



### Article Research on the Ecological Restoration Effects of a Vallisneria natans (Lour.) Hara-Dominated Multitrophic Level Ecosystem

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**Abstract:** This study aims to assess the ecological restoration effects of *Vallisneria natans* in a multitrophic level ecosystem. The water-purification effects of two hierarchical configuration modes of *V. natans-Bellamya aeruginosa* and *V. natans-B. aeruginosa-Hyriopsis cumingii* were studied. Results show that a *V. natans* and *B. aeruginosa* configuration ratio of 15:2 stabilizes water quality at Grade IV (TN  $\leq$  1.5 mg/L, TP  $\leq$  0.3 mg/L), and increasing *B. aeruginosa* density significantly reduces total phosphorus. The *V. natans, B. aeruginosa*, and *H. cumingii* configuration at 15:2:10 stabilizes water at Grade III (TN  $\leq$  1.0 mg/L, TP  $\leq$  0.2 mg/L), with a positive correlation between *H. cumingii* density and chlorophyll-a removal. Furthermore, the filtration and biocycling actions of *B. aeruginosa* (snails) and *H. cumingii* (mussels) significantly reduce levels of Total Nitrogen (TN), Total Phosphorus (TP), and Ammonium (NH<sub>4</sub><sup>+</sup>-N) in water, thus enhancing the self-purification capacity of the water bodies. However, the bioturbation effect of *H. cumingii* can temporarily increase phosphorus release from sediments, leading to a short-term rise in TP concentration in the water. Overall, the study concludes that multitrophic level ecosystems are effective in purifying water quality and offer significant ecological restoration benefits. This research provides crucial data support for future construction and ecological restoration projects involving multitrophic level approaches in China's rivers and lakes.

**Keywords:** aquatic plants; *Vallisneria natans*; multitrophic level; ecological restoration; freshwater ecosystem

### 1. Introduction

In recent years, rapid economic and urban development in China, coupled with excessive water resource exploitation and disorderly wastewater discharge, has exacerbated lake eutrophication. This issue is prevalent in over 80% of Chinese lakes, where excessive nutrient input leads to algal blooms and oxygen depletion, destabilizing lake ecosystems. Monitoring data from 2021 revealed that 27.1% of 210 major Chinese lakes and reservoirs were below Grade III (TN  $\leq 1.0 \text{ mg/L}$ , TP  $\leq 0.2 \text{ mg/L}$ ) water quality, including 5.2% at below Grade V (TN  $\leq 2.0 \text{ mg/L}$ , TP  $\leq 0.4 \text{ mg/L}$ ). Moderate and mild eutrophication were recorded in 62.2% and 23.0% of 209 major lakes and reservoirs, respectively [1]. Consequently, the restoration of eutrophic lake waters is crucial for water ecological protection



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**Copyright:** © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). and holds significant importance for the sustainable development of the Earth's ecological environment [2].

Previous studies primarily focused on using single-species aquatic plants for eutrophication restoration in rivers and lakes [3]. However, this approach could not establish stable aquatic ecosystems and might lead to secondary pollution due to overgrowth or decay of plants. Vallisneria natans is a perennial stemless submerged plant, which has the ecological functions of preventing sediment resuspension, absorbing nutrients such as nitrogen and phosphorus [4], and maintaining the stability of the aquatic ecosystem due to its strong tolerance, long lifespan, and root oxygen secretion. In addition, the V. natans likes shade and does not grow out of the water surface, which is easy to manage, and the water body it builds is cleaner [5], so it has become the preferred plant for creating a grass-shaped clear water body. Current research has shifted towards building aquatic ecosystems based on multitrophic level techniques [6,7]. This involves utilizing aquatic plants to absorb nitrogen and phosphorus, reducing eutrophication; providing oxygen through photosynthesis for aquatic animals; and using plants as food and breeding grounds for aquatic animals. Moderately introducing Bellamya aeruginosa, Hyriopsis cumingiis, and fish helps establish a relatively stable ecosystem [8]. The decomposition of their waste by microbes further assists in nutrient absorption by plants. The introduction of large mollusks can delay eutrophication and aid ecosystem recovery [9]. The CO<sub>2</sub> released through respiration is effectively utilized by aquatic plants, forming a self-cleansing ecosystem [10]. Hence, combining submerged plants with mollusks improves water quality, continually optimizing plant and mollusk communities to establish stable aquatic ecosystems [11,12]. Li et al. [13] found that reasonable densities of *B. aeruginosa* can promote the growth of *V. natans*. Li et al. [14] found that co-culturing Anodonta woodiana with V. natans significantly reduced water nitrogen, phosphorus, and phytoplankton chlorophyll levels, also enhancing V. natans growth. Wang et al. [10] demonstrated that specific ratios of Typha angustifolia L. and Margarya melanioides significantly lower water COD, total nitrogen, total phosphorus, ammonia nitrogen, and nitrite, effectively controlling water eutrophication. Xu et al. [15] found that ecological water purification systems combining Hygrophila ringens (L.) R. Br. ex Spreng., V. natans with Macropodus opercularis (Linnaeus) and Cipangopaludina chinensis effectively improved water quality in eutrophicated water bodies. Thus, restoring and managing aquatic ecosystems necessitates multi-species combinations to enhance pollutant removal efficiency and inhibit harmful organism growth [16]. Therefore, the biomanipulation restoration technique is viewed as a multidimensional strategy integrating physical, chemical, and biological methods [12,17]. These findings provide direction for the development and integration of the biomanipulation restoration technique, offering broad application prospects.

Recent studies have shown that using *B. aeruginosa* or *H. cumingii* combined with submerged plants can effectively decrease the concentration of nitrogen and phosphorus in polluted water bodies. However, there is limited research on the quantitative configuration of *B. aeruginosa* and *H. cumingii* with submerged plants and its impact on river and lake purification. This study builds a multi-tiered aquatic ecosystem, based on the action of nutrient absorption by aquatic plants and the flocculation of benthic animals. It uses commonly used species in river and lake ecological restoration projects: *V. natans, B. aeruginosa*, and *H. cumingii*. The study explores different biological configurations and density combinations for eutrophication water purification, aiming to find the optimal configuration and water quality balance range. This provides a theoretical reference for aquatic flora and fauna in restoring nutritious water bodies, contributing to the construction of a healthy and stable multi-tiered bio-ecosystem.

### 2. Materials and Methods

### 2.1. Materials

In the experiment, *V. natans*, *B. aeruginosa* (with an average weight of  $2.20 \pm 0.02$  g), and *H. cumingii* (with an average weight of  $40 \pm 0.4$  g) were sourced from the Fengjing base of Shanghai Taihe Water Environment Science and Technology Co., Ltd. in the Jinshan

District. These specimens were cleaned and temporarily maintained after acquisition. The water used in the experiment was sourced from the Minghu Lake at the Lingang campus of Shanghai Ocean University. The lake water was adjusted to a mildly polluted level for the initial experimental water by adding ammonium chloride (NH<sub>4</sub>Cl), potassium nitrate (KNO<sub>3</sub>), and dipotassium hydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>). The water quality indicators after treatment are shown in Table 1.

Table 1. Concentrations of nutrients of lake water at the beginning of this experiment.

Nutritive Salt	Nutrient Salt Concentration (mg/L)	Degree of Eutrophication
TN	$2.639\pm0.032$	V class [18]
TP	$0.289\pm0.006$	IV class [18]
NH4 <sup>+</sup> -N	$1.123\pm0.011$	IV class [18]

In the experiment, white plastic barrels with a capacity of 100 L (40 cm in diameter and 62 cm in height) were used as environmental containers for constructing underwater micro-ecosystems. Aeration devices were installed in these containers and used to disturb the water inside the device to simulate the natural water flow. Each experimental barrel was filled with 5 cm of quartz sand to stabilize the plant root system, and then 60 L of the prepared initial experimental water was added. The water quality conditions of each system were controlled to be essentially consistent. During the experimental period, water samples from each treatment group were collected every two days, the 500 mL sampling bottle was used, a GF/F glass fiber filter membrane with a pore size of 0.45  $\mu$ m was used for filtration, and various physicochemical indicators of the water were measured.

### 2.2. Experimental Design

The experiment was conducted indoors with water temperature controlled at  $24 \pm 2$  °C. The LED light source was set on the water surface, the illumination intensity was 5000 lx, and the illumination time was 12 h:12 h. There were two treatment groups: the *V. natans-B. aeruginosa* group and the *V. natans-B. aeruginosa-H. cumingii* group. The biological materials used in this experiment were similar in size, with three replicates in each group. The experimental period lasted for 24 days.

In the *V. natans-B. aeruginosa* experimental group, the biomass of *V. natans* was fixed at 180 g/group. This group included one blank control group (CK), one *V. natans* control group, and three *B. aeruginosa* density groups, each with three replicates. The density gradients of *B. aeruginosa* in the control and experimental groups were 0, 12, 24, and 48 g/group, denoted as L0, L1, L2, and L3 groups respectively.

In the *V. natans-B. aeruginosa-H. cumingii* experimental group, the biomass of *V. natans* and *B. aeruginosa* was fixed at 180 g (about 60 plants) and 24 g/group respectively. This group also included one blank control group (CK), one *V. natans-B. aeruginosa* control group, and three density gradient groups, each with three replicates. The density gradients of *H. cumingii* in the control and experimental groups were 0, 60, 120, and 180 g/group, denoted as B0, B1, B2, and B3 groups respectively.

### 2.3. Measurement Methods

In the study, the following analytical methods were employed, adhering to specific standards and protocols. Total nitrogen was measured using the alkaline potassium persulfate oxidation UV spectrophotometry method as per HJ636-2012 [19]. Total phosphorus was determined using the ammonium molybdate spectrophotometric method according to GB 11893-89 [19]. Ammonium nitrogen was analyzed using the Nessler's reagent spectrophotometric method as outlined in HJ 535-2009 [19]. Chlorophyll a was measured using the ethanol spectrophotometry method [20]. Chlorophyll fluorescence parameters are helpful in analyzing the parts of plant photosynthetic structure that are affected, and the photochemical efficiency of the photosystem (PS) is an important parameter to indicate the status of photochemical reaction. The chlorophyll fluorescence parameter Fv/Fm reflects

the conversion efficiency of primary light energy in the PS center, which can be directly used as an indicator of photochemical efficiency, representing the maximum quantum yield of plants. Y(II) reflects the light quantum yield for conversion and dissipation generated by the PS reaction center, namely the effective quantum yield of the plant [21]. The chlorophyll fluorescence parameters were measured by a two-channel chlorophyll fluorometer (Dual-Pam-100, WALZ, Effeltrich, Germany), and the main indexes were Fv/Fm, Y(II).

### 2.4. Data Analysis

In this study, data preprocessing was conducted using Microsoft Excel 2024 v4.3.4.28 software. Origin 2023b software was employed for data analysis, processing, and graphing.

### 3. Results

### 3.1. The Purification Effect of V. natans and B. aeruginosa Configuration on TN in Water Bodies

Figure 1 presents the results of TN removal in eutrophic water by the configuration of V. natans and B. aeruginosa. As shown, the concentration of TN in the experimental water generally showed a decreasing trend over time. In the CK control group, the TN concentration initially increased to 2.806 mg/L within the first four days and then gradually decreased to 2.267 mg/L, with a self-purification rate of 14.13%. The L0 group exhibited the best purification effect. The TN concentration in this group decreased rapidly within the first 8 days, reaching its lowest on the 12th day at 1.178 mg/L, which corresponds to a purification rate of 55.92%. However, it increased again, reaching 1.525 mg/L by day 24, and the purification rate was 42.91%. The L1 and L2 groups showed TN concentration changes similar to L0. The L1 group's concentration decreased to 1.318 mg/L on the 12th day, with a purification rate of 49.90%, and then slightly increased, reaching the lowest level of 1.121 mg/L on the 24th day. The L2 group's concentration decreased to 1.162 mg/L on the 16th day, with a purification rate of 55.48%, and then slightly increased, reaching the lowest level of 1.152 mg/L on the 24th day. In contrast, the L3 group showed a noticeably lower TN purification efficiency than the L1 and L2 groups, reaching its lowest concentration of 1.406 mg/L on the 16th day, with a purification rate of only 47.16%, and then gradually increasing.



**Figure 1.** The effect of *V. natans-B. aeruginosa* matching on TN purification. The ordinate represents the average value of the nutrient concentration, and the abscissa represents the number of experiment days. All data were expressed as mean  $\pm$  standard deviation.

From the above, it can be observed that on the 16th day, the TN concentration in the L2 group was significantly lower than in the L1 and L3 groups (p < 0.05), and there was no significant difference compared to the L0 group (p > 0.05). At the full 24 days of the experiment, the total nitrogen concentrations of the L1 and L2 groups were significantly lower than those of the L0 and L3 groups, and there was no significant difference in the

total nitrogen concentrations between the L1 and L2 groups (p > 0.05). On the 24th day, the total nitrogen removal rate of the L2 group was 12.95% higher than that of the L0 group. This indicates that the L2 group had a better TN removal effect than the other experimental groups. Thus, it is evident that a combination of *V. natans* and *B. aeruginosa* at a low or medium density has a more effective TN removal capability.

### 3.2. The Purification Effect of V. natans and B. aeruginosa Configuration on NH4<sup>+</sup>-N in Water Bodies

Figure 2 shows the purification results of  $NH_4^+$ -N in eutrophic water bodies by the configuration of *V. natans* and *B. aeruginosa*. As depicted, the  $NH_4^+$ -N concentration in the water bodies of all experimental groups generally showed a declining trend over time. In the CK control group,  $NH_4^+$ -N concentration slowly decreased to 0.873 mg/L, with a self-purification rate of 22.19%. The  $NH_4^+$ -N concentrations in the L1 and L2 groups gradually decreased, whereas in the L0 group, the  $NH_4^+$ -N concentration significantly decreased to 0.476 mg/L by day 12 (p < 0.05), performing better than other groups, but showed little change thereafter, and the purification of ammonia nitrogen removal rate at 24 days was 57.64%. Among these, the L2 group exhibited the best purification effect, with the  $NH_4^+$ -N concentration decreasing from 1.134 mg/L to 0.294 mg/L within 24 days, achieving a purification rate of 74.28%. The L3 group had the least effective purification, almost similar to the control group, where the  $NH_4^+$ -N concentration initially increased before decreasing.



**Figure 2.** The effect of *V. natans-B. aeruginosa* matching on  $NH_4^+$ -N purification. The ordinate represents the average value of the nutrient concentration, and the abscissa represents the number of experiment days. All data were expressed as mean  $\pm$  standard deviation.

From these results, it is evident that by day 24 of the experiment, the NH<sub>4</sub><sup>+</sup>-N concentration in the L2 group was significantly lower than that in the L0 group and L3 groups (p < 0.01), and the ammonia nitrogen removal rate in the L2 group was 16.64% higher than that in the L0 group, indicating that the combination of *V. natans* and *B. aeruginosa* at a moderate density was most effective in absorbing ammonia nitrogen.

3.3. The Purification Effect of V. natans and B. aeruginosa Configuration on Total Phosphorus (TP) in Water Bodies

Figure 3 presents the results of TP purification in eutrophic water bodies by the configuration of *V. natans* and *B. aeruginosa*. As indicated in Figure 3, the TP concentration in the CK control group slightly decreased, with a self-purification rate of only 9.22%. In the L0 group, the TP concentration initially decreased and then increased, significantly dropping to 0.083 mg/L within the first 8 days, followed by a gradual rise and a marked increase on day 24 (p < 0.05), and the removal rate was 58.42%. In the L1, L2, and L3 groups, the TP concentration consistently declined over time. Within the first 12 days, the concentration rapidly decreased in each group. Subsequently, the L3, L2, and L1 groups showed a slow decline, with the L3 group had decreased to the lowest level of 0.024 mg/L, corresponding to a purification rate of 91.61%. The lowest TP concentrations and purification rates for the L2 and L1 groups were 0.043 mg/L at 85.12% and 0.063 mg/L at 78.79%, respectively.



**Figure 3.** The effect of *V. natans-B. aeruginosa* matching on TP purification. The ordinate represents the average value of the nutrient concentration, and the abscissa represents the number of experiment days. All data were expressed as mean  $\pm$  standard deviation.

From these results, it is evident that the TP concentration in the L0 group decreased to the lowest within the first 8 days, but subsequently was significantly higher than the other experimental groups (p < 0.05). Over the 24 days, the TP purification rates of the L1, L2, and L3 groups progressively increased, and the total phosphorus removal rate of the L3 group was 33.19% higher compared to the L0 group, indicating that introducing *B. aeruginosa* helps reduce the TP concentration in water bodies, with higher densities yielding better results.

# *3.4. The Impact of V. natans and B. aeruginosa Configuration on the Photosynthetic Physiology of V. natans*

Figure 4 illustrates the changes in Fv/Fm and Y(II) of *V. natans* leaves under the configuration with *B. aeruginosa*. As shown in Figure 4a, there were no significant differences in Fv/Fm values between different experimental groups (p > 0.05). However, there was a general decline in Y(II) values (Figure 4b). On day 24, the Y(II) value of *V. natans* leaves in the control group decreased to 0.136, which was significantly lower than that in the

experimental groups (p < 0.05), representing a decrease of 27.27% compared to day 0. In the L1 group, the Y(II) value on day 24 was 0.154, a decline of 17.20% compared to day 0. While there was a decrease in Y(II) in the L2 and L3 groups, it was not as pronounced, with the overall trend showing a decrease over time, more noticeably in groups with lower densities.





From these results, it is evident that the presence of *B. aeruginosa* promotes the PSII photochemical efficiency of *V. natans* leaves.

# 3.5. The Purification Effect of V. natans, B. aeruginosa, and H. cumingii Configuration on TN in Water Bodies

Figure 5 depicts the purification results of TN in eutrophic water bodies under the configuration of *V. natans*, *B. aeruginosa*, and *H. cumingii*. As indicated in Figure 5, the TN concentration in all experimental groups showed a decrease. In the CK control group, the TN concentration initially increased to 2.938 mg/L within the first four days, then

gradually decreased to 2.227 mg/L by day 24, resulting in a self-purification rate of 24.20%. The TN concentrations in the experimental groups decreased rapidly in the first six days. The TN removal rates for the B0, B1, B2, and B3 groups were 39.9%, 39.4%, 41.7%, and 37.1%, respectively, with no significant difference from the B0 group (p < 0.05). On day 24, the TN removal rates for the B0, B1, B2, and B3 groups reached 55.70%, 69.75%, 67.33%, and 49.04%, respectively. The B1 group exhibited the best TN removal effect, reducing the TN concentration to 0.780 mg/L, followed by B2 and B0, with the B3 group being the least effective.



**Figure 5.** Effect of *V. natans-B. aeruginosa-H. cumingii* matching on TN purification. The ordinate represents the average value of the nutrient concentration, and the abscissa represents the number of experiment days. All data were expressed as mean  $\pm$  standard deviation.

From these results, at 24 days, the total nitrogen concentration in the B1 group was the lowest, significantly lower than the B0 and B3 groups (p < 0.01), with no difference from the B2 group (p > 0.05), and the B3 group was significantly higher than the B1 and B2 groups (p < 0.01). It can be concluded that adding an appropriate density of *H. cumingii* to the *V. natans* and *B. aeruginosa* base helps in TN removal. However, an excessively high density of *H. cumingii* is counterproductive for TN removal.

# 3.6. The Purification Effect of V. natans, B. aeruginosa, and H. cumingii Configuration on $NH_4^+$ -N in Water Bodies

Figure 6 displays the results of  $NH_4^+$ -N purification under the configuration of *V. natans, B. aeruginosa*, and *H. cumingii*. As shown in Figure 6, the  $NH_4^+$ -N concentration in all experimental groups showed a decreasing trend, while the CK control group exhibited a slight decline, with  $NH_4^+$ -N concentration dropping to 0.883 mg/L by day 24, resulting in a self-purification rate of 25.92%. Comparatively, the B0, B1, and B2 groups showed better purification effects. There was a slight increase in  $NH_4^+$ -N concentration on day 2 (except for B0), followed by a gradual decrease, with  $NH_4^+$ -N removal rates of 74.34%, 71.45%, and 71.45% respectively, by day 24. In contrast, the B3 group showed a slower downward trend in  $NH_4^+$ -N concentration, with a removal rate of only 54.23% by day 24.



**Figure 6.** Effect of *V. natans-B. aeruginosa-H. cumingii* matching on  $NH_4^+$ -N purification effect. The ordinate represents the average value of the nutrient concentration, and the abscissa represents the number of experiment days. All data were expressed as mean  $\pm$  standard deviation.

At 24 days, there was no significant difference in ammonia nitrogen concentration between the B0, B1, and B2 groups (p < 0.05), and the ammonia nitrogen concentration in the B3 group was significantly higher than that in other experimental groups (p < 0.01). From these results, it can be inferred that adding *H. cumingii* to the *V. natans* and *B. aeruginosa* base had no significant effect on the removal of NH<sub>4</sub><sup>+</sup>-N.

# 3.7. The Purification Effect of V. natans, B. aeruginosa, and H. cumingii Configuration on TP in Water Bodies

Figure 7 presents the results of TP purification under the configuration of *V. natans*, *B. aeruginosa*, and *H. cumingii*. As indicated in Figure 7, the TP concentration in the CK control group showed almost no decline, with a self-purification rate of only 9.22%. In contrast, the B0 group exhibited a continuous downward trend, achieving the best TP removal effect with a removal rate of 83.07%. For the B1, B2, and B3 groups, the TP concentration initially decreased and then increased. In the B1 and B2 groups, the TP concentration reached its lowest on day 8, with the lowest TP concentrations and purification rates being 0.137 mg/L at 49.45% and 0.116 mg/L at 59.86%, respectively. On day 20, the TP concentrations increased to 0.193 mg/L and 0.215 mg/L, respectively, and showed a slight decrease on day 24, with the lowest TP concentrations and purification rates being 0.187 mg/L at 31.00% and 0.199 mg/L at 31.42%, respectively. In the B3 group, the TP concentration decreased to its lowest at 0.141 mg/L on day 6, with a purification rate of 48.91%, and then continuously increased to 0.270 mg/L.



**Figure 7.** Effect of *V. natans-B. aeruginosa-H. cumingii* matching on TP purification. The ordinate represents the average value of the nutrient concentration, and the abscissa represents the number of experiment days. All data were expressed as mean  $\pm$  standard deviation.

# 3.8. The Removal Effect of V. natans, B. aeruginosa, and H. cumingii Configuration on Chla in Water Bodies

Figure 8 illustrates the changes in *Chla* concentration in water bodies within the *V. natans, B. aeruginosa,* and *H. cumingii* configuration systems. As shown in Figure 8, the *Chla* concentration in all experimental groups demonstrated a decreasing trend, with higher densities of *H. cumingii* leading to more effective *Chla* removal. In the B0 control group, the *Chla* concentration at 24 h was 8.055  $\mu$ g/L, with a removal rate of 15.70%. The B3 group showed the best *Chla* removal effect, with a *Chla* concentration of 3.276  $\mu$ g/L at 24 days, translating to a removal rate of 65.71%, which was significantly lower than the control group.



**Figure 8.** Effect of *V. natans-B. aeruginosa-H. cumingii* matching on *Chla* concentration change in water bodies. The ordinate represents the average chlorophyll concentration in the water, and the abscissa represents the number of experimental days. Different lowercase letters indicate significant differences between the treatment groups (p < 0.05). All data were expressed as mean  $\pm$  standard deviation.

### 4. Discussion

### 4.1. Mechanism of Nutrient Removal in V. natans and B. aeruginosa Configuration

This study found that in an artificially constructed ecosystem with *V. natans* at a density of 3 g/L, when the plant biomass: the surface area of the lake is 1.4:1, that is, the planting area is  $1.4 \text{ kg/m}^2$ , the optimal removal rate of total nitrogen and total phosphorus is achieved. the maximum removal rates for TN and TP were 55.92% and 72.05%, respectively. These findings align with Huang et al. [22], which showed that the removal rates of nitrogen and phosphorus nutrients by submerged plants increased with planting density in the range of 0.5–3.0 g/L. Aquatic plants play a dominant role in nutrient removal in artificially constructed ecosystems [23,24]. The addition of benthic animals, such as *B. aeruginosa*, further enhances the water purification capability of the ecosystem [25,26]. After the addition of 24 g of *B. aeruginosa* in an artificially constructed ecosystem with a *V. natans* and *B. aeruginosa* was 15:2, the system removal rate of TN was 55.86%, and the TP removal rate was 85.12%. This efficiency is attributed to the flocculation effect of *B. aeruginosa* and the filtration and scraping actions of benthic animals as primary phosphorus removal methods, consistent with the findings of Pu et al. [27].

### 4.2. Mechanism of Nutrient Removal in V. natans, B. aeruginosa, and H. cumingii Configuration

After the addition of *H. cumingii*, the experimental group showed no significant difference in TN removal compared to the B0 group but demonstrated a noticeable effect compared to the control group (CK). This aligns with the findings of Li et al. [28], who reported average removal rates of TN and  $NH_4^+$ -N by different densities of river *H. cumingii* and *B. aeruginosa* as 31.98% and 20.97%, respectively. In our experiment, the addition of medium-density *B. aeruginosa* and *H. cumingii* led to removal rates of 67.3% for TN and 71.5% for  $NH_4^+$ -N, indicating the role of submerged plants in the system. After introducing *H. cumingii*, TP concentration in the system initially decreased and then increased, likely due to phosphorus absorption by phytoplankton and subsequent release upon their death [29].

Additionally, at the beginning of the experiment, the bioturbation effect of *H. cumingii* was not strong, but after it had adapted to the environment, the bioturbation became strong, promoting the release of phosphorus from sediment into the water, similar to findings by Li et al. [30], resulting in an initial decrease and subsequent increase in total phosphorus content in the water. It has also been found that the secretion, excretion, and metabolic behaviors of mussels lead to the release of dissolved nitrogen and phosphorus nutrients [31]. In the process of filter-feeding suspended solids, the unabsorbed parts of the body settle to the bottom of the water in the form of excreta and "fake feces", and the nitrogen and phosphorus nutrients from the overlying water also enter the sediment, changing the physical and chemical properties of the sediment [32]. However, the sediment disturbance behaviors such as mussel burrowing and migration promoted the resuspension of sediment, resulting in the rerelease of nitrogen and phosphorus nutrients in the sediment. Zhang et al. [33] found that the bioturbation behavior of Anodonta woodiana accelerated the release of phosphorus nutrients from the sediment and increased the total phosphorus content of the overlying water. In addition, dissolved oxygen also determines the deposition and release of phosphorus between water and sediment. When the dissolved oxygen content of the water body decreases at night, the sediment also releases phosphorus, causing the total phosphorus content of the water body to rise [34].

### 4.3. Interactions and Synergistic Restoration Effects among Organisms

*Fv/Fm*, representing the maximum photochemical efficiency of PSII, changes minimally under non-stress conditions and is a key indicator of the extent to which plant photosynthetic light reactions are inhibited under various stresses [35,36]. In PSII reaction centers, absorbed light energy mainly contributes to three types of transformation and dissipation, including Y(II), the effective quantum yield [37]. The interaction between *V. natans* and *B. aeruginosa* is evident in the removal of algae attached to leaves. In the

L0 group without *B. aeruginosa*, Y(II) of *V. natans* leaves began decreasing on the 12th day, possibly due to the impact of *V. natans* on photosynthesis, leading to leaf decay and increased TN concentration [11]. In contrast, *B. aeruginosa* introduced in the experimental group facilitated photosynthesis by grazing on the algae and provided oxygen for the *B. aeruginosa*, aiding in algae removal. Therefore, no significant decrease in Y(II) was observed in the L2 and L3 groups.

The interaction between *V. natans* and *H. cumingii* is reflected in the removal of phytoplankton, with increasing *H. cumingii* density significantly enhancing algal removal, because the mussels can feed on planktonic algae in water through filter feeding [38]. By the 24th day, the B3 group with *H. cumingii* showed a significantly higher algal removal rate of 65.71% than other groups, and the *Chla* concentration decreased from 9.555  $\mu$ g/L to 3.276  $\mu$ g/L. This is consistent with the finding of Xu et al. [38] that algal removal by *H. cumingii* increased with *H. cumingii* density. However, higher *H. cumingii* density is not necessarily better; while high-density *H. cumingii* aids in *Chla* removal, it is less effective for nutrient removal, aligning with the results of Hu et al. [39], suggesting an optimal density range for effective control of phytoplankton growth. The decay of aquatic plants produces residues that can cause secondary pollution to the aquatic environment.

However, the presence of a decomposer community helps mitigate the secondary pollution caused by plant decay in the aquatic environment. Microorganisms and invertebrates in the aquatic environment are the main decomposers of aquatic plant residues. Insoluble substances in aquatic plant residues are absorbed through the decomposition by microorganisms and invertebrates [40]. On one hand, microorganisms can promote the transfer of nutrients to invertebrates [41]; on the other hand, microorganisms may also compete with invertebrates by absorbing detritus through mineralization [42]. Furthermore, appropriate amounts of plant residues can improve the cycling process of nutrients such as nitrogen and phosphorus in the water environment, preventing the deterioration of water quality. Aquatic plant residues can serve as a carbon source, addressing the imbalance of the carbon-to-nitrogen ratio in ecosystems when dealing with non-point source pollution [42,43]. Wu et al. [44] found that the decomposition of *Typha orientalis* C. Presl might facilitate the removal of nitrogenous nutrients in wastewater in constructed wetlands. Zhuang et al. [45] discovered that residues of sweet flag (Acorus calamus) could be used as an external carbon source in wetlands, with 48.43% of the released TOC being utilized by microorganisms for denitrification.

In addition to the filter-feeding effect of *H. cumingii*, submerged plants also play an important role in controlling the biomass and community structure of planktonic algae. Submerged plants can compete with planktonic algae in nutrients, light, and space, and can control planktonic algae biomass and community structure through their allelopathic algae inhibitions. Xian et al. [46] extracted algae-inhibitory substances such as 2-ethyl-3-methylmaleimide from the leaves of *V. spiralis*, and the phenolic acids extracted from the leaves of *V. spiralis* could significantly inhibit the growth of *Microcystis aeruginosa* [47].

The grass-snail combination can not only improve water quality but also help promote the growth of bitter grass. The coexistence of grass-snail-mussel has a better effect on reducing the biomass of planktonic algae in water. Therefore, the introduction of submerged plants and filter-feeding shellfish at the same time in the restoration of eutrophication water bodies can accelerate the ecological restoration of eutrophicated shallow lakes. The combination forms of submerged plants and benthic organisms are diverse, and different biological combinations play different roles in the restoration of water bodies. Therefore, in practical application, attention should be paid to the selection of submerged plants and benthic animals, avoiding the introduction of invasive species, and selecting suitable biological materials according to local climate and hydrological conditions. The high productivity and biomass of submerged plants can quickly and stably absorb nitrogen and phosphorus nutrients in the environment, effectively alleviating eutrophication in water bodies [48]. However, when the nutrients in the water environment continue to increase, the nitrogen and phosphorus content in plants will reach their saturation content [49], and the absorption capacity of nitrogen and phosphorus will also decrease. Therefore, nitrogen and phosphorus can be removed from the water environment by mowing, and these submerged plants can also be used as aquaculture raw materials and fertilizers [50].

### 5. Conclusions

In this study, we simulated eutrophication water in the natural environment and compared the purification effects of the combination of submerged plants, snails, and mussels at different densities on water quality by simulating eutrophication water bodies in the natural environment and using the common local *V. natans* and *B. aeruginosa* and *H. cumingii* as experimental materials, and screened out the density combinations with the best treatment effect. The experiment aimed to create a comprehensive water purification system in lakeside wetlands using "Aquatic Plants-Benthic Organisms" by combining different densities of aquatic plants and benthic organisms. The results indicated:

- 1. In the *V. natans* and *B. aeruginosa* configuration, the optimal biomass ratio of 15:2 resulted in the highest total nitrogen removal rate of 55.86%, ammonia nitrogen removal of 74.28%, and total phosphorus removal of 91.61%. The *B. aeruginosa* can scrape the attached algae on the leaves of *V. natans*, promote the photosynthesis of *V. natans*, and improve the absorption capacity of *V. natans* to nutrients in the water body.
- 2. In the *V. natans*, *B. aeruginosa*, and *H. cumingii* configuration, the optimal biomass ratio of 15:2:10 achieved a total nitrogen removal rate of 69.75%, ammonia nitrogen removal of 71.45%, and a *Chla* removal rate of 50.48%, showing significant inhibition of phytoplankton.
- 3. The *V. natans, B. aeruginosa,* and *H. cumingii* configuration demonstrated superior water quality compared to the *V. natans* and *B. aeruginosa* configuration, with a 13.89% increase in total nitrogen removal following the addition of *H. cumingii*.
- 4. After adding the *H. cumingii* to the system, there was no significant effect on the removal of ammonia nitrogen in the water body, and the total phosphorus concentration showed a changing trend of first increasing and then decreasing; the higher the density of the *H. cumingii*, the greater the upward trend. Therefore, in the practical application stage, the density of *H. cumingii* should be carefully selected.

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