Article

Using Freshwater Bivalves (*Corbicula Fluminea*) to Alleviate Harmful Effects of Small-Sized Crucian Carp (*Carassius Carassius*) on Growth of Submerged Macrophytes during Lake Restoration by Biomanipulation

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Abstract: Increased recruitment of small-sized fish following biomanipulation by reducing the biomass of plankti-benthivorous fish, not least in (sub)tropical lakes, may deteriorate water quality and thereby potentially hamper the recovery of submerged macrophytes. Filter-feeding bivalves remove suspended particles from the water and may, thereby, somewhat or fully counteract this negative effect of the increasing abundance of small-sized fish. So far, only few studies have investigated the interactive effects of fish and bivalves on water clarity and macrophyte growth. We conducted a 2×2 factorial designed outdoor mesocosm experiment with two densities of small crucian carp *Carassius carassius* (low 10 g m^{-2} and high 40 g m^{-2}) and two densities of bivalves *Corbicula fluminea* (low 204 g m^{-2} and high 816 g m^{-2}). We found significant interactive effect of fish and bivalves on the growth of the macrophyte *Vallisneria natans*. In the low density bivalve regime, the relative growth rates, root mass, root:shoot ratio and number of tubers were 30.3%, 30.8%, 21.6% and 27.8% lower in the high than in the low density fish treatments, while the decrease was less pronounced in the high density bivalve regime: 1.2%, 8.7%, 2.1% and 13.3%, respectively. Thus, bivalves reduced the negative effects of fish, not least when bivalve density was high. The weaker effects of small fish on plants in the high- than in the low-density *C. fluminea* regime can be attributed to lower total suspended solids (TSS) and Chl *a* in the first week of the experiment. Better light conditions further stimulated the growth of benthic algae, potentially increasing the removal of nutrients from the water and reducing fish-driven resuspension of the sediment. In addition, high densities of *C. fluminea* also enriched the sediment total nitrogen (TN) and total phosphorus (TP) content, favouring plant growth as indicated by an increase in leaf tissue TN and TP contents. Our results demonstrate that

filter-feeding bivalves can alleviate harmful effects of small fish by prolonging a clear-water state that facilitates submerged macrophyte growth. Addition of the bivalve *C. fluminea* can be a promising tool for the restoration of submerged macrophytes in shallow eutrophic lakes, in particular lakes containing small, rapidly reproducing fish that due to their small sizes are not capable of controlling the bivalves.

Keywords: small-sized fish; *Corbicula fluminea*; submerged macrophytes; restoration

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1. Introduction

Biomanipulation, targeted at obtaining a substantial reduction of planktivorous and benthivorous fish, has been widely used to improve water quality and promote re-establishment of submerged macrophytes [1–3]. Submerged macrophytes are considered to play a fundamental structuring role in shallow lakes [4–6], and when abundant help to sustain a long-term clear-water state after restoration [7,8], as they provide a refuge for zooplankton against fish predation, with a consequently higher grazing pressure on phytoplankton; they further protect young piscivores from predation, reduce sediment resuspension and inhibit phytoplankton growth via nutrient and light competition and allelopathy. Successful biomanipulation achieved in some temperate lakes (e.g., in the Netherlands and Denmark), has been attributed to increased development and cover of submerged macrophytes [9–11]. However, fish manipulation may not have the same positive effects on macrophyte re-establishment in warm as in temperate lakes [12,13].

Subtropical and tropical lakes are often dominated by small omnivorous fish [2,14,15], who recruit rapidly after fish removals due to ample food availability and frequent reproduction [14,16], and they may create a return to a turbid state before the submerged plants have been established. Examples are the only short term high water clarity after a >80% natural fish kills in Lake Naini Tal, India [17], and a limited success of submerged macrophyte transplantation in some Chinese lakes (e.g., Huizhou West Lake, Wuli Lake) [18–20]. To control small fish, a few European experiments have used pike (*Esox lucius*) stocking [21,22], but the pike generally do not develop populations large enough to control the small fish [7]. How to control the abundance is thus of key relevance restoration of warm lakes, but so far not well studied.

Freshwater bivalves, including clams and mussels, remove suspended particulate matter (algae, bacteria, particulate organic carbon) and improve the water quality through filter-feeding [23–25] and they may, therefore, potentially be used as a tool in lake restoration. Experiments with *Anodonta* have shown that they can filter up to 1.1 mL g^{-1} min^{-1}, which significantly reduced phytoplankton biomass [23]. Additionally, bivalves transfer the filtering particles from the water to the sediments as faeces and pseudofaeces, potentially enhancing the growth of submerged plants [26,27]. Field observations from Oneida Lake, North America, showed a deeper shoot expansion and increased diversity of submerged macrophytes with the presence of invasive zebra mussels (*Dreissena polymorpha*) [28,29]. However, whether stocking of native bivalves can be used to counteract the negative effects of small fish on the water quality is still unknown, and in shallow lakes with high abundance of small fish, the role of bivalves for macrophyte growth needs further elucidation.

Wuli Bay (31°30′07″ N, 120°15′11″ E to 31°32′48″ N, 120°13′54″ E), situated in the north of Taihu Lake, China, became severely eutrophic in the 1960s with increased nutrient loading, decreased water transparency and disappearance of submerged macrophytes [30,31]. To improve water quality, large-scale ecological restorations with coarse fish removal and reestablishment of submerged macrophytes were carried out in the north of Wuli Bay in 2010 [19,32,33]. However, high abundances of small-sized fish after fish removal, dominated by benthivorous species such as crucian carp, led to limited distribution of plants as a consequence of a poor Secchi depth (Z. Liu, unpublished data). *Corbicula fluminea*, originating from China and Southeast Asia, is the predominant

benthic filter-feeder in nearby Lake Taihu [34] and can be abundant in rivers, lakes, estuaries and shallow coastal waters around the world [35,36]. The species has a higher filtration rate than other bivalves [37] and can remove particle sizes typically ranging from 1 μm to 20–25 μm [38], but even up to 170 μm [39]. However, until now, *C. fluminea* has not received much attention as a potential tool in water management and its possible effects remain largely unknown.

In this study, outdoor mesocosms were used to explore the interactions between bivalves and submerged macrophytes at different densities of small fish. Our hypothesis was that filter-feeding bivalves are able to alleviate harmful effects (i.e., sediment resuspension, nutrient release, poor light availability) caused by small fish and to enrich the nutrient contents of sediments, potentially facilitating plant growth.

2. Materials and Methods

2.1. Experimental Materials

The outdoor mesocosom experiment was carried out from August to September 2018 in 16 circular polyethylene tanks positioned at the north shore of Lake Taihu (Lake Taihu Experimental Station, Wuxi, China) (Figure 1). The tanks had a top diameter of 97 cm, a bottom diameter of 77 cm and a height of 95 cm; they contained 10 cm sediment and approximately 500 L water collected from Lake Taihu. The sediment was sieved through a 5 mm mesh and stirred to ensure complete uniformity, and the water, initially containing 1.47 ± 0.25 mg L^{-1} of total nitrogen (TN) and 0.08 ± 0.02 mg L^{-1} of total phosphorus (TP), was passed through a 380 μm mesh and then manually stirred to develop a homogenous mixture of zooplankton and invertebrate communities among all replicates before being added into the tanks.
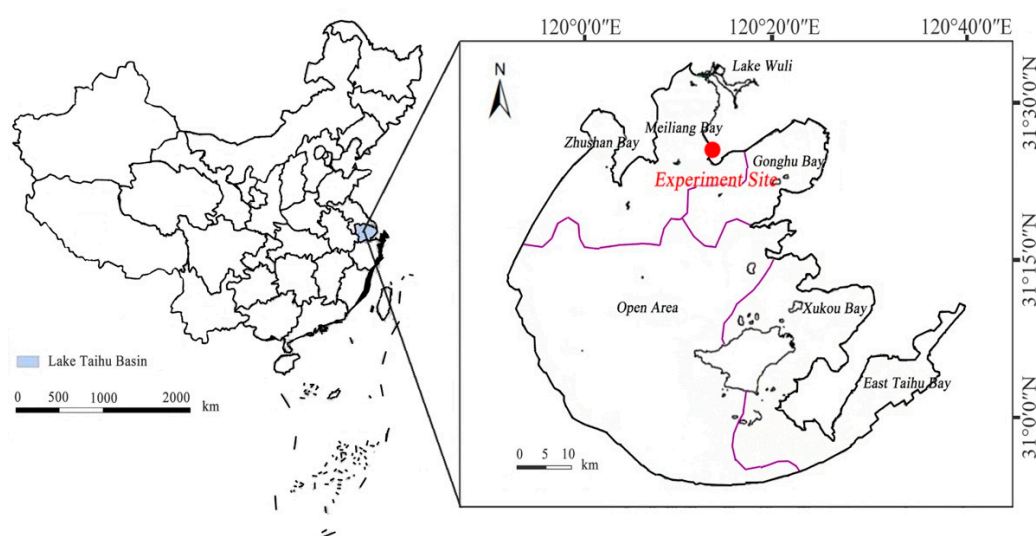

Figure 1. Location of Lake Taihu and experimental site (Lake Taihu Experimental Station).

Vallisneria natans is a perennial submerged plant that is widely distributed in China and it is commonly used in restoration experiments aimed at reestablishing macrophytes in Wuli Bay [40,41]. Young plant individuals were collected from the littoral zone of the lake and cultivated in tanks for two weeks until initiation of the experiment. Specimens of *C. fluminea* were obtained from Meiliang Bay where also small crucian carp (*C. carassius*) were caught by electrofishing from the Freshwater Fisheries Research Center of the Chinese Academy of Fishery Sciences. They were kept in the field in separate oxygenated bins filled with lake water to allow adaptation to the experimental environment.

2.2. Experimental Design

The experiment had a 2 × 2 factorial design including two densities of small fish (low, high) and two densities of bivalves (low, high), with four treatments as follows: low fish density + low bivalve density; high fish density + low bivalve density; low fish density + high bivalve density; high fish density + high bivalve density (Figure 2). Each treatment included four replicates; 2 individuals (10 g m^{-2}) and 8 individuals (40 g m^{-2}) of small crucian carp, with an average length of 5.2 ± 0.09 cm and a mean wet weight of 2.5 ± 0.03 g, were added to the mesocosms in low and high fish densities, reflecting the range recorded in restored areas of Wuli Bay (Liu, unpublished data) or other turbid shallow lakes [42,43]. The highest observed density was used to evaluate the most extreme expected effects. Low and high density of bivalves in the mesocosms were obtained by stocking 40 and 160 similar-sized individuals (length: 3.1 ± 0.03 cm, weight: 2.5 ± 0.02 g), respectively, approximating densities of 204 g m^{-2} (or 80 ind m^{-2}) and 816 g m^{-2} (320 ind m^{-2}), which is comparable with the field values of 0–820 ind m^{-2} or 0–522.9 g m^{-2} in Lake Taihu reported by Cai et al. [44]. All fish survived the experiment and the survival rates of bivalves were all above 97%.
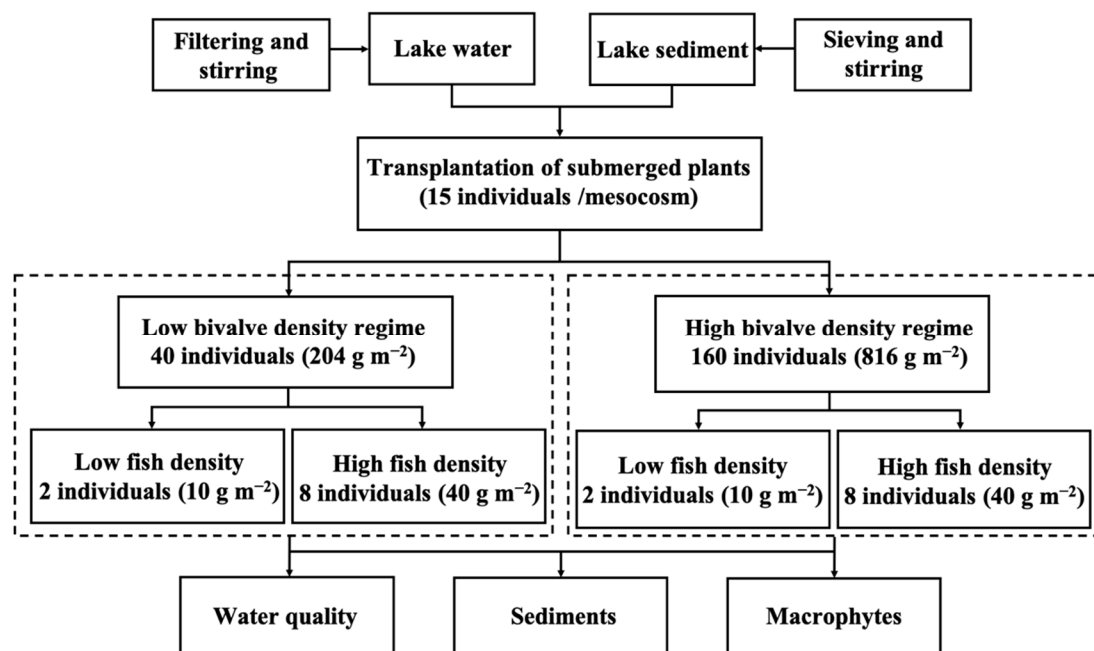

Figure 2. The experimental design and scheme.

One week before the start of the experiment, a total of 240 young plants (15/mesocosm) with similar morphology (length: 23.4 ± 1.5 cm, total wet weight: 28.0 ± 2.0 g) were separately transplanted into 16 mesocosms, corresponding to the mean density of submerged macrophytes planted in a restoration site in Wuli Bay, Lake Taihu (30 plants per square meters) [41]. Before crucian carp and bivalve introduction, an artificial plastic substrate of 80 cm^2 was placed vertically at 35 cm water depth in each mesocosm to enable colonisation of periphyton, and the periphyton biomass on *V. natans* was also determined.

2.3. Samplings

In the morning of day 0 (before fish and bivalve introduction), 7, 14, 21 and 28 of the experiment, turbidity was measured with a portable instrument (YSI 9500) and the illumination ratio of 20 cm water depth to the water surface (0 cm) was measured between 9 and 12 am using an illuminometer (ZDS-10W) to calculate the changes in light over time. Afterwards, approximately 2 L depth-integrated water samples for water chemical analysis and phytoplankton and suspended solids determination were taken with a tube sampler extending to mid-depth of the water column. Water nutrient contents

were analysed using standard methods applied in Chinese lake eutrophication surveys [45]. Unfiltered water samples were analysed for total nitrogen (TN) and total phosphorus (TP), filtered water samples being analysed for total dissolved phosphorus (TDP) and total dissolved nitrogen (TDN), ammonia nitrogen (NH_4-N) and soluble reactive phosphorus (SRP). TN and TDN were determined using an alkaline potassium persulfate digestion-ultraviolet (UV) spectrophotometric method, TP and TDP using the ammonium molybdate spectrophotometric method after digestion with $K_2S_2O_8$ solution. NH_4-N was detected by the Nessler's reagent colorimetric method and SRP was determined using the molybdenum blue method. Chl *a* was used as a surrogate measure of phytoplankton biomass and determined spectrophotometrically from matter retained on a GF/C filter over 24 h and extracted into 90% ethanol in a 75 °C water bath for 2 min. Suspended solids (TSS) were collected after filtering 100–200 mL water subsamples through a pre-weighed paper filter (Whatman) and weighed after 4 h drying at 105 °C. Residue on ignition was subsequently determined after 2 h heating at 550 °C in a muffle furnace to measure inorganic suspended solids (ISS). Zooplankton samples were collected on the first and last day of the experiment from a 5 L integrated water sample and filtered through a 64-μm mesh net and stored in 4% formaldehyde solution. Species were identified and counted under a microscope following the method described by Wang [46], Chiang and Du [47] and Shen and Du [48]. The zooplankton (wet weight): Chl *a* ratio, an indicator of zooplankton predation on phytoplankton, was calculated [49].

At the end of the experiment, all substrata were collected and stored separately in plastic boxes for laboratory analysis. The attachments were washed from substrata into 20 mL distilled water that was subsequently filtered through glass fibre filters and dried at 105 °C for 2 h to accumulate periphyton biomass per substratum surface. Fish were caught by electrofishing and stomachs were dissected to identify gut contents under a 100× magnification microscope. The specific food items were divided into macrophytes, zooplankton and particulate matter (including periphyton and seston); no macroinvertebrates were found. Frequency of occurrence (FO%) of each prey item was calculated as: FO% = number of stomachs containing prey item i/total number of stomachs × 100.

In order to sample benthic algae, the overlying water in each tank was removed by siphon and the upper 0–1 cm sediments in the centre of each mesocosm were collected using a plexiglass tube with an inner diameter of 1.6 cm. Then, the sediments were mixed with distilled water and filtered through a GF/C (Whatman International Ltd., Maidstone, UK) filter. The Chl-*a* concentration of benthic algae was measured by spectrophotometry using the same method as for phytoplankton. Afterwards, the sediment in each tank was collected, air-dried in a dark place and ground into powder to measure the content of nitrogen, phosphorus and organic matter in the sediment.

Ecological traits of *V. natans* samples, including wet weight of the whole plant, root biomass (the entire under-surface part), leaf biomass, mean leaf length, root:leaf ratio and the number of tubers, were determined immediately after removing all the plants from each mesocosm. The relative growth rate (RGR) was calculated as: RGR (mg g^{-1} d^{-1}) = ln (W_f/W_i)/days, where W_f (g) and W_i (g) were final and initial total wet weights of the plants in each tank, respectively. After that, plant leaves were dried for 48 h at 60 °C in a dry oven and then ground to fine powder to determine nitrogen (N), phosphorus (P) and carbon (C) concentrations according to Huang [50], and the C:P ratio and C:N ratios of the leaves were calculated. After plant sampling, the bivalves were fished out, counted and weighed wet.

2.4. Data Analyses

For time series data, including water chemical parameters, water clarity and light intensity, repeated measures analysis of variance (rANOVA) was used. Data on periphyton biomass, benthic algae, zooplankton abundances, zooplankton:Chl *a*, sediments and plants characteristics were analysed by two-way analysis of variance (two-way ANOVA), with fish and bivalve as treatment variables. In case of a significant interaction term, a further simple test (Bonferroni procedure), using bivalve density as a categorical factor and fish density as a quantitative factor, was performed to determine where the differences occurred. A *t*-test was used to analyse the differences between replicates of

zooplankton biomass and community before the start of the experiment. Data were log transformed to satisfy ANOVA requirements. The analyses were performed using the statistical package SPSS 16.0 with a level of significance of $p < 0.05$, and all figures were plotted by Prism 8.0.

3. Results

3.1. Nutrients

Both fish and bivalves had a significant effect on the TN and TP concentrations ($p < 0.01$), and also the interactions were significant ($p < 0.05$) (Figure 3, Table 1). Mean TN and TP concentrations at high fish density were higher than at low fish density but decreased significantly with increasing density of bivalves. In the low-density bivalve regime, high fish density led to rapid increases in TN and TP concentrations during the first week, with mean concentrations of 27.7% and 25.4%, respectively, which was significantly higher than at low fish density ($p < 0.01$). However, in the high-density bivalve regime, TN and TP concentrations decreased significantly and there was no obvious upward fish-induced trend in the first week. These values were only 4.7% and 8.5% higher at high than at low fish density, the changes between the two fish densities were not significant ($p > 0.05$). No significant effects of fish and bivalves or their interactions were found on TDN and TDP ($p > 0.05$). NH$_4$-N and SRP only responded significantly to fish ($p < 0.05$), with higher concentrations in the high-density fish treatments, whereas the effects of bivalves and interactions were insignificant ($p > 0.05$).
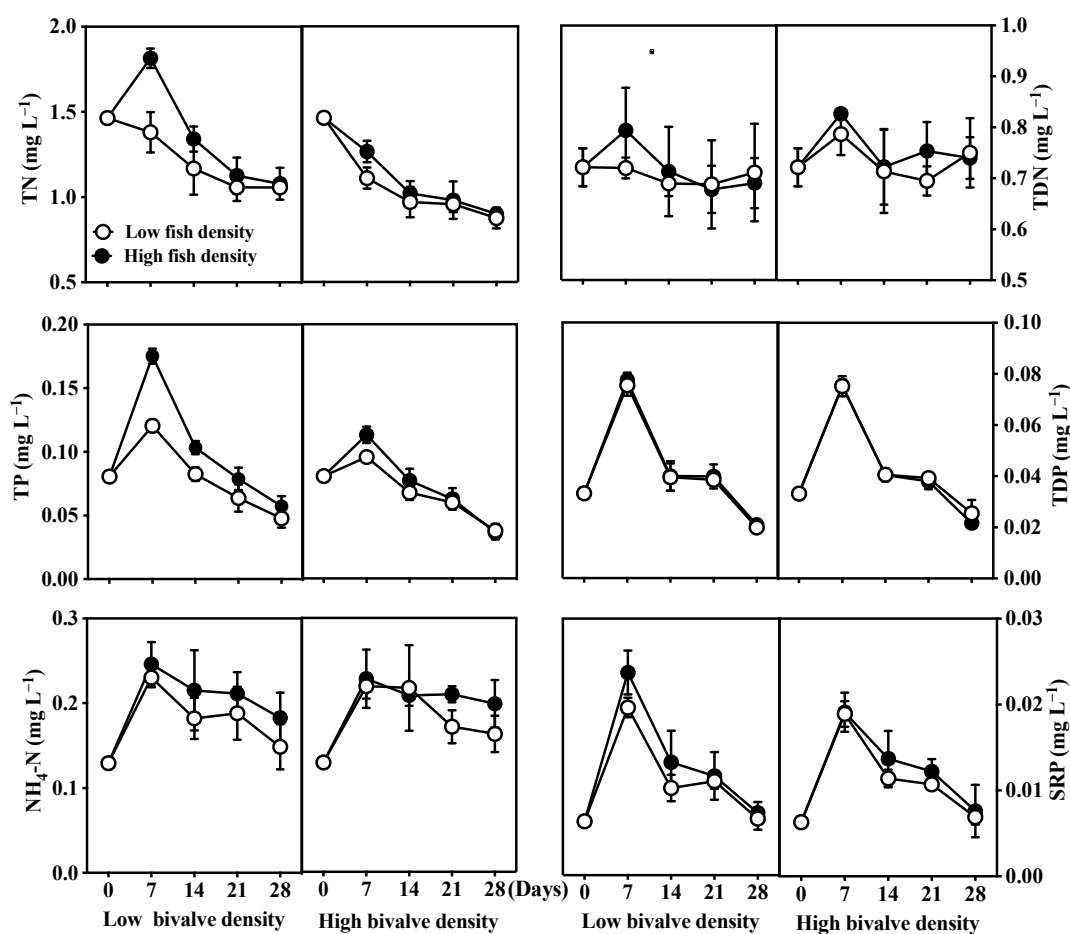

Figure 3. Time series of TN, TP, TDN, TDP, NH$_4$-N and SRP in the different treatments during the experiment (means ± standard deviation (SD)).

Table 1. Results of repeated measures analysis of variance (rANOVA) for water chemical parameters, water clarity and light intensity based on time-weighted data recorded during the experiment.

	Effect	DF	*F* Value	Pr > *F*		DF	*F* Value	Pr > *F*
TN	Fish (F)	1	37.80	**	Chl *a*	1	14.59	**
	Bivalve (B)	1	154.55	**		1	51.27	**
	F × B	1	8.54	*		1	11.74	*
TP	Fish (F)	1	48.80	**	Turbid	1	28.78	**
	Bivalve (B)	1	94.43	**		1	151.89	**
	F × B	1	14.66	**		1	11.81	**
TDN	Fish (F)	1	1.12	n.s.	TSS	1	25.20	**
	Bivalve (B)	1	3.37	n.s.		1	98.46	**
	F × B	1	1.28	n.s.		1	5.12	*
TDP	Fish (F)	1	0.01	n.s.	ISS	1	16.07	**
	Bivalve (B)	1	1.26	n.s.		1	61.65	**
	F × B	1	3.76	n.s.		1	2.53	n.s.
NH$_4$-N	Fish (F)	1	6.07	*	Lux	1	19.11	**
	Bivalve (B)	1	0.05	n.s.		1	239.7	**
	F × B	1	0.24	n.s.		1	17.90	**
SRP	Fish (F)	1	10.60	**				
	Bivalve (B)	1	0.54	n.s.				
	F × B	1	0.81	n.s.				

* $p < 0.05$, ** $p < 0.01$, n.s. = not significant. TN, total nitrogen; TDN, total dissolved nitrogen; TP, total phosphorus; TDP, total dissolved phosphorus; NH$_4$-N, ammonia nitrogen; SRP, soluble reactive phosphorus; Chl *a*, chlorophyll *a*; Turbid, turbidity; TSS, total suspended solids and ISS, inorganic suspended solids; Lux, light intensity.

3.2. Water Clarity and Light Intensity

Water clarity responded significantly to both fish and bivalves. Turbidity, TSS and ISS demonstrated a negative relationship with fish density and a significant decrease with bivalves, with lower mean values at high than at low density ($p < 0.01$) (Figure 4, Table 1). A significant interaction term showed that the effects of fish on turbidity, TSS and ISS differed between the two densities of bivalves ($p < 0.05$). In the low-density bivalve regime, fish led to significant increases in turbidity, TSS and ISS in the first week with mean values at high fish density of 35.2%, 31.0% and 33.7%, which was significantly higher than at low fish density ($p < 0.05$), while in the high-density bivalve regime, values increased slightly during the first week, followed by a rapid decline with no marked differences between the two fish densities ($p > 0.05$). Both fish and bivalves had a significant effect on the Chl-*a* concentrations, and the interactions were also significant ($p < 0.05$). In the low-density bivalve regime, the concentration of Chl *a* was 16.4% higher at high than at low fish density, while in the high-density bivalve regime, the Chl *a* concentration decreased sharply right from the first week but with no significant difference between the two fish treatments.

Fish, bivalves and their interactions all distinctly affected the water light intensity ($p < 0.05$) (Figure 5, Table 1). In the low-density bivalve regime, the ratio of light depth at 20 cm water depth to the water surface significantly declined with increasing density of fish within the first week of the experiment and was with 26.5% lower at high than at low fish density. Although the light intensity tended to rise slowly after 7 days, it was still lower than the initial value at the end of the experiment. However, in the high-density bivalve regime, the ratio of light depth at 20 cm water depth to the water surface increased significantly with no obvious differences between the two fish densities ($p > 0.05$). In summary, a high density of bivalves can control the decrease in light intensity caused by small fish, reducing the difference in impact between high and low fish density.
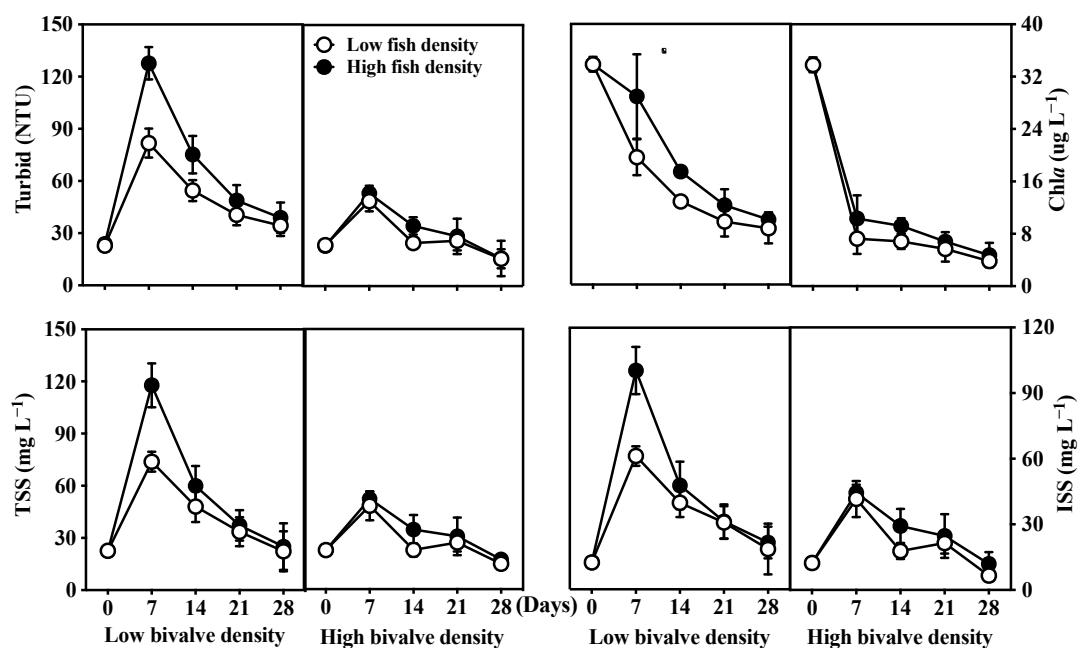
Figure 4. Time series of turbidity, Chl *a*, TSS and ISS in the different treatments during the experiment (means ± SD).
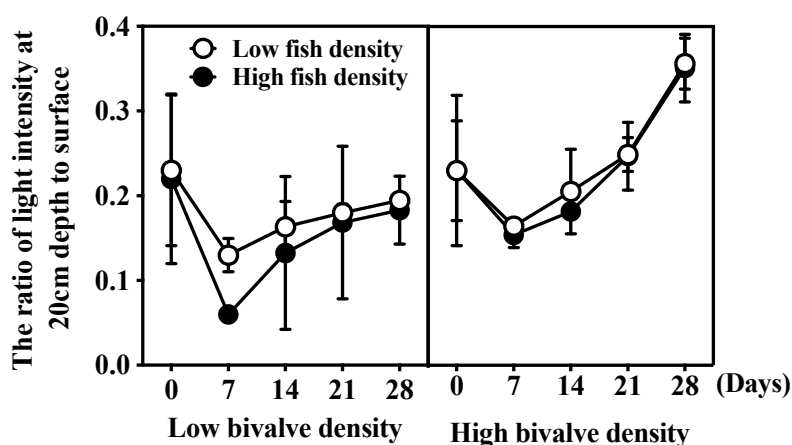

Figure 5. Variations in the ratio of light intensity at 20 cm water depth relative to the water surface in the different treatments during the experiment (means ± SD).

3.3. Macrophytes

High bivalve density significantly increased the TN and TP contents of the plants ($p < 0.01$) and reduced the C:N and C:P ratios compared with low bivalve density ($p < 0.05$) (Figure 6, Table 2). At high fish density, the TN contents of the plants increased significantly (14.3%) compared with low fish density in the high-density bivalve regime ($p < 0.05$), whereas no significant effects were observed on TP and the C:N and C:P ratios ($p > 0.05$). However, the interaction terms of fish and bivalves were not significant ($p > 0.05$).

Both fish and bivalves greatly affected the growth and reproduction of *V. natans* ($p < 0.05$) (Figure 7, Table 2). All the plant indices significantly decreased proportionally to the increasing fish density (except leaf mass) but were promoted by increased bivalve density. The interaction terms were only significant for relative growth rates, root mass, root:shoot ratio and tuber numbers with differences in fish-induced changes in the two bivalve regimes. Thus, in the low-density bivalve regime, the relative

growth rates, root mass, root:shoot ratio and tuber numbers were 30.3%, 30.8%, 21.6% and 27.8% lower at high than at low fish density, while the decrease was alleviated in the high bivalve density regime: 1.2%, 8.7%, 2.1% and 13.3%, respectively. Accordingly, bivalves reduced the negative effects caused by fish.

Table 2. Two-way analysis of variance (ANOVA) of plants and sediments characteristics, periphyton biomass, benthic algae, zooplankton abundances, zooplankton:Chl *a* at the end of the experiment.

	Fish			Bivalve			Fish × Bivalve		
	DF	F	Pr > F	DF	F	Pr > F	DF	F	Pr > F
Plant N concentration	1	10.65	*	1	197.66	**	1	1.36	n.s.
Plant P concentration	1	0.78	n.s.	1	370.36	**	1	0.35	n.s.
Plant C:N ratio	1	1.23	n.s.	1	174.11	**	1	0.70	n.s.
Plant C:P ratio	1	2.29	n.s.	1	18.04	*	1	0.38	n.s.
Relative growth rate	1	30.94	**	1	147.13	**	1	20.22	**
Mean leaf length	1	48.62	**	1	53.48	**	1	1.29	n.s.
Leaf mass	1	20.82	**	1	95.36	**	1	2.24	n.s.
Root mass	1	9.12	*	1	47.34	**	1	8.18	*
Root:shoot ratio	1	10.59	*	1	20.5	**	1	7.62	*
Tuber numbers	1	80.66	**	1	726.00	**	1	10.67	**
Periphyton biomass	1	6.25	n.s.	1	29.90	**	1	6.10	n.s.
Chl *a* of benthic algae	1	32.70	**	1	58.60	**	1	12.97	*
Zooplankton biomass	1	44.60	*	1	0.41	n.s.	1	0.07	n.s.
Zooplankton:Chl *a*	1	26.18	**	1	0.37	n.s.	1	0.01	n.s.
Sediment TN content	1	0.002	n.s.	1	6.21	*	1	0.04	n.s.
Sediment TP content	1	0.01	n.s.	1	8.24	*	1	0.39	n.s.
Sediment OM content	1	0.27	n.s.	1	0.22	n.s.	1	2.90	n.s.

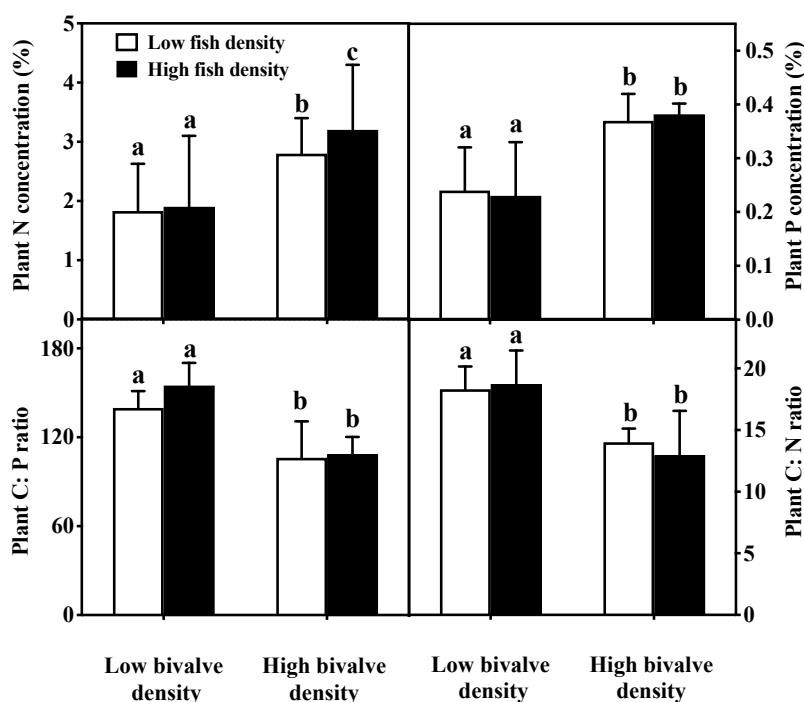$* p < 0.05$, $** p < 0.01$, n.s. = not significant.

Figure 6. Nitrogen (N), phosphorus (P) and the carbon:phosphorus (C:P) and carbon:nitrogen (C:N) ratios of *Vallisneria natans* in the different treatments at the end of the experiment (means ± SD). Different letters show significant differences among treatments.
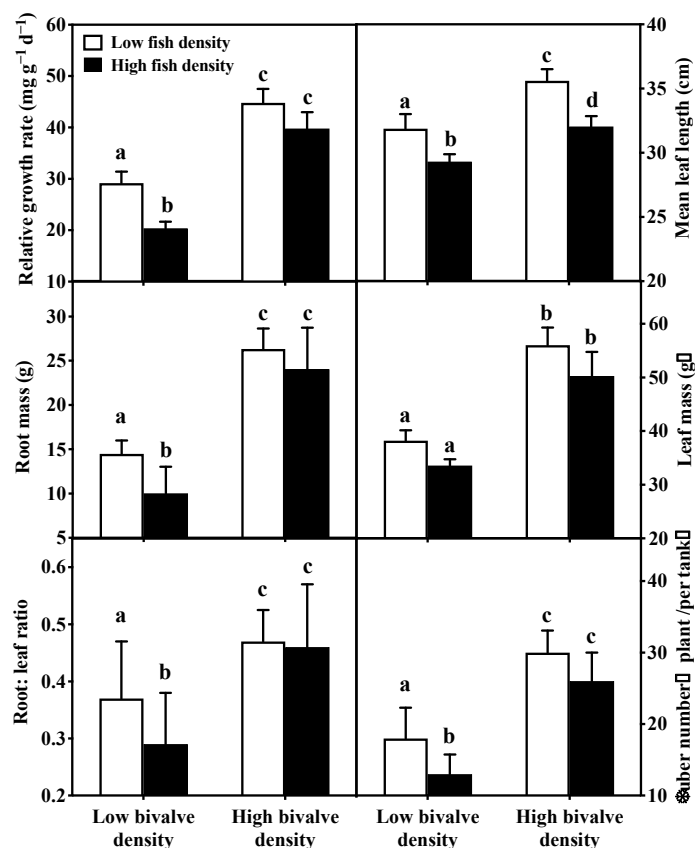
Figure 7. Relative growth rate (entire study period), mean leaf length, leaf mass, root mass, root: shoot ratio and tuber number of *Vallisneria natans* in the different treatments at the end of the experiment (means ± SD). Different letters show significant differences among treatments.

3.4. Periphyton and Benthic Algae

High bivalve density significantly increased periphyton biomass compared with low bivalve density. However, the effect of fish and interactions were insignificant ($p > 0.05$) (Figure 8, Table 2). Both fish and bivalves had a significant effect on the Chl *a* of benthic algae, and interactions were also detected ($p < 0.05$). In the low-density bivalve regime, the Chl *a* of benthic algae decreased significantly (33.8%) at high-density fish compared with low fish density, while values increased significantly with high bivalve density without obvious difference between the two fish densities, though.
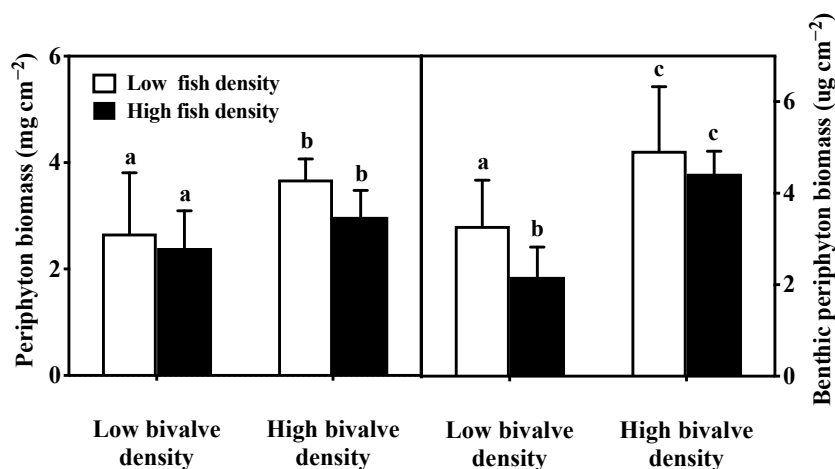

Figure 8. Periphyton biomass and Chl *a* of benthic algae in the different treatments at the end of the experiment (means ± SD). Different letters show significant differences among treatments.

3.5. Zooplankton

Before the addition of fish and bivalves, mean total zooplankton biomass was 1.35 mg L^{-1} and the zooplankton community was predominantly comprised of copepods (71%), mainly *Limnoithona*, *Sinocalanus*, *Mesocyclops* and nauplii species. The second most important group was cladocerans (22%), represented by *Ceriodaphnia* and *Bosmina* species, followed by rotifers (7%), represented by *Brachinonus*, *Polyarthra* and *Lecane* species (Figure 9, Table 2). A *t*-test did not show the significant differences in the biomasses and communities of zooplankton between the four treatments. At the end of the experiment, zooplankton biomass was significantly lower at high-density fish than at low-density fish in both bivalve regimes ($p < 0.05$). High fish density significantly decreased the biomasses of cladocerans and copepods and significantly increased that of rotifers. Absence of *Sinocalanus* and a significantly lower biomass of large *Bosmina longispina* (>400 um) were observed at high fish density where the communities were dominated by small-sized nauplii and rotifers. Consequently, the zooplankton:Chl *a* ratio was significantly lower at high than at low fish density in both bivalve regimes ($p < 0.05$). However, bivalves only significantly increased rotifer biomass ($p < 0.05$), whereas they did not affect total zooplankton biomasses and zooplankton: Chl *a* ratio ($p > 0.05$). The interaction terms of fish and bivalves were not significant ($p > 0.05$).
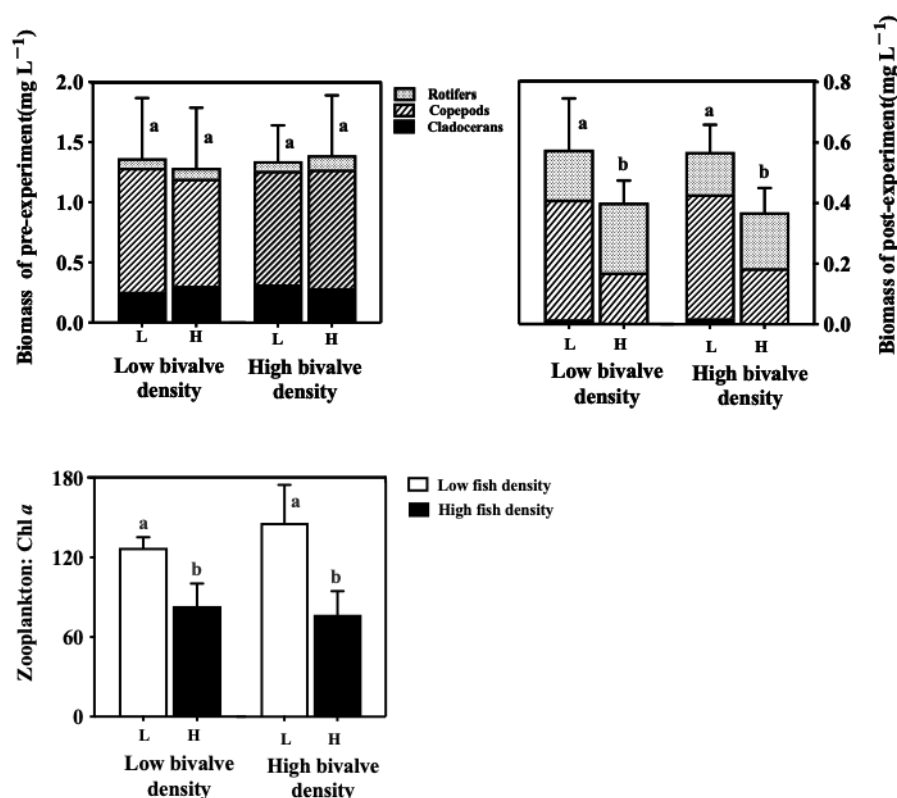

Figure 9. Total zooplankton biomass before (**left**) and after the experiment (**right**) and zooplankton: Chl *a* after the experiment in the different treatments (means ± SD). L = low density of fish; H = high density of fish. Different letters show significant differences among treatments.

3.6. Fish Guts

Zooplankton was the main food source of fish in all treatments and they appeared less frequently at high than at low fish density fish (Table 3). Macrophytes were the next most frequent item in the fish guts, with similar percentage occurrences ranging from 50% to 60%. Filamentous algae appeared occasionally at low bivalve density and frequently at high bivalve density.

Table 3. Frequency of occurrence (%) of main prey items in the fish diet.

Prey Items	Low Bivalve Density		High Bivalve Density	
	Low Fish Density	High Fish Density	Low Fish Density	High Fish Density
Macrophytes	50	56.3	50	62.5
Zooplankton	100	81.3	100	75
Filamentous algae	25	31.3	50	56.3

3.7. Sediments

Bivalves had a significant effect on the nutrient content of sediments ($p < 0.05$), whereas the effect of fish and their interactions were insignificant ($p > 0.05$) (Tables 2 and 4). At the end of the experiment, the TN and TP contents in the high-density bivalve regime were noticeably higher than at low density. Fish and bivalves and their interactions had no significant effect on the OM content of sediments ($p > 0.05$).

Table 4. The content of total nitrogen (TN), total phosphorus (TP) and organic matter (OM) in the different treatments at the end of experiment (Means ± SD).

Prey Items	Low Density Bivalve		High Density Bivalve	
	Low Density Fish	High Density Fish	Low Density Fish	High Density Fish
TN (mg kg^{-1})	1508 ± 119	1525 ± 102	1688 ± 253	1744 ± 99
TP (mg kg^{-1})	235 ± 17	237 ± 60	266 ± 33	260 ± 37
OM%	1.51 ± 0.10	1.72 ± 0.05	1.66 ± 0.06	1.77 ± 0.13

4. Discussion

Our results support the hypothesis that high abundance of small-sized crucian carp decreases the growth of the submerged macrophyte *V. natans*, while filtration by the bivalve *C. fluminea* partly alleviates the negative effects of the fish on plant growth.

In the low-density bivalve regime, high density of small fish supported a higher turbidity and led to a substantially lower root mass and relative growth rate of *V. natans* than in the low-fish density treatments. This may partly be attributed to sediment resuspension by the fish when foraging in the sediment, resulting in less light availability for the plants. Although this effect is considered to be weaker for small fish due to their gape limitation of foraging options [51,52], we recorded significantly higher TSS and ISS in the high-density fish treatment than at the low fish density. Higher phytoplankton biomass, expressed as Chl *a*, also contributed to the higher turbidity at high fish density. Besides increasing nutrient concentrations by sediment disturbance, the fish through excretion may also indirectly have contributed to the higher TP, TN, and NH$_4$-N at the high fish density treatment. TDN, TDP and SRP, however, did not differ significantly between the two fish densities, likely due to a higher uptake by phytoplankton at the high fish densities (as more algae in the water). A higher fish predation pressure on zooplankton may further have boosted phytoplankton growth in the high density treatment, as seen in other studies with fish [53,54]. Zooplankton was frequently found in the fish stomachs and the zooplankton biomasses, especially those of cladocerans and calanoid copepods such as *Bosmina longispina* and *Sinocalanus*, were significantly lower in the high fish density treatment and dominated by small-sized nauplii and rotifers. Accordingly, the zooplankton:Chl *a* ratio was lower in the high than low fish density treatment, also indicating strong size-selective planktivory and a low grazing pressure on the phytoplankton [49]. Similar results were obtained in studies of other warm lakes where many fish species (e.g., crucian carp and tilapia) spawn several times per year, leading to large abundances of young-of-the-year fish [55,56].

Direct uprooting or consumption of plants has been reported for larger omnivorous fish, hampering the growth of submerged vegetation [32,57,58]. We did not detect direct physical uprooting of plants

by the small crucian carp, but macrophyte materials occurred in the fish stomachs, although it may have an incidental uptake while searching for animal food [59,60]. Yu et al. [61], however, showed that small omnivorous fish, like *Acheilognathus macropterus,* not only grazed on small-leaf macrophytes such as *Hydrilla verticillata*, *Ceratophyllum demersum* and *Myriophyllum spicatum* but also on large-leaf macrophytes like *Vallisneria denseserrulata*, thereby considerably reducing the biomass and RGR of submerged macrophytes. Therefore, we cannot discard a direct consumption effect by the fish in our experiment, although indirect mechanisms mediated by light limitation, due to higher growth of phytoplankton and sediment stirring by fish in high densities, are a more likely explanation.

A high density of the bivalve *C. fluminea* significantly facilitated growth of *V. natans*, even at high densities of small crucian carp, as seen also in natural lakes after the invasion of zebra mussels [26,62]. Benthic bivalves can remove significant quantities of organic and inorganic suspended particles from the water column by filtration, as shown by, for example, Hwang et al. [63], and we found continuously decreasing concentrations of Chl *a* concentration, suspended solids and total phosphorus in the water at high densities of *C. fluminea.* We also recorded a proportional decrease in TSS, primarily of ISS and Chl *a*, with increasing bivalve density. This decrease, in turn, led to better light conditions and enhanced relative growth rate but also changes the biomass allocation of plants. The plants reduced shade adaption (i.e., elongating above-ground) and allocated more resources to the belowground biomass. Accordingly, the number of tubers and root biomass increased significantly in the high-density bivalve regime, resulting in a higher root/leaf ratio. These morphological changes of plants are collectively indicators of improved light conditions [64].

However, some studies have shown that the disturbance of *C. fluminea* will increase the amount of nutrients in water [65], but only at densities >1000 ind m^{-2} [66]. The density of *C. fluminea* in our experiment was <320 ind m^{-2} (816 g m^{-2}), which explains why they improved rather than deteriorated the water quality. Filtration of bivalves is believed to be influenced by food quality and particle size [67] and cyanobacteria is considered as a low-quality food item [68]. However, the phytoplankton in our study was mainly composed of *Raphidiopsis curvata*, *Aphanizomenon* and *Euglena gracilis*, accounting for 87.5%, 12.4% and 0.06% of the entire phytoplankton community (H. Jin, unpublished data) and the particle size of these three algae types does not hamper *C. fluminea* filtration [39].

The filtered phytoplankton can be transferred to the benthos via faeces and pseudofaeces by bivalves, elevating the nutrient concentration of the sediments that are available in the rhizosphere [69,70]. In a study evaluating interactions between suspension-feeding bivalves and seagrass conducted by Peterson and Heck [71], the mussel density manipulations resulted in a doubling of the TN and TP levels of sediments as well as a significant increase in shoot growth and productivity. We found that both the sediments and leaf tissue were enriched with N and P in the high-density *C. fluminea* regime, whereas leaf tissue C:N and C:P ratios were lower than in the low-density regime as seen in other studies with elevated nutrient levels (e.g., Fourqurean and Zieman [72]). In our study, TN and TP in water column were lower in the high-density bivalve regime than at low bivalve density, while no differences were found for dissolved nutrients. This seems to exclude higher sediment nutrient uptake from the water at high bivalve density, and suggest that the higher N and P concentrations in the plants were a result of nutrient enrichment of the sediment by the bivalves.

We found that high bivalve density significantly counteracted the negative effects (high concentrations of nutrients, Chl *a* and suspended solids in the water column) even at high fish density and the shading inhibition on the plants was modest and only of short duration. Moreover, the increase in light penetration also led to an enhanced biomass of benthic periphyton, which may have precipitated the nutrient reduction through uptake. High benthic production may also suppress the nutrient release from sediments caused by fish disturbance [73]. Lastly, bivalve biodeposits are considered to be an organic nutrient-rich and easily assimilated food source [74]. This potentially supports a higher availability of food for small fish, preventing them from digging deeper into sediments when foraging and thus causing deteriorating water quality. However, the present study did not provide data allowing any firm conclusion about this process, but this effect has been found for

other filter-feeding fish species like bighead carp in an experiment conducted by Shen et al. [75] and of silver carp in an investigation undertaken by Yan et al. [76].

Most cases of biomanipulation conducted for the purpose of restoration have revealed a clear-water phase and high abundance of macrophytes following nutrient and fish reduction [3,77]. However, the clear-water phase may be transient although critical for macrophyte development [16], but if established, the macrophytes may reinforce their water-clearing effect and prolong clear-water state through a number of positive feedback mechanisms. Our four-week study revealed that filter-feeding bivalves can reduce suspended matter concentrations caused by sediment-disturbing foraging of small crucian carp prolonging the early clear-water state that benefits rapid expansion and large coverage of submerged macrophytes [2,15]. Compared with filter-feeding fish such as silver carp, filter-feeding bivalves exert limited predation pressure on large zooplankton [78], thus avoiding a reduction in the top-down control of zooplankton on phytoplankton. In addition, freshwater bivalves have a relatively stable community structure that may exert a long-term grazing pressure on phytoplankton.

However, the small scale and short duration of our experiment limit the extent of our conclusions. Thus, long-term and large-scale field studies are needed to ensure the effectiveness of bivalve stocking as a lake restoration method. In addition, as our principal aim was to experimentally elucidate the interaction terms of small fish and bivalves, we did not establish control treatments without fish and bivalves. However, our previous study provided basic data regarding the density-dependent effect of small crucian carp [79] and *C. fluminea* [80], J. Gu et al., unpublished data] on plant growth. In addition, some studies showed that the presence of mussels did not substantially affect the biomass of pelagic algae in a clear lake, but significantly affected the water quality by reducing the phytoplankton biomass (Chl-*a*) in a turbid lake, emphasizing that the mussel effect likely depends on the trophic state of the lake [61,81]. The conditions prevailing at low density fish reflect the present eutrophic conditions of Lake Taihu, China [18,82], and probably also many eutrophic aquatic systems with frequent reproduction of small fish. Thus, the low density of fish is used as background levels of our study, allowing evaluation of the bivalve effect on the most extreme eutrophic expected effects with high densities of fish on the restoration of Taihu Lake. Indeed, we found that high-density filter-feeding bivalves alleviated the negative effects of small fish, compared with a low density, which is more representative of biomasses observed in natural water bodies.

5. Conclusions

Our study revealed that a high density of small-sized crucian carp can reduce light in the water triggered by fish-induced high concentrations of phytoplankton biomass and inorganic suspended solids, delaying or hampering the growth of submerged macrophytes. Introduction of the bivalve *C. fluminea* might be used as an effective tool to mitigate the harmful effects of small fish and create sufficient transparency for macrophytes to develop, providing that they are exposed to low predation by large fish (as it will be after biomanipulation).

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