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Comparison of algal harvest and hydrogen peroxide treatment in mitigating cyanobacterial blooms via an in situ mesocosm experiment

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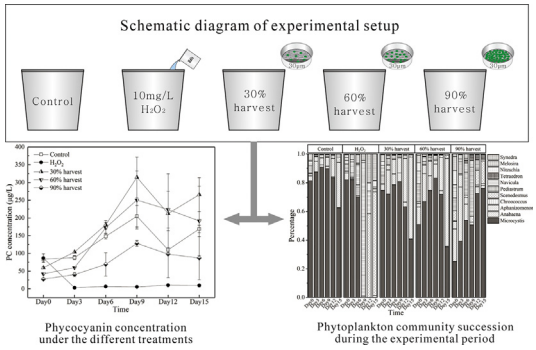
HIGHLIGHTS

- Comparison of algal harvest and hydrogen peroxide treatment in mitigating cyanobacterial blooms was performed via an in situ mesocosm experiment.
- The cyanobacterial biomass can rapidly recover at low harvest intensities, while remained relatively low under H_2O_2 treatment.
- The application of curative methods results in a shift of phytoplankton community.

GRAPHICAL ABSTRACT



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ABSTRACT

The use of short-term, fast-acting curative treatments to rapidly suppress the proliferation of upcoming cyanobacterial blooms without negative side effects on overall water quality is important for environmental regulatory agencies. A 15-day in situ mesocosm experiment was conducted to evaluate the effects of algal harvest at different intensities and the effect of hydrogen peroxide on the mitigation of cyanobacterial blooms, subsequent algal growth and phytoplankton community structure. The results indicate that filtration through a 30-μm-pore-size net could remove most of the *Microcystis* colonies, leading to a decline in algal biomass. However, algal harvest at 30% and 60% intensities tended to promote cyanobacterial growth under nutrient-replete conditions, and the mitigation effect only lasted a few days, since cyanobacteria biomass exhibited no significant difference between the control and those two treatments on Day 6. When the algal harvest intensity was 90%, the cyanobacterial biomass remained at a relatively low level for 15 days. The average *Microcystis* colony size rapidly returned to the initial level after an initial decline across all the algal harvest intensities, indicating that algal harvest should be repeatedly performed within a short time period to mitigate *Microcystis* blooms. Furthermore, removing *Microcystis* colonies by filtration led to increased diversity in the phytoplankton community, as the proportion of non-*Microcystis* cyanobacteria increased with harvest intensity. This result might pose a challenge for cyanobacterial bloom control over the long term if filamentous cyanobacteria become dominant. The 10.0 mg L^{-1} H_2O_2 treatment selectively suppressed cyanobacteria throughout the experimental period, leading

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to succession from a cyanobacteria-dominated to a Chlorophyta-dominated community after Day 9. Overall, using hydrogen peroxide is more effective than algal harvesting as a one-time quick curative measure.

1. Introduction

Excessive nutrient loading is the major cause of water blooms of cyanobacteria (Conley et al., 2009). Water blooms may cause high turbidity, oxygen deficiency and malodor of the water and may have significant detrimental impacts, e.g., decreased biodiversity, drinking water shortages, and potent toxin production (Paerl and Otten, 2013). The reduction in external nutrient loading is a prerequisite for controlling harmful cyanobacterial blooms. A nearly 90% reduction of both phosphorus and nitrogen loads is required to prevent phytoplankton blooms, with 20 mg L^{-1} chlorophyll *a* found throughout Lake Taihu (Janssen et al., 2017). However, internal loading and nutrient input caused by flooding make nutrient reduction difficult to achieve in shallow lakes in the lower reaches of the Yangtze River (Qin et al., 2006). Meanwhile, controlling harmful cyanobacterial blooms may be more challenging in the future under a changing climate, which includes increases in temperature and a high frequency of extreme weather events (Paerl et al., 2016; Yang et al., 2016).

Despite water authorities indicating a great need for preventive measures leading to nutrient reduction and thus to the reduction in cyanobacterial blooms over the long term, they also need short-term, fast-acting curative treatments to mitigate cyanobacterial nuisance. The effectiveness of several end-of-pipe measures, such as the application of microorganisms, predators of cyanobacteria, plant extracts, ultrasound and artificial mixers in nonstratifying lakes, has been evaluated. However, none of these methods seem to be a widely applicable solution to cyanobacterial nuisance, while mechanical removal or algaecide seem more feasible (Lurling et al., 2016). The direct removal of cyanobacterial scums from the water column by filtration and magnetic separation, which are commonly applied during algal harvesting (Milledge and Heaven, 2013; Wang et al., 2015), has been utilized for mitigating cyanobacterial blooms in China over the last ten years (Gao et al., 2009; Liu et al., 2013). For example, dozens of large algal harvesting boats, using filtration with a 30-μm-mesh net have been put into operation in Lake Taihu, Lake Chaohu, and Lake Dianchi, which are the most notorious lakes in terms of algal blooms (Fan et al., 2013). Hydrogen peroxide (H_2O_2) has the potential to be an effective and ecofriendly algaecide without producing harmful residues, and have been extensively studies in the laboratory and in lakes (Lurling et al., 2016; Matthijs et al., 2012). Furthermore, the dosage of H_2O_2 to control cyanobacteria bloom is dependent on the dominant species, the biomass and the colony size of cyanobacteria, as well as the potential ecological risk (C. Chen et al., 2016; Liu et al., 2017; Matthijs et al., 2012; Yang et al., 2018). Our field mesocosm study indicated 10 mg L^{-1} is the reasonable dosage for mitigating heavy *Microcystis* bloom, when the efficiency of elimination and the ecological influence are comprehensively considered.

However, it is not clear whether the direct removal of cyanobacterial biomass actually works to mitigate algal blooms under the pressure of eutrophication. The question of under what harvest intensity can algal growth and blooms be inhibited remains. Additionally, the subsequent ecological effects of algal harvest in comparison to the utilization of algaecide are unknown. In this study, we performed a 15-day mesocosm experiment to assess the capability of algal harvest and H_2O_2 treatment to control natural cyanobacterial blooms. Specifically, we recorded the dynamics of algal biomass, phytoplankton community structure and microcystin (MC) concentration to evaluate the potential influence of those two curative methods on subsequent algal regrowth, phytoplankton community succession and the water environment.

2. Materials and methods

2.1. Experimental setup

Mesocosm experiments were carried out from 23 July 2017 to 6 August 2017 at Lake Taihu Ecosystem Station (31°04′N, 120°26′E). The duration of the experiments was 15 days. Fifteen open plastic containers (200 L) were placed in an artificial pool at the station that simulated the temperature and light conditions of the lake. Lake water (150 L) with a heavy algal bloom was pumped directly from the lake to fill the containers. Algal harvest intensities of 30%, 60% and 90% were achieved by filtering 30% (45 L), 60% (90 L) and 90% (135 L) of the total mixed lake water volume through the 30-μm mesh net. An H_2O_2 stock solution (30%) was added to the containers to achieve a final concentration of 10 mg L^{-1} in the H_2O_2 treatment group. Each treatment had three replicates. Ammonium (NH_4^+), nitrate (NO_3^-), and phosphate (PO_4^{3-}) were added daily to ensure that the dissolved nutrient levels were similar to those in the lake. In situ measurements and the collection of samples to determine chemical and biological parameters were carried out every third day through the experimental period.

2.2. Physiochemical parameter analysis

The physiochemical parameters of the water in each container including pH, temperature, dissolved oxygen and conductivity, were monitored using a multiparameter probe (Model 6600; Yellow Spring Instruments, USA). The concentrations of NH_4^+, NO_3^-, and PO_4^{3-} were measured with a continuous flow analyzer (Skalar SA 1000, Breda, Netherlands).

2.3. Pigment analysis

Chlorophyll *a* (Chl-*a*) was determined spectrophotometrically by measuring absorption after 100 mL of sample was filtered through GF/C filters (Whatman, Maidstone, England), frozen, and extracted with 90% acetone following the procedure of Yan et al. (2004).

To quantify phycocyanin (PC), 100 mL of well-mixed water samples from each plastic container was filtered, and filters (GF/C, Whatman) were fully ground in 10 mL of 50 mM phosphate buffer using a smooth Teflon grinder in a centrifuge tube. Then, the grinder was rinsed with another 10 mL aliquot of phosphate buffer, bringing the total extract volume to 20 mL (Kasinak et al., 2015). The centrifuge tube was mixed by hand and immediately stored in darkness at 4 °C for 24 h. Thereafter, the extract was centrifuged (20 min, 2800 ×*g*, Beckman, GS-6R) to remove the filter components and cell debris. A 2-mL aliquot of supernatant was analyzed for PC using a spectrofluorophotometer (Shimadzu RF-5301, Japan), and the concentration was calculated using the equation employed by Horvath et al. (2013) & Kasinak et al. (2015).

2.4. Microcystis colony size measurement

The colony size of *Microcystis*, expressed as the equivalent spherical diameter (ESD), was determined with image analysis software (Olympus DP Soft), using an Olympus BX 51 light microscope together with

an Olympus DP 71 digital camera, as described by Wilson and colleagues (Wilson et al., 2006). A minimum of 50 colonies were randomly chosen from each sample and measured at three magnifications (100×, 200× and 400×). However, the *Microcystis* colonies became rare as H_2O_2 began functioning, rendering the practical observed colony number <50 from Day 3 in the H_2O_2 group; therefore, it was not possible to measure 50 colonies from this group after Day 3.

2.5. Microcystin concentration

After 24 h of incubation, 80-mL water samples were collected from each container and filtered through GF/C glass microfiber membranes (pore size, 1.2 μm; Whatman, UK). The membranes were used to analyze the intracellular MCs, and the filtrate was used to analyze the extracellular MCs. For intracellular MC extraction, the filters and their contents were ground with 1 mL 5% aqueous acetic acid, using a Fast Prep-24 automated homogenizer (MP Biomedicals, Santa Ana, CA, USA) with 0.5-mm silica beads. Then, the intracellular MCs were extracted using 20 mL of 80% aqueous methanol for 30 min, while shaking. After centrifugation (9500 rpm, 10 min), the supernatant was diluted at a 1:5 ratio with distilled water. The diluted supernatant was then concentrated using a solid-phase extraction cartridge (C18, 0.5 g) that had been rinsed with 10 mL of distilled water and 10 mL of 20% methanol. Ten milliliters of elute obtained from the cartridge using (0.1% TFA) methanol was blown to dryness with nitrogen at 40 °C. The resulting residues were all dissolved in 1 mL of 100% methanol and transferred to a small brown bottle and then blown dry with nitrogen. Then, the residues were dissolved in 200 μL of 50% aqueous methanol before HPLC analysis. For extracellular MC extraction, 80 mL of filtrate was directly concentrated using a solid-phase extraction cartridge (C18, 0.5 g). The subsequent steps of blow drying with nitrogen and dissolving in methanol were performed as described for the intracellular MC extraction (Rodriguez et al., 2008).

The extracted intracellular MCs and extracellular MCs were analyzed using a high-performance liquid chromatography (HPLC) system equipped with an ODS column (Agilent EclipseXDB-C18, 5 μm, 4.6 × 150 mm) and a photodiode array detector (Agilent 1200, Agilent, Palo Alto, CA, USA). The mobile phases were Milli-Q water and acetonitrile, both containing 0.05% (v/v) trifluoroacetic acid (TFA). Chromatographic separation was achieved at a flow rate of 1 mL min^{-1} using a gradient starting at 30% aqueous acetonitrile and increasing to 35% over the next 10 min, followed by an increase to 70% over the next 30 min. The concentrations of the MCs were quantified based on their retention time and characteristic UV spectra. The standards of MC-RR, MC-YR, and MC-LR were purchased from Sigma (München, Germany). The order of the three peaks was MC-RR, MC-YR, and MC-LR, and the retention times were 5.79, 10.05, and 11.19 min, respectively. The maximum absorption peak was at 239 nm. The total MC concentration was quantified as the sum of all MC peaks.

2.6. Phytoplankton analyses

Integrated 500-mL phytoplankton samples were collected and fixed with acid Lugol's iodine solution every three days during our microcosm experiments. Phytoplankton identification and cell counts were conducted in random fields using the standard Utermhöl inverted microscope technique on a Zeiss Axio Vert. A1 (Jena, Germany). Because the sampling frequency was limited, the focus was on the dominant groups in the community, and phytoplankton was identified to the species/genus level. Average algal cell volumes were calculated for each species/genus in each sample with reference to the method of Hillebrand et al. (1999). The biovolume of each species in each sample was calculated by multiplying the cell density by the average cell volume.

2.7. Data analysis

A repeated-measures ANOVA (RM-ANOVA) was conducted to identify significant differences among treatments over time. To improve the statistical power of the RM-ANOVA, we combined the PC data for two adjacent time points to satisfy the basic requirements of the method. Accordingly, the new time series was comprised of Day 0–3, Day 6–9 and Day 12–15 after data merging on the basis of the assumption that growth changes over 3 days were negligible in comparison to those over 6 days. One-way analysis of variance (one-way ANOVA) was used to test for significant differences in the MC concentration under the different treatments. The least significant difference (LSD) test was used to perform pairwise multiple comparisons across time points and treatments, considering that this test is sensitive in the context of small sample size. The specific growth rates of the cyanobacteria were estimated via linear regression of the natural logarithm-transformed PC concentration data against time (Lurling, 2006).

The RM-ANOVA and the LSD test were carried out using SPSS 23 (IBM Corporation), and the linear regression was implemented with Origin 2017 (OriginLab Corporation). For all analyses, a p-value <0.05 was considered a statistically significant level.

3. Results

3.1. Nutrient concentrations

Concentrations of ammonium, nitrate and phosphate were approximately 0.2 mg L^{-1}, 0.1 mg L^{-1} and 0.02 mg L^{-1} respectively. The concentrations were relatively stable and almost identical among the control group and the three algal harvest groups during the entire experiment, since the three dissolved nutrients were added to the experimental vessels daily to simulate the lake water nutrients. Along with the algal growth, TN and TP increased continuously under the treatments of algal harvest, which tended to be highest for the control and the treatment with 30% harvest intensity, reaching to 2.7 mg $L^{-1}$and 0.28 mg L^{-1}, respectively. In comparison, the H_2O_2 treatment caused a rapid increase in the dissolved nutrient concentrations due to cell lysis followed by a decrease along with algal growth (Fig. 1).

3.2. Temporal dynamics of phytoplankton biomass

Filtration through a 30-μm-pore-size net was found to remove algal biomass. The Chl-*a* concentration declined by 27.9%, 46.7% and 57.0% under harvest intensities of 30%, 60% and 90%, respectively, while the PC concentration decreased by 28.7%, 49.2% and 67.2%. Due to the heavy cyanobacterial bloom, the Chl-*a* and PC concentrations were still high, with values of 39.0 μg L^{-1} and 27.4 μg L^{-1}, respectively, even when 90% of the water was filtered. Phytoplankton showed continuous growth after the algal harvest, with the maximum Chl-*a* concentration reaching approximately 180 μg L^{-1}. The PC concentrations continuously rose during the first 9 days in the control group and three algal harvest groups and then fluctuated over the next 6 days, akin to the scenario of the Chl-*a* concentration. In the H_2O_2 group, the PC concentration dropped sharply from 86.6 μg L^{-1} to 3.2 μg L^{-1} within the first 3 days and remained extremely low over the rest of the experimental period, while Chl-*a* started to increase after Day 6 from an approximately nil concentration to 151.7 μg L^{-1}, catching up with other groups on Day 12 (Figs. 2 and 3).

The cyanobacterial biomass under the 30% harvest intensity kept pace with that under the 60% harvest intensity throughout the experimental period despite the significant difference between the two at the very beginning (Day 0–3, $PC_{30\%} = 81.76 \pm 24.40$ μg L^{-1}, $PC_{60\%} = 51.24 \pm 9.83$ μg L^{-1}, $p_{30\%-60\%} = 0.001$). The biomass in these two treatments overtook the cyanobacterial biomass in the control group within 6 days (Day 6–9, $PC_{control} = 176.56 \pm 36.82$ μg L^{-1}, $PC_{30\%} = 247.72 \pm 81.84$ μg L^{-1}, $PC_{60\%} = 211.63 \pm 67.79$ μg L^{-1}, $p_{30\%-60\%} = 0.306$, $p_{control-}$

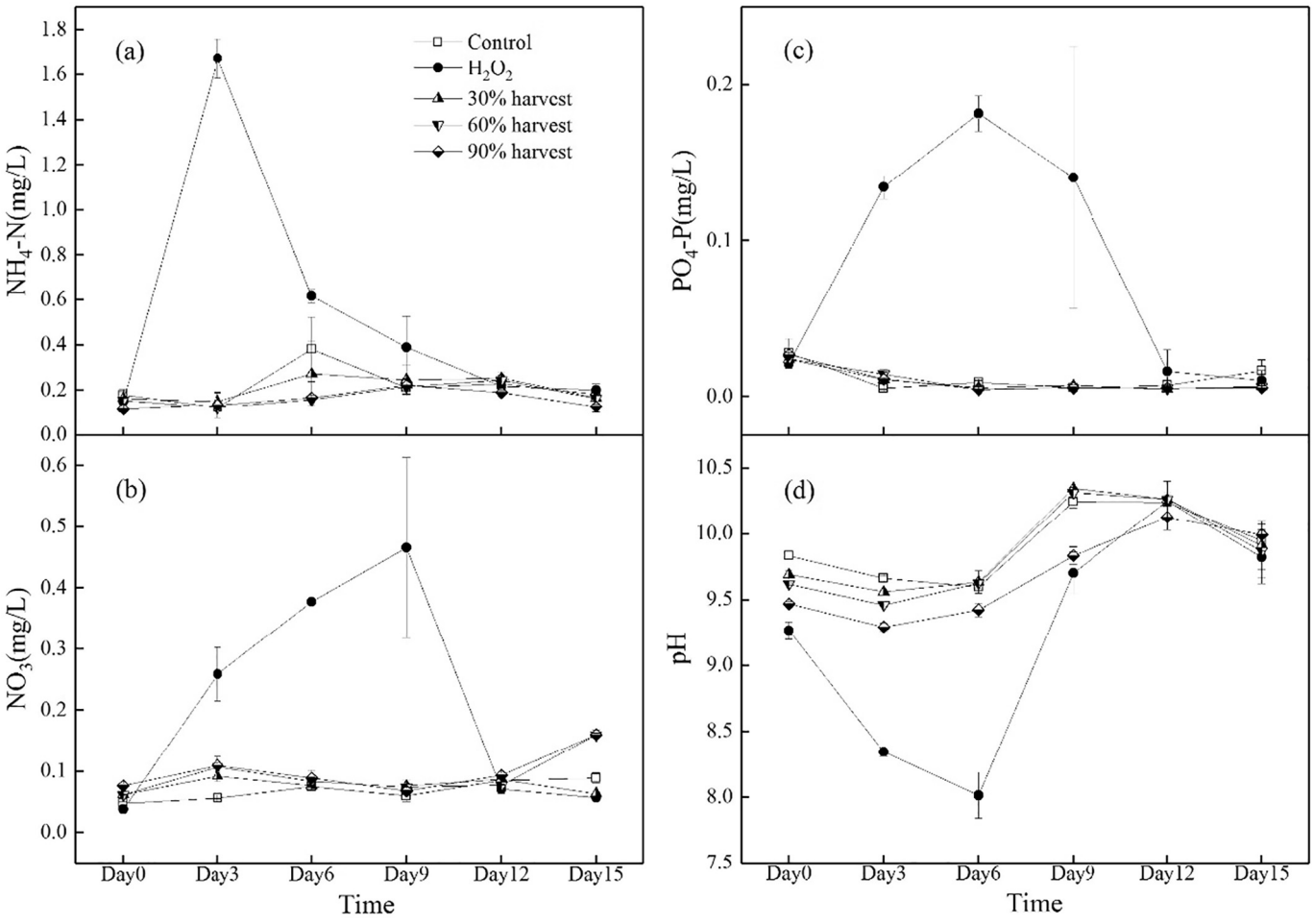

Fig. 1. Nutrient concentrations under the different treatments during the experiment. (a) NH^{4+}, (b) NO_3^-, (c) PO_4^{3-}, (d) pH. Values are represented as the mean ± SD, n = 3.

$_{30\%} = 0.052$, $p_{control\text{-}60\%} = 0.320$), especially in the 30%-intensity harvest group. However, cyanobacterial biomass was maintained at a significantly higher level after Day 9 (Day 12–15, $PC_{control} = 139.62 \pm 33.12$ μg L^{-1}, $PC_{30\%} = 239.20 \pm 82.34$ μg L^{-1}, $p_{control\text{-}30\%} = 0.014$). The PC concentrations under the 90% harvest intensity were significantly lower than those in the control group and the other two harvest groups until the last three days, exhibiting no significant differences from those in the control group (Day 12–15, $PC_{90\%} = 92.52 \pm 57.22$ μg L^{-1}, $p_{control\text{-}90\%} = 0.217$) (Fig. 3).

3.3. Microcystis colony size

The *Microcystis* colony size was approximately 70 μm in the treatment with 30% algal harvest. When the harvest intensity increased,

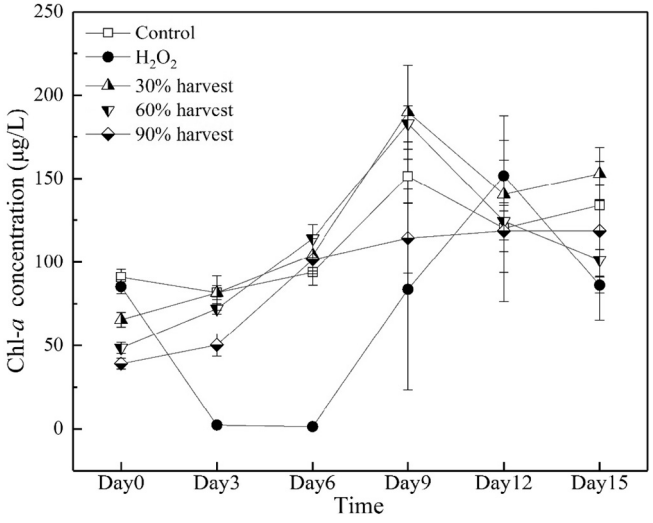

Fig. 2. Chlorophyll *a* concentration under the different treatments. Values are represented as the mean ± SD, n = 3.

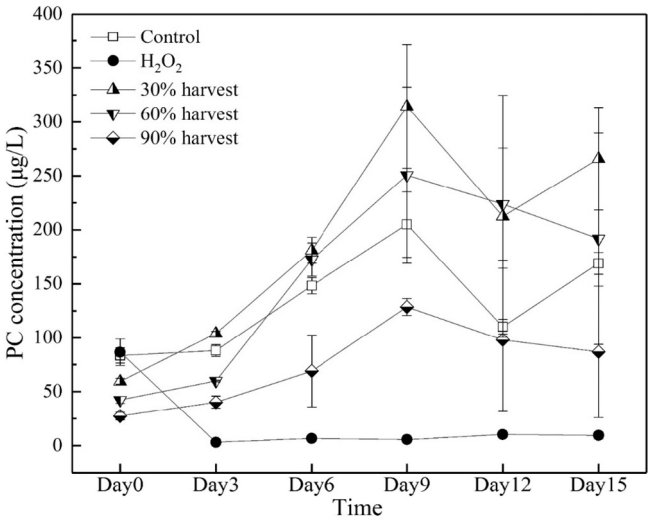

Fig. 3. Phycocyanin concentration under the different treatments. Values are represented as the mean ± SD, n = 3.

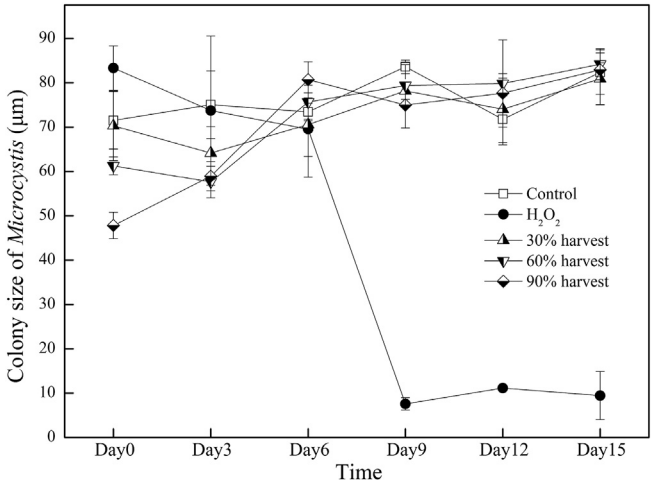

Fig. 4. Colony size of *Microcystis* under the different treatments. Values are represented as the mean ± SD, n = 3.

the colony size decreased, and was approximately 48 μm when 90% of the water was filtered. With growth of the algae, the size of the colony returned to 70 μm after Day 6 under all harvest intensities, similar to that in the control. In comparison, the H_2O_2 treatment caused a decline in cell size on Day 6, dropping to 10 μm after Day 9 (Fig. 4).

3.4. Microcystin concentration

At the beginning of the experiment, the total intracellular MC concentration decreased in the treatment with a high harvest rate, with a value of 0.05 μg L^{-1} at the 90% harvest rate. The total extracellular MC concentration was initially low among all treatments (approximately 0.01 μg L^{-1}). During the experimental period, the intracellular and extracellular MC concentrations increased with algal growth. When the cyanobacterial blooms were exposed to H_2O_2, the intracellular MC concentrations decreased, and the extracellular MC concentrations increased (Fig. 5). This shift from intracellular to extracellular presence of the toxin is presumably the result of the release of toxins due to cell lysis.

3.5. Succession in phytoplankton community composition

Microscopic observations showed that the phytoplankton community was mainly composed of Cyanobacteria, Chlorophyta and Bacillariophyta at the phylum level. In total, approximately 11 genera were observed during the mesocosm experiments. It is evident that after algal harvest and H_2O_2 treatment, the phytoplankton community showed dramatic succession. The proportion of non-*Microcystis* cyanobacteria, diatoms and chlorophytes increased with harvest intensity. When the harvest intensity was 90%, the percentage of *Microcystis* decreased from an initial value of 80% to 20%. Meanwhile, the contributions of diatoms and chlorophytes reached >25% and 20% of the total algal biomass, respectively. The present diatoms mainly included *Synedra*, *Melosira*, *Nitzschia*, *Tetraedron* and *Navicula*, and Chlorophyta included *Pediastrum* and *Scenedesmus*. Afterwards, with the growth of phytoplankton, the cyanobacteria community underwent succession, changing from the dominance of *Microcystis* to *Aphanizomenon* in the control group and the treatments with 30% and 60% harvest intensity. When the harvest intensity was 90%, the phytoplankton community structure seemed more dynamic, changing from an abundant occurrence of *Microcystis* and *Aphanizomenon* on Day 3 to *Microcystis* and *Pediastrum* on Day 9 and to a final composition dominated by *Microcystis* and *Scenedesmus*. The H_2O_2 treatment completely shifted the algal community from *Microcystis* to *Navicula* dominance on Day 9 and then to *Scenedesmus* dominance at the end of the experiment (Fig. 6).

4. Discussion

4.1. The effects of algal harvest and H_2O_2 treatment on the growth of cyanobacteria

Algal harvest has been reported to be an efficient way to reduce cyanobacterial biomass, based on laboratory incubation and field enclosure experiments (B. Chen et al., 2016; Zhou et al., 2012). However, it is difficult to use these results to guide the performance of algal harvest in practice. First, the appropriate harvest intensity to apply to control the potentially subsequent growth of cyanobacteria and to efficiently mitigate cyanobacterial blooms is not clear. Second, nutrients were not supplemented during the experimental period; thus, the subsequent growth of cyanobacteria might be nutrient limited, which would cause an overestimation of the efficiency of cyanobacterial mitigation. In contrast, in natural eutrophic lake ecosystems, nutrients are not limited, due to continuous input from either inflowing rivers or sediment.

Our results suggest that when nutrients are replete, the total cyanobacterial biomass could recover to its original level after 3 days under 30% and 60% harvest intensities in summer. The slopes of the linear regression of the natural logarithm-transformed PC data showed that the harvest of algae at different intensities can boost cyanobacterial growth to varying degrees, yielding greater growth rates than those observed for the algal community without interferences (Fig. 7). This is consistent with the finding that a higher specific growth rate of *Microcystis* is associated with a smaller colony size caused by filtration (Li et al., 2013). It is notable that a 30% harvest intensity helped the algae to break through the bottleneck and even reach a higher maximum biomass during the experimental period. It is clear that water ecosystems have a carrying capacity, since the maximum supportable algal biomass is determined by the most scarce resource, relative to demand (Reynolds, 2006). As a result, the removal of some algal biomass from a batch culture could create living space for new algae and promote subsequent algal growth. Only when a harvest intensity of 90% was performed could the algal growth be efficiently suppressed during the experimental period, due to the low algal biomass. This implies that the implementation of algal harvesting to mitigate cyanobacterial blooms in eutrophic lakes would carry risks if the fact that the relatively low and intermediate algal harvest intensities can promote the potential growth of cyanobacteria were not considered.

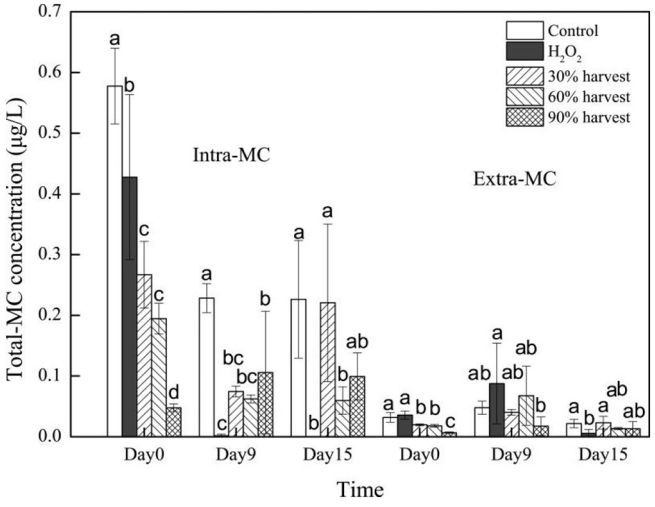

Fig. 5. Microcystin concentration. Values are represented as the mean ± SD, n = 3. Different alphabet letters or Arabic numerals indicate a significant difference between treatment groups at each exposure time at $p < 0.05$, according to a one-way ANOVA test.

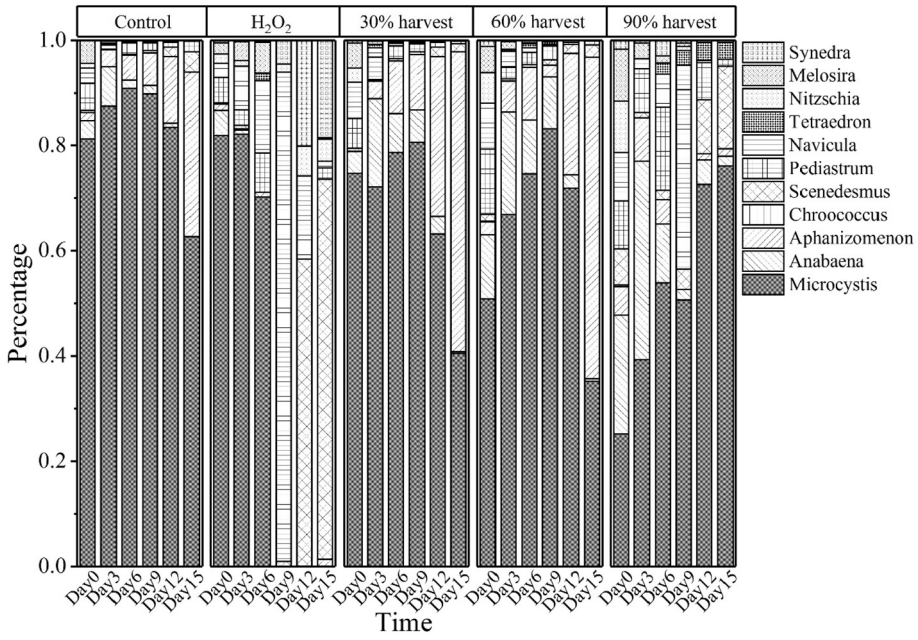

Fig. 6. Phytoplankton community succession during the experimental period.

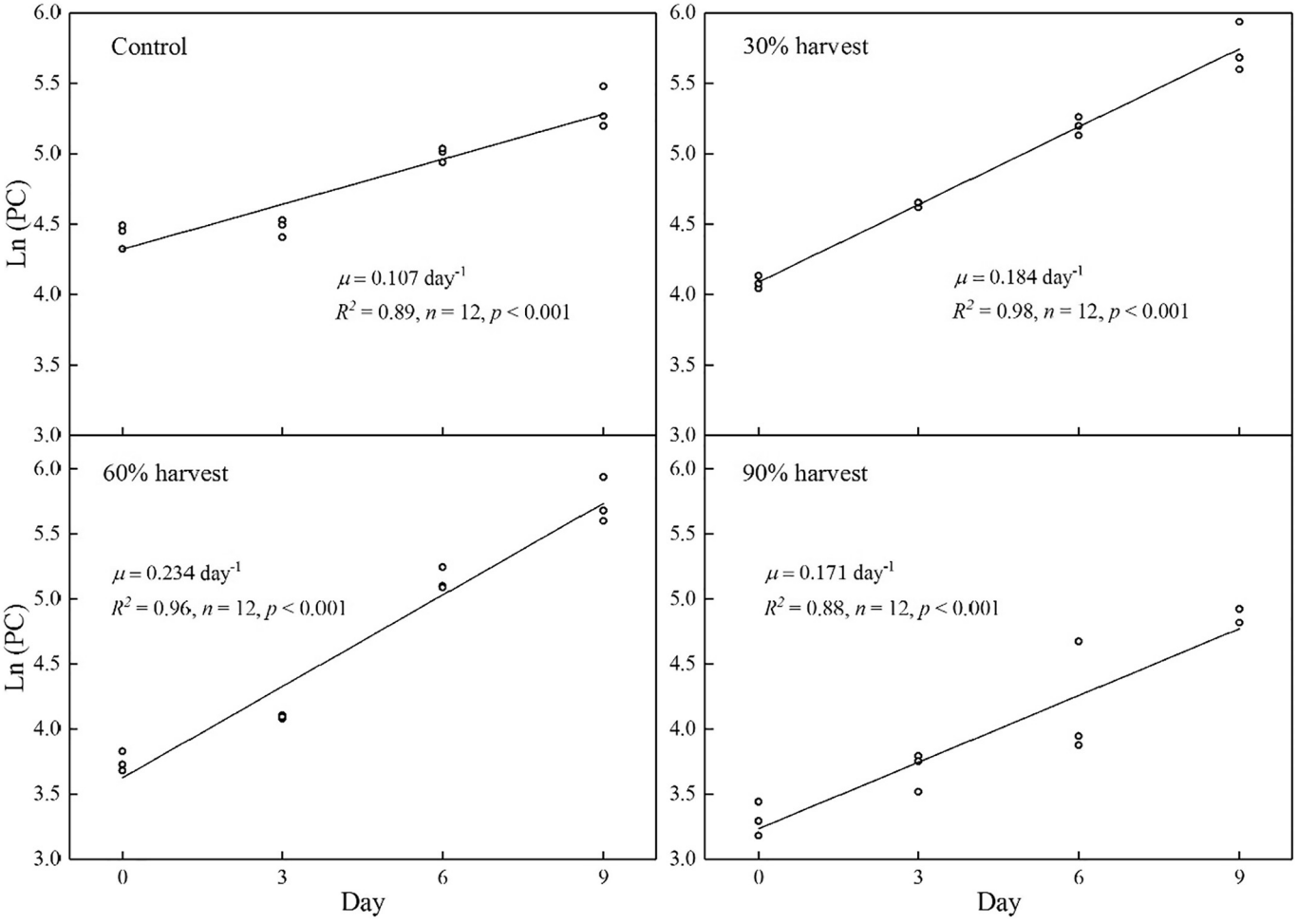

Fig. 7. The specific growth rates (μ) in the control group and the three algal harvest groups estimated via linear regression of the natural logarithm-transformed PC concentration data against time (Day 0–Day 9).

Hydrogen peroxide has been reported to be an effective algaecide and a good option for the removal of cyanobacterial blooms. First, H_2O_2 can degrade rapidly, and its concentrations return to background levels within 48 h without causing any harmful residues to be released into the surrounding environment (Liu et al., 2017). Second, cyanobacteria can be selectively inhibited in mixed phytoplankton communities, since cyanobacteria are affected by H_2O_2 at concentrations 10 times lower than those that affect green algae and diatoms (Huo et al., 2015). Considerable research has been performed to validate the treatment effects of hydrogen peroxide (HP) on bloom-forming cyanobacteria, including studies that not only address the mechanism of action (Drábková et al., 2007; Latifi et al., 2009; Samuilov et al., 2004), but also practical cases involving lab-scale tests, mesocosms and even experiments in entire lakes (Matthijs et al., 2012; Weenink et al., 2015). Our results indicate that the application of H_2O_2 at a concentration of 10 mg L^{-1} effectively inhibited cyanobacterial growth during a 15-day experiment, which is a much more efficient approach than algal harvest.

4.2. Change in Microcystis colony size under the different treatments

Colony formation has been considered to be a strategy used by *Microcystis* to reduce the occurrence of photoinhibition under high light intensities (Wu et al., 2011). Large colonies of *Microcystis* have also been shown to be more competitive than small ones under low phosphorus or low nitrogen conditions (Shen and Song, 2007; Yang and Kong, 2013). In fact, the size of *Microcystis* colonies is highly dynamic in eutrophic lakes throughout the year. Large-sized colonies (>125 μm) dominate in July and August but decrease rapidly after August (Li et al., 2014; Li and Li, 2012). In this study, the average size of the *Microcystis* colonies was approximately 70 μm; however, the size declined with the increase in harvest intensity because filtration with the 30-μm-pore-size net could remove most of the colonial *Microcystis*, as the colony size was 50 μm when the harvest intensity was 90%. Afterwards, the *Microcystis* colony size rapidly returned to 70 μm, and it was almost the same under all harvest intensities on Day 3. It has been reported that once it reaches a certain level, *Microcystis* can form colonies via adhesion to previously existing cells under appropriate environmental conditions regardless of the algal biomass (Yang and Kong, 2013). Therefore, large colonies could form after a short time period, which suggests that the performance of algal harvesting by using 30-μm nets can be repeated every two days to further mitigate cyanobacterial blooms. In comparison, the use of H_2O_2 caused cell lysis, leading to a clear drop in colony size after Day 6.

4.3. Variation of microcystin concentration

The MC concentration in our experimental mesocosms was generally low, with a value of approximately 0.7 μg L^{-1}, which is lower than the provisional guideline of 1 μg L^{-1} set by the World Health Organization (WHO). Furthermore, both toxigenic and nontoxigenic strains of the same species within a genus can coexist in the same water source, and their abundances and proportions vary spatially and temporally (Li et al., 2014). Thus, the low MC concentration might be caused by the relatively low ratio of toxic *Microcystis* genotypes in this study. MC can cause serious health and environmental problems; thus, MC release into the water is an important concern in terms of the application of approaches for bloom control (Mackintosh et al., 1990). Previous studies have shown that chemical algaecides can cause the release of intracellular MCs (Zhang et al., 2011). Our results also showed that the extracellular MC concentration showed a dramatic increase after H_2O_2 treatment. It is also suggested that the application of H_2O_2 as an algaecide should be performed at the early stages of a cyanobacterial bloom, when the cell densities are low and the *Microcystis* colonies are small, to limit the release of intracellular metabolites (C. Chen et al., 2016; Liu et al., 2017). The influence of harvest intensity on MC is complicated. On the one hand, algal harvest can remove a large proportion of large *Microcystis* colonies, which could reduce the concentration of MC, since large *Microcystis* colonies (>100 μm) have been reported to show relatively high MC production and a high proportion of MC-producing genotypes. On the other hand, the removal of algal biomass causes the rapid growth of *Microcystis*, which promotes an increase in MC. Because the extracellular MC concentration increased while cells were dividing, it remained constant or decreased only slightly during the stationary and death phases (Orr and Jones, 1998). Our results revealed that extracellular MC decreased when large colonies were removed by algal harvest on Day 0. Afterwards, *Microcystis* recovered in terms of growth, and the cell size became large, causing an obvious increase in extracellular MC concentration on Day 9.

4.4. Phytoplankton succession under the different treatments

Microcystis contributed >93% of the plankton biomass in the control treatment. Algal harvest with a 30-μm pore size net removed most of the *Microcystis* colonies, whereas the remaining taxa of phytoplankton could easily pass through the net. As a result, the phytoplankton community structure showed succession across the different algal harvest intensities. The contribution of *Microcystis* decreased, and the proportion of other cyanobacteria, chlorophytes and diatoms increased with increasing harvest intensity. Afterwards, phytoplankton showed continuous growth. Compared with chlorophytes and diatoms, cyanobacteria is more competitive under eutrophic, high temperature and low light conditions (Huisman et al., 2018, 1999; O'Neil et al., 2012); thus, cyanobacteria outcompeted chlorophytes at the end of the experiment. Meanwhile, the community structure within Cyanobacteria also changed, shifting from a *Microcystis*-dominated to an *Aphanizomenon*-dominated community. It might take a longer time to see this transition for the treatment with 90% algal harvest intensity because even the succession from Chlorophyta to Cyanobacteria lagged behind in comparison to that under the control and the other two low harvest intensity treatments. In fact, *Aphanizomenon* and *Microcystis* are common dominant species of cyanobacterial blooms in many lakes. Normally, *Aphanizomenon* blooms of occurs earlier than those of *Microcystis* due to its fitness at relatively low temperatures (Wu et al., 2010). During the mesocosm experiment, the TN:TP ratio was approximately 10, and heterocystous *Aphanizomenon* could replace nonheterocystous *Microcystis* to become the dominant species under low TN concentrations and/or ratios of TN to TP (McDonald and Lehman, 2013; Wu et al., 2016). Furthermore, *Aphanizomenon* exhibited higher photosynthetic activities and a stronger competitive ability under high phosphate levels than *Microcystis*, which could also be one of the reasons for this transition. In contrast, H_2O_2 treatment selectively suppressed cyanobacteria, leading to the dominance of eukaryotic phytoplankton, including diatoms and chlorophytes (Matthijs et al., 2012). Our results were consistent with this point and further indicated that *Scenedesmus* and *Synedra* were the dominant genera of these two phyla.

5. Conclusion

Physical removal and H_2O_2 treatment are regarded as feasible approaches to rapidly suppress cyanobacterial blooms without negative side effects on water ecosystems. Our study revealed that algal harvest by filtration through a 30-μm-pore-size net could remove large *Microcystis* colonies, leading to a decline in cyanobacterial biomass and succession of the phytoplankton community. However, when the harvest intensity was 30% and 60%, the mitigating effect could only last for a short period, since cyanobacterial growth was promoted under nutrient-replete conditions. Although large colonies were removed by filtration, they could reform after a short period, indicating that algal harvest by filtration can be repeatedly performed for *Microcystis* bloom control. Meanwhile, the phytoplankton community tended to become more diversified after algal harvest, as the percentage

of non-*Microcystis* cyanobacteria increased. This would lead to a more challenging filtration approach, since filamentous cyanobacteria could pass through the 30-μm net. In comparison, the 10 mg L^{-1} H_2O_2 treatment was found to selectively suppress cyanobacteria throughout the experimental period, leading to a Chlorophyta-dominated phytoplankton community. Therefore, in comparison with algal harvest, hydrogen peroxide treatment is a more effective approach as a one-time quick curative measure.

Declaration of Competing Interest

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

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