# Switching ~~Transforming~~ Harmful Algal Blooms to Submerged Macrophytes by Lake Geo-Engineering Methods

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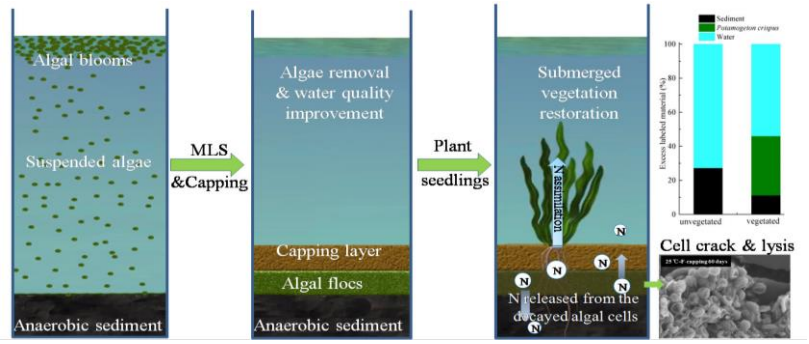
**ABSTRACT:** The switch of dominance from algae to macrophytes is crucial for ~~sustainabl~~sustainability of lake ecosystems~~e management of lakes~~ subject to human-induced eutrophication. The ~~transformation~~transition of algal blooms into macrophytes can transfer excess algae-sourced nutrients ~~into healthy food chain~~, thereby mitigating eutrophication. This process, however, ~~hardly~~ rarely occurs in an ~~established~~ algal bloom dominated waters. Here, we examined a hypothesis under different temperatures that the ~~trans~~transition~~formation~~ of algal bloom into ~~macraphyte~~ macrophyte can be facilitated by using in-lake geo-engineering methods~~,~~ through~~which can~~ reestablishing the growing conditions of macrophytes and ~~subsequently~~ triggering their uptake of algal-sourced nutrients. The results showed that flocculation-capping could not only remove *Microcystis aeruginosa* blooms from water column ~~and~~ but also facilitate algal ~~the algae's~~ decomposition and incorporation into submerged macrophyte (~~-~~*Potamogeton crispus*) biomass ~~at different temperatures after 60 days of controlled experiment~~. The Chl-a concentrations could be significantly reduced after using flocculation-capping technology. ~~-~~Photosynthesis and respiration of *M. aeruginosa* cells were ~~obviously~~ inhibited following flocculation and cell autolysis occurred in the treated systems compared with the control. The labeling test using $^{15}N$ revealed that between 3.3% and 34.8% of *Microcystis*-derived nitrogen could be assimilated ~~3.3% and 34.8%~~ by *P. crispus* ~~under~~ at 8 ℃ and 25 ℃, respectively~~, throughout the experiment~~. The study demonstrated that flocculation-capping ~~geo-engineering~~ method can facilitate the switch from algal to macrophyte-dominated~~s~~ state, which is crucial

for restoring the aquatic ecosystem ~~suffering from algal blooms~~.

**TOC**




## INTRODUCTION

Harmful algal blooms (HABs) ~~spread~~ in natural waters throughout the world ~~and~~ pose serious threats to the aquatic ecosystem, environment and public health.[1, 2] The formation of algal blooms restricts the light penetration into the bottom water, which ~~and then~~ could suppress the growth of ~~results in the loss or degradation of~~ submerged macrophytes due to the decreased photosynthetic rates.[2, 3] It is well known that reduction of nutrient concentrations is often insufficient to restore the vegetated clear state even at substantially lower nutrient concentrations where ~~levels than those at when the massive~~ degradation of the vegetation occurred.[4, 5] Some studies suggest that

~~improving the submerged macrphytes growing condition~~restoration of clear water can~~to~~ trigger the growth of submerged macrophytes, which cause the clear state to be self-stabilizing ~~of self-stabilizing in~~ alternative~~the~~ to the undesirable situation~~,~~ ~~thereby recovering the ecosystem resilience~~.[6, 7] However, this is thought to be difficult to achieve in an ~~established~~ algae~~l~~-dominated ~~bloom~~ waters in where photosynthesis and seed germination in submerged plants are usually suppressed by ~~due to its stable situation including low~~ reduced transparence and low dissolved oxygen.[7]

Over the past few decades, many efforts have been made to reduce the amount of phosphorus or to decrease the abundance of algal blooms directly in the water bodies.[8] In-lake geo-engineering methods have preferably tackled both controlling eutrophication and mitigating HABs by adding solid-phase P sorbents[9] or other metal salts[10], chemical substances[11] and algaecides[12] into waters. However, the side-effects from the use of non-biodegradable metal salts or other chemical substances become increasingly concerned.[10, 13] Some studies eliminated the HABs out of water column through flocculation and sedimentation with the modified clay/soil.[14-16] Considering that a substantial proportion~~massive part~~ of P in water is ~~mainly~~ stored in algal cells during algal blooms~~the algae growing season~~,[17] the modified clay/soil methods can speed up the algal blooms together with nutrients inside the cells settling onto sediment in environmentally-friendly way.[16, 18] However, many settled algal cells may tolerate the low light at lower temperatures and survive on the lake bottom in a certain period,

which may ~~be the "~~ seed ~~bank" of the~~ algal blooms in the following years.[19] Otherwise, the release of excess nutrients form the decayed algal cells together with oxygen consumption may sustain the eutrophic status of lakes.[20] All these factors may favor algal blooms which seriously hinder the reconstruction of the submerged macrophytes, especially for their initial germination or introduction period.

~~Actually, the~~The settling HABs in the sediments can also become major sources of nutrients for the growth of submerged macrophytes once the macrophytes can be reconstructed successfully, thereby transferring excess algae-sourced nutrients into food web.[21-23] Unfortunately, ~~amounts of~~ HAB~~s~~ sedimentation is a slow and uncontrolled process in natural waters during~~by the consequences of~~ bloom die-off or overwintering.[24, 25] Thus the transformation of HABs into macrophytes cannot occurs effectively in an established algal bloom waters, which aggravates the difficulty of lake transformation from dominance by algae to macrophytes. Capping with natural soils after settling HABs has been suggested to prevent algal floc/sediment resuspension and reduce nutrient release into the water column.[26] In addition to enhancing the transparence and oxygen level in water column,[27] this make it possible to construct suitable habitats for restoring submerged macrophytes,[28] in which deposited algal blooms can be buried and decomposed under the capping layer. The reconstruction of submerged vegetation, which is considered the most important for restoring aquatic ecosystems suffering from serious eutrophication,[23] would be potentially facilitated by utilizing the nutrients from both the decomposed algal blooms and sediment. The

redistributed nutrients can reduce the nutrient transfer into the water column and turn them into the food chain via incorporation in plant biomass, and ~~finally provide~~provide opportunities to intercept and partially repair the ~~broken~~ nutrient biogeochemical cycle. Some studies have found that reconstruction of submersed vegetation could be facilitated by using modified soil~~s~~ methods~~flocculation~~,[27, 28] however, to our knowledge,~~ few studies have been reported to investigate~~ the effects of such geo-engineering methods on the nutrient transformation from ~~an~~ established algal blooms to submerged macrophytes remain largely unexplored. Moreover, whether the switch from algal bloom to macrophyte state in waters can be triggered by in-lake geo-engineering method, if it is, the efficiency of nutrients derived from the algae that can be used by the submerged vegetation need to be quantified.

In this study, the HABs in simulated water-sediment columns were treated by using combination of modified soil and capping with natural soils. The morphology and metabolism of the deposited algal cells in control, Flocculation-treated (F-only), and Flocculation-capping-treated (F-capping) systems were investigated at ~~8℃, 25℃ and 35℃~~different temperatures~~, respectively~~. The temperature 8℃, 25℃, and 35℃ were selected to simulate the stage of early spring, early summer, and midsummer in Lake Taihu, where the dominant cyanobacteria appear in the surface water at the end of spring, bloom during summer, and then sink onto the sediment during the late autumn and winter. The assimilation of *Microcystis*-derived nitrogen by the submerged vegetation (i.e., *Potamogeton crispus*) was tracked using $^{15}N$. We hypothesized that the flocculation-

capping technology could accelerate the removal of algal blooms from water, trigger swift ~~death and~~ decomposition of the deposited algal biomass~~blooms in the sediment~~, and ~~then~~ facilitate transference of the excess algae-sourced ~~nitrogen~~ nutrients into the submerged vegetation. The objectives of this study are to quantify the efficiency of nitrogen transferred from algae into macrophyte biomass and to examine the synergetic effects of the flocculation and capping treatment on switching~~transforming~~ HABs into submerged macrophytes~~vegetation~~ at different temperatures. ~~, and to quantify the efficiency of nitrogen transferred from an established algal blooms into submerged vegetation, and to explore the effects of temperature on both the dynamics of the algae's morphology and metabolism and the assimilation of *Microcystis* derived nitrogen.~~

## MATERIALS AND METHODS

**Algae, soils, and flocculants.** *M. aeruginosa* is a well-known freshwater bloom-forming cyanobacteria. The *M. aeruginosa* strain (FACHB-905) was obtained from the Institute of Hydrobiology, Chinese Academy of Sciences, and cultivated in autoclaved BG11 medium with 98% $^{15}N$ as $Na^{15}NO_3$ (Sigma-Aldrich) in the laboratory. Algal batch cultures were maintained at 25℃ ± 1℃ under cool white fluorescent light of 2000-3000 lx on a 12-h light/12-h darkness regimen in an illuminating incubator (LRH-250-G, Guangdong Medical Apparatus Co.Ltd., China). The BG11 medium with 98% $^{15}N$ as $Na^{15}NO_3$ (Sigma-Aldrich) was supplemented ~~added~~ in algal batch cultures on the 3 days before the algae were used for the assimilation experiment.

Soil ~~The soil~~ was collected from the bank of Lake Taihu (China), washed with

deionized water and dried for 10 h at 90℃. The soils used for flocculation and capping were grounded and sieved through 180 meshes (<90 μm) and 40 meshes (>380 μm), respectively. ~~The chitosan~~Chitosan (~~solids,~~ $C_{56}H_{103}N_9O_{39}$, Qingdao Haisheng Bioengineering Co. Ltd., China) was dissolved by adding 100 mg of chitosan into 100 mL of 0.5% HAc (1 g/L) and stirring until all chitosan was dissolved. To modify the soil, 100 ml soil suspension (100 g/L) was added to 300 ml chitosan solution (1 g/L). The mixture was prepared freshly and well stirred for each experiment. All the containers and materials were autoclaved together with BG11 medium.

**Algal biomass v~~V~~itality experiment.** Algal cultures in the mid- to late-exponential growth phase were used. The experiment was conducted in 27 plexiglass cylinders with an inner diameter of 8.4 cm and height of 50 cm (Figure 1 a). 1L bloom water (7.~~29~~3-7.~~69~~7×$10^7$ cells/mL) was filled into the columns and stable for 1 hours; 18 columns were then selected randomly for flocculation with modified soils. ~~–~~The modified soil suspension was added to the bloom water and stirred by using a glass rod. The final concentrations of the modified soils in each column were consisted of 3 mg/L chitosan and 100 mg/L soil. The flocculated columns were kept standing for 3 hours to allow the algal flocs sedimentation, and then, ~~(3 mg/L chitosan and 100 mg/L soil) and~~ nine columns out of the flocculated columns were ~~labeled "F-only". Three hours after sedimentation, 9 flocculated columns were~~ covered with 1-cm-thick layer of natural soil and labeled "F-capping". ~~The flocculation-only columns labeled "F-only" and the remaining~~ The nine~~nine~~ flocculated columns without capping treatment were labeled

as "F-only" and the remaining nine columns without any ~~no~~ treatment were set as the "control". These columns were encircled with a light tight cloth about 15 cm from the bottom. After that, nine columns consisted of triplicated columns for each treatment were separately incubated in the illuminated incubator at 8℃, 25℃ and 35℃ under fluorescent light (2000-3000 lx, 12-h light/12-h darkness). The initial samples were collected to measure morphology, photosynthesis, and respiration rate of *M. aeruginosa* cells after 10 hours.~~All columns were cultured in the illuminated incubator at 8℃, 25℃ and 35℃, respectively, under fluorescent light (2000-3000 lx, 12-h light/12-h darkness).~~ The 8℃, 25℃ and 35℃ conditions were set according to the temperatures in the early spring, early summer, and midsummer, respectively, in Lake Taihu (China). ~~All incubation experiments were conducted in triplicate.~~ Chl-a concentrations were measured at 15 days intervals. The morphology, photosynthesis, and respiration rate of *M. aeruginosa* cells were measured at 0 and 60 days after the incubation experiment.

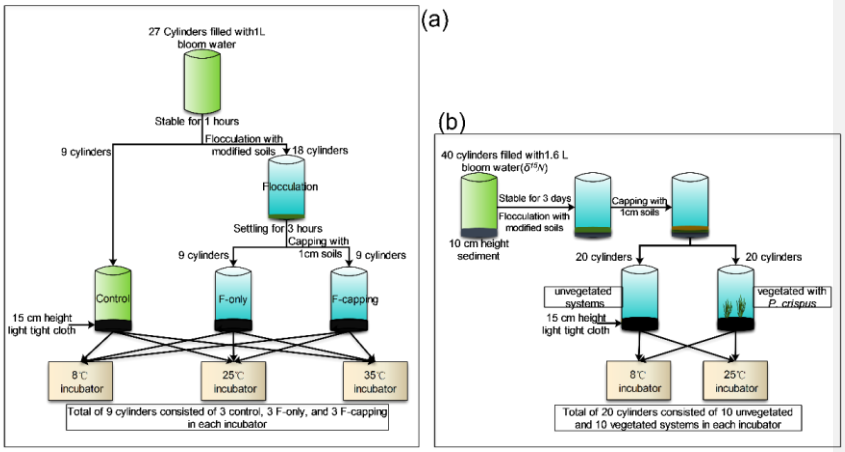



**Figure 1.** The conceptual diagram for the design of the algal biomass vitality

experiment (a) and nitrogen assimilation experiment (b).

–**Chlorphyll-a (Chl-a):** The algal water in the control and algal flocs in the treated systems were siphoned out of the columns at 15 days intervals and filtered with a 0.45-μm membrane. The flocs and membrane were extracted by acetone (90%) for 24 h at 4℃ and measured with a spectrophotometer.[5]

**Morphology:** The samples were collected at 0 and 60 days and centrifuged at 6000 rpm for 3 min. The algal cells were pre-fixed with 2.5% glutaraldehyde for 4 h, washed with phosphate buffer solution (centrifuged and then resuspended into the phosphate buffer solution for 20 min, repeated three times), post-fixed with 1% osmium tetraoxide for 2 h, and again washed with phosphate buffer solution. The washed samples were dehydrated twice through a series of 30%, 50%, 70%, 85%, 95%, and 100% ethanol solutions and dried with a vacuum drier. Completely dry samples were then mounted on a copper stub, coated with gold, and examined with a SEM (S-3000N, HITACHI, Japan).

**Photosynthesis and respiration**: The algal aggregates in the control and flocs in the treated systems were gently siphoned at 0 and 60 days, added to the micro-breathing bottle (4 ml), and cultured at 25℃ ± 1℃ under cool white fluorescent light of 2000-3000 lx with a 10-h light/10-h darkness regimen in the illuminating incubator. After transferring sampling bottles into the incubator, photosynthetic and respiratory rates were measured with a micro-respiration system (MRS, Unisense, Danmark). The $O_2$ concentration was measured continuously for 60s every 2 min in each sample by an $O_2$

189 microsensor within a whole culture cycle (i.e., 10-h light/10-h darkness regimen).
190 ~~(Please see more details about the method in Supporting Information (SI)).~~
191 **Nitrogen assimilation~~Assimilation~~ experiment.** After incubation, ~~The~~ the $^{15}$N-
192 labeled *M. aeruginosa* cells were collected with a 30- μ m net and rinsed at least ten
193 times with deionized water to remove unassimilated $^{15}N$-$NO_3$~~15N-labeled algal cultures~~
194 ~~were washed repeatedly with deionized water to remove unassimilated $^{15}N$-$NO_3$~~. The
195 resulting $\delta^{15}N$ value of the labeled *M. aeruginosa* was 1072 ± 13‰ (n=2), and a certain
196 dosage of algae was used to form bloom water (7.~~29~~3-7.~~69~~7×$10^7$ cells/mL). 40
197 columns~~Columns~~ with the same size as mentioned above were filled with 10 cm of
198 sediment (collected from Lake Taihu, China) and 1.6 L of bloom water and stabled for
199 3 days before the experiment (Figure1 b). A 15-cm above the bottom of the column was
200 encircled with a light tight cloth to avoid the effects of ambient light on the sediment.
201 The sediments were capped with 1 cm of natural soil after flocculation with modified
202 soil. Half of the columns were planted with *Potamogeton crispus* seedlings after
203 capping treatment (vegetated systems), and the remaining columns remained
204 unvegetated. The total columns were divided into two groups and each group consisted
205 of 10 vegetated and 10 unvegetated systems. Each group (including unvegetated and
206 vegetated systems) was cultured in the illuminated incubator at 8°C and 25 ℃,
207 respectively, under fluorescent light (2000-3000 lx, 12-h light/12-h darkness). 8 ℃
208 and 25 ℃ were set up to compare the *P. crispus* biomass and its assimilation of algal-
209 sourced N between germination and rapid growth period.~~Each treatment had 10~~
210 ~~duplicates.~~ Plant and sediment samples (the top 5 cm) were taken on the day of capping
211 (day 0) and on days 10, 17, 27, and 45 after capping treatment.~~;~~ The ~~the~~ samples~~–~~ taken
212 on day 0 were considered as controls. During each sampling event, two random
213 columns (treated as duplicates) were visited, and the entire plant biomass was harvested
214 from them.
215 The sediment and plants were homogenized, dried, and analyzed for stable nitrogen

isotope ratio ($^{15}N/^{14}N$) using a Delta Plus Advantage mass spectrometer (Finnigan MAT) connected to a Flash EA1112 elemental analyzer. Water samples were also analyzed. $^{15}N$ abundance was expressed using the conventional delta notation against the atmospheric nitrogen standard:

$$\delta^{15}N_{(‰)} = (^{15}N/^{14}N_{\text{sample}} / ^{15}N/^{14}N_{standard} - 1) \times 1000 \quad (1)$$

Moreover $^{15}N$ data are presented as excess μmol of $^{15}N$ per gram of dry sample (the absolute amount of $^{15}N$ incorporated in the plant), calculated according to:[29]

$$Excess\ ^{15}N(\mu mol/g) = \frac{\mu mol\ of\ N\ in\ sample \times \frac{at\%^{15}N_{sample} - at\%^{15}N_{control}}{100}}{gram\ of\ dry\ sample} \quad (2)$$

$$at\%^{15}N_{sample} = \frac{100 \times R_{air} \times (\frac{\delta^{15}N_{sample}}{1000} + 1)}{1 + R_{air} + R_{air} \times \frac{\delta^{15}N_{sample}}{1000}} \quad (3)$$

The analytical error between repeated measurements was typically within ±0.1‰. where $at\%^{15}N_{control}$ represents the value on day 0, and $\delta^{15}N$ is expressed as an excess value relative to the atmospheric nitrogen ratio, $R_{air}$=0.0036765.

**Statistical analysis.** Mean value and standard error calculations and construction of graphics were ~~all~~ carried out using ~~origin~~ Origin 8.0. Duncan's multiple range test was conducted to determine significant differences among different treatments using SPSS16.0 software, and the Pearson correlations among different parameters were also analyzed. A two-way ANOVA were used to compare Chl-a and assimilation of nitrogen between different treatment systems under different temperatures at each corresponding

sampling point, with differences accepted at a significance level <0.05.

**RESULTS**

**Algal biomass vitality experiment.**~~Changes in Chl-a concentrations.~~ The Chl-a concentrations in the water from the control systems showed some increasing stages during the experiment with the highest concentrations of 7397μg/L, 9778μg/L, and 9224 μg/L at 8℃, 25℃ and 35℃, respectively (Figure 2). Whereas in the treated systems, the concentrations of Chl-a showed continuous declines at each temperature in the following order: 8 ℃ -F-only >8℃-F-capping >25 ℃ -F-only >25 ℃ -F-capping >35 ℃ -F-only>35 ℃ -F-capping. Moreover, the higher water temperature accelerated the decrease of the Chl-a concentrations in both the F-only and F-capping treated systems (P< 0.05) (Figure 2).~~In the control samples, the concentrations of Chl-a decreased before 15 days of incubation and then increased until the end of the experiment at 8℃ incubation (Figure 1), whereas the Chl-a concentrations in the samples incubated at 25℃ and 35℃ increased to peak values of 9778.4 and 9224.4μg/L on days 30 and 15, respectively, and decreased rapidly until the end of the experiment. Compared with the control samples, the concentrations of Chl-a showed continuous declines in the treated systems at all incubation temperatures in the following order: 8℃ F-only >8℃ F-capping >25℃ F-only >25℃ F-capping >35℃ F-only>35℃ F-capping. A higher water temperature significantly accelerated the decrease of the Chl-a concentrations in both the F-only and F-capping treated systems (P< 0.05) (Figure 1).~~

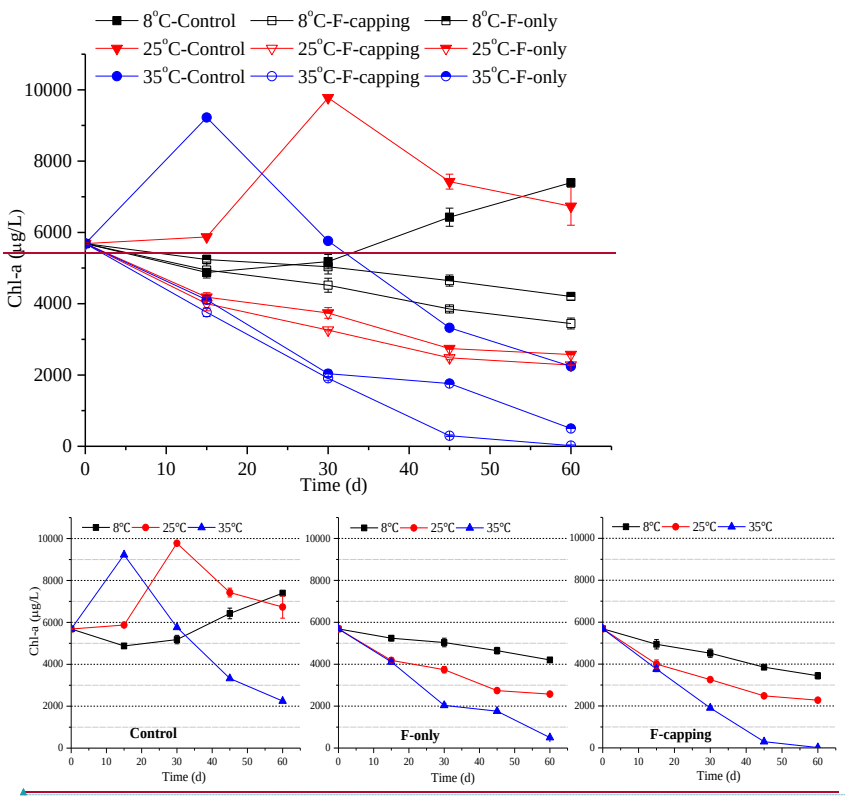



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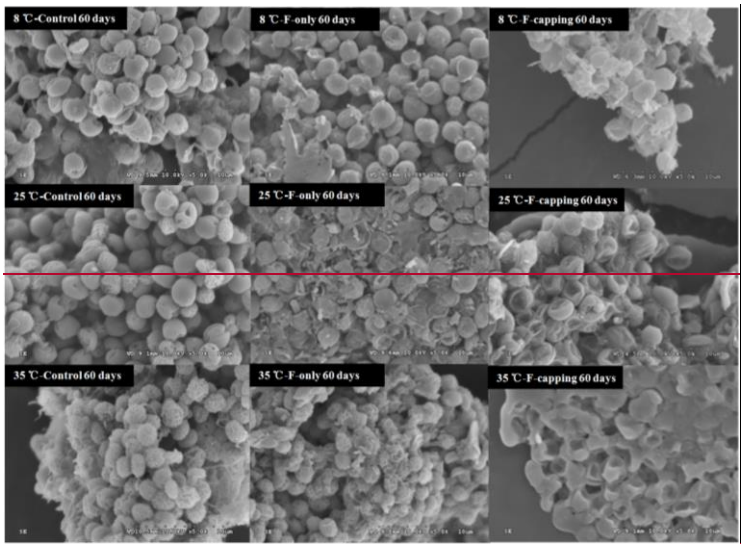
**Figure ~~1~~2.** Concentrations of Chl-a in different treatment systems. Chl-a in the control represents the Chl-a concentrations in the water collected from 10 cm below the surface of water columns, whereas Chl-a in treated systems represents flocs.

**~~Changes in morphology.~~** The algal cells collected on day 0 of the vitality experiment showed intact morphology with no obvious differences among the control, F-only and F-capping systems (Figure S1). At the end of the experiment, most algal cells collected from the three systems also showed intact morphology at 8℃ (Figure 3). However, many algal cells collected from the F-capping systems incubated at 25℃ were obviously deformed and lysed compared with those collected from control systems. More

importantly, obvious cell autolysis occurred in the F-capping incubated at 35℃ compared with those in control and F-only systems (Figure 3). The results also show that the increasing temperature exacerbated the cell destruction in the treated systems.~~The SEM images of algal cells collected on day 0 of the vitality experiment showed no obvious differences among the control, F-only and F-capping systems (Figure S1) in which intact cells were found at the beginning of the experiment.~~

~~Most algal cells collected from the three systems sustained their normal morphology at the end of incubation at 8℃ (Figure 2). Many algal cells collected from the F-capping systems were obviously wizened and cracked at 25℃, whereas no obvious changes in cell morphology were seen in the control and F-only systems. Especially in the F-capping, obvious cell autolysis occurred at 35℃ (Figure 2). In addition, the results also show that the increasing temperature exacerbated the~~



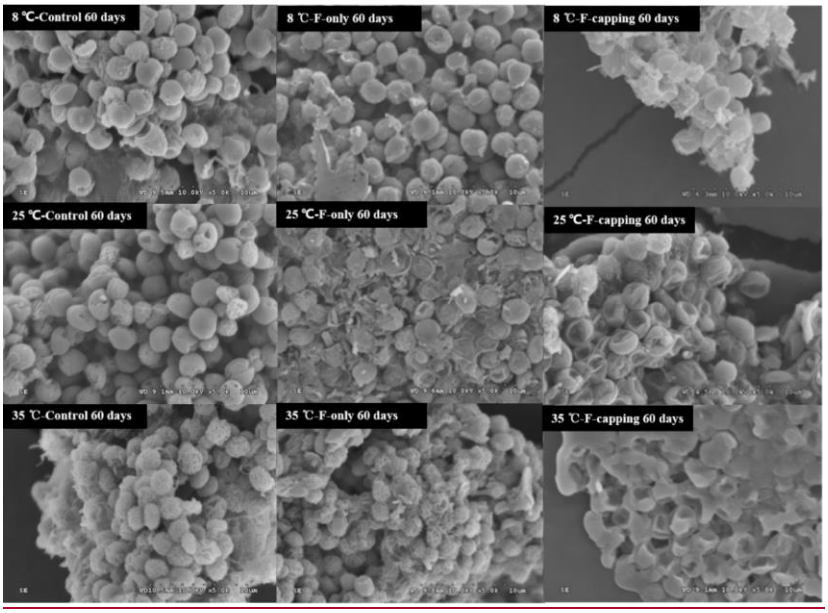
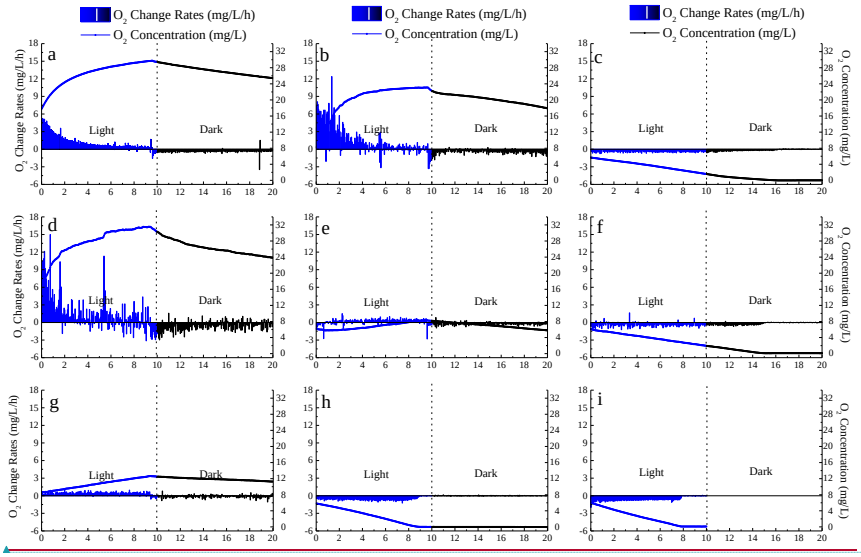




**Figure ~~2~~3.** SEM images of algal cells in different treatment systems incubated for 60 days at 8℃, 25℃ and 35℃, respectively.

At the beginning of the experiment, ~~**Changes in photosynthesis and respiration.**~~

the *M. aeruginosa* cells collected from all the three systems could sustain their normal photosynthesis and respiration which could be reflected by that the oxygen produced in the light stage was sufficient to maintain algae's respiration during the dark phase (Figure S2). The algal cells collected from both control and F-only systems still sustained their photosynthesis incubated at 8℃ and 25℃ after 60 days, reflected by the positive oxygen change rates (Figure 4 a, b and c, d). However, the photosynthesis efficiency was eight times lower in the F-only systems at 25℃ than that in control systems. Although the cells collected from F-capping could sustain photosynthesis in the light phase, the efficiency was much lower than those in control. It should be noted that the $O_2$ change rates for the cells collected from F-capping incubated at 25℃ showed negative values even in the light incubation phase after incubation for 60 days (Figure 4h), indicating that the death of algal biomass had occurred. The death and decay of algal cells were found in all three systems after 60 d of incubation at 35℃, which was reflected by negative $O_2$ change rates (Figure 4 c, f and i). However, the consumption rates of $O_2$ showed significant differences among the three systems in the order: (F-capping >F-only >control; $P<0.05$). These results indicated that the flocculation-capping treatment accelerated algal cell death and decay. ~~The *M. aeruginosa* cells sustained their normal photosynthesis and respiration at the beginning of the experiment (Figure S2). The algal cells collected from both control and F-only systems sustained their normal photosynthesis and respiration after 60 days of incubation at 8℃ and 25℃, and photosynthesis and respiration were 8 times higher in~~

~~the control systems under 25℃ than that in F only systems (Figure 3 d and e). The $O_2$ change rates showed negative values, even in the light incubation phase, for the cells collected from F capping after incubation for 60 d at 25℃ (Figure 3 h), indicating that the death and decay of algal cells had occurred. The death and decay of algal cells were also found at the beginning of measurement in all three systems after 60 d of incubation at 35℃, which was reflected by negative $O_2$ change rates (Figure 3 c, f and i). However, the rate of $O_2$ consumption showed obvious differences between the three systems (P<0.05; F capping >F only >control), which suggests that the flocculation capping treatment accelerated algal cell death and decay at same temperatures.~~

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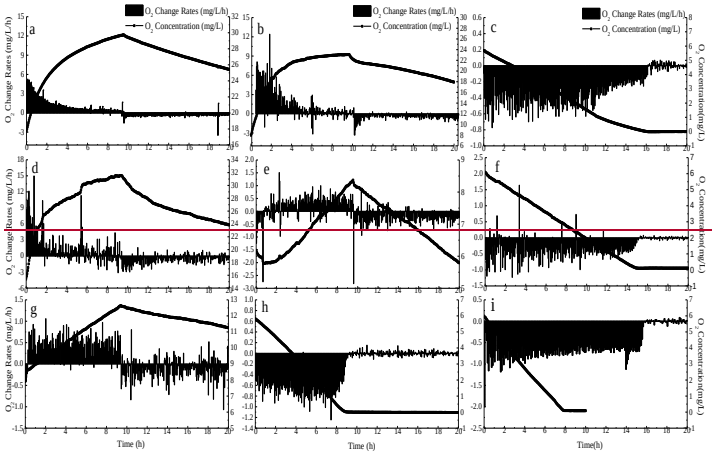


**Figure ~~34~~.** Photosynthesis and respiration of *M. aeruginosa* cells in different systems: a: 8℃-control-60d, b: 25℃-control-60d, c:35℃- control-60d; d: 8℃-F-only-60d, e: 25℃-F-only-60d, f: 35℃-F-only-60d; g: 8℃-F-capping-60d, h: 25℃-F-capping-60d, i:35℃-F-capping-60d.

**Nitrogen assimilation experiment.** ~~**Assimilation of nitrogen in *P. crispus*.**~~ 1.38μmol$^{15}$Ng$^{-1}$ labeled algae was filled in each column before flocculation. The δ$^{15}$N enrichment in the sediment increased from 4.46‰ to 18.47‰ after sinking algal blooms into the sediment. During the experiment, a decreasing trend in the $^{15}$N values was found in the sediment from all four systems (Figure 5). Whereas, at the same temperature, the vegetated sediment lost more *Microcystis*-derived nitrogen than that in the unvegetated systems due to plant uptake. Excess $^{15}$N was found in *P. crispus* collected at both 8℃ and 25℃, whereas the excess $^{15}$N (0.48μmol$^{15}$Ng$^{-1}$) in *P. crispus* collected at 25℃ was 10 times greater than that at 8℃ (0.045 μmol$^{15}$Ng$^{-1}$) (Figure 6). This was consistent with the results the higher temperature could promote more

*Microcystis*-derived nitrogen loss from the sediment whether in the vegetated or unvegetated system (Figure 5).~~The $\delta^{15}$N value in the sediment increased from 4.46‰ to 18.47‰ after flocculation of algal bloom water with 1.38μmol$^{15}$Ng$^{-1}$ labeled algae. During the experiment, a decreasing trend in the $^{15}$N values was found in the sediment from all four systems (Figure 4); however, more *Microcystis* derived nitrogen was removed from the sediment incubated at 25℃ than from that incubated at 8℃ for both vegetated and unvegetated systems (Figure 4). Moreover, the amount of *Microcystis*-derived nitrogen in the sediment of the vegetated columns decreased more than that in the unvegetated systems when the columns were incubated at the same temperature (Figure 4).~~

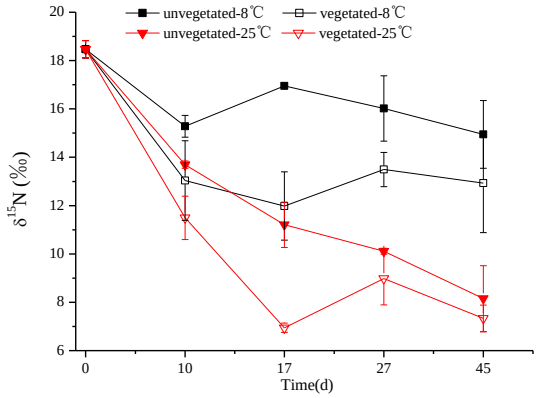


**Figure 45.** Time series of $\delta^{15}$N in sediment collected from different columns during the 45-d experiment.

~~– At the end of the experiment, excess $^{15}$N was concentrated in both of the vegetated and unvegetated sediments (Figure 5). In the unvegetated systems, $^{15}$N retained in the sediments comprised 80.7% (1.11μmol$^{15}$Ng$^{-1}$) and 27.2% (0.38μmol$^{15}$Ng$^{-1}$) of the initial~~

~~excess $^{15}$N at 8℃and 25℃, respectively (Figure 5). Excess $^{15}$N was found in *P. crispus* collected at both 8℃ and 25℃, whereas the excess $^{15}$N (0.48μmol $^{15}$Ng$^{-1}$) in *P. crispus* collected at 25℃ was 10 times greater than that at 8℃ (0.045 μmol $^{15}$Ng$^{-1}$) (Figure 5). Lower amounts of excess $^{15}$N were found for both sediment and water in the vegetated systems than in the unvegetated systems (Figure 5).~~

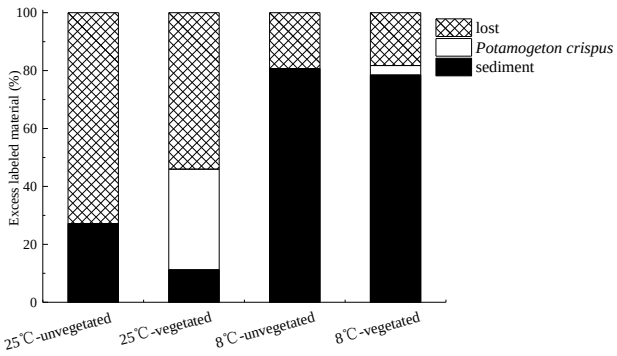


**Figure ~~5~~6.** Labeled N retained by sediments, *P. crispus*, and water in different systems at the end of experiment.

In addition, the increase rate of $\delta^{15}$N in *P. crispus* at 25℃ was 5 times higher than that at 8℃ during the 45-d experiment (Figure 7). The increase in $^{15}$N enrichment significantly correlated with the increase in the *P. crispus* biomass ($p<0.05$) where the biomass of *P. crispus* grown at 25℃ was double that grown at 8℃ (Figure 7).~~Moreover, the increase rate of $\delta^{15}$N in *P. crispus* at 25℃ was 5 times higher than that at 8℃ during the 45-d experiment (Figure 6). Compared to the initial value of $\delta^{15}$N (6.65‰) in *P. crispus*, the $\delta^{15}$N values were 13.87‰ and 46.97‰ at 8℃ and 25℃, respectively, at the end of the experiment. The increase in $^{15}$N enrichment significantly~~

~~correlated with the increase in the *P. crispus* biomass ($p<0.05$) where the biomass of *P. crispus* grown at 25℃ was double that grown at 8℃ (Figure 6).~~

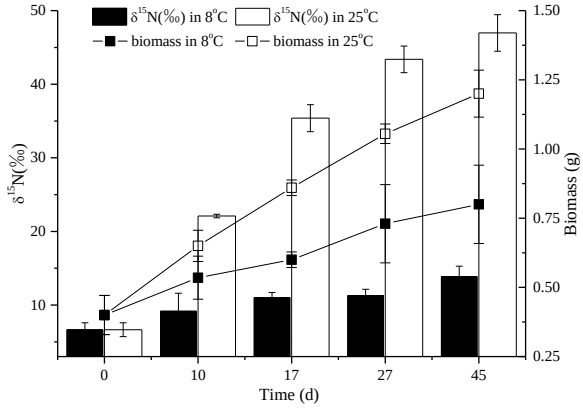


**Figure ~~6~~7.** Time series of $\delta^{15}N$ and dry biomass of *P. crispus* in 8℃ and 25℃ conditions during the 45-d experiment.

## DISCUSSION

**HABs sedimentation using modified clay/soils.** The removal and management~~control~~ of the growth of blooms, especially cyanobacterial blooms, is an important step in the recovery of eutrophic lakes~~ suffering eutrophication~~ before the re-emergence~~reconstruction~~ of macrophytes. In this study, the modified soil was selected to accelerate~~speed up the established~~ algal blooms sedimentation~~settling onto the sediment~~. The soil particles provided the algal biomass~~algal flocs~~ with sufficient ballast to counteract the buoyancy of the *M. aeruginosa* cells in the water columns, so most flocs in the treated systems remained at the bottoms of the columns in this experiment, whereas, in the controls, *M. aeruginosa* mainly were suspended ~~accumulated at the~~

~~water surface or was suspended~~ in the water column (Figure ~~1~~2). ~~Recently, the use of clays as ballast to sink algal blooms has been widely applied in both freshwater and marine environment.[9, 16, 27]~~ Although the Chl-a concentrations in F-only and F-capping treatments showed similar declining trends under each temperature after application of the modified soils, the Chl-a concentration in each sampling point from F-only systems was higher than those in F-capping systems. This was attributed to that~~However,~~ more M. *aeruginosa* cells survived~~is supposed to survive~~ in the F-only columns than in the F-capping columns at various temperatures, which can be partly reflected by the higher Chl-a concentrations in the flocs (Figure ~~1~~2). These surviving algal ~~colonies~~ biomass may return to the water columns together with the sediments, especially in shallow waters, where wind and wave-induced turbulence could be substantial.[26]

The flocculation and capping treatment by modified soil caused little damage to the *M. aeruginosa* cells, as reflected by the intact cell morphology and normal photosynthesis and respiration at day 0 (Figure S1 and S2). This results contributed to the ~~hypothesis could be confirmed by the~~ visually observation~~fact~~ that no homogeneously green or yellow occurred for the flocs in the water and the sediment of the columns, which is suggested as an indicator of cell lysis in this type of laboratory experiment.[9, 30] Given that cell lysis usually leads to the release of cell contents into water, including both cyanobacterial toxins and excess nutrients,[15, 32] this result may be important for preventing the intracellular cyanotoxins or excess nutrients released abruptly to the environment in practice. ~~As a consequence, the intracellular cyanotoxins~~

~~or excess nutrients should not be released abruptly to the environment.~~ ~~Likewise,~~ ~~modified clay/soils has been shown to be environmentally friendly to aquatic organisms in previous reports.[16, 31]~~ However, the chitosan, which was used to modify natural soils in this study, may possess antimicrobial activities against some bacteria,[32, 33] including cyanobacteria species.[30,34,35] Although Miranda et al. (2017) found no detrimental effects of chitosan on the *Microcystis*, [34] other studies still found evidence for cell lysis of *M. aeruginosa* at a relatively high dose of chitosan (e.g., >8 mg/L).[35] In our previous study, the combination of chitosan with natural soils could lower the toxic risk on the aquatic organisms exerted by chitosan alone by using a bioassay battery.[31] Although the modified clay/soil has also been shown to be environmentally friendly to aquatic organisms in other reports,[16] ~~Likewise, modified clay/soils has been shown to be environmentally friendly to aquatic organisms in previous reports.[16, 31]~~ ~~However, other reports have still found some evidence for cell lysis of *M. aeruginosa* using a relatively higher dose of chitosan modified kaolinite flocculation.[30]~~ ~~Cell lysis usually leads to the release of cell contents into water, including both cyanobacterial toxins and excessive nutrients.[15, 32]~~ ~~Thus,~~ the dose of flocculants as well as the dominant species ~~used for the precipitation of cyanobacteria~~ should be seriously considered~~–~~ in practice.

**Vitality changes in settled *M. aeruginosa*.** Capping with soils can keep the settled *M. aeruginosa* cells out of the light, which is a key factor affecting the photosynthetic rates. When light stress was induced by flocculation-capping treatment, metabolism of the deposited *M. aeruginosa* cells in our study was severely hindered due to reduction

in photosynthesis and respiration efficiency and subsequently triggered the degradation of algal cells. Significant photo-inhibition occurred for the *M. aeruginosa* cells in the F-capping systems compared with those in control and F-only systems, which was indicated by the continuous consumption of $O_2$ at 25℃, even under the light incubation (Figure ~~3~~ 4 h). The photosynthesis and respiration effects of *M. aeruginosa* cells could also be inhibited in F-only systems as reflected by the ~~fact that~~ significantly lower change rate of $O_2$ respiration than those in control. The results above mentioned confirmed the hypothesis that flocculation-capping treatment can accelerate the deposited algal bloom die-off. It should be noted that although the interference of bacteria (e.g., respiration of heterotrophic bacteria) could be minimized in our study, the influence of bacterial activity on the algal cell vitality should be further considered especially for the practical implementation of lake restoration.

In addition, ~~the~~ temperature is a crucial factor in the living activities of cyanobacteria in natural waters. In our studies, the three temperatures (8℃, 25℃ and 35℃) were established ~~according t~~to simulate~~the temperatures in winter~~ early spring, early summer and midsummer, respectively, in Lake Taihu, China, where the dominant cyanobacteria appear in the surface water at the end of spring, bloom during summer, and then ~~sink onto the~~ sediment during the late autumn and winter.[3~~6~~3] Moreover, our results showed that the deposited algal ~~blooms~~ biomass tend to be tolerant of low light at lower temperatures, as reflected by the normal morphology and photosynthesis and respiration in the control system (Figure ~~2~~ 3 and ~~3~~4). Similarly, Ma et al (2016) found

that most cyanobacteria sank to the sediment and remained dormant as viable inoculants (akinetes) below 12.5℃.[37,74] These deposited algal cells ~~will~~ can return to the water column as a potential source of bloom formation due to stimulation of their growth by ~~the~~ higher temperatures.[38,85] The higher temperature stimulated the growth of *M. aeruginosa* cells, as reflected by the faster and higher increasing rates of Chl-a in controls at 35℃ than at 25℃ before 15 days (Figure ~~3~~4). The consumption of $O_2$ in F-capping systems also increased as the temperature increased, and the $O_2$ ~~change rates~~respiration became negative ~~values~~ at 25℃, especially at 35℃, after 60 days of incubation (Figure ~~3~~4), indicating that higher temperatures accelerate the respiration rate of algal blooms buried under the capping layer. This contributed to the lysis of ~~fact that many~~ algal cells collected from the F-capping systems ~~were obviously wizened and cracked~~ at 25℃, as compared to no obvious changes in cell morphology from the control and F-only systems (Figure ~~2~~3). This is consistent with ~~the fact that~~ the decomposition of organic matter is ~~intrinsically~~ sensitive to increased temperature.[39,96,40,37]

**Assimilation of labeled nitrogen by submerged vegetation.** In lakes, most metabolic activities (i.e, organic matter mineralization and nutrient cycling) occur in the sediment, with~~and~~ algae sedimentation ~~strongly influences~~a strong influence on these biogeochemical processes ~~in sediments~~. The decomposition of algal blooms can directly release nutrients and ~~pollutants~~ toxins ~~into the surrounding environment~~, which leads to changes in nutrient composition cycling in sediment and water.[38,41-43,0] In this

study, nitrogen was ~~obviously~~ released into the sediment from the settled *M. aeruginosa* during the experiment via lysis of algal biomass (Figure ~~4~~5). The loss of $\delta^{15}N$ from the sediment increased as the incubation time increased for all systems. A portion of the loss of $\delta^{15}N$ from the sediment may be ~~resulted from~~due to benthic perturbation and mineralization,[4~~41~~] which could transform organic nitrogen into inorganic fractionation with net loss via denitrification reactions into gaseous phases~~and hence out of water in gas forms~~ (e.g., $N_2$ and $N_2O$).[4~~52~~] A higher proportion of the loss of labeling N was due to its release into the water column (Figure ~~5~~6). Moreover, higher temperatures could trigger greater *Microcystis*-derived nitrogen release from sediments, which is consistent with other reports that the nutrient cycling rates increase with the addition of settled algal blooms and elevation of temperatures.[3~~85~~]

The desirable growing conditions for submerged macrophytes including light penetration and higher dissolved oxygen concentrations~~oxygen level~~ can be reestablished after removal algal blooms using modified clay/soils.[16, 27] Macrophytes uptake plays a vital role in the mitigation of internal nutrient loads in vegetated sediments in lakes.[7] In this study, ~~the vegetated system retained more *Microcystis*-derived nitrogen than the unvegetated systems (Figure 5). From Figure 4 to 6, we can find~~we found that the excess $^{15}N$ was indeed assimilated by the *P. crispus*, which contributed to less excess $\delta^{15}N$ in both sediment and water in the vegetated systems than in the unvegetated systems, thereby reducing release of algae-sourced N into water (Figures 5-7~~5~~). This is the accepted way to restore a healthier ecological system

dominated by submerged vegetation in shallow waters in previous studies.[27, 46] The rapid uptake of $\delta^{15}N$ at both 8℃ and 25℃ mainly occurred within the first 10 d in this study (Figure 45), which is similar to the finding of rapid uptake of labeled ammonium and nitrate by common reeds.[47] This phenomena may be attributed to both uptake of mineralized (inorganic) nitrogen and organic nitrogen in the sediment, which may play important roles in assimilation of algae-sourced nitrogen by aquatic plants.[48, 49] In addition, the assimilation of nitrogen by submerged vegetation may also ~~be resulted~~ result from directly uptake of nitrogen from water column.[50] This can be inferred from the decrease of labeling N in the water (Figure 56); nevertheless, the contributions of these uptake pathways cannot be identified for the *P. crispus* in this study due to the limitations of the experimental set-up. Further study should focus on the mineralization rate of deposited algal blooms and the utility of inorganic and organic nitrogen by *P. crispus* or other macrophytes.

Our results indicate that increasing of temperature ~~obviously~~ facilitates the assimilation of *Microcystis*-derived nitrogen into *P. crispus*. This could be partially attributed to that higher temperature could facilitate the decomposition of deposited algal biomass (Fig.2 and 3), thus release more algal-sourced N. In addition, ~~Because that~~ most aquatic plants grow from the early spring to midsummer in temperate lakes, which is consistent with the result in this study that the growing rate of *P. crispus* was twice as high at 25℃ as at 8℃ (Figure 67). The growing rate significantly affects the incorporation of $\delta^{15}N$ in the *P. crispus*, as reflected by the 5 fold higher $\delta^{15}N$ ‰ found

at 25℃ than at 8℃. This is also attributed to the fact that a higher temperature accelerates the processes of death, decay, and decomposition of the deposited algal cells.

**Implications for lake restoration.** Generally, switch of lakes subject to human-induced eutrophication from the dominancy by algae to macrophyte can be difficult to~~hardly~~ achieve under natural conditions due to persistent~~the stable undesirable situation caused by~~ excessive growth of algae biomass~~ in water column~~. Moreover, restoration of such lakes from an established algal bloom to a desired state dominated by submerged macrphytes requires significant~~drastic and expensive~~ intervention, even after reducing external nutrient inputs. For this reason, many in-lake geo-engineering methods have been widely used as environmentally-friendly, efficient and economical way to accelerate removal of algal blooms from waters.[16, 18, 27] The improvement of transparency and dissolved oxygen concentrations~~transparence and oxygen level~~ in bottom water resulted from the application of modified clay/soil technology[16, 27] can facilitate~~pave the way~~ (e.g., establishing a certain period for plant germination and growth)~~ for reconstructing~~ reconstruction of submerged macrophytes. Flocculation-capping methods, as shown in this study, can not only effectively eliminate the algal biomass~~blooms out of water columns~~, but~~and~~ also facilitate their degradation~~,~~ with released nutrients~~and subsequently to be~~ absorbed by the growth of submerged vegetation. Higher temperatures~~ obviously~~ accelerated both the algae's decomposition and incorporation into plant biomass, implying that application of these method during the outbreak period of algal blooms can also facilitate such

transformation due to the overlap of growing seasons between algae and submerged vegetation, especially in temperate lakes. However, the effectiveness in the laboratory test cannot be representative of field-scale application because of differences in scale and environmental and hydraulic conditions. A field pilot experiment is necessary to test the potential effects of such in-lake geo-engineering methods for both control algal blooms and facilitate switch from algal to macrophyte state in lakes.

## ASSOCIATED CONTENT

### Supporting Information

~~Details on the methods for monitoring the morphology, photosynthesis and respiration rate of M. aeruginosa cells.~~ Figures showing SEM images of algal cells at the beginning of the experiments (0 day), Phtotosynthesis and respiration of M. aeruginosa cells in different systems: a: 25 ℃-control-0d, b: 25 ℃-F-only-0d, c:25 ℃-F-capping-0d.

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### Notes

The authors declare no competing financial interest.

## ACKNOWLEDGEMENTS

The research was supported by the National Natural Science Foundation of China (41401551); the Strategic Priority Research Program of CAS (XDA09030203); the National Key Research and Development Program of China (2017YFA0207204); and

Natural Science Foundation of Beijing (8162040).

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