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Interannual and Seasonal Shift between *Microcystis* and *Dolichospermum*: A 7-Year Investigation in Lake Chaohu, China

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Abstract: The shifts among bloom-forming cyanobacteria have attracted increasing attention due to the reductions in nitrogen and phosphorus during the eutrophication mitigation process. However, knowledge is limited regarding the pattern and drivers of the shifts among these cyanobacterial genera. In this study, we performed a 7-year long, monthly investigation in Lake Chaohu, to analyze the interannual and seasonal shifts between *Microcystis* and *Dolichospermum*. Our results showed that *Microcystis* was the dominant cyanobacterium in the western lake region in summer, whereas *Dolichospermum* was dominant in the other regions and seasons. The *Microcystis* biomass and ratio were driven primarily by total phosphorus and temperature. The sensitivity of *Dolichospermum* to nutrients and temperature was relatively weak compared to that of *Microcystis*. The shifts between *Microcystis* and *Dolichospermum* might be led by *Microcystis*. If the temperature and phosphorus level were relatively high, then *Microcystis* grew rapidly, and competitively excluded *Dolichospermum*. If the nutrient level, especially the phosphorus level, was low, then the exclusive power of *Microcystis* was weak, and *Dolichospermum* maintained its dominance, even in summer. The key temperature (~17 °C) determined the dominance of the two cyanobacteria. *Microcystis* never dominated, while *Dolichospermum* was always dominant below the key temperature. *Microcystis* and *Dolichospermum* had different means of responding to the interaction of temperature, nitrogen and phosphorus. The *Dolichospermum* biomass was sensitive to the variation in nitrogen level, and the sensitivity depended on temperature. While the *Microcystis* biomass was sensitive to the variation in phosphorus level, and the sensitivity depended on temperature and total nitrogen. The different ways might contribute to the succession of the two cyanobacteria. Our findings will be helpful for improving the understanding of the shift process between *Microcystis* and *Dolichospermum*.

Keywords: *Microcystis*; *Dolichospermum*; Lake Chaohu; seasonal shift

1. Introduction

Cyanobacteria often dominate a phytoplankton community, and even form harmful algal blooms in eutrophic freshwaters, such as lakes, reservoirs and estuaries. The bloom-forming cyanobacteria are principally gas-vacuolate species. They are distributed across a number of genera, and vary in form and size from small filaments, such as *Dolichospermum* or *Oscillatoria*, to large globular colonies, such as *Microcystis* [1]. The most common bloom-forming cyanobacteria in these water bodies are *Microcystis*, *Dolichospermum* (*Anabaena*) and *Aphanizomenon* [2]. Generally, different bloom-forming cyanobacteria can co-occur or successively dominate at an intra-annual scale in a specific water body [3,4]. The temporal and spatial dynamics of bloom-forming cyanobacteria is considered to be a

result of the synergistic effects of multiple driving forces [5,6]. However, our understanding of the succession patterns of bloom-forming species, and their underlying mechanics, is limiting.

Temperature is usually considered the primary driver of seasonal succession among cyanobacterial genera or species, due to the differences in their temperature niches [4,7,8]. The average temperatures at which *Microcystis* dominated were significantly higher than the temperatures at which *Dolichospermum* and *Aphanizomenon* were the most abundant [9]. Li and Watanabe [10] found that most of the *Dolichospermum* species had optimal growth when the temperature was above 20 °C. The optimal growth temperatures of some *Dolichospermum* species are similar to those of *Microcystis* under optimal nutrient conditions, but the maximal growth rates of *Microcystis* are higher than those of *Dolichospermum* [11,12]. In addition, low temperatures limited *Microcystis* more severely than *Dolichospermum* [13,14]. These findings regarding temperature niches were closely correlated with these species seasonally occurring or dominating in the field. For example, N₂-fixing *Aphanizomenon* and *Dolichospermum* generally proliferate in early spring or late fall when the temperature is relatively low. Nondiazotrophic *Microcystis* occurs principally in midsummer, when the temperature is relatively high [4,15]. However, the seasonal correlation was also often altered by some exceptional observations. For example, dominant *Dolichospermum* was often observed in the eastern region of Lake Chaohu in summer [9,16]. The diazotrophic cyanobacteria *Aphanizomenon* appeared and formed a bloom in summer, and a *Microcystis* bloom was formed in winter in Lake Kinneret [17]. Therefore, the intra-annual shift in dominant cyanobacteria cannot be completely explained by temperature.

The availability of nutrients also plays an important role in this shift. Diazotrophic cyanobacteria can fix atmospheric N₂ into biologically available NH₃ in order to satisfy their nitrogen requirements for growth [18]. They are usually dominant when the total nitrogen to total phosphorus (TN/TP) ratio is low, or when nitrogen is limiting [19]. Nondiazotrophic cyanobacteria require chemically bound nitrogen source, and generally dominate when it is in excess [20]. Hence, controlling N inputs could actually aggravate the dominance of N-fixing cyanobacteria [18,21], and result in the shift from nondiazotrophic to diazotrophic cyanobacteria. However, at the intra-annual scale, the nitrogen concentration is high in spring or early summer, and low in summer and autumn, in productive temperate lakes [22]. During this period, a typical pattern involves diazotrophs, such as *Aphanizomenon* or *Dolichospermum*, occurring first, followed by nondiazotrophic genera, most notably *Microcystis* [23]. This seems to be in contrast with the pattern by which diazotrophs are expected to dominate when nitrogen levels are low [24]. In addition, the phosphorus strategy of bloom-forming cyanobacteria (*Dolichospermum* and *Microcystis*) is also considered one of the reasons for their shift. Rapid uptake and polyphosphate body accumulation in *Microcystis* guarantees their growth under low phosphorus conditions, and increases the phosphorus deficiency in *Dolichospermum* [25]. However, this mechanism is not able to explain the dominance of diazotrophic cyanobacteria in summer, which were not replaced by *Microcystis* [9,17].

There is strong evidence that the relative importance of temperature and nutrients in promoting cyanobacteria is taxon-dependent. Some taxa, such as *Dolichospermum*, are more sensitive to nutrients, while others, such as *Microcystis*, are more sensitive to temperature [26]. The strategic difference might explain the counterintuitive patterns in the shifts among cyanobacterial genera in response to temperature and nutrients. However, our understanding of how the responses of these cyanobacterial genera to one variable depend on another variable is limiting.

To gain more insight into the relationship between temperature, nutrients, and the dominance of specific bloom-forming cyanobacterial genera, we conducted a monthly investigation from 2012 to 2018 in Lake Chaohu, to analyze the interannual and seasonal shift between *Microcystis* and *Dolichospermum*. Our aim was to answer the following two questions: (1) What is the difference between the two cyanobacteria with regards to their response to temperature and nutrients? (2) What are the key values of these variables that result in their shift? First, the variations in their biomass and ratios to total biomass were determined. Second, the main predictors, and the key values of the ratios of *Microcystis* and *Dolichospermum* to total algal biomass, were measured with classification and regression

tree analysis. Finally, the mutual dependence of their responses to nitrogen or phosphorus, and the dependence on temperature, were analyzed and discussed.

2. Materials and Methods

2.1. Study Lake

Lake Chaohu is the fifth largest freshwater lake in China, and is located in central Anhui Province (Figure 1). It covers a catchment area of approximately 9200 km², and has a surface of approximately 750 km². The volume of Lake Chaohu can change dramatically from 17.2×10^8 m³ in the dry season to 32.3×10^8 m³ in the rainy season [27]. The maximum lake depth is approximately 6 m and mean depth is approximately 3 m. In the lake, the submerged vegetation abruptly disappeared in 1963 due to the installation of the Chaohu sluice, and strong cyanobacterial blooms (primarily *Microcystis* and *Dolichospermum*) have been observed, attributing to aggravated eutrophication since the 1980s [28–31].

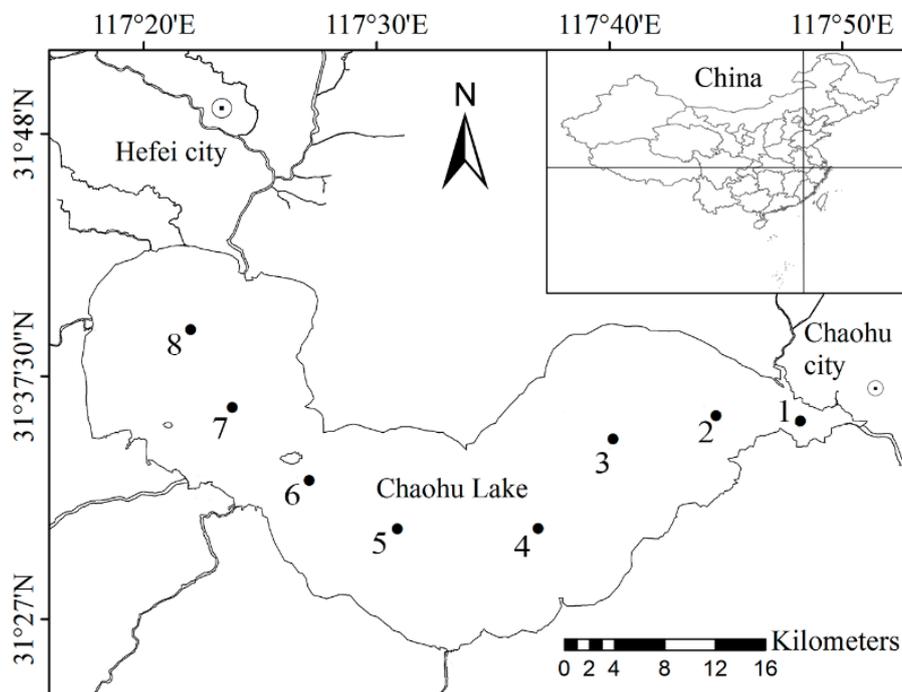


Figure 1. Map of Lake Chaohu showing the locations of 8 sampling sites.

2.2. Sampling and Environmental Analyses

To investigate the spatial-temporal distribution of bloom-forming cyanobacteria, we performed a monthly field investigation of 8 sites (eastern region: site 1, 2 and 3; central region: site 4, 5 and 6; western region: site 7 and 8) from 2012 to 2018. Water samples were collected by mixing the surface (50 cm below the surface), middle and bottom (50 cm above the bottom) samples taken with an Uwitec water sampler (Uwitec, Mondsee, Austria). A total of 672 samples have been collected over the past seven years. Environmental parameters (temperature, pH, conductivity and dissolved oxygen) were measured at every sampling site using a multiparameter meter. Total nitrogen (TN) and total phosphorus (TP) were analyzed by peroxydisulfate oxidation and spectrophotometric methods [32]. Ammonium (NH₄), nitrate (NO₃), nitrite (NO₂) and orthophosphate (PO₄) were measured using a continuous flow analyzer (Skalar SA 1000, Breda, the Netherlands). Transparency (SD) was measured with a Secchi disk.

2.3. Phytoplankton Analyses

Integrated 500-mL samples were collected for phytoplankton analysis at each site. The samples were fixed with acid Lugol's solution. Phytoplankton were identified to genus or species based on the most recent literature [33]. Identification and counts were performed in more than 30 random fields in sedimentation chambers (30 mL) using a microscope [34]. The biovolume of each species at each site was calculated based on the mean value of the linear measurements in 30 cells and converted to volumes after approximation to standard geometric solids [35]. The biomass was determined and converted to fresh weight, assuming a specific gravity of 1 g/cm³.

2.4. Data Analysis

Temporal variations in environmental variables (TN, TP, NH₄, NO_x including NO₃ and NO₂, PO₄ and SD) in three lake regions (eastern, central and western regions) were shown based on a 3-month moving average. The ratios of *Microcystis* and *Dolichospermum* biomass to total algal biomass and these environmental variables among lake regions or sites were compared with the Tukey HSD method. Prior to the analyses, data were checked for homogeneity of variances and normality.

To identify the main predictor variables of the *Microcystis* and *Dolichospermum* ratios in the total algal biomass, their fractions were determined and used to perform classification and regression tree analysis with the caret package in R. Before the analysis, we identified the collinear variables and selected a subset of variables based on inspection of the variance inflation factors (VIFs). Those variables that had a VIF > 10 (i.e., multiple collinear variables) were removed [36]. We trained the model with the machine learning algorithm 'rpart'. The models were tuned using the 'tuneGrid' function with maxdepth = 3 and mincriterion = 0.95. In addition, the dependence of the relationship between the ratios and TP, or TN, on TN or TP and temperature was also presented with the 'coplot' function.

3. Results

3.1. Spatial-Temporal Variation in *Microcystis*, *Dolichospermum* and Environmental Variables

3.1.1. Spatial-Temporal Variation in Environmental Variables

From 2012 to 2018, the TP and PO₄ concentrations increased, and the increasing magnitude was higher in the central and eastern lake regions than in the western lake region (Figure 2a,c and Figure S1a,c). The TN concentration decreased in the western lake region and increased in the central and eastern lake regions (Figure 2b and Figure S1b). The NH₄ concentration decreased remarkably in the three regions in 2015 (Figure 2d and Figure S1d). The trends in NO_x in the central and eastern lake regions were similar to those in TN. However, the NO_x concentration increased obviously before 2015, and remained relatively stable after that (Figure 2e and Figure S1e). The transparency in the three lake regions presented a decreasing trend, and the decreasing magnitude in the eastern lake region was higher than that in other regions (Figure 2f and Figure S1f).

Spatially, the TP, PO₄, TN and NO_x concentrations gradually decreased from the western to the eastern lake region. In contrast, the transparency gradually increased. There was no obvious difference in the NH₄ concentration among the lake regions (Figure 3).

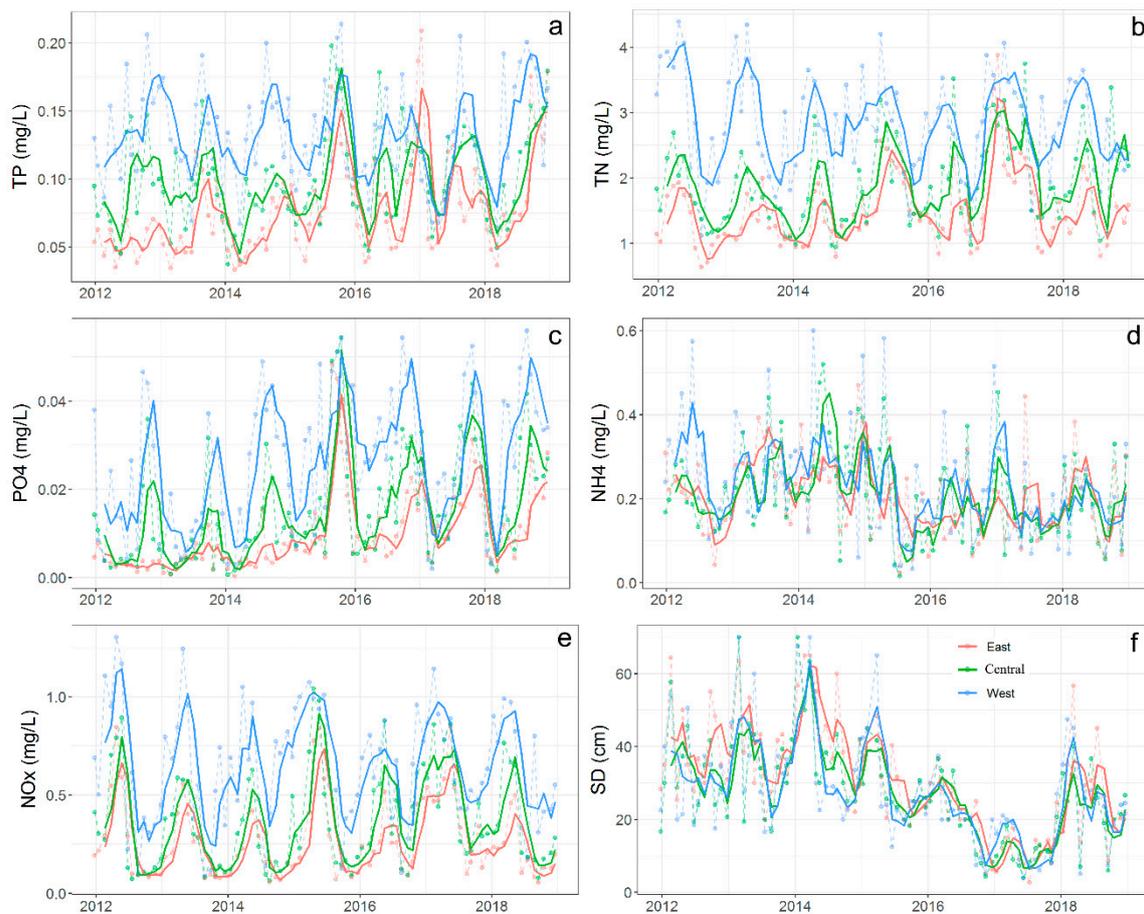


Figure 2. Long-term variations in the mean values of (a) total phosphorus (TP); (b) total nitrogen (TN), (c) orthophosphate (PO_4); (d) ammonium (NH_4); (e) nitrate + nitrite (NO_x) and (f) transparency (SD) in the three lake regions of Lake Chaohu from 2012 to 2018. The solid lines represent the temporal trajectories based on a 3-month moving average.

3.1.2. Spatial-Temporal Variation in *Microcystis* and *Dolichospermum*

Dolichospermum flos-aquae and *Microcystis* spp. were identified as the dominant cyanobacteria in Lake Chaohu. For the past seven years, the *Dolichospermum* ratio has been higher than that of *Microcystis* in the whole lake. *Dolichospermum* dominated the eastern and central lake regions and *Microcystis* dominated the western lake region. The biomass of *Microcystis* showed obvious seasonal variation, and peaked in summer in all lake regions. Generally, the *Microcystis* ratios in the western region were higher than those in the central and eastern regions. However, the ratio showed an increasing trend in the eastern region (Figure 4a,b). The biomass of *Dolichospermum* generally peaked in late autumn, winter and early spring. There was a remarkable difference among the lake regions. The trend in the *Dolichospermum* ratio showed a decreasing trend (Figure 4c,d).

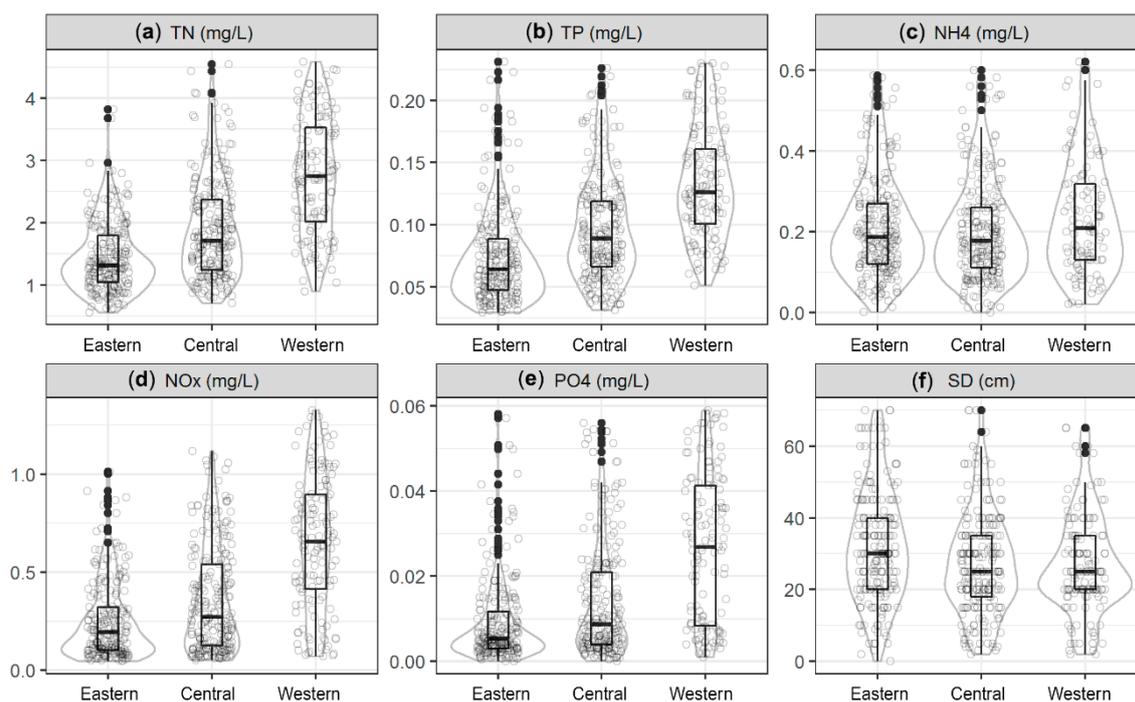


Figure 3. Values of (a) total nitrogen (TN), (b) total phosphorus (TP), (c) ammonium (NH_4), (d) nitrate + nitrite (NO_x), (e) orthophosphate (PO_4) and (f) transparency (SD) in the three lake regions of Lake Chaohu from 2012 to 2018. Whiskers represent the minimum and maximum values. Boxes symbolize the 25th and 75th percentiles. Lines inside the boxes show the median values. The black points show the outliers. The grey polygons show the distribution of the data along the Y-axis.

3.2. Predictors and their key values of *Microcystis* and *Dolichospermum* Ratios to Total Biomass

Water temperature (WT) was the dominant factor for the *Microcystis* ratio (Figure 5; node 1). When the WT was lower than $17.24\text{ }^\circ\text{C}$, the *Microcystis* ratio was generally low. Within this partition, an additional separating factor was TP concentration (Figure 5; nodes 2, 3). Low concentrations of TP ($\leq 0.13\text{ mg/L}$) reduced the *Microcystis* ratio to less than 10%. If the TP concentrations were higher than 0.13 mg/L , then the *Microcystis* ratio was approximately 0–30%. When the WT exceeded $17.24\text{ }^\circ\text{C}$, the *Microcystis* ratio in most samples was high. TP and TN were additional separating factors in this section (Figure 5, nodes 7, 8). If the TP concentrations were higher than 0.125 mg/L , then the *Microcystis* ratio could reach approximately 30–80%. At a TP concentration of $\leq 0.125\text{ mg/L}$, TN or TN/TP was important to the *Microcystis* ratio. When $\text{TN} > 1.51\text{ mg/L}$ or $\text{TN/TP} > 12.08$, the *Microcystis* ratio in most samples was limited to $< 15\%$. When $\text{TN} \leq 1.51\text{ mg/L}$ or $\text{TN/TP} \leq 12.08$, the *Microcystis* ratio in most samples was approximately 10–60%.

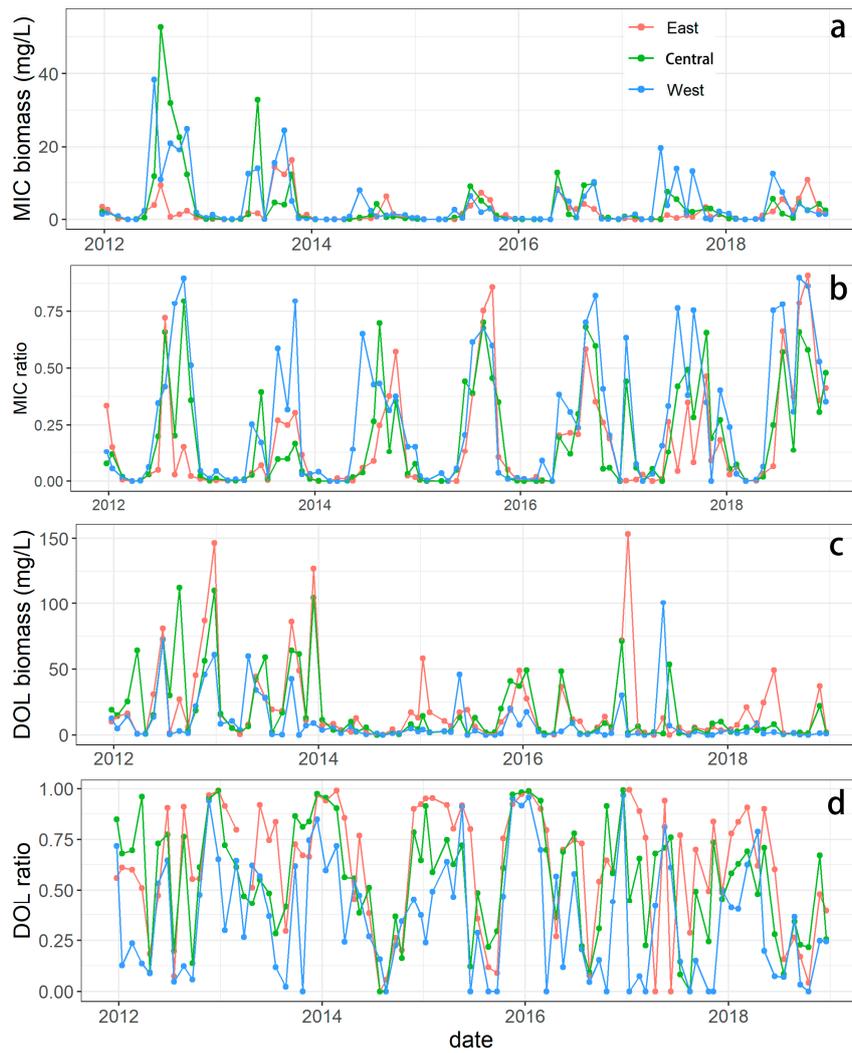


Figure 4. Variations in *Microcystis* (MIC) biomass (a), *Dolichospermum* (DOL) biomass; (c) and their ratios to the total biomass (b) and (d) from 2012 to 2018 in Lake Chaohu. The colored points and lines indicate different lake regions.

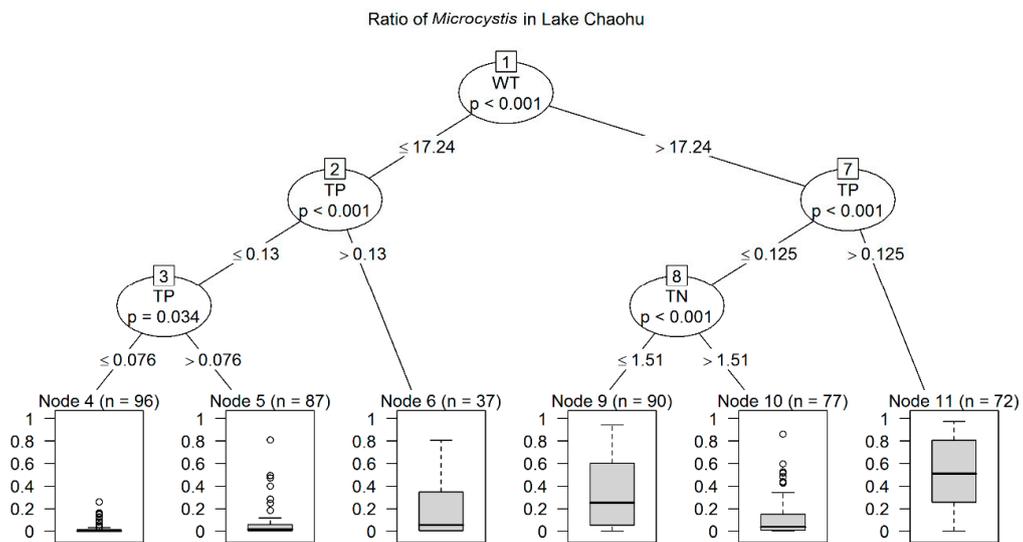


Figure 5. Classification trees for the ratio of *Microcystis* to total biomass. The extracted predictor variables represent the link: TN, total nitrogen (mg/L); TP, total phosphorus (mg/L); and WT, water temperature (°C).

WT was also a crucial factor for the *Dolichospermum* ratio (Figure 6; node 1). When the WT was lower than 16.63 °C, the *Dolichospermum* ratio was generally high. The TN concentration was an additional separating factor within this partition (Figure 6; nodes 2). Low concentrations of TN (≤ 2.173 mg/L) increased the *Dolichospermum* ratio to more than 70%. When the TN concentrations were higher than 2.173 mg/L, the *Dolichospermum* ratio was approximately 20–80%, and the median was approximately 50%. When WT exceeded 16.63 °C, TP and WT became additional separating factors. If $TP > 0.063$ mg/L and $WT > 25.285$ °C, then the *Dolichospermum* ratio was limited to $< 40\%$. If $TP > 0.063$ mg/L and $WT \leq 25.285$ °C, then the median *Dolichospermum* ratio increased to 40%. At a TP concentration of ≤ 0.063 mg/L, the median *Dolichospermum* ratio was as high as 60%.

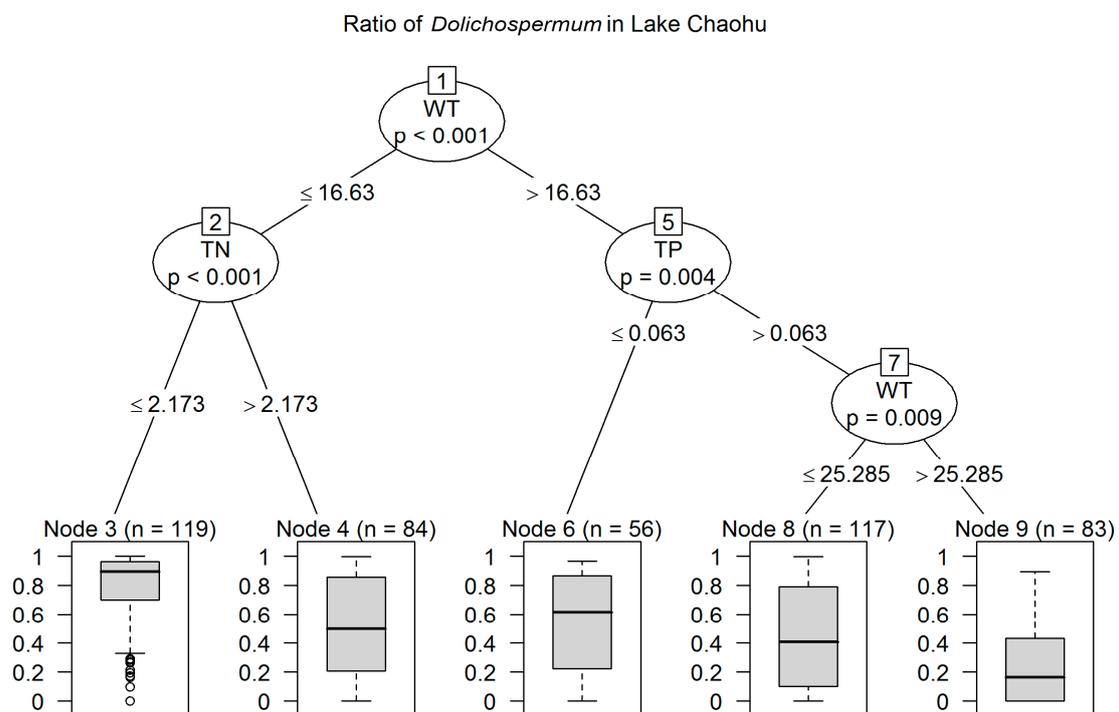


Figure 6. Classification trees for the ratio of *Dolichospermum* to total biomass. The extracted predictor variables represent the link: TN, total nitrogen (mg/L); TP, total phosphorus (mg/L); and WT, water temperature (°C).

WT was the most important factor for the two cyanobacterial ratios, and TN and TP were also key regulating factors. When the WT was less than 17 °C, the *Dolichospermum* ratio gradually decreased from the eastern to western lake region, and the *Microcystis* ratio was still low. When the WT was 17–25 °C, the *Dolichospermum* and *Microcystis* ratios were close to each other in the western lake region, where the nutrient level was high. In the lake regions with relatively low nutrient levels, the *Dolichospermum* ratio was higher than the *Microcystis* ratio. When the WT was more than 25 °C, the *Microcystis* ratio exceeded that of *Dolichospermum* in the central and western lake regions. However, the *Dolichospermum* ratio was still higher than that of *Microcystis* in the eastern lake region (Figure 7).

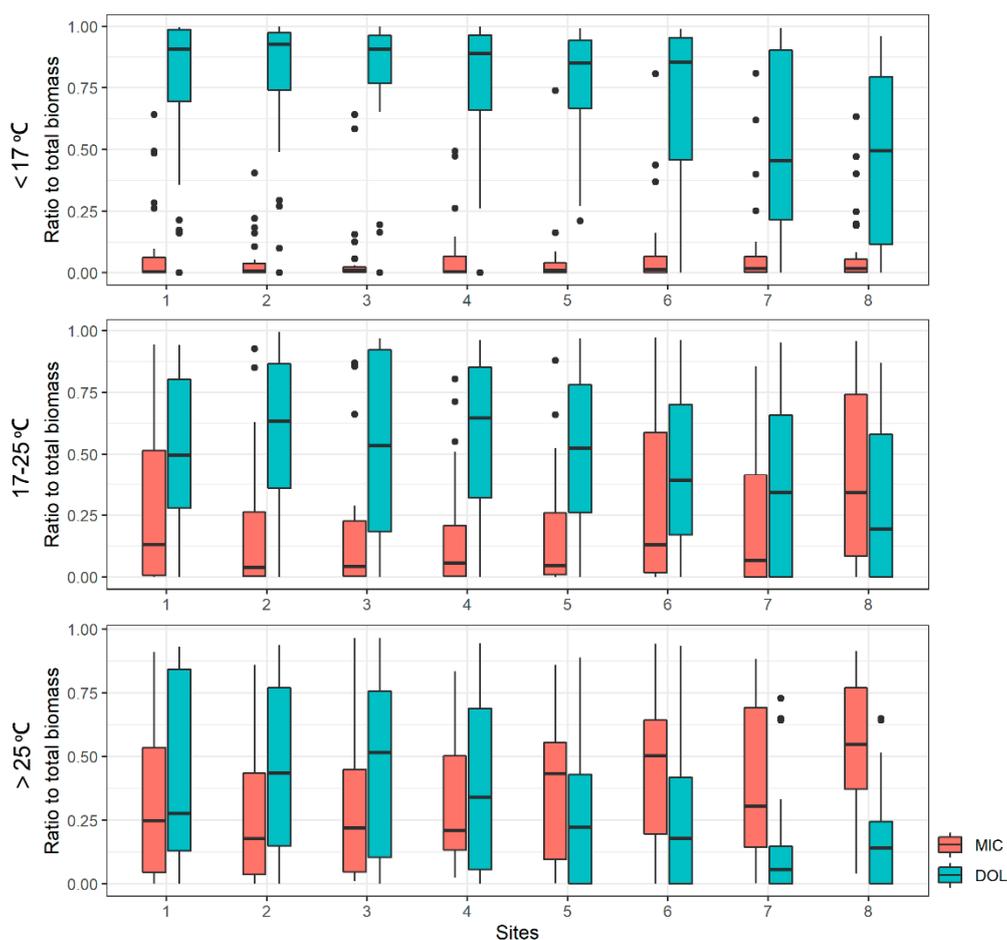


Figure 7. Ratios of *Microcystis* (MIC) and *Dolichospermum* (DOL) to total biomass at the difference sites of Lake Chaohu under different temperature conditions.

3.3. Responses of *Microcystis* and *Dolichospermum* to Water Temperature, Nitrogen and Phosphorus

There were significantly positive relationships between WT and *Microcystis* biomass at all TN and TP levels ($p < 0.05$, Figure 8), and the slopes of the fitting lines were similar. The positive response of *Microcystis* biomass to TP increased with the increasing WT and TN level (Figure S2), while the negative biomass response to TN decreased with the increasing WT and TN level (Figure S3). The *Microcystis* ratios increased significantly with the increasing WT. The slopes of the relationship between the *Microcystis* ratio and WT increased with the increasing TP level, and almost did not change with the TN level. The relationship between the *Dolichospermum* biomass and WT only showed a significantly negative trend when the TP concentration was high (>ca. 0.10 mg/L) and the TN concentration was low (<ca. 1.5 mg/L) (Figure 9). When the WT was more than 20 °C, the *Dolichospermum* biomass was positively correlated with TN under high TP conditions, and had no relationship with TN under low TP conditions (Figure S4). There was no significant relationship between *Dolichospermum* biomass and TP under any of the TN conditions (Figure S5). The relationships between the *Dolichospermum* ratio and temperature were negative when TP was less than approximately 0.075 mg/L ($p < 0.05$), or when TP was more than this value and TN was less than approximately 2.25 mg/L.

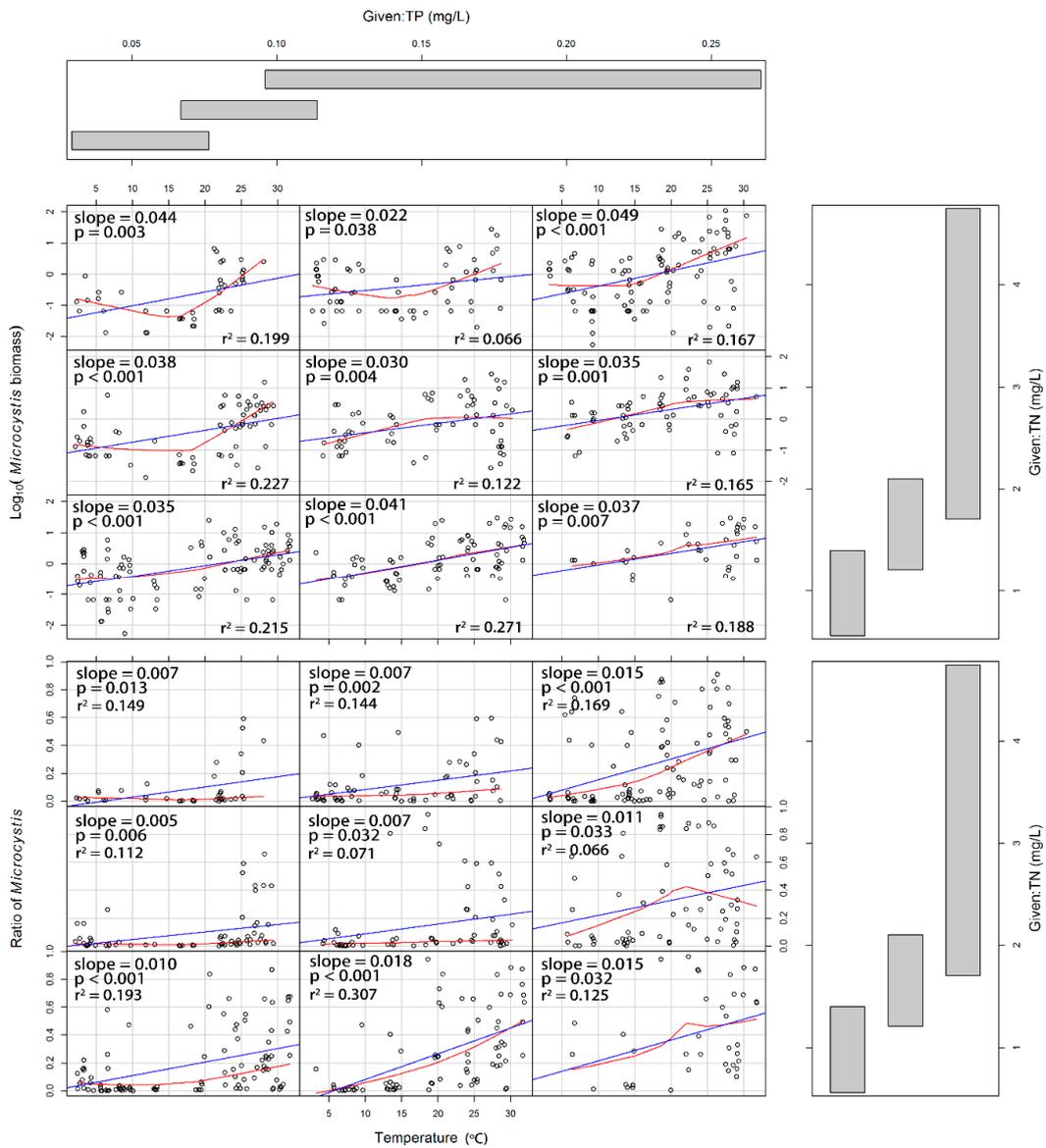


Figure 8. Relationships between temperature and *Microcystis* biomass (top) or ratios (bottom) depending on total nitrogen (TN) and total phosphorus (TP), which was divided into three segments, on average, of the data numbers with 30% overlap. The red and blue lines represent the LOESS and linear fits, respectively. The parameters of linear fit were shown in each panel.

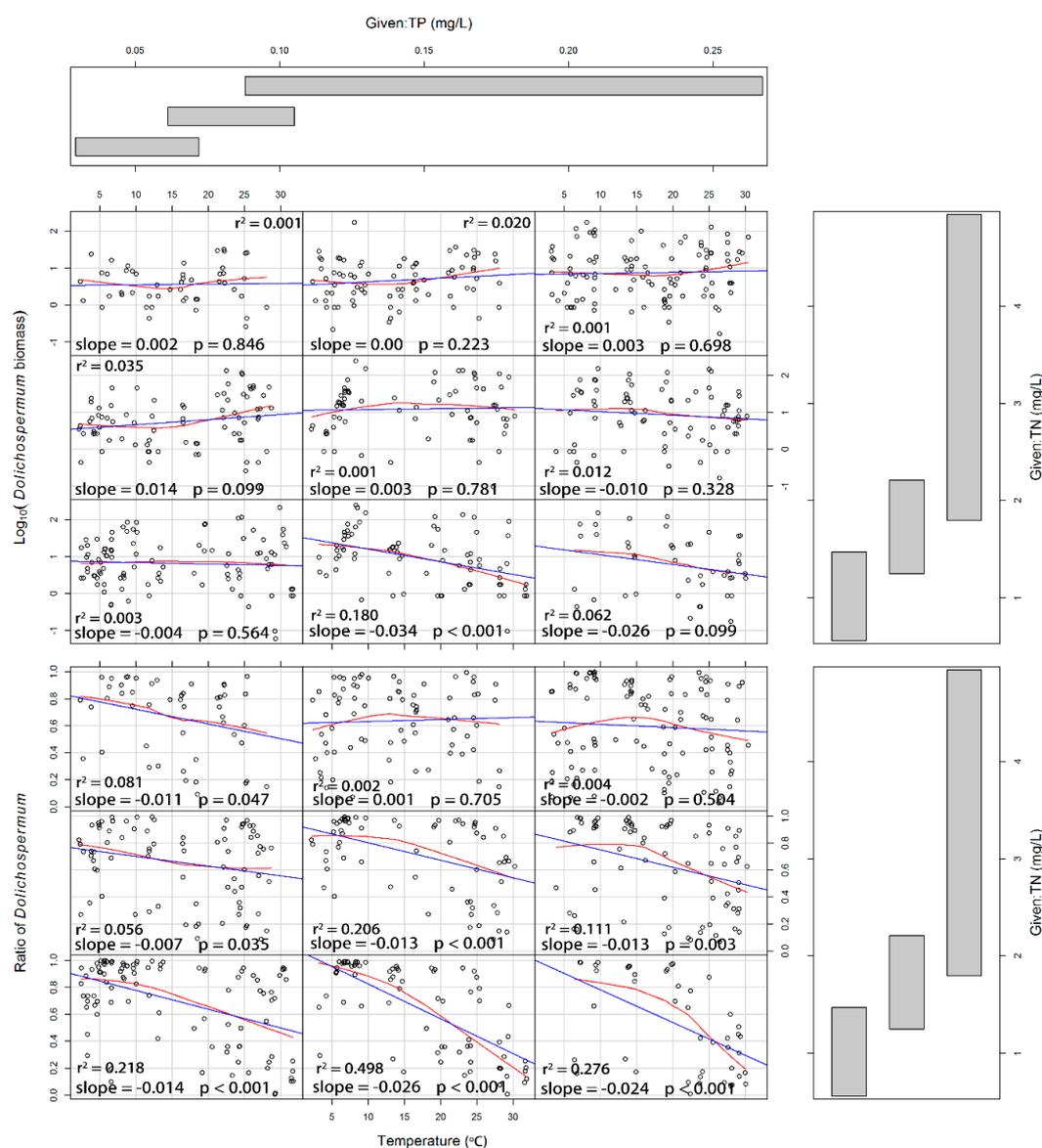


Figure 9. Relationships between water temperature and *Dolichospermum* biomass (top) or ratios (bottom) depending on total nitrogen (TN) and total phosphorus (TP), which was divided into three segments, on average, of the data numbers, with 30% overlap. The red and blue lines represent the LOESS and linear fits, respectively. The parameters of linear fit were shown in each panel.

4. Discussion

Recently, species succession among bloom-forming cyanobacteria has attracted increasing attention from researchers and managers of water bodies, and this succession varies with reductions of nitrogen and phosphorus during eutrophication mitigation process [4,23,25]. In this study, we performed a 7-year investigation of the spatial and temporal shifts between *Microcystis* and *Dolichospermum* in Lake Chaohu. We found that *Microcystis* mainly dominated the western region of the lake in summer, whereas *Dolichospermum* dominated in other regions and seasons. The shift between *Microcystis* and *Dolichospermum* in Lake Chaohu might be mostly led by *Microcystis*. If the temperature and phosphorus levels were relatively high, then *Microcystis* grew rapidly and competitively excluded *Dolichospermum*. If the nutrient levels, especially the phosphorus level, were low, then the exclusive power of *Microcystis* was weak, and *Dolichospermum* maintained its dominance, even on hot summer days.

4.1. Temperature

Diazotrophs, such as *Aphanizomenon*, were replaced seasonally by nondiazotrophs, such as *Microcystis*, which have been observed in many waterbodies [8,21,37]. However, the shift from *Dolichospermum* to *Microcystis* often showed different patterns. Generally, the two species co-exist or dominate different regions of one water body [6]. Sometimes, their succession was also not complete, and even *Dolichospermum* could not be replaced by *Microcystis* in Lake Chaohu [9,25]. In the study, we confirmed the failed succession with a 7-year investigation. Generally, the shift in *Microcystis* may be attributed to temperature adaptations [7,23], and that of filamentous N₂-fixing exhibited lake-specific responses [4]. We found that the *Microcystis* ratio increased, and the *Dolichospermum* ratio decreased, with increasing temperature and phosphorus levels, which was consistent with the previous findings that high temperature and phosphorus levels favor *Microcystis* dominance [13,14]. Li and Watanabe [10] found that most strains of *Dolichospermum* have a high optimal growth temperature, which was even similar to that of *Microcystis*, but the maximal growth rate of *Microcystis* was higher than that of *Dolichospermum* [11,12]. This may partially explain the high *Microcystis* biomass and the decrease in *Dolichospermum* biomass in summer. Bormans et al. found that the *Dolichospermum* population collapsed when the temperature was greater than 26 °C in the field [38]. However, the collapse did not occur in the eastern lake region, even in summer, when the surface water temperature was over 26 °C. Therefore, in Lake Chaohu, temperature could explain the shift from *Dolichospermum* to *Microcystis* in the western region of the lake, but was not able to explain the dominance of *Dolichospermum* in the eastern region in summer. In the study, we also found that a key temperature (~17 °C) will determine dominance of the two cyanobacteria. When the temperature was lower than the key temperature, *Microcystis* did not dominate the phytoplankton community. Whereas *Dolichospermum* was always the dominant cyanobacteria, and the ratio of *Dolichospermum* to total phytoplankton biomass might depend on nitrogen and phosphorus levels. Because the *Dolichospermum* ratio was low in the western lake region, where nitrogen and phosphorus levels were relatively high, the dominance of the two cyanobacteria appears to be determined by the interaction of nutrients and temperature, as the temperature here was higher than the key temperature.

4.2. Nutirents

The difference in the responses of the two cyanobacteria to phosphorus was also used to explain the shift from *Dolichospermum* to *Microcystis*. Wan et al. [25] found that during the process of replacement by *Microcystis*, *Dolichospermum* was adversely affected by phosphorus deficiency, while *Microcystis* never experienced P limitation due to its ability to rapidly take up and store phosphorus at low phosphorus levels. However, the phosphorus level was lower in the eastern region of Lake Chaohu than that in the western region. Although *Microcystis* had the advantage of using phosphorus, and increased the phosphorus deficiency of the coexisting phytoplankton species, the strategy did not support the pattern of *Microcystis* replacing *Dolichospermum*. Therefore, the phosphorus strategy might be insufficient to explain the dominance of *Dolichospermum* in the eastern lake region in summer. In addition, *Dolichospermum* was considered dominant when nitrogen was limiting or the TN/TP ratio was low, because *Dolichospermum* is able to fix N₂ when nitrogen is in short supply [19]. However, few heterocysts were observed in the sample dominated by *Dolichospermum*, suggesting that the bioavailable nitrogen might have been sufficient, so it was unnecessary to fix nitrogen by heterocysts [9]. Therefore, the variations in the TN/TP ratio might not be the primary factor affecting the shifts between the two cyanobacteria in Lake Chaohu.

According to our results, there was a significant positive relationship between TP and *Microcystis* biomass. It is possible that *Microcystis* biomass was limited by the relatively low TP in the eastern lake region. In addition, the *Microcystis* ratio increased and the *Dolichospermum* ratio decreased over the long-term, which was mainly due to the increase of the *Microcystis* ratio in the eastern lake region [39]. Furthermore, the increase in the *Microcystis* ratio might have been the result of the increase in TP and PO₄. The response of *Dolichospermum* biomass to temperature, TN and TP was weak when

nutrients were relatively low. The differences of the two cyanobacteria responding to phosphorus might maintained the dominance of *Dolichospermum* in the eastern region of Lake Chaohu in summer. The accumulation of biomass might be more important than other factors in terms of the shift between *Microcystis* and *Dolichospermum*, which depends on their phosphorus uptake kinetics. The phosphorus kinetic parameters of *Microcystis*, including the maximal uptake rate (V_{\max}) and half-saturation constant for uptake (K), were higher than those of *Dolichospermum* [40]. The higher V_{\max} and K indicate that *Microcystis* has a higher phosphorus requirement, and its growth response is more sensitive to a relatively large range of phosphorus concentrations. This explained the increase in *Microcystis* biomass and the ratio at high phosphorus levels in the study. In addition, microcystin is considered the primary factor that controls the growth of *Dolichospermum* [41]. In the study, the microcystin level produced by *Microcystis* might not have been the adequate dose to inhibit *Dolichospermum* growth, because the microcystin content might have been reduced by low nitrogen and *Dolichospermum* [42]. Therefore, the relatively low *Microcystis* biomass was not able to overcome *Dolichospermum* dominance, even with the microcystin.

4.3. Interaction among Temperature, Nitrogen and Phosphorus

The interaction among nitrogen, phosphorus and temperature plays an important role in dominance formation and the succession of specific bloom-forming cyanobacteria [5,26,43]. As mentioned above, when temperature was lower than the key temperature (~ 17 °C), the *Dolichospermum* ratio might depend on the nitrogen and phosphorus levels. According to the relationship between *Dolichospermum* biomass and nutrients under low temperature conditions, the low *Dolichospermum* ratio might be mainly correlated to the high nitrogen level, not the phosphorus level. However, when the temperature was more than approximately 20 °C, the *Dolichospermum* biomass and ratio increased with the increasing TN concentration, but had no significant relationship with TP concentration. This result indicated that the responses of *Dolichospermum* to TN were opposite under low and high temperature conditions, and the response of *Dolichospermum* to TP was not sensitive (Figure S5). Regosi et al. found that *Dolichospermum* was more sensitive to nutrients [26]. Our findings further indicated that it was sensitive to nitrogen, and its response to TN depended on temperature.

The response of *Microcystis* biomass to TP depended on TN and temperature, and *Microcystis* biomass increased faster with increasing TP concentrations under a higher temperature and TN concentration condition. However, its responses to TN and temperature did not depend (or did, but only weakly) on the other two variables according to the variation in the slopes of the relationships between *Microcystis* biomass and TN or temperature. These results suggested that although *Microcystis* biomass was affected directly by TN, TP and temperature, only the relationship between *Microcystis* biomass and TP was associated with TN and temperature. Our findings indicated that both *Microcystis* and *Dolichospermum* had different ways of responding to the interaction of temperature, nitrogen and phosphorus, which might contribute to their seasonal succession in Lake Chaohu.

5. Conclusions

Microcystis and *Dolichospermum* responded to variations in multiple environmental variables in multifaceted ways in the complex aquatic ecosystem of Lake Chaohu. From the perspective of the primary variables affecting cyanobacterial dynamics, it is evident that the shift between *Microcystis* and *Dolichospermum* in Lake Chaohu might be mostly led by *Microcystis*, depending on its response to temperature and phosphorus. However, the response of *Dolichospermum* was passive, due to its weak sensitivity to the variation in temperature and nutrients during the shift. Our results indicate that in eutrophic Lake Chaohu, minimizing phosphorus levels will lead to significant reductions in nondiazotroph *Microcystis* biomass. In addition, minimizing the nitrogen level will lead to significant reductions in the diazotroph *Dolichospermum* biomass at high temperatures and high phosphorus levels, and significant increases at low temperatures and low phosphorus levels. Our findings improve

the understanding of the shifts between *Microcystis* and *Dolichospermum* in eutrophic lakes, and will be helpful for the control of eutrophication and the management of nutrient loadings.

Supplementary Materials: The following materials are available online at <http://www.mdpi.com/2073-4441/12/7/1978/s1>, Figure S1: Long-term trends in total phosphorus (TP), total nitrogen (TN), orthophosphate (PO₄), ammonium (NH₄), nitrate + nitrite (NO_x) and transparency (SD) in the three lake regions of Lake Chaohu from 2012 to 2018. The solid lines represent the regression lines fitted with the LOESS. Figure S2: Relationships between total phosphorus and *Microcystis* biomass depending on total nitrogen and temperature, which was divided into three segments on average of the data numbers with 30% overlap. The red and blue lines represent the LOESS and linear fits, respectively. The parameters of linear fit were shown in each panel. Figure S3: Relationships between total nitrogen and *Microcystis* biomass depending on total phosphorus and temperature, which was divided into three segments on average of the data numbers with 30% overlap. The red and blue lines represent the LOESS and linear fits, respectively. The parameters of linear fit were shown in each panel. Figure S4: Relationships between total phosphorus and *Dolichospermum* biomass depending on total nitrogen and temperature, which was divided into three segments on average of the data numbers with 30% overlap. The red and blue lines represent the LOESS and linear fits, respectively. The parameters of linear fit were shown in each panel. Figure S5: Relationships between total nitrogen and *Dolichospermum* biomass depending on total phosphorus and temperature, which was divided into three segments on average of the data numbers with 30% overlap. The red and blue lines represent the LOESS and linear fits, respectively. The parameters of linear fit were shown in each panel.

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