Manipulating nutrient limitation using modified local soils: a case study at Lake Taihu (China)

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Abstract

The effect of geo-engineering materials of chitosan modified local soil (MLS) on nutrient limitation was studied in comparable whole ponds in Lake Taihu in October 2013. After 20 kg MLS were sprayed onto the whole water pond (400 m^2), the chlorophyll-a (Chl-a) concentration was decreased from 42 to 18 μg L^{-1} within 2 hours and remained below 20 μg L^{-1} in the following 15 months, while the average Chl-a was 36 μg L^{-1} in the control pond throughout the experiment. In situ nutrient addition bioassay experiments indicated that the nutrient limitation was shifted from nitrogen (N) and phosphorus (P) co-limitation to P limitation after MLS treatment from October 2013 to March 2014 compared to the control pond. In the cyanobacterial bloom season of June 2014, N and P co-limitation remained and N was the primary limiting nutrient and P was a secondary one in the control pond, because phytoplankton biomass (as Chl-a) showed significant increase by N addition and

further increase by N+P additions, while both N and P became the limiting nutrient for phytoplankton growth on the basis that only combining N and P additions showed significant Chl-a stimulation in the treatment pond. In the next summer (June 2014), a cyanobacteria-dominated state still remained in the control pond but chlorophytes, bacillariophytes and cyanophytes distributed equally and submerged vegetation was largely restored in the treatment pond. Meanwhile, the upper limiting concentration of DIN was enhanced from 0.8 to 1.5 mg L^{-1} and SRP from 0.1 to 0.3 mg L^{-1} compared to the control pond. This study indicates that nutrient limitation can be manipulated by using MLS technology.

Keywords

Nutrient limitation, Phytoplankton biomass and composition, Modified local soil，Submerged vegetation restoration, Whole lake experiment

1. Introduction

Cyanobacterial blooms caused by anthropogenic nutrient input to aquatic ecosystems are expanding worldwide, which is a serious threat to drinking water supplies, integrity of food webs and ecological and economic sustainability of some freshwater ecosystems (Huisman 2005; Paerl et al. 2001; Ryther and Dunstan 1971). Reducing external nutrients loading has been widely accepted as the first step to control cyanobacterial blooms in eutrophic ecosystems (Conley et al. 2009; Lewis et al. 2011). However, some shallow eutrophic lakes may show little response to reduced external nutrient inputs due to the nutrient release from already enriched sediment (Cooke et al. 2005; Sondergaard et al. 2001). Hence, effective in-lake technologies together with

appropriate management strategies are crucial for accelerating lake restoration.

Phosphorus (P) has been traditionally regarded as the key limiting nutrient of cyanobacteria blooms formation in freshwaters (Carpenter 2008; Paerl 1988; Schindler et al. 2008). Controlling P input is a basic goal for managing eutrophication, and the eutrophication rates are indeed slowed and algal blooms are reduced due to P input reduction in some cases (Jeppesen et al. 2005; Schindler 1977). However, excessive N loads can promote non-N_2 fixing cyanobacteria production in some shallow lakes (Galloway et al. 2008; Paerl 2008). Inorganic N deposition has resulted in eutrophication and increased phytoplankton biomass in some naturally unproductive lakes (Bergstrom and Jansson 2006). Hence, to control external N loading may also become necessary in mitigating eutrophication. In fact, many lakes, reservoirs and rivers exhibit N and P co-limitation, either simultaneously or in seasonally-switching patterns (Elser et al. 2007; Havens et al. 2001; Muller and Mitrovic 2015; Paerl et al. 2011).

Reducing nutrient concentrations below the threshold of phytoplankton growth has been suggested to restore a system to its pre-human impact status, for instance, 20 μg L^{-1} chlorophyll-a (Chl-a) was suggested as a control target to ensure acceptable water quality (Xu et al. 2015), but it is a very slow process for eutrophication restoration even if the external N and P inputs are under control (Jeppesen et al. 2005; Scheffer et al. 2001; Sondergaard et al. 2007). However, the tasks imposed by environmental legislation for improving water quality are close to the deadlines, such as the Water Framework Direction (WFD) in Europe and Clean Water Act in USA. In China, two

national programs (“Water Pollution Prevention Action Plan” and “Eco-civilization Construction”) have been promulgated by Chinese government in 2015, which targets on improving water quality to good status by 2020. Very large financial budgets have been approved for these programs. Since the natural restoration processes are far slower than the time scale of these management targets, ecological safe/effective and environmental friendly in-lake technologies for quick control of internal nutrient loads are required. Recently, geo-engineering in lakes has caused much interest for eutrophication control, which can offer the promise of rapid effects (Mackay et al. 2014; Spears et al. 2013). Some conventional geo-engineering materials, such as aluminum-/iron- salts and solid-phase P sorbents (aluminum, iron and lanthanum modified soils/clays etc.) have been tested in fields, which can reduce bioavailable P in water column or sediments (Cooke et al., 1993; Douglas et al. 2012; Egemose et al. 2010; Lurling and van Oosterhout 2013; Reitzel et al. 2005; Robb et al. 2003). However, long-term records of N and P concentrations in 12 large lakes indicate that reducing P consistently is not conductive to N removal (Finlay et al. 2013). Over the last several years, Pan and his colleagues developed a “Modified Local Soil Induced Ecological Restoration” (MLS-IER) technology, which could remove algal blooms in situ and transform algal biomass and its nutrients into submerged vegetations in shallow lakes (Pan et al. 2012a; Pan et al. 2011; Pan et al. 2006; Zou et al. 2006). During a bloom, bioavailable N and P can be largely absorbed and reduced by algal biomass. The particulate forms of N and P, especially those contained in the algal cells, can be quickly and largely flocculated from water columns and buried under the

sediment by capping treatment. The buried nutrient may be more available for the growth of submerged vegetation than for the growth of phytoplankton after the water clarity is improved by MLS in shallow waters. The MLE-IER and the triggered processes (e.g. submerged vegetation) may result in changes in nutrient concentrations, N:P ratios, and phytoplankton composition, hence affect the nutrient limitation. However, little is known on whether the nutrient limitation can be altered or manipulated by using MLS-IER, and if so, what the mechanism is and how much it can affect the subsequent nutrient management strategy.

Small scale of laboratory or mesocosm experiments might not be able to reflect all the complex interactions in the ecological system (Carpenter 2008). Quantitative experiments of lake geo-engineering should be carried out when frequent and continuous monitoring is possible in multiple comparable whole water systems (Waajen et al. 2015). Nutrient limitation of phytoplankton growth can be assessed in many ways, including nutrient availability analysis, algal physiological characters, nutrient enrichment culture and long-term observation data etc. (Beardall et al. 2001; Hecky 1988). In situ nutrient addition bioassays can be used to measure the immediate response of algae growth to enhanced nutrient concentrations in short term (Paerl and Bowles 1987).

In this work, we applied MLS in two comparable whole water ponds in Lake Taihu. High frequency monitoring in short term together with the monthly or quarterly monitoring were conducted to characterize the dynamics of nutrient concentrations, phytoplankton biomass, phytoplankton composition, and macrophyte restoration, as

111 the responses of the whole water system. In situ nutrient addition bioassay
112 experiments were carried out to identify the changes in nutrient limitation and
113 thresholds of phytoplankton growth after MLS-IER treatment in short and long terms.
114 The objective of the study is to explore the nutrient limitation effect of
115 geo-engineering method (MLS-IER) and to provide new insights for eutrophication
116 management.

117 ## 2. Methods and materials

118 ### 2.1. Study site

119 The whole water ponds situated in Meiliang Bay in the north of Lake Taihu (China),
120 where cyanobacterial blooms dominated throughout the year except winter (Fig. 1).
121 These ponds were created by dividing the original lake beach through dams with
122 sandbags and stones in October 2012. The water inside the whole ponds is
123 exchangeable with outside open lake through pipes (Φ20 cm) fitted on the dams. Two
124 ponds with similar surface area (~ 400 m^2) and mean depth (~ 1.5 m) were selected as
125 treatment and control pond (Fig. 1), whose physical, chemical and biological
126 characters were highly comparable. After 1 year stabilization of these water ponds, we
127 closed the pipes and took measures to remove fish and vegetations. Then, we carried
128 out MLS experiment on 12 October 2013 in the treatment pond.

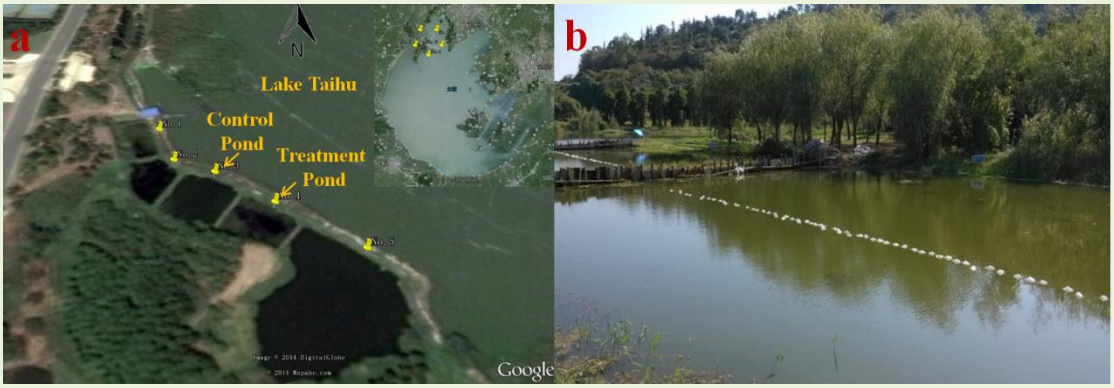

Fig. 1-a: Location of the whole water ponds in Meiliang Bay of Lake Taihu (google map was taken in 2014). b: In situ nutrient addition bioassays field experiment (photo taken on 13 October 2013).

2.2. Nutrient manipulation experiment

Local soils collected from the lakeside were washed and screened with a self-designed floatation facility to remove floating substances and to select the right particle size fraction of the soil particles. The washed and selected soil particles were prepared as the stock suspension (100 g L^{-1}) using lake water. Chitosan, the soil modifier, was purchased from Qingdao Yunzhou bioengineering Co. Ltd. The chitosan solution was obtained by adding 200 mg chitosan to 200 mL 0.5% HAc and stirred until all the chitosan was dissolved. In the field, the chitosan solution were added to the local soil suspension in a controlled ratio ($V_{chitosan\ solution}$: $V_{local\ soil\ suspension}$ = 1:1000), which was mixed with a stirrer. Then, the mixture was sprayed onto the water surface by a pumping machine. The average dosage used in the treatment pond was approximately 50 g MLS m^{-2}.

2.3. Nutrient limitation bioassay experiments

Seasonal in situ nutrient addition bioassay experiments were conducted after MLS treatment in October 2013, March 2014, June 2014 and December 2014. Water samples were collected from 0.2 m below the surface using a pre-cleaned (0.01N HCl

149 and then lake water) 5-L Perspex water sampler from the treatment and control pond,
150 respectively. The water samples were screened through a 180-μm mesh to remove
151 large zooplankton grazers (Xu et al. 2010) and distributed into prepared (acid-washed
152 and then lake water-rinsed) 1-L polyethylene Cubitainers® (Hedwin Corporation)
153 which are considered chemically inert, unbreakable and transparent (80% PAR
154 transmittance). The method and procedures of in situ cubitainer bioassays were
155 detailed in Paerl and Bowles (1987).
156 At start of the experiment, water samples were analyzed for Chl-a and nutrients. N
157 additions (+N), P additions (+P), N and P combined additions (N+P) and the lake
158 water with no addition of N or P (NA) bioassays were conducted in the treatment
159 pond and the control pond, respectively. N was added as KNO_3 and P was added as
160 $K_2HPO_4 \cdot 3H_2O$. In addition, 285.6 mg L^{-1} $NaHCO_3$ as an inorganic carbon source was
161 added to all treatments including the NA to avoid C limitation during incubations
162 (Redfield 1958; Rudek et al. 1991). HNO_3 and $K_2HPO_4 \cdot 3H_2O$ were dissolved with the
163 corresponding lake water to form N and P solutions, respectively, and added to 1 L
164 container according to the pre-designed concentration gradients. To determine the
165 effects of varying N and P concentrations on phytoplankton growth, a range of N
166 additions (i.e. 0, 0.05, 0.1, 0.5, 1.0, 1.5, and 2.0 mg N L^{-1}) with and without a fixed P
167 level (0.5 mg P L^{-1}), a range of P additions (i.e. 0, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, and
168 1.0 mg P L^{-1}) with and without a fixed N level (2.0 mg N L^{-1}) were carried out in the
169 nutrient addition bioassays.
170 All the N and P addition treatments were conducted in triplicate. The containers were

171 incubated in situ near the surface for 4 (autumn and summer) or 6 (winter and spring)
172 days by fastening them on the string fixed on both sides of the pond (Fig.1), allowing
173 for natural light, temperature and water surface wave action conditions. The
174 containers were sampled every 2 days for Chl-a and nutrients analyses. The growth
175 rate (μ) of each treatment was calculated according to the modified exponential
176 growth equation,

177 $\mu = \ln(X_2/X_1)/(T_2-T_1)$

178 Where X_1 is the concentration of *Chl-a* at the initial incubation stage (T_1), and X_2 is
179 the concentration of *Chl-a* at the peak incubation stage (T_2).

180 The maximum growth rate (μ_{max}) and half–saturation constant (K_u) were calculated
181 according to the Monod kinetic equation (Monod 1950).

182 **2.4. Physical, chemical and biological conditions of the water**

183 In situ water temperature (WT), dissolved oxygen (DO), pH, oxidation reduction
184 potential (ORP) and electrical conductivity (EC) were measured in the field using a
185 Yellow Springs Instruments (YSI) 556 MPS. The water transparency was measured
186 with Secchi disc. Chemical analyses of water samples included total nitrogen (TN),
187 total dissolve nitrogen (TDN), ammonium (NH_4^+-N), nitrate (NO_3^--N), total
188 phosphorus (TP), total dissolve phosphorus (TDP) and soluble reactive phosphorus
189 (SRP). TN and TDN were determined using alkaline potassium persulphate
190 digestion-ultraviolet spectrometer; NH_4^+-N was measured by nessler's colorimetric
191 method and NO_3^--N by ultraviolet colorimetric method; TP and TDP were determined
192 using potassium persulfate digestion-Mo-Sb-Vc colorimetric method and SRP by

193 Mo-Sb-Vc colorimetric method. Chl-a was extracted by acetone (90%) for 24h at 4℃
194 from algal cells collected with 0.45 μm membrane filters and measured by
195 spectrophotometer. Phytoplankton samples were fixed with Lugol's iodine solution
196 (1.5% final conc.) and settled for 24h. Cell density was measured with a
197 Sedgwich-Rafter counting chamber under microscopic magnification ×400.
198 Phytoplankton species were identified according to Hu (2006).

199 **2.5. Statistical analysis**

200 The difference in growth responses between the various additions were analyzed by
201 one way-ANOVA. Post-hoc multiple comparisons of treatments means were
202 performed by LSD (L) and S-N-K(S) procedures. The data in all the cases met the
203 homogeneity of variance. Statistical analysis was performed with SPSS 19.0 (SPSS
204 software, IBM), and the level of significance used was $p < 0.05$ for all the tests.

205 **3. Results**

206 **3.1. The dynamics of phytoplankton biomass (Chl-a)**

207 The Chl-a concentration was reduced from 42 to 18 μg L^{-1} two hours after MLS
208 treatment, which remained lower than 20 μg L^{-1} throughout the monitoring period.
209 The average Chl-a concentration in the control pond was more than double of that in
210 the treatment pond within the first 12 months, with a maximum in May 2014 that was
211 3.5 times higher than the treatment (Fig. 2).

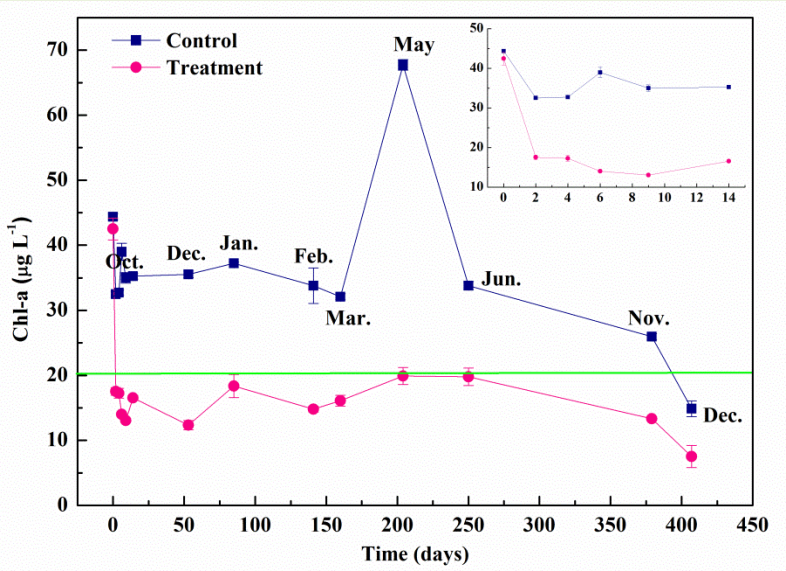

Fig. 2- Dynamics of Chl-a in control and treatment pond from October 2013 to December 2014.

3.2 The dynamics of nutrient concentrations and N:P ratios

In the short term, TP was reduced from 0.40 to 0.32 mg L^{-1} and remained at a similar concentration for the next two weeks after MLS treatment, while the average TP in control pond remained 0.42 mg L^{-1} during this period (inserted chart in Fig. 3a). SRP was reduced from 0.025 to 0.019 mg L^{-1} and maintained this concentration within the next two weeks (inserted chart in Fig. 3b). Both TP and SRP showed seasonal changes, which were lower in the winter and higher in the summer. The differences between the two ponds were reduced during the winter (December 2013 to February 2014) but diverged again in the next summer where TP in the control pond was about 1.5 times higher than that in the treatment pond (Fig. 3a).

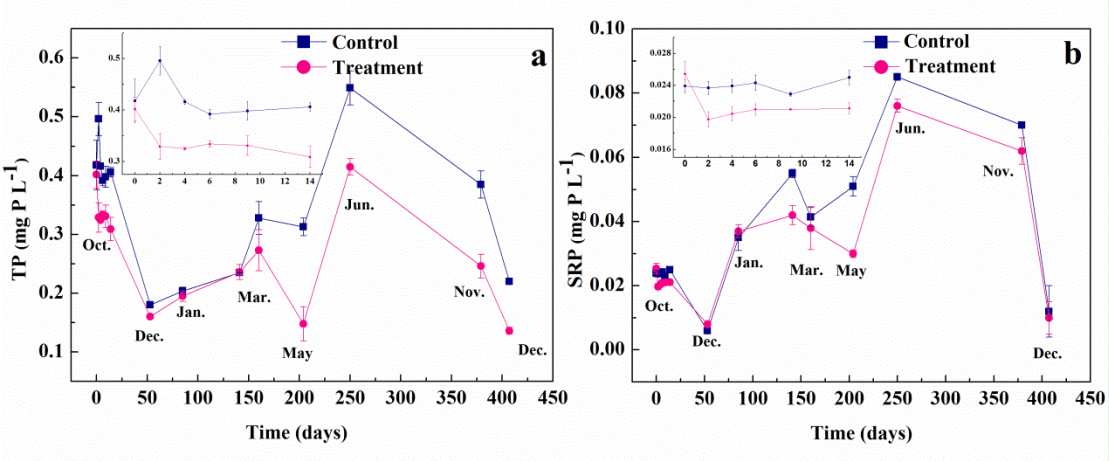

Fig. 3- Dynamics of TP (a) and SRP (b) in control and treatment pond from October 2013 to December 2014.

In the short term, TN reduced from 5.15 to 1.92 mg L^{-1} one day after MLS treatment and kept dropping down to 1.13 mg L^{-1} within the first two weeks (inserted chart in Fig. 4a), and maintained this concentration until January 2014. In the control pond, TN showed a slight decrease but remained 2 times higher than that in the treatment pond until January 2014. TN showed seasonal changes in both ponds, with low concentrations appeared in summer and winter and peaks in spring and fall, while, TN concentration in the control pond were more than 1.5 times higher than that in the treatment pond from March to December 2014 (Fig. 4a).

Both NO_3^--N and NH_4^+-N are the primary forms of DIN. In the treatment pond, NO_3^--N was reduced from 2.96 to 0.75 mg L^{-1} within the first two weeks (inserted chart in Fig. 4b) and declined to 0.37 mg L^{-1} in January 2014. In the control pond, NO_3^--N also exhibited decrease but kept higher than that in the treatment pond (Fig. 4b). The short and long term treatment effect on NH_4^+-N was most remarkable, where NH_4^+-N was reduced from 0.84 to 0.22 mg L^{-1} and remained at an average of 0.40 mg L^{-1} within the next 15 months without apparent seasonal change, while in the control

pond NH_4^+-N showed large seasonal change with peaks in both winter and summer with an average value of 0.71 mg L^{-1} (Fig. 4c).

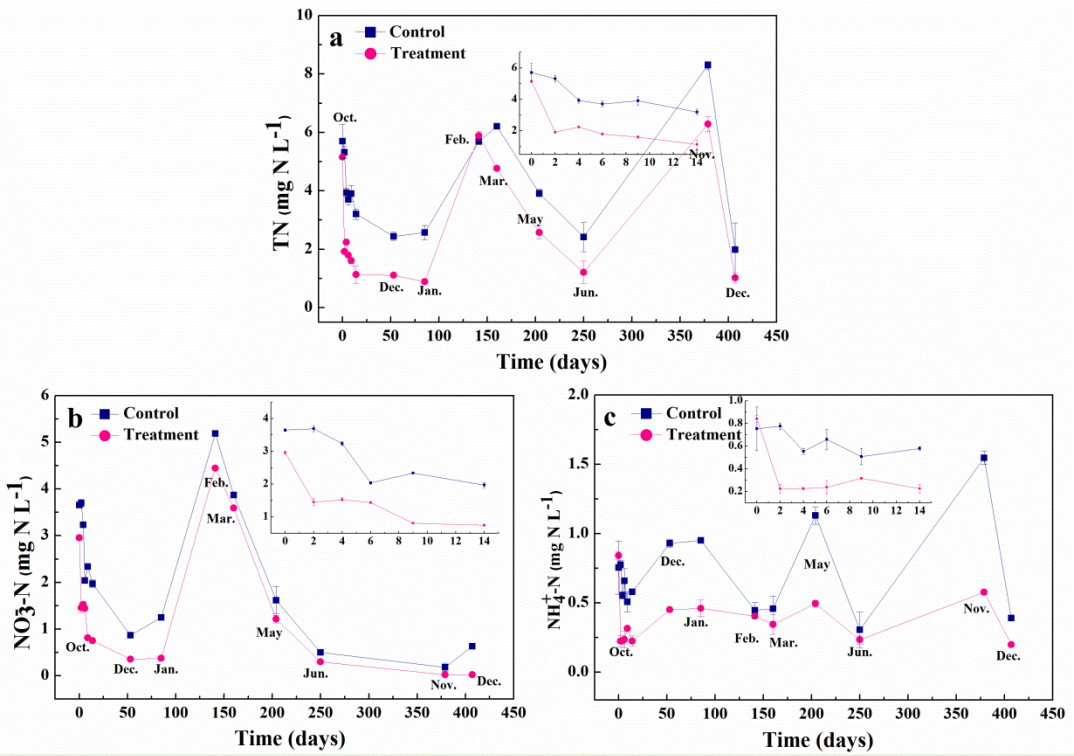

Fig. 4- Dynamics of TN (a), NO_3^--N (b) and NH_4^+-N (c) in control and treatment pond from October 2013 to December 2014.

The mass ratio of TN:TP reduced from 13:1 to 6:1 one day after algal blooms removal and continually reduced to 4:1 two weeks after MLS treatment. TN:TP ratios in the control pond also showed decrease within the first two weeks but higher than 7:1 (inserted chart in Fig. 5). In the long term, TN:TP ratio exhibited similar seasonal variations in the two ponds, and TN:TP > 25:1 in the spring and autumn but TN:TP < 7:1 in the summer (Fig.5).

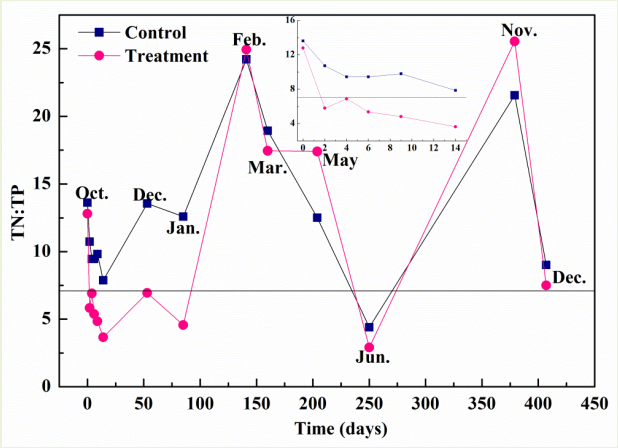

Fig. 5- Dynamics of ratio of TN:TP in control and treatment pond from October 2013 to December 2014. The line represents the Redfield ratio (7:1, mass ratio)

3.3. Composition structure of phytoplankton community

Changes of phytoplankton composition in both ponds are shown in Fig. 6. One day after MLS treatment, about 65% algal cells were removed from the water column, with cyanophytes, bacillariophytes and euglenophytes accounting for 71%, 50% and 50%, respectively (Support Information (SI), Fig. S2), but cyanophytes was still the dominant species. In the next spring (Mar. 2014), chlorophytes became the dominant species in the treatment pond, bacillariophytes and cryptophytes were the dominant and sub-dominant species in the control pond. In the next summer (Jun. 2014), phytoplankton biodiversity increased in the treatment pond (Table. S1), and the percentages of cyanophytes, bacillariophytes and chlorophytes were 33%, 33% and 23%, respectively, while cyanophytes recaptured the dominant position in the control pond with high percentage and cell numbers (Fig.6 and Fig. S3d). In the next winter (Dec. 2014), cyanophytes almost disappeared and bacillariophytes, chlorophytes and cryptophytes were the major species in the treatment pond. Meanwhile, cyanophytes and bacillariophytes were the major species in the control pond.

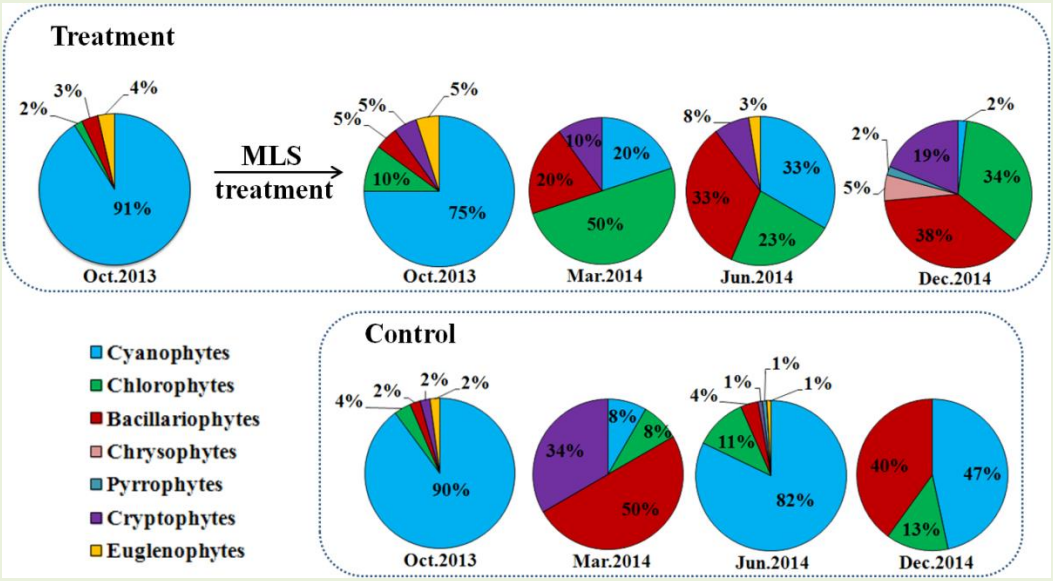

Fig. 6- The initial phytoplankton composition in bioassays experiments of the control and treatment pond.

3.4. Submerged vegetation restoration

Before the experiment there was no submerged vegetation in both control and treatment pond. The submerged vegetation appeared in next spring (Mar. 2014) in the two ponds. The coverage of submerged vegetation increased gradually from about 40% in March 2014 to 63% in December 2014 in the treatment pond, while it increased from 8% to 19% in the control pond (Table 1). The maximum biomass of submerged vegetation (mean wet weight) in the treatment pond was 3.6 times higher than that in the control pond (Table 1) in December 2014. The succession of dominant submerged vegetation was from *Potamogeton crispus* in March to *Elodea canadensis* in December.

Table 1- Coverage and biomass of submerged vegetation in the two ponds.

Water Pond	Time	Coverage	Biomass of submerged vegetation
			Mean wet weight (g m^{-2})
Control	Oct.2013	-	-
	Mar.2014	8%	176 ± 126

	Jun.2014	5%	108 ± 59
	Dec.2014	19%	329 ± 238
Treatment	Oct.2013	-	-
	Mar.2014	40%	676 ± 125
	Jun.2014	49%	849 ± 204
	Dec.2014	63%	1179 ± 357

285 "-" means there has no submerged vegetation.

286 **3.5. Responses of phytoplankton growth to nutrient addition**

287 The initial physical, chemical and biological properties of the pond water used for
288 bioassays are shown in Table S2 and the phytoplankton responses to various nutrient
289 additions in different season are shown in Fig. 7. For the October 2013 bioassay
290 experiment, N addition had no effect on Chl-a, whereas P and N+P additions led to
291 significant higher Chl-a concentrations than NA ($F_{3, 8}$ = 5.671, P < 0.001) in the
292 treatment pond (Fig. 7a). In the control pond, the Chl-a concentration showed
293 significant increase ($F_{3, 8}$ = 14.945, P < 0.001) under individual and combined N and
294 P additions (Fig. 7a). In March 2014 bioassay experiment, the response pattern of
295 phytoplankton to N, P and N+P additions in both ponds (Fig. 7b) was similar to that of
296 October 2013 bioassay experiment (Fig. 7a). In the next summer (Jun. 2014), Chl-a
297 exhibited no significant difference compare to NA by either N or P addition but
298 increased significantly only by combining N and P additions in the treatment pond.
299 Different from the Chl-a response of treatment pond, N addition showed higher Chl-a
300 stimulation than P addition, while N+P additions showed the strongest stimulatory
301 effect on Chl-a biomass in the control pond (Fig. 7c). In the next winter (Dec. 2014),
302 although Chl-a showed increase by nutrient additions in both ponds, the maximum

values (< 40 μg L^{-1}) were much lower than those in other seasons bioassays (Fig. 7d).

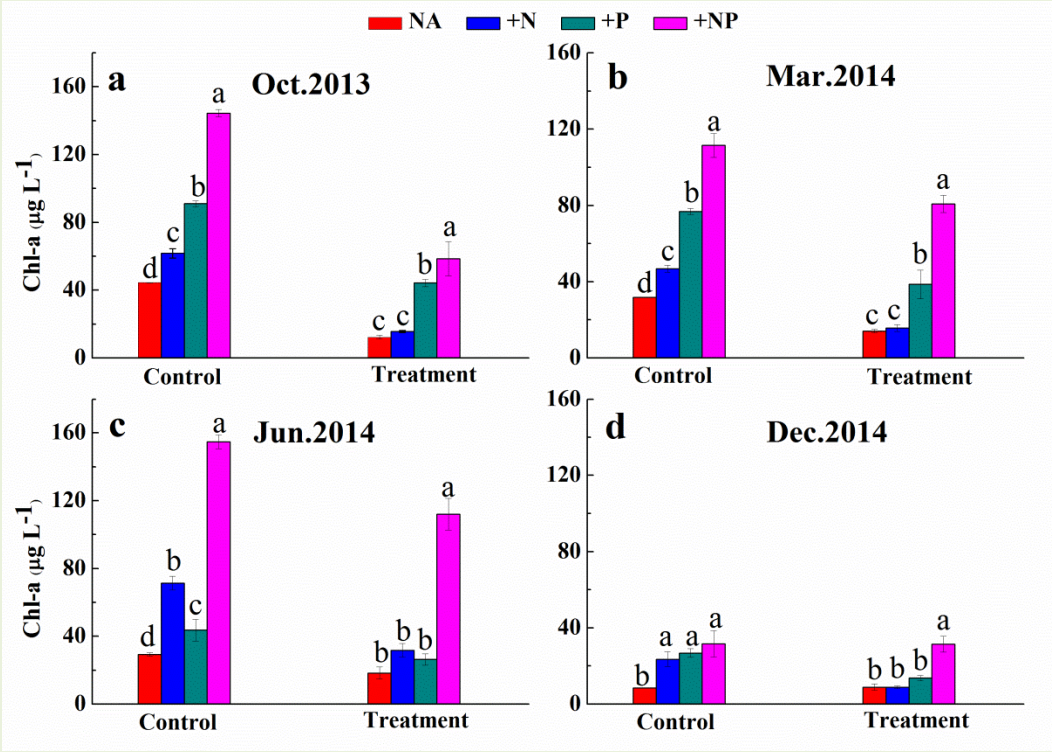

Fig. 7- The maximum phytoplankton biomass (Chl-a) responses to N and P additions in bioassays. Error bars represent ± SD of triplicate samples. Difference among various N and P addition are shown on the basis of ANOVA post hoc tests (a>b>c>d; $p < 0.05$) ("NA" represented pond water with no addition of nutrient).

3.6. Responses of phytoplankton growth at different N and P concentration

The specific relationship between nutrient concentration and phytoplankton growth was studied by examining the growth rate as a function of nutrient concentration using non-linear regression. In October 2013, the growth rates increased consistently until the SRP concentration reached approximately 0.2 mg L^{-1} in the treatment pond and 0.1 mg L^{-1} in the control pond. The maximum growth rate and half-saturation concentration from the fitted results of Monod equation were 0.325 d^{-1} and 0.180 mg L^{-1} in the treatment pond, which were higher than 0.260 d^{-1} and 0.054 mg L^{-1} in the control pond (Fig. 8a). In March 2014, the phytoplankton growth was no longer P limited when SRP concentrations reached about 0.3 mg L^{-1} in both ponds (Fig. 8b). A

0.3 mg L^{-1} of upper limiting SRP concentration maintained in the treatment pond but 0.1 mg L^{-1} in the control pond in the next summer cyanobacteria blooms period. The maximum growth rate and half-saturation concentration obtained from the Monod equation were 0.525 d^{-1} and 0.155 mg L^{-1} in the treatment pond, which were higher than 0.424 d^{-1} and 0.062 mg L^{-1} in the control pond (Fig. 8c). In the next winter (Dec. 2014), the upper limiting SRP concentrations reduced to 0.03 and 0.05 mg L^{-1} in the treatment and control pond, respectively (Fig. 8d).

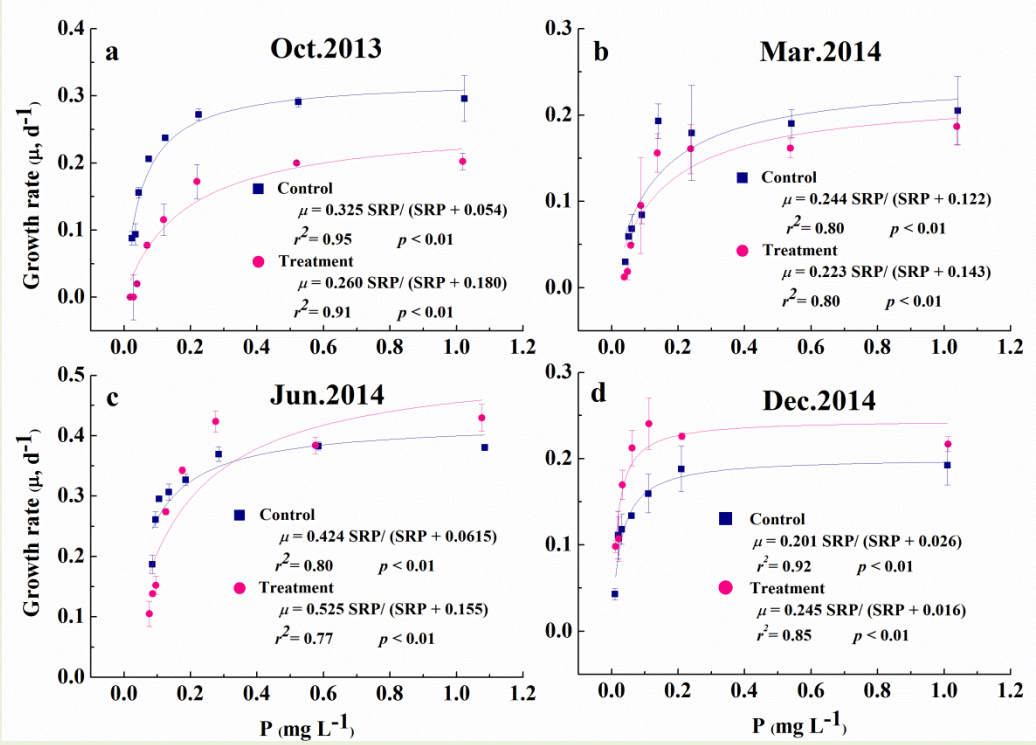

Fig. 8- Responses of growth kinetics of phytoplankton to different P concentrations. Curves were fitted by nonlinear regression. Error bars represent ±1 SD of triplicate samples.

In the short and mid-term, the relationship between the growth rate and DIN concentration was almost linear in the treatment pond (Fig. 9a and 9b), but, an addition of 0.25 mg L^{-1} was enough to satisfy the phytoplankton growth in October 2013 and March 2014 in the control pond (Fig. 9a and 9b). In the long term, the growth rate of phytoplankton from both ponds exhibited increase with the increase of

DIN concentration (Fig. 9c) due to the relatively low N concentration in the next summer (Jun. 2014). The upper limiting DIN concentrations obtained from the growth curves were 1.5 and 0.8 mg L^{-1} in the treatment and control pond, and the corresponding half-saturation concentrations for DIN were 0.790 and 0.407 mg L^{-1}, respectively (Fig. 9c). In the next winter (Dec. 2014), the upper limiting DIN concentrations reduced to 0.028 and 0.180 mg L^{-1} in the treatment and control pond respectively (Fig. 9d), and the maximum growth rate in the treatment pond was higher than that in the control pond (Fig. 8d and 9d).

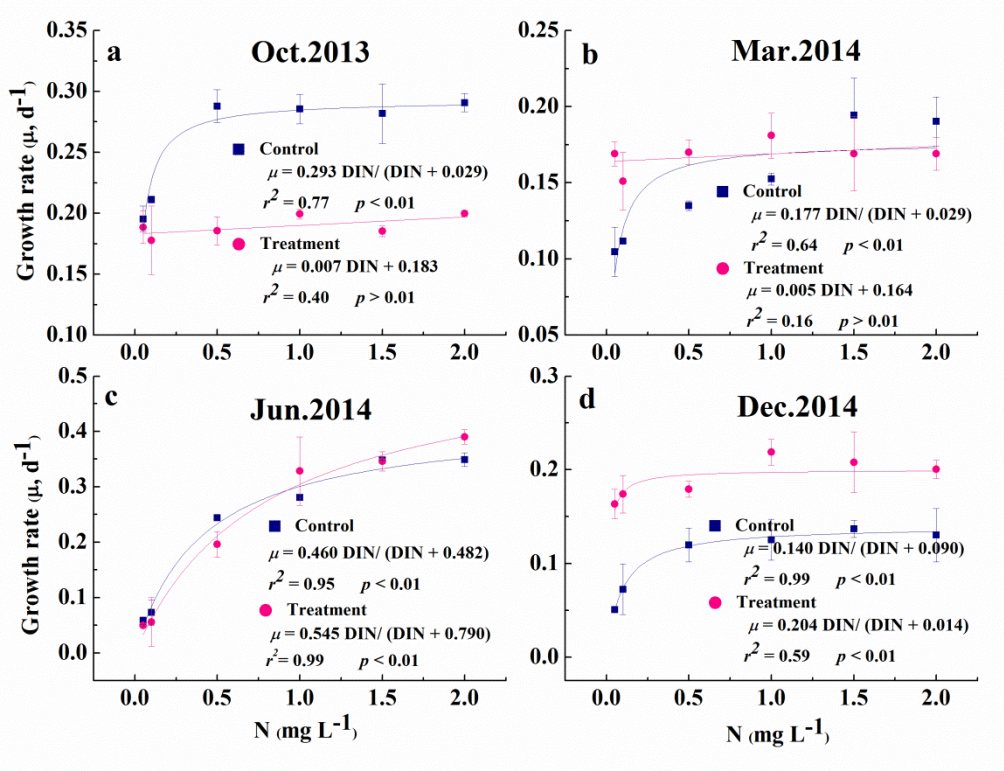

Fig. 9- Responses of growth kinetics of phytoplankton to different N concentrations. Curves were fitted by nonlinear regression. Error bars represent ±1 SD of triplicate samples.

4. Discussion

4.1. Effects of nutrient concentration and its ratio on nutrient limitation

After MLS treatment, about 20% TP was removed from water column within the first two weeks (inserted chart in Fig. 3a), which mainly depended on the flocculation of

algae and suspended particles. The initial removal rate for TP in this work appeared much less than our previous works (Pan et al. 2012b; Pan et al. 2011), which may be caused by insufficient treatment activities in the field (e.g. spraying and stirring) due to the lacking of labor at start of the experiment. The slight decrease of SRP (24%) within the initial two weeks (inserted chart in Fig. 3b) may be related to the P adsorption of MLS materials. In this experiment, chitosan was used as local soil modifier, which has the P absorption ability (Jung et al. 2013; Xian et al. 2008). In contrast to the low initial TP removal, more than 63% of TN was removed from water column (inserted chart in Fig. 4a), which depended not only on algal removal but also, perhaps more importantly, on the denitrification processes. NO_3^--N removal was the major contributor to TN reduction, but the removal mechanism was complicated. The NO_3^--N in the treatment pond showed more decrease than that in the control pond within the first two weeks (inserted chart in Fig. 4b). This indicated that the denitrification might exist in the control pond and it was reinforced with MLS intervention in the treatment pond. The settled algae cells might provide abundant biodegradable carbon for the denitrifying bacteria and exacerbate the N removal (Schipper et al. 2010) in the treatment pond. However, it still needs further studies to identify the response of denitrification to the MLS treatment.

Nutrient concentration reduction and change of TN:TP ratio caused by the MLS-IER may be responsible for changes in nutrient limitation, particularly in the first few months after MLS treatment. One day after MLS treatment, DIN reduced from 3.80 to 1.68 mg L^{-1} and SRP decreased from 0.025 to 0.019 mg L^{-1}. However, N was still not

371 a limiting element due to the fact that Chl-a had no increase by N alone addition (Fig.
372 7a), suggesting that DIN of 1.68 mg L^{-1} was sufficient for the residual algae growth.
373 SRP of 0.019 mg L^{-1} was lower than the upper limiting concentration of 0.20 mg P L^{-1}
374 (Fig. 8a), and Chl-a exhibited increase with P addition (Fig. 7a), hence, a P limitation
375 was obtained immediately after MLS treatment. In the control pond, P addition led to
376 a higher Chl-a increase than N addition (Fig. 7a), indicating that P was the major
377 limiting nutrient. The SRP of 0.025 mg L^{-1} was lower than the upper limiting
378 concentration of 0.1 mg L^{-1} of phytoplankton growth (Fig. 8a), which provided further
379 evidence for P limitation. In addition, Chl-a also exhibited increase by N addition,
380 which might be related to the dominant species of non-N_2 *microcystis* that compete
381 for DIN (Paerl et al. 2011). Based on the above discussion, both N and P co-limitation
382 for phytoplankton growth existed in the control pond in October 2013. In the
383 following monitoring period, the TN and TP in both ponds showed gradual decrease
384 until December 2013, but TN:TP > 7:1 in the control pond and TN:TP < 7:1 in the
385 treatment pond (Fig. 5) due to relatively higher TN concentrations in the control pond,
386 indicating that N might be the limiting nutrient in the treatment pond during this
387 period. In the mid-term (Mar. 2014), although SRP concentrations rose in both ponds
388 (Fig. 3b), they were lower than the upper limiting SRP concentration of 0.3 mg L^{-1}
389 (Fig. 8b), suggesting that P was still the limiting nutrient. This observation agreed
390 with the phytoplankton response to P addition in March 2013 bioassay experiment
391 (Fig. 7b). However, N addition showed no effect of Chl-a in the treatment pond but
392 increase in the control pond, which mainly depended on phytoplankton composition

393 (discussed in section 4.2).

394 In the long term, P and N concentrations exhibited seasonal variations similar to
395 previous studies on Lake Taihu (Xu et al. 2015). The maximum concentrations of P
396 (TP and SRP) (Fig. 3) and the minimum concentrations of N (TN, NO_3^--N and
397 NH_4^+-N) (Fig. 4) occurred in the next summer, meanwhile, the ratio of TN:TP was
398 less than 7:1 due to N reduction, which might lead to a N limitation in both ponds. In
399 the control pond, N addition showed a higher Chl-a increase than P addition and N+P
400 additions led to the strongest Chl-a stimulation (Fig. 7c), indicating that N was the
401 major limiting nutrient and P was secondary one. In the treatment pond, only
402 combining N and P additions could increase Chl-a biomass significantly (Fig. 7c).
403 This difference might be related to phytoplankton composition in both ponds. It is
404 noteworthy that TN and TP concentrations in the control pond maintained about 1.5
405 times higher than those in the treatment pond from March to December 2014, even if
406 seasonal variations of them observed in both ponds (Fig. 3a and 4a). In the treatment
407 pond, the high water clarity (Fig. S1c) provided suitable restoration conditions for
408 submerged vegetation, and the *Potamogeton crispus* were observed abundantly during
409 the spring while *Elodea canadensis* became the dominant species from September to
410 December 2014 (Fig. S6). A large amount of submerged vegetation not only compete
411 with phytoplankton for available nutrients but also may release alleopathic
412 compounds with the potential to inhibit phytoplankton growth (Erhard and Gross
413 2006; Wu et al. 2007), which might be responsible for the low level of Chl-a (< 20 μg
414 L^{-1}). The averaged NH4+-N concentration of 0.40 mg L^{-1} in the treatment pond

remained less than that in the control pond, which also might depended on the restored submerged vegetation, because NH_4^+-N was the directly assimilate form of N for macrophytes. The submerged vegetation was also observed in the control pond in the next year, but the coverage and biomass were much less than those in the treatment pond (Table. 1). In the next winter, N and P co-limitation occurred in both ponds due to the low concentrations of both N and P.

4.2. Effect of phytoplankton composition on nutrient limitation

In the short term, although a large proportion of non-N_2 fixing cyanobacteria (*microcystis*.spp) were removed from the water column of treatment pond (Fig. S2), the phytoplankton composition had no significant changes before and after MLS treatment (Fig. 6). The nutrient limitation of phytoplankton growth mainly depended on the dissolved N and P concentrations (discussed in section 4.1). However, the non-N_2 fixing cyanobacteria (*microcystis*. spp) decreased and N_2 fixing cyanobacteria (*chroococcus.* spp) increased gradually in the treatment pond from November 2013 to January 2014 (Fig. S5). This change of phytoplankton community together with the relatively low DIN concentration and TN:TP ratio (< 7:1) may further explain why N became the limiting nutrient during this period. In the control pond (Oct. 2013), about 90% algae were *microcystis* dominance of cyanobacteria, and whose cell numbers were about three times higher than the total cell numbers in the treatment pond (Fig. S4b), hence, more bioavailable N would promote the algae growth. As a result, phytoplankton growth was also controlled by N, providing further information of N and P co-limitation in the control pond.

437 In the mid-term, cyanophytes decreased greatly and other algae species appeared in
438 both ponds in March 2014 (Fig. 6), which might be due to the relatively low water
439 temperature (< 15℃) (Fig. S1a). The optimal temperature for cyanophytes growth is
440 often above 25℃ (Bell 2006), and hence, the growth of cyanophytes would be
441 inhibited in the bioassay experiment in March 2014. Compared to cyanophytes,
442 chlorophytes and bacillariophytes can endure the low water temperature of 7-14℃
443 (Tan et al. 2009). In the treatment pond, the domiant chlorophytes embrace high N
444 and P concentrations (Xu et al. 2014), the DIN concentration of 4.0 mg L^{-1} might
445 meet the demand of the algae growth because of Chl-a increase with N addition,
446 however, the SRP concentrations of 0.038 mg L^{-1} did not reach the staruate
447 concentration for the algae growth (Fig. 9b). This explained that P limitation of
448 phytoplankton growth in the treatment pond in March 2014. In the control pond, the
449 dominant bacillariophytes are significantly related to the PO_4^{3-}-P (Liu et al. 2012a).
450 The sub-dominant cryptophytes are often correlated to NO_x-N in winter and spring
451 (Liu et al. 2012b) and the nitrogen source that we added in our bioassay experiments
452 was NO_3^--N. Both of the algae species responses to N and P nutrient coincided with
453 the N and P co-limitation in the control pond.
454 In the long term, bacillariophytes, cyanophytes and chlorophytes distributed equally
455 in the treatment pond and the non-N_2 fixing cyanobacteria recaptured the dominant
456 position in the control pond in cyanobacteria blooms season of June 2014 (Fig. 6).
457 DIN concentrations were almost the lowest values in both ponds in June 2014 (Fig. 4),
458 and non-N_2 cyanobacteria would proliferate rapidly only if the adequate N supply. It

was fully embodied in the control pond due to the fact that N addition led to significant Chl-a stimulation (Fig. S4d). Hence, N was the major limiting nutrient for phytoplankton growth in the control pond. However, the cell numbers of high nutrient (N and P) favored bacillariophytes and chlorophytes were 1.7 times higher than that of cyanophytes in the treatment pond, and the Chl-a concentration increased significantly only with N+P additions (Fig. 7c). This indicated both N and P was limiting nutrient for phytoplankton growth in the treatment pond. In the next winter (Dec. 2014), the different phytoplankton composition in both ponds (Fig. 6) might affect the different responses of phytoplankton to nutrient addition but would not determine nutrient limitation of phytoplankton growth.

4.3. Nutrient threshold of phytoplankton growth

In addition to nutrient limitation manipulation, nutrient threshold can also serve as the basis for setting nutrient criteria after geo-engineering. The Monod equation described the relationship between algal growth rates and dissolved nutrient concentrations. The true nutrient-growth kinetics cannot be derived by bioassay addition experiments, because they do not account for reductions in concentration that occurred within the incubation period due to algae uptake. However, addition experiments provide an upper nutrient concentration for saturating phytoplankton growth. Compared to the control pond, the upper SRP threshold enhanced from 0.1 to 0.2 mg L^{-1} one day after MLS treatment, which might be caused by the part removal of particulate nutrients and dissolved nutrients. For N, the growth rates showed no difference as the added N concentration increased, because DIN was saturated for the phytoplankton growth

soon after MLS treatment. The maximum growth rates in the control pond were higher than those in the treatment pond (Fig. 8a and 9a). The flocculation and sedimentation after MLS treatment may largely inhibit the division and proliferation of algae cells (Li and Pan 2013, Wang et al. 2015). In the mid-term (Mar. 2014), the high nutrient favored algae species increased in both ponds, the upper threshold of SRP in both ponds exhibited similar concentrations of about 0.3 mg L^{-1} (Fig. 8b). In the long term, a 0.3 mg L^{-1} of SRP threshold maintained in the treatment pond and 0.1 mg L^{-1} of SRP in the control pond in the cyanobacterial blooms season of June 2014 (Fig. 8c). Likewise, the upper threshold of DIN increased from about 0.8 to 1.5 mg L^{-1} compared to the control pond (Fig. 9c). This might be caused by the different phytoplankton composition in both ponds. The cyanobacteria generally predominated in low nutrient concentrations (Zhu et al. 2008), which were the dominant species in the control pond. Bacilliariophytes and chlorophytes favored high nutrient concentration, and both of them took more than 50% in the treatment pond. The higher growth rates in the control pond when the SRP < 0.3 mg L^{-1} and DIN < 0.8 mg L^{-1} (Fig. 8a and 9a), which might be caused by the dominant cyanobacteria growth. Conversely, the bacilliariophytes and chlorophytes might lead to higher growth rates in the treatment pond when the SRP > 0.3 mg L^{-1} and DIN > 0.8 mg L^{-1}. In the next winter (Dec. 2014), both N and P thresholds reduced in both ponds (Fig. 8d and 9d) compared to those in the other seasons, and the low-water temperature (< 10 ℃, Fig. S1a) might limit nutrient assimilation of algae growth.

4.4. Implications

503 Reducing external loading of both N and P is often the first step to control
504 cyanobacterial blooms in eutrophic waters, but it is a very slow or costly process in
505 already enriched nutrient shallow lakes. However, the demand of society and the
506 governments for good water quality is urgent. Some countries have set compulsory
507 goals for water quality within short period of time and large amount of funds were
508 attached (Mackay et al. 2014). In-lake measures of geo-engineering may be useful to
509 meet this urgent need, although long term ecological safety must be taken into
510 consideration. Most of geo-engineering materials so far only focus on the P reduction.
511 The MLS materials (Li and Pan 2013; Shi et al. 2015; Zou et al. 2006) can
512 simultaneously manipulate N and P by removing algal blooms in short term, affecting
513 redox environment in the sediment, and restoring submerged vegetation in long term
514 in shallow waters (Pan et al. 2012a; Pan et al. 2011). High water clarity and the low
515 nutrient concentrations can be quickly obtained by MLS flocculation. The MLS
516 materials can also be modified by P locking modifiers and MLS-capping can be used
517 to maintain a mid-term effect in anti-resuspension and internal loads control (Pan et al.
518 2012a). The improved water quality conditions accelerate the restoration of
519 submerged vegetation in shallow waters, which could, in turn, assimilate nutrient and
520 inhibit algae growth. In this test, Chl-a can be maintained at an “acceptable” low level
521 (< 20 μg L^{-1}) and the phytoplankton biodiversity increased significantly, where the
522 *microcystis* dominance of cyanobacteria were replaced by multiple algae species.
523 These changes are likely to affect the property of nutrient limitation in the aquatic
524 system. The change of nutrient limitation and the enhancement of upper N and P

thresholds for phytoplankton growth may alleviate pressures of external nutrient loading after MLS treatment. These effects need further verifications in the future, which may provide new insights for eutrophication management.

5. Conclusions

Based on the results of this study, some conclusions can be described as follows.

- Nutrient limitation of phytoplankton growth was shifted from N and P co-limitation to P limitation shortly after MLS treatment. In the long term, both N and P became the limiting nutrient.
- The upper threshold of DIN for phytoplankton growth was enhanced from 0.8 to 1.5 mg L^{-1}, while SRP was increased from 0.1 to 0.3 mg L^{-1} during the cyanobacterial blooms period.
- Phytoplankton biomass (Chl-a) remained less than 20 μg L^{-1} after MLS treatment within the monitoring period.
- The cyanobacteria-dominated state was switched to the multi-algae coexisted state and submerged vegetation restored after MLS treatment.

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