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Reducing nutrient increases diatom biomass in a subtropical eutrophic lake, China–Do the ammonium concentration and nitrate to ammonium ratio play a role?

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ABSTRACT

Response of aquatic organisms to eutrophication have been well reported, while less studies are available for the recovery of eutrophic lakes following a reduction in the external loading, especially for systems where nitrogen is reduced but the phosphorus concentration is maintained high due to internal loading. Diatoms are nitrate (NO₃-N) opportunists but can also use ammonium (NH₄-N). They may, therefore, be more sensitive to nitrogen reduction than other algae that typically prefer NH₄-N. We document the variations of nutrients and diatoms in subtropical, eutrophic Lake Taihu over 28 yr during which a reduction of the external loading resulted from lake management. According to the results of change point analysis, data on environmental variables were divided into two periods (P1: 1992-2006; P2: 2007-2019) with two different seasons (WS: Winter-Spring; SA: Summer-Autumn), respectively. Compared with P1-WS, the concentration of NH₄-N decreased significantly whereas NO₃-N showed no significant change in P2-WS. In contrast, NH4-N concentrations were low and showed no significant changes in P1-SA and P2-SA and NO₃-N decreased significantly in the latter period. Accordingly, NO₃-N: NH₄-N mass ratios in P1-SA and P2-WS were all significantly higher than those in P2-SA and P1-WS, respectively. The biomass of WS diatom increased significantly and the timing of the peak biomass shifted from P1-SA to P2-WS since 2007. The SEM analysis showed that NO₃-N was retained as a statistically significant predictor for diatom biomass in P1-SA and significant effects of windspeed, zooplankton and NH₄-N on diatom biomass in P2-WS. Windspeed and zooplankton have further changed the biomass of diatoms in the case of declining inorganic nitrogen. We conclude that the magnitude of vernal suppression or stimulation of diatom assemblages has increased, concomitant with the variations of NH₄-N and NO₃-N: NH₄-N mass ratios. Diatoms response to NH₄-N or NO₃-N is apparently changing in response to water temperature in this eutrophic shallow lake. Thus, parallel reductions in external nitrogen loading, along with variations in dominant inorganic nitrogen, will stimulate the growth of diatom and therefore increase the total biomass of phytoplankton in still high internal phosphorus loading, which is should be regarded as a good sign of restoration measures.

1. Introduction

Aquatic ecosystems in densely populated areas have been negatively affected by eutrophication, many of them driven by increasing external nutrient loading from domestic sewage, industrial waste water and agricultural diffuse sources (Michalak et al., 2013; Tong et al., 2020). Common symptoms of eutrophication include turbid water, dense algal blooms, loss of biodiversity and loss of recreation and esthetic value (Huisman et al., 2018; Ho et al., 2019). To mitigate these negative

effects of eutrophication, the Chinese government is working to reduce nutrient inputs and restore the functionality of lake ecosystems since 2000 (Zhou et al., 2017; Fu et al., 2021). However, eutrophication remains a major problem, either because external loading has not been reduced to sufficiently low levels or because of internal lake mechanisms, chemical or biological, that prevent or delay recovery (Søndergaard et al., 2007; Van Meter et al., 2018; Xu et al., 2021).

Reduction in external loading of phosphorus (P) and nitrogen (N) might be accompanied by a delay in lake recovery because internal

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loading continuously replenishes the P pool in the water column (Jeppesen et al., 2005a, b; Anneville et al., 2019; Ibáñez and Peñuelas, 2019). Based on previous studies, a quick response of the total nitrogen (TN) concentration has been observed in shallow lakes, because N loss by denitrification results in negligible internal N loading (Søndergaard et al., 2007; Shi et al., 2022). There have also been several summaries of biotic responses to reductions in nutrient loading, such as the biomass of planktivorous fish declined and the proportion of potential piscivores increased, zooplankton biomass decreased, Secchi transparency increased and therefore submerged macrophytes appeared (Mitchell and Perrow, 1997; Jeppesen et al., 2005a, b; Ibáñez and Peñuelas, 2019). Typically, the phytoplankton community has responded as well. The contribution of non-heterocystous cyanobacteria decline substantially, while N₂-fixing cyanobacteria, dinophytes, cryptophytes and chlorophytes increased (Jeppesen et al., 2002, 2005a, b; Moss et al., 2005). An open question is how a delay in the P reduction in lakes but fast response of N will affect the phytoplankton community response. N₂-fixing cyanobacteria can fix sufficient N to offset a reduction of N-inputs (Schindler et al., 2008; Shatwell and Köhler, 2019). Thus, emphasis has been placed on N₂-fixing cyanobacteria how it responds to N reduction or low external N concentration, whereas other phytoplankton taxa received much less attention.

Anthropogenic activities are altering both total nutrient loads, and they are also changing the dominant form of N nutrient delivered to aquatic ecosystems (Glibert et al., 2016). Whether different chemical forms of N influence primary producers differentially, are well documented in marine and freshwater ecosystems (Berg et al., 2003; Donald et al., 2011; Andersen et al., 2020). Diatoms are widely distributed in almost all aquatic habitats, and have been estimated to contribute approximately 20% of global primary productivity (Falkowski et al., 1998; Field et al., 1998; Kong et al., 2021). It has been suggested that diatoms were the only phytoplankton group significantly associated with the uptake of nitrate (NO3-N), because they may have enhanced NO3-N handling and assimilatory efficiency relative to other algae (Lomas and Glibert, 2000; Needoba and Harrison, 2004; Andersen et al., 2020). While the classical physiological literatures conclude that ammonium (NH₄-N) is the preferred form of N for phytoplankton (McCarthy, 1981; Raven et al., 1992). The favorability for NH₄-N by phytoplankton is a function of both the preferential use of NH₄-N and its favorable energetics, and the repressive effect of NH4-N on NO3-N uptake and assimilation (Dortch, 1990; Lomas and Glibert 1999a; 1999b). It has also been documented that under conditions of highly elevated NH₄-N, both the TN taken up and overall growth with NH₄-N enrichment can be suppressed rather than enhanced (Glibert et al., 2014; Swarbrick et al., 2019).

Lake Taihu is the third largest shallow eutrophic lake, where non N2fixing cyanobacteria (Microcystis spp.) dominated in summer over the past three decades (Liu et al., 2011; Guo et al., 2019). Until recently, the large diatoms (e.g., Aulacoseira spp., Synedra spp. and Asterionella spp.) and small cryptophytes (Cryptomonas spp. and Chroomonas spp.) and green algae (Dictyosphaerium sp.) increased in spring (Guo et al., 2019). Since the highly publicized drinking water crisis in 2007 (Guo, 2007), a variety of restoration measures were implemented to control eutrophication, including construction of sewage pipelines and new wastewater treatment plants (Qin et al., 2019). To date, TN has declined significantly and total phosphorus (TP) has slightly increased, and cyanobacterial blooms have not decreased in size and frequency as expected from abatement efforts (Qin et al., 2019; Qin et al., 2020). At the same time, the frequency of spring diatom bloom increased in Lake Taihu (Liu X., unpublished data). Previous studies have emphasized that the changes in the spring phytoplankton assemblage in the lake are influenced by climate change (Guo et al., 2019; Deng et al., 2018; 2019; 2021). In addition, diatoms can provide most of the high quality food for zooplankton growth in spring and thus their abundance depend strongly on the reproduction of predators (Pagano, 2008).

However, it is generally agreed that N and P are linked to core

biology process, which constrain biotic production of aquatic ecosystems (Elser et al., 2007; Conley et al., 2009; Liang et al., 2020). Glibert et al. (2016) confirmed that diatoms appear to be disproportionately affected by ratios of NO₃-N with NH₄-N. In Lake Taihu, the concentration of dissolved inorganic N (mainly composed by NO₃-N and NH₄-N) gradually decreased since 2007 (Zhu et al., 2020). Thus, we hypothesized that: (1) the effects of N concentration on diatom in the different seasons varied with dominant inorganic N (NO₃-N and NH₄-N) in Lake Taihu; (2) diatoms as reliable indicators of ecosystem response to N reduction, especially to changing of dominant inorganic N in eutrophic lakes. Here, a large, long-term intensive monitoring data set of nutrient concentrations and phytoplankton for the period 1992–2019 available in the northern part of Lake Taihu were analyze. An investigation of 48 shallow lakes on the Yangtze River Plain was also utilized to examine these hypotheses.

2. Methods

2.1. Description of the study area

The Middle-Lower Yangtze plain, one of the three largest plains in China, contains numerous shallow eutrophic lakes driven by nutrient enrichment (Yang et al., 2008). Lake Taihu is located in the eastern region of the Yangtze River delta $(30^{\circ}56' - 31^{\circ}34'N)$ $119^{\circ}54'-120^{\circ}35'E$). It is a polymictic lake, with a mean depth of 1.95 m, a surface area of 2338 km², a catchment area of 36,500 km². Annual freshwater input to the lake averages 88×10^8 m³, and the average water retention time is approximately 220-309 days (Qin et al., 2007). Meiliang Bay is located in the northern part of Lake Taihu near the suburbs of Wuxi city and has an area of 132 km². The Liangxi and Lujiang Rivers, which are connected to Meiliang Bay, discharge effluents from the cities of Wuxi and Changzhou into the lake. In response to heavy industrial and agricultural pollution, bloom-forming cyanobacteria in Meiliang Bay have been frequently observed in recent decades. The lake basin is influenced by the East Asian monsoon and exhibits a typical subtropical climate with four distinct seasons: spring (March--May), summer (June-August), autumn (September-November) and winter (December-February).

In the present study, the biomass of diatom in 48 shallow lakes with different nutrient levels were surveyed in spring 2012. The lake area and mean depth ranged from 1.3 to 80.4 km² and 0.6 to 7.1 m, respectively. There were three sampling sites in each lake. Detailed information on the sample locations and the geographical information can be found in Fig. S1 and Table S1.

2.2. Sample collection and laboratory analysis

Data originate from an ongoing long-term monitoring program in the Taihu Laboratory for Lake Ecosystem Research. The analysis focuses on the period 1992–2019, during which the physical, chemical, and biological data have been collected at monthly intervals. Samples of water quality and diatoms were collected from 2 sites (S1 and S2; Fig. S2) in Meiliang Bay of northern Lake Taihu. The water samples were collected with a 2.5 L modified Patalas bottle sampler. At each site, water samples were taken by mixing the surface (0.5 m), middle, and bottom (0.5 m above the bottom) layers of the water column.

Monitored water quality parameters included water temperature, total nitrogen (TN), TP, nitrate (NO₃-N), ammonium (NH₄-N), orthophosphate (PO₄-P), silicate (Si), and chlorophyll *a* (Chl *a*). Water transparency was determined using a Secchi disk. Detailed analytical methods for water quality parameters are described in Jin and Tu (1990). The radiation network in eastern China comprises three stations, Shanghai, Nanjing, and Hangzhou (Zhang et al., 2004), and mean global radiation values for Lake Taihu were calculated. Air temperature and windspeed were obtained from the China Meteorological Data Sharing Service System (http://data.cma.cn/, Wuxi meteorological station

(31.58°N, 120.32°E)).

Phytoplankton samples (1 L) were fixed with Lugol's iodine solution (2% final concentration) and settled for 48 h. Then, the supernatant was slowly removed by siphoning, and 30 mL was retained for measuring cell density. Cell density was microscopically determined in a 0.1 mL Sedgwick-Rafter counting chamber at × 400 magnification (Olympus BX51, Japan). The mean cell volume was calculated using appropriate geometric calculations and volume values were converted to biomass assuming that 1 mm³ of volume was equivalent to 1 mg of fresh-weight biomass (Hillebrand et al., 1999). In these samples, the total biomass of phytoplankton from 2015 to 2019 was calculated by the formula 0.003 Chl *a* + 8.76, which was recorded in Chen et al. (1998). All diatom sample preparations followed standard procedures (Battarbee et al., 2002). Nomenclature and taxonomy mainly followed Krammer and Lange-Bertalot (1988a, b; 1991a, b; 2000).

Zooplankton samples (7.5 L) were concentrated with a 64- μ m mesh net and preserved in 4% formaldehyde at a final volume of 50 mL. The zooplankton samples were analyzed at \times 40 magnification with a microscope (Olympus Corporation, Tokyo, Japan) and identified to species or genus level (Zhang and Huang, 1991).

The detailed methods for sampling, water quality analysis and diatom identification used in 48 shallow lakes were the same as those in Lake Taihu.

2.3. Data analysis

Annual mean air temperature showed a significant increase (p < 0.05) and water temperature did not correspond to the air temperature in Lake Taihu (Fig. S3). Compared with air temperature, the growth of phytoplankton was much more directly influenced by water temperature in lakes (Anderson, 2000). Thus, water temperature was used in our study.

The threshold of Si concentration was selected as: 0.5 mg/L. This corresponded to the Si concentration at which the growth of diatom was limited (Lund, 1964). Prior to analysis, a range of lakes with Si concentrations lower than 0.5 mg/L was excluded (Fig. S4).

The total diatom biomass mainly covered the samplings from late December to April and June to October. Thus, "Winter-Spring (WS)" was defined as late December to April and "Summer-Autumn (SA)" as June to October in our analysis. We estimated the timing of the seasonal peak throughout the entire year (the central tendency, *T*) using the month coordinate of the center of gravity of the area below graphs of monthly means:

$$T = \frac{\sum_{i=1}^{12} M.x_m}{\sum_{i=1}^{12} x_m}$$

where x_m is the mean biomass in month *M* (January = 1, ..., December = 12) (Edwards and Richardson, 2004).

We examined whether diatom influence where the thresholds occur responded to changes in TN and TP in the water column, using a change point analysis (the package "*strucchange*" in *R*) of diatom biomass and the timing of the peak in their biomass. A function named "breakpoints" was used to determine the locations of the changes. The sequential *F*-statistic was performed to test for a change in the mean and the peak *F* value indicated a shift. The present analysis focuses on the variations of total diatom.

Trends in plankton biomass and abundance were evaluated using the nonparametric Mann-Kendall (MK) trend test (Helsel and Hirsch, 1992), because changes over time did not appear to be linear. Multiple comparison analysis of ANOVA and box plots were used to test whether significant differences observed in environmental factors between seasons. The *agricolae* and *stats* packages in *R* were conducted in this analysis. The linear regression was used to estimate the differences between the peak of total diatom in 1992 and 2019. A strong negative difference indicates the peaks are becoming earlier (Edwards and

Richardson, 2004). Theil-Sen regression was used to robustly explore the effects of NO3-N: NH4-N mass ratios on total diatom biomass in Lake Taihu and the 48 other lakes. Theil-Sen estimation is a robust method that determines the slope of the regression line via the median of the slopes (Su et al., 2020). The mblm package in R was used to perform this analysis. Structural equation modeling (SEM) was used to explore the pathways by which windspeeds, nutrients and zooplankton affects diatom biomass and to test diatom respond to which forms of inorganic N in different seasons. We first constructed a full model that included possible pathways; then, most of nonsignificant pathways were eliminated to optimize the model. SEM, which involved sets of multiple regression analyses, allowed a rigorous estimation of the causal relationship network (Grace, 2006). The standardized path coefficients between two variables represented the relative strength of a relationship. We used a *chisq* test and *p* values to evaluate the fit of the models (e.g. models were considered to have a good fit when the *p* values ranged from 0.1 to 1). Higher temporal variation of phytoplankton communities than spatial heterogeneity in Meiliang Bay of Lake Taihu (Guo et al., 2019). The SEM thus was performed based on the spatial average data pooled from the two sampling sites. lavaan and semPlot packages were used in the SEM analysis. Biological data were $\log_{10}(x + 1)$ -transformed before the Theil-Sen analysis and all the variables were $\log_{10}(x + x)$ 1)-transformed before the SEM analysis. We conducted hierarchical partitioning to estimate the percent contribution to the explained variance by the individual predictors examined using the hier.part package within R (Mao et al., 2020).

3. Results

3.1. Long-term trends in environmental variables

From 1992 to 2019, mean WS and SA water temperatures were 9.3 \pm 1.2 °C and 25.7 \pm 0.8 °C, respectively (Fig. S5). Water temperature in Lake Taihu was generally significantly higher in SA season than in WS season (Fig. 1). No pronounced changes were found in water temperature in any of the seasons (p > 0.05, Table S2). Windspeeds declined significantly both in WS and SA seasons. Mean radiations around Lake Taihu were 972 \pm 96 MJ/m² in WS season and 1112 \pm 81 MJ/m² in SA season. It was significantly higher in SA than in WS. Increasing trends appeared in radiations in WS season.

Concentrations of TP averaged 0.10 \pm 0.04 mg/L and 0.15 \pm 0.04 mg/L, respectively, in Lake Taihu during WS season and SA season in all years (Fig. S6). TP decreased significantly in WS season, whereas it increased significantly in SA season (p < 0.001, Table S2). The seasonal variation of PO₄-P exhibited the same trends as TP.

Concentrations of TN averaged 3.96 \pm 1.40 mg/L and 2.37 \pm 0.50 mg/L, respectively, in the lake during WS season and SA season in all years (Fig. S6). TN exhibited significant seasonal and interannual variations, and the concentration was substantially higher in WS seasons than in SA seasons (Fig. 2). Both NO₃-N and NH₄-N concentrations were significantly higher in WS seasons (mean NO₃-N = 1.11 \pm 0.48 mg/L; mean NH₄-N = 1.18 \pm 0.91 mg/L) than in SA seasons (0.40 \pm 0.20 mg/L; 0.29 \pm 0.14 mg/L). Decreasing trends appeared in TN and NH₄-N in WS season. For NO₃-N, decreasing trends appeared in SA season.

3.2. Change point analysis and comparison of environmental variables in different periods

Changes in diatom biomass and the timing of the peak in their biomass were detected through change point analysis and sequential *F*statistic (Fig. S7). Results showed that breakpoints of diatom biomass and the timing of peak biomass were in 2008 and 2007, respectively. Thus, data on environmental variables were divided into two periods ("P1" and "P2") for the subsequent analysis. "P1" refers to the years from 1992 to 2006 and "P2" refers to the years from 2007 to 2019.



Fig. 1. Multiple comparison analysis of ANOVA and box plots for physical and meteorological factors (water temperature, windspeed and global radiation) in the Meiliang Bay of Lake Taihu. Letters "a, b, ab and ba" indicate significant differences between seasons (p < 0.01), and letters "ns" indicate non-significant differences between seasons (p > 0.05). Labels "WS" and "SA" mean "Winter-Spring" and "Summer-Autumn", respectively.

For WS seasons, mean windspeed was 2.8 ± 0.2 m/s during P1 and significantly lower (2.4 ± 0.3 m/s) in P2. It was significantly higher in P1 (mean value = 2.8 ± 0.2 m/s) than in P2 (2.3 ± 0.2 m/s) for SA seasons. Windspeeds exhibited no significant difference between WS and SA seasons in the same period (Fig. 1). Compared to the WS season

in P1, radiation was significantly higher than that in the same season in P2, while there was no significant difference among periods in the SA season.

There was no significant difference in TP concentration between P1-WS and P1-SA, but it showed significantly lower concentration in P2-WS (mean value = 0.08 ± 0.02 mg/L) than in P2-SA (0.17 ± 0.03 mg/L) (Fig. 2). During WS season, TP concentration was significantly higher in P1 than that in P2. For SA season, it was much lower in P1 than in P2. PO₄-P concentration showed a similar pattern to that of TP concentration, but it was no significant difference between P1-WS and P2-WS.

During P1-WS, the mean value of TN concentration was 4.74 ± 1.17 mg/L, which was significantly higher than that during P2-WS (3.07 \pm 1.06 mg/L). No significant difference was observed in TN between the two SA seasons. NO₃-N concentration showed significantly higher value during SA season in the P1 (mean value = 0.52 ± 0.16 mg/L) than in the P2 (0.26 ± 0.14 mg/L), while there was no significant difference between WS seasons. The concentration of NH₄-N averaged 1.73 ± 0.81 mg/L in the P1-WS, which was significantly higher than in the P2-WS and other two SA seasons. In SA season, NH₄-N concentrations showed no significant difference between P1 and P2.

Mass ratios of NO₃-N: NH₄-N were usually much higher in P1-SA and P2-WS than in the other periods (Fig. 2). Average values for P1-SA, P2-WS, P2-SA, and P1-WS were 2.3 \pm 1.6, 2.7 \pm 1.8, 1.0 \pm 0.4 and 1.0 \pm 0.8 by weight.

3.3. Temporal change in plankton biomass and abundance

The main genus of diatom Asterionella spp., Aulacoseira spp., Cyclotella spp., Synedra spp., and Nitzschia spp. were observed and identified in Lake Taihu from 1992 to 2019 (Fig. S8). The WS diatom biomass in all years ranged from 0.01 to 7.08 mg/L, with an average of 1.40 mg/L. The SA diatom biomass varied from 0.09 to 2.78 mg/L, with an average of 0.89 mg/L. Seasonal and interannual changes in diatom showed that WS biomass was low from 1992 to 2006, after which an increasing trend was apparent (Fig. 3a). The trends of total phytoplankton biomass followed the same pattern as diatom, increasing significantly in the WS season (Fig. 3b). No pronounced changes were found in SA season (MK trend test: p > 0.05). The contribution of diatom to total phytoplankton biomass varied from 7% to 62% in WS, with no clear trend over time (Fig. 3c). Significant decline in the proportion of diatom biomass appeared in SA season. However, during the study period the timing of peak diatom biomass showed a significant advancement (Fig. 4b). It fluctuated widely, but generally appeared in SA season prior to 2007. Thereafter, the timing of the peak biomass advanced from the SA season to the WS season, except 2016 and 2018. During P1, the peak diatom biomass appeared in June (Summer-Autumn, Fig. 4a), but in March (Winter-Spring) during P2. The peak in P1-SA was significantly lower than in P2-WS (F-test, p < 0.001). There was also a slight peak of diatom biomass in August (Summer-Autumn) during P2, but no significant difference was found in peak biomass between August (P2-SA) and June (P1-SA) (*F*-test, p = 0.25).

The abundance of zooplankton in WS fluctuated between 100 and 1363 ind./L from 1999 to 2019, without showing any clear pattern (Fig. 5). In SA season, however, the zooplankton abundance decreased significantly (p < 0.001).

3.4. Potential drivers of changes in diatom biomass

Because the timing of the peak diatom biomass appeared in the P1-SA and P2-WS (Fig. 4b), they were chosen in the SEM analysis, which tried to show the effects of N on diatom in different temperature. Significant effects of windspeed, NH₄-N concentration, and zooplankton abundance on diatom biomass were found for the P2-WS (Fig. 6). Only NO₃-N was retained as a statistically significant predictor for diatom biomass in SA in the SEM analysis. NO₃-N: NH₄-N ratios had significant and positive effects on diatom biomass, with biomass advancing linearly



Fig. 2. Multiple comparison analysis of ANOVA and box plots for nutrients (TP–Total phosphorus, PO₄-P–Orthophosphate, TN–Total nitrogen, NO₃-N–Nitrate, NH₄-N–Ammonium) and mass ratios of NO₃-N:NH₄-N in the Meiliang Bay of Lake Taihu. Letters "a, b, ab and ba" indicate significant differences between seasons (p < 0.01), and letters "ns" indicate non-significant differences between seasons (p > 0.05). Labels "WS" and "SA" mean "Winter-Spring" and "Summer-Autumn", respectively.

with increasing NO₃-N: NH₄-N ratios in Lake Taihu and in the 48 other lakes (Fig. 7).

4. Discussion

Globally, diatoms are used in biomonitoring to assessing the ecological status of aquatic systems because they have a cosmopolitan nature, short life span and quick response to environmental and anthropogenic disturbances (Malviya et al., 2016; Pandey et al., 2017). Here, we found that diatom increased greatly in WS season and apparently responded differently to different dominant inorganic N and seasons during the process of the external loading reduction of a eutrophic lake.

4.1. Diatom responses to the N reduction in different seasons

The N but not P concentrations changed markedly in Lake Taihu after 2007, confirming that TN can respond quickly to nutrient management in the watershed (Jeppesen et al., 2005a, 2005b; Søndergaard et al., 2007). The concentrations of TN as well as NO₃-N and NH₄-N declined, especially in WS season. Despite the significant reduction of TN, the lake was still eutrophic with the mean value above the hypereutrophic of TN and TP concentration thresholds (TN > 1.4 mg/L, TP > 0.05 mg/L, USEPA, 2009). Moss et al. (2005) and Schindler et al. (2008) demonstrated that N₂-fixing cyanobacteria appeared in significant abundance within weeks of reducing inputs of TN at constant inputs of TP. A recently analysis of data from Lake Taihu has also shown the biomass proportion of N₂-fixing cyanobacteria increased, which corresponded to deceased TN: TP mass ratios after 2007 (Zhang et al., 2021). Klausmeier



Fig. 3. Long-term changes of diatom biomass, total phytoplankton biomass, and contribution of diatom to total phytoplankton biomass in Winter-Spring (WS) and Summer-Autumn (SA) in the Meiliang Bay of Lake Taihu. Trends of biomass estimated by Mann-Kendall (MK) trend tests (τ). Significant effects are marked by asterisks (p < 0.001 (***), p < 0.01 (**), and p < 0.05 (*)) and without asterisk (p > 0.05).

et al. (2004) reported that Redfield TN: TP ratio of 7.5 is not a universal biochemical optimum, and the optimal TN: TP stoichiometry of algae ranges from $3.7 \sim 20.3$ by weight. Although annual mean TN: TP mass ratios reduced to 21 (near to optimal TN: TP) in P2, TN: TP mass ratios averaged 39 (higher than optimal TN: TP) in WS season and showed no significant difference between P1-WS (averaged 42) and P2-WS (averaged 37) (p > 0.05, Fig. S9). Our results revealed that it was not changes in TN: TP that triggered an increase in the biomass of spring diatoms in Lake Taihu. In the early study of diatoms, they did not find the significant relationships between diatom proportion of total phytoplankton and TN: TP in the lake (McCarthy et al., 2009). Thus, we can speculate that a decrease in N loading, and the concomitant decrease in NH₄-N concentrations in WS season, may have driven spring increase in diatoms in Lake Taihu.

The lower energetic costs of uptake and assimilation of NH₄-N has led to the common assumption that NH₄-N is generally used first by phytoplankton, especially when N is in low concentrations, but the preference for NH₄-N is not universal (Dugdale et al., 2012; Glibert et al., 2016). Hence, phytoplankton inhibition by NH₄-N enrichment has been reported in many aquatic ecosystems such as the Delaware Estuary in the United States (NH₄-N: 0.18 mg/L, Yoshiyama and Sharp, 2006), the San Francisco Bay Delta (NH₄-N: $0.02\sim0.29$ mg/L, Wilkerson et al., 2006), Buffalo Pound Lake, and Wascana Lake in Canada (NH₄-N: ca. 1.5 mg/L, Waiser et al., 2011; Swarbrick et al., 2019) and it has been confirmed experimentally (Parker et al., 2012). NH₄-N as one of the most important predictors with negative effects on total algae and cyanobacteria, which was also suggested in the previous study of Lake Taihu (Mao et al., 2020). Our data indicate a suppression of diatoms at high concentrations of NH₄-N (1.73 ± 0.81 mg/L) during P1-WS, being more pronounced than for other phytoplankton, including cryptophytes and green algae (Fig. S10, Swarbrick et al., 2019). Exposure to high NH₄-N reduce the growth of diatoms by enhancement of energetic costs in cells through photorespiration (Raven, 2011). Importantly, when the diatom is provided with NH₄-N only, photorespiration responses to other stress factors, such as cold temperatures, can increase significantly (Kangasjärvi et al., 2012).

By contrast, diatoms are recognized as being NO₃-N opportunists. They can take up NO₃-N as nutritional requirement or use it as an oxidant to dissipate the periodic overflow of electron energy and prevent photoinhibitory (Lomas and Glibert, 1999a). This is attributed to the special N transport and assimilation pathways in diatom cells, such as an easily induced nitrate reductase (Blomqvist et al., 1994), higher density



Year

Fig. 4. Changes in diatom biomass in the Meiliang Bay of Lake Taihu. (a) seasonal cycles for diatom during the periods 1992–2006 and 2007–2019; (b) inter-annual variability of the seasonal peak for diatom from 1992 to 2019. The linear regression in (b) was used to estimate the difference between the seasonal peak in 1992 and 2019. A negative difference between 1992 and 2019 indicates seasonal peaks are becoming earlier.

of NO₃-N uptake transporters (Glibert et al., 2016), a capacity to store NO₃-N in internal vacuoles (Lomas and Glibert, 2000), and the ability to respire NO₃-N under dark or anoxic conditions (Kamp et al., 2011). However, the pathway of NO₃-N uptake and assimilation varies with environmental conditions. For example, low temperature may contribute to excess in electrons because the light reactions of photosynthesis are relatively temperature-insensitive, but Calvin Cycle reactions are temperature-sensitive leading to low electron transfer rates

in cells (Lomas and Glibert, 1999a). Assimilative NO_3 -N reduction can be used as a protection mechanism to buffer these excess electrons and dissipate high energy from light harvesting (Glibert et al., 2016).

Under low water temperature (9.3 \pm 1.2 °C) in P1-WS in Lake Taihu, high NH₄-N with low NO₃-N: NH₄-N may, therefore, have disfavored the growth of diatoms. With the decrease of NH₄-N and increase of NO₃-N: NH₄-N in P2-WS, NO₃-N use may not have been inhibited by NH₄-N and NO₃-N uptake might have been an important pathway for energy



Fig. 5. Long-term changes of zooplankton abundance in Winter-Spring (WS) and Summer-Autumn (SA) in the Meiliang Bay of Lake Taihu. Trends of biomass estimated by Mann-Kendall (MK) trend tests (τ). Significant effects are marked by asterisks (p < 0.001 (***), p < 0.01 (**), and p < 0.05 (*)) and without asterisk (p > 0.05).



Fig. 6. Structural equation model (SEM) of windspeed, zooplankton and nutrients (PO₄-P–Orthophosphate, NO₃-N–Nitrate, NH₄-N–Ammonium) effects on diatom biomass in Summer-Autumn (SA) P1 (1992–2006) and Winter-Spring (WS) P2 (2007–2019). The thickness of each significant path represents the magnitude of the standardized regression coefficient or effect size; positive or negative values are given on the arrows. Significant effects are marked by asterisks (p < 0.001 (***), p < 0.01 (**), and p < 0.05 (*)). The dashed arrows represent non-significant paths (p > 0.05 (n.s.)).

dissipation at low temperatures (Glibert et al., 2016). Low NH₄-N thus have been used as nutritional requirement instead. Thus, we speculate that the absorption of NH₄-N and the protection mechanism of NO₃-N jointly promoted the increase of diatom biomass in P2-WS. Under high water temperature (25.7 ± 0.8 °C) in P1-SA, photorespiration increases under conditions of high light and high temperature (Kangasjärvi et al., 2012). Calvin Cycle reactions of photosynthesis are no longer limited by temperature and able to utilize the higher daily light doses for higher photosynthetic productivity (Smith and Platt, 1985; Descolas-Gros and

de Billy, 1987; Lomas and Glibert, 1999a). It means that electrons from light harvesting can be used promptly in Calvin Cycle reactions. The protection mechanism of NO₃-N may be no longer needed in cells. NO₃-N use is therefore not expected to be inhibited by low NH₄-N concentrations at high NO₃-N: NH₄-N. Under the condition of low N in summer, cyanobacteria and chlorophytes were more strongly stimulated by NH₄-N whereas diatoms were often equally, or more strongly, stimulated by NO₃-N (Andersen et al., 2020). Diatoms may have weak competition to utilize NH₄-N in summer when the cyanobacteria



Fig. 7. Effects of the NO₃-N–NH₄-N mass ratios on diatom biomass in the Meilaing Bay of Lake Taihu and 48 other shallow lakes along the Yangtze River. Slopes of the regression line were determined by the Theil-Sen slope estimator.

prevailed in Lake Taihu. NO_3 -N thus have been used as nutritional requirement for diatoms in this period.

We also found that the increased spring diatoms biomass coincided with the increased NO₃-N: NH₄-N mass ratios in 48 shallow lakes in the Yangtze River region being concurrent with the findings for Lake Okeechobee (McCarthy et al., 2009). Our findings on N suppression or stimulation are consistent with results of short-term nutrient enrichment studies (Lomas and Glibert, 1999; Glibert et al., 2014; Shangguan et al., 2017) and month-long mesocosm experiments (Donald et al., 2011; Swarbrick et al., 2019), all of which identify unique effects of N in P-rich ecosystems.

4.2. Other potential drivers of diatom biomass

Light availability and resources conditions (e.g., P and Si) potentially control diatom have been well characterized (Reynolds, 1984). Diatom can grow at very low irradiances (< 1 μ E/m² s, Richardson et al., 1983), showing physiological advantage in waters with high levels of mixing. During our study period, the light intensities in spring were always higher than 46 μ E/m² s (Liu X., unpublished data), implying that the light demand of diatoms was met. Although light radiation increased in P2-WS and a reduced windspeed may also have increased the light availability in the water column in spring (Guo et al., 2019; Deng et al., 2021), water transparency (indicated as underwater light conditions, Fleming-Lehtinen and Laamanen, 2012) was not significantly improved in Lake Taihu (Fig. S11). Our results confirmed that the conditions of decreased windspeed and increased light radiation could favor the growth of diatoms when the concentration of NH₄-N was reduced in Lake Taihu.

Diatom, being a good competitor at low PO₄-P concentrations (< 0.001 mg/L), benefit of low P concentrations (Tilman and Kilham, 1976; Sommer, 1983; Interlandi et al., 1999; Bertrand et al., 2003; Rühland et al., 2010). Helliwell et al. (2021) showed that P-limited diatoms use a Ca²⁺-dependent signaling pathway to sense and respond to the critical macronutrient P. Diatom recovery from P limitation requires rapid and substantial increases in N assimilation and this process is dependent on $P-Ca^{2+}$ signaling. $P-Ca^{2+}$ signaling thus mediates fundamental cross-talk between the vital nutrients P and N (Thamatrakoln, 2021). Because of P release in sediments (Jeppesen et al., 2005 a, b), TP has remained stable in the water column and even increased in SA season after external loading reduction since 2007 in Lake Taihu. Thus, it is not likely that diatom growth was limited by P and inorganic N assimilation was affected in P enriched water column. Additionally, Si concentrations were above the growth limiting level for diatoms of 0.5 mg/L in the lake (Lund, 1964).

The effects of zooplankton grazing on biomass and composition of phytoplankton, including diatoms are well-established (e.g., Winder and Schindler, 2004; Pagano, 2008) as also indicated by the SEM analysis, showing a significant grazing pressure on the diatoms in Lake Taihu. Nevertheless, compared with inorganic N, the effect of zooplankton appeared to be of modest importance, having relatively weaker independent effects on WS diatom biomass (Fig. S12). Our results indicate that zooplankton was one of the important factors in influencing the biomass of diatom in the case of declining NH₄-N in Lake Taihu.

5. Conclusion

This study investigated the variations of nutrients and diatoms in subtropical, eutrophic Lake Taihu over 28 yr (1992–2019) during a decrease of N and slightly increase of P. Our results highlighted the onset of increased spring diatoms and the timing of the peak biomass shifted from SA to WS season coincides with the significantly decreased NH₄-N concentrations at the winter-spring low temperatures. We conclude that the magnitude of vernal suppression or stimulation of diatom assemblages has increased, concomitant with NO₃-N: NH₄-N mass ratios and climatic changes in this eutrophic shallow lake. Thus, the parallel reductions in external N loading, along with variations in dominant inorganic N, will stimulate the growth of diatoms. Diatom biomass increase in spring is a valuable indicator during the process of the external



Fig. 8. A conceptual model of diatoms responds differently depending on the forms of dominant inorganic nitrogen (NO₃-N and NH₄-N) in Winter-Spring (WS) seasons. (a) P1–1992–2006; (b) P2–2007–2019. The various pathways are exaggerated or minimized under each condition to illustrate their relative importance as well as how these proportions change under the different conditions. Note that under low temperature and high NH₄-N conditions, the pathway of photorespiration increases, and of the NO₃-N concentrations used as an additional electron sink (energy dissipation) to prevent photoinhibitory damage is depressed. These pathways have been confirmed by Lomas and Glibert (1999a), Raven (2011) and Glibert et al. (2016).

loading reduction in eutrophic lakes.

Authors' contributions

X. Liu and F. Chen conceived the ideas and designed the methodology. X. Liu, Y. Li, R. Shen and M. Zhang collected the data. X. Liu analyzed the data. X. Liu and F. Chen led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

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