sites and 0.99 for River sites, with partial overlap between the two groups in ordination space.

Table 2. Strong Spearman correlations among environmental variables after FDR correction.

Variable 1	Variable 2	ρ	Unadjusted p	FDR-Adjusted p	Direction
Fe	S^{2-}	0.828	0.0002532	0.0205	Positive
pH	TDS	−0.824	0.0002874	0.0205	Negative
TDS	CON	0.815	0.0003838	0.0205	Positive
pH	CON	−0.811	0.0004314	0.0205	Negative

Note: Strong correlations were defined as $|\rho| \geq 0.70$ with an FDR-adjusted $p < 0.05$ ($n = 14$).

No individual environmental variable showed a significant Lake–River difference after FDR correction. The unadjusted p values for SiO_2 , Fe, and S^{2-} were 0.0049, 0.0105, and

0.0239, respectively, whereas the corresponding FDR-adjusted p values were 0.1076, 0.1157, and 0.1750. Kruskal–Wallis tests across sampling periods likewise detected no significant differences in individual environmental variables after FDR correction.

3.2. Phytoplankton Taxonomic Composition and Dominant Groups

A total of 54 phytoplankton genera were recorded, and their occurrence across the 14 sampling events is listed in Table S1. Based on the current phylum-level aggregation, Bacillariophyta was detected in all 14 samples and had a mean relative cell density of 69.4%, a mean relative biomass of 83.5%, and an occurrence frequency of 100%. Cyanobacteria and Chlorophyta were the subdominant groups, with mean relative cell densities of 16.2% and 10.8%, respectively, and mean relative biomasses of 5.8% and 9.1%, respectively (Figure 3).

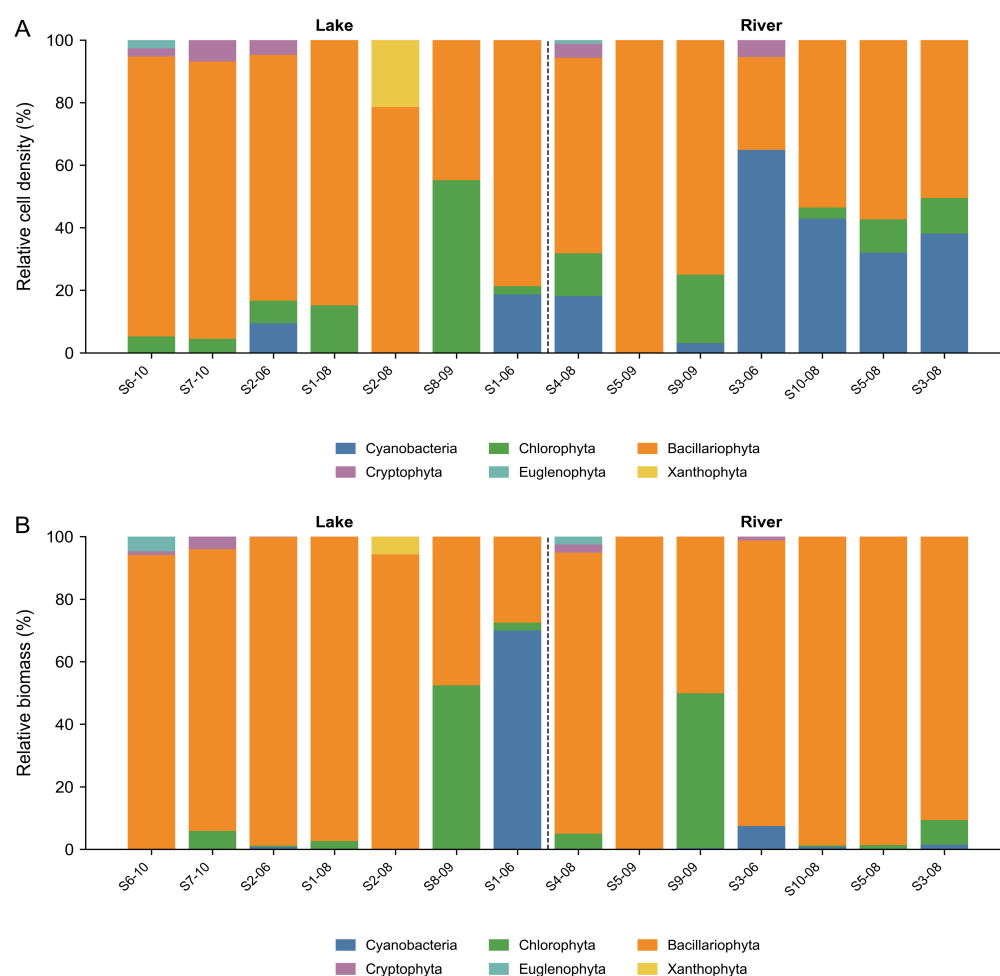


Figure 3. Phylum-level relative composition of phytoplankton in the Siling Co lake–river confluence zone. (A) Relative cell density. (B) Relative biomass. Samples were grouped as Lake or River and ordered within each group by scores on the first axis of the environmental PCA.

Bacillariophyta was the dominant group in both Lake and River samples. Based on relative cell density, the mean contribution of Bacillariophyta was 77.7% in Lake samples, exceeding that in River samples (61.2%). Cyanobacteria had a higher mean relative cell density in River samples (28.5%) than in Lake samples (4.0%). Based on relative biomass, the mean contributions of Bacillariophyta were 78.5% and 88.5% in Lake and River samples, respectively.

At the genus level, *Melosira* had the highest mean relative cell density (10.7%), followed by *Oscillatoria* (7.7%) and *Navicula* (6.9%). *Diatoma* had the highest mean relative biomass (11.7%), followed by *Peridinium* (10.7%), *Cymatopleura* (7.8%), *Cocconeis* (7.7%),

A

Relative cell density (%)

B

Relative biomass (%)

Lake

River

S1_2023_06, S2_2023_06, S1_2023_08, S2_2023_08, S8_2024_09, S6_2023_10, S7_2023_10, S3_2023_06, S10_2023_08, S3_2023_08, S4_2023_08, S5_2023_08, S5_2024_09, S9_2024_09

Legend:

- Melosira
- Diatoma
- Pinnularia
- Cymatopleura
- Cocconeis
- Eunotia
- Navicula
- Achnanthes
- Oocystis
- Oscillatoria
- Merismopedia
- Ceratium
- Peridinium
- Others

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3.3. Variation in Phytoplankton Abundance, Biomass, and α -Diversity Across Sampling Periods

Across the 14 sampling events, total cell density ranged from 5.8×10^2 to 3.7×10^5 cells L⁻¹, with a median of 1.9×10^5 cells L⁻¹. Total biomass ranged from 0.0008 to 2.7 mg L⁻¹, with a median of 0.52 mg L⁻¹. The coefficients of variation for total cell density and total biomass were 65.3% and 107.8%, respectively. The median values of the Margalef richness index (d), Shannon diversity index (H), Pielou evenness index (J), and Simpson dominance index (S) were 0.97, 2.26, 0.91, and 0.14, respectively (Table 3).

Table 3. Kruskal–Wallis test results for phytoplankton abundance, biomass, and α -diversity metrics across sampling periods.

Metric	H	Unadjusted <i>p</i>	FDR-Adjusted <i>p</i>	ϵ^2	Effect Size
Total cell density	7.019	0.0713	0.0933	0.402	Large
Total biomass	9.305	0.0255	0.0510	0.630	Large
<i>D</i>	6.752	0.0802	0.0933	0.375	Large
<i>H</i>	9.952	0.0190	0.0510	0.695	Large
<i>J</i>	6.410	0.0933	0.0933	0.341	Large
<i>S</i>	9.952	0.0190	0.0510	0.695	Large

Note: *p* values were adjusted using the Benjamini–Hochberg FDR procedure; no metric reached the adjusted significance threshold of *p* < 0.05.

Across sampling periods, the Kruskal–Wallis test for total cell density yielded *H* = 7.019, an unadjusted *p* = 0.071, an FDR-adjusted *p* = 0.093, and ϵ^2 = 0.402. The corresponding results for total biomass were *H* = 9.305, an unadjusted *p* = 0.026, an FDR-adjusted *p* = 0.051, and ϵ^2 = 0.630. In August 2023, the median total cell density and total biomass were 3.1×10^5 cells L⁻¹ and 0.96 mg L⁻¹, respectively, compared with 2.4×10^3 cells L⁻¹ and 0.0053 mg L⁻¹ in September 2024 (Figure 6).

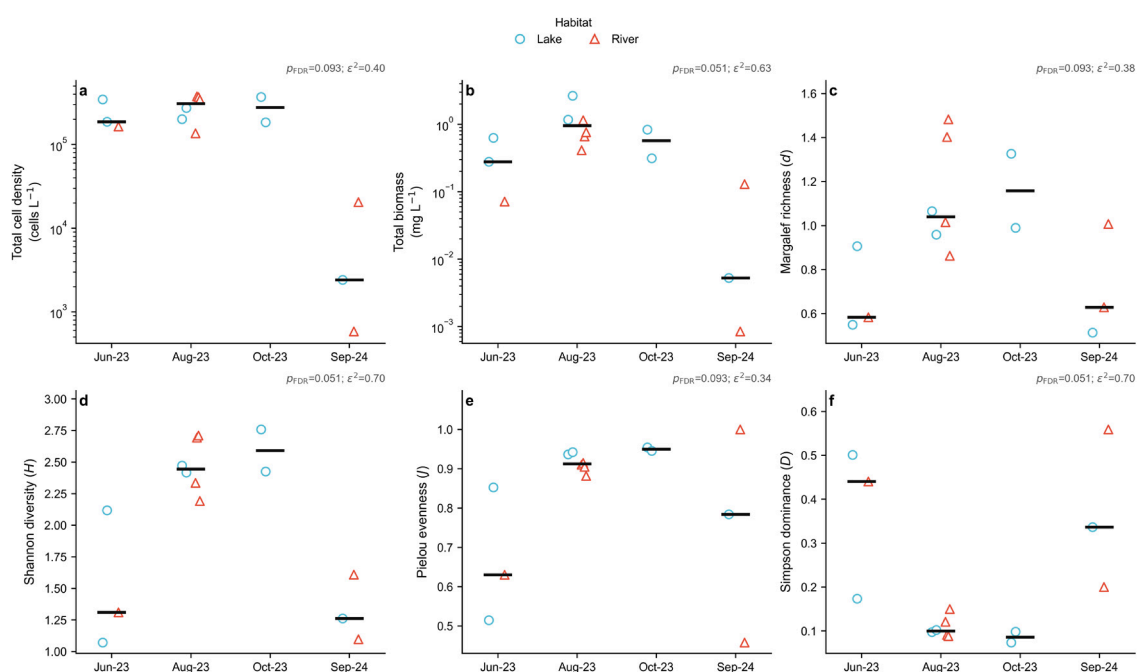


Figure 6. Total phytoplankton cell density, total biomass, and α -diversity metrics across sampling periods. Circles and triangles represent Lake and River samples, respectively, and black lines indicate the median for each sampling period. Because month and year were not fully crossed in the sampling design, the results represent differences among sampling periods rather than strictly seasonal effects. Note: (a) Total cell density; (b) Total biomass; (c) Margalef richness (*d*); (d) Shannon diversity (*H*); (e) Pielou evenness (*J*); (f) Simpson dominance (*D*).

For both the Shannon diversity index and Simpson dominance index, the unadjusted Kruskal–Wallis results were $H = 9.952$ and $p = 0.019$; the FDR-adjusted p values were 0.051 and ϵ^2 was 0.695 for both indices. In October 2023, the median Shannon, Pielou, and Simpson indices were 2.59, 0.95, and 0.09, respectively, compared with 1.31, 0.63, and 0.44 in June 2023. The remaining α -diversity metrics also did not reach an FDR-adjusted significance threshold of 0.05.

In the observational Lake–River comparison, total cell density, total biomass, and all four α -diversity metrics did not reach FDR-adjusted significance. Sensitivity analyses that aggregated repeated samples into 10 independent spatial sites yielded FDR-adjusted p values of 0.841 for all metrics. The directions of effect were consistent for all six metrics relative to the sampling-event-level comparison (Table 4).

Table 4. Mantel test results for associations between grouped environmental matrices and phytoplankton community dissimilarity.

Community Matrix	Environmental Variable Group	Mantel r	Permutation p	FDR q	FDR Result
Cell density	All environmental variables	0.313	0.0560	0.1120	ns
Cell density	Basic physicochemical variables	0.154	0.3289	0.3947	ns
Cell density	Salinity-related variables	0.390	0.0183	0.0549	ns
Cell density	Nutrients	0.197	0.2981	0.3947	ns
Cell density	Major ions	0.417	0.0071	0.0426	*
Cell density	Other variables	0.128	0.4356	0.4356	ns
Biomass	All environmental variables	0.238	0.1538	0.3076	ns
Biomass	Basic physicochemical variables	0.072	0.6483	0.7780	ns
Biomass	Salinity-related variables	0.319	0.0400	0.1200	ns
Biomass	Nutrients	0.194	0.3170	0.4755	ns
Biomass	Major ions	0.369	0.0167	0.1002	ns
Biomass	Other variables	−0.023	0.8945	0.8945	ns

Notes: Bray–Curtis distances were calculated from Hellinger-transformed community matrices, whereas Euclidean distances were calculated from z-score-standardized environmental matrices. Tests used 9999 two-sided permutations (ns indicates $p > 0.05$, * indicates $p < 0.05$).

3.4. Genus-Level Community β -Diversity and Effects of Water-Body Type and Sampling Period

In PCoA based on genus-level cell density, the first two axes explained 19.29% and 19.11% of community variation, respectively. In biomass-based PCoA, the first two axes explained 21.16% and 18.69%, respectively. Lake and River samples overlapped in both ordinations. Two-dimensional NMDS yielded stress values of 0.168 for cell density and 0.174 for biomass, showing overall distribution patterns consistent with the PCoA results (Figures 7 and S1).

In single-factor PERMANOVA, Type explained 10.42% and 9.75% of variation in cell-density and biomass communities, respectively, with pseudo-F values of 1.396 and 1.296 and p values of 0.1292 and 0.1956. PERMDISP showed no significant differences in within-group multivariate dispersion between Lake and River samples for either cell-density ($F = 0.258$, $p = 0.677$) or biomass-based communities ($F = 0.289$, $p = 0.667$). The mean distances to group centroids (mean $\pm$ SD) were 0.50 ± 0.10 for Lake samples and 0.48 ± 0.06 for River samples in the cell-density dataset. The corresponding values in the biomass dataset were 0.53 ± 0.08 and 0.51 ± 0.08 , respectively (Table S2). These results indicate comparable within-group β -diversity heterogeneity between Lake and River habitats. In the site-level sensitivity analysis, repeated observations were aggregated into 10 site-level community profiles, comprising five Lake and five River sites. Water-body type explained 12.54% of variation in cell-density composition and 10.46% in biomass composition, with p values

of 0.3438 and 0.5592, respectively. These analyses did not detect statistically significant differences between Lake and River communities, but do not establish equivalence between the two habitat types.

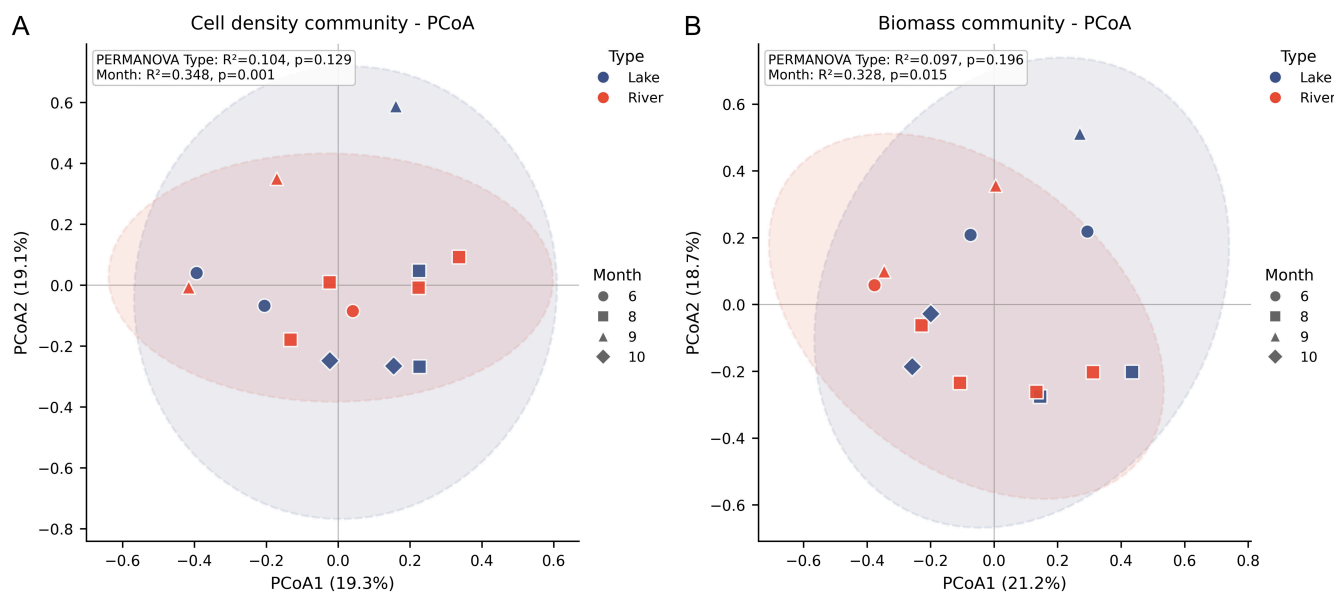


Figure 7. PCoA of phytoplankton communities based on genus-level cell density (A) and biomass (B). Bray–Curtis dissimilarities were calculated from relative-abundance and Hellinger-transformed community data. Colours indicate water-body type, point shapes denote sampling periods, and shaded ellipses represent the 95% distribution range.

ANOSIM provided a consistent result for habitat-level community differentiation. Based on genus-level cell-density data, the ANOSIM R statistic was 0.1020 ($p = 0.1392$), whereas the biomass-based analysis yielded an R statistic of 0.0418 ($p = 0.3004$), based on 9999 permutations in both cases (Table S3). These low and non-significant R values indicate that between-habitat dissimilarities were only slightly greater than, or comparable to, within-habitat dissimilarities, providing no evidence of clear phytoplankton community separation between Lake and River habitats.

Sampling period explained 34.76% and 32.79% of the variation in cell-density and biomass communities, respectively, with pseudo-F values of 1.776 and 1.627 and nominal p values of 0.0014 and 0.0148. The additive Type + Period model explained 44.90% and 44.45% of community variation, with nominal overall model p values of 0.0006 and 0.0024, respectively. These results describe variation among the observed sampling periods and do not establish independent seasonal or interannual effects.

The Bray–Curtis distance matrices for cell density and biomass were significantly correlated ($\rho = 0.811$, $p = 0.0002$). Both community data types showed a higher proportion of explained variation for Period than for Type (Table 5).

Table 5. PERMANOVA results for differences in phytoplankton community structure.

Community Matrix	Model	R^2	Pseudo-F	p	Result
Cell density	Type	0.104	1.396	0.1292	ns
Cell density	Period	0.348	1.776	0.0014	Significant
Cell density	Type + Period	0.449	1.834	0.0006	Significant
Cell density	Environmental PC1 + PC2	0.201	1.379	0.0754	ns
Biomass	Type	0.098	1.296	0.1956	ns
Biomass	Period	0.328	1.627	0.0148	Significant

Table 5. Cont.

Community Matrix	Model	R ²	Pseudo-F	p	Result
Biomass	Type + Period	0.445	1.800	0.0024	Significant
Biomass	Environmental PC1 + PC2	0.195	1.333	0.1190	ns

Notes: Period denotes the four sampling periods: June 2023, August 2023, October 2023, and September 2024. *p* values were calculated using 4999 unrestricted permutations and are interpreted as exploratory because within-site dependence was not explicitly accounted for in these event-level tests. ns indicates $p \geq 0.05$.

3.5. Univariate and Constrained-Ordination Evidence for Community–Environment Associations

In Spearman analyses with within-family FDR correction, only the associations of cyanobacterial relative biomass with TN and SO_4^{2-} reached the predefined threshold for statistical significance. Cyanobacterial relative biomass was positively correlated with TN ($\rho = 0.885$, FDR-adjusted $p = 0.0040$) and negatively correlated with SO_4^{2-} ($\rho = -0.836$, FDR-adjusted $p = 0.0151$). In analyses of independent sites aggregated by sample_id, the association between Cyanobacterial relative biomass and TN remained significant ($\rho = 0.929$, FDR-adjusted $p = 0.0155$). The complete correlation structures for overall community metrics and phylum-level composition are presented in Figures 8 and 9.

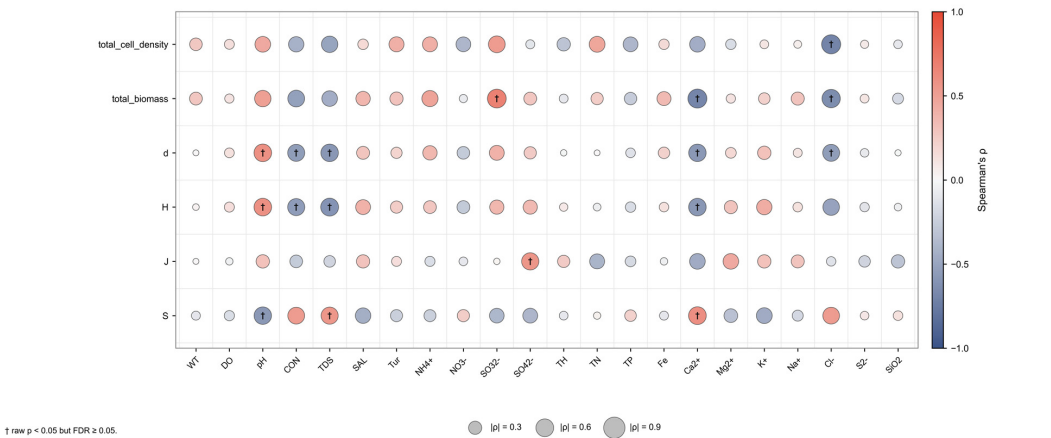


Figure 8. Spearman correlations between overall phytoplankton metrics and environmental variables.

Other associations that met the effect-size and unadjusted *p*-value thresholds but did not pass FDR correction were treated as exploratory. Total cell density was negatively correlated with Cl^- ($\rho = -0.702$, $p = 0.0051$). Total biomass was negatively correlated with Ca^{2+} ($\rho = -0.693$, $p = 0.0060$) and positively correlated with SO_3^{2-} ($\rho = 0.677$, $p = 0.0078$). The Shannon index (H) was negatively correlated with TDS ($\rho = -0.607$, $p = 0.0213$), and the relative abundance of Oscillatoria was positively correlated with SiO_2 ($\rho = 0.754$, $p = 0.0018$).

The final db-RDA model for cell density retained pH and SiO_2 . It explained 25.17% of genus-level community variation, with an adjusted R^2 of 11.56%, pseudo- $F = 1.850$, and model $p = 0.0007$. The sequential R^2 contributions of pH and SiO_2 were 0.1358 and 0.1159, with conditional permutation p values of 0.0076 and 0.0294, respectively. The final biomass db-RDA model retained only Cl^- and explained 15.34% of community variation, with an adjusted R^2 of 8.29%, pseudo- $F = 2.174$, and model $p = 0.0027$. The conditional permutation p value for Cl^- was 0.0030 (Figure 10; Table 6).

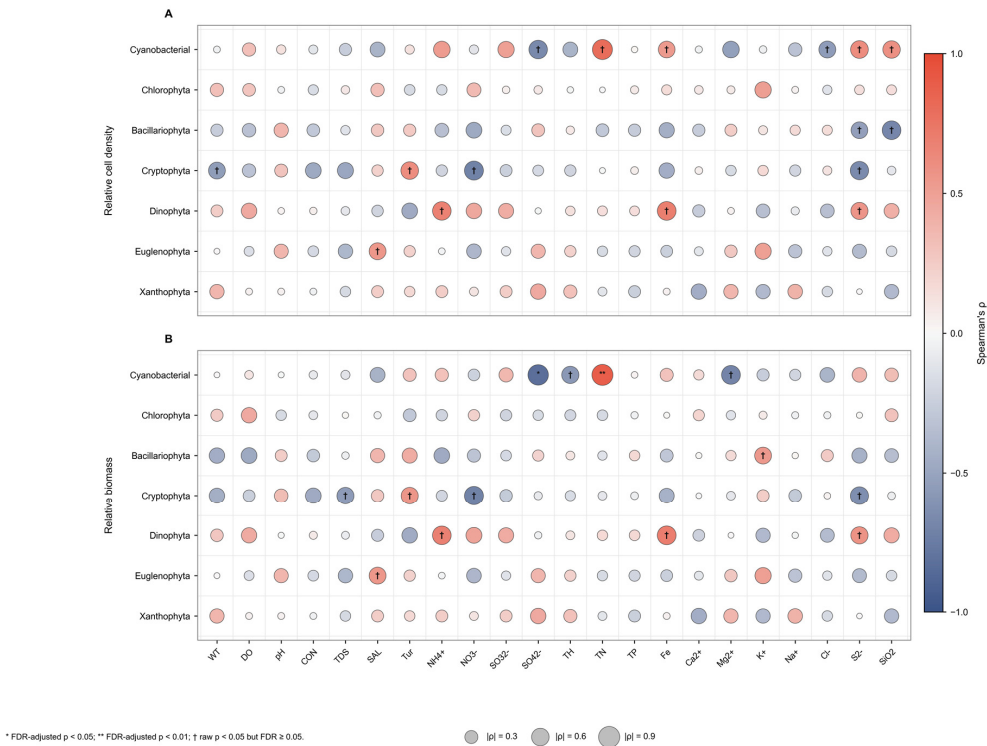


Figure 9. Spearman correlations between phylum-level phytoplankton composition and environmental variables. (A) Cell density. (B) Biomass.

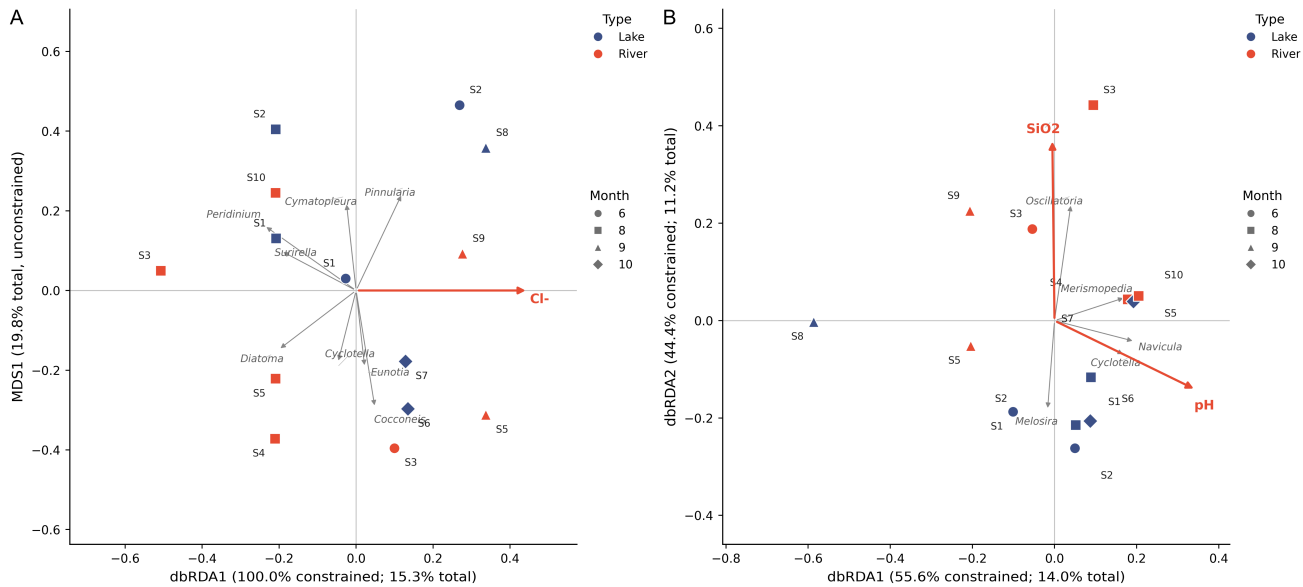


Figure 10. Distance-based redundancy analysis of environmental variables and genus-level phytoplankton community composition. (A) Biomass db-RDA, with Cl^- retained in the final model. (B) Cell-density db-RDA, with pH and SiO_2 retained in the final model. Red arrows indicate retained environmental variables, whereas grey arrows indicate the correlation directions of major genera.

Table 6. Final db-RDA models for genus-level phytoplankton community composition.

Community Matrix	Final Environmental Variables	R^2	Adjusted R^2	Pseudo- F	Model p
Cell density	pH; SiO_2	0.252	0.116	1.850	0.0007
Biomass	Cl^-	0.153	0.083	2.174	0.0027

Notes: Conditional permutation p values for pH and SiO_2 in the cell-density model were 0.0076 and 0.0294, respectively. The conditional permutation p value for Cl^- in the biomass model was 0.0030.

3.6. Associations Between Grouped Environmental Matrices and Community Dissimilarity

Mantel tests showed that the major-ion matrix was positively correlated with Bray–Curtis dissimilarity in cell-density communities (Mantel $r = 0.417$, $p = 0.0071$, FDR $q = 0.0426$). After controlling for Type, the partial Mantel result was $r = 0.424$, $p = 0.0054$, and FDR $q = 0.0324$. The Mantel correlation between the salinity-related matrix and cell-density community dissimilarity was 0.390 ($p = 0.0183$, FDR $q = 0.0549$). After controlling for Type, the correlation was 0.391 ($p = 0.0161$, FDR $q = 0.0483$).

For biomass communities, the Mantel correlations for major-ion and salinity-related matrices were 0.369 and 0.319 , with unadjusted p values of 0.0167 and 0.0400 , respectively. However, the corresponding FDR q values were 0.1002 and 0.1200 . Associations of all environmental variables, basic physicochemical variables, nutrients, and other variables with both community dissimilarity matrices did not reach FDR-adjusted significance (Figure 11).

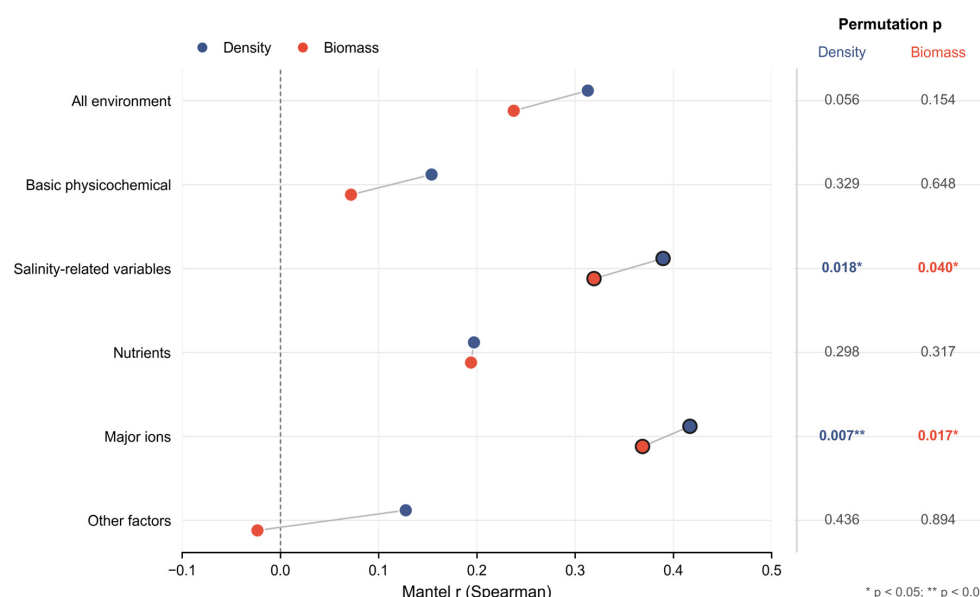


Figure 11. Mantel relationships between environmental variable groups and phytoplankton community dissimilarity. The salinity-related group comprises conductivity (CON), total dissolved solids (TDS), and salinity (SAL). Bubble size represents the absolute value of Mantel r , and colour indicates significance after FDR correction.

The method-specific analyses identified associations of pH and SiO_2 with cell-density composition and of Cl^- with biomass composition, together with correlations of TN and SO_4^{2-} with cyanobacterial relative biomass and Mantel associations involving major ions. Supplementary exploratory analyses summarize these results using a heuristic integrated ranking and random forest models (Figures S2 and S3; Tables S4 and S5). Random forest predictive performance was limited for most responses, and its variable-importance estimates were not treated as independent confirmation of these associations.

4. Discussion

4.1. Continuous Environmental Gradients Explain Community Variation Better than the Lake–River Dichotomy

The central finding of this study is that phytoplankton communities in the Siling Co river–lake confluence zone did not form two stable, mutually independent clusters along Lake and River labels. Lake and River samples overlapped in ordination space, and Type did not independently explain variation in either cell-density or biomass communities. After controlling for Type, associations of major-ion and salinity-related matrices with cell-density community dissimilarity were retained. Together, these findings are more

consistent with community turnover along continuous hydrochemical gradients than with patterns determined solely by water-body type. Previous studies of river–lake confluence zones have similarly shown that hydrodynamics, inflow properties, and mixing can jointly structure dynamic ecological ecotones [3,4,15].

This interpretation accords with the physical structure of river–lake confluence zones. River inputs, lake–water mixing, evaporative concentration, and local ion exchange can jointly alter ionic composition, nutrient forms, and water-residence conditions among adjacent sites. Consequently, substantial heterogeneity may occur within the same water-body category. Lake and River should therefore be treated as contextual site classifications rather than independent ecological mechanisms that substitute for continuous environmental information [3,4].

Importantly, a continuous-gradient interpretation does not exclude contributions from spatial position or hydrodynamic processes. The present partial Mantel analyses controlled only for the binary Type matrix. They did not control for geographic distance, discharge, water depth, or mixing intensity. Associations involving major ions and salinity-related variables may therefore reflect direct environmental filtering, but may also contain information from unmeasured hydrological or spatial processes.

4.2. Major-Ion Composition and Salinity-Related Variables Characterize Hydrochemical Gradients Associated with Community Turnover

The major-ion matrix received FDR-significant support from Mantel and partial Mantel tests for cell-density communities, and Cl^- entered the final biomass db-RDA model. Together, these findings indicate a consistent association between phytoplankton community variation and ionic composition. Compared with individual metrics such as SAL, TDS, or CON, the major-ion matrix retains information on ionic strength, ion ratios, and potential differences in water sources. It may therefore better represent the hydrochemical setting of this confluence zone. Freshwater-salinization studies have shown that changes in ionic composition and concentration can restructure plankton communities through osmotic stress, competitive interactions, and food-web processes [5–10,13].

Cl^- , SO_4^{2-} , SAL, TDS, CON, and pH share substantial information. The statistical effect of any one variable should therefore not be equated with an independent physiological action. For example, the association of Cl^- with biomass composition may reflect chloride concentration itself. It may also reflect evaporative concentration, lithological sources, water mixing, or other conditions that covary with ionic ratios. The negative correlation between SO_4^{2-} and cyanobacterial relative biomass represents a statistical association within the sampled dataset and does not demonstrate direct physiological inhibition by sulfate. This observational analysis cannot separate a sulfate-specific effect from the effects of covarying environmental conditions, and relative biomass does not directly measure cyanobacterial growth or absolute biomass. Establishing a direct inhibitory effect would require controlled experiments that manipulate sulfate while accounting for other relevant environmental conditions.

An analytical framework centred on ion assemblages rather than a single salinity metric helps explain why SAL did not enter the final db-RDA model. Nevertheless, salinity-related and major-ion matrices remained associated with community dissimilarity. Previous studies likewise show that interactions of salinity with nutrient status, temperature, and hydrodynamic conditions can reshape phytoplankton diversity and dominant groups. A single salinity metric is often insufficient to represent the full set of environmental constraints [5–9,13].

4.3. Nutrient and Physicochemical Associations Vary Among Taxa and Response Metrics

The nutrient matrix was not significantly associated with overall genus-level community dissimilarity. However, TN, SiO_2 , and pH retained clear signals at specific response

levels. This pattern indicates that associations with environmental variables may not emerge simultaneously across all taxonomic levels and community metrics. The positive association between TN and cyanobacterial relative biomass remained significant in the independent-site sensitivity analysis. It was therefore not fully attributable to repeated sampling. Nevertheless, this relationship concerns the relative contribution of Cyanobacteria to total biomass, rather than a universal response of total phytoplankton abundance to TN. Previous studies also indicate that nitrogen status, community turnover, and nutrient stoichiometry can jointly influence resource use and community structure in plateau lakes [14,44,45].

The cell-density db-RDA model retained pH and SiO₂, whereas the biomass model retained Cl[−], indicating different environmental associations for the two community metrics. Differences in cell size and per-cell biomass among taxa offer a possible explanation, but this interpretation remains untested in the present study. Cell size, cell volume, and related functional traits were not directly measured in the sampled communities; biomass was estimated using reference mean wet-weight coefficients rather than sample-specific cell-volume measurements. The contrasting model results therefore do not establish a trait-mediated mechanism. Future monitoring should pair cell counts with direct measurements of cell dimensions and volume to test whether size-related traits account for these differences.

The role of SiO₂ also requires careful definition. Its inclusion in the cell-density db-RDA model does not imply that silicon directly promotes all diatoms. In the relative-abundance data, Bacillariophyta did not show an FDR-significant positive association with SiO₂, and response directions varied among genera. SiO₂ may therefore encode environmental information related to runoff inputs, water sources, mixing conditions, and nutrient status. Confirming a physiological limitation or facilitation mechanism will require absolute-abundance data, high-frequency observations of dissolved silica, and controlled experiments.

4.4. Sampling Period Explains More Community Variation than Water-Body Type

Sampling period accounted for a greater proportion of variation in both genus-level community matrices than water-body type, although the associated permutation *p* values are exploratory. Total cell density, total biomass, and several α -diversity metrics also showed large effect sizes across sampling periods. These results indicate that phytoplankton community variation was closely associated with sampling period. In the present data, this temporal signal was stronger than the Lake–River spatial grouping. Lake heat-waves, stratification phenology, and the timing of ice formation and thaw may influence community dynamics through effects on light, mixing, and nutrient redistribution [46–48].

The temporal signal may integrate concurrent changes in water temperature, runoff, mixing, light, nutrient inputs, and ionic concentration. In high-altitude river–lake confluence zones, seasonal hydrology and local weather can jointly affect community turnover rates and the replacement of dominant taxa. All samples from 2024 were collected in September, whereas the 2023 samples were collected in June, August, and October. No calendar month was sampled in both years, preventing separation of seasonal and interannual variation. Temporal patterns are therefore interpreted only as associations with the observed sampling periods.

Cell density and biomass provide complementary information. Cyanobacterial relative cell density was higher in River samples, but its mean relative biomass did not increase correspondingly. In contrast, estimated biomass in some samples was dominated by low-frequency genera assigned higher reference mean wet-weight coefficients; this pattern does not demonstrate larger cell volumes in the sampled populations. Analyses based

only on cell density may therefore overestimate the contribution of small but numerically abundant taxa to biomass accumulation. Analyses based only on biomass may overlook rapid turnover in numerical community structure.

4.5. Study Boundaries and Future Validation

The strength of evidence in this study was constrained by sample size and sampling design. The 14 sampling events included repeated spatial sites. Although the independent-site sensitivity analysis did not alter the overall Lake–River comparison, it cannot replace spatial replication across additional river reaches, lake bays, and confluence zones. The sample size also limited the number of explanatory variables that could be included simultaneously in multivariate models. Consequently, some associations with large effect sizes did not remain significant after FDR correction. Site-level sensitivity analyses and site-grouped random forest resampling addressed repeated observations in selected analyses, but did not correct within-site dependence across all tests. The event-level PERMANOVA, db-RDA, Mantel and ANOSIM analyses used unrestricted permutations, while the temporal Kruskal–Wallis tests did not explicitly account for repeated observations. Their *p* values should therefore be interpreted as exploratory. Site-level sensitivity results support only the specific comparisons examined and do not validate the sampling-period effects.

Future studies should establish balanced Lake, River, and confluence-zone replicates across the same months and years. Geographic coordinates, discharge, water depth, transparency, light availability, and mixing conditions should be recorded concurrently. High-frequency hydrochemical observations combined with phytoplankton absolute abundance, cell volume, and functional traits would help distinguish direct filtering by ionic composition from indirect covariation generated by hydrological processes. Gradient experiments or semi-natural incubation experiments targeting TN, SiO₂, Cl[−], and SO₄^{2−} could further test the mechanistic basis of the current statistical associations.

Selective retention by the 64 µm mesh may underrepresent small phytoplankton and alter taxonomic proportions [27]. Using the same protocol across samples does not establish equal retention efficiency across sites or sampling periods. The resulting environmental associations should therefore be interpreted as relationships within the net-collected assemblage. Paired whole-water sampling is needed to evaluate whether these patterns extend to the complete phytoplankton community.

4.6. Ecological Significance and Uniqueness of Siling Co as an Extremely High-Altitude River–Lake Confluence Zone

The value of studying Siling Co and its inflowing river confluence zones lies in the convergence of three environmental features: extremely high elevation, an endorheic lake basin, and river–lake confluence processes. In this study, genus-level communities from Lake and River samples did not form completely separate clusters. Major-ion and salinity-related matrices remained associated with cell-density community dissimilarity after controlling for Type. In addition, pH, SiO₂, and Cl[−] entered different community-response models. Siling Co is therefore not a system that can be summarized by a single water-body label. Instead, it provides a natural window for observing continuous hydrochemical gradients generated by riverine source waters, lake evaporative concentration, and local mixing over relatively small spatial scales. Lake-scale studies from the Tibetan Plateau likewise show that elevation, salinity or conductivity, and nutrient status are often coupled with phytoplankton community structure and assembly processes [49–52].

This uniqueness arises not only from geographic setting, but also from the amplification of river–lake connectivity and hydrochemical conditions by plateau hydroclimatic change. Sampling period explained more variation in both community matrices than water-body type. This pattern describes differences among the observed sampling periods; the

present design cannot attribute these differences to specific hydrological or meteorological processes. However, because month and year were not fully crossed, this signal can only be interpreted as a difference among sampling periods. Studies from high-altitude regions indicate that precipitation inputs, elevation-related changes in water temperature and flow velocity, and shifts in lake water balance, salinity, and primary productivity may restructure plankton communities. These findings support the use of Siling Co as a sensitive observation unit for the ecological responses to hydroclimatic disturbance on the Tibetan Plateau [53–57].

At the regional scale, lakes across the Tibetan Plateau are undergoing coordinated changes in lake area, water storage, thermal regime, and biogeochemical properties. Recent syntheses and empirical studies indicate that climate-driven lake expansion, ice-phenology shifts, warming, and nutrient fluctuations can alter mixing, transparency, salinity, and phytoplankton ecological networks [58–65]. Monitoring at the Siling Co confluence zone can therefore connect hydrochemical continua, paired cell-density and biomass metrics, and the broader hydroclimatic context of the plateau. The most defensible extension of this study is to treat Siling Co as a representative case of environment–community coupling in high-altitude river–lake confluence zones. It should not be used to infer that all plateau lakes share the same ion-driven mechanisms.

This study also showed that environmental responses based on cell density and biomass were not fully consistent. Cyanobacterial relative cell density increased in River samples, whereas mean relative biomass did not increase correspondingly. This pattern indicates that ecological monitoring in extremely high-altitude confluence zones should not rely solely on community abundance or chlorophyll proxies to infer environmental risk. Under joint changes in salinity and nutrients, seasonal community turnover, and organic-matter inputs, different community metrics may not convey the same ecological implications [66–72].

The present inferences are limited to the net-collected assemblages sampled in this study. Future monitoring should combine phytoplankton and hydrochemical measurements at fixed river, confluence, and lake stations across matched sampling periods. Broader spatial replication and paired whole-water sampling are needed to test whether the observed associations extend beyond the sampled assemblages. Targeted observational or experimental studies are also needed to assess their causal basis in Siling Co and other high-altitude river–lake systems.

5. Conclusions

This study provides an initial characterization of phytoplankton communities in the extremely high-altitude Siling Co river–lake continuum. Shaped jointly by extremely high-altitude climatic conditions, evaporative concentration in an endorheic lake, and inflow and mixing of tributary waters, this confluence zone represents a natural setting for examining how hydroclimatic change on the Tibetan Plateau may be transmitted through hydrochemical gradients to primary-producer communities. The results indicate that community variation in this system is more usefully interpreted in relation to continuous hydrochemical gradients and sampling-period differences than to Lake and River classifications alone. The partly distinct patterns obtained from cell density and estimated biomass further support their joint use in ecological monitoring.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/w18192378/s1>.

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