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RESEARCH ARTICLE

Intermittent disturbance combined N and P adding favor colony size and abundance of *Microcystis flos-aquae*

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Abstract – Nutrients and disturbance are both important environmental factors in the freshwater ecosystem. Here we present data on the effects of intermittent disturbance (ID), nitrogen and phosphorus adding (+N+P), and intermittent disturbance combined N and P adding (ID+N+P) on colony size and abundance of *Microcystis flos-aquae* in lab experiments. Results showed that the mean colony sizes of *M. flos-aquae* in ID+N+P group were1.68, 1.56, 1.17 times that in the control, +N+P, ID groups, respectively. Moreover, the average number of *M. flos-aquae* in ID+N+P group were 1.47, 1.19, 1.42 times those in the control, +N+P, ID groups, respectively. The average concentration of EPS (extracellular polysaccharides) in ID and ID+N+P group was significantly higher than those in control and +N+P groups. Results demonstrated that intermittent disturbance combined N and P adding promoted colony size and abundance of *M. flos-aquae*. These results suggest that intermittent disturbance combined N and P adding plays an important role in the formation of *Microcystis* blooms in freshwater ecosystems.

Keywords: Intermittent disturbance / phosphorus / nitrogen / *Microcystis* / EPS

1 Introduction

Cyanobacterial blooms dominated by *Microcystis* spp. occur frequently in lakes, rivers, and reservoirs around the world (Xiao *et al.*, 2018). Many species of *Microcystis* are toxic (Chorus and Bartram, 1999), thus blooms dominated by members of this genus can threaten drinking water safety and potentially harm human health (Guo, 2007). The high biomass of *Microcystis* during blooms can disturb the natural structure and function of microbial food webs, and degrade services provided by aquatic ecosystems (Paerl *et al.*, 2011; Plaas and Paerl 2021).

When *Microcystis* blooms outbreak, lots of colonies of *Microcystis* assemble at the surface of water in a natural water (Qin *et al.*, 2018; Zhu *et al.*, 2016). Colonial *Microcystis* has faster vertical migration velocity (Nakamura *et al.*, 1993), and has advantage in natural water when competition with other algae for more tolerant to high light intensity (Xu *et al.*, 2017). Besides, colonial *Microcystis* is also considered a strategy for resisting zooplankton grazing (Yang *et al.*, 2006), has advantages in the absorption of nutrients (Xiao *et al.*, 2018), and is more resistant to physical water disturbances (Wu and Kong, 2009). Colonies play an important role in the formation of *Microcystis* blooms in river, reservoir, and lakes in freshwater ecosystem (Yamamoto *et al.*, 2011; Zhu *et al.*, 2014). Nevertheless, colonial *Microcystis* usually changes to single or two cells after several generations when isolated and cultured in lab (Yang *et al.*, 2008). Thus, studies on the effects of various environmental factors on colony formation and colony size of *Microcystis* can provide a better understanding of *Microcystis* bloom formation (Zhu *et al.*, 2016).

The mechanism how *Microcystis* shift from a single-cell to colony morphology has attracted a lot of attention. Many factors can induce the formation of colony *Microcystis*, including grazing by zooplankton (Burkert *et al.*, 2001, Jang *et al.*, 2003), the existence of heterotrophic bacteria (Shen *et al.*, 2011), growth under nitrogen (Chu *et al.*, 2007) or phosphorus (Yang, 2010) restriction, high concentrations of lead ions (Bi *et al.*, 2013), the microcystin (Gan *et al.*, 2012), low temperature and light intensity (Xu *et al.*, 2016), and water disturbance (Zhong *et al.*, 2019).

Hydrodynamic conditions play an important role in the horizontal and vertical distribution of *Microcystis* blooms (Qin *et al.*, 2018). Besides, it was revealed that colony size and the abundance of *Microcystis* by disturbance depend on mode, intensity, and time of disturbance (O'Brien *et al.*, 2004; Li *et al.*, 2018; Zhong *et al.*, 2019; Yang *et al.*, 2020).

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Table 1. The mean value of nutrient paramenters and Chla in different treatments.

Parameters	Control	+N+P	ID	ID+N+P
DTN (mg/L)	2.810^{b}	5.103^{a}	2.691^{b}	4.795^{a}
DTP (mg/L)	0.094^{b}	0.202^{a}	0.083^{b}	0.189^{a}
NO_3^--N (mg/L)	2.333^{b}	4.219^{a}	2.272^{b}	4.025^{a}
NH_4^+-N (mg/L)	0.133^{b}	0.163^{a}	0.174^{a}	0.120^{b}
PO_4^{3-}-P (mg/L)	0.064^{c}	0.215^{a}	0.063^{c}	0.183^{b}
Chla (μg/L)	160.778^{c}	206.041^{b}	156.481^{c}	231.659^{a}

Note: The same letter indicates no significant difference and the different letters indicate significant difference ($P < 0.05$). ID = Intermittent disturbance, N = nitrogen, P = phosphorus.

Moreover, it was reported the mechanical action produced by water disturbances can cause changes in the physiology of *Microcystis* cells (Han *et al.*, 2018; Liu *et al.*, 2017). Nutrient is another important factor in the formation of *Microyctis* bloom. Numerous studies have focused on *Microcystis* bloom from the perspective of nutrients (mainly N and P), and have indicated N and P adding benefitted *Micrcoysits* bloom (Tang *et al.*, 2018). The formation of a cyanobacterial bloom is determined by sufficient algal biomass, cellular buoyancy and hydrodynamic conditions (Reynolds, 2006). To date, the formation mechanism of *Microcystis* blooms is not completely clear. Based on field observations, *Micrcoysits* blooms do occur frequently in slow-flowing rivers and intermittently disturbed eutrophic lakes and reservoirs (Reynolds, 2006). How the *Microcystis* respond to intermittent disturbance, particularly associated with N and P adding, remains to be explored.

The purpose of this study had two objectives: (1) to determine the effect of intermittent disturbance combined N and P adding on the colony size of *Microcystis*, (2) to determine the effect of intermittent disturbance combined N and P adding on the abundance of *Microcystis*. This research results will helpful in understanding the formation mechanism of *Microcystis* blooms.

2 Materials and methods

2.1 Experiment setup

Microcystis flos-aquae is one of the dominant species of *Microcystis* in Lake Taihu in China. In Jun. 2017, we isolated single colony of *M. flos-aquae* from lake water in Meiliang Bay (dominated by *Microcystis* bloom) in Lake Taihu and cultured in BG-11 medium (Rippka *et al.*, 1979). After Sept. 2017, unialgal cultures of *M. flos-aquae* were transferred to modified BG-11 medium (where TN=10 $mg \cdot L^{-1}$, TP = 0.5 $mg \cdot L^{-1}$). *M. flos-aquae* cultures consisted of a mixture of single-cells, double-cells and small colonies ($\sim 0.773 \times 10^6$ cells mL^{-1}) until the beginning of the experiment. Taking 0.15 L *M. flos-aquae* in logarithmic growth period to 0.50 L Erlenmeyer flask. Four treatments were established in triplicate: control, N and P adding (+N+P) group, intermittent disturbance (ID) group, intermittent disturbance combined N and P adding (ID+N+P) group. Then, 0.15L BG-11 medium without N and P was added to the control and ID group. And 0.15L BG-11 medium with N and P (TN = 10 mg/L, TP = 0.5 mg/L) was added to the +N+P and ID+N+P group, respectively.

Next, control and +N+P group were put in incubator at 25 °C under cool fluorescent lights at an intensity of 40.5 mol m^{-2} s^{-1} with a light-dark period of 12:12 h until the end of experiment. In ID and ID+N+P group, disturbance intensity is set as 100 r/min (which approximate current velocities of 0.32m/s) (reference Zhong *et al.*, 2019). ID and ID+N+P group were put on a shaker incubator (100 rpm) for 24 h at 25 °C under dark to simulate the effect of the disturbance induce by wind-wave on *M. flos-aqua*. Disturbance was generated on four horizontally oscillating shaking incubators (Zhong *et al.*, 2019). After disturbance, ID and ID+N+P group were shift to the incubator. During the whole experiment period, ID and ID+N+P group were disturbed in the 1st, 5th, 9th, and 13th days for 24 h, and no disturbance in other time (reference Yang *et al.*, 2020). The whole experiment lasted 17 days

Samples were collected on the 0th, 1st, 5th, 9th, 13th and 17th days in this experiment to measure total soluble nitrogen (DTN), total dissolved phosphorus (DTP), and orthophosphate (PO_4^{3-}-P), EPS (extracellular polysaccharide), colony size and abundance of *M. flos-aquae*. The determination methods of the above indicators may be found in our previous studies (Zhong *et al.*, 2019).

2.2 Statistical analysis

All the data obtained from the lab experiment was analyzed using SPSS 24.0 software. Statistically significant differences between the control and treatments were compared using one-way analyses of variance (ANOVA). In this study, *P*-value <0.05 is considered as statistically significant level.

3 Results

3.1 The nutrient paramenters and Chla

The mean value of DTN, DTP, NH_4^+-N, PO_4^{3-}-P in +N+P and ID+N+P group were significantly higher than those in control and ID group (Tab. 1). However, the mean value of NH_4^+-N in +N+P and ID group were significantly higher than those in control and ID+N+P group (Tab. 1). The mean concentration of Chla in ID+N+P group was the highest among four treatment groups.

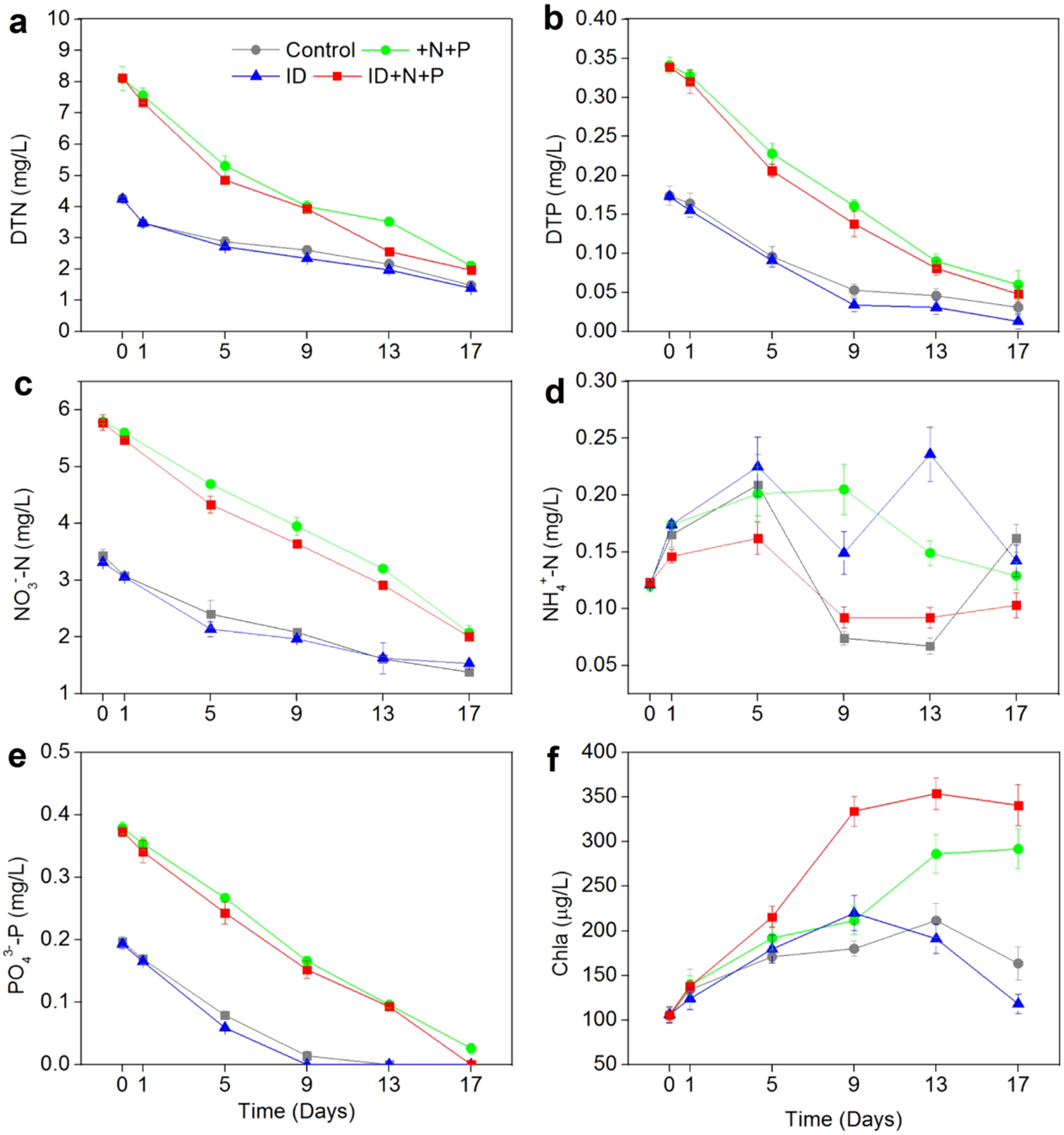

Fig. 1. The variation of DTN (a), DTP (b), PO_4^{3-} (c) and Chla (d) with time during this lab experiment. ID = Intermittent disturbance, N = nitrogen, P = phosphorus. a = DTN, b = DTP, c = NO_3^--N, d = NH_4^+-N, e = PO_4^{3-}-P, f = Chla.

During this experiment, the concentration of DTN, DTP, NH_4^+-N, PO_4^{3-}-P in all treatment group decreased (Fig. 1a, b, c, e). However, the concentration of Chla in +N+P and ID+N+P group increased during this experiment (Fig. 1f). No obvious law was found about the concentration variation of NH_4^+-N in all treatment group (Fig. 1d).

3.2 Colony size of *Microcystis flos-aquae*

As shown in Figure 2a, the average colony size of *M. flos-aquae* was 32.22 (±1.65), 34.65 (±1.71), 46.56 (±2.11), 54.32 (±2.82) µm in control, +N+P, ID, and ID+N+P groups, respectively. The average colony size of *M. flos-aquae* in ID and ID+N+P group were significantly higher than those in the control and +N+P group ($P < 0.05$). Moreover, the mean colony size of *M. flos-aquae* in ID+N+P group was significantly higher than that in ID group ($P < 0.05$).

In addition, the biggest coloy sizes of *M. flos-aquae* in ID and ID+N+P group was 52.56, 65.31 µm on 9th day, respectively (Fig. 2b). However, in control and +N+P group, the biggest coloy sizes of *M. flos-aquae* in control (35.59 µm) and +N+P (36.53 µm) group was found on 1st day (Fig.2b). At the end of the experiment, the mean size of *M. flos-aquae* colony was 30.17 (±1.62), 33.67 (±1.67), 50.27 (±2.28), 63.91 (±3.21) µm in control, +N+P, ID, and ID+N+P group, respectively (Fig. 2b). The mean colony size of *M. flos-aquae*

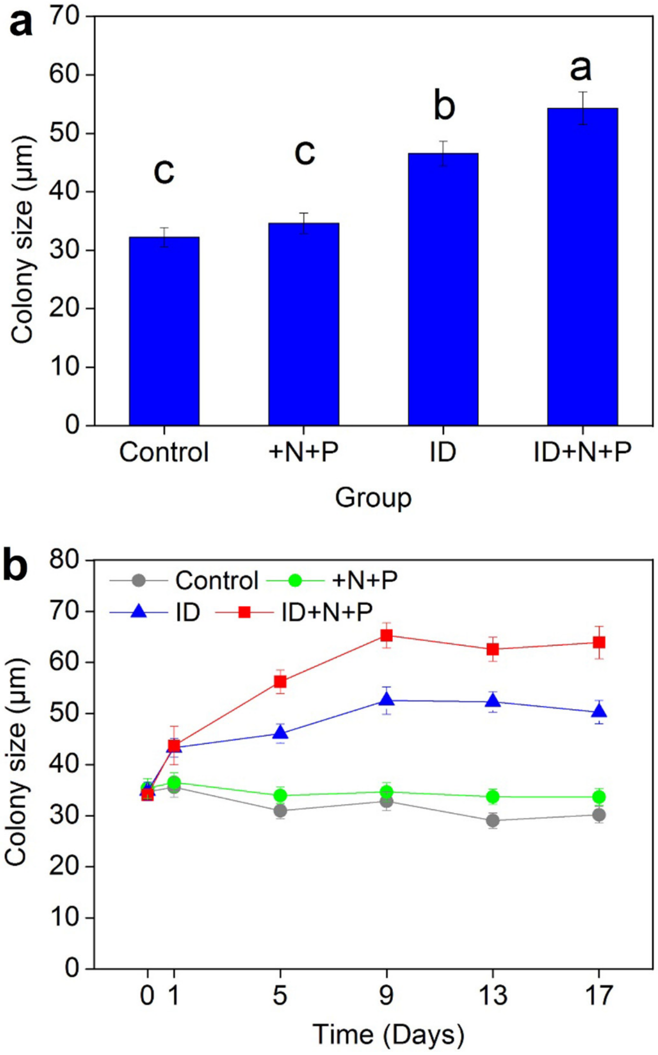

Fig. 2. The average colony size (a) and colony size variation (b) of *M. flos-aquae* with time during the lab experiment. ID = Intermittent disturbance, N = nitrogen, P = phosphorus. The different letters a, b, c indicates significant differences ($P < 0.05$).

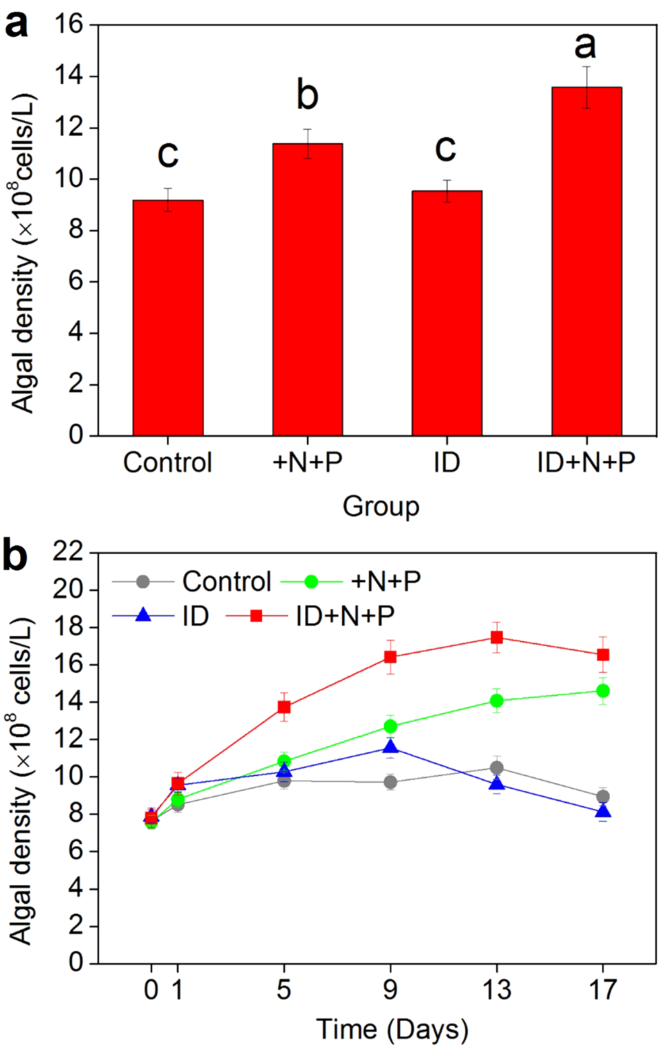

Fig. 3. The average algal density (a) and algal density variation (b) of *M. flos-aquae* with time during this lab experiment. ID = Intermittent disturbance, N = nitrogen, P = phosphorus. The different letters a, b, c indicates significant differences ($P < 0.05$).

in ID+N+P group was significantly higher than those in control, +N+P, and ID group ($P < 0.05$). Our result indicated that intermittent disturbance benefited the colony size of *M. flos-aquae* in this experiment.

3.3 Abundance of *M. flos-aquae*

In the lab experiment, the mean algal density of *M. flos-aquae* was 9.19 (±0.45), 11.37 (±0.57), 9.53 (±0.43), 13.57 (±0.81) × 10^8 cells/L in control, +N+P, ID, and ID+N+P group, respectively (Fig. 3a). The average algal density of *M. flos-aquae* in ID+N+P group was significantly higher than those in other three treatment groups ($P < 0.05$).

Besides, the algal density of *M. flos-aquae* in ID+N+P group increased 2.24 times from 7.81 × 10^8 cells/L to 17.47 × 10^8 cells/L on 13 days. However, the algal density of *M. flos-aquae* in control and ID group increased 1.17 and 1.03 times at the end of experiment, respectively. This result showed that intermittent disturbance combined N and P adding promoted the abundance accumulation of *M. flos-aquae* in this experiment.

3.4 EPS concentration of *M. flos-aquae*

It was confirmed that EPS (extracellular polysaccharides) was important in colony formation of *Microcystis* (Yang *et al.*, 2008). In this lab experiment, the average concentration of EPS was 1.07 (±0.012), 0.99 (±0.009), 1.78 (±0.074), 1.56 (±0.044) pg/cell in control, +N+P, ID and ID+N+P group, respectively (Fig. 4a). ANOVA analyzed showed that the average concentration of EPS of *M. flos-aquae* in ID and ID+N+P group was significantly higher than those in control and +N+P groups ($P < 0.05$) (Fig. 4a, b). Similarly, the sEPS (soluble extracellular polysaccharides) and bEPS (bound extracellular polysaccharides) of *M. flos-aquae* in ID and ID+N+P group were significantly higher than those in control and +N+P

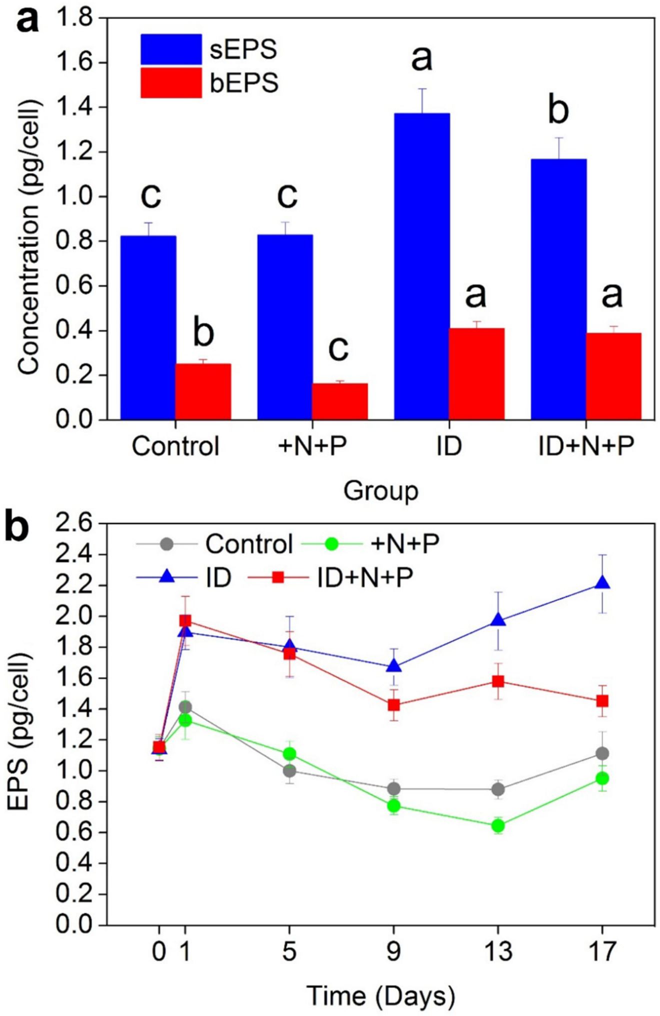

Fig. 4. The average concentration of sEPS and bEPS of *M. flos-aqua* during the lab experiment (a). The concentration variation of EPS of *M. flos-aqua* with time during the lab experiment (b). sEPS= soluble extracellular polysaccharides, bEPS = bound extracellular polysaccharides, ID = Intermittent disturbance, N= nitrogen, P = phosphorus. The different letters a, b, c indicates significant differences ($P < 0.05$).

groups ($P < 0.05$) (Fig. 4a). The maximum concentration of EPS in ID group was 2.21 (±0.19) pg/cell at the end of the experiment (Fig. 4b). And the maximum concentration of EPS in ID+N+P group was found in the first day, with the concentration 1.97 (±0.16) pg/cell (Fig. 4b). The results showed that intermittent disturbance significantly promoted the release of EPS from *M. flos-aquae* cells.

4 Discussion

4.1 Intermittent disturbance benefit the colony size of *Microcystis*

It was reported that colony size of *Microcystis* in Lake Taihu increased from 20.19 to 70.11 µm after disturbance for 24 h at current velocity of 0.53 cm/s in field condition (Yang *et al.*, 2017). Besides, it was found that under lab culture disturbance (current velocity = 0.16–1.28 m/s) for one day, prompting an increase in colony size of *M. flos-aquae* at current velocity <0.32 ms^{-1} (Liu *et al.*, 2017). It was confirmed that under lab culture conditions at current velocity <0.64 ms^{-1} is favorable for the colony aggregation of *M. aeruginosa* to form large colonies (Zhong *et al.*, 2019). In recent, it was revealed that intermittent disturbance (turbulent dissipation rate = $2.98 \times 10^{-6}\ \mathrm{m}^2\ \mathrm{s}^{-3}$) prompted an increase in colony size of *Microcystis* in microcosm condition (Yang *et al.*, 2020). Above studies indicated the intermittent disturbance with appropriate intensity favor the colony size of *Microcystis*.

EPS are found in mucilage or the cell's sheath, and it is very important in colony formation in *Microcystis* (Yang *et al.*, 2008; Li *et al.*, 2013; Zhu *et al.*, 2014). It was found that the concentration of EPS in *Microcystis* colonies was significantly higher than in single cells (Li *et al.*, 2013). It was reported that the bEPS (bound extracellular polysaccharides) may prevent daughter cells from separating after cell division, while sEPS (soluble extracellular polysaccharides) may increase cell adhesiveness (Li *et al.*, 2013). The average concentration of EPS of *M. flos-aquae* in ID and ID+N+P group was significantly higher than those in control and +N+P groups, which may explain why the average colony size of *M. flos-aquae* in ID and ID+N+P group were significantly higher than those in the control and +N+P group.

4.2 Intermittent disturbance combined N and P adding favored the abundance accumulation of *Microcystis*

Disturbance, a ubiquitous hydrodynamic feature of all inland waters and a highly variable environmental parameter, was generated at the surface or by natural processes (Kang *et al.*, 2019). Disturbance promotes or inhibits the primary production of phytoplankton by changing the light environment and nutrient supply (Macintyre and Jellison, 2001; Zhang *et al.*, 2004).

Disturbance can reduce the concentration of waste products or metabolites released from cells, which reduced their potential inhibitory effects to algae. However, disturbance will bring harm for growth of algae when disturbance exceeds a certain threshold. On the one hand, disturbance produces an unstable water movement environment, which is disadvantage for the growth of algal cells (Yan *et al.*, 2008). On the other hand, considering the shear forces, disturbance can lead to mechanical damage to algal cells, inhibit or reduce the division of algal cells, and change cell morphology and physiological activities (Karp-Boss *et al.*, 2000). It was confirmed that the disturbance intensity (0.32 m/s in this experiment) was favorable for the abundance accumulation of *Microcystis* in lab condition (Zhong *et al.*, 2019). Moreover, it was found that intermittent disturbance benefited the abundance increasing of *Microcystis* in field (Yang *et al.*, 2020). In addition, N and P concentration ($\mathrm{TN} = 10\ \mathrm{mg \cdot L^{-1}}$, $\mathrm{TP} = 0.5\ \mathrm{mg \cdot L^{-1}}$ in this experiment) are favorable for cell division and growth of *Microcystis*, including the single-cells, paired-cells and colonies *Microcystis* (Xu *et al.*, 2014). Therefore, intermittent disturbance combined N and P adding promoted abundance increase of *M. flos-aquae* in this lab experiment.

4.3 Significance for *Microcystis* in natural phytoplankton community

Microcystis can float or sink to obtain the optimal light by gas vacuolation according to the underwater light intensity (Reynolds, 1984). Colonial *Microcystis* has faster vertical migration velocity (Nakamura *et al.*, 1993), and has advantage in natural water when competition with other algae for more tolerant to high light intensity (Xu *et al.*, 2017). Besides, colonial *Microcystis* has advantages in the absorption of nutrients (Xiao *et al.*, 2018), and is more resistant to physical water disturbances (Wu and Kong, 2009). In this experiment, the larger colony morphology of *Microcystis* in ID+N+P group has advantage for *Microcystis* to get the dominant position in the competition with other algae.

Zooplankton is the main predators of phytoplankton and prefers to feed on the small individuals of algae. In this study, ID +N+P group were associated with larger *Microcystis* colonies. Large colonies of *Microcystis* are so big that it is impossible to be fed by zooplankton (Oliver and Ganf, 2000), which bring further advantage for *Microcystis* in competition with others algae. Nevertheless, because of single cell and small colony morphology of *Microcystis* was easily grazed by zooplankton in the control and +N+P group. Thus, large colonial *Microcystis* can have an advantage relative to other algae under pressure from grazers. In the ID+N+P group, enough N and P support the growth and reproduction of *Microcystis* (Fig. 2a, b). Therefore, intermittent disturbance combined N and P adding was important in ensuring the dominance of *Microcystis* in natural phytoplankton community.

5 Conclusions

The effects of intermittent disturbance, N and P, intermittent disturbance combined N and P adding on *M. flos-aquae* were studied in lab condition. The abundance, colony size of *M. flos-aquae* were determined. The main results are as followings: (1) ID+N+P favored the colony size increasing of *M. flos-aquae*, (2) ID+N+P benefited the abundance accumulation of *M. flos-aquae*. Our research results finally confirmed that intermittent disturbance combined N and P adding played important effect during the formation of *Microcystis* blooms in freshwater ecosystem.

Declaration of interest statement

No potential conflict of interest was reported by the authors.

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