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Nitrogen limitation affects the sinking property of *Microcystis* by promoting carbohydrate accumulation

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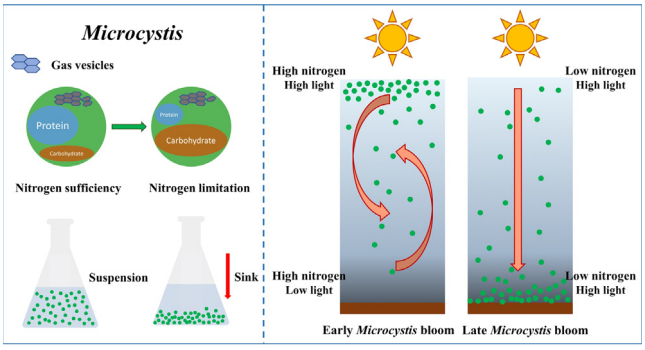
HIGHLIGHTS

- Nitrogen limitation increased the specific density of *Microcystis*.
- The higher specific density was attributed to the accumulation of carbohydrates.
- Rapid carbohydrate accumulation was due to its lower consumption rate in darkness.
- Nitrogen limitation negatively affected the buoyancy regulation.
- This study complements the disappearance mechanisms of *Microcystis* blooms.

GRAPHICAL ABSTRACT



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ABSTRACT

Nitrogen limitation has been proven to inhibit *Microcystis* proliferation, and the significant decline in *Microcystis* blooms in late summer or autumn has been considered to be related to the nitrogen depletion in water. Sinking loss is another factor that influences the dynamics of cyanobacteria in lakes. However, to date, it is still unclear how the sinking property of *Microcystis* responds to nitrogen availability. Our results suggest that nitrogen limitation would directly influence sinking property of *Microcystis*, through a significant increase in the specific density of cells. In the short term, carbohydrate accumulation was mainly responsible for the high specific density, showing a high correlation among the NO_3^--N concentration, specific density and carbohydrate content. Furthermore, carbohydrates could rapidly accumulate after one light/dark cycle, which was mainly due to the reduction in carbohydrate consumption in the darkness under nitrogen limitation. Under nitrogen-light coupling conditions, the specific density ranged from 1.060 to 1.068, except for the treatment with high-nitrogen plus low-light, which showed the value of 1.032. More importantly, when coupled with low nitrogen, the low light did not decrease the carbohydrate content and the specific density, which implied that the sinking cells could not migrate back to the surface. Accordingly, a hypothesis was proposed that the carbohydrate accumulation induced by low nitrogen availability caused an increase in specific density, which invalidates the buoyancy

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regulation, and cells sink continually out of the water column. This study explores a new understanding on the disappearance mechanisms of *Microcystis* blooms in the late summer and fall.

1. Introduction

In recent years, non-nitrogen (N_2) -fixing cyanobacteria, especially *Microcystis*, have been the dominant species in many eutrophic lakes, such as Taihu Lake and the Great Lakes, and form cyanobacterial blooms (Qin et al., 2010; Stumpf et al., 2012; Paerl, 2014). Studies have shown the link between nitrogen availability and the increasing frequency of *Microcystis* (cyanobacterium) blooms (Xu et al., 2010; Paerl and Otten, 2013). Laboratory and field studies have demonstrated that nitrogen reduction can effectively control the proliferation of *Microcystis* (Paerl et al., 2011; Xu et al., 2015). The significant decline in *Microcystis* biomass in the late summer or early autumn has also been considered to be related to the depletion of bioavailable nitrogen in water bodies (Michalak et al., 2013).

Sinking loss is another factor which influences the dynamics of cyanobacteria in lakes (Diehl, 2002; Pannard et al., 2007; Huang et al., 2016). In the summer, during cyanobacterial blooms, the sinking fluxes of cyanobacteria can exceed 10^9 cells m^{-2} d^{-1}, with a maximum value of 3.7×10^{10} cells m^{-2} d^{-1} (Cirés et al., 2013). Visser et al. (1995) found that during September and October, cyanobacteria have a cumulative percentage in the sedimentation traps compared to that in the summer. Moreover, the sinking rates of *Microcystis* (cyanobacteria) can increase sharply from 0.0045 m d^{-1} in June–August to 0.24 m d^{-1} in October (Takamura and Yasuno, 1988). The sinking property of *Microcystis* can be assessed by the specific density of cells, which depends on the extent to which the lift provided by gas vesicles is counteracted by cell ballasts, such as carbohydrates (density ~1550 g L^{-1}) and proteins (density ~1300 g L^{-1}). There has been evidence to show that the production of gas vesicles is limited by low nitrogen availability (Chu et al., 2007). Due to diminishing gas vesicles, *Microcystis* might lose its resuspension ability and begin to sink, eventually being trapped by the bottom sediment. However, to date, it is still unclear how the sinking property of *Microcystis* responds to nitrogen availability. Furthermore, when the sinking cells move downward, they experience low light availability. Our previous study has demonstrated that the decrease in available light may negatively influence the specific density of *Microcystis*, thus lowering the sinking loss rate (Huang et al., 2016). The decrease in specific density in response to low-light conditions was due to the consumption of carbohydrates (Kromkamp and Walsby, 1990). Therefore, relying on buoyancy regulation through the reduction of carbohydrate contents, the sinking cells might be able to float upwards back to the water column (Carey et al., 2012). However, these studies were conducted under conditions of nitrogen sufficiency. Therefore, it is critical to understand the coupling effect of the nitrogen and light on the sinking property of cyanobacteria.

We assumed that nitrogen availability, especially nitrogen limitation would affect the sinking property of *Microcystis*. To validate the hypothesis and determine the underlying mechanism, batch tests were conducted to investigate the sinking property of *Microcystis* during nitrogen-limited growth by analyzing of the specific density and the regulating factors, including intracellular carbohydrate content, intracellular protein content, and relative gas vesicle content. Further experiment was conducted to investigate the coupling effect of the availability of nitrogen and light on the sinking property of *Microcystis*.

2. Materials and methods

2.1. Cyanobacteria strain

Microcystis aeruginosa (No. FACHB 942) was obtained from the Freshwater Algae Culture Collection of the Institute of Hydrobiology, Chinese Academy of Sciences. The tested cyanobacteria were incubated with an autoclaved BG-11 medium and maintained in an artificial climate chamber at 25 ± 1 °C, with a light-dark cycle of 14:10 h and an illumination of 50 μmol photons $m^{-2} \cdot s^{-1}$.

Microcystis cells in the exponential phase were harvested by centrifugation for 15 min at 3000×*g* (Sorvall ST 16 R, Thermo, Unit States). Next, the culture medium was decanted and the cells were suspended in pure water. The cells were washed two more times using the process detailed above and suspended in pure water for later use.

2.2. Nitrogen limitation experiment

2.2.1. Batch tests I

Nitrate modified BG-11 medium was inoculated with *Microcystis* cells mentioned above. $NaNO_3$ was used for NO_3^--N. The treatments included an addition of NO_3^--N at initial concentrations of 0 mg L^{-1}, 0.5 mg L^{-1}, 1 mg L^{-1}, 2 mg L^{-1} and 5 mg L^{-1}, using three replicates for each concentration. The initial cell density was approximately 5×10^5 cells ml^{-1}. The other conditions of cultivation were the same as used for *Microcystis* cultivation. The experiment lasted 10 days. During the cultivation, culture samples were taken to measure cell density, intracellular carbohydrate content, intracellular protein content, relative gas vesicle content, specific density of cells, and the NO_3^--N concentration in the culture medium.

2.2.2. Batch tests II

The nitrate modified BG-11 medium was inoculated with *Microcystis* cells mentioned above. $NaNO_3$ was used for NO_3^--N. The treatments included an addition of NO_3^--N at initial concentrations of 0 mg L^{-1}, 0.2 mg L^{-1}, 0.5 mg L^{-1}, 1 mg L^{-1}, 2 mg L^{-1} and 3 mg L^{-1}, using three replicates for each concentration. The initial cell density was approximately 5×10^5 cells ml^{-1}. The other conditions of cultivation were the same as used for *M. aeruginosa* cultivation.

The experiment lasted 24 h, including one light cycle (14 h) and one dark cycle (10 h). After the light cycle and the dark cycle, culture samples were taken to measure the cell density, intracellular carbohydrate content, intracellular protein content, specific density of cells, and the NO_3^--N concentration in the culture medium.

2.3. Nitrogen-light coupling experiment

The nitrate modified BG-11 medium was inoculated with *Microcystis* cells as mentioned above. The experiment consisted of four treatments, including high-nitrogen plus high-light (10 mg NO_3^--N L^{-1} and 50 μmol photons $m^{-2} \cdot s^{-1}$), high-nitrogen plus low-light (10 mg NO_3^--N L^{-1} and 1 μmol photons $m^{-2} \cdot s^{-1}$), low-nitrogen plus high-light (0.5 mg NO_3^--N L^{-1} and 50 μmol photons $m^{-2} \cdot s^{-1}$), low-nitrogen plus low-light (0.5 mg NO_3^--N L^{-1} and

1 μmol photons $m^{-2}\cdot s^{-1}$). $NaNO_3$ was used for NO_3^--N, and each treatment had three replicates. The initial cell density was approximately 5×10^5 cells ml^{-1}. The other cultivated conditions were the same as for *Microcystis* cultivation.

The experiment lasted 10 days. After the cultivation, culture samples were taken to measure cell density, intracellular carbohydrate content, intracellular protein content, relative gas vesicle content, specific density of cells, and the NO_3^--N concentration in the culture medium.

2.4. Analyses

Cell density was determined under a microscope. The specific density of *Microcystis* was measured by the Percoll density gradient centrifugation method according to our previous study (Huang et al., 2016). The *Microcystis* cells were harvested by centrifugation for 15 min at 3000×*g*. The supernatant was used to measure the NO_3^--N concentration according to standard methods (APHA, 1995). The harvested cells were suspended in 5 ml of pH = 7.4, 10 mmol L^{-1} phosphate buffer solution (PBS), and the cells were then homogenized by an Ultrasonic Cell Homogenizer (96-II, BILON, China) at 80 W for total time of 200 s (ultrasonic time: 10 s; rest time: 10 s) under cooling in an ice-bath. Next, the homogenate was centrifuged at 10,000×*g* for 15 min at 4 °C. The supernatant was used for protein and carbohydrate measurements by the Coomassie brilliant blue method (Bradford, 1976) and modified phenol method (Chow and Landhäusser, 2004), respectively. These two methods give a standard deviation of 1.2% and 3%, respectively. Intracellular carbohydrate content and intracellular protein content were calculated by dividing the quantity of protein and carbohydrate by cell counts in 5 ml of PBS.

Relative gas vesicle content of *Microcystis* was estimated by a turbidity measurement using a pressurization device according to our previous study (Huang et al., 2018). When the *Microcystis* suspension was held under 0.6 MPa for 5 min, all the gas vesicles collapsed. According to the study of Walsby (1971), the collapse of gas vesicles in a suspension of cyanobacteria under pressure causes a decrease in turbidity, which is explained by the disappearance of gas-filled spaces that have a lower refractive index than the surrounding aqueous medium. The ratio of the decrease in the turbidity is proportional to the quantity of gas vesicles. According to the turbidity meter (WGZ-3B, Xin Rui Instrument, China), the standard deviation of turbidity value was 6%. Relative gas vesicle content was calculated according to the study of Beard et al. (2002):

$$\text{Relative gas vesicle content} = (T - T_c)/T_c \quad (1)$$

where T is the initial turbidity of the suspension, and T_c is the turbidity remaining after all the gas vesicles had collapsed.

All the estimated parameters in the treatments were compared using an ANOVA, followed by an LSD test. *P*-values less than 0.05 were considered as significant.

3. Results

3.1. Growth and specific density of Microcystis under nitrogen-limited conditions

The NO_3^--N in the culture medium was gradually consumed (Fig. 1a). After 10 days, the NO_3^--N was nearly completely consumed, even at the treatment with 5 mg NO_3^--N L^{-1}. However, for the *Microcystis* treated with different initial concentrations of NO_3^--N, continued growth was maintained, although the treatments with higher NO_3^--N concentrations showed higher cell density values (Fig. 1b). The results suggested that *Microcystis* could

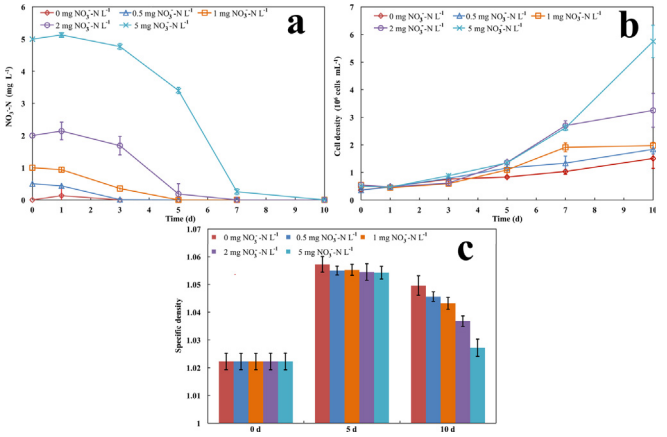

Fig. 1. *Microcystis* cultivation under different concentrations of initial nitrate. a. NO_3^--N concentration in culture medium; b. Cell density of *Microcystis*. c. Specific density of *Microcystis*. Data show the means (n = 3) with associated error bars (SD).

maintain its growth even when the NO_3^--N in the medium is depleted.

Additionally, as shown in Fig. 1c, the change of specific density obviously lagged behind the change of NO_3^--N concentration. After five days of cultivation, the specific density of *Microcystis* increased significantly from 1.022 to approximately 1.055 for all of the treatments ($p < 0.05$), with no obvious difference among the treatments ($p > 0.05$). However, after 10 days, the specific density of all the treatments decreased. Higher specific density values were observed with the decrease in the initial concentration of NO_3^--N, suggesting that nitrogen limitation could lead to higher specific density of *Microcystis*.

3.2. The response of specific density-regulatory factors of Microcystis to nitrogen limitation

As shown in Fig. 2a, in the first three days, there was no significantly difference in the intracellular carbohydrate content among the treatments ($p > 0.05$). Subsequently, the intracellular carbohydrate content increased except for the treatments with 5 mg NO_3^--N L^{-1}. Interestingly, the increase in intracellular carbohydrate content was related to the depletion of NO_3^--N in the culture medium. Taking the treatments with 2 mg NO_3^--N L^{-1}as an example, the intracellular carbohydrate content fluctuated from 4.2 pg $cell^{-1}$ to 6.5 pg $cell^{-1}$ in the first seven days. Upon the depletion of NO_3^--N, the intracellular carbohydrate content reached 10.6 pg $cell^{-1}$on the 10th day and increased by 77.6% compared with that at the 7th day. After 10 days, the highest value of intracellular carbohydrate content was observed in the treatments with 0 mg NO_3^--N L^{-1} and 0.5 mg NO_3^--N L^{-1}, at approximately 13 pg $cell^{-1}$, while the value for the treatments with 5 mg NO_3^--N L^{-1} was

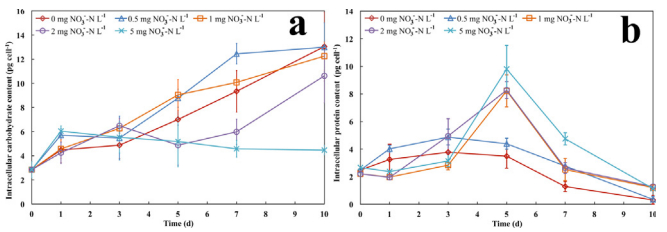

Fig. 2. Carbohydrate and protein in *Microcystis* cells cultivated under different concentrations of initial nitrate. a. Intracellular carbohydrate content; b. Intracellular protein content. Data show the means (n = 3) with associated error bars (SD).

only 4.5 pg cell^{-1}. The results suggested that the intracellular carbohydrate content of *Microcystis* increased with the decrease in initial concentration of NO_3^--N in the culture medium. During the experiment, the intracellular protein content for all the treatments initially increased, showing higher peak values in the treatments with a higher initial concentration of NO_3^--N (Fig. 2b). The highest value of 9.8 pg cell^{-1} was observed on the 5th day in the treatments with 5 mg NO_3^--N L^{-1}. As the NO_3^--N in the culture medium was consumed, the intracellular protein content decreased, which, at the 10th day for the treatments with 5 mg NO_3^--N L^{-1} and 0 mg NO_3^--N L^{-1}, was 44.9%, or only 12.5% of its initial value.

Before inoculation in the nitrate modified BG-11 medium, the *Microcystis* cells had a high relative gas vesicle content of 1.06 (Fig. 3). After cultivation for 10 days under different concentrations of initial NO_3^--N, the relative gas vesicle content reduced significantly to less than 0.2. This value increased with the initial concentration of NO_3^--N in the culture medium (Fig. 4). The relative gas vesicle content was reduced by 93.07% for the treatments with 0 mg NO_3^--N L^{-1} and by 85.57% for the treatments with 5 mg NO_3^--N L^{-1}. However, there was no significant difference between the treatments ($p > 0.05$).

The correlation coefficients (R) among the initial NO_3^--N concentration, the specific density and its regulatory factors are shown in Table 1. Under nitrogen-limited conditions, a highly negative correlation ($|R| > 0.8$) was found between the initial NO_3^--N concentration and the specific density. The specific density-regulatory factors, the intracellular carbohydrate content and the relative gas vesicle content are also highly correlated with the initial NO_3^--N concentration. There was a moderate correlation with intracellular protein content ($0.8 > |R| > 0.3$). Between specific density and its regulating factors, the intracellular carbohydrate content and the relative gas vesicle content also showed higher correlation coefficients than that for the intracellular protein content. Considering that there was no significant difference in the relative gas vesicle content among the treatments, the change in the response of intracellular carbohydrate content to the initial NO_3^--N concentration was the main reason for the change in the specific density under nitrogen-limited conditions.

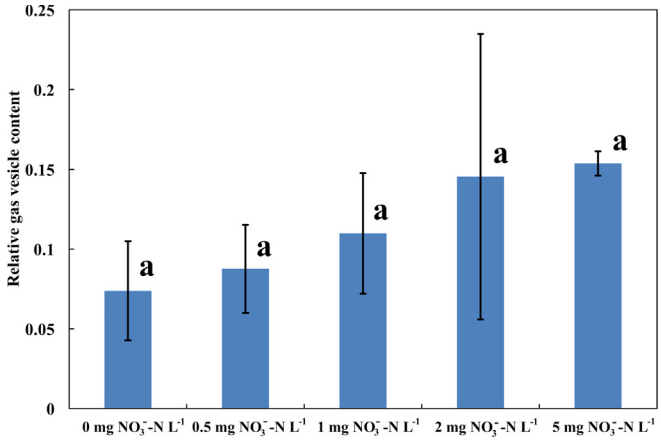

Fig. 3. Relative gas vesicle content in *Microcystis* cells cultivated for 10 days under different initial concentrations of nitrate. Data show the means (n = 3) with associated error bars (SD). The different letters indicate significant differences.

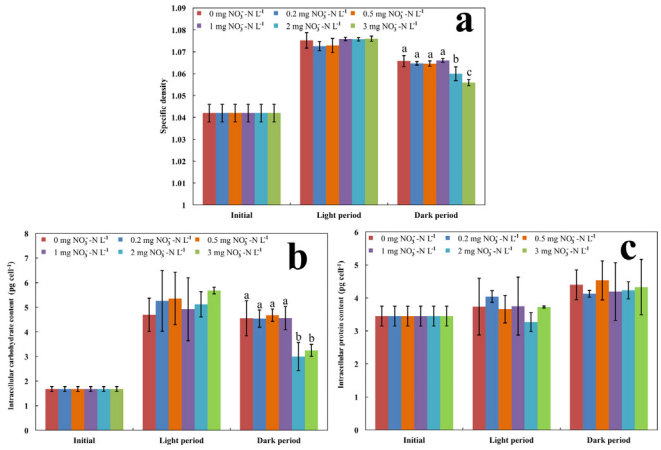

Fig. 4. The specific density (a) and intracellular carbohydrate content (b) of *Microcystis* cultivated under different concentrations of initial nitrate for one light/dark cycle. Data show the means (n = 3) with associated error bars (SD). Different letters indicate significantly difference.

3.3. Short-term carbohydrate accumulation in Microcystis cells under nitrogen-limited conditions

As shown in Fig. 4, the specific density and intracellular contents of carbohydrates and proteins increased after one light period. However, there was no significant difference among the treatments ($p > 0.05$). After one dark period, the specific density and the intracellular carbohydrate content decreased for all the treatments and showed significantly lower values for the treatments with higher initial concentrations of NO_3^--N (2 mg NO_3^--N L^{-1} and 3 mg NO_3^--N L^{-1}, $p > 0.05$). A highly positive correlation ($R = 0.89 > 0.8$) was also observed between the specific density and the intracellular carbohydrate contents. At the same time, a slight increase in the intracellular protein content was observed among all the treatments but without significant differences ($p > 0.05$). The results indicated that under nitrogen-limited conditions, the decrease in the carbohydrate consumption in the dark period might be the main reason for the increase in specific density.

3.4. The response Microcystis specific density and its regulatory factors under different nitrogen-light coupling conditions

As shown in Table 2, it was shown that the specific density ranged from 1.060 to 1.070 for the treatments with high-nitrogen plus high-light, low-nitrogen plus high-light and low-nitrogen plus low-light, with the value in the treatment with high-nitrogen plus low-light being much lower. The highest values of intracellular carbohydrate content were observed under low-nitrogen plus high-light conditions, which was 8.7 times that of the smallest value in the treatment with low-nitrogen plus high-light. Intracellular protein content was higher for both treatments with high-nitrogen. Additionally, except for the treatment with high-nitrogen plus high-light, the relative gas vesicle content for the other three treatments was below 0.10.

4. Discussion

4.1. The reasons for high specific density under nitrogen-limited conditions

The sinking property of a given cyanobacteria taxon may vary due to the change in cell (or colony) shape and size, as well as the specific density, and it may further influence the loss process (Jezberová and Komárková, 2007; Stoyneva et al., 2007; Chen et al., 2011). The specific density of cyanobacterial cells depends mainly on the carbohydrate and protein content and gas vesicle volume. Recent studies have measured the ballasts and gas vesicles, and

Table 1
The correlation coefficient (R) between the parameters after 10 days of cultivation.

	Specific density	Intracellular carbohydrate content	Intracellular protein content	Relative gas vesicles content
Initial NO_3^--N	−0.98	−0.99	0.61	0.88
Specific density	/	0.95	−0.70	−0.94
Intracellular carbohydrate content	/	/	−0.52	−0.81
Intracellular protein content	/	/	/	0.86

Table 2
The specific density and its regulating factors for *Microcystis* cells cultivated for 10 days under different nitrogen-light coupling conditions. Data show the means (n = 3) with associated error bars (SD).

	High-nitrogen High-light	High-nitrogen Low-light	Low-nitrogen High-light	Low-nitrogen Low-light
Specific density	1.064 ± 0.001	1.033 ± 0.001	1.068 ± 0.001	1.060 ± 0.003
Intracellular carbohydrate content (pg $cell^{-1}$)	18.3 ± 2.4	2.9 ± 0.6	25.2 ± 0.9	10.9 ± 0.5
Intracellular protein content (pg $cell^{-1}$)	7.9 ± 0.3	8.0 ± 1.8	5.3 ± 0.9	4.3 ± 0.6
Relative gas vesicles content	0.34 ± 0.14	0.01 ± 0.00	0.09 ± 0.03	0.01 ± 0.00

focused on floating cells/colonies and their buoyancy. The study of Brookes and Ganf (2001) suggested that nitrogen-limited cells of *Microcystis* were negatively affected both from the dilution in gas vesicle volume and the increase in carbohydrate content, which resulted in a loss of floating cells. Chu et al. (2007) calculated the net buoyancy of *Microcystis* cells, and found the ratio of floating colonies increased during the nitrogen-limited cultivation. Furthermore, they inferred that, in the long term, the sinking and floating of cells were mainly determined by changes in gas vesicle volume, whereas in the short term, they were mainly determined by carbohydrate accumulation and consumption. In this study, we directly assessed the sinking property by measuring the specific density and analyzed the correlation coefficients between the NO_3^--N concentration, the specific density and its regulating factors. The decrease in protein content had a negative effect on the specific density, and, after 10 days, the protein content for the treatment with 0 mg NO_3^--N L^{-1} decreased up to 87.6%, which was probably due to the active and specific degradation of the phycobilisomes, the main nitrogen storage material of cyanobacteria (Hu, 2004). Similarly, in response to nitrogen limited stress, a study of *Nitzschia closterium* found that it degraded nonessential proteins (such as chloroplast proteins) to provide a temporary nitrogen pool (Liu et al., 2012). Gas vesicle in buoyant cyanobacteria varies widely due to its species, even subspecies, and the environmental factors. For instance, the relative gas vesicle content of different strains of *Planktothrix* ranged from 3.2 to 10.5 (Beard et al., 2002), and the relative gas vesicle volume of *Microcystis* displayed a range of 209–2036 in response to light and nutrient (Brookes and Ganf, 2001). In this study, the relative gas vesicle content of *Microcystis* was much lower than *Planktothrix* mentioned above, and decreased significantly with a decrease in the NO_3^--N concentration, from 1.06 to 0.07–0.15; however, there was no significant difference among the treatments ($p > 0.05$). In contrast, the carbohydrate content showed a highly positive correlation with the specific density. Furthermore, a significant increase in specific density and rapid accumulation of carbohydrate content was also observed for the treatments with a lower NO_3^--N concentration (≤1 mg NO_3^--N L^{-1}, $p > 0.05$). Our results suggested that, even after only one day, nitrogen limitation could induce carbohydrate accumulation in *Microcystis* cells, leading to an increase in their specific density in a short period of time, which implied a stronger sinking property of cells under nitrogen-limited conditions.

4.2. The mechanisms of carbohydrate accumulation under nitrogen-limited conditions

In cyanobacteria, nitrogen and carbon metabolisms are functionally bridged and consequently, a nitrogen limitation results in excess carbon (Hasunuma et al., 2013; Yue et al., 2015). The excess carbon is rerouted into carbon storage granules, such as the polyhydroxyalkanoate and glycogen granules (Deschoenmaeker et al., 2016). Glycogen can serve as a carbon and energy source, and sustain the synthesis of protein and other cell components at the same rate as cells growing in darkness (Post et al., 1985; Visser et al., 2005). As nitrogen is an essential constituent of all functional proteins in cyanobacterial cells (Hu, 2004), its deficiency would inhibit the conversion process from carbohydrate into protein, leading to carbohydrate accumulation. Recently, an *in vivo* labeling study provided another underlying mechanism for carbohydrate accumulation under nitrogen limited conditions. In *Arthrospira* cells (non-diazotrophic cyanobacteria), glycogen was biosynthesized with carbon derived from amino acids released from proteins via gluconeogenesis (Hasunuma et al., 2013). Deschoenmaeker et al. (2014) supported this statement by proteomic and cellular analyses and found that the upregulation of carbohydrate synthesis and the downregulation of glycogenolysis were accompanied by the downregulation of photosynthetic activities and inorganic carbon fixation, as well as the degradation of proteins and cyanophycin. In this study, the increase in carbohydrate was also followed by the decrease in protein, and there was a negative correlation between them. Overall, it is conceivable that nitrogen availability might regulate the metabolism and partitioning of carbon because in growing cells, nitrogen metabolism represents a significant sink for carbon (Klein, 1987; Yang et al., 2014).

Studies have shown that the carbohydrate content of cyanobacterial cells can rapidly respond to the environment. The carbohydrate content of a diatom assemblage was 104 ± 6 pg $cell^{-1}$ in low CO_2, increasing by almost 70% in high CO_2 after 48 h incubation (Shaik et al., 2017). More rapidly, after 12 h incubation, the glycogen content of *Arthrospira* cells increased from 18% to 40%, and by 45% of cell dry weight incubated at 270 μmol photons m^{-2} s^{-1} and 700 μmol photons m^{-2} s^{-1}, respectively (Aikawa et al., 2012). In this study, a rapid response of carbohydrate to nitrogen availability was observed. More importantly, the responses during the light cycle and dark cycle were obviously different. After one light/dark cycle, the carbohydrate content of *Microcystis* increased significantly with the decrease in the NO_3^--N concentration but without significant differences in all treatments ($p > 0.05$). However, significant differences between the specific density and carbohydrate content

could only be observed after the dark cycle. Although dark period would lead to carbohydrate reduction in all the treatments, carbohydrate content remained stable or increased significantly in long-term experiment. It seemed that carbohydrate synthesis could supplement its consumption, even promoting carbohydrate accumulation in a low nitrogen availability. Additionally, the protein content maintained an increasing trend in short-term experiment. In spite of the depletion of the exogenous nitrogen, the endogenous nitrogen might be sufficient. In cyanobacterial cells, besides intracellular NO_3^--N, formamides, cyanates and urea were also potential endogenous nitrogen sources (Deschoenmaeker et al., 2014), which could also affect the biosynthesis and metabolism of intracellular components. Therefore, the biosynthesis of proteins was maintained using the endogenous nitrogen, and with such a short time scale, different mechanisms of carbohydrate accumulation could exist. This scenario might be due to the reduction in respiration in darkness, which consumes carbohydrate to provide energy for cell activities. In the darkness, the energy requirements of nitrogen absorption and assimilation constitute a significant portion of respiration (Bloom et al., 1992), and the nitrogen availability has a positive influence on respiration (Zou and Gao, 2013). Increased respiration under high nitrogen conditions might support the increased costs associated with higher maintenance demands (e.g., increased Rubisco contents) and greater absorption and assimilation of exogenous nitrogen, which seems to be unnecessary under low nitrogen condition (Zou et al., 2011). For instance, a significant decrease in the dark respiratory rate of *Ulva conglobata* was observed. In comparison with high-nitrogen-grown algae (total inorganic nitrogen = 2.8 mg N L^{-1}), the dark respiratory rate decreased by 33.3% and 28.1% for the low-nitrogen-grown algae (total inorganic nitrogen <0.14 mg N L^{-1}) under 15 °C and 25 °C, respectively (Zou and Gao, 2014).

4.3. The implication of the sinking property of Microcystis in the water column

Nutrient limitation has been proven to inhibit cyanobacterial proliferation, leading to a decline in biomass (Paerl et al., 2011; Xu et al., 2015). A significant decline of *Microcystis* biomass in late summer or early autumn has also been considered to be related to the depletion of bioavailable nitrogen in water bodies (Michalak et al., 2013). This study found that nitrogen limitation would directly influence the sinking property of *Microcystis*, showing a significant increase in the specific density of the cells. The increase in specific density further accelerates the sinking velocity, thus raising the sinking loss rate. Furthermore, it would influence the dynamics of cyanobacteria in the water column and thus this mechanism should be taken into account while studying the dynamics of cyanobacteria in eutrophic lakes, especially in a nitrogen-limited condition.

Light attenuates exponentially through the water column (Loiselle et al., 2008). The buoyancy regulation theory suggests that in response to lower light availability in deep water, the stored cellular carbohydrate would decrease continuously, the buoyancy of the cell would be restored (Wallace and Hamilton, 1999), and the sinking cells would migrate back to the water surface. However, in the field, an increase in the sinking biomass of cyanobacteria has been observed in late summer and fall (Takamura and Yasuno, 1988; Visser et al., 1995; Cirés et al., 2013). Therefore, there should be other reasons to invalidate the buoyancy regulation. Nitrogen dynamics in eutrophic lakes usually change seasonally (Pina-Ochoa and Alvarez-Cobelas, 2009). In the spring, the available nitrogen in a natural water column was sufficient, and the floating cells complemented the surface biomass, thus maintaining the development of cyanobacterial blooms. In the summer and fall, when temperature and meteorological conditions favor the formation of cyanobacterial blooms, a condition of nitrogen limitation commonly occurs (Michalak et al., 2013; Bullerjahn et al., 2016). This study suggests that when the nitrogen was insufficient, the specific density of the *Microcystis* cells increased to a higher value, and the cells tended to sink, migrating downwards to low-light conditions. Moreover, as shown in this study, the specific density of the *Microcystis* cells that sank to low-light conditions maintained a high value in response to the low available nitrogen, indicating that the cells would sink continually. Eventually, the surface cyanobacterial blooms would disappear due to the continuously sinking of biomass under the nitrogen limitation. This study indicates that a nitrogen limitation can invalidate buoyancy regulation, and complements studies on the disappearance mechanisms of cyanobacterial blooms in the late summer and fall, which should be further investigated in pilot-scale field studies.

5. Conclusion

Our study investigated the sinking property of *Microcystis* in response to nitrogen limitation. A nitrogen limitation could directly influence the sinking property of *Microcystis*, with a significant increase in the specific density of cells. The carbohydrate content showed a highly positive correlation coefficient with the specific density. Additionally, the decrease in intracellular protein content had a negative effect on the specific density, and there was no significant difference in the relative gas vesicle content between the treatments. It has been suggested that under nitrogen-limited conditions, on short time-scales, carbohydrate accumulation is mainly responsible for the increase in specific density. Furthermore, carbohydrate can accumulate rapidly after one light/dark cycle, which was mainly due to the reduction of carbohydrate consumption in darkness under nitrogen limitations. This study explores a new understanding on the disappearance mechanisms of cyanobacterial blooms in the late summer and fall. It posits that carbohydrate accumulation induced by low nitrogen availability causes an increase in specific density, which invalidates buoyancy regulation and leads to cells continually sinking out of the water column. Therefore, while studying the dynamics of cyanobacteria in eutrophic lakes, especially in nitrogen limited conditions, the sinking property should be taken into account.

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