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RESEARCH ARTICLE

Influence of *Daphnia magna* and *Ceratophyllum demersum* on the control of algae under different phosphorus concentrations

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Abstract – In order to evaluate the influence of *Daphnia magna* and *Ceratophyllum demersum* on the control of algae under different phosphorus concentrations, *Cyclotella* sp., *Microcystis aeruginosa*, and *Chlorella vulgaris* were selected to establish an aquatic microcosmic model. When the phosphorus concentration ranged from 0.05 to 2 mg L^{-1}, *D. magna* significantly inhibited the three species of algae from different phylum, particularly *M. aeruginosa*, and the total growth rates of the three species of algae decreased with the increase of phosphorus concentration. When the phosphorus concentration ranged from 0.05 to 2 mg L^{-1}, *C. demersum* imparted a significant inhibition of the three species of algae, particularly *M. aeruginosa*. The total growth rates of the three species of algae were reduced with higher phosphorus concentrations; however, the effect was lower than that of *D. magna*, with *C. vulgaris* as the dominant species. When the phosphorus concentration ranged from 0.05 to 2 mg L^{-1}, *D. magna* combined with *C. demersum* inhibited the growth of the three species of algae to a considerable degree, which was an improvement over that of other experimental groups using only *D. magna* or *C. demersum* by themselves. The total growth rates of algae were reduced with higher phosphorus concentrations. When the phosphorus concentration ranged from 0.05 to 0.1 mg L^{-1}, the removal rates of phosphorus exceeded 90%, and the phosphorus concentration became the limiting factor in the culture system. We observed that under higher initial phosphorus concentrations, the nitrogen removal rate increased, whereas the phosphorus removal rate decreased.

Keywords: *Cyclotella* sp. / *C. vulgaris* / *M. aeruginosa* / phosphorus concentration / biomanipulation

1 Introduction

Eutrophication is currently the primary environmental issue that negatively impacts natural waterways on a global scale. The over-enrichment of ambient water with nitrogen and phosphorus quickly produces the following symptoms: (a) the rapid proliferation of plankton and other aquatic plants; (b) a decrease of water transparency and a serious shortage of dissolved oxygen in deeper water; and (c) a range of adverse effects on aquatic animals, particularly the massive death of fish (Carpenter *et al.*, 1998). An obvious and major manifestation of eutrophic freshwater is the frequent occurrence of cyanobacteria algae blooms, the control of which has become a hot topic in the study of aqueous ecological environments (Xie and Sliver, 1998). Significant nutrient inputs such as nitrogen and phosphorus, lack of aquatic plants and their unreasonable utilization, overfishing of aquatic animals and the unbridled expansion of aquaculture leads to algae blooms that cause great damage to lake associated ecosystems. Many years of theoretical research and practice have shown that ecological restoration is the optimal strategy for the control of algal blooms, which includes two important and effective measures: algae control through biomanipulation, and the use of aquatic macrophyte (Moss, 1990; Ji and Wang, 2013; Liu *et al.*, 2005).

Biomanipulation controls the growth of phytoplankton through the direct ingestion of zooplankton. Multiple studies have revealed that phytoplankton productivity was lower in lakes that were dominated by Daphnia and many other large

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Daphnia hyaline animals (Ekvall *et al.*, 2014; Wang *et al.*, 2017). Aquatic macrophyte not only compete with phytoplankton for nutrients, light, and space (Zhang, 1998; Dong *et al.*, 2017; Lv *et al.*, 2016), but also secrete allelopathic substances that inhibit phytoplankton growth (Zhu *et al.*, 2010; Elsheekh *et al.*, 2017). Both of them remediation supplement each other toward the efficient suppression of algae. Certain threshold concentrations of nitrogen and phosphorus in aqueous ecosystems will influence the impact of ecological restoration. Nitrogen may be directly absorbed from the ambient atmosphere by several types of lake dwelling organisms, which include certain species of cyanobacteria, and nitrogen is often not a limiting factor for plant growth. In terms of urban wastewater treatment, the control of nitrogen requires higher investments and operating costs, which significantly increases the costs of lake bloom control in China. Several researchers have claimed that the capacity of some types of cyanobacteria to fix di-nitrogen, and thus potentially compensate via nitrogen reduction, makes N-control an unnecessary cost for society (Schindler *et al.*, 2008; Wang and Wang, 2009; Welch, 2009). The control of phosphorus inputs, in accordance with Liebig's Law of Minimum (Ploeg *et al.*, 1999), is therefore a prerequisite for the regulation of freshwater eutrophication. The concentration of phosphorus is a key factor in eutrophication as it plays a primary role in the control of algae growth in lakes (Chen, 2008). Healthy clear water ecosystems may easily become turbid if algae propagate rapidly in eutrophic water due to excessive phosphorus concentrations (Qin *et al.*, 2006). When water bloom phenomena occurs, the success of a given ecological restoration strategy is contingent on whether it may influence phosphorus concentrations in the water. Benndorf (1987) initially put forward the phosphorus threshold for biological manipulation; however, investigations of different phosphorus threshold ranges have not been consistent. Some researchers define this threshold to range from 0.05 to 0.15 mg L^{-1} (Jørgensen and Bernardi, 1998), whereas others have set it as 0.25 mg L^{-1} (Scheffer *et al.*, 2001). Therefore, it is necessary to further study phosphorus concentrations for water bodies in China.

Much research has been conducted by our lab, which shows that within the range of certain phosphorus concentrations, the combination of biological manipulation and the reestablishment of aquatic vegetation, may effectively inhibit algae growth to reduce nitrogen and phosphorus concentrations in aqueous ecosystems (Jin *et al.*, 2016; Ma *et al.*, 2014; Ma *et al.*, 2012). This experiment was conducted to elucidate the inhibitory effects of zooplankton and aquatic macrophyte on mixed three types of common bloom microalgae from different phylum under different eutrophication levels.

For this study, three species of common water blooms microalgae (*Cyclotella* sp., *Microcystis aeruginosa*, and *Chlorella vulgaris* as a representative of diatom, cyanobacteria and chlorophytes, respectively), zooplankton (*Daphnia magna*) and submerged macrophyte (*Ceratophyllum demersum*) were selected to establish a microcosm model, and culture solutions with different concentrations of phosphorus were prepared. The phytoplankton was co-cultured with zooplankton or submerged macrophyte to quantitatively investigate and analyze the relationships among of phytoplankton, zooplankton and submerged macrophyte under different phosphorus concentrations. The results of the study will provide an important reference for the control of water blooms.

2 Materials and methods

2.1 Experimental algae, zooplankton, and aquatic macrophyte

For this experiment, *Cyclotella* sp. (FACHB-1654), *C. vulgaris*, and *M. aeruginosa* (FACHB-573) were obtained from the Freshwater Algae Culture Collection, at the Institute of Hydrobiology, Chinese Academy of Sciences, which were preserved in B+D medium (50% BG11+ 50% D1 medium) and grown in an incubator under a temperature of 25 °C, at a light intensity of from 2600 to 3000 Lx, and a light/dark exposure ratio of 14 h:10 h.

Daphnia magna was collected from Muye Lake in Xinxiang City, and the purification culture was prepared in the laboratory. *D. magna* with active life phenomena was selected to be cultured and purified separately, which was fed on *C. vulgaris* with cultivation conditions similar to that of the algae. *D. magna* from the same mother, which was bred 6–24 hours following birth, was used in this experiment.

Ceratophyllum demersum, collected from Muye Lake in Xinxiang City, was rinsed with distilled water to remove any detritus, and then cultured in a large aquarium with B+D culture medium, with sufficient sunlight of 2600 Lx light intensity under a suitable temperature of 25 °C. For this experiment, we selected the top portion of *C. demersum* with consistent growth and rinsed it with distilled water multiple times, and if required, with hydrogen peroxide to remove any attached surviving organisms on the *C. demersum*, so as not to affect the experimental results.

2.2 Pretreatment of algae

The three species of algae were inoculated into the B+D medium, respectively, to expand the culture to the adequate population density. Centrifugation was performed at 3500 r min^{-1}, for 10 min. The supernatant was removed, whereafter the algae cells deposited at the bottom of the centrifuge tube were suspended with sterile distilled water, which was repeated in triplicate to remove any residual nutrients in the solution. The final precipitated algae species was inoculated into the B+D medium without nitrogen and phosphorus, and malnourished for a week. Subsequently, centrifugation was performed again (3500 r min^{-1} for 10 min.) to remove the supernatant, and the precipitated algae species was suspended with sterile distilled water, which was repeated in triplicate. Using this method, the nutrients in the culture media and within the algae were removed, to minimize experimental error. Finally, the three species of algae were extracted and inoculated into the media according to the experimental requirements.

2.3 Preparation of culture medium

Standard solutions of nitrogen and phosphorus were prepared using $NaNO_3$ and KH_2PO_4, respectively, whereas

B+D medium without nitrogen or phosphorus was selected as the mother solution. The B+D medium without N and P was prepared into culture medium with a concentration of 11 mg L^{-1} of N, and concentrations of P at 0.05, 0.1, 0.5, and 2 mg L^{-1}, respectively.

2.4 Co-culture of three species of algae

An 800 mL volume of the B+D culture solution with designated phosphorus concentration (0.05, 0.1, 0.5, and 2 mg L^{-1}) was introduced into a 1 L conical flask and autoclaved. *Cyclotella* sp., *C. vulgaris*, or *M. aeruginosa* were inoculated into a 1 L conical flask that contained 800 mL of the B+D medium to attain a concentration of 1×10^5 cells mL^{-1} (The cell density of each alga were 1×10^5 cells mL^{-1}), which was cultured for 16 days under the same cultivation conditions as described the above, with the volume of the culture medium maintained at 800 mL during culturing. The processing sets were performed in triplicate. At the conclusion of the experiment, the population density of each of the three species algae were calculated, the concentrations of nitrogen or phosphorus in the culture medium was measured, and the growth rates of the three species of algae were assessed.

2.5 Co-culture of *D. magna* and three species of algae

An 800 mL volume of the B+D culture solution with designated phosphorus concentration (0.05, 0.1, 0.5, and 2 mg L^{-1}) was introduced into a 1 L conical flask and autoclaved. *Cyclotella* sp., *C. vulgaris*, or *M. aeruginosa* were inoculated into a 1 L conical flask that contained 800 mL of the B+D medium to attain a concentration of 1×10^5 cells mL^{-1} (The cell density of each alga were 1×10^5 cells mL^{-1}), five *D. magna* with similar morphological characteristics were introduced into the medium. Each of the processing sets was performed in triplicate, which was cultured for 16 days under the same cultivation conditions as described above, and the volume of the culture medium was maintained at 800 mL during culturing. At the conclusion of the experiment, the population densities of the three species of algae or *D. magna* were counted, respectively, the concentrations of nitrogen or phosphorus in the culture solution were measured, and the growth rates of the three types of algae or *D. magna* and the removal rates of nitrogen and phosphorus were calculated.

2.6 Co-culture of *C. demersum* and three species of algae

An 800 mL volume of the B+D culture solution with designated phosphorus concentration (0.05, 0.1, 0.5, and 2 mg L^{-1}) was respectively added into each of the 1 L conical flasks and autoclaved. *Cyclotella* sp., *C. vulgaris*, or *M. aeruginosa* were then inoculated into each of the 1 L flasks to attain a concentration 1×10^5 cells mL^{-1} (the cell density of each alga were 1×10^5 cells mL^{-11}), whereafter a 0.1 g top portion of *C. demersum* was also added (1.25 kg FW m^3, which was same as the water body with abundant submerged plants). Each of the processing sets was performed in triplicate, which was cultured for 16 days under the same cultivation conditions as described above, and the volume of culture medium was maintained at 800 mL during culturing. At the end of the experiment, the populations of the three species of algae or *D. magna* were counted, respectively, the nitrogen or phosphorus concentrations in the culture solution were measured, and the growth rate of the three types of algae or *C. demersum* and the removal rates of nitrogen and phosphorus were calculated.

2.7 Co-culture of *D. magna*, *C. demersum* and the three species of algae

An 800 mL volume of the B+D culture solution with designated phosphorus concentration (0.05, 0.1, 0.5, and 2 mg L^{-1}) was respectively added into each of 1 L conical flasks and autoclaved. *Cyclotella* sp., *C. vulgaris*, or *M. aeruginosa* was inoculated into each of the 1 L flasks to attain a concentration 1×10^5 cells mL^{-1} (the cell density of each alga were 1×10^5 cells mL^{-1}), after which five of *D. magna* with similar morphological characteristics, and 0.1 g of the top portion of *C. demersum* were also added into the medium. Each of the processing sets was performed in triplicate, which was cultured for 16 days under the same cultivation conditions as described above, and the volume of culture medium was maintained at 800 mL during culturing. At the end of this experiment, the population densities of the three species of algae and *D. magna* were counted, respectively, the quality of *C. demersum* was determined, and the nitrogen and phosphorus concentrations in the culture solution was measured. Subsequently, the growth rates of the three species of algae, *D. magna*, *C. demersum*, and the removal rates of nitrogen and phosphorus were calculated.

2.8 Sample analysis

To determine the cell density of the algae: Algae samples (1 mL) were extracted, and the cells were counted by using a XB-K-25 cell counting plate under a microscope. The cell density of the three species of algae was calculated.

The number of *D. magna*: Visual counts.

The fresh weight of *C. demersum*: *C. demersum* was taken from the culture medium, the surface moisture was dried with water absorbent paper, followed by weighing with an electronic balance.

The growth rates of algae, *D. magna* and *C. demersum* were calculated as follows:

$$R_1 = \frac{D_t - D_0}{D_0} \times 100\% \quad (1)$$

$$R_2 = \frac{N_t - N_0}{N_0} \times 100\% \quad (2)$$

$$R_3 = \frac{W_t - W_0}{W_0} \times 100\% \quad (3)$$

In formula (1), D_0 and D_t were the cell densities of the initial and *t*-times of the algae cells, respectively. In formula (2), N_0 and N_t were the number of the initial and t-times of the *D. magna*, respectively. In formula (3), W_0 and W_t were the

fresh weight of the initial and *t*-times of *C. demersum*, respectively. Total nitrogen and total phosphorus: At the conclusion of the experiment, a defined volume of culture medium was centrifuged and the supernatant was extracted, followed by the determination of the content of nitrogen and phosphorus in the culture medium using the standard method (State Environmental Protection Agency, 2002).

2.9 Statistical analysis

SPSS 19.0 was utilized to process the variance analysis and Tukey multiple comparison ($P < 0.05$) of the growth rates of *Cyclotella* sp., *C. vulgaris*, and *M. aeruginosa*, *D. magna* and *C. demersum* at the end of the experiment.

3 Results and analysis

3.1 Co-culture of the three types of algae

In the co-culture experiments of *Cyclotella* sp., *C. vulgaris*, and *M. aeruginosa*, the growth rates of *Cyclotella* sp., *C. vulgaris*, or *M. aeruginosa* were −100%, 3808%, and 92233%, respectively. Meanwhile the total cell density and the total growth rate were 9.6×10^7 mg L^{-1} and 64000%, respectively. Among them, *M. aeruginosa* had the most rapid proliferation and dominated, and while *Cyclotella* sp. was significantly inhibited, which was the result of competition between the three species algae.

3.2 Influence of *D. magna* on the control of algae under different phosphorus concentrations

When *D. magna* and the three species of algae were co-cultured in the medium under different phosphorus concentrations, there were changes in the growth rates of *D. magna* and the three species of algae, as shown in Figure 1. With higher phosphorus concentrations, the population density of *D. magna* was increased. For the population density of *D. magna*, there was a significant difference between the different phosphorus concentrations ($P < 0.05$). When the initial phosphorus concentration was 2 mg L^{-1}, the highest population size of *D. magna* was 907. The growth rates of the three species of algae decreased under higher phosphorus concentrations, and their growth rates were far lower than that of *D. magna*. Under all phosphorus concentrations, the growth rate of *Cyclotella* sp. was negative, with none of them surviving when the phosphorus concentration was 0.5–2 mg L^{-1}, which was not significantly different from the results when the three species of algae were co-cultured. When the phosphorus concentration was 0.05–0.1 mg L^{-1}, the cell density of *C. vulgaris* increased slightly more than that of the initial inoculation. When the phosphorus concentration was 0.05 mg L^{-1}, the growth rate or cell density of *C. vulgaris* was the highest, with values of 150% and 4×10^5 cells mL^{-1}, respectively. The growth rate of *M. aeruginosa* was positive under a phosphorus concentration of 0.05 mg L^{-1}, whereas under the other phosphorus concentrations, the growth rate of *M. aeruginosa* was negative, and the values were significantly lower than for when the three species of algae were co-cultured ($P < 0.05$). The results revealed that when the phosphorus concentration ranged from 0.05 to 2 mg L^{-1}, *D. magna* significantly inhibited the proliferation of the three species of algae, particularly for *M. aeruginosa*.

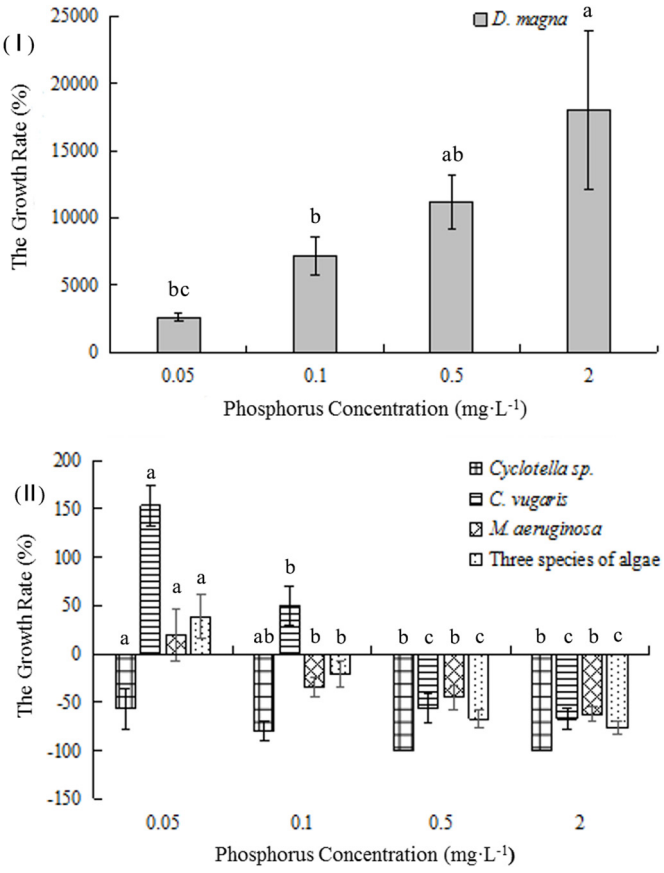

Fig. 1. Change in the growth rate of (I) *D. magna*, (II) *Cyclotella* sp., *C. vugaris*, *M. aeruginosa*, and the total algae.

3.3 Influence of *C. demersum* on the control algae under different phosphorus concentrations

Changes in the growth rates of *C. demersum* and the three species of algae, subsequent to being co-cultured in the medium under different phosphorus concentrations, are depicted in Figure 2. For each phosphorus concentration, the growth rate of *C. demersum* initially increased and then declined. When the phosphorus concentration were 0.05–0.5 mg L^{-1}, the growth rates of *C. demersum* increased with higher phosphorus concentrations, and changed significantly ($P < 0.05$). Under a phosphorus concentration of 2 mg L^{-1}, the growth rate of *C. demersum* was significantly lower than that when the phosphorus concentration was 0.5 mg L^{-1} ($P < 0.05$), which had several potential explanations. It may have been that the high phosphorus concentration imparted stress on the growth of *C. demersum*, and algae proliferation competed with *C. demersum* for resources to survive. The maximum growth rates of both *M. aeruginosa* and *Cyclotella* sp. were lower than 31% under each phosphorus concentration, which indicated that the growth of *M. aeruginosa* or *Cyclotella* sp. was significantly inhibited.

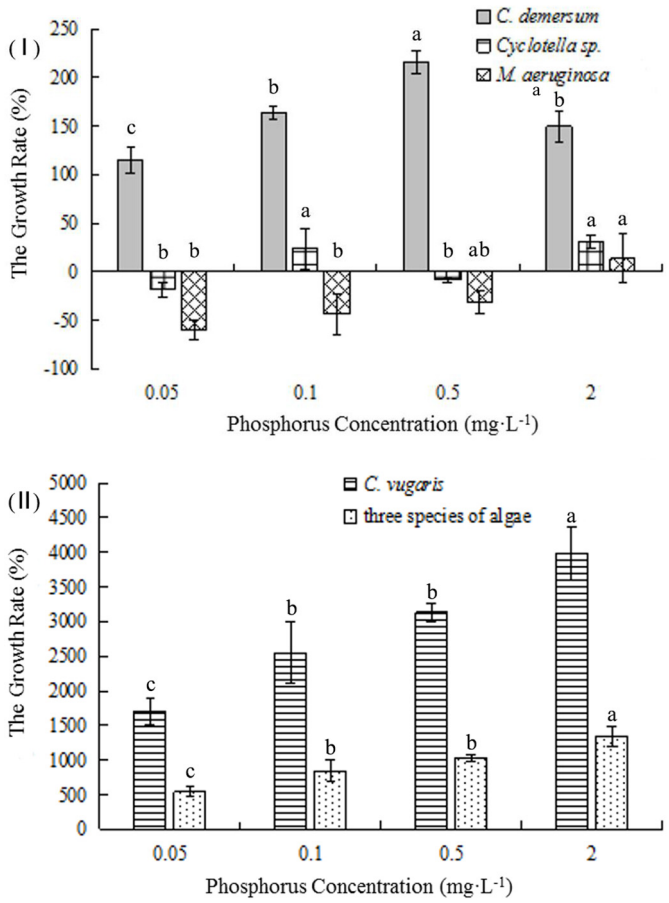

Fig. 2. Change in growth rate of (I) *C. demersum*, *Cyclotella* sp., *M. aeruginosa*, (II) *C. vugaris*, and the total algae.

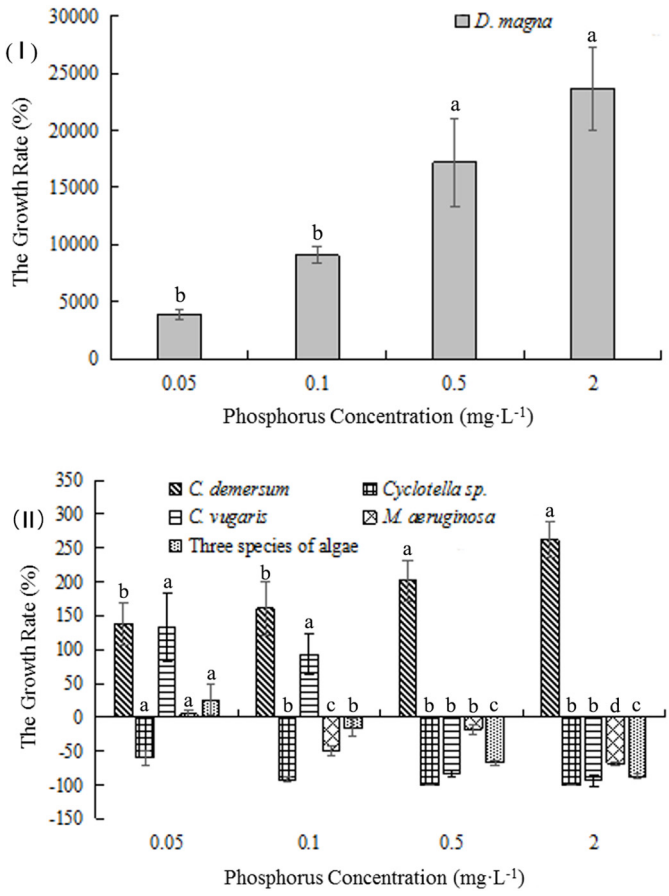

Fig. 3. Changes in the growth rate of (I) *D. magna*, (II) *C. demersum*, *Cyclotella* sp., *C. vugaris*, *M. aeruginosa*, and the total algae.

The cell density of *C. vulgaris* increased with higher phosphorus concentrations, and its growth rate was much better than that of *C. demersum* and the other two species of algae, which led to its becoming the dominant species. This was because *C. demersum* significantly inhibited *M. aeruginosa* and reduced its density, which weakened the competitive inhibition of *C. vulgaris*. When the phosphorus concentration was 2 mg L^{-1}, the growth rate or cell density of *C. vulgaris* was the highest, with a value of 3983%, or 4.08×10^6 cells mL^{-1}. Simultaneously, the total cell density was also the highest, with a value of 4.33×10^6 cells mL^{-1}. When the phosphorus concentration was 0.05 mg L^{-1}, the total algal cell density was the lowest, with a value of 1.9×10^6 cells mL^{-1}.

Compared with the experiment where the three species of algae were co-cultured (with a total algal cell density of 9.6×10^7 cells mL^{-1}), although the water remained green (with *C. vugaris* as the dominant species), *C. demersum* had significant inhibitory effects on the algae, particularly on *M. aeruginosa*, which indicated that when phosphorus concentration ranged from 0.5 to 2 mg L^{-1}, the use of *C. demersum* alone had a certain inhibitory effect. Compared with the impact of *D. magna* on algae control, both had the most potent inhibitory effect on *M. aeruginosa*, which exhibited negative growth. The growth rate of *C. vulgaris* was much higher in contrast to when they were co-cultured with *D. magna*, which indicated that the overall inhibitory effect of *C. demersum* on the three species of algae was lower than that of *D. magna* under the same conditions.

3.4 Influence of *D. magna* and *C. demersum* on the control of algae under different phosphorus concentrations

As shown in Figure 3, when *D. magna*, *C. demersum*, and the three species of algae were co-cultured in the medium under different phosphorus concentrations, there were changes in the growth rates of *D. magna*, *C. demersum*, and the three species of algae. The population density of *D. magna* increased with higher phosphorus concentrations, and there was a significant difference in the growth rate under the different phosphorus concentrations ($P < 0.05$). Under a phosphorus concentration of 2 mg L^{-1}, the population size of D. *magna* was highest (1180). With higher phosphorus concentrations, the weight of *C. demersum* increased to a maximum value of 0.362 g, and there were no significant differences between the other phosphorus concentrations ($P > 0.05$). The growth rates of the three types of algae decreased with higher phosphorus concentrations. When the phosphorus concentration was 0.05 mg L^{-1}, the total growth rate of the algae was greatest, at 26%, whereas the total algae cell density was 3.78×10^5 cells mL^{-1}. When the phosphorus concentration ranged from

0.1 to 2 mg L^{-1}, there was negative growth for overall of the algae, and the culture medium was very clear. This revealed that both of *D. magna* and *C. demersum* could efficiently inhibit the algae when the phosphorus concentration ranged from 0.05 to 2 mg L^{-1}, particularly when the phosphorus concentration was 2 mg L^{-1}. The overall inhibitory effect on the algae was significantly improved over that of the other experimental groups, which involved only *D. magna* or *C. demersum*.

3.5 Removal efficiency of total nitrogen and total phosphorus in the culture medium

The removal rates of nitrogen and phosphorus in different co-cultures are shown in Figure 4. For this experiment, the nitrogen removal rate was the lowest (<40%), which revealed that nitrogen was not the limiting factor. When the three species of algae were co-cultured with *C. demersum*, the nitrogen and phosphorus removal rates were higher than for the other two experimental groups, as *C. vugaris* grew and multiplied in large numbers, utilizing the elemental nitrogen and phosphorus in the water. When the phosphorus concentration ranged from 0.05 to 0.1 mg L^{-1}, the phosphorus removal rates for the three experimental groups attained >90%; hence, the phosphorus in the culture solution was effectively removed. When the phosphorus concentration ranged from 0.5 to 2 mg L^{-1}, the phosphorus removal rates ranged from 20% to 70% across the three experimental groups, which revealed that the phosphorus concentration was not a limiting factor for algae growth. In the experimental co-cultured group that included *D. magna*, *C. demersum*, and the three species of algae, the consumption of nitrogen and phosphorus was lower than that of the other groups, which was due to the significantly inhibited proliferation of algae; therefore, large quantities of nitrogen and phosphorus were not consumed. When the initial phosphorus concentration was high, the nitrogen removal rate was lower, but the removal amount of phosphorus was also high.

For each of the three experimental groups, when the phosphorus concentrations were 0.05 mg L^{-1} and 0.1 mg L^{-1}, there was no significant differences between the nitrogen or phosphorus removal rates ($P > 0.05$), while the differences were significant under other different phosphorus concentrations ($P < 0.05$). The reason was that when phosphorus concentrations were 0.05 mg L^{-1} and 0.1 mg L^{-1}, all of the phosphorus in the culture solution was essentially utilized. The consumption of nitrogen and phosphorus by aquatic plants may be correlated to a certain extent; hence, the difference between the nitrogen and phosphorus removal rate was not significant.

4 Discussion

4.1 The influence of eutrophication on the control of the biological manipulation of algae

There was an intimate relationship between the effects of biological manipulation and the concentration of phosphorus in water. When *D. magna* and the three species of algae were co-cultured in the culture solution under different phosphorus

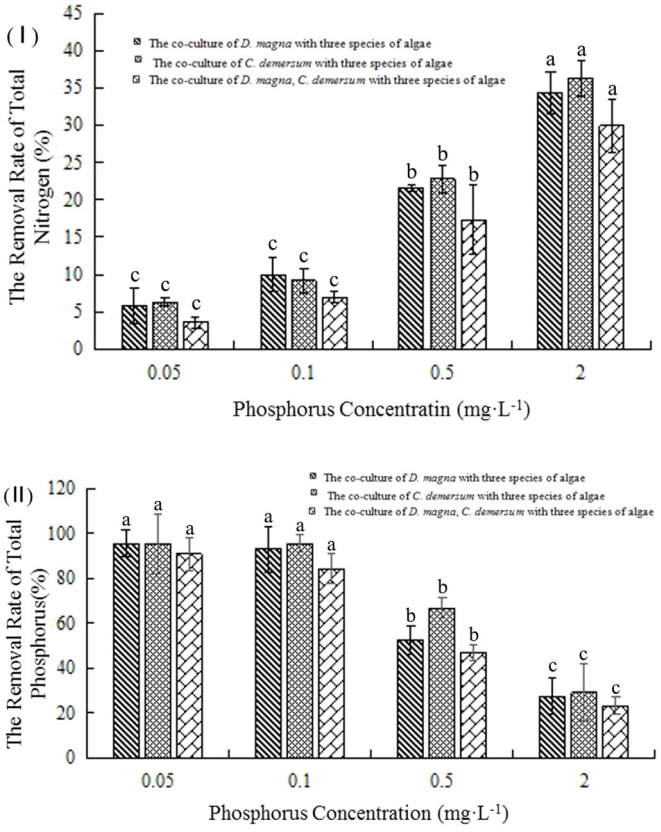

Fig. 4. Removal rates of (I) total nitrogen and (II) total phosphorus.

concentrations, the population density of *D. magna* was positively correlated with the phosphorus concentration. Under each of the phosphorus concentrations, the *D. magna* growth rate was obviously dominant. The higher concentration of phosphorus, the higher inhibitory effect of *D. magna* on the three species of algae, particularly the inhibition of *D. magna* on *M. aeruginosa*. This was contrary to the results of an experiment that was conducted in the author's laboratory, where *D. magna* was cultured with only one species of algae (Ma *et al.*, 2013). A potential rationale was that algae-algae competition inhibited each other's proliferation, which facilitated the survival and reproduction of *D. magna*. Under high phosphorus concentrations, *D. magna* reproduced rapidly, which in turn more effectively inhibited algal growth. It might have also been the case that *D. magna* was selective in its feeding on algae, with its algae selection criteria primarily related to size, shape, nutritional composition, and so on. Therefore, when the phosphorus concentration ranged from 0.05 to 2 mg L^{-1}, *D. magna* inhibited the growth of algae, with higher concentrations showing further improvements. Some researchers put forward that the biological control was optimal when the phosphorus concentration ranged from 0.05 to 0.15 mg L^{-1} (Jørgensen and Bernardi, 1998); however, the results obtained under the experimental conditions went beyond this range. For this experiment, the maximum cell density of all algae was only 4×10^5 cells mL^{-1}, the culture solution was clear since the population density required for water-bloom formation could not be attained. This indicated that *D. magna* could effectively control algal blooms. During

the management of Nanhu Lake in Changchun, China, the water quality could be enhanced through the direct introduction of *D. magna* and *Ceriodaphnia quadrangular* into the lake (Zhang *et al.*, 1998). In this experiment, if the concentration of phosphorus increased, some algae likely regained dominance and inhibited the growth of zooplankton and formed algal blooms, which was confirmed via the experiment of Yang Peiyun (Yang, 2016). Therefore, to effectively control algae, external nutritional inputs must be controlled. Algal growth may be utilized to effectively remove the nitrogen and phosphorus content of aqueous ecosystems. It is feasible to employ an appropriate population of zooplankton or fish to filter the algae to control its growth, which was verified through the algal daphnia system studied by Ma Jinfeng (2014) for the removal of phosphorus in the water.

4.2 The influence of eutrophication on the controlling effect of submerged plants on algae

The inhibition of algal growth via submerged plants was closely related to the degree of eutrophication. In an experiment that involved the co-culturing of *C. demersum* with the three species of algae, the growth rate of *C. demersum* was lower under a phosphorus concentration of 2 mg L^{-1} than from 0.1 to 0.5 mg L^{-1}, which may have been due to two reasons: (1) on one hand, the high phosphorus concentration had a stressing effect on the growth of *C. demersum.* When the nutrient concentration was too high and exceeded the tolerance of C. *demersum*, its physiological activities would be seriously affected, which inhibited its growth (Scheffer *et al.*, 2001). (2) On the other hand, algae proliferation competed with *C. demersum* for resources (nutrition, light, space, etc.) to survive.

For this experiment, *C. demersum* had a good inhibitory effect on the proliferation of the three types of algae. The inhibition of *C. demersum* on *C. vulgaris* was the weakest, the inhibition of *C. demersum* on *M. aeruginosa* was the strongest, and the inhibition of *C. demersum* on *C. vulgaris* was less than that of *C. demersum* on *C. vulgaris* when they were co-cultured (Ma *et al.*, 2013), which revealed that there were different degrees of mutual inhibition between them. The significant inhibition of *C. demersum* on *M. aeruginosa* reduced the density of *M. aeruginosa*, which in turn reduced the competitive inhibition on *C. vulgaris*, while the planktonic algae inhibited the growth of *C. demersum*, which slowed the growth of *C. demersum*. Due to the proliferation of a large population of *C. vulgaris*, the removal rate of nitrogen and phosphorus in water was the highest, which led to a large quantity of nitrogen and phosphorus in eutrophic water being removed by *C. vulgaris*, with an absorption rate that attained 80%. The inhibitory effect of *C. demersum* on algae was less effective than that of *D. magna*. The large-scale breeding of algae eventually causes serious turbidity in ambient waterways, which makes it difficult to develop a healthy aquatic ecosystem when large aquatic plants do not grow well.

4.3 The combination of biological manipulation and the re-establishment of submerged macrophyte

The combination of biological manipulation and the re-establishment of submerged plants can improve the inhibition of algae growth (Ma *et al.*, 2014). For this experiment, the co-culturing of *D. magna*, *C. demersum*, and the three species of algae, resulted in increases in the populations of both *D. magna* and *C. demersum* under higher phosphorus concentrations. The growth rates of both *D. magna* and *C. demersum* were greater than that of the other two groups, when *D. magna* or *C. demersum* were co-cultured with the three species of algae alone. When *D. magna*, *C. demersum*, and the three species of algae were co-cultured, the inhibitory effect on the growth of algae was improved over that of the other groups involving only *D. magna* or *C. demersum*, where the effect of algae inhibition was as follows: grass-daphnia-algae > daphnia-algae > grass-algae. The reason was that the mutual inhibition between algae and algae, or between algae and grass promoted the rapid multiplication of *D. magna*, which fed on large quantities of the three types of algae. Simultaneously, the algae reduced the utilization of sustaining resources, which was conducive to the growth of *C. demersum*. The existence of *C. demersum* also created favorable conditions for the survival and reproduction of *D. magna*, while having an allelopathic effect on the algae. Compared with the experiment of *C. demersum* and the three species of algae were co-cultured, in where *D. magna,* C. *demersum* and the three species of algae were co-cultured, when the concentration of phosphorus was 2 mg L^{-1}, *C. demersum* growth was not inhibited, which might have been due to a portion of the nitrogen and phosphorus in the culture medium being absorbed by the planktonic algae and *C. demersum*, which reduced the damage of the high phosphorus concentration to C. *demersum*. Further, the growth of the algae was inhibited, which reduced the competition inhibition on *C. demersum*. For this experiment, the ratio between *D. magna* and *C. demersum* was 5/0.1 g, and both *D. magna* and *C. demersum* survived well and inhibited the growth of algae. This revealed that under specific conditions, a certain proportion of zooplankton and large submerged plants could enhance their advantages for survival, increasing the predation and inhibition of phytoplankton, which in turn improved the purity, quality and transparency of the water body. For this experiment, the phosphorus concentration threshold ranged from 0.05 to 2 mg L^{-1}, the culture medium was clear, and the algae was significantly inhibited. However, there remained a threshold at which the excessive growth of algae could not be controlled by the combination of zooplankton and submerged plants, which will require further research.

5 Conclusion

The following conclusions could be obtained under a temperature of 25° C, light intensity 2600–3000 Lx, light/dark exposure ratio of 14 h: 10 h, using a B + D medium.

1 When phosphorus concentration ranged from 0.05 to 2 mg L^{-1}, *D. magna* exhibited a significant inhibition on the three species algae, particularly *M. aeruginosa*. The total growth rate of the three species of algae decreased with higher phosphorus concentrations.
2 When the phosphorus concentration ranged from 0.05 to 2 mg L^{-1}, *C. demersum* demonstrated a significant inhibition on the three species of algae, particularly *M. aeruginosa*. The total growth rate of the three of

species algae decreased with higher phosphorus concentrations; however, the inhibition effect was worse than that of *D. magna* (*C. vugaris* was the dominant species).

3 When phosphorus concentration ranged from 0.05 to 2 mg L^{-1}, *D. magna* and *C. demersum* imparted a significant inhibition on the three species of algae, especially *M. aeruginosa*. The total growth rate of the three species of algae decreased with higher phosphorus concentrations. The inhibition effect was improved over that of the other two groups, with only *D. magna* or *C. demersum* under the same phosphorus concentration.

4 For all the experimental groups, under phosphorus concentrations that ranged from 0.05 to 0.1 mg L^{-1}, the phosphorus removal rate was > 90%, where the phosphorus concentration became the limiting factor. When the phosphorus concentration ranged from 0.5 to 2 mg L^{-1}, the phosphorus removal rate was from 20% to 70%. When *C. demersum* was co-cultured with the three species algae, the nitrogen and phosphorus removal rate was higher than the other two groups, which was due to the very high *C. vugaris* cell density. Larger quantities of nitrogen were removed under higher initial phosphorus concentrations. The removal rate of phosphorus decreased under higher phosphorus concentrations; however, the removal amount of phosphorus increased with higher initial phosphorus concentrations.

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