Nitrogen availability affects the dynamics of Microcystis blooms by regulating the downward transport of biomass

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ABSTRACT

Nitrogen availability is one of the key factors affecting the dynamics of non-diazotrophic cyanobacterial blooms in eutrophic lakes. While previous studies mainly focused on the promoting effect of nitrogen on the growth of cyanobacteria, this study aimed to investigate the role of nitrogen availability in the downward transport of biomass and its effects on the dynamics of Microcystis blooms. We performed field enclosure experiments which demonstrated that nitrogen availability negatively affects the downward transport of biomass. With a nitrogen loading of 0.02 g N m$^{-2}$ d$^{-1}$, the Microcystis biomass in the water column decreased by 56.2% over a 4-day period. During the same period of time, the average sinking ratio was 0.23 d$^{-1}$; moreover, the termination of biomass growth was detected. At the notably higher nitrogen loading of 0.5 g N m$^{-2}$ d$^{-1}$, the downward transport of biomass could still compensate for the biomass growth, although the average sinking ratio was lower at 0.16 d$^{-1}$. Additional laboratory culture experiments demonstrated that the increase in the downward transport of Microcystis occurred in parallel to an increase in the carbohydrate content and a decrease in gas vesicle content. Further proteomic analysis indicated that the carbohydrate accumulation induced by nitrogen deficiency was a result of the slowing down of catabolic consumption, especially the downregulation of glycolysis. Thus, our study suggests that increased intracellular carbohydrate accumulation at low nitrogen availability causes a higher sinking ratio of Microcystis, indicating that nitrogen limits the duration of Microcystis blooms; thus, decreased nitrogen availability may lead to increased sinking of biomass out of the water column, accelerating the dissipation of Microcystis blooms.

1. Introduction

Harmful cyanobacterial blooms are promoted by nutrient loading and climate change, and excessive nitrogen significantly increases the abundance of non-diazotrophic genera (Jankowiak et al., 2019; Paerl, 2014; Paerl et al., 2016). Nitrogen availability is one of the main factors that drives the dynamics of non-diazotrophic cyanobacterial blooms (Gobler et al., 2016). Several field investigations have suggested that certain cyanobacteria, such as Microcystis sp., are sensitive to the nitrogen availability and that their biomass tends to decline with a reduction in the external nitrogen loading (Michalak et al., 2013; Xu et al., 2010). Recent studies emphasized that, owing to the high nitrogen demand of non-diazotrophic cyanobacteria, low nitrogen availability reduces their proliferation by affecting intracellular physiological processes (Erratt et al., 2018; Forchhammer and Schwarz, 2019).

There are numerous factors affecting the dynamics and termination of a bloom, including zooplankton grazing, virus infection, and fungal parasitism (Choi et al., 2017; Frenken et al., 2016; Weynberg, 2018). Field investigations identified the blooming cyanobacterium Microcystis from sediment samples, indicating that downward transport of biomass is another important factor influencing the dynamics of cyanobacteria in the water column (Feng et al., 2019a; Kitchens et al., 2018; Magonono et al., 2018; Pannard et al., 2007). It was reported that the downward transport of biomass was affected by abiotic factors such as temperature and light availability (Huang et al., 2016; Visser et al.,...
1995). However, few studies have directly linked the downward transport of Microcystis biomass to nitrogen availability. Our recent study suggested that low nitrogen availability promoted the carbohydrate accumulation of Microcystis, and might further enhanced its downward transportation (Huang et al., 2019). However, these results were obtained in the laboratory with batch cultures, and it is remains unclear whether nitrogen availability could affect the downward transport of Microcystis colonies under field conditions and, thus, affect the duration of the Microcystis bloom period. In eutrophic lakes, cyanobacteria, specifically Microcystis sp., do not remain unicellular but always form colonies in which cells are embedded in extracellular polymer substances (EPS) or a so-called “matrix” (Cai et al., 2014; Xu et al., 2013). Whether low nitrogen availability can induce carbohydrate accumulations in Microcystis colonies is still questionable. Besides, several studies performed on certain cyanobacteria have shown that the accumulation of carbohydrates is associated with low nitrogen availability, which affects metabolic processes and carbon partitioning (e.g. gluconeogenesis, polyhydroxyalkanoate and glycogen granule biosynthesis, and the tricarboxylic acid (TCA) cycle) (Deschoenmaeker et al., 2016; Hasunuma et al., 2013; Osanai et al., 2014). However, the mechanism of carbohydrate accumulation in the Microcystis population and its response to nitrogen availability still needs further exploration.

In the present study, we investigated the manner in which nitrogen availability affects the dynamics of Microcystis blooms as well as the downward transport of biomass. We further linked the downward transport of Microcystis colonies in the water column with carbohydrate accumulation in Microcystis cells; moreover, the mechanisms underlying carbohydrate accumulation were investigated via proteomic analysis.

2. Materials and methods

2.1. Enclosure experiments

Enclosure experiments were conducted to study the manner in which nitrogen availability affects the dynamics of Microcystis blooms. The enclosure experiments were conducted in the northeast part of Lake Dianshan (Qingpu District, China, 31°14′ 94° N, 121°02′ 40° E) during a Microcystis bloom in August-September 2018.

The experimental enclosures were made of cylindrical columns (polyvinyl chloride pipes; internal diameter, 40 cm) that were vertically inserted into the bottom sediment at a depth of 50 to 80 cm depth. The enclosures were filled with lake water. The depth of the water columns in each enclosure was about 1.5 m. A honeycomb-structured sediment trap (10 cm in height, 9 cm in diameter, and 2 cm in honeycomb cells) was inserted into the bottom sediment at a depth of 50 to 80 cm depth. The experimental enclosures (Fig. 1b), such that the lower boundary of the tube was located approximately 10 cm above the sediment.

Before we modified the nitrogen concentrations, the dissolved inorganic nitrogen (DIN) levels in the enclosures were as follows: nitrate 0.3–0.7 mg L\(^{-1}\), nitrite 0.02–0.08 mg L\(^{-1}\), and ammonia -0.36 mg L\(^{-1}\). Before sampling, the water in the tube was mixed vertically for 1–2 min with an airflow rate of 40–50 mL min\(^{-1}\). The flow rate and distance of the tube from the bottom was selected in such a manner as to prevent possible resuspension of the bottom sediments as well as biomass trapped in the sediment traps. At each sampling time, approximately 5 mL of water was collected from the tube to determine the chlorophyll a (Chl a) concentration in the water column. Then, the sediment traps were lifted slowly, and the water with the sunken Microcystis colonies was collected to measure the Chl a concentration accumulated in the traps. The sediment traps were filled with filtered lake water and then slowly returned to the bottom of the enclosures to avoid the suspension of the sediment.

2.1.1. Enclosure experiment I

The experiment continued for 3 days, and the Microcystis biomass collected from the experimental site was added such that the Chl a concentration approached 200–250 mg m\(^{-2}\) (150–180 μg L\(^{-1}\)). Nitrate (as NaNO\(_3\)) as a nitrogen source was added to the enclosures as there were three treatments with different initial DIN concentrations: 2.5 g N m\(^{-2}\) (1.8 mg N L\(^{-1}\)), 3.6 g N m\(^{-2}\) (2.6 mg N L\(^{-1}\)), and 6.1 g N m\(^{-2}\) (4.4 mg N L\(^{-1}\)). Each treatment was set up as three replicates.

2.1.2. Enclosure experiment II

This experiment continued for 4 days, and the Microcystis biomass was added such that Chl a concentration approached 100–150 mg m\(^{-2}\) (90–110 μg L\(^{-1}\)). There were three treatments with different levels of nitrogen loading. To simulate pulsed nitrogen inputs, NaNO\(_3\) was added each morning at approximate concentrations of 0.02 g N m\(^{-2}\) d\(^{-1}\) (0.014 mg N L\(^{-1}\) d\(^{-1}\)), 0.2 g N m\(^{-2}\) d\(^{-1}\) (0.143 mg N L\(^{-1}\) d\(^{-1}\)) and 0.5 g N m\(^{-2}\) d\(^{-1}\) (0.357 mg N L\(^{-1}\) d\(^{-1}\)). The initial DIN concentrations were 0.39 g N m\(^{-2}\) (0.28 mg N L\(^{-1}\)), 0.46 g N m\(^{-2}\) (0.33 mg N L\(^{-1}\)), and 0.60 g N m\(^{-2}\) (0.43 mg N L\(^{-1}\)), respectively. Each treatment was set up as three replicates.

2.1.3. Analyses

Concentrations of dissolved inorganic nitrogen (nitrate, nitrite, ammonium) and dissolved total nitrogen in the overlying water were measured using standard methods (APHA, 1995).

Chl a was used as a proxy for the Microcystis biomass as it is difficult to count cells in colonies. The Chl a concentration was calculated using the OD\(_{680nm}\) and OD\(_{720nm}\) values that were measured using a hand-held AquaPen (AP-C 100, Photon Systems Instruments, Czech Republic). It should be noted that when the heterogeneity of samples is obviously different or the light scattering is strong, the Chl a concentration will be less precise.

To further analyze Microcystis dynamics in the enclosure experiments, we quantified different components of biomass balance using the Chl a concentrations in the water column (subscript “W”) and in the sediment trap material (subscript “S”) as proxies of the respective concentrations of Microcystis. The following components of biomass balance on a certain day (the ith day) were calculated: the biomass in the enclosure water column (column biomass) \(B_{Wi}\) (mg Chl a m\(^{-2}\)), multiplying the Chl a concentration in the water by the water column depth in the enclosure), daily sinking biomass \(S_{1,i}\) (mg Chl a m\(^{-2}\) d\(^{-1}\)), multiplying the Chl a concentration in the sediment trap in the sediment trap biomass, sinking ratio \(SS_{1,i}\) (d\(^{-1}\)), daily sinking biomass expressed per unit of the mean biomass in the water column, total biomass in the whole enclosure (gross biomass) \(B\) (mg Chl a m\(^{-2}\)), the sum of the column biomass and the total of the biomass accumulated in the trap since the beginning of the experiment, daily growth biomass \(G_{1,i}\) (mg Chl a m\(^{-2}\) d\(^{-1}\)), the sum of the water column biomass increase during one day \(B_{Wi} - B_{Wi-1}\) and \(S_{1,i}\), and growth ratio \(G_{1,i}\) (d\(^{-1}\)), the daily growth biomass expressed per unit of the mean biomass.

The following parameters were calculated for the entire duration of our experiments: the average specific growth rate (rate of gross biomass change, GR\(_{0,E}\) d\(^{-1}\)), the average specific rate of column biomass change (rate of net biomass change, NR\(_{0,E}\) d\(^{-1}\)), the overall change in column biomass (Δ \(B_{Wi-1}\) mg Chl a m\(^{-2}\)), the overall sinking biomass (Δ \(S_{1,i}\) mg Chl a m\(^{-2}\)), and the overall change in gross biomass (Δ \(B_{Wi}\) mg Chl a m\(^{-2}\)). Details of calculations of the Microcystis biomass balance in the enclosure are presented in the Supplementary Material.

All the estimated parameters were compared using an ANOVA with an LSD test. P-values less than 0.05 were considered significant.
The water column was collected and immediately delivered to the lab. The biomass was diluted with lake water to approximately 1 mg L$^{-1}$. K$_2$HPO$_4$ was added as a phosphorus source to provide a concentration of approximately 1 mg L$^{-1}$. The bottles contained approximately 1000 mL of *Microcystis* culture medium. The *Microcystis* colonies were incubated in an artificial climate chamber at 28 ± 1°C, with a light:dark cycle of 16:8 h. During light hours, the illumination was 50 μmol photons m$^{-2}$ s$^{-1}$. The experiment lasted 3 days. Before and after the experiment, *Microcystis* samples were collected to measure dry weight, specific density, carbohydrate concentration, protein concentration, relative gas vesicle volume, and the floating ratio of the *Microcystis* colonies.

Dry weights, carbohydrate concentrations and protein concentrations were measured using standard methods (APHA, 1995), the Coomassie brilliant blue G 250 method (Bradford, 1976), and the modified phenol method (Chow and Landhäusser, 2004), respectively. The carbohydrate content and protein content were calculated by dividing the carbohydrate and protein concentrations by the dry weight. The specific density of *Microcystis* was measured using the Percoll density gradient centrifugation method (Huang et al., 2016). The relative gas vesicle volume of *Microcystis* was estimated using a turbidimeter measurement using a pressurization device according to our previous study (Huang et al., 2018). The relative gas vesicle volume of *Microcystis* was calculated by dividing the carbohydrate and protein concentrations by the dry weight. The specific density of *Microcystis* was measured using the Percoll density gradient centrifugation method (Huang et al., 2016). The relative gas vesicle volume of *Microcystis* was estimated using a turbidimeter measurement using a pressurization device according to our previous study (Huang et al., 2018). The floating ratio of *Microcystis* colonies was measured as follows. The *Microcystis* culture was mixed well, and the Chl a concentration was determined using a hand-held AquaPen (AP-C 100, Photon Systems Instruments, Czech Republic). The mixed sample (15 mL) was placed into a 25 mL graduated cylinder and left to stand for 1 h. Then, 2 mL of the surface sample was collected to determine the Chl a concentration, which is considered as the floating biomass. The floating ratio (FR, %) of *Microcystis* colonies was calculated using the formula:

$$\text{FR} = \left( \frac{C_{\text{floating}} \times V_{\text{floating}}}{C_{\text{mixing}} \times V_{\text{total}}} \right) \times 100\%$$

where $C_{\text{floating}}$ is the Chl a concentration of the upper sample, $C_{\text{mixing}}$ is the Chl a concentration of the mixed sample, and $V_{\text{floating}}$ and $V_{\text{total}}$ are 2 mL and 15 mL, respectively.

All the estimated parameters were compared using an Independent T-test. P-values less than 0.05 were considered significant.

### 2.2. Nitrogen-deficient cultivation experiment in the laboratory

This experiment was conducted in September 2018. The sampling site was the same as in the previous experiments (2.1). Water with a high *Microcystis* biomass at the surface and the water in the middle of the water column was collected and immediately delivered to the lab. The *Microcystis* biomass was diluted with lake water to approximately 2 mg Chl a L$^{-1}$. Microalgae Brilliant Blue G 250 method (Bradford, 1976), and the modifications were measured using standard methods (APHA, 1995), the Coomassie Brilliant Blue G 250 method (Bradford, 1976), and the modified phenol method (Chow and Landhäusser, 2004), respectively. The carbohydrate content and protein content were calculated by dividing the carbohydrate and protein concentrations by the dry weight. The specific density of *Microcystis* was measured using the Percoll density gradient centrifugation method (Huang et al., 2016). The relative gas vesicle volume of *Microcystis* was estimated using a turbidimeter measurement using a pressurization device according to our previous study (Huang et al., 2018). The floating ratio of *Microcystis* colonies was measured as follows. The *Microcystis* culture was mixed well, and the Chl a concentration was determined using a hand-held AquaPen (AP-C 100, Photon Systems Instruments, Czech Republic). The mixed sample (15 mL) was placed into a 25 mL graduated cylinder and left to stand for 1 h. Then, 2 mL of the surface sample was collected to determine the Chl a concentration, which is considered as the floating biomass. The floating ratio (FR, %) of *Microcystis* colonies was calculated using the formula:

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where $C_{\text{floating}}$ is the Chl a concentration of the upper sample, $C_{\text{mixing}}$ is the Chl a concentration of the mixed sample, and $V_{\text{floating}}$ and $V_{\text{total}}$ are 2 mL and 15 mL, respectively.

All the estimated parameters were compared using an Independent T-test. P-values less than 0.05 were considered significant.

### 2.3. Proteomic analysis

The *Microcystis aeruginosa* (provided by the Freshwater Algae Culture Collection of the Institute of Hydrobiology, the Chinese Academy of Sciences) was incubated with an autoclaved BG-11 medium and maintained in an artificial climate chamber at 28 ± 1°C, with a light:dark cycle of 16:8 h. During light hours, the illumination was 50 μmol photons m$^{-2}$ s$^{-1}$.

A nitrogen-free BG-11 medium was inoculated with *Microcystis* in an exponential growth phase, and *Microcystis* cultivated in a standard BG-11 medium was used as the control. The nitrogen-deficient treatment and the control were repeated as three replicates. The experiment lasted 48 h.

*Microcystis* cells from three replicates of the nitrogen-deficient treatment and the control were obtained to extract protein. The protein was digested and labeled using TMT 10 plex Mass Tag Labeling Kits and Reagents (Thermo Scientific, United States). Nano LC-MS/MS analysis was performed on TMT-labeled samples. Quantification was based on the ratio of the summed areas across the eluting chromatographic peak. For quantification, the average of the control was used as an internal reference, and the ratios of treatment/control were calculated. Ratios < 0.833 and > 1.2 were applied to identify significantly down- and up-regulated proteins, respectively. Significantly different proteins were further analyzed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway annotation.

Detailed experimental processes are shown in Supplementary Material.
showed significantly with the increase in the overall sinking biomass (ΔS0,E) among the three treatments (Fig. 4a), which could be attributed to a decrease in the column biomass in lower nitrogen loading treatments. However, the average sinking ratio was higher than the average specific rate of net biomass change (NR0,E). The gross biomasses (B) increased rapidly by 92.2 ± 11.0%, 22.9 ± 6.1%, and 73.7 ± 14.3% compared to the initial biomasses in the enclosures with low, middle, and high initial DIN concentrations, respectively. The ratio of overall sinking biomass to overall change in gross biomass was 0.93 ± 0.05, 0.77 ± 0.03, and 0.48 ± 0.06 in the enclosures with low, middle, and high initial DIN concentrations, respectively. The sinking biomasses were lower than the growth biomasses, leading to the increase in the column biomasses. The results suggested that increasing the nitrogen input would reduce the downward transport of Microcystis colonies and further affect column biomass.

### 3.2. Responses of column biomass and sinking biomass to different nitrogen loading

Enclosure Experiment II aimed to investigate the effects of the pulsed nitrogen input on Microcystis dynamics. During the experiment, the DIN concentrations ranged from 0.08 - 0.65 mg N m$^{-2}$ (Fig. 3b). As shown in Fig. 3, in the enclosures with 0.02 g N m$^{-2}$ d$^{-1}$, the column biomasses (Bc) decreased from 153.6 ± 16.5 mg Chl a m$^{-2}$ to 67.3 ± 27.4 mg Chl a m$^{-2}$, with the highest reduction ratio of 57.1 ± 12.8%. In the enclosures with higher nitrogen loading of 0.2 g N m$^{-2}$ d$^{-1}$ and 0.5 g N m$^{-2}$ d$^{-1}$, the decrease was also observed in the column biomasses; however, their reduction ratios were lower, at approximately 34.6 ± 16.3% and 20.6 ± 17.2%, respectively. These results suggested that the decrease in nitrogen loading could increase the reduction in the Microcystis biomass in the water column under low nitrogen available conditions.

Downward transport and growth of biomass are two important factors that influence the dynamics of Microcystis blooms in the water column. Daily sinking biomass (S) of Microcystis fluctuated greatly during the experiment (Fig. S2), and there was no significant difference ($f = 2.5, p > 0.05$) in the overall sinking biomass (ΔS0,E) among the three treatments (Fig. 4a), which could be attributed to a decrease in the column biomass in lower nitrogen loading treatments. However, the average sinking ratio showed that the downward transport of Microcystis increased with decreasing nitrogen loading (Table 1). The average sinking ratio was significantly higher in lower nitrogen loadings ($f = 6.8, p = 0.03$). In the enclosures with 0.02 g N m$^{-2}$ d$^{-1}$, the average sinking ratio was 2.3 times that in the enclosures with 0.5 g N m$^{-2}$ d$^{-1}$. During the same period, the growth of Microcystis was positive in the enclosures with 0.2 g N m$^{-2}$ d$^{-1}$ and 0.5 g N m$^{-2}$ d$^{-1}$; whereas, the growth nearly terminated in the enclosures with the lowest daily nitrogen loading of 0.02 g N m$^{-2}$ d$^{-1}$ and the average...
3.3. Sinking properties of Microcystis colonies in response to nitrogen-biomass.

would be terminated owing to the increasing downward transport of biomass. Continued under low nitrogen loading, the bloom in the water column was much higher than the growth of biomass. These results indicated that although the growth of Microcystis colonies under nitrogen limitation.

Average daily growth biomass (mg Chl a m\(^{-2}\) d\(^{-1}\))

Overall daily growth biomass (mg Chl a m\(^{-2}\) d\(^{-1}\))

Relative gas vesicle volume 7.2 ± 0.4 4.2 ± 0.8 *

Data are presented as mean ± standard deviation (SD). The mark * indicates significant differences from initial values by an independent T-test at p < 0.05.

accumulated the most near the water surface with floating ratios of more than 80%, and the specific density was lower than that of water, whose value is approximately 1. After a 3-day cultivation period without any additional nitrogen sources, the floating ratio decreased significantly by 17.1% (p = 0.01), and the specific density increased to 1.009 ± 0.001. Although the protein content decreased by 55.5% (p = 0.002), exerting a negative effect on the specific density, the carbohydrate content increased by 2.2 times (p = 0.002) and the relative gas vesicle volume decreased to 58.8% (p = 0.01), which positively affected the specific density. These results confirmed that the downward transport of Microcystis colonies increased without available nitrogen, which was in parallel to an increase in the carbohydrate content and a decrease in the gas vesicle content.

3.4. Differential protein expression in Microcystis under nitrogen-deficient conditions and carbon metabolism toward carbohydrate accumulation

The proteomic measurement identified a total of 2786 proteins. Among these, 955 proteins were significantly different in abundance between the nitrogen-deficient treatment and the control; of these, 504 proteins were upregulated and 451 proteins were downregulated in the nitrogen-deficient treatment. KEGG analysis indicated that the pathways involved in carbohydrate synthesis and metabolism included photosynthesis (Rich Factor, RF = 0.578), carbon fixation in photosynthetic organisms (RF = 0.529), starch and sucrose metabolism (RF = 0.400), pentose phosphate pathway (RF = 0.529), glycolysis (RF = 0.414), pyruvate metabolism (RF = 0.367), and citrate cycle (RF = 0.188). The identified proteins related to carbohydrate metabolism pathways are grouped in Table 3 and Figs. S3–S7. All these data indicate that both the synthesis and metabolism of carbohydrates were downregulated, induced by nitrogen deficiency; moreover, the main reason for carbohydrate accumulation may be the decrease in carbohydrate consumption, especially by glycolysis. Additionally, gas vesicle proteins are also shown in Table 3, which showed a decrease in the abundance of GvpA, C, J, K, N, V, and X.

4. Discussion

In eutrophic shallow lakes such as the Lake Taihu, the dissipation of Microcystis blooms usually occurs in late summer or autumn, and the underlying mechanisms might involve the depletion in bioavailable

<table>
<thead>
<tr>
<th>Daily nitrogen loading</th>
<th>0.02 g N m(^{-2}) d(^{-1})</th>
<th>0.2 g N m(^{-2}) d(^{-1})</th>
<th>0.5 g N m(^{-2}) d(^{-1})</th>
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</thead>
<tbody>
<tr>
<td>Average daily sinking biomass (mg Chl a m(^{-2}) d(^{-1}))</td>
<td>-26.4 ± 4.3 *</td>
<td>-22.0 ± 3.3 *</td>
<td>-20.9 ± 0.6 *</td>
</tr>
<tr>
<td>Average sinking ratio (d(^{-1}))</td>
<td>-0.23 ± 0.04 *</td>
<td>-0.19 ± 0.01 ab</td>
<td>-0.16 ± 0.01 b</td>
</tr>
<tr>
<td>Average daily growth biomass (mg Chl a m(^{-2}) d(^{-1}))</td>
<td>4.8 ± 1.3 a</td>
<td>10.1 ± 6.0 ab</td>
<td>14.5 ± 5.6 ab</td>
</tr>
<tr>
<td>Average growth ratio (d(^{-1}))</td>
<td>0.02 ± 0.04 a</td>
<td>0.08 ± 0.05 a</td>
<td>0.10 ± 0.05 a</td>
</tr>
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</table>

Data are presented as mean ± standard deviation (SD). Different letters indicate significant differences using an ANOVA with an LSD test at p < 0.05.
nitrogen in water bodies (Michalak et al., 2013). In inland waters, low nitrogen availability induces a decrease in non-diazotrophic cyanobacterial biomass by suppressing their growth (Forchhammer and Schwarz, 2019; Paerl et al., 2011; Xu et al., 2015). According to field surveys, the DIN concentration can be as high as 0.76 mg L\(^{-1}\) in inland waters, low nitrogen availability induces a decrease in non-diazotrophic cyanobacterial biomass. In natural lakes, the nitrogen concentration can be enriched incidentally from Enclosure Experiment I (Fig. 2) indicated that when short-term nitrogen input increases nitrogen availability, it can further enhance Microcystis biomass in the water column not only by promoting their growth but also by reducing the downward transport of biomass. Therefore, Microcystis can maintain their growth with DIN concentrations decreasing to 0.12 mg N L\(^{-1}\) (Orr and Jones, 1998). Therefore, Microcystis can maintain their growth under low-nitrogen availability; and “growth suppression” seems unlikely to be the complete explanation for bloom dissipation, as the low nitrogen availability only reduces the growth rate of Microcystis but does not directly lead to the decline in column biomass.

From an ecological point of view, the dynamics of Microcystis biomass in a closed system should be regulated by both growth (outcome of cell reproduction and mortality/lysis) and downward transport. Previous studies also showed that the downward transport of cyanobacteria is a widespread phenomenon and can regulate cyanobacterial dynamics in waters (Kitchens et al., 2018; Magonono et al., 2018). The results of the present study confirmed that the downward transport of biomass should be considered while studying Microcystis dynamics. In natural lakes, the nitrogen concentration can be enriched incidentally by short-term events such as rainfall or wastewater discharge. Results from Enclosure Experiment I (Fig. 2) indicated that when short-term nitrogen input increases nitrogen availability, it can further enhance Microcystis biomass in the water column not only by promoting their growth but also by reducing the downward transport of biomass.

<table>
<thead>
<tr>
<th>Pathway: Starch and sucrose metabolism (KEGG_id: ko00500)</th>
<th>Gene name</th>
<th>Fold Change Ratio</th>
<th>t test p value</th>
<th>Identified Protein IDs</th>
</tr>
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<tbody>
<tr>
<td>Glycogen synthase</td>
<td>glgA2</td>
<td>1.32</td>
<td>0.023</td>
<td>A0A1X9L649</td>
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<tr>
<td>1,4-alpha-glucan branching enzyme</td>
<td>gglB</td>
<td>1.24</td>
<td>0.015</td>
<td>A0A1X9L5P6</td>
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<td>Alpha-1,4 glucan phosphorylase</td>
<td>gglP</td>
<td>1.21</td>
<td>0.0063</td>
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<td>Glucose-6-phosphate isomerase</td>
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<td>0.80</td>
<td>0.0071</td>
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<td>Fructokinase</td>
<td>scrK</td>
<td>0.80</td>
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<tr>
<td>Phosphoglucomutase</td>
<td>pgm</td>
<td>0.70</td>
<td>0.0050</td>
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<tr>
<td>Fructose-1,6-bisphosphate aldolase</td>
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<tr>
<th>Pathway: Glycolysis (KEGG_id: ko00010)</th>
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<td>Pyruvate-flavodoxin oxidoreductase</td>
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<td>6-phosphofructokinase</td>
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<td>Diphosphate-dependent phosphofructokinase</td>
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<tr>
<td>Pyruvate kinase</td>
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<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
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<td>Pyruvate kinase</td>
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<tr>
<td>Glucose-6-phosphate isomerase</td>
</tr>
<tr>
<td>Phosphoenolpyruvate carboxykinase (ATP)</td>
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<tr>
<td>Fructose-1,6-bisphosphate aldolase</td>
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<tr>
<td>Phosphoglucomutase</td>
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<td>Phosphoglycerate kinase</td>
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<td>Pyruvate-flavodoxin oxidoreductase</td>
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<tr>
<td>Fumarate reductase flavoprotein subunit</td>
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<tr>
<td>Succinate dehydrogenase flavoprotein subunit</td>
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<tr>
<th>Pathway: Pyruvate metabolism (KEGG_id: ko00620)</th>
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<tr>
<td>Phosphatase acyltransferase</td>
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<td>Pyruvate-flavodoxin oxidoreductase</td>
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<td>Aldehyde dehydrogenase</td>
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<td>Pyruvate kinase</td>
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<td>Acetyl-coenzyme A carboxylase carboxyl transferase subunit</td>
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<td>Phosphoenolpyruvate carboxykinase (ATP)</td>
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<td>2-isopropylmalate synthase</td>
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Moreover, surface runoff brings continual nitrogen input to lakes. When pulsed nitrogen inputs were simulated in Enclosure Experiment II, a decline in the *Microcystis* biomass in the water column was observed, especially in the enclosures with the daily nitrogen loading of 0.02 g N m$^{-2}$ d$^{-1}$ (Figs. 3 and 4). Although *Microcystis* maintained positive growth ratios, the sinking ratios were much higher and increased with the decrease in nitrogen loading (Table 1). Therefore, with low nitrogen loading, the increase in downward transport, rather than cell mortality, was mainly responsible for the decline in the *Microcystis* biomass in the water column. These results pinpoint that the downward transport of biomass should not be ignored as it greatly affects the dynamics of *Microcystis* blooms. Remarkably, with a decrease in nitrogen availability, once the downward transport exceeded the gross growth, the dissipation of *Microcystis* blooms in the overlying water occurred.

The specific density of cyanobacterial cells can influence their downward transport processes and is mainly regulated by their ballast (carbohydrate and protein) content and gas vesicle volume. Previous studies showed that low nitrogen availability could lead to an increase in the specific density of *Microcystis* cells, mainly through the promotion of carbohydrate accumulation and the reduction in gas vesicles; however, these studies were conducted using unicellular strains and not *Microcystis* colonies (Brookes and Ganf, 2001; Chu et al., 2007; Huang et al., 2019). In the present study, we found a clear response of *Microcystis* colonies to nitrogen deficiency. A decrease in the floating ratio and an increase in specific density was observed with a 2.2 times increase in carbohydrate content and a decrease in relative gas vesicle volume to 58.8% of its initial value. Thus, under nitrogen-deficient conditions, *Microcystis* colonies lost their floating ability and began to sink in parallel to the accumulation of carbohydrate and the decrease in gas vesicle volume.

By analyzing the expression of proteins in *Microcystis* cells between nitrogen-sufficient and nitrogen-deficient conditions, it was shown that the changes in the sinking properties of *Microcystis* cells under nitrogen-deficient conditions were driven by a series of physiological processes. Previous studies showed that for some cyanobacteria, nitrogen deficiency results in excess carbon (Hasunuma et al., 2013; Yue et al., 2015). The excess carbon flows into carbon storage granules, such as polyhydroxyalkanoate and glycogen granules (Deschoenmaeker et al., 2016; Hickman et al., 2013), and further increases the specific density of cells. Likewise, our proteomic analysis suggested that, for starch and sucrose metabolism, the upregulated proteins were involved in the process of biosynthesis of glucose-1-phosphate (G1P) into amylose and glycogen and that the downregulated proteins were involved in the process of degradation of sucrose into G1P, indicating less G1P entered the glycolysis pathway. In glycolysis, nine important proteins were downregulated, suggesting less pyruvate production from G1P or glucose. Consistent with this, pyruvate metabolism and the TCA cycle were also downregulated. Furthermore, their reduction negatively influenced the biosynthesis of amino acids and fatty acids. Additionally, proteins involved in the pentose phosphate pathway were also downregulated, suggesting that oxidative decomposition of glucose had been blocked. Similar results have been observed for other species of cyanobacteria. For instance, the downregulation of glycolysis was accompanied by a decrease in photosynthetic activities and inorganic carbon fixation in *Arthrospira* sp. (Deschoenmaeker et al., 2014). A reduction in the proteins involved in glycolysis and the TCA cycle was also detected in *Synechocystis* sp. (Huang et al., 2013) as well as a significant decrease in the TCA cycle intermediates, cis-aconitate and fumarate (Hickman et al., 2013). Accordingly, it can be inferred that carbohydrate accumulation induced by nitrogen deficiency is due to the slowing down of catabolic consumption of carbohydrates.

Apart from carbohydrate accumulation, the proteomic analysis also showed a decrease in the abundance of gas vesicle proteins under nitrogen-deficient conditions. Gas vesicles are constructed from a homologous protein filled with several gasses (Walsby, 1994). The main mass of the structure is formed by GvpA, and GvpC located on the outside of the structure can strengthen the gas vesicle (Walsby, 1994). This was consistent with the decrease in the relative gas vesicle content (Table 2), which implied that the improvement in downward transport of biomass partially originated from the loss of gas vesicles.

Over the last decades, cyanobacterial blooms have gradually become a common and harmful nuisance in eutrophic shallow lakes. Earlier studies suggested that phosphorus was the main limiting factor for the growth of cyanobacteria (Carpenter, 2008; Schindler, 1977). However, present studies have found that nitrogen may be one of the limiting factors for the eutrophic lakes, such as Lake Taihu, Lake Müggelsee and Lake Erie, whose dominant species happen to be non-diazotrophic cyanobacteria (e.g., *Microcystis*) (Harke et al., 2016a; Harke et al., 2016b; Shatwell and Köhler, 2019). It is expected that when the nitrogen availability is low, the growth rate of non-diazotrophic cyanobacteria will decrease significantly, resulting in termination of blooms (Erratt et al., 2018; Paelr and Otten, 2013). The present study explored a nitrogen-driven factor regulating the dynamics of *Microcystis* blooms and further indicated that it should play an important role in determining the duration of the bloom period. It was shown that, apart from population growth, the downward transport of *Microcystis* biomass is also sensitive to nitrogen availability. Without this availability, there would be increased biomass sinking out of the water column and, thus, accelerated the dissipation of *Microcystis* bloom.

In the present study, we considered *Microcystis* biomass balance and the role of downward transport in enclosures in practically still water; a similar condition occurs in *Microcystis* scum-gathering areas. In contrast, in natural lakes, turbulent motions play a very important role in the distribution of *Microcystis* colonies (Hozumi et al., 2019; Ostrovsky et al., 2018) and may thus affect the sinking rates of colonies, especially in the open waters of the shallow lakes. Therefore, investigations are required to evaluate the effects of turbulence on the downward transport of *Microcystis* colonies. Remarkably, *Microcystis* cells are relatively tolerant to dark and anoxic conditions, such that the cells can survive for a period of time at the sediment-water interface after downward transport (Bouchard and Purdie, 2011; Chen et al., 2018). Living *Microcystis* cells might have an opportunity to float or reach the euphotic zone when the wind or turbulent motion is strong (Ostrovsky et al., 2017; Wu et al., 2013), thus compensating for their sinking biomass (Feng et al., 2019b; Zhu et al., 2018). Additionally, the present study used Chl a as the proxy for *Microcystis* biomass, which usually only represents the total biomass but not a specific species. In the enclosure experiments, the dominant species was *Microcystis* sp., accounting for more than 99% of the abundance observed under the microscope. Therefore, the total Chl a concentration can be considered as *Microcystis* biomass. However, under the conditions of different nitrogen availability, whether the production of chlorophyll is synchronous with the production of organic material is unclear; thus, the bias of using Chl a as the biomass proxy needs to be discussed in further studies.

5. Conclusion

This study provides a better understanding of the effect of nitrogen availability on the dynamics of *Microcystis* blooms as well as a deeper insight into the contribution of the downward transport of biomass to the dissipation of *Microcystis* blooms. The results showed that when short-term nitrogen input increases nitrogen availability, it will further enhance the *Microcystis* biomass in the water column not only by promoting their growth but also by reducing the downward transport of biomass. Moreover, when nitrogen loading decreased, *Microcystis* showed positive growth ratios, and the increase in sinking ratios was mainly responsible for the decline in *Microcystis* biomass in the water column. The increased downward transport of *Microcystis* colonies was driven by a series of physiological processes, in parallel with an increase in the carbohydrate content and a decrease in the gas vesicle
volume. The proteomic analysis suggested that carbohydrate accumulation induced by nitrogen deficiency resulted from the slowing down of the catabolic consumption of carbohydrates rather than the increasing biosynthesis, especially the downregulation of glycolysis. Further investigations are needed to evaluate the role of physical forces (wind, currents, turbulence) in the downward transport and resuspension of Microcystis.

Declaration of Competing Interest

None.

Acknowledgments

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Supplementary materials


Appendix A. Supplementary data

Additional experimental process and figures are given in the Supplementary Material.

References


