Effects of different aquaculture methods for introduced bivalves (*Hyriopsis cumingii*) on seston removal and phosphorus balance at the water–sediment interface

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**ABSTRACT**

A field simulation experiment was conducted to test the hypothesis that altering bivalve aquaculture methods could promote the restoration of eutrophic waterbodies. We used three aquaculture methods – benthic aquaculture, suspended aquaculture, and macrophytes/bivalve combined aquaculture – to (1) investigate their effects on seston removal through monitoring water turbidity, pelagic and benthic algae, and (2) compare their impacts on the phosphorus (P) balance at the water–sediment interface by determining different forms of P contents in the water and sediments. The results showed that the seston removal effects did not differ significantly among these three aquaculture methods. Furthermore, the changes of all investigated P parameters in the water and sediments showed that P release occurred in the benthic and suspended aquaculture treatments. The suspended aquaculture strengthened the regeneration of P from sediments into the water compared with the benthic aquaculture. In addition, the results of a principal component and classification analysis showed that macrophytes/bivalve combined aquaculture promoted the maintenance of restoration effects and P balance at the water–sediment interface. In conclusion, benthic aquaculture coupled with replanting submerged macrophytes is a better choice for water managers when using biomanipulation of bivalves to remedy eutrophic waterbodies.

**KEYWORDS**

Aquaculture method; biomanipulation; eutrophication; *Hyriopsis cumingii*; phosphorus loading

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**INTRODUCTION**

Benthic animals act as a link between a waterbody and its sediment by affecting the phytoplankton community structure and nutrient exchange at the water–sediment interface (Vaughn and Hakenkamp 2001; Lucas et al. 2016). Introducing filter-feeding bivalves at an appropriate density can effectively reduce the phytoplankton biomass (Richard and Sergej 2003; Hwang et al. 2004; Stadmack and Conley 2011). Many researchers are attempting to use this measure to restore eutrophic waterbodies worldwide, especially to control the overgrowth of phytoplankton (Ackerman et al. 2001; Hakenkamp et al. 2001; Souchu et al. 2001; Fulford et al. 2007).

Reducing external nutrient loading is key to restoring eutrophic waterbodies and should have the highest priority (Cooke et al. 2005), but internal nitrogen (N) and phosphorus (P) loadings also significantly influence the restoration of eutrophic waterbodies. Søndergaard et al. (2003, 2007) reported that inhibiting the release of internal P loading from the sediment pool accumulated during

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a high P loading period has become an important factor determining the success of lake ecological restoration. Some previous studies have proved that introducing bivalves into eutrophic water could promote the release of P, thereby triggering a shift from a clear to a turbid water state (Paolo et al. 2000; Nizzoli et al. 2011; Zhang et al. 2014). Therefore, further research on improving the seston removal effects and how to reduce the release of P loading caused by bivalves is necessary to the application of bivalve manipulation technology.

Although there are many relevant studies on the restoration effects of bivalves on eutrophic waterbodies, few investigations focus on the seston removal and P transport and transformation as influenced by different bivalve aquaculture methods. The common practical aquaculture methods mainly involve suspended aquaculture and benthic aquaculture. For the benthic aquaculture method, the filtering and assimilation of natural seston by bivalves can reduce total phosphorus (TP) content in the aquaculture water. The feces and pseudofeces of bivalves, containing undigested or decomposed remains, will be excreted directly at the sediment surface, thereby promoting the algal biomass and the nutrient transport from overlying water to the sediments (Strayer et al. 1999; Gergs et al. 2009; van Broekhoven et al. 2015). Furthermore, bivalve respiration activity and decomposition of feces and pseudofeces deplete sediment oxygen content, thereby stimulating anoxic conditions in the sediments and P release by redox-sensitive iron dynamics (Bartoli et al. 2001; Nizzoli et al. 2005). For the suspended aquaculture method, suspending bivalves in the water may release less P by weakening the bioturbation of sediments (Widdows et al. 1998; Sgro et al. 2005). Improving bivalve contact with water may improve filtration efficiency. The direct excretion of feces and pseudofeces into water by bivalves, however, may increase the re-suspension of nutrient P. Overall, each method has advantages and disadvantages regarding the reduction of P release. Future research that aims at higher filtration efficiency on seston and less P release by the aquaculture method is needed.

Submerged macrophytes, which are an important part of the biological community owing to their effect on both water and sediments, greatly impact both nutrient cycling and the plankton community. Many previous studies have found that replanting submerged macrophytes can effectively reduce almost all forms of suspended nutrients and promote a shift in the water state from turbid to clear (Scheffer et al. 1993; Qiu et al. 2001; Pan et al. 2011). Introducing bivalves coupled with replanting submerged macrophytes may effectively reduce the P release and improve the restoration effects. We therefore studied the effects of bivalves/macrophytes aquaculture method on biomanipulation of bivalves.

To test our hypotheses that altering bivalve aquaculture methods could (1) improve seston removal and (2) reduce the P release at the water–sediment interface, a low-cost, easy-controlling field simulation experiment was conducted to compare the effects of three aquaculture methods on seston removal through monitoring turbidity, pelagic, and benthic algae. Meanwhile, the changes of different forms of P content in the water and sediments in this experiment were also determined to investigate the transformation of P loading between water and sediments in different aquaculture methods. This research could provide a reference for water managers when biomanipulation of bivalves to remedy eutrophic waterbodies. Due to the limited experimental conditions, and to exclude the interference of other biotic or abiotic factors as much as possible, the scale of the field simulation experimental systems was relatively small and the monitoring time only lasted two months. Real ecosystems are often complex and evolving, however, and because our experimental results were obtained under experimental conditions, more whole-lake scale experiments are needed to test their potential.

**Materials and methods**

**Study site**

Lake Donghu (30°33’N, 114°23’E; surface area 33 km²) is the largest urban lake in China. In recent years, the lake has become increasing eutrophic because of the sewage input from the surrounding
residents, and sediment P loading (ranging from 0.50 to 2.78 g/kg) has become a threat to water quality improvement (Xie et al. 2001). Our experiment was conducted near Miao Lake (Figure 1), a part of Donghu Lake where algal blooms occurred frequently every year (Lin et al. 2005). The sediments were collected from the top 0–10 cm of the sediments in a pond near the bank of Miao Lake (Figure 1). The contents of TP, inorganic phosphorus (IP), and organic matter (OM) in the sediments were 2.45 ± 0.02, 2.24 ± 0.03, and 106.00 ± 8.49 g/kg, respectively. Sediment TP content used in the experiment was in accordance with the characteristics of lakes in Wuhan (Xie et al. 2001). Before added to the experimental systems, the sediments were completely mixed and sieved (mesh size = 5 mm) to remove coarse debris. The water was collected at the same site as the sediment. Before filling the experimental systems, the water was filtered through a zooplankton net (mesh size = 86 μm) to reduce the interfering effects of macrozooplankton. The basic physicochemical characteristics of the water for turbidity, pelagic algal chlorophyll a (Chl-a) concentration, TP, total dissolved phosphorus (TDP), and soluble reactive phosphorus (SRP) were 9.8 ± 0.3 mg/L, 14.51 ± 0.58 mg/m³, 0.070 ± 0.030 mg/L, 0.022 ± 0.001 mg/L, and 0.017 ± 0.001 mg/L, respectively (n = 9).

**Experimental setup**

The experiment was performed during 84 d from September to December 2015 in 12 circular ceramic cylinders (total volume ~120 L, upper diameter ~65 cm, bottom diameter ~45 cm, height ~50 cm) containing ~10 cm-thick layers of sediments and ~35 cm-deep layers of water (volume ~85 ± 1 L). Meanwhile, a rain-proof shelter was constructed using translucent corrugated plastic, approximately 2 m above the experimental systems. The experimental light and temperature conditions were maintained to be consistent with the local natural light and temperature. These experimental systems were allowed to equilibrate for two weeks after adding the sediments and water. The 12 systems were then randomly divided into four experimental treatments named control, benthic, suspended, and combined treatments. Subsequently, the filter feeding bivalves and submerged macrophytes (*Ceratophyllum demersum* L.) were introduced into different treatment groups. As shown in Figure 2, the control treatments were without bivalves and macrophytes, and the benthic and suspended treatments introduced only bivalves (amount ~1 ind., length ~10 cm, weight ~80 ± 2 g). The bivalve in the benthic treatments was stocked at the sediment surface, whereas in the suspended treatments they were suspended in the water by a plastic net (mesh size = 5 mm). In the combined treatments, the same weight of bivalves were stocked at the sediment surface, and submerged macrophytes (untrimmed, length ~15 ± 3 cm, wet weight ~60 ± 1 g) were replanted.

The triangle sail mussel (*Hyriopsis cumingii*), a Chinese native bivalve used in this study, was purchased from a freshwater mussel breeding site in Hubei province, China; the macrophytes...
(C. demersum L.) were collected from Donghu Lake located in Wuhan, China. Before being used in the experiment, all the aquatic organisms were pre-cultured for two weeks using water collected from the pond which the experimental water and sediments were collected from. Dead bivalves were immediately removed and replaced with the same weight of bivalves.

**Sampling and analysis**

To investigate the seston removal effects, the water turbidity, and Chl-α concentration of pelagic and benthic algae were detected. The turbidity was measured with a turbidimeter (2100P; Hach, USA). To represent pelagic algal biomass, the Chl-α concentration in the water was determined. Every 7 d, 0.5 L water samples from the middle of each experimental system were siphoned into respective clean bottles and taken to the laboratory within an hour where they were immediately filtered through Whatman GF/C glass filters (0.45 μm). The filters were kept in the dark at −20 °C until spectrophotometric analysis after a 24 h extraction with 90% ethanol (Chen et al. 2006). The water volume was corrected using running water for the sampling and evaporative losses every week.

To collect the benthic algae samples, 12 granite blocks (10 × 10 × 1 cm) were set close to the sediment surface of each system to allow benthic algae to colonize. These artificial substrate granites were carefully removed every other week and cleaned thoroughly with a toothbrush and distilled water. The benthic algae samples accumulated on the substrates were collected into 12 respective plastic bottles. The Chl-α content of benthic algae was also determined spectrophotometrically after extraction by hot ethanol. Besides the turbidity and pelagic and benthic algal Chl-α, some other physico-chemical characteristics of the water, including temperature, pH, dissolved oxygen (DO), and oxidation–reduction potential (ORP), were detected at 25 cm below the surface of the water using the portable Multimeter (YSI ProPlus) every 7 d.

In addition to the above water quality indexes, water TP, TDP, and SRP concentrations as well as sediment TP, IP, and OM contents were also detected to reflect the effects of bivalves on P balance at the water–sediment interface. Every two weeks, 0.5 L water samples were collected for water quality analysis. Concentrations of TP, TDP, and SRP were determined according to the standard methods (State Environmental Protection Agency of China 2002). Particulate phosphorus (PP) was calculated as TP–TDP. To reduce disturbance, the sediment samples were collected fortnightly using a hollow plastic pipe (inner diameter ~5.0 cm, length ~60.0 cm). We gently inserted the plastic pipe into the sediments, blocked the upper end, and slowly removed it to extract the sediment. Five replicates were collected of each sample. In addition, to ensure the samples were representative, the sampling points were uniformly distributed throughout the experimental system, and each sample was fully mixed before analysis. The mixed samples were air-dried and sieved with a 100-mesh sieve. To determine TP content, the sediment sampling was heated at 450 °C for 3 h, extracted with
20 mL of 3.5 M HCl for 16 h, and then determined using the ascorbic acid method (APHA, 15th edition, 1980). Sediment IP content was directly extracted with 20 mL of 1 M HCl for 16 h and then determined in the extract, as described for TP. Organic phosphorus (OP) in the sediments was calculated by the difference between TP and IP content. OM was measured as loss in muffle furnace at 450 °C for 3 h.

It is worth noting that we mainly focused on the effects of bivalve aquaculture methods on internal P loading, but we also recognize the importance of nutrient N in water restoration. Some existing paradigms identify N as the primary limiting nutrient in terrestrial and marine ecosystems and P as the main limiting nutrient in lakes (Schindler 1977; Vitousek and Howarth 1991; Howarth and Marino 2006). Recent works have showed that N and P limitation are equivalent in lakes (Downing et al. 1999; Francoeur 2001; Elser et al. 2007), however, and the patterns of ecological nutrient limitation can be shifted (Elser et al. 2009). Further research is therefore needed to study the effects of bivalves on N cycling, which will be the main focus of our future experiments.

Data analysis

One-way analysis of variance (ANOVA) was performed to determine significant differences between treatments. Non-parametric tests (Kruskal–Wallis test) were used when the data distribution was skewed, with \( P < 0.05 \) considered significant. If a significant difference was found, \textit{post hoc} tests for multiple comparisons between treatments were also conducted (Kruskal–Wallis test followed by all pairwise multiple comparisons).

To better analyze the relationships among environmental variables and detect the stability of experimental treatments, a principal component and classification analysis (PCCA) were performed on the mean changing rates of all detected environmental variables. PCCA plots, which provide correlations between samples and the first two factors, were used to demonstrate the degree of crowding near the coordinate origin for dots of each experiment treatment. The higher the crowding degree, the more stable the treatment effects. Furthermore, the Spearman’s correlation coefficients among different environmental variables were calculated using the data expressed for PCCA.

The ANOVAs and PCCA were performed with SPSS 20.0 for Windows. The figures were constructed with Origin 8.0. For constructing of PCCA models, the software Statistica 6.0 (Statsoft 2001) was used.

Results

During the experiment, the water temperature fell gradually from an average 15.4 to an average 8.7 °C. By the end of the experiment, the wet weight of submerged macrophytes in the combined treatments increased from 60 g to more than 500 g. The final coverage of submerged macrophytes reached to 100%. Besides, two bivalves from the combined treatments died on the sixth day. These dead bivalves were replaced with a same weight of bivalves. The survival status of other bivalves was good, and there were no obvious changes in the bivalve body weight or size before and after the experiment.

Changes in turbidity and the pelagic and benthic algal Chl-a

The values of turbidity and pelagic and benthic algal Chl-a (Figure 3) show that compared with their initial concentrations, the mean concentration of turbidity in the control, benthic, suspended, and combined treatments decreased by 36.6%, 40.2%, 33.6%, and 49.4%, respectively; the pelagic algal Chl-a in the control, benthic, suspended, and combined treatments decreased by 71.9%, 70.9%, 61.2%, and 40.9%, respectively; and the benthic algal Chl-a in the control, benthic, suspended, and combined treatments decreased by 33.1%, 16.9%, 22.6%, and 58.5%, respectively. The results of \textit{post hoc} tests showed that turbidity and benthic algal Chl-a significantly varied with treatment (Figure 3,
P < 0.05), but differences in the pelagic algal Chl-a concentrations among treatments were not observed (Figure 3). The turbidity in the control was significantly higher than in the combined treatments (Figure 3, P < 0.05), and there was no significant difference among other treatments. Furthermore, the benthic algal Chl-a concentration in the control treatments was the highest among all treatments. The concentrations of benthic algal Chl-a in the control and benthic treatments were not significantly different, but values in both treatments were higher than in the combined treatments.

Changes in different forms of P contents in the water

The concentrations of TP, TDP, PP, and SRP in the water significantly varied with time (P < 0.05). Compared with their initial concentrations, the mean water TP concentrations in all treatments increased by 28.4%, 12.9%, 27.3%, and 42.1%, respectively; the mean water TDP concentrations in the control, benthic, suspended, and combined treatments increased by 56.7%, 82.9%, 376.5%, and 47.2%, respectively; the mean water PP concentrations in the control and combined treatments increased by 5.4% and 34.8%, respectively; the mean water PP concentrations in the benthic and suspended treatments decreased by 34.0% and 13.8%, respectively; the mean water SRP concentrations in the control, benthic, suspended, and combined treatments increased by 580%, 258%, 3150%, and 470%, respectively.

TP increment in the overlying water was calculated according to the experimental water volume (85.0 ± 1.0 L) and the difference in TP concentration before and after treatment. After the 84 d treatment, the TP contents in the suspended and combined treatments increased by 2.55 and 2.72 mg, respectively, more than in the control (1.62 mg) and benthic (0.94 mg) treatments. Similarly, the TDP concentrations in the control, benthic, suspended, and combined treatments
increased by 1.45, 2.47, 5.44, and 2.13 mg, accounting for 89.5%, 263.6%, 213.3%, and 78.1% of their TP increment, respectively. Correspondingly, the PP concentrations in the control, benthic, suspended, and combined treatments increased by 0.17, −1.53, −2.89, and 0.59 mg, respectively.

The results of post hoc tests for different forms of P among treatments showed that water TP concentration in the suspended and combined treatments was significantly higher than in the control and benthic treatments (Figure 4); TDP in the suspended and combined treatments was significantly higher than in the control but was not significantly different from the benthic treatments. PP in the suspended treatments was significantly higher than in the benthic and combined treatments; SRP concentrations in the suspended and combined treatments were higher than in the control treatments; and SRP in the benthic treatments was not significantly different from other treatments.

**Changes in different forms of P and OM in the sediments**

The concentrations of TP, IP, OM, but OP in the sediments varied significantly with time (Figure 5, $P < 0.05$). Compared with their initial concentrations, the mean sediment TP, IP, OP, and OM contents in the control, benthic, and suspended treatments all decreased. The reduction rates of sediment TP contents in the control, benthic, and suspended treatments were 8.4%, 5.9%, and 11.4%, respectively; the sediment IP contents decreased by 7.5%, 1.0%, and 12.0%, respectively; the sediment OP contents decreased by 22.7%, 25.3%, and 7.5%, respectively; the OM contents decreased by 1.1%, 1.6%, and 9.2%, respectively; the sediment TP, IP, and OM contents in the combined treatment increased by 2.1%, 8.1%, and 4.4%, respectively; and the sediment OP content decreased by 25.3%.

![Figure 4. Values of TP, TDP, PP, and SRP in the water for all samples. The box plots indicate the variation around the median value. The mean values are shown using '■'. The outlier values are shown using '•.' Letters a, b, and c indicate significant differences ($P < 0.05$).](image)
The results of post hoc tests showed that sediment TP in the benthic and suspended treatments were not significantly different from that in the control (Figure 5). Sediment TP and IP in the combined treatments were both significantly higher than in other treatments. Furthermore, OM in the control was the lowest among all treatments, whereas the combined treatment was the highest. There was no significant difference in OM between the benthic and suspended treatments.

**PCCA analysis on all environmental variables**

The first two principal factors were extracted by PCCA analysis (Figure 6(a)). Factor 1 reflected the effects of different treatments on the P transport and transformation, which mainly extracted the variable information of DO, pH, TP, TDP, PP, and SRP in the water, and TP, IP, and OM contents in the sediments. Factor 2 reflected the effects of different treatments on seston removal, which was mainly related to the variables of water temperature, turbidity, pelagic and benthic algal Chl-a, and sediment OP. Their eigenvalues explained for 51.5% of the total variance.

The angles between the arrows in the Figure 6(a) indicated the relationships among different environmental variables, which were confirmed by the Spearman’s correlation coefficients matrix. The pelagic algal Chl-a was positively related to water temperature, pH, turbidity, PP, and benthic algal Chl-a as well as sediment TP, OP, and OM, while negatively correlated with water DO, ORP, TDP, and SRP. The water TP was positively correlated with water pH, TDP, PP, SRP, and sediment OM contents, while negatively correlated with water DO, ORP, turbidity, benthic algal Chl-a, and sediment OP. In addition, sediment TP was positively correlated with water DO, pH, and pelagic
algal Chl-a as well as sediment IP and OM contents, while negatively correlated with water ORP and benthic algal Chl-a.

The effects of different aquaculture treatments were confirmed by the results of PCCA. As shown in Figure 6(b), most of dots of the combined aquaculture treatments gathered around the coordinate origin. The crowding degree of dots of the combined aquaculture treatments was higher, indicating
that the treatment promotes the maintenance of the restoration effect under our experimental conditions.

**Discussion**

**Effects of aquaculture methods on seston removal**

The changes of the turbidity and pelagic algal Chl-\(a\) showed that altering aquaculture methods did not improve the seston removal effects, although all three aquaculture methods reduced turbidity. This result differs from our original expectation that altering bivalve aquaculture methods could improve the effects of bivalve on seston removal. The possible reason is that ambient temperature and its density are the key factors determining filtration efficiency of bivalves (Vanderploeg et al. 1995; Kotta et al. 2005). However, the aquaculture method for *H. cumingii* has little influence on its filtration activity. Fei et al. (2005) and Li et al. (2006) improved water transparency and reduced Chl-\(a\) content using different aquaculture methods in different waterbodies. Many researchers (Fei et al. 2005; Naddafi et al. 2007) have shown that the filtration efficiency of bivalves will increase with temperature, and that the seston removal rate of *H. cumingii* at 17 °C was one-third its rate at 29 °C. In our experiment, the lack of significant difference in turbidity and pelagic algal Chl-\(a\) may be mainly due to the low experimental temperature. The clearance rates of bivalves and pelagic algal growth rate were weak at low ambient temperature (Kotta et al. 2005). In addition, a reasonable increase in bivalve density can improve the control of algal biomass (Oganjan and Lauringson 2014). In our experiment, the bivalve density was \(\sim 340 \text{ g/m}^2\), a value consistent with the field value of 0–500 g/m\(^2\) in TaiHu (Cai et al. 2010) and higher than that in the study by Fei et al. (2005), who stocked 1200 individuals of *H. cumingii* in a large pond (1470 m\(^2\)) and significantly reduced the pelagic algal Chl-\(a\) concentration. Accordingly, we suggest selecting an appropriate density and considering fully these factors affecting the clearance rate are the premises for successful restoration of eutrophic water by introducing bivalves.

Our experimental results showed that the suspended and combined treatments significantly depressed the benthic algal primary productivity compared with the control treatment, possibly caused by high P concentrations in the suspended and combined treatments (Figure 4). Previous studies (Vadeboncoeur et al. 2001; MacIntyre et al. 2004) have demonstrated an inverse relationship between pelagic and benthic algae, mediated by nutrient availability. When nutrient concentrations in overlying water exceed a critical value or threshold, the community may shift to a turbid state dominated by pelagic algae. Another possible reason is that submerged macrophytes in the combined treatments inhibited the growth of benthic algae by competition for nutrient and light resources (van Donk and van de Bund 2002; Erhard and Gross 2006). Therefore, we believe that the suspended aquaculture method did not promote the seston removal, but increased the migration of nutrient P from sediments to the water, thereby accelerating the pace of eutrophication. Thus, stocking bivalves at the sediment surface may be a better aquaculture method when introducing only bivalves to remedy eutrophic waterbodies.

**Effects of aquaculture methods on P balance**

The changes of all investigated P parameters in the water and sediments showed that P release at water–sediment interface occurred in the control, benthic, and suspended treatments, possibility caused by temperature variation. Compared with their initial values, the water P concentrations increased, and sediment P contents decreased. Comparing their final TP increments, we believe that, compared with the benthic aquaculture method, the suspended aquaculture method promoted the release of nutrient P from sediments into the water. By the end of the experiment, the water TP increment in the suspended treatments was as much as 2.73 times the benthic treatments. Correspondingly, the sediment TP reduction rate of the suspended treatments (11.4%, from 2.54 ± 0.06
to 2.26 ± 0.23 mg/L) was higher than that of the benthic treatments (5.9%, from 2.50 ± 0.01 to 2.37 ± 0.21 mg/L), possibly because the biodeposition of bivalves is a key factor affecting the dynamics of nutrient P (Newell 2004; Gergs et al. 2009). The excretion of undigested remains as feces and pseudofeces could promote the regeneration of water P loading (Newell et al. 2005). In the benthic treatments, the feces and pseudofeces were buried at the sediment surface, whereas they were excreted into water in the suspended treatments, which could also explain why the concentrations of water TP and PP in the suspended treatments were significantly higher than those of the benthic treatments in our experiment (Figure 4). In addition, Nizzoli et al. (2011) believe that suspended mussels, as the high sediment OM content below the mussel ropes, could stimulate heterotrophic microbial metabolism and mineralization of organic N and P. Many studies on bivalve effects showed that they can also affect nutrient cycling by many other mechanisms, such as assimilation and absorption, bioturbation of sediments through bivalve movements, oxygen depletion and others (Newell 2004; Sgro et al. 2005). Vaughn and Hakenkamp (2001) posit that if bivalve biodiversity is declining and populations release more nutrients than they absorb, bivalves may serve as a nutrient source, whereas they may serve as a nutrient sink while a population is growing or if biomass is being lost from the ecosystem by export or permanent burial.

Previous studies have suggested that biodeposition by benthic filter feeders in freshwater conveys high-quality pelagic resources to the sediment, resulting in changes in benthic species composition and abundance (Izvekova and Lovova-Katchanova 1972; Roditi et al. 1997; Strayer et al. 1999). However, we found that the suspended treatment in our experiment may increase the re-suspension and reproduction of the undigested algae as feces and pseudofeces, thereby stimulating primary productivity. Our results showed that water PP concentrations decreased while water TDP concentrations increased in the both benthic and suspended treatments (Figure 3), indicating that bivalve promoted the transformation of PP to dissolved P. Moreover, the nutrients excreted by bivalves could support further phytoplankton production through ‘bottom-up’ effects, especially for the dissolved inorganic nutrient, which could be directly utilized by algae. In our experiment, the SRP concentration in the suspended aquaculture method was significantly higher than in the control. Overall, we suggest that the benthic aquaculture method may be more suitable compared with the suspended aquaculture method for the biomanipulation of bivalves because it slows down the progress of water eutrophication.

**Effects of submerged macrophytes on biomanipulation of bivalves**

The PCCA biplot showed that the combined aquaculture method promoted the maintenance of the restoration effect under our experimental conditions. Based on the variation around the median values, we found that the water and sediment P contents in the combined treatments did not fluctuate strongly in contrast to other treatments with only bivalves. These results show that the appearance of submerged macrophytes in the combined treatments promoted the maintenance of P balance at the water–sediment interface.

Previous studies have proved that submerged macrophytes have an important role in weakening the exchange of nutrient P at the water–sediment interface (Horppila and Nurminen 2003; Wu et al. 2003). In our previous experiment, we compared the effects of bivalves, silver carp, and macrophytes as well as their combination on controlling the water nutrient content and the algal biomass, and finally found that stocking bivalves was more applicable to regulate the phytoplankton community structure while replanting macrophytes was more efficient in reducing the water nutrient content. However, a combination of filter-feeders and submerged macrophytes was the most effective at remediating eutrophic waterbodies (Wang et al. 2017). The complementary mechanisms between bivalves and macrophytes could promote the restoration of eutrophic waterbodies. First, submerged macrophytes could inhibit the primary productivity of pelagic and benthic algae by nutrition competition and allelopathy (Hilt and Gross 2008; Vanderstukken et al. 2011). Our previous experiment proved that submerged macrophytes could promote the increasing of large zooplankton, thereby
intensifying the removal of small sized algae that could not be grazed by bivalves (Wang et al. 2017). These may further consolidate the effects achieved by bivalves on controlling the algal biomass. Furthermore, submerged macrophytes could reduce the sediment re-suspension and the internal P load release (Horppila and Nurminen 2003; Nurminen and Horppila 2009). Newell and Koch (2004) developed a model indicating that bivalve filtration and sea grass sediment stabilization could efficiently regulate turbidity and decrease the re-suspension caused by waves. Moreover, submerged macrophytes can not only absorb the nutrient from water and sediments, but they also significantly decrease the ability of P desorption on the sediments (Wang et al. 2007). Conversely, biomanipulation of bivalves is often applied to remove seston in eutrophic waterbodies. The improvement of water transparency could promote the replanting of submerged macrophytes (He et al. 2014).

In summary, we suggest that stocking bivalves directly at the sediment surface, coupled with replanting submerged macrophytes, is a better choice for water managers when introducing bivalves to remedy eutrophic waterbodies. It is noteworthy that owing to the death of bivalves in our experiment, the water TP content in the combined treatments increased more significantly than in the control, indicating that the effect of submerged macrophytes nutrient absorption may be limited in the short term. Therefore, the density factor should be considered in the practical application of replanting submerged macrophytes (Dai et al. 2012). Excessive nutrient concentration will influence the success of replanting submerged macrophytes (Hilt et al. 2006). The densities of bivalves and submerged macrophytes in our study were about 340 g/m² and 0.7 g/L, respectively, both reasonably realistic compared with field values (Duarte and Kalff 1990; Cai et al. 2010). Our study provides additional evidence for successful outcomes when stocking bivalves at the sediment surface, and more studies on artificially optimized collocation of their density are needed.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Qiaohong Zhou, Zhenbin Wu, and Feng He are senior scientists. Their research ranges from water ecological restoration to constructed wetland.
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