

Article

Research on the Relationship between Eukaryotic Phytoplankton Community Structure and Key Physiochemical Properties of Water in the Western Half of the Chaohu Lake Using High-Throughput Sequencing

Bingbing Zhao ^{1,2,*}, Wei Peng ², Xinhao Zhu ², Hua Zhang ^{1,2}, Xingmei Zhuang ², Jinhua Wang ^{1,2}, Shanshan Xi ^{1,2} and Tao Luo ²

¹ Anhui Institute of Ecological Civilization, Anhui Jianzhu University, Hefei 230601, China

² School of Environment and Energy Engineering, Anhui Jianzhu University, Hefei 230601, China

* Correspondence: bing_zhao12@163.com

Abstract: Eukaryotic phytoplankton play a major role in the circulation of material and energy in a lake's ecosystem. The acquisition of information on the eukaryotic phytoplankton community is extremely significant for handling and regulating the ecosystems of lakes. In this study, samples were collected from the western half of Chaohu Lake in the summer and winter periods. Analyses revealed that the eukaryotic phytoplankton in this region comprised 70 genera, 34 orders, and 7 phyla. There were 61 genera, 29 orders, and 7 phyla in summer, and 25 genera, 14 orders, and 5 phyla in winter. The dominant genus was *Chlamydomonas* of Chlorophyta in summer. In contrast, the dominant genus was *Mychonastes* of Chlorophyta in winter. The diversity index analysis revealed that the eukaryotic phytoplankton community exhibited greater fluctuation in the summer than in the winter. Moreover, analysis of the physiochemical properties of the water samples showed considerable spatial and temporal differences in the water quality. This paper focusses primarily on analysing the influence of the physiochemical properties of water on the eukaryotic phytoplankton community. In particular, the effects of the major physicochemical properties of water on the community evolution of eukaryotic phytoplankton classes were evaluated using the redundancy analysis method. The findings demonstrated that total phosphorus (TP), $\text{PO}_4^{3-}\text{-P}$, $\text{NH}_4^+\text{-N}$, and total nitrogen (TN) were the primary influencing factors in summer, whereas $\text{NO}_3^-\text{-N}$, DO, and water temperature (WT) were the major influencing factors in winter. Subsequently, the Mantel test revealed that the phylum level of the eukaryotic phytoplankton community was significantly correlated with WT, DO, $\text{NH}_4^+\text{-N}$, TN, TP, and Chlorophyll a. Variance partitioning analysis indicated that seasonal factors accounted for a large proportion of the variation in the eukaryotic phytoplankton community, reaching 48.4%. Subsequently, co-occurrence network analysis demonstrated that most families of eukaryotic phytoplankton were facilitated mutually, with the proportion of promotion being 94.1%. This study provides insight into the crucial factors that influence the phytoplankton communities and a reasonable control direction for the positive evolution of the eukaryotic phytoplankton community in the western half of Chaohu Lake.

Keywords: high-throughput sequencing; lake ecosystem; eukaryotic phytoplankton; physicochemical factors; community structural information



Citation: Zhao, B.; Peng, W.; Zhu, X.; Zhang, H.; Zhuang, X.; Wang, J.; Xi, S.; Luo, T. Research on the Relationship between Eukaryotic Phytoplankton Community Structure and Key Physiochemical Properties of Water in the Western Half of the Chaohu Lake Using High-Throughput Sequencing. *Water* **2024**, *16*, 2318. <https://doi.org/10.3390/w16162318>

Academic Editor: Hodon Ryu

Received: 24 July 2024

Revised: 11 August 2024

Accepted: 16 August 2024

Published: 18 August 2024



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1. Introduction

The ecosystems of lakes play crucial roles in maintaining the stability of regional and global ecosystems. They also serve as regulators of climate change and play a vital role in water supply, agricultural irrigation, ecological habitats, landscaping, and flood control [1]. However, these ecosystems are facing accelerated lake eutrophication due to industrial and agricultural development accompanied by rapid urbanisation. This has ultimately

increased the frequency and severity of algal blooms to some extent [2]. Algal blooms may severely and negatively affect biodiversity in water ecosystems, impacting surface water safety. Eutrophication and algal blooms have emerged as global concerns [3]. Chaohu Lake is located in the lower reaches of the Yangtze River. It is the fifth-largest freshwater lake in China and is considered to be a precious, valuable lake [4]. However, Chaohu Lake is also one of the three top eutrophication lakes in China; it has garnered widespread attention worldwide, along with Tai Lake and Dian Lake [5]. Because the western half of Chaohu Lake is close to Hefei City and receives abundant industrial and municipal wastewater, eutrophication of the lake gradually intensifies from east to west [6].

As a leading producer in water ecosystems, phytoplankton accounts for 50% of global net primary productivity. They play vital roles in material circulation and energy flow in aquatic ecosystems [7–10]. Moreover, phytoplankton are more sensitive to environmental changes in water than other organisms. Hence, the abundance of phytoplankton can be used as an essential biological index to reflect water conditions [11,12]. Phytoplankton might be affected by multiple factors in water ecosystems. For example, N, P, and other nutrients in water may have crucial effects on phytoplankton [13,14]. Water temperature (WT) can influence the mutual competition among phytoplankton and indirectly affect the diversity of phytoplankton [15]. Aside from water quality factors, extreme weather and human activities may also impact phytoplankton communities to a certain extent [16]. Moreover, biology [17], climate hydrology [18], geography, environment [19,20], and other factors may affect the dynamic variations in phytoplankton communities.

The regulation of phytoplankton is increasingly complicated because of the mutual impacts of various influencing factors. Hence, studying the relationships between multiple environmental factors and the phytoplankton community is crucial for the governance of lake ecosystems [21]. To address the phytoplankton issue in Chaohu Lake, many scholars have thoroughly examined the Cyanophyta community; however, there is insufficient research on other eukaryotic phytoplankton [22,23]. Moreover, early studies mainly examined the phytoplankton community using a microscope; as a result, many microplankton species, which are difficult to distinguish with respect to morphology, may have been neglected. Thus, this study monitored the eukaryotic phytoplankton community of Chaohu Lake via high-throughput sequencing [24]. Thus, this study addressed the limitations of previous studies with the ability to investigate micro-eukaryotic phytoplankton in Chaohu Lake. This research focussed on the relationships between eukaryotic phytoplankton and the physicochemical properties of the lake water and offsets shortages of previous studies on eukaryotic phytoplankton. Due to the unique geological position of the west half of Chaohu Lake, the division of the study area along the banks is next to the wetland, and the peripheral regions cover urban and rural areas as well as 7 rivers in the jurisdiction. There are many types of nutrient substances in water, mainly including $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, total nitrogen (TN), $\text{PO}_4^{3-}\text{-P}$, total phosphorus (TP), and so on. A survey on the west half of Chaohu Lake was carried out in the present study. The specific research objectives were as follows: (1) To analyse the effects of multiple environmental factors on the dynamic changes in the eukaryotic phytoplankton community. This analysis can facilitate the development of a mutual constraint relationship model between the physiochemical properties of the water and the eukaryotic phytoplankton. The findings are expected to inform control schemes for different phyla and genera of eukaryotic phytoplankton in Chaohu Lake. (2) To determine the key eukaryotic phytoplankton in the community via co-occurrence network analysis and examine their inter-relationships. It was expected that these findings would inform the development of appropriate genetic engineering projects and provide references for the application and promotion of 18S rDNA to aquatic ecological environmental monitoring.

2. Materials and Methods

2.1. Study Area

Chaohu Lake is located in Anhui Province, Eastern China (E:117°16'~117°51', N:31°25'~31°43'). It is a subtropical shallow lake with a water area of 780 km² and an

average depth of 3 m. The local climate is affected by the East Asian monsoon, and there are four distinctive seasons [25]. A total of 10 sampling points were established in the study area to explore the water quality and eukaryotic phytoplankton community features in the western half of Chaohu Lake. These points were divided into Group A (sampling points 1–5) and Group B (sampling points 6–10) according to the intensity of human activities. Sampling points in Group A were close to urban areas and were significantly affected by human activities. Specifically, points 1–5 were located in Zhongmiao, Nanfei River, Shiwuli River, Tangxi River, and Pai River. The sample points in Group B were far away from urban areas and were less impacted by human activities. Specifically, points 6–10 were located in Gushan, Hangbu River, Baishitian River, Zhao River, and Mushan Island. Figure 1 depicts the distribution of the sampling points.

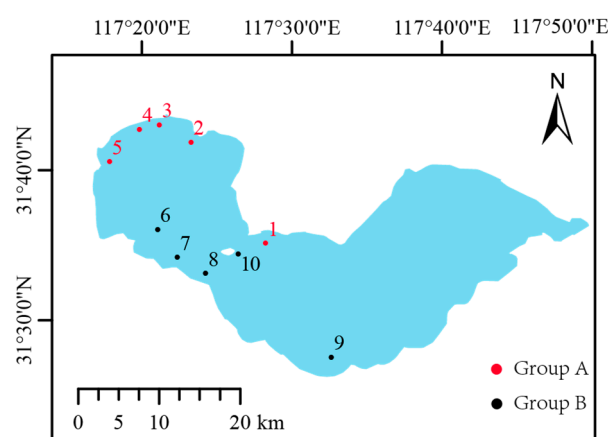


Figure 1. Distribution of the sampling points in the western half of Chaohu Lake. Notes: Group A includes sampling points 1~5; Group B includes sampling points 6~10.

2.2. Sample Collection

The samples were collected in August 2022 (summer) and February 2023 (winter). A total of 10 sampling points were set in the study area in the winter and summer, respectively. Water samples and phytoplankton samples were collected. The locations of the sampling points were kept consistent (Figure 1). Water samples of 2 L were collected with glass water samplers 0.5 m below the water surface to analyse the physiochemical properties of the water. Moreover, eukaryotic phytoplankton community features were identified. A piece of No.25 plankton filter screen with a mesh diameter of 64 μm was dragged in a “ ∞ ” motion 0.5 m below the water surface in order to collect the eukaryotic phytoplankton in the water body. The concentrated samples were placed into centrifuge tubes and brought back to the laboratory in a refrigerated container. In the laboratory, the water samples were filtered using a cellulose acetate fibre filter membrane (Shanghai Xingya Purification Material Plant, Shanghai, China, diameter: 50 mm; pore size: 0.22 μm) to collect the eukaryotic phytoplankton. The filter membrane was placed in a centrifuge tube and kept in a liquid nitrogen tank for subsequent high-throughput sequencing.

2.3. Test of Physicochemical Factors and Chlorophyll a Concentration

Water temperature (WT), pH, and dissolved oxygen (DO) were tested at the sampling sites using a multi-parameter water quality analyser (Hach, HQ2100). The collected water samples were poured into polyethylene bottles and brought to the laboratory in dark conditions. The concentrations of ammonia nitrogen ($\text{NH}_4^+\text{-N}$), nitrate nitrogen ($\text{NO}_3^-\text{-N}$), total nitrogen (TN), phosphate ($\text{PO}_4^-\text{-P}$), total phosphorus (TP), and Chlorophyll a (Chl.a) in the water samples were tested [26,27]. The Chl.a concentration was tested by the acetone method [27] using an ultraviolet spectrophotometer (Beijing Purkinje GENERAL Instrument Co., Ltd, Beijing, China, Puxi TU-1950). Specifically, water samples (0.3 L) were filtered with a glass fibre filter membrane (Shanghai Xingya Purification Material Plant,

Shanghai, China, diameter: 50 mm; pore size: 0.45 µm), and the filter membrane was then ground using 90% acetone. A constant volume of 15 mL was established, followed by 6 h immersion and extraction. The supernatant was collected in a cuvette after centrifugation. A 90% acetone solution was used as the reference solution, and the absorbance was tested at wavelengths of 750 nm, 664 nm, 647 nm, and 630 nm. The formula for calculating the Chl.a concentration was as follows:

$$\rho_1 = 11.85 \times (A_{644} - A_{750}) - 1.54 \times (A_{647} - A_{750}) - 0.08 \times (A_{630} - A_{750})$$

$$\rho = \frac{\rho_1 \times V_1}{V}$$

where ρ_1 is the Chl.a concentration of the sample (mg·L⁻¹), A_{750} is the absorbance of the sample at 750 nm, A_{644} denotes the absorbance of the sample at 644 nm, A_{647} is the absorbance of the sample at 647 nm, A_{630} is the absorbance of the sample at 630 nm, ρ is the Chl.a concentration in the water (µg·L⁻¹), V_1 is the constant volume (mL), and V refers to the volume of the filtered water sample (L).

2.4. DNA Extraction and Sequencing of Plankton

Nucleic acid was extracted with a DNA extraction kit using the TGuide S96 paramagnetic particle method. The extracted nucleic acid concentration was tested by ELISA (Synergy HTX, Gene Company Limited, Hong Kong, China). Electrophoresis of the amplified polymerase chain reaction (PCR) products was conducted using agarose with a concentration of 1.8%, and the integrity was tested. PCR amplification and sequencing were based on the V4 interval of the 18S rDNA. F:CCAGCASCYGC GGTAATTCC and R:ACTTTCGTTCTTGATYRA were used as primers, and the amplification system was 10 µL. The amplification programme was as follows: pre-denaturation for 5 min at 95 °C, 25 cycles (denaturation for 30 s at 95 °C, annealing for 30 s at 50 °C, and extension for 40 s at 72 °C), and finally, extension for 7 min at 72 °C. The built library was sequenced on an Illumina Novaseq6000 platform (novaseq6000, Illumina, San Diego, CA, USA).

2.5. Sequencing Control and Data Analysis

First, the raw reads were filtered using Trimmomatic 0.33 software. Next, the primer sequences were recognised and eliminated using cutadapt 1.9.1 software. Then, the reads were spliced, and the chimera was eliminated using USEARCH 10.0. Finally, clean reads were collected for subsequent analysis. The sequences were clustered using USEARCH 10.0 at a similarity level of 97% to obtain the OTUs. The sequencing results and the Silva 138 database were compared. Taxonomic annotation of characteristic sequences was performed using the Naive Bayes classifier, thus obtaining species taxonomic information corresponding to each characteristic. In total, 20 samples were collected in the summer and winter. A total of 1,594,142 pairs of reads were obtained following the amplified sequencing of the V4 interval of 18S rDNA. The quality of double-end reads was controlled and spliced, producing a total of 1,402,904 clean reads. Each sample produced at least 51,468 clean reads.

After yielding sufficient OTUs, the α diversity of the eukaryotic phytoplankton community was calculated using the vegan package of the R(4.3.1) language, including the Shannon–Wiener index and Pielou index. The eukaryotic phytoplankton diversity at different points was analysed. Moreover, the diversity index was used to ascertain the water quality, determining the boundary value and corresponding water quality conditions [28,29]. The details are listed in Table 1. Meanwhile, the dominance index (y') was calculated according to the following formula:

$$y' = P_i \cdot f_i$$

where P_i is the proportion of individual species i among the total individuals and f_i is the frequency of occurrence of species i at different points [28,29].

Table 1. Water quality evaluation indexes of Shannon–Wiener index and Pielou index.

Shannon Index	Pielou Index	Water Quality
>3	0.8~1	no pollution
2~3	0.5~0.8	light pollution
1~2	0.3~0.5	moderate pollution
0~1	0~0.3	heavy pollution

All parameters were subjected to a $\lg(x + 1)$ transformation to achieve normal distributions. Significant differences in the physiochemical properties of the water samples in the different groups were analysed using analysis of variance (ANOVA). IBM SPSS Statistics 26 software was used to perform the analyses. Principal component analysis (PCA) of the physiochemical properties of the water samples in the different groups was conducted to obtain the sample point distribution characteristics of the physiochemical properties at the different sampling points. The inter-group differences in the physiochemical properties were analysed by PERMANOVA. These analyses were performed using the R(4.3.1) language vegan package.

Co-occurrence network analysis using eukaryotic phytoplankton relative abundance data. Co-occurrence network analysis was performed using the R(4.3.1) language psych package and Gephi 0.9 software to examine the co-occurrence relationships between the eukaryotic phytoplankton.

Based on the physicochemical properties of the water and the relative abundance data of the eukaryotic phytoplankton, detrended correspondence analysis (DCA) and redundancy analysis (RDA) were performed using Canoco 5 software to analyse the relationships between the relative abundance of the eukaryotic phytoplankton classes and the physiochemical properties of the water. When the calculation gradient is higher than 4, canonical correlation analysis (CCA) is applied. When the calculation gradient is between 3 and 4, both RDA and CCA are optional. When the calculation gradient is lower than 3, RDA is applied [11]. According to DCA results, the maximum first-axis lengths in summer and winter were 0.98 and 1.25, both of which were lower than 3. Hence, RDA was applied to investigate the influences of the physicochemical properties of the water on the class evolution of the community. The Mantel test was performed using the R language vegan, linkET, dplyr, and ggplot2 packages to examine the influences of the physicochemical properties on the eukaryotic phytoplankton classes. Variance partitioning analysis (VPA) was performed using the R(4.3.1) language vegan package to examine the contributions of environmental factors, seasonal changes, and human influences to changes in the eukaryotic phytoplankton community.

3. Results

3.1. Spatial and Temporal Differences in Physiochemical Factors of Water

Figure 2 illustrates the variations in the physiochemical properties of the water. Specifically, Group A and Group B in summer, as well as Group A and Group B in winter, represent water samples collected at sampling points of Group A and Group B (Figure 1) in summer and winter, respectively, which were denoted as Summer A, Summer B, Winter A, and Winter B. Seasonal changes may influence the physiochemical properties of water to some extent. Here, WT and DO fluctuated significantly in the different seasons ($p < 0.01$). Human activity may also affect the physiochemical properties of water to a certain extent. Here, there were extremely significant differences in $\text{NH}_4^+\text{-N}$ and TN between Summer A and Summer B ($p < 0.01$). $\text{NO}_3^-\text{-N}$ differed significantly between Winter A and Winter B ($p < 0.01$). In the study area, $\text{PO}_4^{3-}\text{-P}$ and TP exhibited no significant differences among the different groups. The average maximum concentrations of $\text{NH}_4^+\text{-N}$, TN, TP, and Chl.a in Summer A were $0.90 \text{ mg}\cdot\text{L}^{-1}$, $2.87 \text{ mg}\cdot\text{L}^{-1}$, $0.12 \text{ mg}\cdot\text{L}^{-1}$, and $109.81 \mu\text{g}\cdot\text{L}^{-1}$, respectively. The average concentration of $\text{NO}_3^-\text{-N}$ in Winter A was the highest, and the average concentration of DO in Winter B was the highest.

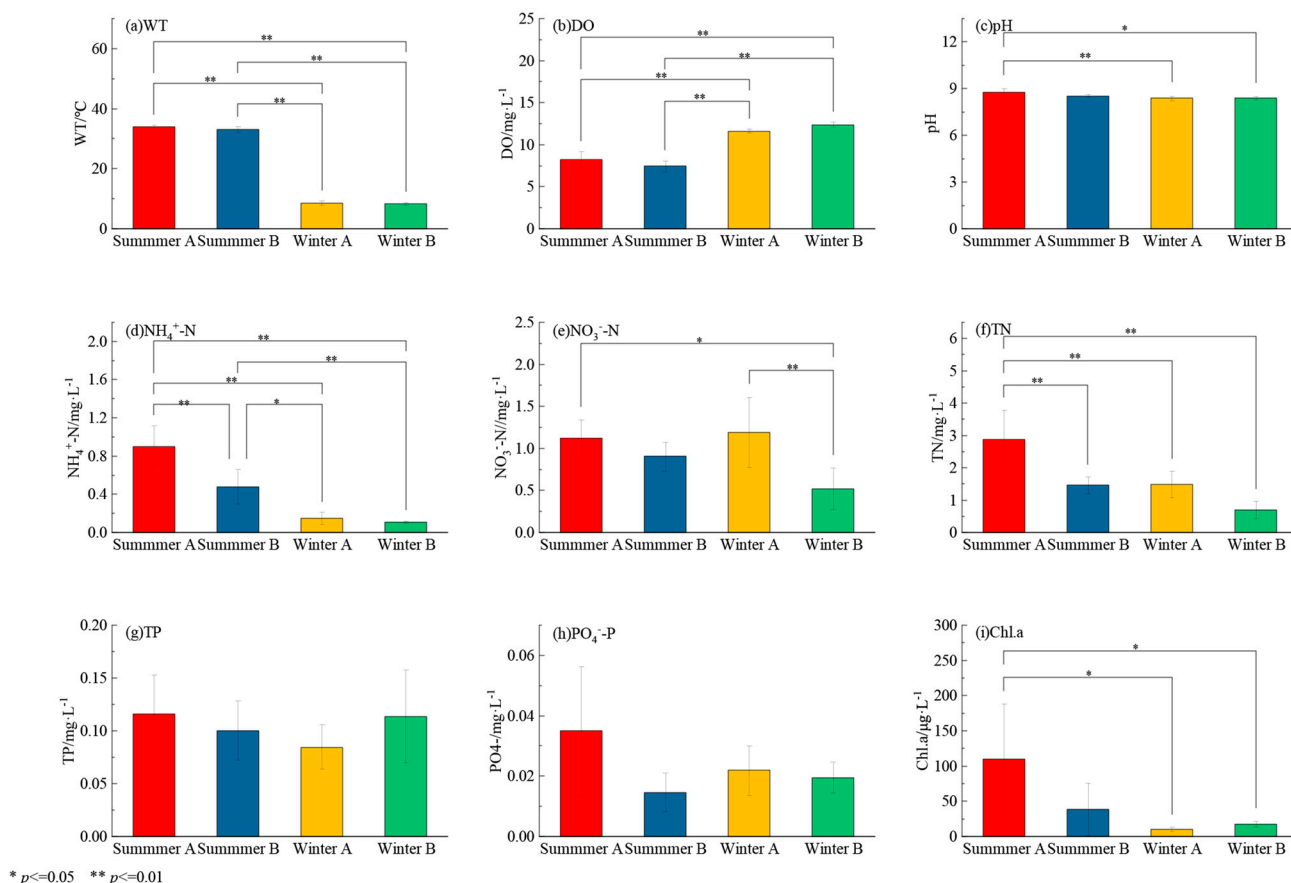


Figure 2. Analysis of the differences in the physiochemical properties of the water in the western half of Chaohu Lake as a function of group. Notes: Group A is close to urban areas, and Group B is far away from urban areas. WT—water temperature; DO—dissolved oxygen; TN—total nitrogen; NH₄⁺-N—ammonia nitrogen; NO₃⁻-N—nitrate nitrogen; TP—total phosphorus; PO₄³⁻-P—phosphate; Chl.a—Chlorophyll a.

A PCA of the physiochemical properties of the four groups of water samples was conducted. The results are presented in Figure 3. If the straight distance between the coordinates of two points is shorter, there is a smaller difference in the physiochemical properties of the water. According to the PCA results, two factors accounted for 70.9% of the variation in the water quality. The physiochemical properties of the water, such as the TN concentration, TP concentration, and WT, were positively related to the PCA factors to some extent, while DO was negatively related to the PCA factors. With respect to the distribution of the sampling points, the physiochemical properties of the water at all sampling points were within the 95% confidence interval. In summer, the sampling points were concentrated in the first and fourth quadrants, whereas they were concentrated in the second and third quadrants in winter. A PERMANOVA was conducted due to the overlapping of the confidence intervals of the different groups after PCA. According to the analysis, the physiochemical properties of the water in the four groups were significantly different ($R^2 = 0.793$, $p = 0.001$). An inter-group analysis of the four groups was performed. The findings are shown in Table S1. Differences were observed among the different groups.

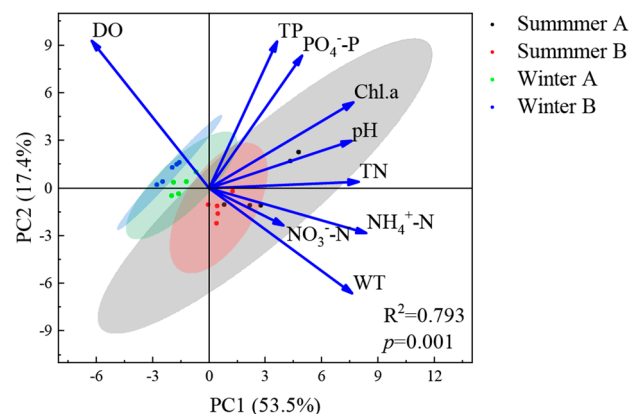


Figure 3. PCA of the physiochemical properties of the water for the different sample groups in the study area. Notes: WT—water temperature; DO—dissolved oxygen; TN—total nitrogen; NH_4^+ -N—ammonia nitrogen; NO_3^- -N—nitrate nitrogen; TP—total phosphorus; PO_4^{3-} -P—phosphate; Chl.a—Chlorophyll a.

3.2. Structure of the Eukaryotic Phytoplankton Community

In this study, a total of 70 genera, 34 orders, and 7 phyla of eukaryotic phytoplankton were detected. Among them, 61 genera, 29 orders, and 7 phyla of eukaryotic phytoplankton were detected in the summer, whereas 25 genera, 14 orders, and 5 phyla of eukaryotic phytoplankton were detected in the winter. The diversity index variation in eukaryotic phytoplankton is presented in Table 2. The mean Shannon diversity index of Summer A, Summer B, Winter A, and Winter B was 1.97, 1.83, 1.88, and 1.89, respectively. The maximum mean was observed in Summer A. The mean Pielou diversity index of the four groups was 0.78, 0.76, 0.84, and 0.84, respectively. Both the Shannon diversity index and the Pielou diversity index fluctuated more significantly in the summer than in the winter.

Table 2. Diversity index of phytoplankton in summer and winter in the study area.

Season	Diversity Index	1	2	3	4	5	6	7	8	9	10
Summer	Shannon	2.33	2.52	1.99	1.99	1.04	1.95	1.2	1.59	2.13	2.27
	Pielou	0.91	0.83	0.8	0.83	0.53	0.76	0.62	0.89	0.72	0.8
Winter	Shannon	1.86	1.65	1.94	2.04	1.92	1.94	2.28	1.67	1.74	1.76
	Pielou	0.84	0.85	0.88	0.85	0.8	0.84	0.89	0.76	0.79	0.84

The seasonal and inter-group differences in the phyla of the eukaryotic phytoplankton are shown in Figure 4a. The results indicate that Chlorophyta and Bacillariophyta were dominant in summer and winter in the study area. The relative abundances of Chlorophyta in Summer A and Summer B were 35.4% and 60.6%, and the relative abundances of Bacillariophyta were 33.2% and 26.0%, respectively. The relative abundances of Chlorophyta in Winter A and Winter B were 51.1% and 59.4%, respectively, and the relative abundances of Bacillariophyta were 30.6% and 20.5%. In the same season, the relative abundance of Chlorophyta in Group A was lower than that of Group B, whereas the opposite phenomenon was observed in terms of Bacillariophyta. In summer, the relative abundance of Cryptophyta was only next to that of Chlorophyta and Bacillariophyta, but Cryptophyta was not detected in winter. The relative abundance of Dinophyta was relatively low in the summer but increased to a certain extent in the winter.

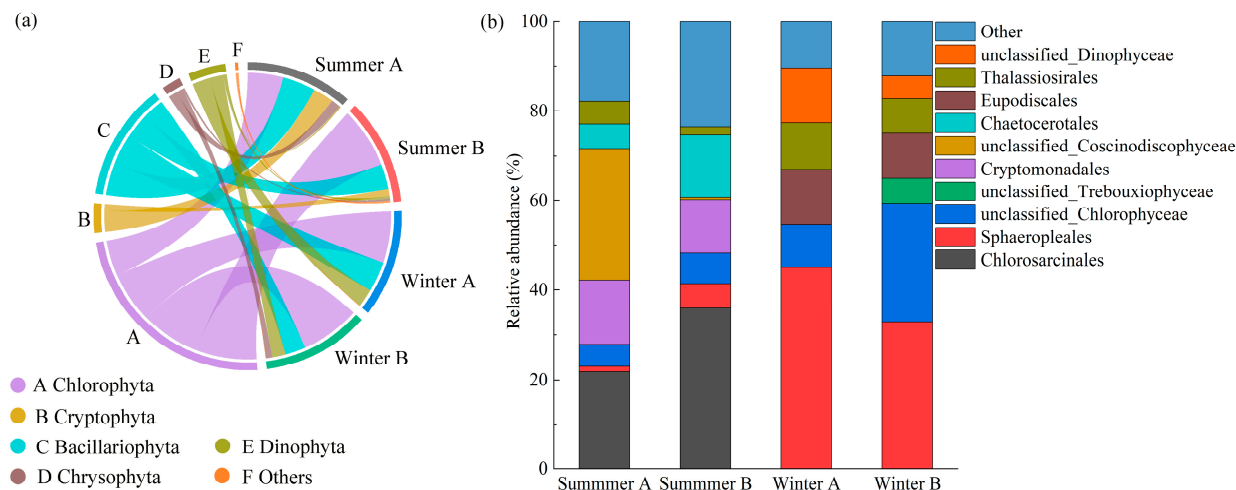


Figure 4. Variations in the phyla (a) and orders (b) of eukaryotic phytoplankton in the study area. Notes: Group A is close to urban areas, and Group B is far away from urban areas.

Figure 4b illustrates the seasonal and inter-group changes in the orders of eukaryotic phytoplankton. The relative abundance of Chlorosarcinales was highest in the summer, but Chlorosarcinales were not detected in the winter. The relative abundance of Sphaeropleales was relatively low in the summer and reached a maximum in the winter. Moreover, Cryptomonadales and Chaetocerotales were only detected in the summer, whereas Eupodisciales were only detected in the winter. The variability between eukaryotic phytoplankton community groups is shown in Table S2.

The numbers of genera in all phyla in winter decreased to some extent as compared to summer. Only 15 genera of eukaryotic phytoplankton were detected in both summer and winter, with great differences in genera. Table 3 presents the results of the analysis of the dominant genera of the eukaryotic phytoplankton. The dominant genera of the eukaryotic phytoplankton in the study area were distributed in Chlorophyta, Bacillariophyta, and Cryptophyta; there were spatial and temporal differences among the different dominant genera. Among all dominant genera, only *unclassified_Chlorophyceae* were considered dominant in all groups. The degree of dominance of *Chlamydomonas* reached a maximum in summer, but *Chlamydomonas* was not detected in winter. A similar phenomenon was observed for *Aulacoseira*, *Chaetoceros*, *Cryptomonas*, and *Anomoeoneis*. Although *Mychonastes* was detected in the summer, the degree of dominance was relatively low. However, *Mychonastes* demonstrated the maximum degree of dominance in winter. Two dominant genera, *Picochlorum* and *Odontella*, were only detected in winter.

Table 3. Dominant genera and the degree of dominance of the different groups in the study area.

Phylum	Genus	Summer A	Summer A	Winter A	Winter B
Chlorophyta	<i>Chlamydomonas</i>	0.199	0.331	-	-
Chlorophyta	<i>unclassified_Chlorophyceae</i>	0.040	0.069	0.096	0.096
Chlorophyta	<i>unclassified_Mamiellophyceae</i>	0.001	0.028	-	-
Chlorophyta	<i>Mychonastes</i>	0.000	0.002	0.323	0.323
Chlorophyta	<i>unclassified_Selenastraceae</i>	0.000	0.001	0.090	0.090
Chlorophyta	<i>Picochlorum</i>	-	-	-	0.023
Diatomea	<i>Aulacoseira</i>	0.294	0.003	-	-
Diatomea	<i>Cyclotella</i>	0.030	0.009	0.104	0.077
Diatomea	<i>Chaetoceros</i>	0.045	0.141	-	-
Diatomea	<i>Anomoeoneis</i>	0.013	0.032	-	-
Diatomea	<i>Odontella</i>	-	-	0.124	0.100
Diatomea	<i>unclassified_Mediophyceae</i>	0.001	0.001	0.034	0.035

Table 3. Cont.

Phylum	Genus	Summer A	Summer A	Winter A	Winter B
Diatomea	<i>unclassified_Diatomea</i>	0.007	0.011	0.049	0.017
Cryptophyta	<i>Cryptomonas</i>	0.088	0.025	-	-

Notes: Group A is close to urban areas, and Group B is far away from urban areas. The genera with a degree of dominance higher than 0.02 were defined as the dominant genera. “-” indicates not detected.

3.3. Effects of the Physiochemical Properties of Water on the Eukaryotic Phytoplankton Community

RDA was applied to evaluate the effects of the physiochemical properties of the water on evolution at the class level. Figure 5a depicts the RDA results in the summer. Two factors accounted for 92.54% of the variation in the eukaryotic phytoplankton community. In particular, TP, $\text{PO}_4^{3-}\text{-P}$, $\text{NH}_4^+\text{-N}$, and TN demonstrated substantial contributions. In summer, all classes were positively related to TP, $\text{NH}_4^+\text{-N}$, and TN and negatively related to $\text{PO}_4^{3-}\text{-P}$. The RDA results in winter are presented in Figure 5b. Two factors accounted for 75.71% of the variation in the eukaryotic phytoplankton community. $\text{NO}_3^-\text{-N}$, DO, and WT, in particular, had the maximum contributions. The effects of the DO concentration were statistically significant ($p \leq 0.05$). Dinophyceae was positively related to $\text{NO}_3^-\text{-N}$. Chrysophyceae and Trebouxiophyceae were positively related to DO and WT. In the same water body, the water quality properties that play a key role in the evolution of the eukaryotic phytoplankton community varied significantly with the seasons.

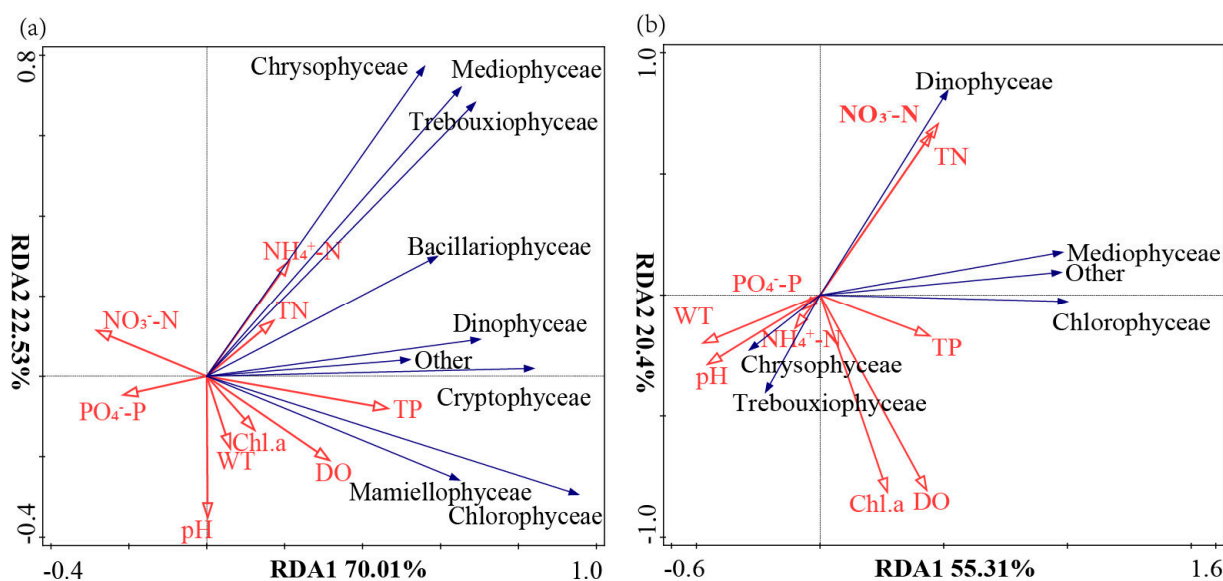


Figure 5. RDA results of the eukaryotic phytoplankton and physiochemical properties of the water in the study area in summer (a) and winter (b). Notes: WT—water temperature; DO—dissolved oxygen; TN—total nitrogen; $\text{NH}_4^+\text{-N}$ —ammonia nitrogen; $\text{NO}_3^-\text{-N}$ —nitrate nitrogen; TP—total phosphorus; $\text{PO}_4^{3-}\text{-P}$ —phosphate; Chl.a—Chlorophyll a.

A mantle test of the relative abundance of the eukaryotic phytoplankton and the physiochemical properties of the water was conducted to further understand the impacts of the physiochemical properties of the water on the eukaryotic phytoplankton community. The results are presented in Figure 6. Correlations were observed between the phyla of eukaryotic phytoplankton and the physiochemical properties of the water. WT, DO, $\text{NH}_4^+\text{-N}$, TN, TP, and Chl.a. in particular had significant effects on the eukaryotic phytoplankton community. Chlorophyta exhibited extremely significant correlations with WT and DO ($p < 0.01$) and a significant correlation with TP ($p < 0.05$). Cryptophyta demonstrated extremely significant correlations with WT, $\text{NH}_4^+\text{-N}$, and TN ($p < 0.01$) and significant correlations with TP and Chl.a ($p < 0.05$). Both Bacillariophyta and Chrysophyta exhibited

significant correlations with $\text{NH}_4^+\text{-N}$ ($p < 0.05$). Dinophyta showed extremely significant correlations with WT and DO ($p < 0.01$). The correlation coefficients are listed in Table S3.

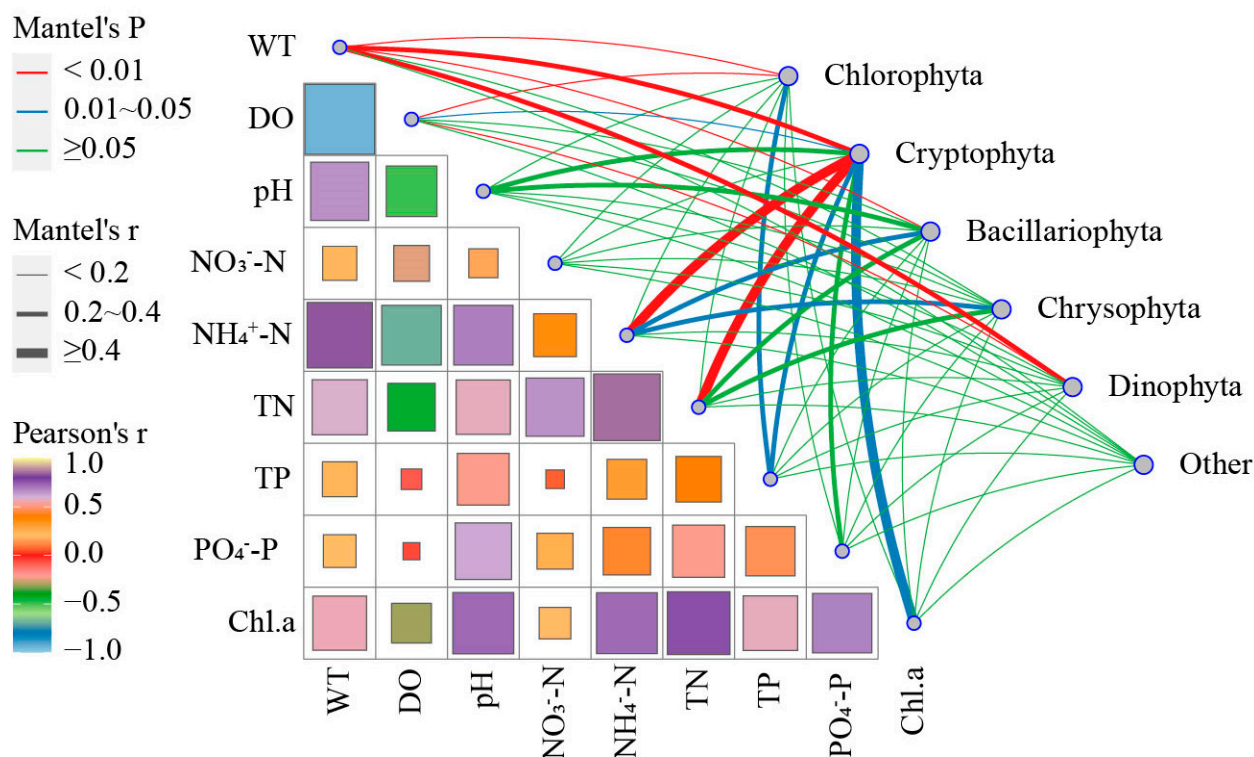


Figure 6. Mantel test of the relative abundances of the eukaryotic phytoplankton and the physiochemical properties of the water in the study area. Notes: WT—water temperature; DO—dissolved oxygen; TN—total nitrogen; $\text{NH}_4^+\text{-N}$ —ammonia nitrogen; $\text{NO}_3^-\text{-N}$ —nitrate nitrogen; TP—total phosphorus; $\text{PO}_4^{3-}\text{-P}$ —phosphate; Chl.a—Chlorophyll a.

3.4. Variance Partitioning Analysis of the Eukaryotic Phytoplankton Community

VPA (variance partitioning analysis) was conducted on the physiochemical properties of the water, the effects of human activities, and seasonal changes to understand the effects of different types of influencing factors on the evolution of the eukaryotic phytoplankton community in the study area. The results are presented in Figure 7. These three types of influencing factors accounted for 53.0% of the variation in the evolution of the eukaryotic phytoplankton community. Seasonal changes accounted for 48.4% of the variation in the evolution of the eukaryotic phytoplankton community, followed by the physiochemical properties of the water (48.3%) and human activities (only 3.3%). Human activity influences only interpreted 3.3%. This is because in the Overall Construction Plan of Wetland Parks Around Chaohu Lake, 10 wetland parks were planned around Chaohu Lake, including Anhui Hefei Binghu National Wetland Park, Anhui Lujiang Maweihe National Wetland Park, and Anhui Feixi Sanhe National Wetland Park, covering a total area of 9664.19 ha. These 10 wetland parks are around the whole of Chaohu Lake, giving a small range for human activities. In addition, seasonal changes and the physiochemical properties of the water accounted for 47.0% of the community changes overall. In other words, seasonal changes and the physiochemical properties of the water play critical roles in the evolution of the eukaryotic phytoplankton community.

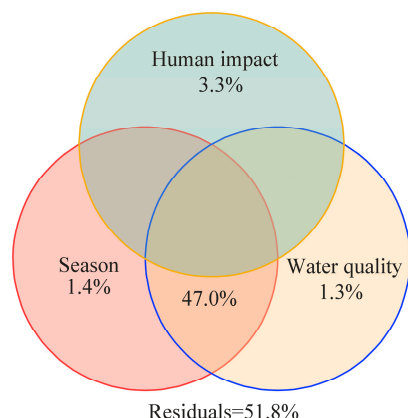


Figure 7. VPA diagram of the eukaryotic phytoplankton community in the study area.

3.5. Co-Occurrence Network Analysis

The correlations among the eukaryotic phytoplankton were visualised by establishing a co-occurrence network. Figure 8 illustrates the co-occurrence network analysis diagram. A Spearman correlation analysis was conducted on the relative abundances of the families of eukaryotic phytoplankton. When the Spearman correlation coefficient was $p < 0.05$ and $R > 0.8$, there was a significant correlation. The co-occurrence network comprised 34 sides and 32 nodes. Nodes belonged to Chlorophyta (9 families), Bacillariophyta (11 families), Cryptophyta (2 families), Chrysophyta (7 families), and Dinophyta (3 families). Among the interaction relationships, promotion accounted for 94.1%, and inhibition accounted for 5.9%. Chlamydomonadaceae, Chrysocapsaceae, Cymatosiraceae, and unclassified_Chaetophorales all had four connection lines with other families. Thus, they may be key eukaryotic phytoplankton in the network. In particular, there was a significant correlation between Chrysocapsaceae and unclassified_Chaetophorales.

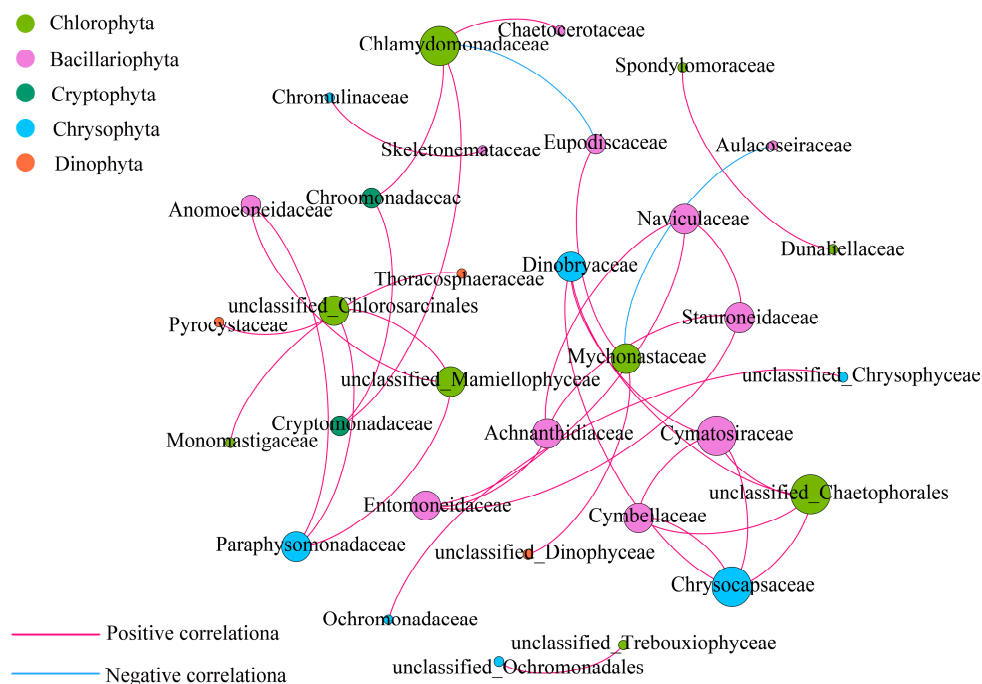


Figure 8. Network analysis of the families of eukaryotic phytoplankton in the study area.

4. Discussion

4.1. Structural Features and Changes in Eukaryotic Phytoplankton in the Chaohu Lake

Eukaryotic phytoplankton in west Chaohu Lake were examined through high-throughput sequencing. These results are more detailed than those obtained in previous microscopic studies of phytoplankton in Chaohu Lake. According to the microscopic observation results of Jiang et al., a total of 37 genera and 5 phyla of eukaryotic phytoplankton were detected in Chaohu Lake in four seasons [13]. However, in the current study, Euglenophyta was not detected, which differs, to some extent, from previous research results. This may be related to the specificity of the primers in the V4 interval of 18S rDNA [30].

Existing studies on Cryptophyta have demonstrated its remarkable adaptation. Hence, it can survive stably in lake ecosystems. The abundance of Cryptophyta might be increased in response to eutrophication or increased WT caused by climate change. In this study, Cryptophyta was detected in the summer but not in the winter. This might be due to the decreased WT in winter [31]. According to existing studies, Bacillariophyta prefer low temperatures. With a decrease in temperature, Bacillariophyta will reproduce quickly to take a dominant role in winter and spring. Some scholars have studied Bacillariophyta in the Shengjin Lake and discovered that its relative abundance was higher than that of Chlorophyta in winter, but its relative abundance decreased gradually from winter to summer. This is consistent with the low-temperature preference of Bacillariophyta [32,33]. In this study, the relative abundance of Bacillariophyta was lower than that of Chlorophyta in summer and winter. This result might be attributed to different detection methods for the abundance of eukaryotic phytoplankton. High-throughput sequencing can detect information that might be lost during microscopic observation. For example, *Mychonastes* in Chlorophyta, with the highest degree of dominance in winter, are challenging to observe under a microscope due to small particle size and lack of unique morphological features [34]. At the genus level, one genus of eukaryotic phytoplankton was considered a dominant genus in all groups. In contrast, other dominant genera only met the requirements for dominance in specific seasons. Hence, there are large differences at the genus level across the seasons. Some scholars have discovered that only a few species dominate the phytoplankton community at specific times and locations, accompanied by many species with relatively low abundances. However, these species with low relative abundances may still exhibit high abundances at least once or twice a year, which is consistent with the changes in the dominant genera [35].

4.2. Water Quality Conditions and Water Quality Evaluation of Chaohu Lake

According to the test results, there were spatial differences in the water quality indices in the study area. The mean $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, and $\text{PO}_4^{3-}\text{-P}$ concentrations in Group A were higher than those in Group B in twice sampling. Some temporal differences were also observed in the water quality of Chaohu Lake. Although there were no significant differences in TP and $\text{PO}_4^{3-}\text{-P}$ in winter and summer, the DO content in summer was far lower than that in winter. The mean TN and $\text{NH}_4^+\text{-N}$ concentrations in the summer were higher than those in the winter. This suggests that the water quality in the study area in the summer was worse than that in the winter [36]. In this study, sampling was performed twice in the summer and winter seasons. The maximum mean concentrations of TN and TP of the four groups were $2.87 \text{ mg}\cdot\text{L}^{-1}$ and $0.12 \text{ mg}\cdot\text{L}^{-1}$, both of which were observed in Summer A. However, these values have significantly decreased compared to previous studies of Chaohu Lake. It is speculated that this is due to the gradual effect of governance measures on Chaohu Lake [37].

In this study, the Shannon index and Pielou index were selected to represent the eukaryotic phytoplankton community. Both indices fluctuated more in summer than in winter, indicating that differences in the eukaryotic phytoplankton community among different water bodies are greater in summer than in winter. Moreover, some scholars have applied phytoplankton diversity indices to freshwater ecosystems to evaluate water quality; however, the applicability of these indices may not be consistent with the research

conclusions of different scholars [29,38]. The findings of this study verify the applicability of the phytoplankton diversity indices for the evaluation of the western half of Chaohu Lake. However, it should be noted that the diversity indices may demonstrate inaccuracies in water quality evaluation. For example, the water quality evaluation based on the Shannon index indicated that the study area was moderately polluted in summer and winter. The detection of the nutrition concentration in the study area showed that the water quality in summer was worse than that in winter, but the Shannon index was not able to effectively distinguish the differences in the water quality in summer and winter. In this study, the water quality was also evaluated using the Pielou index. According to this index, the water quality in the study area was mildly polluted in the summer and not polluted in the winter. Thus, these results indicate that the different phytoplankton diversity indices offer inconsistent results and have different abilities to distinguish differences in the water quality in the same water body. At present, several scholars are suspicious of the validity of phytoplankton water quality evaluations and have offered different perspectives on phytoplankton diversity and biogeographical modes related to water quality [38]. Phytoplankton diversity indices might also be affected by climate factors, which may affect the measurement of phytoplankton diversity indices for water quality evaluation [39]. For ecosystems with great geological spans and complicated terrains (e.g., rivers and lakes), the diversity indices can reflect ecological and environmental changes to some extent. However, water quality indices are considered to provide a more comprehensive evaluation of water quality [28,40].

4.3. Eukaryotic Phytoplankton in the Western Half of Chaohu Lake and the Influencing Factors

The relationship between the eukaryotic phytoplankton community and the physiochemical properties of water has been a key focus in research on water ecology. In this study, the Mantel test results demonstrated that the relative abundance of eukaryotic phytoplankton was correlated with the physiochemical properties of the water. According to the RDA analysis results, differences in the major environmental factors influenced the eukaryotic phytoplankton community in summer and winter. WT is considered a crucial environmental factor affecting phytoplankton. High temperatures not only increase photosynthesis and respiration but also increase the activity of thermally sensitive enzymes to increase the turnover rate of enzymes, thus influencing the phytoplankton community directly or indirectly [41,42]. The growth rate of phytoplankton is impacted by the WT. Many phytoplankton can achieve a maximum growth rate when the WT is about 20 °C [18]. pH is also an essential physiochemical index. Different growth and reproduction behaviours of phytoplankton have different requirements for water pH [43]. During the study period, the water in the western half of Chaohu Lake was generally alkaline, which is beneficial for photosynthesis, thereby increasing the productivity of phytoplankton. Some scholars have found that a low pH may inhibit the development of some algae [44,45]. N and P are also major nutrients that limit phytoplankton growth in water ecosystems [46]. Some scholars have proposed that the N/P ratio is one of the most important indices that influence phytoplankton. A P limitation state is when $N/P > 16$, and an N limitation state is when $N/P < 16$ [47,48]. Insufficient N content will decrease the Chl.a of major photosynthetic pigments and increase the contents of non-photosynthetic pigments. Insufficient P will cause phytoplankton to increase the synthesis of non-phosphorus compounds, using other compounds instead of phospholipids in the cell membrane [49]. The concentration of Chl.a can usually be used to characterise the biomass of phytoplankton. In this study, the Chl.a concentration differed significantly between summer and winter. The results indicated that the biomass of phytoplankton in the summer was higher than that in the winter. This indirectly suggests that conditions for phytoplankton growth are better in the summer [50].

5. Conclusions

(1) Sampling of the western half of Chaohu Lake in the summer and winter seasons revealed a total of 70 genera, 34 orders, and 7 phyla of eukaryotic phytoplankton through

high-throughput sequencing. There were 61 genera, 29 orders, and 7 phyla in summer, and 25 genera, 14 orders, and 5 phyla in winter. The dominant genus was *Chlamydomonas* of Chlorophyta in the summer and *Mychonastes* of Chlorophyta in the winter.

(2) According to PCA and PERMANOVA, the water quality indices varied among the four groups, reflecting spatial and temporal differences in water quality in the study area. Moreover, the Shannon index was not able to satisfactorily distinguish differences in water quality in the study area. Thus, in future evaluations of water quality, multiple methods, such as biological evaluation combined with physiochemical indices, should be used.

(3) The effects of the physiochemical properties of the water on the classes of eukaryotic phytoplankton were analysed during the study period through RDA. TP, $\text{PO}_4^{3-}\text{-P}$, $\text{NH}_4^+\text{-N}$, and TN were the primary physiochemical properties of water that affected the eukaryotic phytoplankton in the summer. $\text{NO}_3^-\text{-N}$, DO, and WT were the primary influencing factors in winter. According to the Mantel test, the eukaryotic phytoplankton community was correlated with the WT, DO, $\text{NH}_4^+\text{-N}$, TN, TP, and Chl.a. VPA revealed that seasonal factors could account for 48.4% of the variation in the eukaryotic phytoplankton community. In addition, the co-occurrence network analysis revealed that the promotion effect accounted for 94.1% of the total correlations among the eukaryotic phytoplankton.

(4) Future studies should further investigate the relationships between changes in the physiochemical properties of the water and changes in the eukaryotic phytoplankton community in Chaohu Lake. To this end, additional sampling points and an increased sampling frequency should be adopted in the study area to more comprehensively observe the variation trends in the water quality and eukaryotic phytoplankton community in different regions.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/w16162318/s1>, Table S1: PERMANOVA on physiochemical factors of water of different sample groups in the study area; Table S2: Results of PERMANOVA analyses of different subgroups of eukaryotic phytoplankton; Table S3: Analysis of the correlations between eukaryotic phytoplankton and physiochemical factors of water in the study area based on the Mantel test.

Author Contributions: Conceptualization, B.Z.; Methodology, B.Z.; Software, X.Z. (Xinhao Zhu); Validation, H.Z. and X.Z. (Xingmei Zhuang); Resources, H.Z., X.Z. (Xingmei Zhuang), J.W., S.X. and T.L.; Data curation, J.W., S.X. and T.L.; Writing—original draft, W.P. and X.Z. (Xinhao Zhu); Funding acquisition, B.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Natural Science Foundation of China (52303360), the Natural Science Foundation of the Universities of Anhui Province (2022AH050241), the Project of Introducing Talents and Doctoral Start-up Fund of the University (2022QDZ10), the Anhui Institute of Ecological Civilisation (AHSWY-2022-02), and the Project of National College Students' Innovative Entrepreneurship Training Program (202310878032).

Data Availability Statement: The datasets and materials used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

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