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Rapid Recovery of Buoyancy in Eutrophic Environments Indicates That Cyanobacterial Blooms Cannot Be Effectively Controlled by Simply Collapsing Gas Vesicles Alone

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Abstract: Many aquatic ecosystems are seriously threatened by cyanobacteria blooms; gas vesicles enable cyanobacteria to form harmful cyanobacterial blooms rapidly. Many lake managers try to control cyanobacterial blooms by collapsing gas vesicle, but it is still unclear whether gas vesicle recovery will cause this method to fail. Through the culture experiments of three cyanobacteria, it was found that all cyanobacteria with collapsed gas vesicles can rapidly regain buoyancy in a few days under nutrient-sufficient environments, and average gas vesicle content was even 9% higher than initially. In contrast, buoyancy recovery of all cyanobacteria under nutrient-limited environments was significantly worse. After culture experiments, the average gas vesicle content of all cyanobacteria in phosphorus-limited environments only reach 49% of the initial value. The gas vesicle content of two non-nitrogen-fixing cyanobacteria in nitrogen-limited environments only reached 38% of initial value. The buoyancy of cyanobacteria in different tropic levels was similar to the gas vesicle content. These results indicate that collapsing gas vesicles can only control cyanobacterial blooms in the short-term. To control cyanobacterial blooms in the long-term, in deep lakes, lake managers should discharge gas vesicles' collapsed cyanobacteria into deep water. In shallow lakes, the disruption of gas vesicles must be combined with nutrient control measures to effectively control cyanobacteria blooms.

Keywords: cyanobacteria; gas vesicles; recovery; buoyancy

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1. Introduction

Cyanobacterial blooms affect public health and ecosystem services globally. Cyanobacterial blooms are caused by the excessive proliferation of cyanobacteria, and global warming and increased nutrients are also important causes of this phenomenon [1,2]. Cyanobacteria have a competitive advantage under eutrophication conditions and become the dominant phytoplankton in many lakes [3]. Cyanobacterial blooms affect humans and ecosystems in a variety of ways. Microcystins produced by cyanobacteria pose a serious threat to drinking water safety and the survival of other organisms in lakes [4,5]. Ingestion of cyanotoxins has been linked to liver and digestive issues, and even death. The odor compounds produced by the metabolism and death of cyanobacteria are difficult to remove by conventional water treatment processes applied in waterworks, resulting in complaints from consumers [6,7]. The lack of oxygen in lakes caused by the respiration and degradation of cyanobacteria is also an important factor affecting the survival of fish and benthic animals. Cyanobacteria develop diverse and highly effective ecophysiological adaptations, which make them dominant in aquatic environments undergoing natural and human-induced environmental change [3]. Bacteria may also interact with cyanobacteria and affect the development and succession of cyanobacterial blooms [2,8]. Climate change is a potent catalyst for the further expansion of

cyanobacteria blooms [9,10]. In order to deal with this problem, many researchers and lake managers have tried to control and use cyanobacteria by a variety of means [11–13].

Gas vesicles enable cyanobacteria to form harmful cyanobacterial blooms and gain an advantage in phytoplankton competition. Studies have shown that the outbreak of cyanobacterial blooms is partly due to the rapid proliferation of phytoplankton, but more often due to the phytoplankton rising to the water surface in a short time [14,15]. Cyanobacteria with gas vesicles can migrate vertically, and change position in the water column as needed [16]. Cyanobacteria have access to more sunlight and CO_2 when they migrate to the water surface and to large amounts of nutrients released by sediments when they migrate to deep water; zooplankton grazing also forces phytoplankton to migrate vertically [4,17]. In some turbid lakes, where light is the main limiting factor for phytoplankton growth, cyanobacteria with strong buoyancy can occupy the surface water and become the dominant species [18].

Collapsing gas vesicles to make cyanobacteria lose buoyancy is an effective method of controlling cyanobacterial blooms, but there are few studies that focus on whether cyanobacteria can regain buoyancy, which may cause a high risk of cyanobacterial bloom. Researchers have pointed out many methods of controlling cyanobacterial blooms by collapsing gas vesicles such as ultrasound and pressurization. Elevated pressure can be generated on cyanobacteria cells by pumping water with cyanobacteria into a constructed deep pipe, as high pressure makes gas vesicles collapse and cyanobacteria sink [19,20]. This technique has been applied in eutrophic lakes such as Lake Taihu. The use of ultrasound close to the resonance frequency of gas vesicles can effectively remove cyanobacteria by collapsing gas vesicles [21,22]. These treatments collapse gas vesicles so that the cyanobacteria lose their vertical migration ability and settle into deep water with low light, low oxygen, low temperature and high hydrostatic pressure [20]. These methods can temporarily affect cyanobacteria, but it is possible that gas vesicle regeneration causes cyanobacteria to regain their vertical migration ability [23]. Therefore, it is important to know whether the gas vesicle of cyanobacteria will recover after being collapsed.

In order to study the recovery of gas vesicles after complete collapse, simulation experiments were carried out on three common phytoplankton. *Dolichospermum spiroides* (non-nitrogen-fixing cyanobacteria), *Microcystis aeruginosa* and *Planktothrix mougeotii* (nitrogen-fixing cyanobacteria) are three common phytoplankton that often form cyanobacterial blooms in lakes. The gas vesicle of these three phytoplankton was completely collapsed and then incubated in different trophic levels to observe the recovery of buoyancy. Cyanobacteria with disrupted gas vesicles will cause greater risk of cyanobacterial blooms if they regain buoyancy. This study will provide useful suggestions for the method of controlling cyanobacterial blooms by collapsing gas vesicles.

2. Materials and Methods

2.1. Phytoplankton and Experiment Conditions

Phytoplankton isolated from lakes were cultured using M11 medium. *Dolichospermum spiroides* was isolated from the Yanghe Reservoir, *Microcystis aeruginosa* was isolated from Lake Taihu, and *Planktothrix mougeotii* was isolated from Lake Kasumigaura. These phytoplankton were cultured in M11 medium (pH 8.0) at 25 ± 1 °C, under a 3000 lux white fluorescent light with a 12/12 h light/dark cycle. M11 medium consisted of 100 mg $NaNO_3$, 10 mg K_2HPO_4, 75 mg $MgSO_4 \cdot 7H_2O$, 40 mg $CaCl_2 \cdot 2H_2O$, 20 mg Na_2CO_3, 6 mg Fe citrate, and 1 mg $Na_2EDTA \cdot 2H_2O$ in 1 L deionized water.

The growth of phytoplankton in different trophic levels was simulated using nutrient-limited medium. Phytoplankton in exponential growth phase were treated at 1.0 MPa pressure for 5 min to completely collapse the gas vesicle, then phytoplankton were inoculated and incubated in 200 mL nutrient-limited M11 medium. Phytoplankton were cultured using M11 medium with 1 mg K_2HPO_4 to simulate a phosphorus-limited environment, while M11 medium with 10 mg $NaNO_3$ was used to simulate nitrogen-limited environment. Phytoplankton cultured in 200 mL M11 medium were set as control groups.

The cyanobacteria were cultured in M11 medium. Cyanobacteria were taken from the medium before the experiment and measured for the gas vesicle content, migration speed and percentage of floating cells; these data indicated the initial state of these cyanobacteria. Next, gas vesicles in cyanobacteria were collapsed and experiments were conducted. Cyanobacteria with collapsed gas vesicles were inoculated into 200 mL nutrient-limited M11 medium as an experimental group. At the same time, cyanobacteria with collapsed gas vesicles were inoculated into 200 mL M11 medium as a control group. The initial phytoplankton abundance of *Dolichospermum spiroides* (*D. spiroides*), *Microcystis aeruginosa* (*M. aeruginosa*), and *Planktothrix mougeotii* (*P. mougeotii*) in medium were more than 2×10^4 cells/mL, 1×10^5 cells/mL and 2×10^3 cells/mL, respectively. All treatments were performed in triplicate and manually shaken three times per day during the experiment. To reduce errors, control experiments were first carried out for nitrogen-limited and nitrogen-sufficient environments, followed by control experiments for phosphorus-limited and phosphorus-sufficient environments. Phytoplankton were cultured in 500 mL conical flasks at 25 ± 1 °C, under a 3000 lux white fluorescent light with a 12/12 h light/dark cycle. The gas vesicle content, migration speed and floating percentage were daily measured.

2.2. Measurement of Gas Vesicle Content

Gas vesicle content was measured under a light microscope using a modified capillary compression tube. The device consisted of a capillary pressure tube within a quartz pressure tube [24,25]. The volumes of the quartz pressure tube and capillary pressure tube were 2 mL and 5 μL, respectively. The 2 mL phytoplankton sample was injected into the capillary compression tube and treated under a pressure of 1.0 MPa for 5 min to make most gas vesicles collapse. The gas vesicle volume was calculated directly from the change in position of the meniscus in the capillary. Meanwhile, the number of cyanobacterial cells (*Dolichospermum spiroides* and *Microcystis aeruginosa*) or the length of algal filaments (*Planktothrix mougeotii*) were measured, andthese data were used to calculate the gas vesicle content per cell or per unit length.

2.3. Measurement of Migration Speed

To measure the migration speed, the phytoplankton samples were well mixed and placed into settling chambers. The chamber height for *M. aeruginosa* was 0.25 mm, and for *D. Spiroides* and *P. mougeotii* were 4 mm. The phytoplankton abundance at the top and bottom of the chambers after every time interval was photographed and counted under a microscope (Olympus BH-2). The photographs were taken under cold light illumination and the light was extinguished immediately thereafter to prevent illumination heat transfer. T_{50} (time when 50% of cells floated or settled) was used to calculate the migration speed as follows:

$$V = d/(2 \times T_{50}) \quad (1)$$

where d is the chamber height.

2.4. Percentage of Floating Cells Calculation

The percentage of floating cells was quantified using the Sedgwick–Rafter chamber and a microscope (Olympus BH-2). The chamber height for *M. aeruginosa* was 0.5 mm, for *D. spiroides* and *P. mougeotii* was 5 mm. The 1 mL phytoplankton sample was injected into the chamber and settling was allowed for 30 min, after which the number of cells at the top of the chamber and total number of cells were counted. The percentage of floating cells was calculated as the number of cells at the top of the chamber divided by the total number of cells.

2.5. Statistical Analysis

Independent t tests were performed with SPSS 22.0 (IBM, Armonk, NY, USA). The line charts and bridge chart were drawn with Origin 2020 (OriginLab, Northampton, MA, USA).

3. Results

3.1. Recovery of Gas Vesicle in Different Trophic Levels

Overall, recovery of gas vesicle was worse in nutrient-limited environments than in nutrient-sufficient environments for almost all these cyanobacteria (Figure 1).

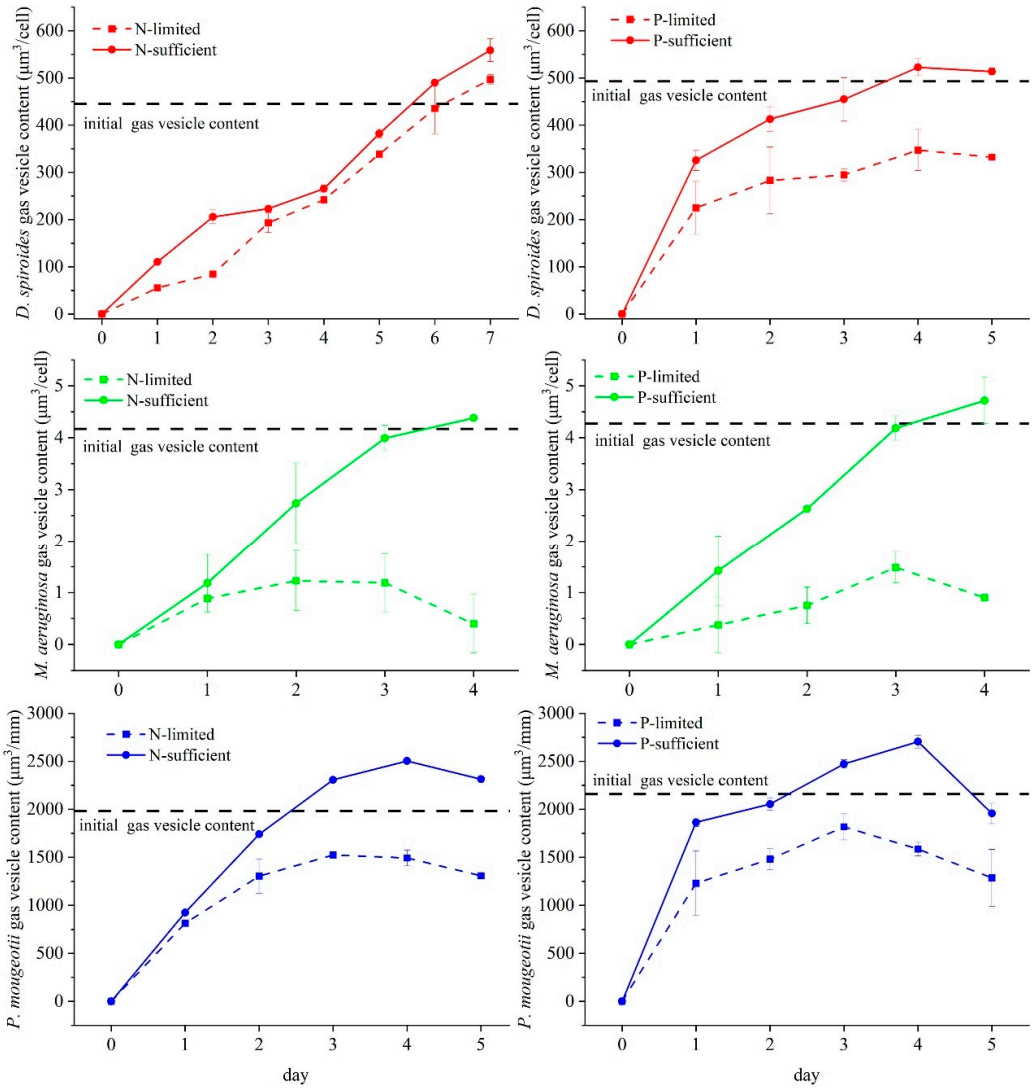

Figure 1. Recovery of cyanobacterial gas vesicle under different nutrient levels. The initial gas vesicle content is indicated by the black dashed line.

Nitrogen-limited environments had little effect on the gas vesicle recovery of *D. spiroides*, while the gas vesicle recovery of *M. aeruginosa* and *P. mougeotii* was much worse in nitrogen-limited environments than in nitrogen-sufficient environments. The gas vesicle content of *D. spiroides* in nitrogen-sufficient environments reached 489 μm^3/cell on day 6, exceeding the initial gas vesicle content 445 μm^3/cell. The gas vesicle content in nitrogen-limited environments also reached 497 μm^3/cell on day 7. There was no significant difference ($p > 0.05$) in gas vesicle content of *D. spiroides* under different nitrogen concentrations. The gas vesicle content of *M. aeruginosa* in nitrogen-sufficient environments reached 4.38 μm^3/cell on 4 d, exceeding the initial content of 4.17 μm^3/cell. The *M. aeruginosa* gas vesicle content in nitrogen-limited environments reached a maximum of 1.23 μm^3/cell on day 2 and then declined. Significant differences ($p < 0.05$) were found in the gas vesicle content of *M. aeruginosa* under different nitrogen concentrations on day 3 and day 4. The gas vesicle content of *P. mougeotii* in nitrogen-sufficient environments exceeded the initial content on day 3. But the gas vesicle content in nitrogen-limited environments reached a maximum of 1524 μm^3/mm on day 3, which

less than the initial content. Significant differences ($p < 0.05$) were found in the gas vesicle content of *P. mougeotii* under different nitrogen concentrations from day 3 to day 5.

Phosphorus-limited environments significantly inhibited the recovery of the gas vesicle content of all three cyanobacteria. The gas vesicle content of *D. spiroides*, *M. aeruginosa* and *P. mougeotii* in phosphorus-sufficient environments exceeded the initial gas vesicle content on day 4 and day 3, respectively. The gas vesicle content reached a maximum of 522 μm^3/cell, 4.72 μm^3/cell and 2705 μm^3/mm, respectively. In contrast, the gas vesicle content of all three cyanobacteria in phosphorus-limited environments were all lower than the initial gas vesicle content. *D. spiroides* reached a maximum gas vesicle content of 347 μm^3/cell on day 4, *M. aeruginosa* increased to 1.49 μm^3/cell on day 3 and *P. mougeotii* reached 1817 μm^3/mm on day 3. Significant differences ($p < 0.05$) were found in the gas vesicle content of *D. spiroides* under different phosphorus concentrations on the last day. From day 2 to day 4, the gas vesicle content of *M. aeruginosa* under different phosphorus concentrations showed significant differences ($p < 0.05$). Significant differences ($p < 0.05$) were found in the gas vesicle content of *P. mougeotii* under different phosphorus concentrations from day 2 to day 4.

3.2. Recovery of Migration Speed in Different Trophic Levels

The migration speed of cyanobacteria could barely return to the initial state in nutrient-limited environments, while their migration speed easily exceeded the initial state in nutrient-sufficient environments (Figure 2).

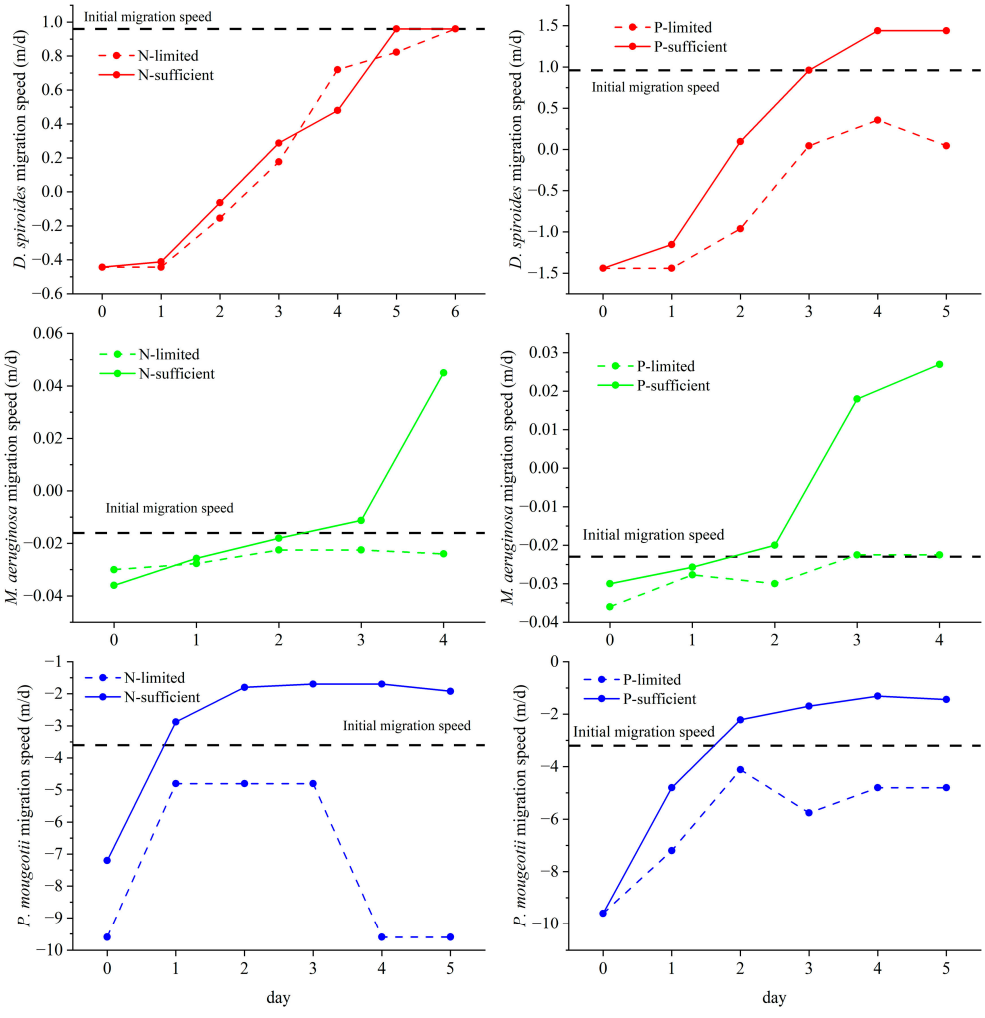

Figure 2. Recovery of cyanobacterial migration speed under different nutrient levels. The initial migration speed is indicated by the black dashed line.

Only *D. spiroides* of the three cyanobacteria could recover to the initial migration speed in a nitrogen-limited environment. In a nitrogen-sufficient environment, nitrogen-fixing *D. spiroides* recovered to the initial migration speed on day 5, at which point *D. spiroides* in a nitrogen-limited environment recovered to 85% of initial migration speed. *D. spiroides* in a nitrogen-limited environment returned to the initial migration speed on day 6 and reached 0.96 m/d. There was no significant difference ($p > 0.05$) in the migration rate of *D. spiroides* under different nitrogen concentrations. Non-nitrogen-fixing *M. aeruginosa* and *P. mougeotii* cannot recover to their initial migration speed in nitrogen-limited environments, while they recovered to their initial migration speed only on day 3 and day 1 in nitrogen-sufficient environments, respectively. The recovery of *M. aeruginosa* migration speed in nitrogen-limited environments was slow and the minimum settling speed of *M. aeruginosa* was 0.024 m/d, reached 1.5 times the initial settling speed. The recovery of *M. aeruginosa* migration speed in nitrogen-sufficient environment was faster, even reaching 0.045 m/d on day 4. *P. mougeotii* migration speed briefly recovered to −4.8 m/d in nitrogen-limited environments, but still reached 1.5 times the initial settling speed. *P. mougeotii* in nitrogen-sufficient environments reached a migration speed of −1.69 m/d on day 3, much slower than the initial settling speed. From day 1 to day 5, the migration speed of *P. mougeotii* under different nitrogen concentrations was significantly different ($p < 0.05$).

None of the three cyanobacteria in phosphorus-limited environments could recover to the initial migration speed. *D. spiroides* in phosphorus-sufficient environments returned to their initial migration speed on day 3 and then reached a maximum of 1.44 m/d on day 4. *D. spiroides* in phosphorus-limited environments reached the maximum migration speed on day 4, which was 37% of the initial migration speed. From day 2 to day 5, the migration speed of *D. spiroides* under different phosphorus concentrations was significantly different ($p < 0.01$). *M. aeruginosa* migration speed in phosphorus-sufficient environments increased continuously, exceeding the initial migration speed on day 2 and then reaching a maximum of 0.027 m/d on day 4. *M. aeruginosa* in phosphorus-limited environments recovered slowly, reaching minimum settling speed of −0.023 m/d on day 3, which was close to the initial settling speed. *P. mougeotii* in phosphorus-sufficient environments exceeded the initial migration rate on day 2, and the minimum settling speed was only 41% of the initial settling rate on day 4. *P. mougeotii* in phosphorus-limited environments reached a minimum settling speed of 1.3 times the initial settling speed on day 2, and maintained a high settling speed of −4.8 m/d after 4 d. From day 1 to day 5, the migration speed of *P. mougeotii* under different phosphorus concentrations was significantly different ($p < 0.01$).

3.3. Floating Percentage in Different Trophic Levels

The floating percentage of *D. spiroides* recovered slowly in nitrogen-limited environments, while *M. aeruginosa* and *P. mougeotii* were unable to float at all. *D. spiroides* in nitrogen-sufficient environments quickly recovered to more than 90% of the initial floating percentage on day 3 and recovery progress slowed down after that. The floating percentage of *D. spiroides* in nitrogen-limited environments slowly recovered to 90% on day 5. Finally, the floating percentage of *D. spiroides* under different nitrogen concentrations all recovered to 100% on day 6. The floating percentage of *D. spiroides* under different nitrogen concentrations was significantly different ($p < 0.01$) only on day 3 and day 4. No floating cells of *M. aeruginosa* and *P. mougeotii* in nitrogen-limited environments were observed during the experiment, while the floating percentage of *M. aeruginosa and P. mougeotii* in nitrogen-sufficient environments ended up at 47% and 11%, respectively. From day 2 to day 4, the floating percentage of *M. aeruginosa* under different nitrogen concentrations was significantly different ($p < 0.05$).

The recovery of floating percentage of all three cyanobacteria in phosphorus-limited environments was not as good as in phosphorus-sufficient environments. *D. spiroides* in phosphorus-limited environments started to recover after 1 d and reached a maximum floating percentage of 69% on day 4, while the floatation percentage continued to increase in phosphorus-sufficient environments and reached 100% on day 4. From day 3 to day 5,

the floating percentage of *D. spiroides* under different phosphorus concentrations was significantly different ($p < 0.05$). *M. aeruginosa* in phosphorus-limited environments started to recover after 3 d, reached a maximum floating percentage of 11% on day 4 and then declined. From day 2 to day 4, the floating percentage of *M. aeruginosa* under different phosphorus concentrations was significantly different ($p < 0.05$). *P. mougeotii* in phosphorus-limited environments failed to resume floating at all, while *P. mougeotii* in phosphorus-sufficient environments started to recover after 2 d and reached a maximum floating percentage of 19% on day 4. From day 3 to day 5, the floating percentage of *M. aeruginosa* under different phosphorus concentrations was significantly different ($p < 0.05$).

4. Discussion

4.1. Effect of Nutrient Limitation on the Recovery of Cyanobacterial Floating Capacity

For nitrogen-fixing cyanobacteria, nitrogen-limited environments had little effect on the recovery of floating capacity. On the other hand, for non-nitrogen-fixing cyanobacteria, nitrogen-limited environments significantly inhibited the recovery of floating capacity. The *gvp* gene cluster is a key gene that regulates gas vesicle synthesis, and nitrogen-limited environments block the synthesis of *gvp* [26,27]. Furthermore, studies have shown that the gas vesicle consists of a single layer of the 7–8 kDa protein gas vesicle protein A (GvpA), and nitrogen is an important component for protein synthesis [16,28]. Therefore, for cyanobacteria that cannot get sufficient nitrogen, it is difficult to synthesize enough gas vesicle quickly to restore buoyancy. *D. spiroides* is a nitrogen-fixing cyanobacteria and can use nitrogen in the air for its growth, so nitrogen-limited environments had little effect on the recovery of floating capacity. There were almost no significant differences in the recovery of gas vesicle content, migration speed and floating percentage of *D. spiroides* under different nitrogen concentrations ($p > 0.05$). In contrast, *M. aeruginosa* and *P. mougeotii* belong to non-nitrogen-fixing cyanobacteria; all the nitrogen they need came from the water, so nitrogen-limited environments had a significant effect on their floating capacity. There were significant differences in the recovery of gas vesicle content and floating percentage of *M. aeruginosa* under different nitrogen concentrations ($p < 0.05$). The recovery of gas vesicle content and migration speed of *P. mougeotii* under different nitrogen concentrations also had significant differences ($p < 0.05$).

Phosphorus-limited environments significantly inhibited the recovery of floating capacity of all three cyanobacteria. Although phosphorus is not a component of gas vesicle, it is closely related to the energy supply of cyanobacteria, so phosphorus limitation may also have an impact on gas vesicle and floating ability. Phosphorus-limited environments can reduce the maximum photosynthetic rate of some phytoplankton, which may limit the energy supply of cyanobacteria and affect gas vesicle recovery [29,30]. Soluble reactive phosphate is essential for the growth of cyanobacteria. Some cyanobacteria such as *Microcystis* need to live in symbiosis with phosphorus-solubilizing bacteria; the phytoplankton provide the bacteria with extracellular organic carbon and the bacteria provide the phytoplankton with soluble reactive phosphate [31,32]. In phosphorus-limited environments, cyanobacteria need more organic carbon in exchange for phosphorus., which may also limit the recovery of gas vesicle.

4.2. Characteristics of Different Cyanobacteria for Restoring Buoyancy

4.2.1. *D. spiroides*

Nitrogen limitation had little effect on buoyancy recovery of *D. spiroides*, while phosphorus limitation affected buoyancy recovery. The buoyancy recovery of *D. spiroides* is more resilient than that of *M. aeruginosa* and *P. mougeotii*. *D. spiroides* is nitrogen-fixing cyanobacteria, and nitrogen-limited environments had almost no effect on the recovery of *D. spiroides*. The gas vesicle recovery in the phosphorus-limited environment reached 65% of that in phosphorus-sufficient environments, much higher than that of *M. aeruginosa* and close to that of *P. mougeotii*. The migration speed in the phosphorus-limited environment reached 51% of that in the phosphorus-sufficient environment, also much higher than that of *M. aeruginosa* and close to that of *P. mougeotii*. Disrupting the gas vesicle of *D. spiroides*

and reducing the nitrogen concentration did not effectively reduce the competitive advantage of *D. spiroides*. Reducing the phosphorus concentration can limit the recovery of *D. spiroides* to some extent.

4.2.2. *M. aeruginosa*

Microcystis has a high migration speed that allows it to occupy the surface water of lakes even when waves are high. In turbid lakes where light is an important limiting factor for cyanobacteria growth, the high migration speed of *Microcystis* helps it to take over the competitive advantage [33,34]. In eutrophic lakes, phytoplankton do not need to sink near the sediment to obtain nutrients, so *Microcystis* with high upward migration speed is more likely to dominate [24].

Gas vesicle is not the only way by which *Microcystis* achieves buoyancy, as the formation of large colonies allows *Microcystis* to migrate faster. The maximum migration speed of *M. aeruginosa* in this experiment was 0.05 m/d, while some field observations found a maximum migration speed of *Microcystis* even exceeding 10 m/d [35,36]. This is due to the fact that *Microcystis* often forms large colonies which may migrate much faster than single cells [37]. Stokes' law describes the vertical migration speed of small and solid particles as follows:

$$v = kgD^2(\rho - \rho_{water})/18\ \mu \quad (2)$$

where v is the vertical migration speed, k is the fractal coefficient, g is the gravitational acceleration, D is the particle diameter, ρ is the particle density, ρ_{water} is the density of water, and μ the is viscosity of surrounding liquid. According to Stokes' law, it can be found that the larger the diameter of the colony, the greater the difference in migration speed between colony and single cell.

Since *Microcystis* can form large colonies, it is important to prevent *Microcystis* from regaining buoyancy, which would cause more severe cyanobacterial blooms. The little difference in migration speed of every single cell can cause a huge difference in colony migration speed. If *Microcystis* with collapsed gas vesicles quickly regain buoyancy and form large colonies, they may float much faster than before and lead to worse cyanobacterial blooms. Only if the buoyancy of *Microcystis* is not enough to float, the formation of colonies will cause *Microcystis* to sink faster and be unable to form cyanobacterial blooms.

4.2.3. *P. mougeotii*

Gas vesicle is one of the ways in which *Planktothrix* regulates buoyancy, and cell ballasts also have a significant effect on buoyancy. Three mechanisms of buoyancy regulation have been reported: change in cell ballasts, synthesis and dilution of gas vesicle and irreversible collapse of gas vesicle [4]. From the experimental results, it can be found that the migration speed and floating rate of *P. mougeotii* were not synchronized with the changes of gas vesicle content in different trophic levels (Figures 1–3). This phenomenon suggests that rapid changes in the content of sugars ($\rho \approx 1550$ kg/m^3), proteins ($\rho \approx 1330$ kg/m^3), nucleic acids ($\rho > 1660$ kg/m^3) and other cell ballasts also have a strong influence on the vertical migration of *P. mougeotii* [38].

Since *Planktothrix* can tolerate insufficient light environments, the collapsed gas vesicle seems to have little effect on the formation of cyanobacterial blooms. *Planktothrix* had a high sinking speed, which may be due to the fact that *Planktothrix* can obtain more nutrients near the sediment and Planktothrix can tolerate insufficient light [39,40]. These features make it unnecessary for *Planktothrix* to accumulate in surface water, but to occupy a different ecological niche from *Microcystis*.

Gas vesicle recovery of nitrogen-fixing cyanobacteria like *D. spiroides* may be less affected in nitrogen-deficient environments. Small differences in the buoyancy of single cells may lead to large differences in the buoyancy of colonies, so single-cell buoyancy is very important for colony-forming cyanobacteria such as *M. aeruginosa*. The effect of collapsing gas vesicle on the buoyancy of filamentous cyanobacteria such as *P. mougeotii* may not be significant.

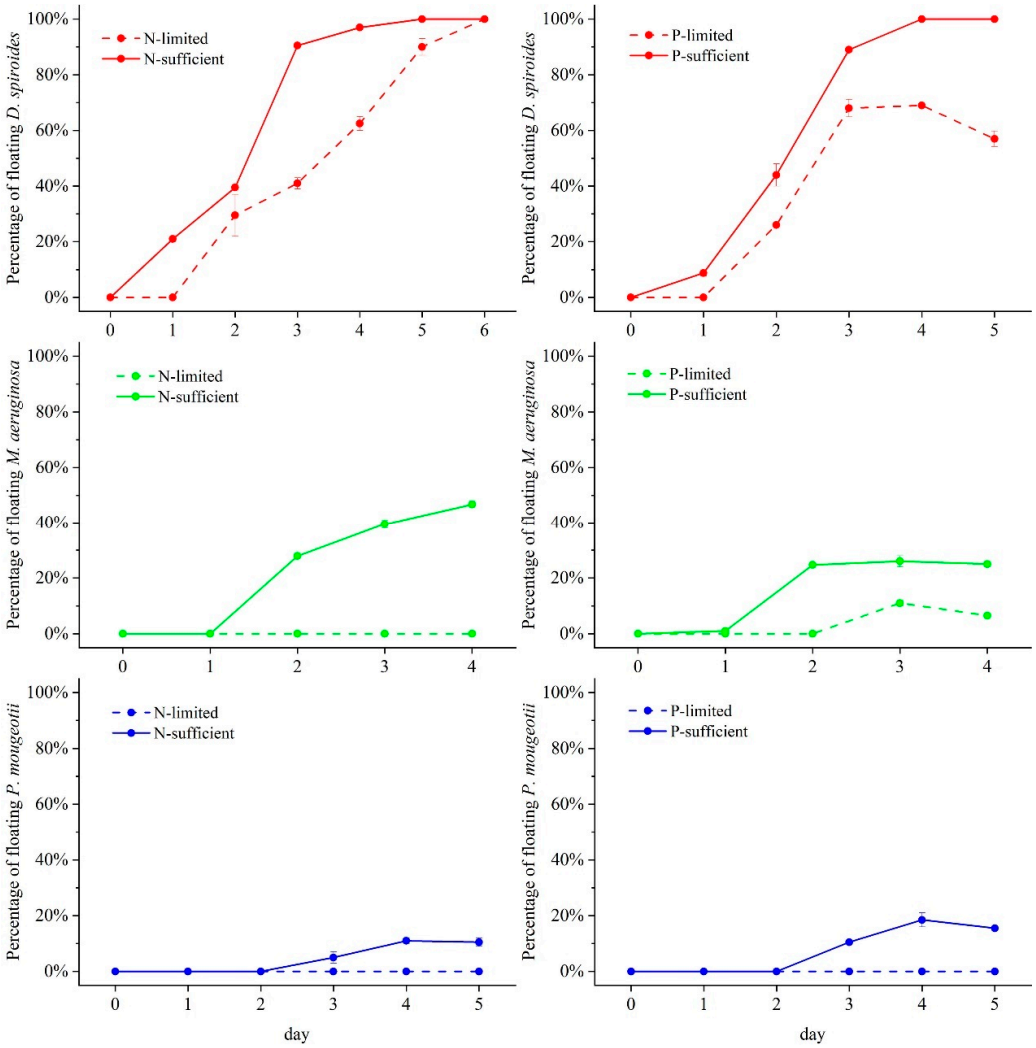

Figure 3. Recovery of cyanobacterial floating percentage under different nutrient levels.

4.3. Implications for Lake Management

Many studies have shown that cyanobacterial blooms are not due to rapid proliferation of phytoplankton, but rather due to rapid upwelling of phytoplankton and gathering in the surface water. Destruction of gas vesicles by water pressure or ultrasound can make phytoplankton temporarily lose buoyancy and control cyanobacterial blooms immediately. However, if the phytoplankton regains buoyancy, cyanobacterial blooms may occur rapidly again. Therefore, phytoplankton must be effectively eliminated by the right methods.

Discharging gas vesicles collapses cyanobacteria into surface water, and not reducing nutrient concentrations may make cyanobacterial blooms worse. Except for *P. mougeotii* which has a low light demand, all cyanobacteria sink less than 2.5 m before resuming upwelling in sufficient light and nutrient environments (Figure 4a,c). Such a small sinking distance will not cause cyanobacteria to be eliminated by lack of light or by the water pressure. The light limitation caused by sinking even increased the buoyancy, making it easier for cyanobacteria to upwell and form cyanobacterial blooms again [41,42]. For all three cyanobacteria, sufficient light and nutrients made the gas vesicle content and migration speed of cyanobacteria eventually higher than the initial state. This will cause more phytoplankton to accumulate on the surface water, forming a more severe cyanobacterial bloom than before.

Collapsing gas vesicles needs to be combined with other measures to effectively control cyanobacterial blooms. Disruption of gas vesicle is an effective method to eliminate cyanobacteria if carried out properly in deep lakes (mixing depth < mean depth). But in shallow lakes (mixing depth > mean depth), nutrient reduction must be done before

using this method. Our experiment has shown that cyanobacteria that have lost buoyancy can recover quickly in an environment with sufficient nutrients and light. Collapsing gas vesicles can only control cyanobacterial blooms in the short term. To control cyanobacterial blooms in the long term, collapsing gas vesicle must be combined with other measures. In deep lakes, lake managers are better to discharge gas-vesicle-ruptured cyanobacteria into deep water where water pressure can make it difficult for cyanobacteria to form gas vesicles (Figure 5a) [43]. In this way, lake managers can completely eliminate these cyanobacteria. Another way to limit the buoyancy recovery of cyanobacteria in eutrophic lakes is to limit light. Lake managers should discharge gas vesicles ruptured cyanobacteria below the euphotic layer, where the cyanobacteria continue to sink (Figure 4b,d) and cannot get enough energy to stay alive. However, this method may not be effective if the energy reserves in cyanobacteria are high. In shallow lakes, frequent hydrodynamic disturbance resuspends the cyanobacteria that lose buoyancy in surficial sediments into the water column. This process makes the gas-vesicle-ruptured cyanobacteria to obtain light and thus regain buoyancy. Therefore, in shallow lakes the disruption of gas vesicle alone cannot eliminate cyanobacteria. and other methods must be used to reduce the nutrient concentrations in the lake, especially phosphorus concentration (Figure 5b). If the nutrient concentration is not limited synchronously, the gas vesicle content and the upwelling speed can even exceed the initial state, making the cyanobacterial bloom worse (Figure 5c).

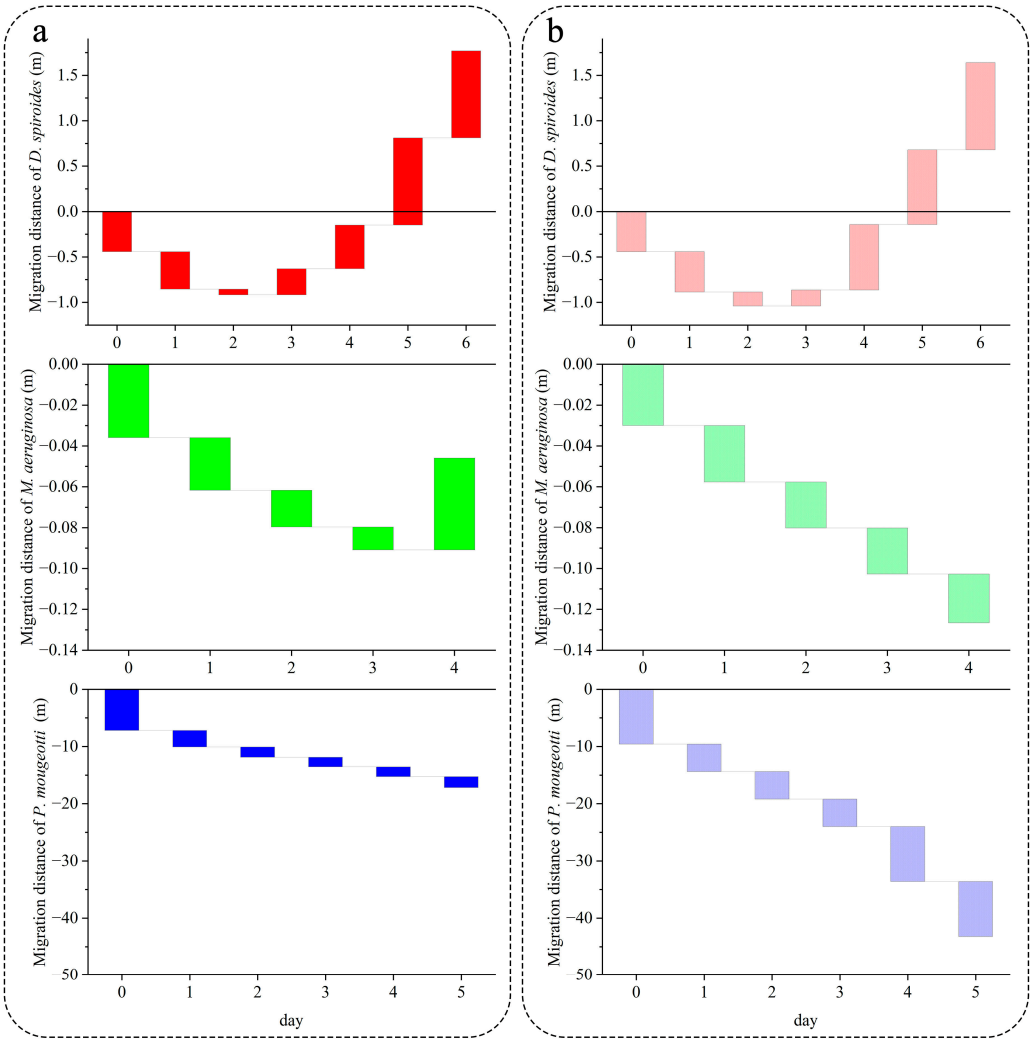

Figure 4. *Cont.*

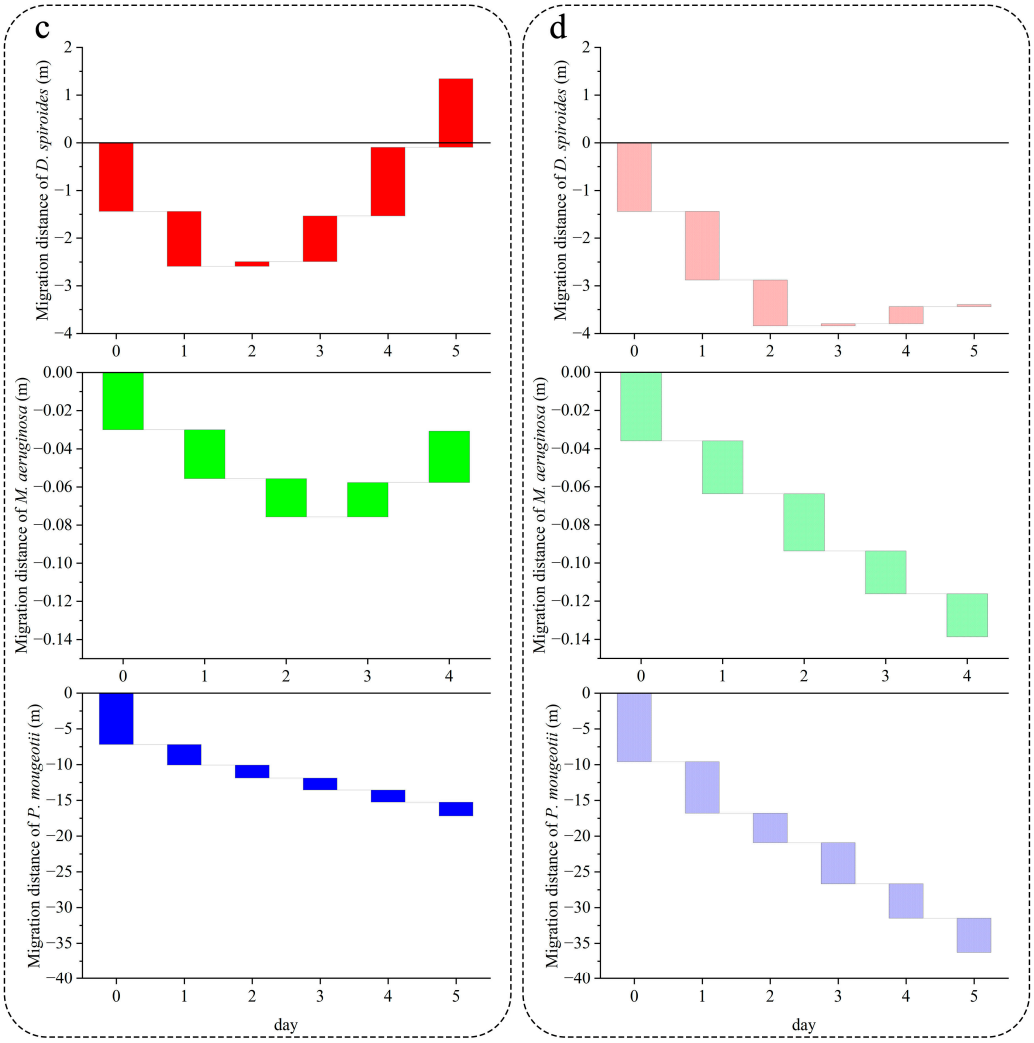

Figure 4. Migration distances of three cyanobacterial species in (**a**) nitrogen-sufficient, (**b**) nitrogen-limited, (**c**) phosphorus-sufficient, (**d**) phosphorus-limited environments.

Disruption of gas vesicle has different effects on different cyanobacteria. Nitrogen-limited environments have little effect on the recovery of nitrogen-fixing cyanobacteria such as *Dolichospermum*. In order to control these phytoplankton blooms, it is necessary to reduce phosphorus concentrations before destroying gas vesicles. The effect of nutrient limitation on the recovery of *Microcystis* is significant, and this effect will amplify when *Microcystis* forms large colonies. Decreasing nutrient concentration along with disrupting gas vesicle may be an effective way of controlling *Microcystis* bloom. Despite the fact that nutrient limitation slows the recovery of *Planktothrix*, *Planktothrix* can rapidly return to its initial migration speed. In addition, *Planktothrix* has a high sink speed and a low floating rate. Therefore, affecting the buoyancy of *Planktothrix* by disrupting gas vesicles may not be an effective way to control *Planktothrix* bloom.

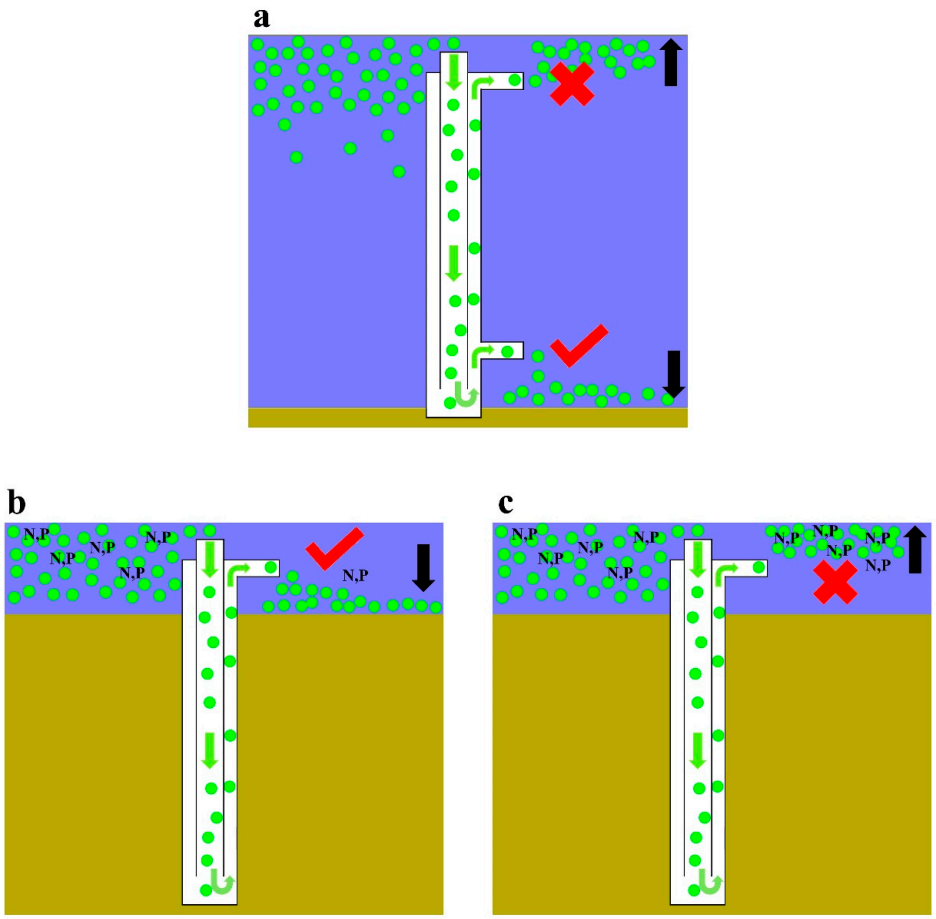

Figure 5. Cyanobacteria control in (**a**) deep lakes and (**b**,**c**) shallow lakes by collapsing gas vesicles. The check marks show the correct handling methods, while the cross marks show the incorrect handling methods.

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