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Determining Critical Nutrient Thresholds Needed to Control Harmful Cyanobacterial Blooms in Eutrophic Lake Taihu, China

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Ⓢ *Supporting Information*

ABSTRACT: Nutrient overenrichment has led to dramatic increases in harmful cyanobacterial blooms, creating serious threats to drinking water supplies, ecological and economic sustainability of freshwater ecosystems. Nutrient-cyanobacterial bloom interactions were examined in eutrophic Lake Taihu, China. In situ microcosm nutrient dilution bioassays and mesocosm nutrient addition experiments were conducted to determine nitrogen (N) and phosphorus (P) concentration and load thresholds needed to control cyanobacterial bloom formation. Blooms were dominated by toxic, non N_2 fixing *Microcystis* spp, from May to December. Dilution bioassays showed seasonality in nutrient limitation, with P-availability controlling prebloom spring conditions and N-availability controlling summer-fall blooms. Nutrient dilution and enrichment bioassays indicated that total nitrogen (TN) and total phosphorus (TP) concentration thresholds should be targeted at below 0.80 mg L^{-1} and 0.05 mg L^{-1}, respectively, to limit intrinsic growth rates of *Microcystis* dominated blooms. Based on estimates of nutrient loading and observed stoichiometry of phytoplankton biomass, 61−71% TN and 20−46% TP reduction are necessary to bring Taihu's phytoplankton biomass to "acceptable" sub-bloom conditions of less than 20 μg L^{-1} chlorophyll *a*.

INTRODUCTION

Harmful cyanobacterial blooms (CyanoHABs) are expanding worldwide and represent a serious threat to water and habitat quality, drinking water supplies, food webs and the overall sustainability of freshwater ecosystems.[1,2] Excessive nitrogen (N) and phosphorus (P) inputs from industrialization, urbanization, and intensive agriculture are linked to CyanoHAB proliferation.[3,4] Accordingly, reducing anthropogenic inputs of both N and P has been recommended to control CyanoHABs in eutrophic ecosystems.[5−7]

Developing effective and quantifiable nutrient reduction targets is essential for restoring impaired water quality. Stressor-response relationships characterize progressive degradation of ecosystems receiving increasing nutrient inputs and help delineate the numerical nutrient criteria that would support desirable ecological attributes. Relationships between nutrient inputs and biological responses in lakes are often nonlinear.[8−10] In such cases, establishing nutrient thresholds, defined as the critical levels of N or P that control an abrupt change or regime shift such as sudden and protracted dominance by CyanoHABs,[11] is a quantifiable and attractive approach. Maintaining nutrients below a threshold level is more cost-effective and may be more practical and achievable than reducing nutrients to historical levels.

Taihu ("large lake" in Mandarin), the third largest freshwater lake in China, is located in Jiangsu province, one of the most densely populated and rapidly growing regions of China. Taihu serves the drinking water needs of approximately 20 million local inhabitants and is a key regional fisheries and tourism resource.[12] Rapid urbanization and agricultural expansion in the Taihu basin have increased nutrient inputs from wastewater discharge, industries, and chemical fertilizer use. These increases have transformed this once diatom-dominated meso-oligotrophic lake in the 1960s into a eutrophic, cyanobacteria-dominated system, with toxic *Microcystis* spp. blooms now occurring regularly from spring to late fall throughout the lake.[13−15] These blooms have caused serious environmental, economic, and societal problems, including unsafe water resources for the local population and reduced fisheries and tourism.[16,17] In May 2007, a very large "cyanobacteria mat" caused the drinking-water plant of the nearby city of Wuxi (population ∼5 million) to fail, leading to a highly publicized drinking water crisis.[17,18] This crisis has increased pressure on local, provincial, and central governments to identify causative factors and initiate solutions.

Seasonal in situ nutrient addition bioassays[19,20] indicate that cyanobacterial proliferation in Lake Taihu is caused by the

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combined effects of N and P inputs, with P availability controlling algal production during spring, and N availability controlling production during the summer-fall when CyanoHABs are most severe. This has led to the recommendation that a dual nutrient (N and P) reduction strategy is needed to control eutrophication and bloom outbreaks in Lake Taihu.[20]

In the present work, water quality monitoring was conducted to characterize nutrient conditions and cyanobacterial bloom dynamics in Taihu. Parallel in situ nutrient dilution and enrichment bioassays were conducted to determine phytoplankton group-specific responses to nutrient manipulations. The maximum acceptable loads for N and P were estimated using a mass balance model that simulated nutrient concentration as a function of external nutrient loads. Nutrient thresholds derived from these studies will allow managers to establish specific nutrient input reduction goals for Lake Taihu.

■ MATERIALS AND METHODS

Lake Description and Field Sites. Lake Taihu is located in the southeastern part of Yangtze River Delta (30°55′40″-31°32′58″N; 119°52′32″-120°36′10″E). It is a large, shallow (mean depth ∼2 m) polymictic lake, with an area of 2340 km^2, a catchment area of 36,500 km^2 and a volume of 4.4 billion m^3.[12,17] The Taihu basin contains 117 rivers and tributaries draining into the lake. The annual freshwater input to the lake is about 88×10^8 m^3,[21] and the water retention time of the lake is approximately 180 days. Generally, freshwater inputs enter the lake from the southwestern mountainous watershed and the main discharge points are Eastern Taihu Bay and the Taipu River, which drains via Shanghai into the East China Sea (Figure 1).

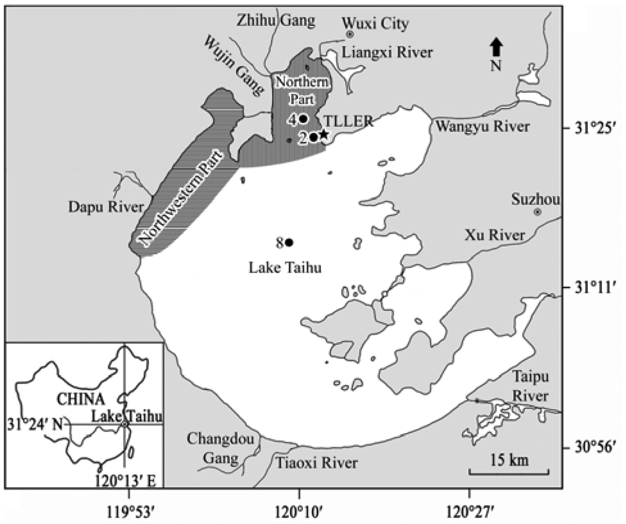

Figure 1. Lake Taihu: Sites 4 and 8 are locations from which water samples were routinely collected and Site 2 shows the location at which in situ nutrient dilution and addition bioassays were incubated. Northern and Northwestern part are hypertrophic and bloom-prone regions.

Meiliang Bay is situated in the northern part of Taihu, and is one of its most eutrophic bays. The surface area of the bay is 132 km^2 with a mean depth of 2.0 m. Monthly sampling was conducted in Meiliang Bay (Site 4), and the central lake (Site 8). In addition, Site 2 was an intermediate location near the Taihu Laboratory for Lake Ecosystem Research (TLLER), Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences. This site served as the incubation site for the nutrient dilution and addition bioassays described below (Figure 1).

Ambient Physical, Chemical, And Biological Conditions. Ambient water quality and biological data were provided by TLLER. Water sampling and measurements were performed quarterly at 32 stations for the entire lake and monthly inside Meiliang Bay and in the central lake during 2009–2012. During the bloom period from May to September 2011–2012, sampling was conducted weekly in Meiliang Bay and in the central lake to obtain higher frequency nutrient and chlorophyll *a* (Chl*a*) data. At each location, depth-integrated samples were collected with a 2 m long, 10 cm diameter clear plastic tube. In situ water temperature was measured using a Yellow Springs Instruments (YSI) 6600 multisensor sonde. Water samples were transported to the laboratory at TLLER within 4 h and filtered using GF/F filters (Whatman, Kent Great Britain). Chemical analyses of water samples included total nitrogen (TN), total dissolved nitrogen (TDN), ammonium (NH_4–N), nitrate (NO_3–N), nitrite (NO_2–N), total phosphorus (TP), total dissolved phosphorus (TDP), and soluble reactive phosphorus (SRP). SRP was determined using the molybdenum blue method.[22] NH_4–N was measured by the indophenol blue method, and NO_3–N and NO_2–N with the cadmium reduction method.[22] TP, TDP, TN, and TDN were analyzed using a combined persulfate digestion,[23] followed by spectrophotometric analyses as for SRP and NO_3–N. Biological parameters included phytoplankton community composition, biomass and Chl*a*. Phytoplankton samples were fixed with Lugol's iodine solution (2% final conc.) and settled for 48 h. Cell density was determined microscopically using a Sedgwick-Rafter counting chamber at magnifications of 320–400×. Phytoplankton species were identified and counted according to Hu et al.[24] Conversion to biomass was based on 1 mm^3 of volume being equivalent to 1 mg of fresh weight biomass. Chl*a* concentrations were determined spectrophotometrically after extraction in 90% hot ethanol.[25]

Nutrient Dilution and Enrichment Bioassay Experiments. Six nutrient dilution bioassays were conducted during spring 2009–2012, August 2009 and October 2010. Lake water samples containing natural phytoplankton assemblages were collected from 0.2 m below the surface with precleaned (0.01 N HCl–washed and then lake water–washed) 20-L polyethylene carboys at Site 4 (Figure. 1). Water samples were diluted over a 30–70% range with a major ion solution (MIS), which contained anions and cations (e.g., Na, K, Ca, Mg, etc.) representative of Taihu water[12] (Table S1, Supporting Information (SI)), but did not contain N and P. Individually or combined, N and P were then added back in incremental amounts to determine which nutrient(s) were most limiting, and levels of input reductions of these nutrients needed to control phytoplankton growth and bloom formation. Using this approach, nutrient threshold levels, below which growth is nutrient limited, could be established.

Undiluted treatments served as controls. Specific triplicate treatments are shown in Table S2, SI. The treatments were incubated in 1 L Cubitainers (Hedwin Corp., Baltimore, MD), which are chemically inert, unbreakable and transparent (80% PAR transmittance) polyethylene vessels. The methodology for Cubitainer bioassays is detailed in Xu et al.[19] Cubitainers were incubated in situ near the surface for 2 days by placing them in a floating frame off the TLERR pier, allowing for natural light, temperature, and surface wave action conditions. Dissolved inorganic carbon (DIC) was added at 560 μmol·L^{-1} $NaHCO_3$ to avoid inorganic carbon limitation during incubations.[26] Cubitainers were sampled after 2 days incubation for Chl*a* and nutrient analyses. This incubation period provided sufficient time to measure phytoplankton growth responses while minimizing container effects.[26,27]

A separate bioassay experiment was conducted to determine the effects of varying N and P concentrations on phytoplankton growth in August 2013, when cyanobacterial blooms were most intense. Triplicated treatments contained a range of P concentrations (i.e., 0, 0.005, 0.01, 0.02, 0.04, 0.06, and 0.08 mg L^{-1}) with a fixed N level (2.00 mg L^{-1}) and a range of N concentrations (i.e., 0, 0.050, 0.10, 0.30, 0.50, 0.80, and 1.00 mg L^{-1}) with a fixed P level (0.20 mg L^{-1}). The *Microcystis* surface scum was swept away prior to sampling to achieve a moderate initial biomass of ~10.0 μg L^{-1} Chl*a*. These experiments were incubated using 100 L translucent polyethylene buckets. Buckets were fixed in a floating frame in the lake to mimic ambient water temperature and light conditions. Nutrients were supplied daily during the first 5 days as KNO_3 and K_2HPO_4 to simulate pulse nutrient inputs. Incubation times lasted up to 10 days to obtain maximum standing biomass. N or P starvation was induced prior to the growth experiments by adding excess P (or N) to buckets for N threshold (or P threshold) experiments to stimulate consumption of intracellular and extracellular N (or P) pools.

The growth rate (μ) under various N or P concentrations was calculated according to the exponential growth equation:

$$\mu = \ln(X_2/X_1)/T_2 - T_1 \tag{1}$$

Where X_1 is the Chl*a* concentration at the initial incubation time point (T_1), and X_2 is the concentration of Chl*a* at end time point (T_2).

Maximum growth rate (μ_{max}) and half saturation constant (*Ku*) were calculated using the Monod kinetic equation.[28] Response curves were established to determine the threshold, that is, the concentration below which growth of the bloom populations was reduced.

Estimating Maximum Acceptable External Nutrient Loading to Lake Taihu. External nutrient loading required to meet the in-lake nutrient concentration can be estimated using a simple mass-balance model (eq 2).

$$L_{in+}L_{atmos} + L_{release} = C_{lake} \times Q_{out} + C_{lake} \times A \times K_{net} \tag{2}$$

where L_{in} is the input load (kg $year^{-1}$) from rivers, L_{atmos} is the input from atmosphere, $L_{release}$ is the input from sediment, C_{lake} is the average in-lake nutrient concentration (g m^{-3}) and is assumed equal to outflow nutrient concentration in a well-mixed water body, Q_{out} is the outflow volume (m^3 $year^{-1}$), A is the lake surface area (m^2), and K_{net} is the in-lake loss constant (m $year^{-1}$). The model assumes that nutrient loads to the lake equal losses due to downstream flow and in-lake processes (e.g., burial, denitrification for N, removal by fisheries export, etc.). All of the model terms can be directly measured except K_{net}, which is calculated by solving eq 2 using data from lake' yearly budget. Annual nutrient input and output loading as well as water flux data of the calendar years 2007−2012 are available from the Taihu Basin Authority, Ministry of Water Resources. Annual sediment input, and atmospheric input data for TN and TP loading are available from the literature.[29−32]

Assuming that K_{net} is invariant, we can substitute into eq 2 the in-lake nutrient targets and then calculate the maximum acceptable nutrient loading required to meet the targeted in-lake nutrient concentration.

Statistical Analyses. The differences in the growth responses between the various treatments were analyzed by one-way ANOVA. Post hoc multiple comparisons of treatment means were performed by Tukey's least significant difference procedure. Statistical analysis was performed using the SPSS 13.0 statistical package (SPSS Inc., Chicago, IL), and the level of significance used was $p < 0.05$ for all tests.

■ RESULTS

Cyanobacterial Bloom Dynamics and Environmental Conditions. Lake water temperatures varied seasonally, ranging from ~2 °C in January−February to ~30 °C in July−August each year (Figure S1A, SI). Throughout our experiments, *Microcystis* comprised more than 50% of phytoplankton biomass from May onward, when water temperatures exceeded 20 °C at the sampling sites. It maintained its dominance until early December, contributing from 60%−90% of total algal biomass during this period (Figure S1B, SI).

Chlorophyll *a* (Chl*a*) concentrations were used to estimate phytoplankton biomass. Based on weekly Chl*a* data from May to September in 2011−2012 and in situ visual observations, when Chl*a* concentrations were less than 10 μg L^{-1}, relatively few *surface Microcystis* colonies were observed. However, *Microcystis* blooms were observed as bright green surface streaks on relatively calm days, when Chl*a* concentrations ranged from 20 to 30 μg L^{-1}. When Chl*a* concentrations exceed 40 μg L^{-1}, surface *Microcystis* scums were observed. *Microcystis* usually started to form surface blooms in May, when Chl*a* concentrations exceeded 20 μg L^{-1}. Blooms were usually most profound from late July to October, when Chl*a* concentrations exceeded 40 μg L^{-1} regularly, and occasionally went up to 100 μg L^{-1} (Figure 2A). Generally, cyanobacterial blooms were more serious in Meiliang Bay than in the Central lake location.

TN and TDN concentrations varied seasonally (Figures 2B, C). Maximum values occurred in winter and spring, while minimum values were confined to late summer and autumn, when cyanobacterial blooms were the most severe. On average, both TN and TDN concentrations remained above 3.0 mg L^{-1} from January to April, and then TN declined to about 2.0 mg L^{-1} and TDN to below 1.0 mg L^{-1} from late July through September. Annual mean concentrations of TP and TDP were 0.10 and 0.03 mg L^{-1} in Meiliang Bay, and 0.08 and 0.03 mg L^{-1} in central lake. TP and TDP showed an opposite seasonal pattern compared to TN, with maximum values occurring in summer and autumn (Figures 2D, E).

NO_3−N and NH_4−N were the primary forms of DIN (Figure S2, SI). NO_3−N concentrations were higher in winter and spring and gradually declined from May to mid-July. During the most severe blooms from August to October, NO_3−N concentrations remained between 0.1 and 0.3 mg L^{-1}. NH_4−N concentrations remained fairly constant at around 0.30 mg L^{-1} from July to October. SRP concentrations showed highest values from August to October, ranging from 0.01 to 0.036 mg L^{-1}. SRP concentrations generally were higher in Meiliang Bay than in the central lake during the bloom period.

Nutrient and Chl*a* concentration data from entire lake were used to assess annual average and summer average character-

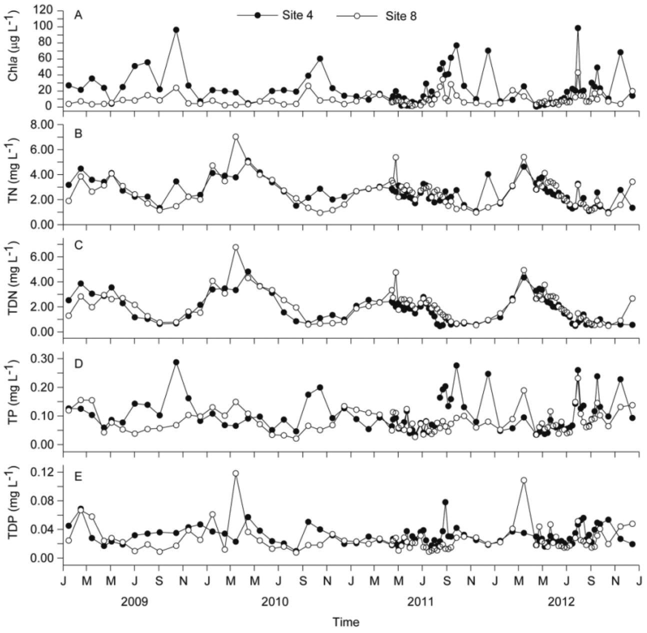

Figure 2. Seasonal variation of chlorophyll *a* (Chl *a*), total nitrogen (TN), total dissolved nitrogen (TDN), total phosphorus (TP) and total dissolved phosphorus (TDP) in Meiliang Bay (Site 4) and central lake (Site 8) locations in Lake Taihu.

istics for the entire lake, northern and northwestern regions of the lake (Table 1). Annual average lake wide Chl*a* concentration was 20.8 μg L^{-1} and this value increased to 35.6 μg L^{-1} during summer months. Annual average Chl*a* concentration was 26.4 μg L^{-1} and the summer average value was 44.2 μg L^{-1} in the northern and northwestern regions of the lake.

Table 1. General Environmental Conditions for the Entire Lake and for the Bloom-Plagued Northern and Northwestern Regions of Lake Taihu for the Calendar Years 2007-2012

	lake-wide		northern and northwestern regions	
	annual average	summer average	annual average	summer average
Chl *a* (μg L^{-1})	20.8	35.6	26.4	44.2
DIN (mg L^{-1})	1.31	0.76	2.51	1.06
SRP (mg L^{-1})	0.011	0.012	0.029	0.030
TN (mg L^{-1})	2.72	2.0	4.60	2.95
TP (mg L^{-1})	0.104	0.101	0.195	0.193
DIN/TN	0.46	0.37	0.52	0.36
SRP/TP	0.11	0.12	0.15	0.16
Chl *a*/TN	6.4	11.9	7.1	15.1
Chl *a*/TP	149.2	244.0	141.7	232.8

Phytoplankton Growth Responses to Nutrient Reductions. Ambient inorganic N and P concentrations for dilution bioassays are shown in Table S3, SI.

The Chl*a* responses to N and P additions at various concentrations imposed by dilution are shown in Figure 3. During spring, N additions alone had no effect on Chl*a* compared to the control over a range of dilutions. The P alone additions and combined P and N additions led to similar significantly ($p < 0.01$) higher Chl*a* concentrations than the control. In contrast to spring, the P alone addition had no effect on Chl*a* compared to the control during summer-fall, while N additions showed varying degrees of Chl*a* stimulation above controls that depended on the dilution level and initial SRP concentrations. In August 2009, SRP concentrations ranged from 0.022, 0.015, 0.010 to 0.007 mg L^{-1} at four dilution levels, N alone addition showed Chl*a* stimulation, while combined N and P addition showed the strongest stimulatory effect on algal biomass over a range of diluted biomasses (36, 25, 18, and 10 μg L^{-1} Chl*a* respectively). In October 2010, N alone additions led to similar Chl*a* stimulation as combined N and P additions at four dilution levels over a range of corresponding diluted biomass values (21.9, 15.9, 10.8, and 7.5 μg L^{-1} Chl*a* respectively) at SRP levels ranging from 0.042, 0.034, 0.019 to 0.014 mg L^{-1}.

Growth rates showed patterns similar to biomass in response to N and P additions after dilution (Figure 4). During spring, P addition showed the same growth rate as combined N and P addition at any dilution level. In August 2009, algal growth rates in N addition treatments declined when P concentrations were reduced by dilution. However, in October 2010 algal growth rates in N addition treatments were similar to combined N and P addition treatments at all four dilution levels.

Phytoplankton Growth Response to Various N and P Concentrations. To specifically examine the relationships between the range of bioassay-amended nutrient concentrations and phytoplankton growth in Taihu, growth rate responses to different N and P concentrations supplied daily were examined during summer (August), when *Microcystis* blooms were profound. Growth curves fitted by nonlinear regression showed that algal growth rates increased consistently until the SRP and DIN concentrations reached about 0.03 mg L^{-1} and 0.40 mg L^{-1}, respectively (Figure 5). The results plotted using the Monod equation indicated a maximum growth rate of 0.58 d^{-1}, with a half saturation concentration of 0.015 mg L^{-1}for P. For N, maximum growth rate and half saturation concentration were 0.53 d^{-1} and 0.17 mg L^{-1}, respectively.

Final yield of phytoplankton biomass measured as Chl*a* was linearly related to the quantities of N and P added during these experiments. Relationships between increases in biomass and total inorganic nutrient concentrations followed equations:

$$\mathrm{Chl}a = 24.38\mathrm{DIN} + 0.43\ (R^2 = 0.97) \tag{3}$$

$$\mathrm{Chl}a = 290.5\mathrm{SRP} + 1.47\ (R^2 = 0.98) \tag{4}$$

Where Chl*a* is maximum chlorophyll *a* concentration (μg L^{-1}), DIN and SRP are total inorganic nitrogen and soluble reactive phosphorus concentrations (mg L^{-1}), respectively.

Nutrient Loss Constants in Lake Taihu. Based on a mass-balance model (eq 2), we solved for K_{net} using the 6-year average water input to the lake (88×10^8 m^3 y^{-1}) and TN and TP input loads from rivers (46429×10^3 kg y^{-1} and 2174×10^3 kg y^{-1}) (Table S4. SI), annual input of TN and TP from sediments (6050×10^3 kg y^{-1} and 390×10^3 kg y^{-1})[29,30] and from atmosphere (8930×10^3 kg y^{-1} and 200×10^3 kg y^{-1}),[31,32] a lake area of 23.4×10^8 m^2, and lake-wide, 6-year area weighted average TN and TP concentrations of 2.72 mg L^{-1} and 0.104 mg L^{-1}(Table 1). Calculated K_{net} were 5.81 m y^{-1} and 7.53 m y^{-1} for TN and TP, respectively.

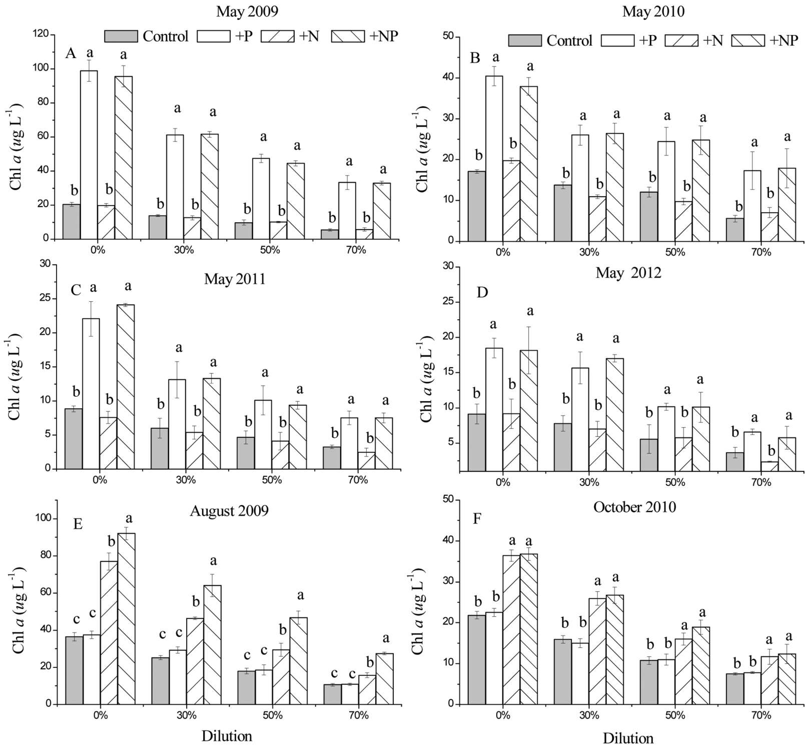

Figure 3. Phytoplankton biomass (as Chl*a*) response to N and P addition after dilution in bioassays conducted in May 2009–2012, August 2009 and October 2010. Water samples for bioassays was collected from the surface at Site 4. Error bars represent ±1SD of triplicate samples. Differences between treatments at individual dilutions are shown based on ANOVA post hoc tests (a > b > c; $P < 0.05$).

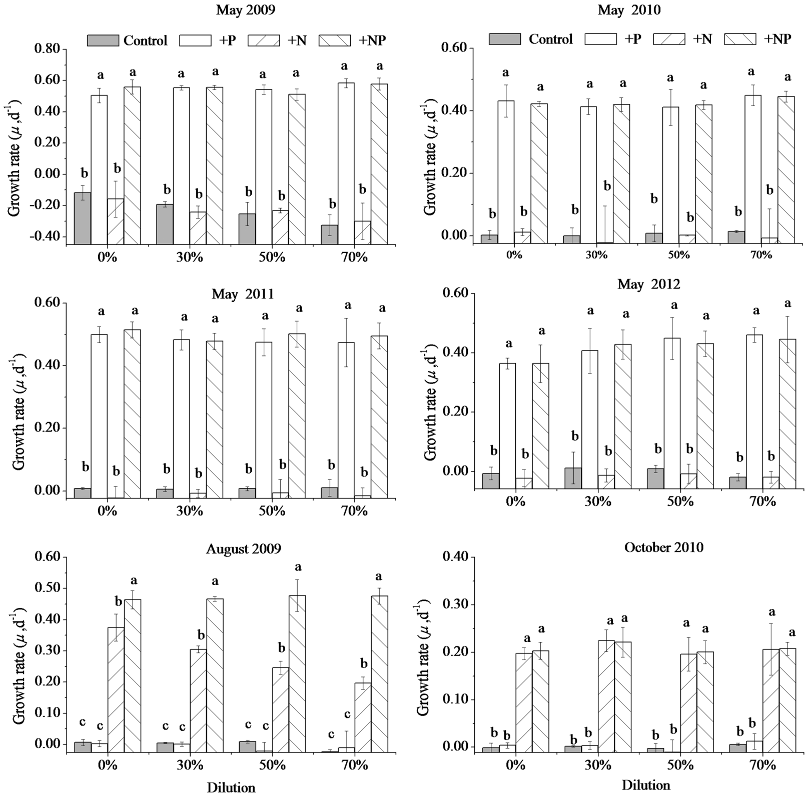

Figure 4. Phytoplankton growth rate response to N and P addition after dilutions in bioassays conducted in May 2009–2012, August 2009 and October 2010. Water samples for nutrient dilution bioassays was collected from the surface at Site 4. Error bars represent ±1 SD of triplicate samples. Differences between treatments at individual dilutions are shown based on ANOVA post hoc tests (a > b > c; $P < 0.05$).

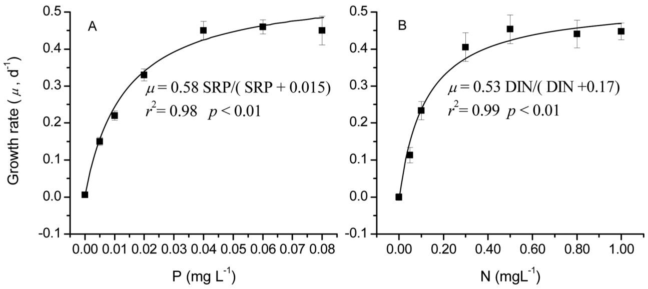

Figure 5. Growth kinetics of natural phytoplankton assemblages in response to a range of (A) daily supplied P concentrations and (B) daily supplied N concentrations during summer 2013. Curves were fitted by nonlinear regression. Water samples for bioassays was collected from the surface at Site 4. Error bars represent ±1 SD of triplicate samples.

DISCUSSION

Nutrient Thresholds Needed for Controlling Cyanobacterial Blooms in Lake Taihu. Cyanobacterial blooms reflect accelerating eutrophication, and are key indicators of impaired lake use.[33] In Taihu, *Microcystis* was dominant throughout the year except winter[34] and accounted for 60−90% of total phytoplankton biomass during bloom periods (SI Figure S1). Developing nutrient thresholds is first needed to establish water quality standard for effective CyanoHAB control.

The dilution bioassay is a useful tool for testing phytoplankton growth responses to reduced nutrient concentrations during high nutrient periods.[35] Dilution bioassays conducted during the spring showed that N enrichment alone failed to increase phytoplankton biomass relative to controls at all dilution levels unless P was also supplied (Figures 3 and 4), indicating P was the most limiting nutrient during that period. When adequate P was supplied to lake water after dilution, DIN concentration dilutions (by MIS) of up to 70% failed to significantly reduce phytoplankton biomass and growth rate compared to combined N and P addition treatments, indicating DIN concentrations exceeded those needed to limit growth by at least 70%.

Once the bloom was well developed during summer, the dilution bioassay indicated that P exceeded phytoplankton demand and N was the primary limiting nutrient (Figures 3 and 4). However, there was some evidence that the extent to which P was in excess during the bloom was lower than the extent to which N was excess during bloom initiation in spring. For example, during the August 2009, once N-limitation was alleviated by adding N, the growth rate of phytoplankton declined with decreasing P concentrations. Also, additions of N and P resulted in higher growth rates than N additions alone at four dilution treatments. This indicates that P availability within the undiluted lake water was present at concentrations close to algal demand.

In dilution bioassays, water samples are incubated in closed containers with a finite supply of nutrients. Thus, nutrient concentrations tend to decline as a consequence of cellular uptake and division to a point where a specific nutrient can limit growth. In contrast, in the field, nutrient fluxes from autochthonous and allochthonous sources are continuous and could potentially balance or exceed phytoplankton uptake rates.[36] Therefore, exhaustion of nutrient supplies in bioassay batch cultures may not reflect limitation by the same nutrient in the field. To address this potential problem, nutrient addition bioassays were conducted during a bloom period by adding various concentrations of N or P daily to lake water samples to test the growth rate response of algae to continuous nutrient pulses. The Monod equation accurately described the relationship between algal growth rates and added dissolved nutrient concentrations. These experiments cannot be used to derive true nutrient-growth kinetics because they do not account for decreases in concentration that occurred within 1 day due to phytoplankton uptake. However, addition experiments provide an upper-bound estimate for nutrient concentrations that saturate algal growth. Phytoplankton growth was saturated at about 0.03 mg L^{-1} SRP and 0.40 mg L^{-1} DIN, indicating that concentrations above these values are likely in excess of algal growth demand. In Taihu, DIN is directly available N, which account for about 50% of TN; and SRP is less than 11% of TP, while about 60% of TP is available phosphorus (AP).[37] Consequently, DIN and SRP concentrations that saturate phytoplankton growth correspond to 0.80 mg L^{-1} TN and 0.05 mg L^{-1} TP respectively, based on the ratios of DIN: TN and AP: TP typical of Taihu. Environmental conditions such as temperature, transparency, depth and phytoplankton community are different among the lakes located in different regions. Therefore, phytoplankton responses to nutrient inputs differ among these lakes. The eutrophication thresholds of TN for lakes range from 0.50 to 1.20 mg L^{-1}, while those for TP range from 0.03 to 0.10 mg L^{-1}.[38] Our nutrient thresholds agree well with these values. This indicates that once nutrient concentrations decline to below these thresholds, the growth rates of phytoplankton should be controllable. These thresholds can serve as the basis for setting nutrient criteria for Taihu. *Microcystis* spp. are the dominant bloom species accounting for about 76% of initial total phytoplankton biomass and 50−70% of total phytoplankton biomass at the end of experiment (data not shown), and they can sustain dominance throughout the duration of the bioassays.[39] Consequently, our results mainly reflect response of *Microcystis* spp. to nutrient supply. The current concentrations of TN and TP in Taihu are far higher than these thresholds, and this helps explain why cyanobacterial blooms can still thrive for much of the year, despite the efforts being undertaken to control nutrient loading.

It is difficult to control nutrient concentrations below the thresholds that limit growth rate of phytoplankton. From a prescriptive standpoint, the goal in developing water quality standards is to prevent excessive algal biomass. In Taihu, cyanobacteria greatly dominate phytoplankton biomass (60−90%) during the bloom season (May-December). Hence, water quality standards can be targeted toward Chl*a*, a simple and easily measured indicator of both total and cyanobacterial biomass.[40] Based on weekly observations, Chl*a* concentrations exceeding 20 μg L^{-1} were defined as blooms and Chl*a* concentrations exceeding 40 μg L^{-1} were defined as especially harmful (e.g., potentially toxic, hypoxia generating, food web disrupting) cyanobacterial blooms, where microcystin concentrations are almost always in excess of the World Health Organization standard of 1 μg L^{-1} total microcystins.[40] In order to ensure acceptable and safe water quality, 20 μg L^{-1} Chl*a* should be set as control target. However, setting targets for water quality based on Chl*a* is complicated in Taihu due to the variable seasonal and spatial patterns of Chl*a* within the lake. On an annual basis, the lake-wide average Chl*a* concentration was 20 μg L^{-1}, which meets water quality targets. However, during the summer, the lake-wide average Chl*a* exceeds 20 μg L^{-1} and the northern and northwestern reaches of the lake experience unacceptable summer bloom conditions with Chl*a* concentrations greater than 40 μg L^{-1} (Table 1). When mitigating harmful summer blooms in the northern and northwestern regions, it is clear that these areas of highest bloom biomass cannot be treated in isolation from the rest of lake. In part, bloom formation in these regions is caused by accumulation of biomass transported by surface currents from other regions of the lake due to consistent southeast winds during summer.[15] Additionally, we have no basis for calculating load reductions to these areas, because estimates of nutrient loading to the lake were made on a whole lake basis. Therefore, we set nutrient targets based on lake-wide summer average Chl*a* with the goal of preventing harmful bloom levels, particularly in the highly impaired northern and northwestward regions. The underlying assumption is that nutrient reductions would cause a proportionate reduction in phytoplankton biomass throughout the lake without affecting the spatial patterns of biomass. Currently, the lake-wide area weighted mean Chl*a* is 35.0 μg L^{-1} during the summer, increasing to 44.2 μg L^{-1} in the northern and northwestern regions. Reducing summertime phytoplankton biomass in these northern and northwestern regions from 44.2 to 20.0 μg L^{-1} Chl*a* is proportional to reducing lake-wide average Chl*a* from 35.0 to 15.8 μg L^{-1}. Based on relationships between nutrient addition and Chl*a* yield (eqs 3 and 4), the concentrations of DIN and SRP required to produce 15.8 μg L^{-1} Chl*a* in the water are 0.63 mg L^{-1} and 0.049 mg L^{-1}, respectively. These values correspond to 1.26 mg L^{-1} TN and 0.082 mg L^{-1} TP, based on the ratios of DIN: TN and AP: TP in Taihu. Of course, phytoplankton will exhibit seasonal difference in response to nutrient supply. The nutrient concentrations produced lower phytoplankton biomass in winter and spring compared with summer period (Figure 2). In order to ensure acceptable water quality during the bloom season, water quality targets for entire year should be set based on thresholds from the summer bloom period. Consequently, in-lake nutrient concentrations in Taihu should be targeted to the thresholds of 1.26 mg L^{-1} TN and 0.082 mg L^{-1} TP in order to control cyanobacterial blooms. The water quality targets set by the Chinese Central Government for Taihu are TN concentration below 1.20 mg L^{-1} and TP concentration below 0.050 mg L^{-1} by 2020.[41] These water quality targets will ensure "safe water quality" according to the nutrient thresholds developed here.

Implications of Nutrient Thresholds for Managing Cyanobacterial Blooms in Lake Taihu. Cyanobacterial blooms are difficult to eliminate completely, given impacts that climatic and hydrologic variability will have on nutrient loading and bloom potentials.[42] Water column transparency has declined significantly in Taihu due to increased nutrient concentrations and phytoplankton blooms. Buoyant cyanobacteria can outcompete subsurface phytoplankton species by reducing photosynthetically available light through shading.[3] Therefore, increased nutrient loading has created a positive feedback loop for CyanoHABs, perpetuating their dominance, until nutrient inputs are reduced.[43,44]

Setting nutrient targets is critically important for managing nutrient loads to the lake. Considering the realities of society's trade-offs between environmental protection and cost, it is best to set nutrient targets according to the threshold for bloom formation in Lake Taihu. Hence, we recommend thresholds of 1.26 mg L^{-1} TN and 0.082 mg L^{-1} TP as water quality targets to maintain bloom-free conditions (i.e., Chl*a* below 20 μg L^{-1}). A target of nutrient loading corresponding to the in-lake water quality target for N and P can be derived from the mass-balance model (eqs 2), assuming that K_{net} is invariant. Calculated loading thresholds for TN and TP based on the mass-balance model were are 15707 × 10^3 kg y^{-1} and 1640 × 10^3 kg y^{-1}, respectively. The current annual external loading from rivers ranged from 40640 to 54450 × 10^3 kg y^{-1} with a mean of 46000 × 10^3 kg y^{-1} for TN and from 2040 to 3060 × 10^3 kg y^{-1}, with a mean of 2170 × 10^3 kg y^{-1} for TP (Table S4, SI). Comparing the current external TN and TP loading with required loading thresholds indicates that load reductions of 61−71% TN and 20−46% TP are necessary in order to control the cyanobacterial biomass and minimize bloom potential. The choices of "acceptable" reductions are value judgments best left to policy makers.[45]

For eutrophic shallow lakes with relatively long hydraulic residence time, like Taihu, a legacy of internal loading, especially for P, is a formidable problem for rapid recovery of water quality. Estimated annual internal TN and TP loadings from sediment are about 5520−8930 × 10^3 kg N y^{-1} and 270−420 × 10^3 kg P y^{-1},[29,30] which are equivalent to 13−20% and 12−19% of external TN and TP loadings. Hence external nutrient reductions are critical for ensuring acceptable water quality. Reduction in external N loading would result in a quick response of in-lake TN concentration, because N loss by denitrification greatly reduces internal N loading.[46] However, in the interim period between P loads reduction and recovery, legacy P loads in the sediments would be expected to maintain high P concentrations in the water column and delay recovery of lake.[47] Changes in external P loading will exert a feedback on sediment P release, leading toward a new equilibrium between external and internal loading, which was typically reached after 10−15 years, or even longer in some cases.[47−50] Therefore, reductions in the external N loads, in addition to reducing external P loads, should speed up the process of de-eutrophication and declining *Microcystis* blooms, which are incapable of N_2 fixation and hence dependent on exogenous combined N sources. This improvement can be accomplished without causing a significant shift to N_2 fixing cyanobacterial species, largely because internal N regeneration is a more attractive N source than energetically expensive N_2 fixation in this highly turbid lake.[20,39] Currently, the local government is using dredging to remove sediments in the heavy polluted northern regions of lake. This may speed the recovery of water quality, as long as dredged materials are deposited outside the basin. Overall, it is difficult to estimate how internal loads will change once external loading is reduced in Taihu. In this regard, our recommendations for TN and TP reductions at current steady-state condition are immediate reduction targets, which most likely will need to be adjusted over time to compensate for changes in internal cycling and meet water quality standards needed to maintain Taihu in a bloom-free condition.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional figures and tables are given in the Supporting Information (Figures S1−S2, Tables S1−S4). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interests.

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■ REFERENCES

(1) Carmichael, W. W. Health effects of toxin producing cyanobacteria: the cyanoHABs. *Hum. Ecol. Risk Assess.* **2001**, *7* (5), 1393−1407.

(2) Huisman, J. M; Matthijs, H. C. P.; Visser, P. M. *Harmful Cyanobacteria*, Springer Aquatic Ecology Series 3; Springer, Dordrecht, The Netherlands, 2005; pp 243.

(3) Paerl, H. W. Nutrient and other environmental controls of harmful cyanobacterial blooms along the freshwater marine continuum. *Adv. Exp. Med. Biol.* **2008**, *619*, 216−241.

(4) Paerl, H. W. Controlling eutrophication along the freshwater-Marine continuum: Dual nutrient (N and P) reductions are essential. *Estuar. Coast.* **2009**, *32*, 593−601.

(5) Lewis, W. M., Jr.; Wurtsbaugh, W. A.; Paerl, H. W. Rationale for control of anthropogenic nitrogen and phosphorus to reduce eutrophication of inland Waters. *Environ. Sci. Technol.* **2011**, *45*, 10300−10305.

(6) Conley, D. J.; Paerl, H. W.; Howarth, R. W.; Boesch, D. F.; Seitzinger, S. P.; Havens, K. E.; Lancelot, C.; Likens, G. E. Controlling eutrophication: Nitrogen and phosphorus. *Science* **2009**, *323*, 1014−1015.

(7) Scott, J. T.; McCarthy, M. J. Nitrogen fixation may not balance the nitrogen pool in lakes over timescales relevant to eutrophication management. *Limnol. Oceanogr.* **2010**, *55*, 1265−1270.

(8) Howarth, W. The progression towards ecological quality standards. *J. Environ. Law* **2006**, *18* (1), 3−35.

(9) Downing, J. A.; Watson, S. B.; McCauley, E. Predicting cyanobacteria dominance in lakes. *Can. J. Fish. Aquat. Sci.* **2001**, *58* (10), 1905−1908.

(10) Vadeboncoeur, Y.; Jeppesen, E. From Greenland to green lakes: Cultural eutrophication and the loss of benthic pathways in lakes. *Limnol. Oceanogr.* **2003**, *48* (4), 1408−1418.

(11) Groffman, P. M.; Baron, J. S.; Blett, T.; Gold, A. J.; Goodman, I.; Gunderson, L. H.; Levinson, B. M.; Palmer, M. A.; Paerl, H. W.; Peterson, G. D.; Poff, N. L.; Rejeski, D. W.; Reynolds, J. F.; Turner, M. G.; Weathers, K. C.; Wiens, J. Ecological thresholds: The key to successful environmental management or an important concept with no practical application. *Ecosystems* **2006**, *9*, 1−13.

(12) Qin, B.; Xu, P.; Wu, Q.; Luo, L.; Zhang, Y. Environmental issues of Lake Taihu, China. *Hydrobiologia* **2007**, *581*, 13−14.

(13) Cai, Q.; Gao, X.; Chen, Y.; Ma, S.; Dokulil, M. Dynamic variations of water quality in Lake Taihu and multivariate analysis of its influential factors. *J. Chin. Geogr.* **1997**, *9* (4), 79−82.

(14) Chen, Y. W.; Qin, B. Q.; Teubner, K.; Dokulil, M. T. Long-term dynamics of phytoplankton assemblages: Microcystis domination in Lake Taihu, a large shallow lake in China. *J. Plank. Res.* **2003a**, *25* (1), 445−453.

(15) Chen, Y. W.; Fan, C. X.; Teubner, K.; Dokulil, M. Changes of nutrients and phytoplankton chlorophyll-a in a large shallow lake, Taihu, China: An 8-year investigation. *Hydrobiologia* **2003b**, *506−509*, 273−279.

(16) Pu, P.; Hu, W. P.; Yan, J.; Wang, G.; Hu, C. A physicoecological engineering experiment for water treatment in a hypertrophic lake in China. *Ecol. Eng.* **1998**, *10* (1), 179−190.

(17) Qin, B.; Zhu, G.; Gao, G.; Zhang, Y.; Li, W.; Paerl, H. W.; Carmichael, W. W. A drinking water crisis in Lake Taihu, China: Linkage to climatic variability and lake management. *Environ. Manag.* **2011**, *45*, 105−112.

(18) Guo, L. Doing battle with the green monster of Lake Taihu. *Science* **2007**, *317*, 1166.

(19) Xu, H.; Paerl, H. W.; Qin, B. Q.; Zhu, G. W.; Gao, G. Nitrogen and phosphorus inputs control phytoplankton growth in eutrophic Lake Taihu, China. *Limnol. Oceanogr.* **2010**, *55* (1), 420−432.

(20) Paerl, H. W.; Xu, H.; McCarthy, M. J.; Zhu, G. W.; Qin, B. Q.; Li, Y. P.; Gardner, W. S. Controlling harmful cyanobacterial blooms in a hypereutrophic lake (Lake Taihu, China): The need for a dual nutrient (N & P) management strategy. *Water Res.* **2011**, *45* (5), 1973−1983.

(21) Yan, S. W.; Yu, H.; Zhang, L. L.; Xu, J.; Wang, Z. P. Water quantity and pollutant fluxes of inflow and outflow rivers of Lake Taihu. *J. Lake Sci.* **2011**, *23* (6), 855−862 in Chinese.

(22) APHA. *Standard Methods for the Examination of Water and Wastewater*, 19th ed. American Public Health Association, American Water Works Association, Water Environment Federation, 1995.

(23) Ebina, J.; Tsutsui, T.; Shirai, T. Simultaneous determination of total nitrogen and total phosphorus in water using peroxodisulfate oxidation. *Water Res.* **1983**, *17*, 1721−1726.

(24) Hu, H.; Li, Y.; Wei, Y.; Zhu, H.; Chen, J.; Shi, Z. *Freshwater Algae in China*; Shanghai Science and Technology Press, 1980.

(25) Papista, E.; Acs, E.; Boeddi, B. Chlorophyll-*a* determination with ethanol - *a* critical test. *Hydrobiologia* **2002**, *485*, 191−198.

(26) Rudek, J.; Paerl, H. W.; Mallin, M. A.; Bates, P. W. Seasonal and hydrological control of phytoplankton nutrient limitation in the lower Neuse River Estuary, North Carolina. *Mar. Ecol.: Prog. Ser.* **1991**, *75*, 133−142.

(27) Paerl, H. W.; Bowles, N. D. Dilution bioassays: Their application to assessments of nutrient limitation in hypereutrophic waters. *Hydrobiologia* **1987**, *146*, 265−273.

(28) Monod, J. Continuous culture technique; theory and aplications. *Ann. Inst. Pasteur* **1950**, *79*, 390−410.

(29) Pang, Y.; Yan, R.; Yu, Z.; Li, Y.; Li, R. Suspension - sedimentation of sediment and release amount of internal load in Lake Taihu affected by wind. *Environ. Sci.* **2008**, *29*, 2456−2464 in Chinese.

(30) Zhang H. J. Studying the self-purification capacityof nitrogen and phosphorous in Lake Taihu. Ph.D thesis, Nanjing, 2014.

(31) Yu, H.; Zhang, L. L.; Yang, S. W.; Li, H. L.; Xu, J. Atmospheric wet deposition characteristics of nitrogen and phosphorus nutrients in Taihu Lake and contributions to the lake. *Res. Environ. Sci.* **2011**, *24* (11), 1210−1219 in Chinese.

(32) Yang, L. Y.; Qin, B. Q.; Hu, W. P.; Luo, L. C.; Song, Y. Z. The atmospheric deposition of nitrogen and phosphorus nutrients in Taihu Lake. *Ocean limnol. Sin.* **2007**, *38* (2), 104−110 in Chinese.

(33) Chorus, I.; Bartram, J. *Toxic Cyanobacteria in Water*; E&F Spon: London, 1999.

(34) Tan, X.; Kong, F. X.; Zeng, Q. F.; Cao, H. S.; Qian, S. Q.; Zhang, M. Seasonal variation of *Microcystis* in Lake Taihu and its relationships with environmental factors. *J. Environ. Sci.* **2009**, *21*, 892−899.

(35) Carrick, H. J.; Schelske, C. L.; Aldridge, F. J. Assessment of phytoplankton nutrient limitation in productive waters: Application of dilution bioassays. *Can. J. Fish. Aquat. Sci.* **1993**, *50*, 2208−2221.

(36) Dodds, W. K.; Johnson, K. R.; Priscu, J. C. Simultaneous nitrogen and phosphorus deficiency in natural phytoplankton assemblages: Theory, empirical evidence and implications for lake management. *Lake Reserv. Manage.* **1989**, *5*, 21−26.

(37) Gao, G.; Zhu, G.; Qin, B.; Chen, J.; Wang, K. Alkaline phosphatase activity and the phosphorus mineralization rate of Lake Taihu. *Sci. China, Ser. D: Earth Sci.* **2006**, *49*, 176−185.

(38) Smith, V. H.; Tilman, G. D.; Nekola, J. C. Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environ. Pollut.* **1999**, *100*, 179−196.

(39) Paerl, H. W.; Xu, H.; Hall, N. S.; Zhu, G.; Qin, B.; Wu, Y.; Rossignol, K. L.; Dong, L.; McCarthy, M. J.; Joyner, A. R. Controlling cyanobacterial blooms in hypertrophic Lake Taihu, China: Will nitrogen reductions cause replacement of non-N_2 fixing by N_2 fixing taxa? *PLoSOne* **2014**, *9* (11), e113123 DOI: 10.1371/journal.pone.0113123..

(40) Otten, T. G.; Xu, H.; Qin, B.; Zhu, G.; Paerl, H. W. Spatiotemporal patterns and ecophysiology of toxigenic *Microcystis* blooms in Lake Taihu, China: Implications for water quality management. *Environ. Sci. Technol.* **2012**, *46* (6), 3480−3488.

(41) Cheng, S. T.; Q, Y. C.; Zhang, H. G. Estimation and application of macroscopic water environmental capacity of total phosphorus and nitrogen for lake Taihu. *Acta Sci. Circumstantiae* **2013**, *33*, 2848−2855 in Chinese.

(42) Paerl, H. W.; Huisman, J. Climate change: A catalyst for global expansion of harmful cyanobacterial blooms. *Environ. Microbiol. Rep.* **2009**, *1* (1), 27−37.

(43) Kumagai, M.; Nakano, S.; Jiao, C.; Hayakawa, K.; Tsujimura, S.; Nakajima, T.; Frenette, J.-J.; Quesada, A. Effect of cyanobacterial blooms on thermal stratification. *Limnology* **2000**, *1* (3), 191−195.

(44) Paerl, H. W.; Otten, T. G. Blooms bite the hand that feeds them. *Science* **2013**, *342*, 433−434.

(45) Reckhow, K. H.; Arhonditsis, G. B.; Kenney, M. A.; Hauser, L.; Tribo, J.; Wu, C.; Elcock, K. J.; Steinberg, L. J.; Stow, C. A.; McBride, S. J. A predictive approach to nutrient criteria. *Environ. Sci. Technol.* **2005**, *39* (9), 2913−2919.

(46) McCarthy, M. J.; Lavrentyev, P. J.; Yang, L.; Zhang, L.; Chen, Y.; Qin, B. Q.; Gardner, W. S. Nitrogen dynamics and microbial food web structure during a summer cyanobacterial bloom in a subtropical, shallow, well-mixed, eutrophic lake (Lake Taihu, China). *Hydrobiologia* **2007**, *581*, 195−207.

(47) Jeppesen, E.; Søndergaard, M.; Jensen, J. P.; Havens, K. E.; Anneville, O.; Carvalho, L.; Coveney, M. F.; Deneke, R.; Dokulil, M.; Foy, B.; Gerdaux, D.; Hampton, S. E.; Hilt, S.; Kangur, K.; Kohler, J.; Lammens, E. H. H. R.; Lauridsen, T. L.; Manca, M.; Miracle, M. R.; Moss, B.; Noges, P.; Persson, G.; Phillips, G.; Portielje, P.; Romo, S.; Schelske, C. L.; Straile, D.; Tatrai, I.; Willen, E.; Winder, M. Lake responses to reduced nutrient loading—An analysis of contemporary long-term data from 35 case studies. *Freshwater Biol.* **2005**, *50*, 1747−71.

(48) Welch, E. B.; Cooke, G. D. Internal phosphorus loading in shallow lakes: Importance and control. *Lake Reserv. Manage.* **2005**, *21*, 209−217.

(49) Søndergaard, M.; Jensen, J. P.; Jeppesen, E. Role of sediment and internal loading of phosphorus in shallow lakes. *Hydrobiologia* **2003**, *506*, 135−145.

(50) Søndergaard, M.; Jeppesen, E.; Lauridsen, T. L.; Skov, C.; van Nes, E. H.; Roijackers, R.; Lammens, E.; Portielje, R. Lake restoration: successes, failures and long-term effects. *J. Appl. Ecol.* **2007**, *44*, 1095−1105.