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Article

Temporal Variation of Plankton Community in a Typical Lake in the Middle Reaches of the Yangtze River: Structure, Environmental Response and Interactions

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Abstract: The Liangzi Lake, a typical shallow lake in the middle reaches of the Yangtze River, is important for water resource and biodiversity conservation. With the development of urbanization, anthropogenic activities have posed serious threats to the water quality and biodiversity of Liangzi Lake. To assess the aquatic ecosystem health of Liangzi Lake, the structure, environmental response, and interactions of plankton were investigated in 2022 and 2023. Results indicated that water temperature was a pivotal factor regulating plankton dynamics, while plankton assemblage patterns were predominantly shaped by phytoplankton species, which was Bacillariophyta in spring and Chlorophyta in summer. In terms of phytoplankton, dissolved oxygen, and N:P ratio significantly affects cyanobacteria distribution, and a high biomass and abundance of them in summer highlighted the potential risk of harmful algal blooms. In contrast to phytoplankton, zooplankton exhibited enhanced resilience to changes of the surrounding environment. Rotifera was the dominant group in summer in terms of both abundance and biomass. Most core genera of plankton were jointly identified by eDNA metabarcoding and microscopical analysis, and eDNA metabarcoding has advantages in revealing a higher diversity. However, some taxa among rotifers, such as Liliiferotrocha, were only identified using the microscopical analysis. Therefore, a combination of both methods is recommended to better understand the structuring mechanisms of plankton assemblages in lake ecosystems.

Keywords: Phyto-zooplankton interaction; Plankton assemblage; Biodiversity; Cyanobacteria; Lake ecosystem

1. Introduction

Plankton are essential components in aquatic ecosystems, playing vital roles in energy flow and material cycling [1,2]. The high genetic diversity and adaptation ability of plankton make them widely distributed in various aquatic environment [1,3]. Among plankton, phytoplankton are crucial contributors to primary production, and zooplankton serve as trophic links between primary producers and higher trophic level aquatic organisms [4,5]. The biodiversity of plankton has non-negligible effects on the stability of food webs, trophic network functioning, and water environmental

quality [6]. However, growing evidence reveals that the biodiversity of plankton is easily affected by environmental and climatic changes, and even anthropogenic activities [7,8]. For example, the outbreak of algal blooms in aquatic ecosystems has been widely reported [9–11]. Therefore, assessing the status and drivers of plankton diversity is vital to reveal the potential environmental problems of aquatic ecosystems.

In recent years, the environmental DNA (eDNA) metabarcoding technique has emerged to monitor the biodiversity of plankton [3,11,12]. Compared to the conventional microscopical method, eDNA metabarcoding can effectively identify species with similar morphological characteristics, such as copepods at the larval stage [8,13]. However, limitations and effectiveness of eDNA metabarcoding still exist in many cases. For example, it is inefficient for revealing cyanobacteria and fish taxa [14]. To date, few studies have compared the biodiversity of both phytoplankton and zooplankton using these two methods in actual aquatic habitats [8].

Liangzi Lake, the second-largest freshwater lake in Hubei province, is located in the middle and lower reaches of the Yangtze River. It is a typical grass-type lake with extensive populations of aquatic macrophytes such as *Potamogeton crispus*, *Bythophyton indicum*, *Egeria densa*, and *Elodea nuttallii* [15]. Due to its high water quality and rich biological resources, Liangzi Lake is considered to be a “fossil lake” and “species gene pool” [16]. Over the past few decades, Liangzi Lake has been overburdened with tourism and the main production modes are aquaculture and natural fisheries. Due to the anthropogenic effects, a series of environmental problems have arisen. For instance, the decline in aquatic biodiversity, and the degradation of aquatic macrophytes [15]. What’s worse, the risk of cyanobacteria blooms has increased in recent years [17]. Therefore, risk assessment and management toward plankton is necessary in Liangzi Lake. Therefore, the objectives of this study are: (1) to assess the structure, environmental response, and interactions of plankton in Liangzi Lake; (2) to compare the biodiversity of plankton using microscopical analysis and eDNA metabarcoding.

2. Materials and Methods

2.1. Sampling and Laboratory Analysis

Liangzi Lake (114°32′~114°43′E, 30°01′~30°16′N) is situated south of Ezhou City, adjacent to Wuhan City. With an average water depth of 4 m, and the lake spans approximately 339 km² within a 2085 km² basin characterized by southern highlands and northern lowlands [18]. Using Liangzi island town, located in the center of Liangzi Lake, as a boundary, Liangzi Lake can be divided into East Lake, the Qianjiang Lakes, West Lake, and Niushan Lake. Notably, West Lake primarily supports aquaculture while East Lake maintains natural fisheries. Four sampling campaigns were conducted during March and June of 2022 and 2023 across key locations (Figure 1). Among these sampling sites, L7 is located at the only outlet of Liangzi Lake, and L10 is located at the estuary of Gaoqiao River, the largest inflowing river of Liangzi Lake. Field measurements of water temperature (WT), pH, and dissolved oxygen (DO) were obtained using a multiparameter controller (Milti3430, WTW, Germany), and transparency (SD) was measured by a Secchi disk. Surface water samples (0 ~ 0.5 m depth) was collected with an organic glass hydrophore and temporarily stored in polyethylene plastic bottles with different volumes for further analysis of water nutrients, chlorophyll a (Chl.a), and plankton. 20 L of surface water was filtered through a NO. 13 zooplankton net and concentrated in 100 mL polyethylene bottles for further analysis of Cladocera, Copepoda, nauplii, and larger Rotifera, and then 4 mL of a 40% formaldehyde solution was added.

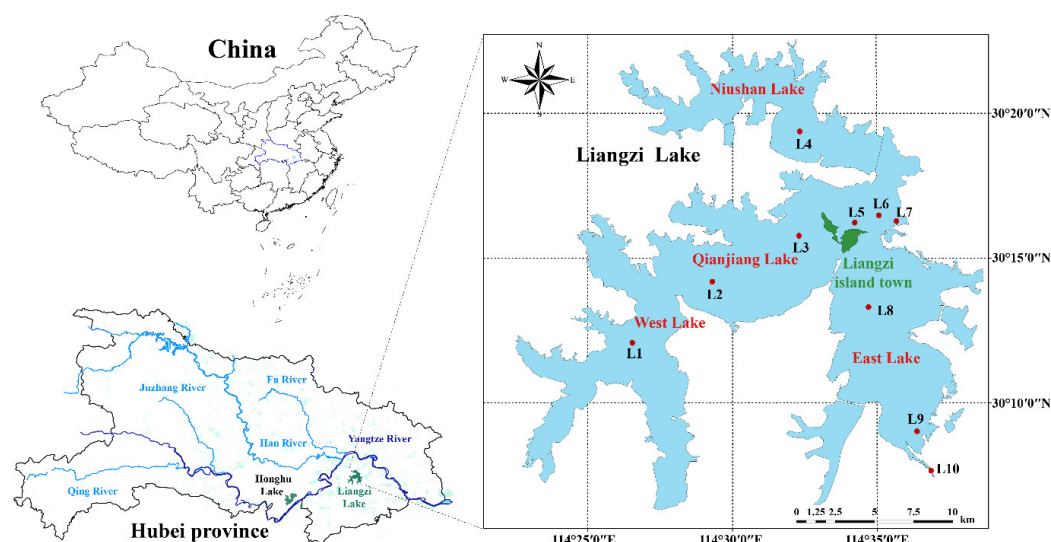


Figure 1. Map of Liangzi Lake with the sampling sites (L1 ~ L10).

After transporting the water samples to the laboratory, the samples for chl.a analyses were filtered through 0.7 μm GF/F filters and stored at -20°C until analysis. 1 L of water sample for phytoplankton, protozoa, and smaller rotifers analyses were fixed with 1.5% (v/v) Lugol's iodine. After sedimentation for 48 h, the supernatant was carefully siphoned off with a 2 mm diameter pipette. The remaining sample was then concentrated to 30 mL. Subsequently, all samples were transported to the Analysis and Testing Center, Institute of Hydrobiology, Chinese Academy of Sciences, for microscopic identification. The concentrated samples were shaken well, and 0.1 mL was transferred to the 0.1 mL counter box for species-level cell counts under a periscope. Protozoa and smaller rotifers were counted from the phytoplankton samples, with counts performed using 0.1 mL and 1 mL whole piece from the concentrated 30 mL sample, respectively [19]. Currently accepted names of phytoplankton species were based on the AlgaeBase [20]. Cladocera and Copepoda were counted from zooplankton samples, with counts performed using a 1 mL counter box. The average values of a triplicate experiment were taken to calculate the density and biomass of the plankton samples. Taxonomic identification of qualitative samples was conducted at species level based on "Atlas of Freshwater Microorganisms" and "Atlas of Freshwater Organisms in China" [19].

The measurements of total nitrogen (TN), total phosphorus (TP), and permanganate index (COD_{Mn}) were conducted based on the Chinese national standard methods of China HJ636-2012, GB11893-1989, and GB11892-89 as described in our previous study [21,22].

In June 2023, surface water sample was also filtered through 0.22 μm filter membranes to collect the eDNA for the analysis of the compositions of plankton in Liangzi Lake. All the filter membranes were temporarily stored at -80°C before further analysis.

2.2. DNA Extraction, and Sequencing Analysis

The total DNA of plankton samples was extracted using the E.Z.N.A. soil DNA kit (OMEGA) based on the manufacturer's protocol. After the quality assessment, the qualified DNA was used for further sequencing analysis. According to the previous studies, the primers A23SrVF2 (CARAAAGACCCTATGMAGCT) and A23SrVR2 (TCAGCCTGTTATCCCTAG) were selected to amplify the 23S rRNA gene region of phytoplankton [23]. The primers V8F (ATAACAGGTCTGTGATGCCCT) and 1510R (CCTTCYGCAGGTTCACCTAC) were used to amplify the 18S rRNA gene region of zooplankton. Subsequently, the PCR products were purified, quantified, pooled, and sequenced on an Illumina MiSeq platform (Illumina, San Diego, USA) at Majorbio Bio-Pharm Technology Co. Ltd., Shanghai, China. All the progress was conducted following our previous study [24]. The raw data were first annotated against the Nucleotide Sequence

Database (NT, v20210917) and then some irrelevant sequences (e.g., bacteria, plant, and fungus sequences) were removed. The filtered sequences were further used to analyze the planktonic community. The sequence raw datasets have been deposited in the NCBI Sequence Read Archive with the Bio-Project accession number PRJNA1114416.

2.3. Calculation Methods

$$\text{Biomass } B_t = \sum_{i=1}^n D_i \times m_i \quad (1)$$

$$\text{Absolute and relative abundances } A = \sum_{i=1}^n D_i, R_i = D_i/A \quad (2)$$

$$\text{Shannon Index } H = - \sum_{i=1}^n \frac{n_i}{N} \ln \frac{n_i}{N} \quad (3)$$

$$\text{Pielou evenness index } J = H/\ln N \quad (4)$$

where B_t is the total biomass of plankton (mg/L), D_i is the density of the taxon (cells/L or inds/L), m_i is the average quality of the taxon (mg/cell), A is the total abundance of plankton (cells/L or inds/L), and R_i is the relative abundance of taxon i , n_i is the number of taxon i , N is the total number of taxa.

Integrated Nutritional Index (TLI)

TLI is used to assess the nutritional state of the lake. The calculation of TLI is based on the principle of "Methods for the assessment of surface water environmental quality of China" (<https://www.mee.gov.cn/gkml/hbb/bgt/201104/W020110401583735386081.pdf>).

$$TLI = \sum_{i=1}^n W_i TLI(i) \quad (5)$$

where $TLI(i)$ is the trophic status index of parameter i , W_i is the relative weight of the nutritional status index of parameter i .

Taking chlorophyll a (chl.a) as the reference parameter, the relevant weights for the normalization of the parameter i are calculated as follows:

$$W_i = r_{ij}^2 / \sum_{i=1}^n r_{ij}^2 \quad (6)$$

where r_{ij} is the correlation coefficient between the parameter i and the reference parameter, n is the number of evaluation parameters. r_{ij} of some parameters is described as follows: r_{ij}^2 (chl.a) = 1, r_{ij}^2 (TP) = 0.7056, r_{ij}^2 (TN) = 0.6724, r_{ij}^2 (SD) = 0.6889, r_{ij}^2 (COD_{Mn}) = 0.6889.

The formula for calculating the nutritional status index of each item is:

$$TLI(\text{Chl.a}) = 10(2.5 + 1.086 \ln \text{Chl.a}) \quad (7)$$

$$TLI(\text{TP}) = 10(9.436 + 1.624 \ln \text{TP}) \quad (8)$$

$$TLI(\text{TN}) = 10(5.453 + 1.694 \ln \text{TN}) \quad (9)$$

$$TLI(\text{SD}) = 10(5.118 - 1.94 \ln \text{SD}) \quad (10)$$

$$TLI(\text{COD}_{\text{Mn}}) = 10(0.109 + 2.66 \ln \text{COD}_{\text{Mn}}) \quad (11)$$

If $TLI < 30$, the lake is in the state of oligotrophy; $30 \leq TLI \leq 50$, mesotrophy; $50 < TLI \leq 60$, light eutrophication; $60 < TLI \leq 70$, moderate eutrophication; $70 < TLI$, severe eutrophication.

2.4. Statistical Analysis

The sequencing data was analyzed and visualized using the online Majorbio Cloud Platform [25]. The data about water quality, and diversity indices were analyzed using one-way ANOVA analysis, followed by Duncan's multiple comparison tests. Data were presented as mean $\pm$ standard deviations (SD), and the letters mean the significant difference in the index between different groups. Redundancy analysis (RDA) and correlation analysis were used to analyze the relationship between environmental factors and planktonic groups using the R package "Vegan" and "Corrplot", respectively. Figures were visible by GraphPad Prism 8, Origin2022, and R 4.03 software [26]. The sampling map in this study was generated by ArcGIS 10.3. Partial least squares path modeling (PLS-PM) was performed in SmartPLS version 4 software using the PLS-SEM algorithm. In addition, the taxa (relative abundance $\geq 1\%$) were considered as core taxa, and only the core taxa were performed with correlation analysis and visualized in network analysis by Gephi (Spearman's $|r| > 0.6$ and BH-adjusted $P < 0.05$).

3. Results

3.1. Environmental Characteristics

Nutrients such as TP showed no noticeable variations across the four sampling campaigns (Figure 2), while TN exhibited significant seasonal differences. Water temperatures ranged from 14.4 to 24.9 °C in spring, and from 26.8 to 31.7 °C in summer. The concentration of Chl.a was significantly higher in summer compared to spring (ANOVA, $P = 0.002$). The TLI of the water sample is approximately 50, a criterion for light eutrophication, with no significant seasonal difference (ANOVA, $P = 0.345$). During the four sampling campaigns, COD_{Mn}, TN, and N:P ratio was significantly higher in summer 2023, while DO was significantly lower at the same time.

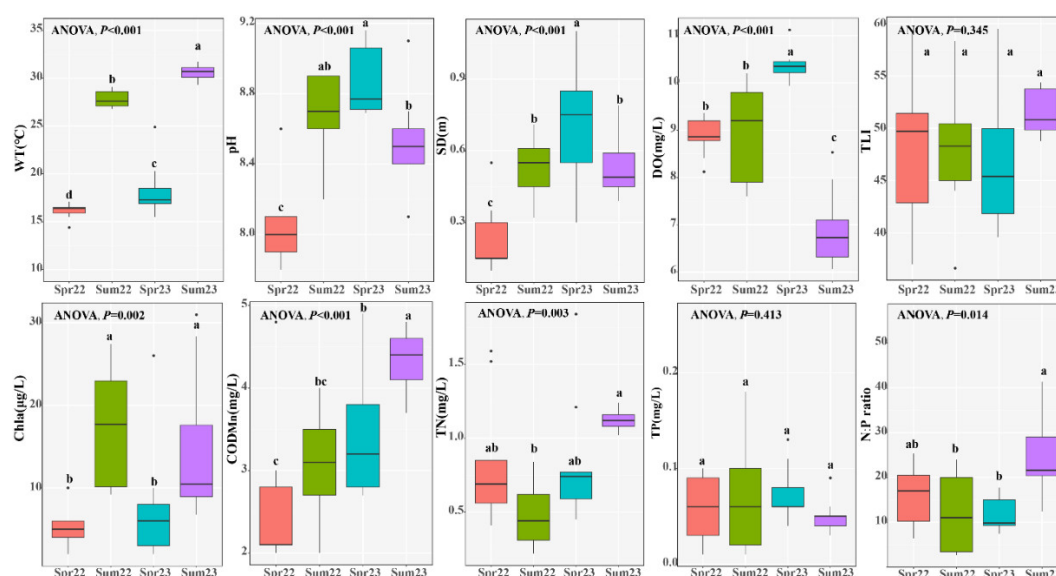


Figure 2. The physical and chemical characteristics of water in Liangzi Lake. The different lowercase letters (a, b, and c) indicate statistically significant differences. Data were presented as Mean $\pm$ SD.

3.2. Biomass of Planktonic Communities

In spring 2022 and 2023, Bacillariophyta dominated the phytoplankton biomass, followed by Chlorophyta (Figure 3). The highest phytoplankton biomass was found in the sampling sites (L6 ~ L9) located in East Lake. In the summer of 2022 and 2023, cyanobacteria became the predominant group, accounting for 49.8% and 61.2% of the total biomass, respectively. Compared to spring, the phytoplankton biomass was significantly higher in summer. For zooplankton (Figure 3b), no obvious variations were observed between spring and summer. Cladocera and Copepoda, belonging to Crustacea, were the dominant groups and accounted for 23.2 ~ 96.6% of the total biomass. In spring 2023, Rotifera contributed the highest proportion of biomass.

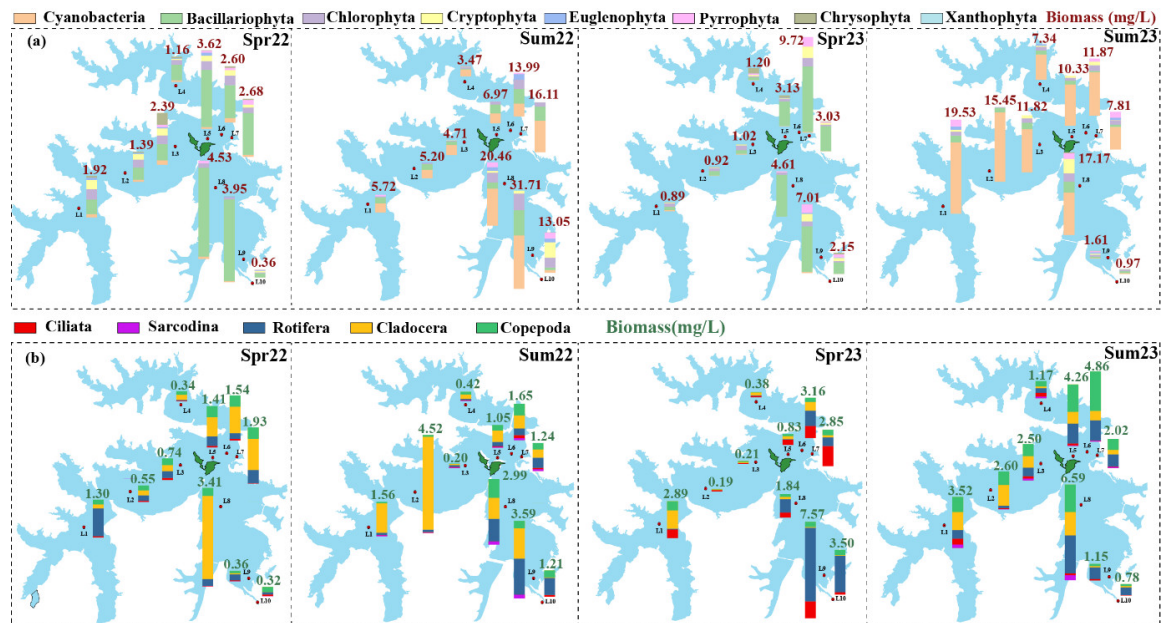


Figure 3. The biomass of phytoplankton (a) and zooplankton (b) at ten sites in March (spring) and June (summer) 2022 and 2023. Spr22: spring 2022; Sum22: summer 2022; Spr23: spring 2023; Sum23: summer 2023.

3.3. Abundance of Planktonic Communities

In the four sampling campaigns, Bacillariophyta and cyanobacteria were the predominant groups in spring (Figure S1), accounting for 32.0 ~ 92.2% of the total abundance, and the relative abundance of these two groups was higher at sampling sites located in East Lake and around Liangzi island town (L5 ~ L9, with an average of 78.83%). The absolute abundance of phytoplankton in summer exceeded 108 cells/L, 1 to 2 orders of magnitude higher than that in spring (Figure S3a). Cyanobacteria was the only predominant group, accounting for 88.8% and 88.2% of the total abundance in summer 2022 and 2023, respectively (Figure S3b). In contrast to the total biomass, Ciliata and Rotifera were always the predominant groups in Liangzi Lake (Figure S2, S3c-d), and in the spring 2023 accounted for 98.5% of the total abundance.

3.4. Environmental Drivers of Planktonic Communities

For phytoplankton at the species level, the diversity (Figure 4a) and evenness (Figure S4) were significantly higher in spring. In terms of zooplankton, the diversity, and evenness were significantly lower in summer 2022 and spring 2023 (Figure 4b and S5). At the genus level, *Aulacoseira* was the dominant genus in the spring 2022 and 2023, but the rest dominant genera in these two sampling times varied greatly (Figure 4c). In summer, *Raphidiopsis*, *Leptolyngbya*, and *Pseudanabena* were the top three genera and accounted for more than 62.0% of the phytoplankton. The dominant genera of zooplankton in spring 2022 were *Tintinnopsis*, *Strobilidium*, and *Conochilus*, and in spring 2023 were *Epistylis* and *Strobilidium* (Figure 4d). In summer, the dominant genera were *Tintinnopsis*, *Liliferotrocha*, *Diffugia*, and *Trichocerca*. RDA analysis further confirmed that the core genera of plankton in summer showed high similarity (Figure 4e and f), while in spring, an obvious distinction was revealed. Among these measured environmental factors, WT and DO significantly affect the distribution of core genera of plankton in Liangzi Lake.

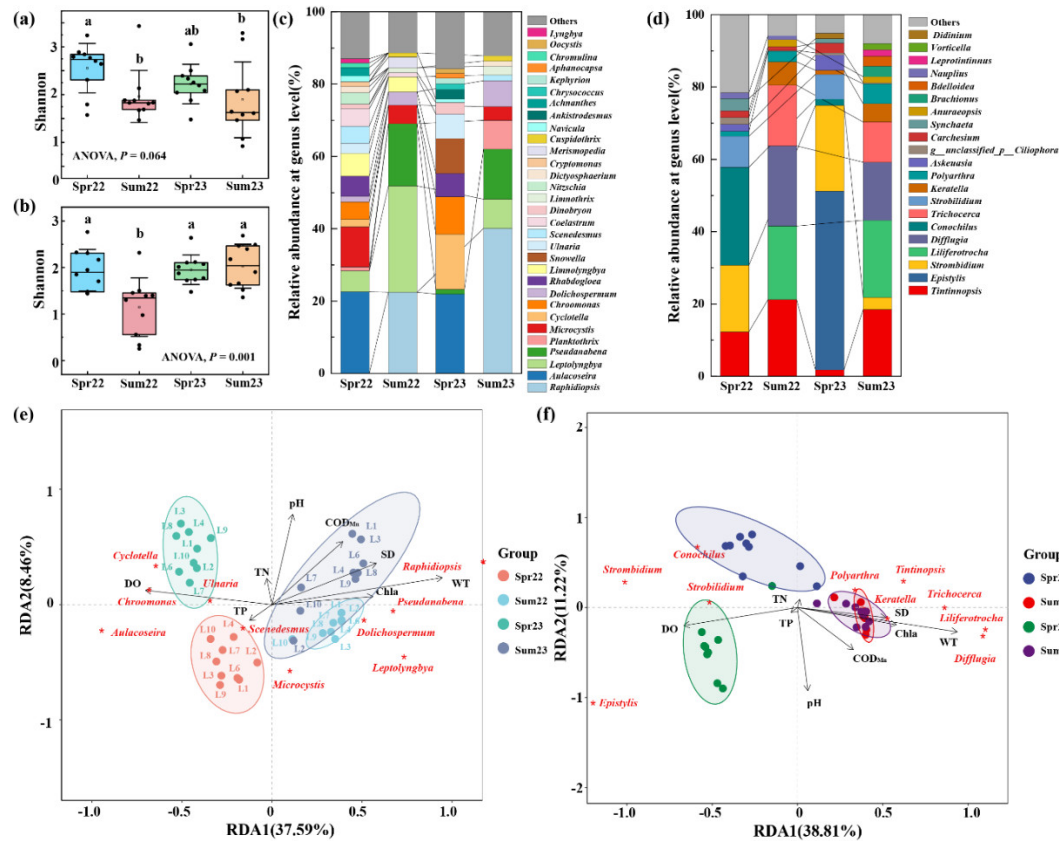


Figure 4. The Shannon index of phytoplankton (a) and zooplankton (b) communities, The different lowercase letters (a and b) indicate statistically significant differences. Data were presented as Mean $\pm$ SD; the relative abundance of core genera of phytoplankton (c) and zooplankton (d) communities in different groups. Those whose relative abundance is greater than 1% in at least one group are identified as core genera, and the rest are classified as others; Redundancy analysis (RDA) of core genera of phytoplankton (e) and zooplankton (f) communities. The lowercase letters represent the significance of the difference between different groups.

Correlation analysis revealed that WT significantly positively correlated with the abundance of cyanobacteria ($P < 0.001$), phytoplankton ($P < 0.001$), Copepoda ($P < 0.001$), Rotifera ($P < 0.001$) and zooplankton ($P < 0.01$) (Figure 5a). In addition, TP significantly negatively correlated with the abundance of phytoplankton ($P < 0.05$) and cyanobacteria ($P < 0.01$). Whereas, a high N:P ratio is beneficial for the growth of phytoplankton and cyanobacteria in that lake ecosystem ($P < 0.05$). As for these planktonic groups, phytoplanktonic groups have significant positive effects on the growth of Rotifera (e.g., cyanobacteria and Rotifera, $P < 0.001$). PLS path modeling further demonstrated that WT, DO, and COD_{Mn} exhibited positive effects on the abundances of phytoplankton, cyanobacteria, zooplankton, and Rotifera (Figure 5b). The effects of environmental factors on them based on these standardized total effects followed the order: Rotifera ($\lambda = 0.719$) > cyanobacteria ($\lambda = 0.697$) $\approx$ phytoplankton ($\lambda = 0.682$) > chl.a ($\lambda = 0.607$) > zooplankton ($\lambda = 0.486$).

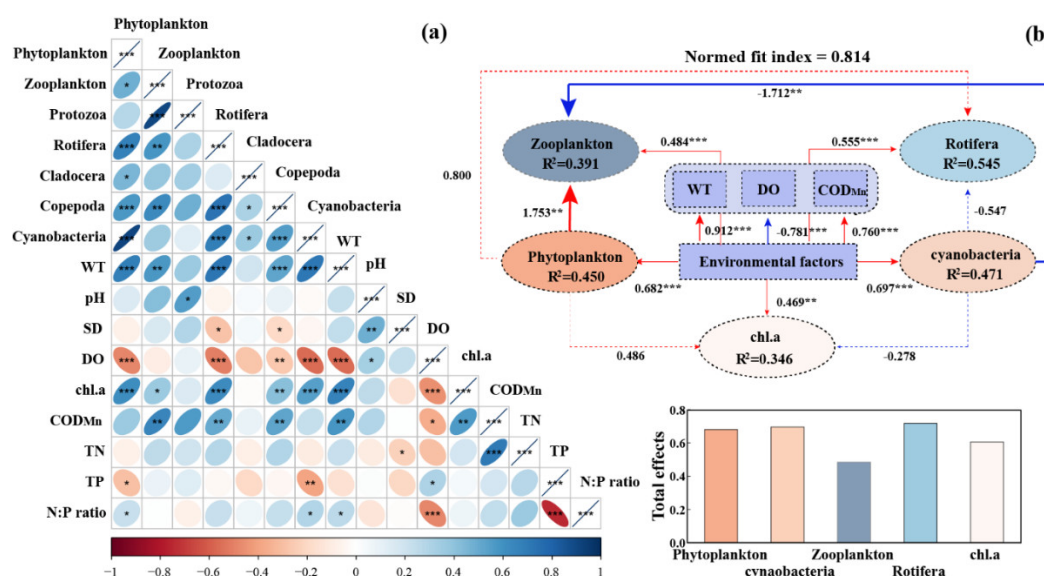


Figure 5. (a) The relationship between planktonic groups and environmental factors. The data of cyanobacteria, phytoplankton (including cyanobacteria), protozoa, Cladocera, Copepoda, Rotifera, and zooplankton (including Rotifera) presented in this figure were transformed with a log 10 scale (Spearman); (b) The final path of partial least squares (PLS) path modeling visualized the direct and indirect effects of environmental factors (WT, DO, and CODMn) on the abundance of phytoplankton, cyanobacteria, zooplankton, and Rotifera. The histogram shows the total effects of environmental factors on phytoplankton, cyanobacteria, zooplankton, Rotifera, and chl.a. The red and blue lines represent positive and negative correlations, respectively. * means " $P < 0.05$ ", ** means " $P < 0.01$ ", and *** means " $P < 0.001$ ".

3.5. Planktonic Co-Existence Patterns

As shown in Figure 5a, the abundance of zooplankton, particularly Rotifera and Copepoda, exhibited significant positive correlations with phytoplankton abundance. To better understand the temporal dynamics of plankton in Liangzi Lake, network analysis was performed with core taxa at module level, due to taxa within the same module are more likely to co-existence [5]. Phytoplankton and zooplankton networks exhibited distinct patterns during different sampling campaigns (Figure 6a). In detail, phytoplankton networks were divided into four dominant modules, and the proportion of dominant modules was higher in summer (98.8% and 95.3%), and the relationships among taxa were tighter in that season, while the proportion of dominant modules in summer 2022 was relatively lower. Further analysis of planktonic co-existence patterns revealed that complex interactions occurred between phytoplankton and zooplankton, and the distribution patterns of dominant modules are similar to those that occurred in phytoplankton. In spring, taxa belonged to Bacillariophyta (phytoplankton), Protozoa, and Rotifera (zooplankton) predominated the dominant modules with highest number of nodes (Figure 6b), while in summer, the predominated taxa in the dominant modules were Chlorophyta and Rotifera.

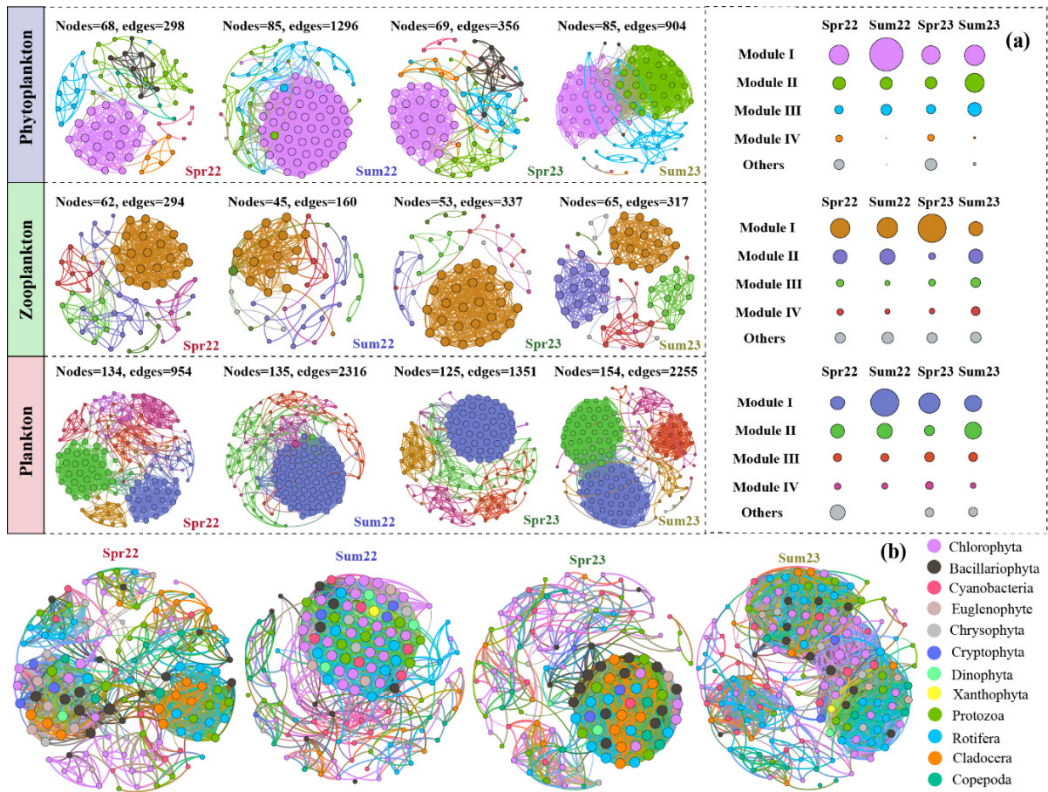


Figure 6. Planktonic species networks constructed based on Spearman's rank correlations between the core taxa in each season. Nodes are colored by modularity (a) and Phylum level (b), respectively. The size of each node is proportional to the degree. The circles in the right panels indicate the relative abundance of each module in corresponding networks, and the size of each circle is proportional to the relative abundance.

3.6. Comparison of eDNA Metabarcoding and Microscopical Analysis

In this study, 8 phyla, 89 genera, and 132 species of phytoplankton were identified by microscopical analysis, and 6 phyla, 95 genera, and 152 species were identified by eDNA metabarcoding (Figure 7a–c). Phyla cyanobacteria, Bacillariophyta, Chlorophyta, Euglenophyta, and Cryptophyta were jointly identified by these two methods. As for zooplankton, more taxa at genera, and species levels were identified using eDNA metabarcoding. However, the number of identified core genera was higher using microscopical analysis (Figure 7d and e). In terms of phytoplankton, most of the jointly identified genera belonged to cyanobacteria, and *Raphidiopsis* was the predominant genus (40.1% and 46.5%). For zooplankton, the unique core genera identified using eDNA metabarcoding mainly belonged to Ciliata, whereas the unique core genera identified by the microscopical analysis mainly belonged to Rotifera.

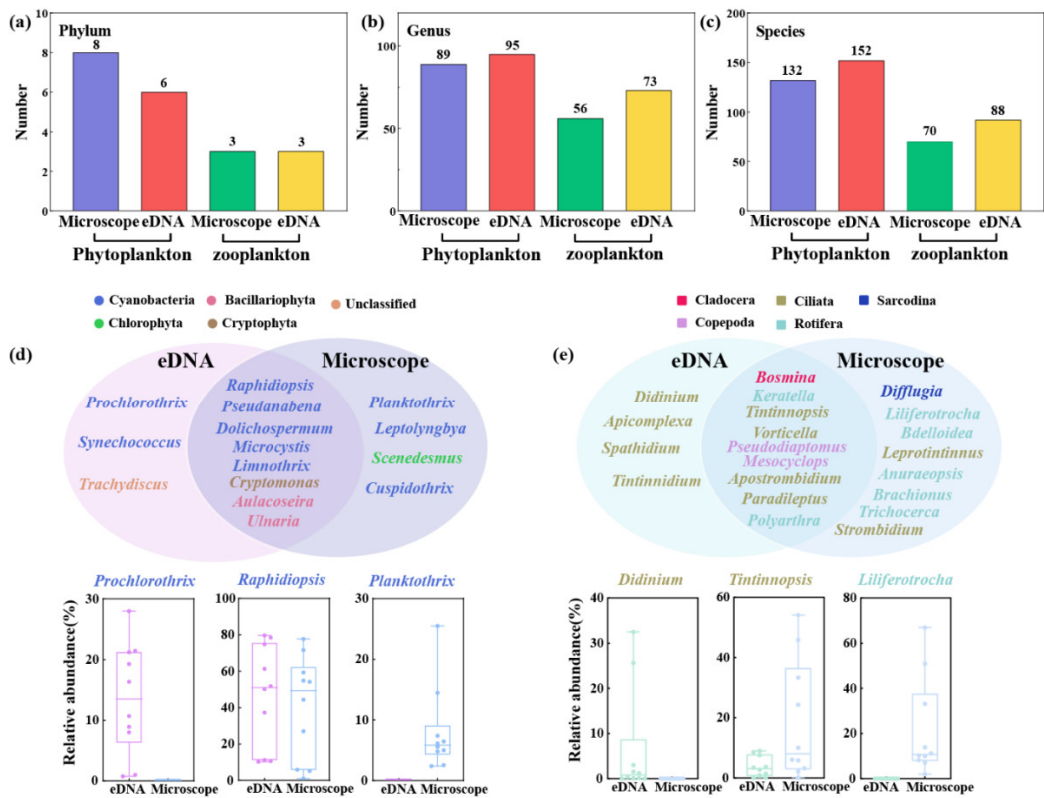


Figure 7. The number of taxa at phylum (a), genus (b), and species (c) levels using microscopical analysis and eDNA metabarcoding; Venn diagrams showing the list of shared and unique species in phytoplankton (d) and zooplankton (e) communities using two methods.

4. Discussion

The biomass of phytoplankton in Liangzi Lake was significantly higher in summer than in spring, while zooplankton biomass exhibited no significant difference between both seasons (Figure S3), highlighting the different lifecycle of phytoplankton and zooplankton. The reason for this difference is that Liangzi Lake is a typical light eutrophic lake (TLI $\approx$ 50) (Figure 2). According to the modified plankton ecology group (PEG) model described by Sommer, Adrian [27], the biomass of phytoplankton in eutrophic waters would reach a peak in summer due to the sufficient nutrients. In contrast, the proliferous of inedible phytoplankton [28], and the predation of filtering-feeding fish such as *Culter alburnus* [29] may further limit the growth of zooplankton.

4.1. Temporal Dynamics and Environmental Response of Planktonic Communities

As a result of adaptation to environmental variations, the composition of phytoplankton in Liangzi Lake changed dramatically from spring to summer. Seasonal dynamics of plankton have been also observed in other freshwater systems [19,30]. In terms of phytoplankton, the abundances of them were significantly higher in summer, this is because the WT of Liangzi Lake in summer ranged from 26.8 to 31.7 $^{\circ}$ C, which fell within the optimal temperature ranges for the growth of most organisms [31]. In Liangzi Lake, Bacillariophyta and cyanobacteria were dominated in spring and summer, respectively (Figure S3). Compared with other species, Bacillariophyta is competent to suffer harsh environmental conditions and thus dominant in low-temperature waters [32]. When it comes to summer, the rising temperature gives cyanobacteria competitive advantages over other species [33], leading to the bloom of cyanobacteria (Figure 3) [34,35]. Similar temporal dynamics of phytoplankton was also reported in Yongli Lake [36]. Particularly, the biomass and proportion (approximately 88% of the total abundance) of cyanobacteria in Liangzi Lake are significantly higher

than in other eutrophic lakes such as Erhai Lake [37] and Hongze Lake [38], indicating the potential risk of cyanobacteria bloom in Liangzi Lake. In addition to WT, P limitation, and nutrient stoichiometry (N:P) are other important determinants that regulate the lifecycle of phytoplankton, especially cyanobacteria [39]. Our result showed that P limitation and high N:P ratios enhanced the community competition of cyanobacteria (Figure 5a), a similar phenomenon was also observed in a gravel-bed urban river [40]. Due to the low concentration of nutrients in the summer 2022 (Figure 2), the proportions of nitrogen-fixing cyanobacteria such as *Leptolyngbya* and *Pseudanabena* [41] were at high levels. In addition, phytoplankton with unicellular or filamentous shapes are efficient in nutrient use, which also contributed to the dominance of these species in nutrient-limited conditions [42].

As for zooplankton, though the Cladocera and Copepoda account for a high proportion of the total biomass in spring and summer (Figure 3), the dominant group in spring was Ciliata (Protozoa) in terms of abundance (Figure S3). This is because the body sizes of most zooplankton belonging to Ciliata are smaller than Cladocera and Copepoda. In addition, the result also indicates that the zooplankton in the eutrophic lake was usually dominated by small- and medium-sized species. Similar phenomena were also revealed in previous studies that small zooplankton taxa are dominated in aquatic environments [43,44]. Similarly to phytoplankton, WT was the most important determinant that drives the succession of zooplankton (Figure 4f and 5). With the increase of WT in summer, Rotifera becomes the dominant group due to their high reproductive rate and environmental adaptability at high-temperature conditions [45–47]. The results of RDA and correlation analyses also showed that chl.a, and DO have significant effects on the distribution of zooplankton. As mentioned in a previous study, low dissolved oxygen in summer synergically enhanced the advantages of rotifers [48]. In addition, rotifers are less affected by planktivorous fishes, which may also further increase their abundance in Liangzi Lake [49].

4.2. Interactions of Planktonic Communities

Results of network analysis implied that the stability and complexity of phytoplankton were higher in summer (Figure 6a), indicating that the increased WT tightened their biological interactions [5,50]. The high proportion of dominant modules in summer further suggested a higher niche differentiation degree, which indicated that co-occurring phytoplankton could use different strategies to utilize the available resources from the surrounding environment to avoid competition and maintain their advantages in eutrophic lakes [3,51]. Therefore, it is no wonder that phytoplankton in summer was only dominated by a few species such as *Raphidiopsis*, leading to low diversity and evenness of phytoplankton (Figure 4c and S4). The high abundance of cyanobacteria and low phytoplanktonic diversity were also previously found in eutrophic shallow lakes [33,52]. Although high WT led to the increased abundance of zooplankton in summer, the biological interaction among zooplankton was inactive (Figure 6a). As revealed in previous studies, phytoplankton is more sensitive to the rise of WT and results in rapid growth rates, while zooplankton can't track the changes in primary production under increased WT [53].

As for the relationship between phytoplankton and zooplankton, the core taxa within the dominant modules in spring belonged to various groups, such as Bacillariophyta, Euglenophyta, Protozoa, Rotifera, and Cladocera (Figure 6b), while in summer, the core taxa within the dominant modules mainly belonged to Chlorophyta and Rotifera. It seems that the main relationship maintained among phytoplankton and zooplankton was changed from competition to predation. As described in a previous study, niche differentiation played a more important role in the community assembly of plankton especially in spring [19]. Therefore, there is no doubt that the temperature bottom-up shapes the phytoplankton to construct the phyto-zooplankton interaction [50]. In addition, the tightened interaction among phytoplankton and zooplankton in summer indicated a higher resistance of planktonic communities to environmental disturbances.

4.3. Difference Between Morphological Analysis and eDNA Metabarcoding

In terms of the plankton that exist in aquatic ecosystems, some species, especially small-sized ones, are difficult to distinguish through morphological characteristics [54,55], and the ignored identification via microscopical analysis would result in a low diversity of plankton [14]. In this study, more species were identified using eDNA metabarcoding compared to microscopical analysis (Figure 7a-c), implying that eDNA metabarcoding has advantages in revealing higher diversity of plankton [3,8,12]. The relative abundance of most unique species identified by eDNA metabarcoding is lower than 1%, which highlighted its high detection sensitivity [56]. Therefore, eDNA metabarcoding is competent to be acted as a useful tool to assess plankton biodiversity.

However, some limitations still existed for this method. Indeed, the biggest barrier to the application of eDNA metabarcoding is that the annotated databases don't contain reference sequences for all of the plankton, resulting in the questionable identification of some species [57]. Substantial intraspecific variability of plankton also affected the accurate annotation of some species. In this study, 54 and 78 OTUs of phytoplankton and zooplankton failed to be annotated and classified as "unclassified". This may explain why some species identified by microscopical analysis are missed via eDNA metabarcoding (Figure 7d and e). For example, *Liliferotrocha subtilis* was detected in the water samples with high detected frequency (100%) and abundance (21.4%) via microscopical analysis, eDNA metabarcoding failed to identify this species due to the lacked corresponding barcode sequence in the NCBI GenBank [46]. A previous study conducted by Liu et al., (2022) revealed that *Asteroplanus karianus* and *Coscinodiscus asteromphalus*, both belong to Bacillariophyta, were not detected in Jiaozhou Bay due to the deficiency of reference sequences [58]. In addition, some decaying biological matters could be also identified by eDNA metabarcoding, which may further affect the understanding of the "true" plankton community [59]. Song and Liang (2023) previously used 18S and COI primer pairs to identify the composition of zooplankton and demonstrated that the choice of primer pairs has significant effects on the identification of zooplankton [8].

In the process of microscopical analysis, the larvae, small-sized species, other uncountable species, and similar morphological species cannot be accurately identified or assigned to appropriate taxonomic levels [58]. In addition, the body sizes of zooplankton varied greatly among different groups, commonly from a few micrometers to a few millimeters. According to previous studies [8,46], the calculation of species abundance in eDNA metabarcoding was based on DNA contents, but in microscopical analysis, the calculation of species abundance was based on cell numbers. Therefore, the increased differences in body sizes would decrease the community similarity between the two methods due to algorithmic differences. As a result, the composition of zooplankton was identified by the two methods with lower similarity (Figure 4d and 7e). In our study, small-sized copepods such as *Pseudodiaptomus* (accounting for 34.8% of the total abundance in eDNA metabarcoding) were identified with low abundances (< 1%) by microscopical analysis. Despite it has some non-negligible disadvantages, microscopical analysis was more suitable for evaluating the response of plankton to environmental stress. As described in a previous study, compared with eDNA metabarcoding, morphological analysis can identify more significant variations across treatments in terms of the number of species and relative abundance [46]. Therefore, though the low matching degree still exists between the two methods, and it is difficult to unify the results of the two methods, the combination of the two methods is suggested to better understand the structuring mechanisms of plankton assemblages.

5. Conclusions

Our study analyzed the structure, environmental response, and interactions of plankton in an typical lake in the middle reaches of the Yangtze River and assessed the difference between eDNA metabarcoding and microscopical analysis in identifying plankton. Plankton composition exhibited strong temporal variations, with water temperature emerging as a critical driver. Phytoplankton especially cyanobacteria in summer was with high biomass and abundance, resulting in a high

potential risk of cyanobacteria blooms. Zooplankton is insensitive to changes in surrounding environments when compared with phytoplankton. Rotifera was the dominant group in terms of both abundance and biomass in summer. Network analysis further revealed that the temperature bottom-up shapes the phytoplankton to construct the phyto-zooplankton interaction. Though eDNA metabarcoding has advantages in identifying the composition of plankton compared to microscopical analysis, supplementary taxa detected by microscopical analysis highlight the benefits of combining both approaches for a comprehensive understanding of plankton occurrence and distribution in water ecosystems.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Figure S1: The absolute (a) and relative (b) abundances of phytoplankton at different sampling points (L1 ~ L10) in different seasons (Spr22, Sum22, Spr23, and Sum23); Figure S2: The absolute (a) and relative (b) abundances of zooplankton at different sampling points (L1 ~ L10) in different seasons (Spr22, Sum22, Spr23, and Sum23); Figure S3: The absolute (a) and relative (b) abundances of phytoplankton in different seasons at the phylum level; the absolute (c) and relative (d) abundances of zooplankton in different seasons; Figure S4: The Pielou index of the phytoplankton community, and the Pielou index of the Zooplankton community. All indices were calculated at the species level. The data were presented as mean $\pm$ standard deviations (SD).

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