Journal of Hazardous Materials xxx (xxxx) xxxx

Contents lists available at ScienceDirect

Journal of Hazardous Materials

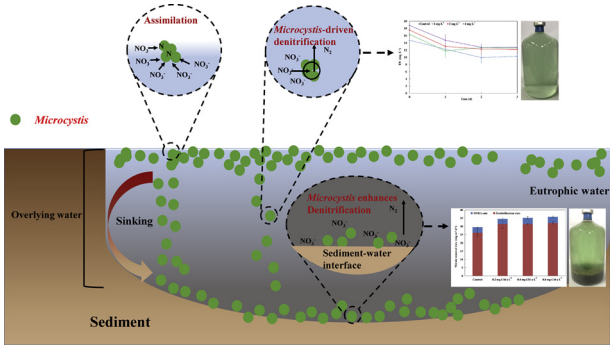
journal homepage: www.elsevier.com/locate/jhazmat

The nitrogen reduction in eutrophic water column driven by *Microcystis* blooms

Yingshi Shen[a], Yingying Huang[a,*], Jun Hu[b], Panpan Li[a], Chen Zhang[a], Lei Li[a], Ping Xu[a], Junyi Zhang[c], Xuechu Chen[a,d,*]

[a] Shanghai Key Lab for Urban Ecological Processes and Eco-Restoration, School of Ecological and Environmental Sciences, East China Normal University, Dong Chuan Road 500, Shanghai, 200241, PR China
[b] Shanghai Qingpu Environmental Monitoring Station, Xi Dayinggangyi Road 15, Shanghai, 201700, PR China
[c] Wuxi Environmental Monitoring Centre, Zhou Xindong Road 123, Wuxi, 214023, PR China
[d] Institute of Eco-Chongming, 3663 N. Zhongshan Road, Shanghai, 200062, PR China

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: R. Debora

Keywords:
Nitrogen reduction
Water column
Denitrification
Microcystis blooms

ABSTRACT

During the bloom seasons, the dissolved inorganic nitrogen declines, which results in the occurrence of nitrogen limitation. It is unclear where the nitrogen goes. Our enclosure experiments and batch tests suggested that *Microcystis* blooms could significantly reduce the nitrogen in water bodies and the key mechanisms for the nitrogen reduction in different layers were different. The assimilation was the main pathway for nitrogen reduction in the surface layer, while denitrification played an important role both at the sediment-water interface and in the overlying water. Stable nitrogen isotope experiments showed that the nitrate reduction efficiency at sediment-water interface was enhanced by *Microcystis*, reaching to 76.5 ~ 84.7 %. Dissimilation accounted for 63.8 ~ 67.3 % of the nitrate reduction, and the denitrification rate was 7.4 ~ 8.5 times of DNRA rate. In the water column, the *Microcystis* bloom facilitated the formation of dark/anoxic condition, which favored the denitrification. The *Microcystis* aggregates collected from the field showed a great potential in removing nitrogen, and the TN in the overly water was reduced by 3.76 ~ 6.03 mg L^{-1} within two days. This study provided field evidences and deeper insights into the relationship between *Microcystis* blooms and nitrogen reduction in the whole water column and gave more details about the enhancing effects of *Microcystis* on nitrogen reduction.

* Corresponding authors at: Shanghai Key Lab for Urban Ecological Processes and Eco-Restoration, School of Ecological and Environmental Sciences, East China Normal University, Dong Chuan Road 500, Shanghai, 200241, PR China.
E-mail addresses: yyhuang@des.ecnu.edu.cn (Y. Huang), xcchen@des.ecnu.edu.cn (X. Chen).

https://doi.org/10.1016/j.jhazmat.2019.121578
Received 19 July 2019; Received in revised form 1 October 2019; Accepted 30 October 2019

1. Introduction

Harmful cyanobacterial blooms occur worldwide due to the excessive external nutrient loading and global climate change (O'Neil et al., 2012; Harke et al., 2016). Cyanobacterial blooms usually exert positive feedback effect on nitrogen cycle (Finlay et al., 2013; Chen et al., 2018a), as when blooms break out, the minimum concentrations of total nitrogen (TN) and dissolved inorganic nitrogen are observed in eutrophic lakes (Paerl et al., 2011). Previous studies suggested that assimilation by cyanobacteria is the major mechanism for the nitrogen reduction (Zhu et al., 2013; Harrison et al., 2009). Recently, more attention was drawn to the interesting link between algae and denitrification. Studies had showed that eutrophic lakes removed over seven times more nitrogen than oligotrophic lakes and were almost three times more efficient in TN removal than unproductive lakes (Finlay et al., 2013); while, the denitrification rate was far higher in the area with severer cyanobacterial blooms (McCarthy et al., 2007). Nevertheless, in eutrophic lakes, the nitrogen reduction would be influenced by the migration of cyanobacteria. During the bloom seasons, the buoyant cyanobacteria float upwards forming dense surface blooms (O'Neil et al., 2012). Meanwhile, cyanobacterial biomass sinks out of water column onto the sediment-water interface (Visser et al., 1995; Diehl, 2002; Pannard et al., 2007), and 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). In surface layer, cyanobacteria proliferates through photosynthesis and assimilate inorganic nitrogen quickly (Cardinale, 2011). At the sediment-water interface, the sinking cyanobacteria might fuel high oxygen consumption through biodegradation or respiration, and algal decomposition produced abundant particulate and dissolved organic carbon (POC and DOC), providing ideal conditions for denitrifiers to convert nitrate to N_2 (Bernhardt, 2013; Chen et al., 2012; McMillan et al., 2010). In addition, some recent studies further indicated that cyanobacteria aggregates might be a hotspot for denitrification (Zhu et al., 2013; Chen et al., 2012). However, the nitrogen reduction process in the whole water column (including overlying water and sediment) with cyanobacterial blooms is still unclear, and rare study has combined the assimilation and denitrification process together to reflect the nitrogen reduction in the whole water column.

To investigate the effects of *Microcystis* blooms on the nitrogen reduction in the whole water column, enclosure experiments were established first in a small pond, and later in Lake Dianshan field site where *Microcystis* bloom occurred. The distribution of nitrogen, dissolved oxygen (DO) and light intensity in the enclosures were measured. Furthermore, batch culture experiments were conducted to evaluate the denitrification rate at the sediment-interface and the enhancing effect of *Microcystis* using stable nitrogen isotope ($Na^{15}NO_3$). The potential of *Microcystis* aggregate on denitrification in the overlying water was also studied under the dark/anoxic condition. The results of this study can provide field evidences for where nitrogen goes during the blooms, and help to gain deeper insights into the relationship between *Microcystis* blooms and nitrogen reduction in the whole water column.

2. Methods and materials

2.1. Enclosure experiment

The experiment was conducted in the July 2017, when *Microcystis* blooms frequently occurred in the northeast of the Lake Dianshan (31°14′ 94″ N, 121°02′ 40″ E). Water with a high *Microcystis* biomass in the surface was collected and immediately delivered to the lab.

Water containing high *Microcystis* biomass was treated with a pressurization device according to our previous study (Huang et al., 2018). When the pressure is under 0.6 MPa for 5 min, all the gas vesicles will be collapsed. After pressurization, a large portion of the biomass sank to the bottom, and the sinking biomass was collected for the enclosure experiment.

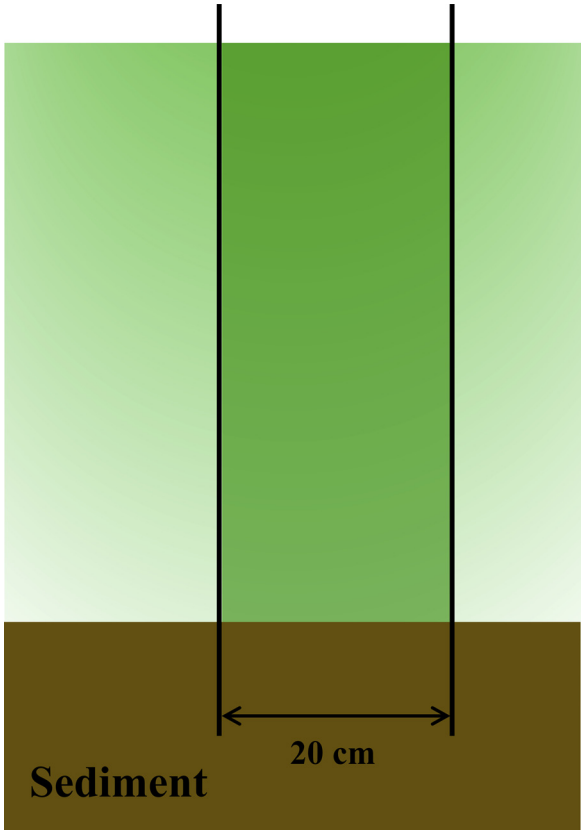


Fig. 1. The schematic diagram of enclosure experiments.

The enclosure experiment was conducted in a small pond of East China Normal University (31°01′37.40″ N, 121°26′51.39″ E). The experimental apparatus was three cylindrical enclosures (20 cm in diameter), which were inserted vertically into the sediment at 50–80 cm. The schematic diagram of enclosure experiments is shown in Fig. 1.

Before the experiment, water in the enclosures was drained, and then the pond water was filtered through the No. 25 (64 μm) plankton net and refilled into the enclosures at approximately 1 m in dept. Nitrate concentrations in the enclosures were modified by $NaNO_3$ at approximately 3 mg N L^{-1}. Untreated *Microcystis* (floating-*Microcystis*) and treated *Microcystis* (sinking-*Microcystis*) was added to two enclosures, respectively, at a concentration of 0.5 mg Chl a L^{-1}. The 3rd enclosure without *Microcystis* addition was used as control. A mixed water sample was taken to measure the initial concentrations of nitrate (NO_3^--N), ammonia (NH_4^+-N), nitrite (NO_2^--N) and dissolved total nitrogen (DTN). One of light meters (HOBO; UA-002-64, Onset, USA) was set to float on the water surface, and the other two meters were set in 60 cm below the water surface and 10 cm above the sediment, respectively, to measure light intensity every 10 min during the experiment.

The experiment lasted for three days. Dissolved oxygen (DO) was measured every day, and then 40 ml of water sample was taken in the surface (10 cm below the water surface), middle (60 cm below the water surface) and bottom (10 cm above the sediment) layers of the water column, respectively. The water was sampled in the same position as the DO measuring. A long soft plastic tube (diameter in 1 cm) was used to sample different layer of water. When sampling the water, one end of the tube extended to a certain depth below the water surface, and water was pumped with a syringe at the other end. The water samples were filtered through a 0.45 μm membrane filter paper, and the filtrates were used to measure NO_3^--N, NO_2^--N, and NH_4^+-N. At the

end of the experiment, a well-mixed sample was also taken to measure DTN.

2.2. The changes of nitrogen at the sediment- water interface

The experiment was conducted in darkened, airtight serum bottles (5 cm in diameter and 9 cm in height) at a temperature of 25 ± 1 °C. The surface sediment samples (0 ~ 10 cm) and lake water were collected from the same place as mentioned above. The gravel and plant and animal residues in sediment samples were removed using tweezers. The sediment samples were mixed and transferred to serum bottles with a sediment layer approximately 2 cm in height. The lake water was filtered with 0.45 μm filter membranes. A unicellular strain of *Microcystis* aeruginosa (cyanobacterium) was used. This strain was provided by the Freshwater Algae Culture Collection of the Institute of Hydrobiology, the Chinese Academy of Sciences. *Microcystis* were incubated optimal growth conditions with an autoclaved BG-11 medium (Rippka et al., 1979) 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} s^{-1}. *Microcystis* cells were collected by centrifugation for 15 min at 3000 × *g*, the culture medium was decanted and the cells were suspended in filtrated lake water. The cells were washed using the process detailed above two more times.

The experiment consisted four treatments with different *Microcystis* biomass, and each treatment had three duplicates. *Microcystis* was added in the three treatments, and the 4th treatment without *Microcystis* addition was used as control. The Chl a concentrations in the three treatments were approximately 0.2 mg L^{-1}, 0.4 mg L^{-1} and 0.8 mg L^{-1}. Isotope $Na^{15}NO_3$ was used to modify the nitrate concentrations at approximately 3 mg N L^{-1}.

All samples were flushed with N_2 for 10 min to remove O_2 (until the DO concentration decreased to approximately 0.2 mg L^{-1}). Under N_2 atmosphere, anoxic samples were injected into each bottle by headspace, slowly to avoid the suspension of sediment. Aluminum caps of silicone seal gasket with PTFE membrane were used in the lid to prevent gas exchange. The experiment lasted three days, and NO_3^--N, NO_2^--N, NH_4^+-N, DTN and ^{15}N-labeled products ($^{29}N_2$, $^{30}N_2$, $^{15}NH_4^+$-N) were measured between the final and initial experimental samples.

2.3. The Changes of nitrogen in the overlying water

The experiment was conducted in darkened, airtight serum bottles (5 cm in diameter and 9 cm in height) at a temperature of 25 ± 1 °C. *Microcystis* and water samples were collected from the same place as mentioned above. The water with high biomass was passed through a 200-mesh sieve and then washed carefully with pure water. The lake water was filtered with 0.45 μm filter membranes.

Microcystis was diluted by the filtered lake water to a Chl a concentration of approximately 1.5 mg Chl a L^{-1}. The experiment consisted four treatments, and each treatment had nine replicated samples. $NaNO_3$ was used to modify the nitrate concentrations at approximately 1 mg N L^{-1}, 2 mg N L^{-1}, and 4 mg N L^{-1}, respectively. The 4th treatment without $NaNO_3$ addition was used as control. All samples were flushed with N_2 for 10 min to remove O_2 (until the DO concentration decreased to approximately 0.2 mg L^{-1}). Under N_2 atmosphere, anoxic samples were injected into each bottle. Aluminum caps of silicone seal gasket with PTFE membrane were used in the lid to prevent gas exchange. The experiment lasted three days. Triplicate bottles of each treatment were chosen at random every day. The concentrations of NO_3^--N, NO_2^--N, NH_4^+-N, TN were measured every day.

2.4. Field experiment

The field experiment was conducted in the same place as sampling on August 2018. The experimental apparatus was nine cylindrical columns (40 cm in diameter), which were inserted vertically into the sediment at 50–80 cm. Before the experiment, water in the enclosures was drained, and then the lake water was filtered through the No. 25 (64 μm) plankton net and refilled into the enclosures at approximately 1.5 m in dept.

The experiment lasted for three days. In each enclosure, $NaNO_3$ was added to a NO_3^--N concentration of approximately 2 mg L^{-1}. There were three treatments with different *Microcystis* biomass, and each treatment had three duplicates. *Microcystis* biomass was added in the two treatments, and the 3rd treatment was without extra *Microcystis* addition. Eventually, the Chl a concentrations in the three treatments were approximately 0.09 mg L^{-1}, 0.18 mg L^{-1} and 0.22 mg L^{-1}.

The experiment lasted three days. When sampling overly water, a plastic tube (160 cm in height and 8 cm in diameter) with a honeycomb structure was used. The tube was situated approximately 5 cm above the sediment. Water in the tube was well mixed for 1–2 min with an airflow rate of 40–50 mL min^{-1}. Sediment would not re-suspend during mixing. A mixed water sample was taken to measure the concentrations of Chl a, NO_3–N, NO_2–N, NH_4^+-N and TN.

2.5. Analyses

NO_3^--N, NO_2^--N, NH_4^+-N and TN was measured according to Standard Methods (APHA, 1995). DTN, DO, light intensity and Chl a was measured by a Total Organic Carbon (TOC) Analyzer (Multi N/C 3100, Analytik Jena, Germany), a Dissolved oxygen meter (HACH LDO101, USA), HoBo (UA-002-64, Onset, USA) and a hand-held AquaPen (AP-C 100, Photon Systems Instruments, Czech Republic), respectively. $^{28}N_2$, $^{29}N_2$, $^{30}N_2$, $^{15}NH_4^+$-N was measured according to the method of Deng et al. (2015) and Yin et al. (2014), analyzed with a membrane inlet mass spectrometer (MIMS). Denitrification rate and dissimilatory nitrate reduction to ammonium (DNRA) rate was calculated according to the following equations:

$$\text{Denitrification rate (mg m}^{-2}\text{ d}^{-1}) = ([^{15}N]_{final} - [^{15}N]_{initial}) \times H / T \quad (1)$$

$$\text{DNRA rate (mg m}^{-2}\text{ d}^{-1}) = ([^{15}NH_4^+\text{-N}]_{final} - [^{15}NH_4^+\text{-N}]_{initial}) \times H / T \quad (2)$$

where $[^{15}N]_{final}$ and $[^{15}N]_{initial}$ (mg L^{-1}) denote the concentrations of ^{15}N (in $^{29}N_2$ and $^{30}N_2$) in the final and initial samples of the experiment, respectively; $[^{15}NH_4^+\text{-N}]_{final}$ and $[^{15}NH_4^+\text{-N}]_{initial}$ (mg L^{-1}) denote the concentrations of $^{15}NH_4^+$-N in the final and initial samples of the experiment, respectively; H (m) denotes the height of the overlying water in the serum bottle; T (d) denotes the experiment duration.

3. Results

3.1. The effects of Microcystis on the nitrogen reduction in different layers of water column

During the experiment, the NO_3^--N in all layers of the three enclosures decreased gradually (Fig.2a–c). In the surface layer of the water column, a sharp decrease of NO_3^--N concentration was observed in the floating-*Microcystis* addition enclosure, from 2.94 mg L^{-1} to 0.11 mg L^{-1}, with a reduction efficiency of 96.2 %. This reduction efficiency was much higher than that value in the surface layer of sinking-*Microcystis* addition enclosure (67.3 %) and the control enclosure (21.3 %). In the middle layer of the enclosures, NO_3^--N concentrations showed similar trends as in the surface layer. On the contrary, in the bottom layer of the enclosures, a highest NO_3^--N reduction efficiency was observed in the sinking-*Microcystis* addition enclosure, which reached 86.8 % on the 1st day and increased to 90.0 % on the 3rd day. It should be noted that the floating-*Microcystis* addition enclosure showed a similar decreasing trend with the control enclosure within the first two days, and on the 3rd day, the decrease of NO_3^--N concentration in its bottom was much faster than the control.

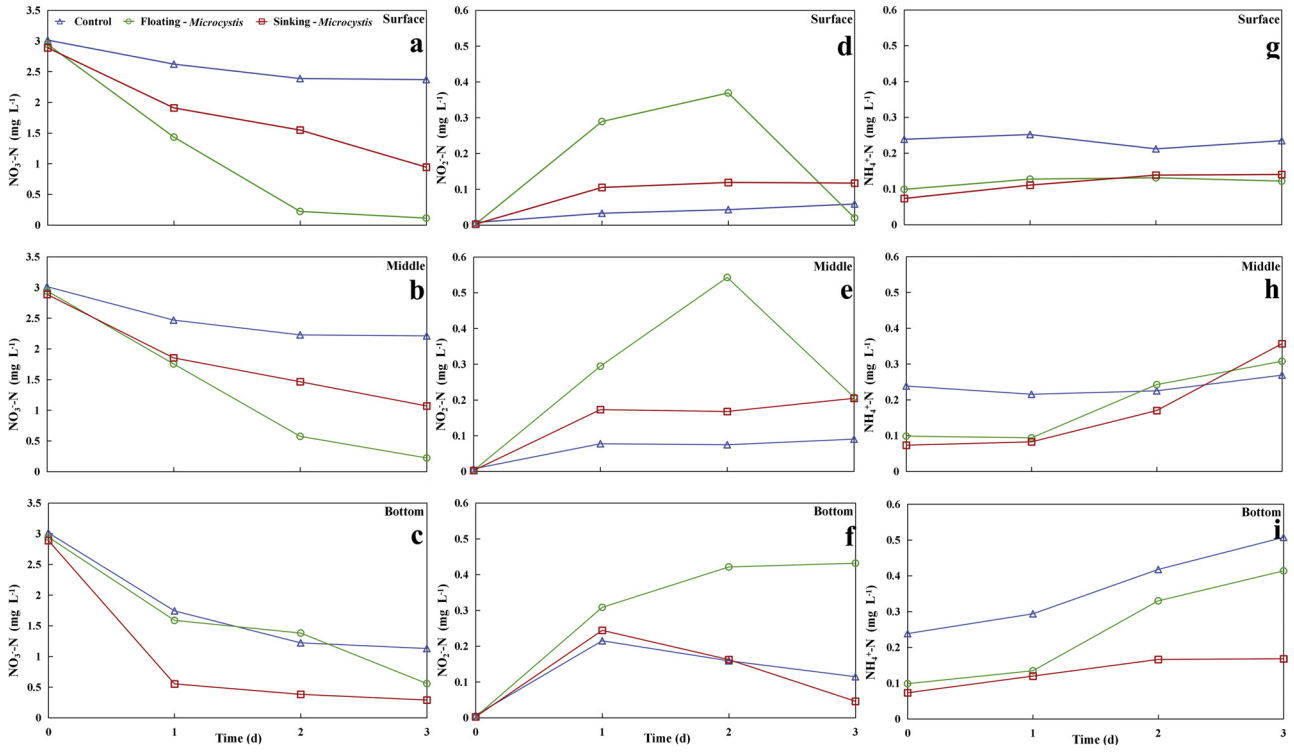


Fig. 2. The changes of nitrogen in different layers of water column.

During the experiment, NO_2^--N accumulation was found in all enclosures (Fig. 2d–f), especially in the floating-*Microcystis* addition enclosure in which the highest value of 0.54 mg L^{-1} was observed in the middle layer on the 2nd day. In the floating-*Microcystis* addition enclosure, the NO_2^--N concentration decreased after two days in the surface and middle layer but remained high level in the bottom layer. Comparatively, in the control enclosure and the sinking-*Microcystis* addition enclosure, the NO_2^--N concentration decreased after one day in the bottom layer but remained still in the surface and middle layer.

NH_4^+-N concentration kept stable in the surface layer and increased gradually in the bottom layer of all enclosures (Fig. 2g and i). In the middle layer, NH_4^+-N concentration kept stable in the control enclosure and increased gradually in the two enclosures with *Microcystis* (Fig. 2h).

As shown in Table 1, the two enclosures with *Microcystis* addition showed higher DTN reduction efficiency. A sharply decrease of DTN was observed in the surface layer of the floating-*Microcystis* addition enclosure, with a reduction efficiency of 91.6 %. This value was much higher than that in the other enclosures. In the middle layer of the enclosures, the floating-*Microcystis* addition enclosure also showed the highest DTN reduction efficiency. Comparatively, in the bottom layer of the enclosure, the highest DTN reduction efficiency was observed in the sinking-*Microcystis* addition enclosure. These results suggested that *Microcystis* enhanced the DTN reduction in the water column.

As shown in Fig. 3, light intensity in the surface and middle layer of the water column showed obvious diurnal variation. The light intensity of the control enclosure and sinking-*Microcystis* addition enclosure showed the similar changes; while the light intensity of the floating-*Microcystis* addition enclosure was lower than that of the other two enclosures, suggesting that the *Microcystis* accumulated on the water surface has a light-shading effect. The bottom layer of the three enclosures was almost dark during the whole day.

During the experiment, the DO in all layers of the floating-*Microcystis* addition enclosure decreased rapidly below 0.4 mg L^{-1} after one day, and afterwards maintained a low level (Fig. 4). The DO in the bottom layer of the sinking-*Microcystis* addition enclosure and control enclosure showed the similar trends as the floating-*Microcystis* addition enclosure. However, in the surface layer of the two enclosures, the DO showed an increasing trend. In the middle layer, the DO in the sinking-*Microcystis* addition enclosure fluctuated; while the DO in the control enclosure showed little changes during the experiment.

Table 1
Nitrogen reduction efficiency in different layers of the water enclosures.

		DTN (mg L^{-1})	DTN reduction efficiency %
Control	Initial	3.26	–
	Surface	2.66	18.28
	Middle	2.57	21.15
	Bottom	1.75	46.30
Sinking-*Microcystis*	Initial	2.96	–
	Surface	1.20	59.46
	Middle	1.63	44.96
	Bottom	0.50	83.00
Floating-*Microcystis*	Initial	3.05	–
	Surface	0.25	91.63
	Middle	0.74	75.78
	Bottom	1.40	53.92

3.2. The effects of Microcystis on nitrate reduction at the sediment-water interface

During the experiment, the Chl a concentrations ranged from 0.2 ~ 0.3 mg L^{-1}, 0.4 ~ 0.5 mg L^{-1} and 0.8 ~ 0.9 mg L^{-1} for the treatments with 0.2 mg Chl a L^{-1}, 0.4 mg Chl a L^{-1} and 0.8 mg Chl a L^{-1}, respectively. Table 2 shows the changes of nitrogen at sediment-water interface. After three days, the NO_3–N reduction efficiency reached 76.5 %, 77.8 %, 84.7 % for the treatments with 0.2 mg Chl a L^{-1}, 0.4 mg Chl a L^{-1}, 0.8 mg Chl a L^{-1}, respectively; while this value was 73.5 % for the control. A slight NO_2–N accumulation was observed for the treatments with 0.4 mg Chl a L^{-1} and 0.8 mg Chl a L^{-1}. Meanwhile, NH_4^+-N in the treatments increased by 1.31 ~ 1.37 mg L^{-1}, and the increasing amount for the control was a little lower at 1.29 mg L^{-1}.

$^{29}N_2$、$^{30}N_2$ and $^{15}NH_4^+$-N was detected for all the treatments and

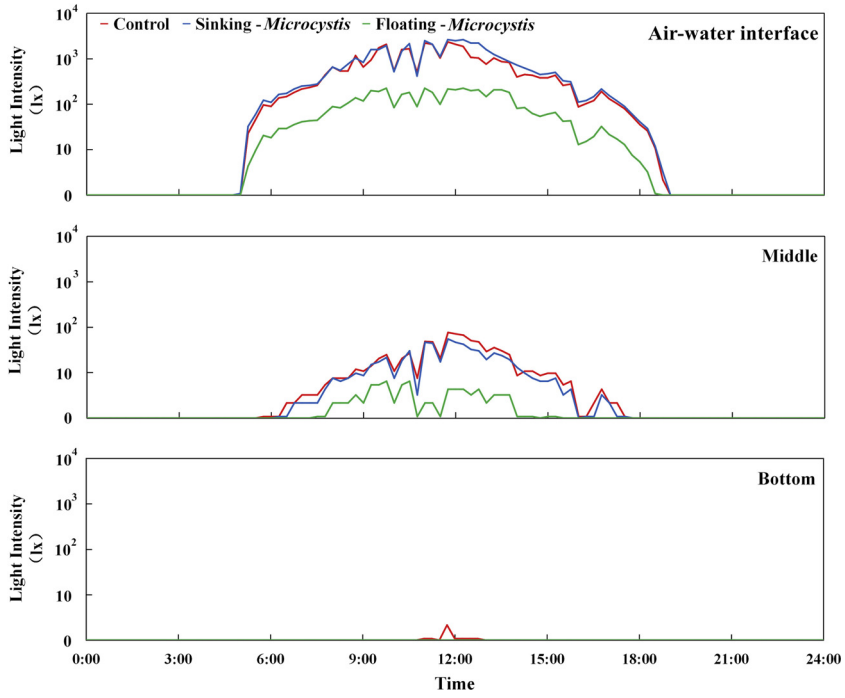


Fig. 3. The diurnal variation of light intensity in the different layers of water column.

control, and the denitrification rate and DNRA rate was calculated accordingly (Fig. 5). Total ^{15}N ($^{29}N_2$、 $^{30}N_2$ and $^{15}NH_4^+$-N) accounted for 59.3 % of the nitrate reduction for the control, and this value reached 63.8 %～67.3 % for the treatments, suggesting that nitrate reduction at the sediment-water interface was dominated by dissimilatory reduction but not assimilatory reduction. The products of denitrification ($^{29}N_2$,$^{30}N_2$) showed a proportion of NO_3^--N dissimilatory reduction at 88.2 % for the control and this value increased slightly to 89.3 %～91.0 % for the treatments, and thus denitrification was considered as the main NO_3^--N reduction process at the sediment-water interface. The denitrification rate significantly elevated with *Microcystis* addition ($p < 0.05$). The highest value was observed for the treatment with 0.8 mg Chl a L^{-1}, which increased by 22.7 % compared to the control. However, there was no significant difference of the DNRA rate between the treatments and control ($p > 0.05$). These results suggested that *Microcystis* enhanced the NO_3^--N reduction process at the sediment-water interface by promoting the denitrification effect but not DNRA effect.

3.3. The effects of Microcystis on nitrate reduction in the overlying water

During the experiment, the Chl a concentration ranged from 1.4～1.6 mg L^{-1}. The results shows the similar trends for the three treatments with different NO_3–N addition (Fig. 6). A sharply decrease of NO_3–N was observed within one day or two days, and maintained a low level less than 0.3 mg L^{-1}. Meanwhile, NO_2–N accumulation was found for the three treatments with NO_3–N addition, and this phenomenon soon disappeared in the second day. The highest NO_2^--N concentration of 2.50 mg L^{-1} occurred for the treatment with 4 mg N L^{-1}. The NH_4^+-N concentration increased in the control and the treatments with NO_3–N addition. Different from NO_3^--N, TN decreased quickly within two days but then maintained a high level greater than 10 mg L^{-1}. After two days, TN was reduced by 4.43 mg L^{-1}, 3.76 mg L^{-1}, 5.33 mg L^{-1} and 6.03 mg L^{-1} for the control and the treatments with NO_3^--N addiction, respectively. These results indicated that the occurrence of *Microcystis* affected the denitrification effect in the overlying water. Additionally, the total amount of TN reduction was much greater than that of NO_3–N reduction, indicating that the nitrogen might partly originated from *Microcystis* cells (e.g. intracellular nitrate and ammonia, microcystin, phycocyanin) (Klotz et al., 2016; Gorl et al., 1998; Forchhammer and Schwarz, 2019).

3.4. The effects of Microcystis blooms on the nitrogen reduction in the field experiment

After three days, NO_3^--N and TN concentrations decreased in the three treatments, and nitrate and TN reduction efficiencies increased with the increasing *Microcystis* biomass in the water column, showing the highest values at 70.5 % and 40.3 %, respectively (Fig. 7a and b). In addition, in the high-biomass enclosure, nitrate and TN reduction rates reached 0.65 g N m^{-2} d^{-1} and 1.08 g N m^{-2} d^{-1}, respectively. *Microcystis* biomass increased in the low- and middle-biomass enclosure, with a specific growth rate at 0.27 d^{-1} and 0.07 d^{-1}, respectively (Fig. 7c). By contrary, *Microcystis* biomass in the high-biomass enclosure remained with no significant change. These results suggested that nitrogen was reduced not only through assimilation of *Microcystis* but also denitrification, especially when the biomass was high.

4. Discussion

4.1. The overall nitrogen reduction affected by Microcystis blooms

Nitrogen dynamics in eutrophic lakes usually change seasonally (Pina-Ochoa and Alvarez-Cobelas, 2009), and the nitrogen limitation commonly occurs in the summer and fall (Bullerjahn et al., 2016; Michalak et al., 2013). Until now, it has been unclear where the nitrogen goes. When surface bloom occurs in the water column, cyanobacteria can assimilate nitrogen efficiently and quickly; however, its short turnover time indicates that it is only a transient storage pool and has a short-term effect on nitrogen reduction (Baulch et al., 2011). On the other side, studies suggest that denitrification takes place at the sediment-water interface, and it is considered to be one of the main mechanisms for the nitrogen reduction in lakes (Xu et al., 2015). Accordingly, there should be different pathways for nitrogen reduction in the whole water column, and the assimilation and denitrification process should be combined together to investigate the relationship

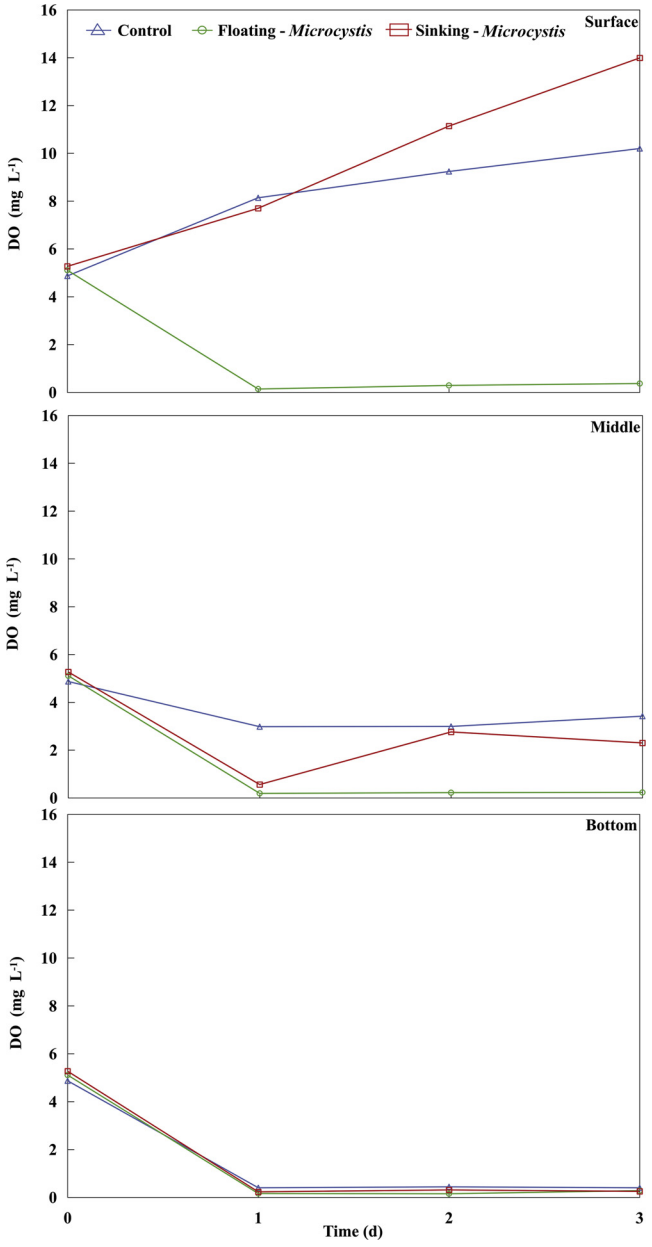


Fig. 4. The changes of DO in the different layers of water column.

between cyanobacterial bloom and nitrogen reduction.

This study clearly showed the *Microcystis* blooms could significantly reduce the nitrogen in water bodies, and illustrated the pathway that how the nitrogen goes with the blooms. Field experiment revealed the impacts of *Microcystis* on the nitrogen reduction in the water column. The high-biomass enclosure showed the highest TN removal rate at 1.08 g N $m^{-2}d^{-1}$, and this value was only 0.72 g N $m^{-2}d^{-1}$ in the enclosure without extra *Microcystis* addition. Moreover, the enclosure experiment indicated that the main mechanisms for the nitrate reduction in different layers were not the same. In control enclosure, low nitrate reduction efficiency was observed at approximate 20 % in the surface and middle layers, and increased to 62.6 % in the bottom due to the denitrification at sediment-water interface. Meanwhile, *Microcystis* showed an enhancing effect on the nitrate reduction. It was showed that the highest nitrate reduction efficiency in the surface was 96.2 %, in the middle was 92.4 % (the floating-*Microcystis* addition enclosure) and in the bottom was 90.0 % (the sinking-*Microcystis* addition enclosure). Assimilation should be the main nitrate reduction pathway in the surface, since biomass mostly accumulated in this layer. However, assimilation only temporarily stores nitrogen in algal tissues (Cardinale, 2011). In aquatic environment, nitrogen can be removed permanently by denitrification (Bruesewitz et al., 2011; Burgin and Hamilton, 2007; Reinhardt et al., 2006). The nitrite accumulation was observed in all different layers of enclosures, suggesting that nitrate dissimilation reduction might occur both at sediment-water interface and in the overlying water, and denitrification might be one of the main pathways. For the denitrification process in the water, the anoxic condition, denitrifiers and the bioavailable carbon source are the three key controlling factors. Based on the previous studies and the results of this research, it could be inferred that the ideal conditions for denitrification could form both at sediment-water interface and in the overlying water during the blooms, and thus favored the nitrogen reduction.

4.2. Denitrification at sediment-water interface

In aquatic environment, denitrification is considered to occur mostly at the sediment-water interface, where denitrifying bacteria are plentiful, DO is consumed by sediment respiration, and organic carbons are sufficient (Sweerts et al., 1991; Jensen et al., 1993; Wilson et al., 2013). During the blooms, there would be a great amount of cyanobacterial biomass sinking onto the sediment-water interface (Visser et al., 1995; Diehl, 2002; Pannard et al., 2007), and the biodegradation of dead cyanobacteria and the respiration of living cyanobacteria consume the DO in bottom water. Under such condition, the oxygen consumption rate of sediment along with cyanobacteria should be much higher than cyanobacterial photosynthetic oxygen evolution rate, and led to an anaerobic condition at the sediment-water interface (Rinke et al., 2010). Meanwhile, the organic substrates originated from the degradation of sinking cyanobacteria could also directly enhance denitrification rates (Chen et al., 2012; McMillan et al., 2010). Furtherly, our previous studies demonstrated that not only dead cells, living *Microcystis* cells could also enhance nitrate reduction at the sediment-water interface through secreting biodegradable hydrophilic DOC as carbon source to denitrifiers (Chen et al., 2018a). However, these results were obtained in lab or under a manipulated dark/anoxic conditions, and it lacks the whole water column and field evidence.

This study provided a deeper insight into the nitrate reduction process. The results of enclosure experiments suggested that sinking-*Microcystis* enhanced the nitrate reduction in the bottom layer, since in

Table 2
The changes of nitrogen at sediment-water interface. Data were presented as mean ± SD.

		3 d			
	Initial	Control	0.2 mg Chl a L^{-1}	0.4 mg Chl a L^{-1}	0.8 mg Chl a L^{-1}
NO_3^--N (mg L^{-1})	2.88 ± 0.06	0.77 ± 0.03	0.68 ± 0.02	0.64 ± 0.08	0.44 ± 0.07
NO_2^--N (mg L^{-1})	0.05 ± 0.02	0.01 ± 0.00	0.01 ± 0.01	0.03 ± 0.01	0.06 ± 0.01
NH_4^+-N (mg L^{-1})	0.25 ± 0.14	1.48 ± 0.07	1.49 ± 0.07	1.55 ± 0.07	1.56 ± 0.04
DTN (mg L^{-1})	3.12 ± 0.18	1.97 ± 0.05	1.90 ± 0.07	1.94 ± 0.08	1.79 ± 0.07
^{15}N ($^{29}N_2$,$^{30}N_2$) (mg L^{-1})	–	1.11 ± 0.11	1.33 ± 0.11	1.33 ± 0.01	1.36 ± 0.03
$^{15}NH_4^+$-N (mg L^{-1})	–	0.15 ± 0.00	0.13 ± 0.02	0.16 ± 0.04	0.16 ± 0.02

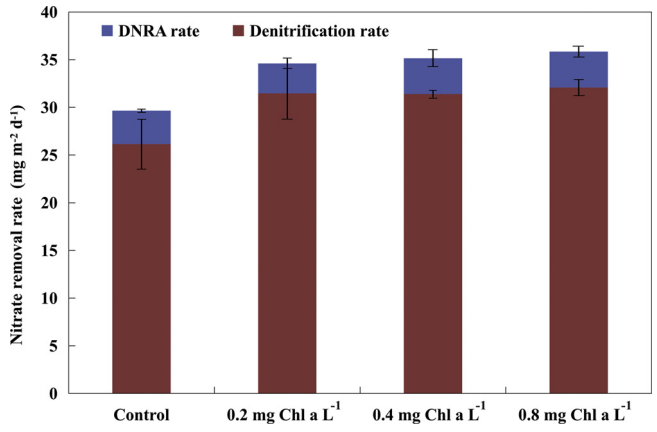


Fig. 5. The denitrification rate and DNRA rate at the sediment-water interface. Data represent the mean (n = 3) with associated error bars (SD).

sinking-*Microcystis* addition enclosure, the nitrate reduction efficiency reached 80.9 % in the bottom layer within only one day, while in the bottom of the control, the value was only 42.2 %. The changes of nitrate concentration in the bottom of the floating-*Microcystis* addition enclosure furtherly supported this idea. The nitrate concentration decreased similarly as the control enclosure during the first two days, and then a rapider decrease was observed. The nitrate reduction efficiency increased from 53.1 % on the 2nd day to 81.0 % on the 3rd day.

There are various pathways for nitrate reduction (Otte et al., 1999; Preisler et al., 2007), and denitrification should be distinguished from other nitrate reduction pathway, such as assimilation and DNRA. In this study, isotope experiment gave more details about the nitrate reduction process at the sediment-water interface. Total ^{15}N ($^{29}N_2$、$^{30}N_2$ and $^{15}NH_4^+$-N) accounted for 59.3 % of the nitrate reduction for the control, and this value reached 63.8 %～67.3 % for the treatments (Table 2), suggesting that nitrate was reduced mainly through dissimilatory reduction but not assimilatory reduction. Denitrification rate was 7.4–8.5 times of DNRA rate (Fig. 5), suggesting that denitrification was the main pathway of dissimilatory reduction. Such results indicated that *Microcystis* could enhance denitrification effect and help permanent nitrate reduction from water column.

4.3. Denitrification in the overlying water

To date, some researches paid attention to the denitrification effect in the presence of anoxic zone in water column (Wenk et al., 2014; Peng et al., 2017). However, there is a lack of understanding on the denitrification effect in the overlying water, especially during cyanobacterial blooms. In this study, the enclosure experiment showed the efficient nitrate reduction, together with a highest nitrite accumulation in the middle of the floating-*Microcystis* addition enclosure (Fig. 1). This result indicated that the denitrification could occur in the overlying water. Furthermore, in the batch tests, a sharp decrease of nitrate and TN was observed with *Microcystis* aggregates collected from a natural lake (Fig. 4). Nitrogen could only be removed from such a closed system through converting to N_2, which indicated that there was denitrification driven by *Microcystis* occurring in the overlying water.

For the denitrification process in the overlying water, the anoxic condition, denitrifiers and the bioavailable carbon source should be three main factors that regulate the whole process. The anoxic condition can occur during the blooms, since the respiration of cyanobacteria can accelerate the oxygen consumption (Chen et al., 2012; Bouffard et al., 2013). Light attenuates exponentially through the water column (Loiselle et al., 2008), and light shading caused by the dense surface blooms aggravated the light attenuation (Rinke et al., 2010). Therefore, even in the overlying water, the low light condition or dark condition can form. Under such condition, the respiratory oxygen consumption rate of cyanobacteria will be much higher than its photosynthetic oxygen evolution rate. As shown in the Figs. 3 and 4, there was a dark/anoxic area in the water column, which providing an ideal condition for denitrification.

In natural lakes, cyanobacteria, especially *Microcystis*, usually aggregated to form colonies, and numerous and varieties of heterotrophic

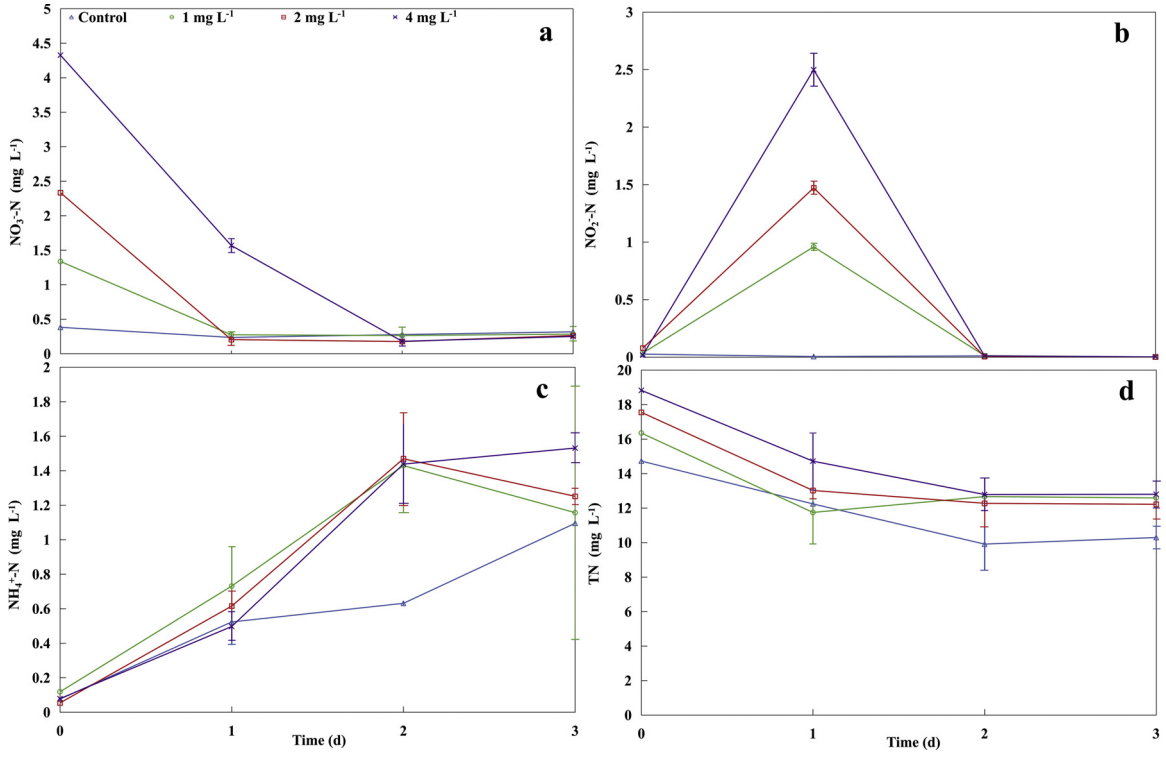


Fig. 6. The changes of nitrogen driven by *Microcystis* under anoxic/dark conditions Data represent the mean (n = 3) with associated error bars (SD).

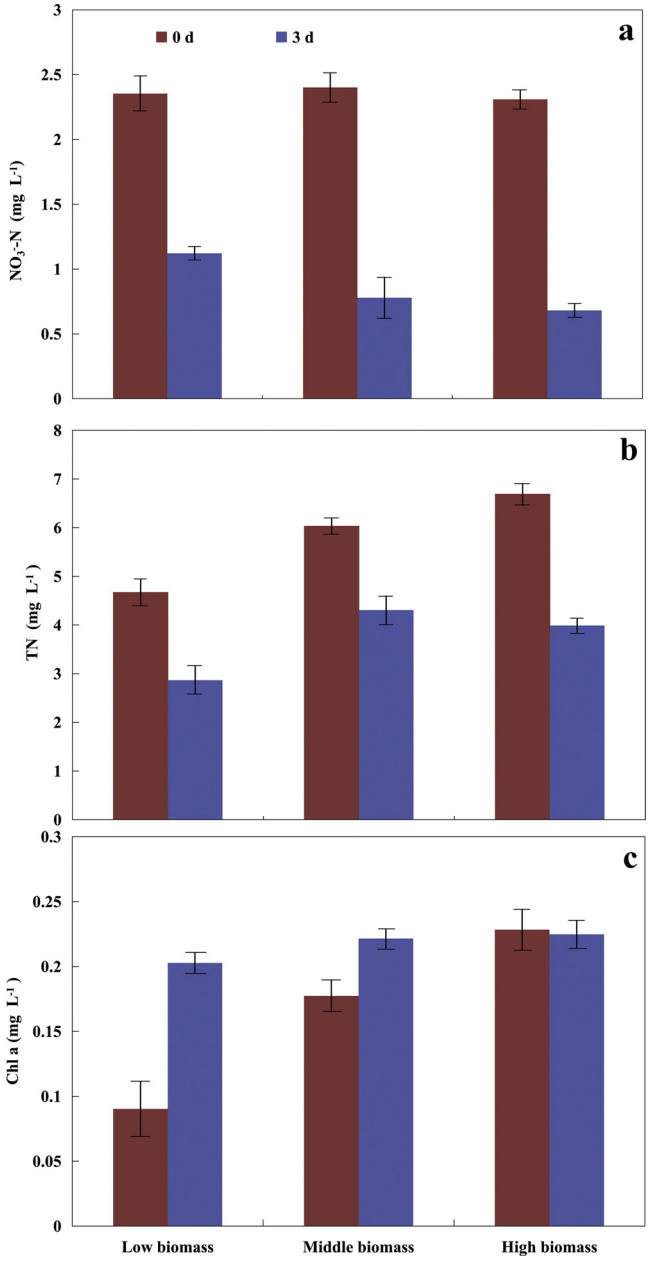


Fig. 7. The changes of NO_3^--N, TN and *Microcystis* biomass in water column. Data represent the mean (n = 3) with associated error bars (SD).

bacteria were found to be associated with cyanobacteria (Li et al., 2011; Cai et al., 2014; Tang et al., 2017). Recent studies have found denitrifiers and denitrifying genes in cyanobacteria aggregates. Cai et al. (2017) isolated a bacterial strain from cyanobacterial aggregates in Taihu Lake (China), which were found to be positive for nitrate reduction and denitrification. Metatranscriptome analysis showed that the denitrifying genes (nirK, nirS, norB, norZ, napA, et al.) were expressed in the *Microcystis* aggregates (Chen et al., 2018b). The results of this study showed that when the *Microcystis* aggregates incubated under ideal condition, a high nitrogen reduction efficiency was observed. Accordingly, it could be assumed that when the *Microcystis* aggregates stayed in the dark/anoxic area of water column for a certain period, its associated bacteria could act as a denitrifiers to reduce nitrogen quickly. However, further studies should be conducted to investigate the community structure of *Microcystis* aggregates, as well as the functional structure and the gene transcriptional activity.

Besides, cyanobacteria could release an abundance of organic matters, which can be used by bacteria as carbon and energy source (Xu et al., 2014; Gonçalves et al., 2017; Xiao et al., 2018). The similar results were also obtained in our previous study (Chen et al., 2018a).

For the above reasons, *Microcystis*-driven denitrification is likely to happen in the water column, and it should be taken into account when studying the nitrogen cycle during the blooms. Further studies should focus on the combined mechanisms and the nitrogen reduction flux driven by cyanobacteria in the whole water column.

4.4. The changes of ammonia reduction in the water column

The experiments of this study and our previous study showed an extra ammonia produced by *Microcystis* at the sediment-water interface with anoxic condition (Chen et al., 2018a). However, in our enclosure experiment, *Microcystis* didn't lead to the ammonia accumulation. This phenomenon was also observed in field experiment with low *Microcystis* biomass. In a natural water column, ammonia is directly released from the sediment and *Microcystis* or produced through DNRA pathway. Thereafter, it diffuses to the up layer and would be assimilated by *Microcystis*, or oxidized to nitrate through nitrification at aerobic layer and then further removed through denitrification (Dalsgaard et al., 2005; Chen et al., 2016). Besides denitrification, the coupled nitrification-denitrification process in water column might also influence by *Microcystis* blooms. Further studies combining with nitrogen stable isotope tracer method were needed to distinguish the ammonia reduction pathway and illustrate their linkage with cyanobacteria.

5. Conclusion

This study investigated the nitrogen reduction in the whole water column and its response to *Microcystis* bloom. Although assimilation was the main pathway of nitrogen reduction in the surface layer of the water column, denitrification played an important role in the nitrogen reduction both at the sediment-water interface and in the overlying water. At the sediment-water interface, nitrate was reduced mainly through dissimilatory reduction, especially denitrification; while *Microcystis* could significantly enhance denitrification effect. In the overlying water, the *Microcystis* bloom could help to form a dark/anoxic condition, and thus the *Microcystis* aggregates would directly affect the nitrate reduction. The field experiments verified the enhancing effects of *Microcystis* bloom on the nitrogen reduction, and the batch tests further proved that the *Microcystis* aggregates had a great potential in removing nitrate. This study illustrated the mechanisms for the nitrogen reduction in the whole water column, and provided field evidences and deeper insights into the relationship between nitrogen cycle and cyanobacterial blooms. Further study should focus on quantifying the partition of nitrogen removal between the overlying water and the sediment-water interface during cyanobacterial blooms, especially in the long-term scale.

Acknowledgments

The present work was supported by the National Natural Science Foundation of China under Grant No. 41601532 and No. 91951104, the Scientific Research Plan Project of Science and Technology Commission of Shanghai Municipality under Grant No. 18295810400, and the Foundation of Shanghai Key Lab for Urban Ecological Processes and Eco-Restoration under Grant No. SHUES2017A05 and SHUES2019A12. We thank Technology Innovation Center for Land Spatial Eco-restoration in Metropolitan area, Ministry of Natural Resource for supporting this study. We also thank Sunset Marina Resort for providing experimental site.

References

O'Neil, J.M., Davis, T.W., Burford, M.A., Gobler, C.J., 2012. The rise of harmful

cyanobacteria blooms: the potential roles of eutrophication and climate change. Harmful Algae 14, 313–334.
Harke, M.J., Steffen, M.M., Gobler, C.J., Otten, T.G., Wilhelm, S.W., Wood, S.A., Paerl, H.W., 2016. A review of the global ecology, genomics, and biogeography of the toxic cyanobacterium, Microcystis spp. Harmful Algae 54, 4–20.
Finlay, J.C., Small, G.E., Sterner, R.W., 2013. Human influences on nitrogen removal in lakes. Science 342, 247–250.
Chen, X., Huang, Y., Chen, G., Li, P., Shen, Y., Davis, T.W., 2018a. The secretion of organics by living Microcystis under the dark/anoxic condition and its enhancing effect on nitrate removal. Chemosphere 196, 280–287.
Paerl, H.W., Xu, H., McCarthy, M.J., Zhu, G., Qin, B., Li, Y., Gardner, W.S., 2011. Controlling harmful cyanobacterial blooms in a hyper-eutrophic lake (Lake Taihu, China): The need for a dual nutrient (N & P) management strategy. Water Res. 45, 1973–1983.
Zhu, M., Zhu, G., Zhao, L., Yao, X., Zhang, Y., Gao, G., Qin, B., 2013. Influence of algal bloom degradation on nutrient release at the sediment-water interface in Lake Taihu, China. Environ. Sci. Pollut. Res. - Int. 20, 1803–1811.
Harrison, J.A., Maranger, R.J., Alexander, R.B., Giblin, A.E., Jacinthe, P.A., Mayorga, E., Seitzinger, S.P., Sobota, D.J., Wollheim, W.M., 2009. The regional and global significance of nitrogen removal in lakes and reservoirs. Biogeochemistry 93, 143–157.
McCarthy, M.J., Lavrentyev, P.J., Yang, L., Zhang, L., Chen, Y., Qin, B., Gardner, W.S., 2007. Nitrogen dynamics and microbial food web structure during a summer cyanobacterial bloom in a subtropical, shallow, well-mixed, eutrophic lake (Lake Taihu, China). Hydrobiologia 581, 195–207.
Visser, P.M., Ibelings, B.W., Mur, L.R., 1995. Autumnal sedimentation of Microcystis spp. As result of an increase in carbohydrate ballast at reduced temperature. J. Plankton Res. 17, 919–933.
Diehl, S., 2002. Phytoplankton, light, and nutrients in a gradient of mixing depths: theory. Ecology 83, 386–398.
Pannard, A., Bormans, M., Lagadeuc, Y., 2007. Short-term variability in physical forcing in temperate reservoirs: effects on phytoplankton dynamics and sedimentary fluxes. Freshw. Rev. 52, 12–27.
Cirés, S., Wörmer, L., Carrasco, D., Quesada, A., 2013. Sedimentation patterns of toxin-producing Microcystis morphospecies in freshwater reservoirs. Toxins 5, 939–957.
Cardinale, B.J., 2011. Biodiversity improves water quality through niche partitioning. Nature 472, 86–89.
Bernhardt, E.S., 2013. Cleaner lakes are dirtier lakes. Science 342, 205–206.
Chen, X., Yang, L., Xiao, L., Miao, A., Xi, B., 2012. Nitrogen removal by denitrification during cyanobacterial bloom in Lake Taihu. J. Freshw. Ecol. 27, 243–258.
McMillan, S.K., Piehler, M.F., Thompson, S.P., Paerl, H.W., 2010. Denitrification of nitrogen released from senescing algal biomass in coastal agricultural headwater streams. J. Environ. Qual. 39, 274–281.
Huang, Y., Chen, X., Li, P., Chen, G., Peng, L., Pan, L., 2018. Pressurized Microcystis can help to remove nitrate from eutrophic water. Bioresour. Technol. 248, 140–145.
Rippka, R., Deruelles, J., Waterbury, J.B., 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J. Gen. Microbiol. 111, 1–61.
Deng, F., Hou, L., Liu, M., Zheng, Y., Yin, G., Li, X., Lin, X., Chen, F., Gao, J., Jiang, X., 2015. Dissimilatory nitrate reduction processes and associated contribution to nitrogen removal in sediments of the Yangtze Estuary. J. Geophys. Res. Biogeosci. 120, 1521–1531.
Yin, G., Hou, L., Liu, M., Liu, Z., Gardner, W.S., 2014. A novel membrane inlet mass spectrometer method to measure 15NH4 + for isotope-enrichment experiments in aquatic ecosystems. Environ. Sci. Technol. 48, 9555–9562.
Klotz, A., Georg, J., Bucinska, L., Watanabe, S., Reimann, V., Januszewski, W., Sobotka, R., Jendrossek, D., Hess, W.R., Forchhammer, K., 2016. Awakening of a dormant cyanobacterium from nitrogen chlorosis reveals a genetically determined program. Curr. Biol. 26, 2862–2872.
Gorl, M., Sauer, J., Baier, T., Forchhammer, K., 1998. Nitrogen-starvation-induced chlorosis in Synechococcus PCC 7942: adaptation to long-term survival. MicrobiologySgm 144, 2449–2458.
Forchhammer, K., Schwarz, R., 2019. Nitrogen chlorosis in unicellular cyanobacteria - a developmental program for surviving nitrogen deprivation. Environ. Microbiol. 21, 1173–1184.
Pina-Ochoa, E., Alvarez-Cobelas, M., 2009. Seasonal nitrogen dynamics in a seepage lake receiving high nitrogen loads. Mar. Freshwater Res. 60, 335–445.
Bullerjahn, G.S., McKay, R.M., Davis, T.W., Baker, D.B., Boyer, G.L., D'Anglada, L.V., Doucette, G.J., Ho, J.C., Irwin, E.G., Kling, C.L., Kudela, R.M., Kurmayer, R., Michalak, A.M., Ortiz, J.D., Otten, T.G., Paerl, H.W., Qin, B., Sohngen, B.L., Stumpf, R.P., Visser, P.M., Wilhelm, S.W., 2016. Global solutions to regional problems: Collecting global expertise to address the problem of harmful cyanobacterial blooms. A Lake Erie case study. Harmful Algae 54, 223–238.
Michalak, A.M., Anderson, E.J., Beletsky, D., Boland, S., Bosch, N.S., Bridgeman, T.B., Chaffin, J.D., Cho, K., Confesor, R., Daloğlu, I., 2013. Record-setting algal bloom in Lake Erie caused by agricultural and meteorological trends consistent with expected future conditions. Proc. Natl. Acad. Sci. U. S. A. 110, 6448–6452.
Baulch, H.M., Stanley, E.H., Bernhardt, E.S., 2011. Can algal uptake stop NO3- pollution? Nature 477, E3.
Xu, H., Paerl, H.W., Qin, B., Zhu, G., Hall, N.S., Wu, Y., 2015. Determining critical nutrient thresholds needed to control harmful cyanobacterial blooms in eutrophic Lake Taihu, China. Environ. Sci. Tech. 49, 1051–1059.
Bruesewitz, D.A., Hamilton, D.P., Schipper, L.A., 2011. Denitrification potential in lake sediment increases across a gradient of catchment agriculture. Ecosystems 14, 341–352.
Burgin, A.J., Hamilton, S.K., 2007. Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways. Front. Ecol. Environ. 5, 89–96.
Reinhardt, M., Müller, B., Gächter, R., Wehrli, B., 2006. Nitrogen removal in a small constructed wetland: an isotope mass balance approach. Environ. Sci. Tech. 40, 3313–3319.
Sweerts, J.-P.R.A., Bär-Gilissen, M.-J., Cornelese, A.A., Cappenberg, T.E., 1991. Oxygen-consuming processes at the profundal and littoral sediment-water interface of a small meso-eutrophic lake (Lake Vechten, The Netherlands). Limnol. Oceanogr. 36, 1124–1133.
Jensen, K., Revsbech, N.P., Nielsen, L.P., 1993. Microscale distribution of nitrification activity in sediment determined with a shielded microsensor for nitrate. Appl. Environ. Microbiol. 59, 3287–3296.
Wilson, R.F., Fennel, K., Paul Mattern, J., 2013. Simulating sediment–water exchange of nutrients and oxygen: a comparative assessment of models against mesocosm observations. Cont. Shelf Res. 63, 69–84.
Rinke, K., Yeates, P., Rothhaupt, K.O., 2010. A simulation study of the feedback of phytoplankton on thermal structure via light extinction. Freshw. Biol. 55, 1674–1693.
Otte, S., Kuenen, J.G., Nielsen, L.P., Paerl, H.W., Zopfi, J., Schulz, H.N., Teske, A., Strotmann, B., Gallardo, V.A., Jørgensen, B.B., 1999. Nitrogen, carbon, and sulfur metabolism in natural Thioploca samples. Appl. Environ. Microbiol. 65, 3148–3157.
Preisler, A., De Beer, D., Lichtschlag, A., Lavik, G., Boetius, A., Jøørgensen, B.B., 2007. Biological and chemical sulfide oxidation in a Beggiatoa inhabited marine sediment. ISME J. 1, 341–353.
Wenk, C.B., Zopfi, J., Gardner, W.S., McCarthy, M.J., Niemann, H., Veronesi, M., Lehmann, M.F., 2014. Partitioning between benthic and pelagic nitrate reduction in the Lake Lugano south basin. Limnol. Oceanogr. 59, 1421–1433.
Peng, Y., Liu, L., Jiang, L., Xiao, L., 2017. The roles of cyanobacterial bloom in nitrogen removal. Sci. Total Environ. 609, 297–303.
Bouffard, D., Ackerman, J.D., Boegman, L., 2013. Factors affecting the development and dynamics of hypoxia in a large shallow stratified lake: hourly to seasonal patterns. Water Resour. Res. 49, 2380–2394.
Loiselle, S.A., Azza, N., Cozar, A., Bracchini, L., Tognazzi, A., Dattilo, A., Rossi, C., 2008. Variability in factors causing light attenuation in Lake Victoria. Freshw. Rev. 53, 535–545.
Li, H., Xing, P., Wu Qinglong, L., 2011. Characterization of the bacterial community composition in a hypoxic zone induced by Microcystis blooms in Lake Taihu, China. FEMS Microbiol. Ecol. 79, 773–784.
Cai, H., Jiang, H., Krumholz, L.R., Yang, Z., 2014. Bacterial community composition of size-fractioned aggregates within the phycosphere of cyanobacterial blooms in a eutrophic freshwater lake. PLoS One 9, e102879.
Tang, X., Chao, J., Gong, Y., Wang, Y., Wilhelm Steven, W., Gao, G., 2017. Spatiotemporal dynamics of bacterial community composition in large shallow eutrophic Lake Taihu: high overlap between free-living and particle-attached assemblages. Limnol. Oceanogr. 62, 1366–1382.
Cai, H., Zeng, Y., Wang, Y., Jiang, H., 2017. Elstera cyanobacteriorum sp. nov., a novel bacterium isolated from cyanobacterial aggregates in a eutrophic lake. Int. J. Syst. Evol. Microbiol. 67, 4272–4275.
Chen, Z., Zhang, J., Li, R., Tian, F., Shen, Y., Xie, X., Ge, Q., Lu, Z., 2018b. Metatranscriptomics analysis of cyanobacterial aggregates during cyanobacterial bloom period in Lake Taihu, China. Environ. Sci. Pollut. Res. - Int. 25, 4811–4825.
Xu, H., Jiang, H., Yu, G., Yang, L., 2014. Towards understanding the role of extracellular polymeric substances in cyanobacterial Microcystis aggregation and mucilaginous bloom formation. Chemosphere 117, 815–822.
Gonçalves, A.L., Pires, J.C.M., Simões, M., 2017. A review on the use of microalgal consortia for wastewater treatment. Algal Res. 24, 403–415.
Xiao, M., Li, M., Reynolds, C.S., 2018. Colony Formation in the Cyanobacterium Microcystis. Biological Reviews of the Cambridge Philosophical Society.
Dalsgaard, T., Thamdrup, B., Canfield, D.E., 2005. Anaerobic ammonium oxidation (anammox) in the marine environment. Res. Microbiol. 156, 457–464.
Chen, X., Jiang, H., Sun, X., Zhu, Y., Yang, L., 2016. Nitrification and denitrification by algae-attached and free-living microorganisms during a cyanobacterial bloom in Lake Taihu, a shallow Eutrophic Lake in China. Biogeochemistry 131, 135–146.