

Bio-manipulation-induced reduction of sediment phosphorus release in a tropical shallow lake

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Abstract Bio-manipulation via fish regulation combined with submerged plant introduction is an effective measure to restore eutrophic shallow lakes. Improved water quality and clarity promote growth of benthic algae, which with submerged plants may limit sediment phosphorus (P) release, thereby reinforce lake recovery. Our study sought to evaluate the effect of such a bio-manipulation on water quality, benthic algal development and sediment P release in a shallow, tropical lake by (1) comparing porewater and lake water quality, light intensity and benthic algal development in restored and unrestored sections; (2) conducting a ^{32}P radiotracer experiment to track P release from sediment cores sampled from both sections. The bio-manipulation led to lower total P, total dissolved P, and soluble reactive P concentrations in lake water, lower phytoplankton biomass, and

increased light intensity at sediment surface, stimulating benthic algal development. Moreover, sediment ^{32}P release was lower in the restored than unrestored section. Concurrently, dissolved oxygen levels in upper layers of the sediment cores were higher in the restored section. Our study indicates that the bio-manipulation improved water quality and enhanced growth of benthic algae, thereby reducing sediment P release, which may be one of the main mechanisms to create successful restoration.

Keywords Bio-manipulation · Submerged plant · Fish manipulation · Restoration · Induced benthic algae · Eutrophication · Shallow lakes

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Introduction

Eutrophication is one of the most common water quality problems in lakes worldwide (Schindler, 2006). As a major nutrient source in the aquatic environment, phosphorus (P) is a key contributing factor to eutrophication and, with it, deteriorating water quality and loss of biodiversity. P reduction is therefore an urgent priority in most lake restoration efforts (Schindler, 1977; Carpenter et al., 1995). However, despite intensive research into how to effectively reduce external loading, P remains a problem in water quality control (Jeppesen et al., 2007; Søndergaard et al., 2013). The most efficient way of improving the ecological status of shallow eutrophic lakes is to reduce the external nutrient loading (Jeppesen et al., 2007). However, when external loading has been reduced, increased release of P from the sediment has been observed, and this internal P loading may decide the trophic status of the lakes (van de Molen & Boers, 1994; Søndergaard et al., 2003, 2013). P sequestered in porewater is of particular concern due to its high mobility (Holdren & Armstrong, 1986), and even after a substantial reduction of the external loading, release of sediment P can contribute to continued high nutrient levels in the water column (Søndergaard et al., 2013), especially in lakes with high density of fish stirring up the sediment when foraging (Zhang et al., 2016). P release from the sediment, latest summarized by Søndergaard et al. (2013), is therefore an important issue to consider in lake restoration strategies.

Submerged plant growth often leads to reduced release of sediment P (Carignan & Kalff, 1980; Jaynes & Carpenter, 1986) through the direct nutrient uptake by the plants and by oxidation of sediment, which increases its P-binding capacity (Barko & James, 1998). Also, the submerged plants stabilize the sediment and reduce resuspension (Jensen et al., 2016). Submerged plants can also enhance the sedimentation of particles in the water column and decrease phytoplankton growth by competing for nutrients and light resources (Carignan & Kalff, 1980; Blindow et al., 1993). Moreover, a reduction of the biomass of coarse fish obtained by removal or stocking of piscivores can also diminish disturbance of the sediment (Drenner & Hambright, 1999; Jeppesen et al., 2012). Consequently, biomanipulation (fish regulation sometimes combined with submerged plant

introduction) has been proposed as an effective ecological measure for improving water quality and clarity and generally promoting the recovery of eutrophic shallow lake ecosystems after an external nutrient loading reduction (Drenner & Hambright, 1999; Qiu et al., 2001; Jin et al., 2006; Jeppesen et al., 2012; Yu et al., 2016).

The increased availability of light at the sediment surface following the restoration allows colonization of benthic algae. Benthic algae have a high affinity for nutrients (Stevenson & Stoermer, 1982; McCormick & O'Dell, 1996; Pérez-Martínez et al., 2010) and may together with rooted macrophytes oxidize the top sediment layers and thus help immobilize nutrients in the sediment (Wetzel, 2001; Zhang et al., 2013), leading to increase in P retention in sediments (Carlton & Wetzel, 1988; Christensen et al., 1997) and lower release of P into the overlying water (Dodds, 2003; Tyler et al., 2003). In a previous study, sediment without benthic algae exhibited P release rates of about $1 \text{ mg P m}^{-2}\text{day}^{-1}$, whereas sediment with benthic algae released only $0.1 \text{ mg P m}^{-2}\text{day}^{-1}$ (van Luijn et al., 1995).

Although numerous studies have investigated the impact of submerged plants (Brenner et al., 1999; Rooney et al., 2003) and benthic algae on the P cycling between the sediment and the water column (Hansson, 1989; Dalsgaard, 2003; Spears et al., 2008; Zhang et al., 2013), little research has been undertaken to elucidate how the sediment P release is affected by biomanipulation in shallow lakes. This is unfortunate as changes in the P release may have profound effects on ecological state. In the present study, we hypothesized that lake biomanipulation (fish removal, stocking of piscivorous fish, plant introduction) would improve water quality and clarity, promoting growth of benthic algae. We further hypothesized that this increase in benthic algae may limit sediment phosphorus (P) release, thereby reinforcing lake recovery. We evaluated the effect of lake biomanipulation on water quality, benthic algae, and sediment P release by (1) comparing sediment porewater and lake water quality, light intensity as well as benthic algal development in restored and unrestored sections of a shallow lake and (2) conducting a ^{32}P radiotracer experiment to track the P release from sediment cores sampled from restored and unrestored lake sections. We expected the P release to be substantially lower from the restored section.

Materials and methods

Lake description

Huizhou West Lake is a shallow eutrophic lake located in Huizhou City, Guangdong Province, South China. The lake is divided into several sub-lakes separated by bridges that do not block the movement of water. The lake covers 1.6 km² and has an average depth of approximately 1.5 m. The average air temperature in the area is 22.0°C and the annual rainfall is 1889 mm. Nutrient levels in the sediment are high, for example in the lake section known as Fenghu TP and TN values are 1.31 and 2.39 mg g⁻¹ as dry weight, respectively (Zhang et al., 2014).

Efforts to address the problem of eutrophication by the local government have been successful in cutting off point sources of external pollution, but the nutrient concentrations in lake water were still high with a Secchi depth of about 35 cm (Table 1) and submerged macrophytes were absent (Liu et al., submitted). In order to restore a section of the lake (as a demonstration), a total of 29.7 t (2.5 t ha⁻¹) fish were removed in the Nanhu section by seine fishing, encompassing 65% tilapia (*Oreochromis niloticus* Linn.), 16% silver carp (*Hypophthalmichthys molitrix* Cuvier et Valenciennes) and bighead carp (*Aristichthys nobilis* Richardson), 12% common carp (*Cyprinus carpio* Linnaeus), and 1.6% long-tailed anchovy (*Coilia grayii* Richardson). Upon removal, transplantation of the submerged macrophytes *Hydrilla verticillata* Linnaeus f. Royle and *Vallisneria natans* Lour. Hara was conducted in 2007. *V. natans* ramets were planted in the sediment at a density of about 10–15 plants per m⁻², and apical sections of young *H. verticillata* shoots were planted at a density of 20–30 plants m⁻². Piscivorous fish (18,000 snakehead *Channa argus* Cantor and 16,000 mandarin fish *Siniperca chuatsi* Basilewsky, 1,500 and 1,333 ha⁻¹, respectively) were also stocked (Liu et al., submitted).

About 85% of the Nanhu section was covered by submerged plants in 2010, with concomitant

improvements in water quality. The restored section was clear, incident light reaching all the way to the lake bottom (Fig. 1). For the purpose of the current study, sampling sites were established in the un-restored, unvegetated Fenghu section and in the restored, macrophyte-rich Nanhu section. Before restoration, water quality in the two sections was similar (Table 1).

Sampling and analysis

Sediment cores and P release experiment

Three 10-cm-deep sediment cores were taken along with overlying water from the selected sites in the un-restored Fenghu section and another three from open plant-free areas of the lake bed near the selected sites in the restored Nanhu section on March 12th, 2010. All cores were collected in perspex core tubes (15 cm length × 6.5 cm internal diameter). The tubes were immediately sealed at the top and bottom with silicon rubber stoppers to preserve the sediment structure. At the same time, 5 l lake water samples were collected in each section for use as experimental water. Both sediment cores and water samples were transported to the laboratory at Jinan University in boxes of ice.

The stoppers on the top of the sediment tubes were removed on arrival at the laboratory to allow gas exchange with air. At the start of the experiments, water overlying each core was siphoned off. The thickness of each sediment core was standardized to 5 cm from the bottom of each tube so that the sediment surface was 2.0 cm above the 5-mm-diameter ³²P injection hole on the side of the tubes (Zhang et al., 2013). The holes were sealed with a silicon rubber stopper through which ³²P could be injected into the sediment. Before initiating the experiment, 200 ml lake water was carefully added to each sediment tube.

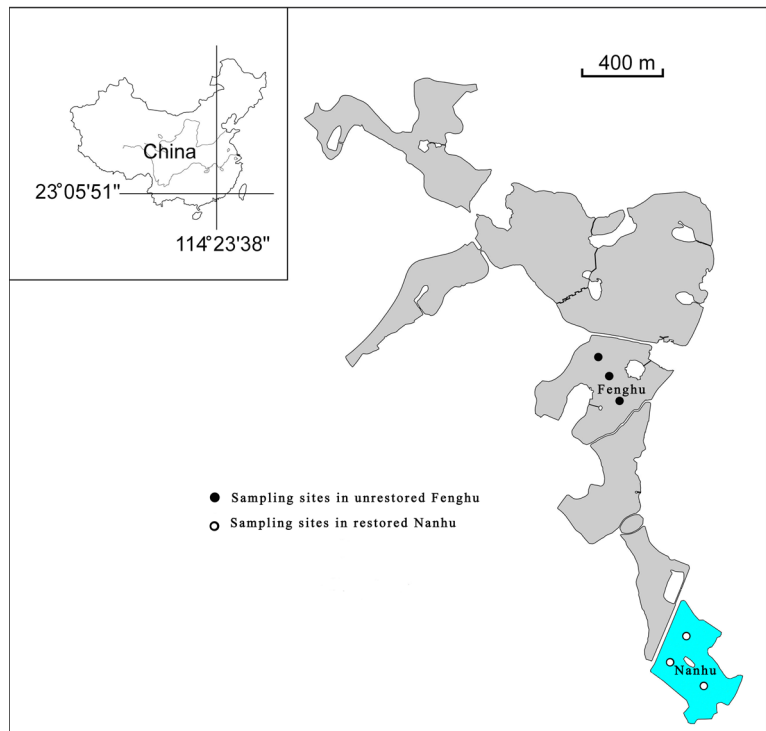
10 μCi NaH₂³²PO₄ was injected into each sediment core using a syringe and a needle piercing the rubber stopper. A 0.5 ml water sample was collected from the

Table 1 Water quality on average in August and October, 2004 of the two sections before restoration conducted (μg l⁻¹)

Section	SD (cm)	Chl <i>a</i>	TN	NH ₄ -N	NO ₃ -N	NO ₂ -N	TP	TDP	SRP
Fenghu	32	37.0	1,164	356	92	7	157	37	10
Nanhu	33	37.7	1,059	309	243	8	128	35	9

Unpublished data of Zhengwen Liu

Fig. 1 West Lake in Huizhou City and the sampling sites in the two sections of the lake



middle of the water column of each tube 3, 6, 24, 48, and 72 h after $\text{NaH}_2^{32}\text{PO}_4$ addition. Water overlying the sediment was not stirred before sampling. The samples were transferred to a scintillation vial containing 10 ml scintillation cocktail (5.0 g 2,5-Diphenyloxazole + 0.5 g 1,4-bis (5-phenyloxazol-2-yl) benzene + 1,000 ml dimethylbenzene + 400 g tritonX-100). The ^{32}P radioactivity (as counts per minute, dpm) of samples was assayed by liquid scintillation (Beckman Model LS6500, Beckman Coulter, Inc., Fullerton, CA) on March 15th and 18th, 2010, and corrected for the radioactive decay rate (Zhang et al., 2013).

The oxidation status of the sediment from both the restored and the unrestored sections was examined by measuring the dissolved oxygen (DO) concentration in the upper sediment layer using Unisense micro sensors (PA2000, Denmark).

Porewater and lake water

Samples from the sediment surface layer (0–5 cm depth) at three sites in the Fenghu and Nanhu sections were collected on March 12th, 2010, by a sediment

sampler (Fig. 1) after which they put into sealed plastic bags and transported to the laboratory in iceboxes. Porewater was extracted from the sediments by centrifugation at 5,000 rpm for 15 min (Kalnejais et al., 2015) for nutrient analysis. Samples for total dissolved phosphorus (TDP) were filtered through GF/C filters (0.45 μm) and determined after persulfate digestion (APHA, 1992), by molybdenum-antimony-ascorbic acid colorimetry with Shimadzu UV 2550 spectrophotometry with 100 mm cuvette at 880 nm. Samples for soluble reactive phosphorus (SRP) determination were also filtered through GF/C filters (0.45 μm) without persulfate digestion.

One liter samples of lake water were collected 0.5 m beneath the surface at each sampling site and analyzed for a range of water quality parameters. All samples were kept cold and transported to the laboratory at the Jinan University in Guangzhou within 3 h of collection. The samples were analyzed for TP, TDP, and SRP, and phytoplankton biomass was measured as chlorophyll *a* (Chl *a*), being determined spectrophotometrically after ethanol extraction at room temperature according to Jespersen & Christoffersen (1987). Total phosphorus (TP)

concentrations were determined after persulphate digestion (APHA, 1992). TDP and SRP were analyzed as described above for porewater.

Light and benthic algae

Light intensity at the bottom of the restored Nanhu and unrestored Fenghu sections was measured at all the sites using an underwater irradiance meter (ZDS-10W) between 9:00 to 12:00 am when collecting the sediment cores. Secchi depth (SD) was also measured using a Secchi disk.

Samples of sediment with benthic algae were taken from open areas of the Nanhu lake bed that were not directly colonized by submerged plants and situated close to the sediment sampling sites when collecting the sediment cores (Fig. 1). The algae were scraped from the sediment surface using a razor blade (Barbour et al., 1999), and their Chl *a* content was measured by spectrophotometry after ethanol extraction at room temperature according to Jespersen & Christoffersen (1987) to obtain a proxy for algal biomass. The dominant algal taxa were identified microscopically (Hu & Wei, 2006).

Statistical analyses

One-way ANOVA was performed to detect differences in TP, TDP, SRP, and Chl *a* concentrations and light intensity between samples from restored and unrestored sections. Repeated measurement ANOVAs were used to test for significant differences between restored and unrestored sections in ^{32}P activity in the water column and DO concentrations in the sediment.

Results

P in porewater

Concentrations of porewater TDP and SRP were significantly lower ($P < 0.05$) in the restored section (TDP was $0.9 \pm 0.3 \mu\text{g l}^{-1}$ and SRP was $0.3 \pm 0.2 \mu\text{g l}^{-1}$) than in the unrestored site (TDP $20.2 \pm 0.7 \mu\text{g l}^{-1}$ and SRP $14.6 \pm 2.4 \mu\text{g l}^{-1}$). These restored section values for TDP and SRP represent about 4%, and 2% of those in the unrestored section, respectively.

P and Chl *a* in lake water

TP concentrations in March were significantly lower ($F = 11.027$, $df = 1$, $P = 0.029$, Fig. 2) in the water column from the restored than from the unrestored section. However, there was no significant between-section difference in water column TDP or SRP ($P > 0.05$).

The water column Chl *a* concentration was $59.9 \pm 19.2 \mu\text{g l}^{-1}$ in the unrestored section compared with $6.1 \pm 2.0 \mu\text{g l}^{-1}$ in the restored section (Fig. 2), indicating a significant reduction ($F = 23.198$, $df = 1$, $P = 0.009$) in phytoplankton biomass through biomanipulation.

Light and benthic algae on the sediment surface

A highly significant difference in light intensity at the sediment surface was observed between the sections ($F = 61.714$, $df = 1$, $P = 0.001$). In the unrestored location, the sediment surface was completely dark and lake water SD was 37 ± 3 cm, while in the restored section light reached the sediment surface at an intensity of $21.6 \pm 4.8 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the time of sampling.

Benthic algae growing on the sediment surface in the restored Nanhu section were dominated by three taxa: *Oscillatoria tenuis* Ag. (Cyanophyceae), *Rhizoclonium hieroglyphicum* Ag. Kütz (Chlorophyceae), and *Navicula* spp. (Bacillariophyta). Mean benthic algal biomass measured as Chl *a* was

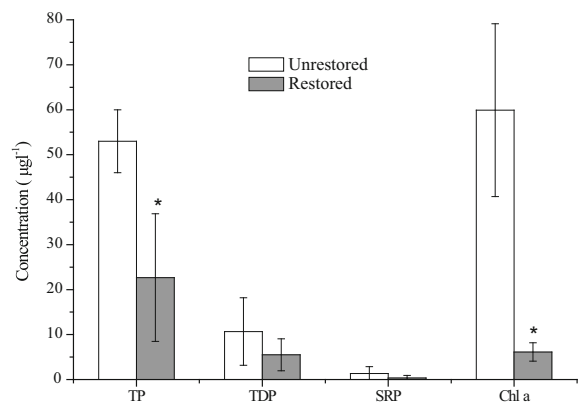


Fig. 2 P and Chl *a* concentrations in lake water (mean \pm SD) from restored and unrestored sections. Asterisk indicates significant ($P < 0.05$) differences between the two sections

$78.7 \pm 52.2 \text{ mg m}^{-2}$. Owing to the complete absence of light, no benthic algae were present on the sediment surface in the unrestored Fenghu section.

Sediment P release

^{32}P activity in the water column was lower in samples overlying sediment cores from the restored section than in sediment from the unrestored section ($F = 13.474$, $df = 1$, $P = 0.021$, Fig. 3). Furthermore, ^{32}P began to appear in the water above the unrestored sediment within 6 h of injection but was almost completely absent in the restored sediment water column until the 24th hour, and low until at least the 48th hour after injection, indicating delayed release of P from the sediments carrying a layer of benthic algae.

DO concentrations in sediment

Average DO concentrations in the uppermost sediment layers (2.0 mm depth) were significantly higher in the restored section ($5.03 \pm 0.19 \text{ mg l}^{-1}$) than in the unrestored section ($1.09 \pm 0.94 \text{ mg l}^{-1}$; $F = 58.740$, $df = 1$, $P < 0.001$, Fig. 4). The maximum depth of the oxic layer in the unrestored sediment was less than 2.5 mm compared with more than 3.5 mm in the restored sediment. Thus, the upper layers of the restored section sediments contained considerably more oxygen than those from the unrestored section.

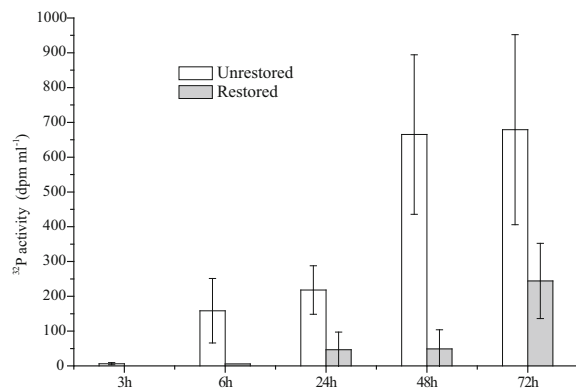


Fig. 3 Appearance of ^{32}P activity (mean \pm SD) in the water overlying the sediment cores during a 72-h period. At time zero, $10 \mu\text{Ci NaH}_2^{32}\text{PO}_4$ was injected into the sediment in each core through an injection hole of 20 mm below the surface

Discussion

We found that the biomanipulation undertaken in parts of shallow eutrophic Huizhou West Lake led to lower TP and Chl *a* concentrations in the water column and higher water clarity, allowing flourishing of benthic algae on the sediment surface of the lake bed. We also found lower TDP and SRP concentrations in porewater and lower release of injected ^{32}P , whereas DO concentrations in the surface sediment were higher in the restored than in the unrestored section.

It has long been known that submerged macrophyte can reduce the concentration of P in sediment porewater by direct absorption of nutrients (Carignan & Kalff, 1980; Shilla et al., 2006) and indirectly by release of oxygen from their roots, promoting formation of oxidized iron crusts in anaerobic sediment layers and enhancing the sorption of P (Hupfer & Dollan, 2003). More P is therefore often retained in the sediment as Fe-bound (Fe-P), thus reducing the P release (Wang et al., 2007). In the present study, P concentrations were substantially lower in the porewater in the restored section sediments –96% for TDP and 98% for SRP compared with the levels in the unrestored section. This reduction is important because porewater TDP and SRP is highly mobile and easily transferred to the overlying lake water (Holdren & Armstrong, 1986). In the restored section, sediment was collected in open plant-free areas, implying that plants can only indirectly (reduced sedimentation and, as discussed below, improved light conditions) have affected the oxygen and P concentrations in the porewater.

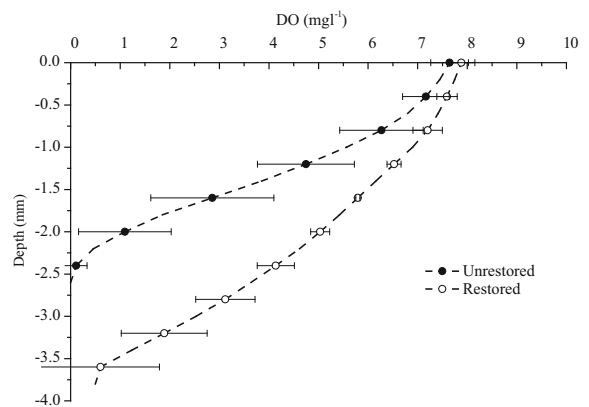


Fig. 4 Microprofile of DO concentration (mean \pm SD) in sediments from unrestored and restored sections of the lake bed

An enhanced benthic algal growth in the restored section may have contributed to the reduced sediment P release, as seen in other studies (Carlton & Wetzel, 1988; Hansson, 1990; Wetzel, 2001). In shallow lakes, even a small increase in light intensity at the sediment surface can promote the growth of benthic algae, some of which can tolerate remarkably low light levels (Steinman & McIntire, 1987). A light compensation point of $4.7 \mu\text{mol m}^{-2} \text{s}^{-1}$ has been recorded for benthic algal communities (Wiencke, 2011); however, the light compensation point may vary for different species of algae within the benthic communities. For example, for *Mougeotia*, the light compensation point falls below $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Graham et al., 1996), whereas *Cladophora* requires $29 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Lorenz et al., 1991). In our study, we measured $21.6 \pm 4.8 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the time of sampling in the restored section, while no light reached the bottom in the unrestored section. The light reaching the sediment surface in the restored section was sufficient to induce benthic algal growth, as evidenced by benthic algae occurrence on the sediment surface in the restored but not in the unrestored section. Benthic algal growth may have contributed to the lower P release in the restored section by enhancing the oxygen concentration in the sediment and by releasing extracellular material that acts as a glue to stabilize the sediment surface, the latter enhancing cohesion and adhesion of sediment particles and thus limiting sediment resuspension (Tolhurst et al., 2008; Lubarsky et al., 2010) and sediment P release. A previous ^{32}P lab study (Zhang et al., 2013) conducted at ambient room light intensity (daytime average: $15.8 \pm 9.4 \mu\text{mol m}^{-2} \text{s}^{-1}$, darkness at night), with a dark/light cycle of about 12:12 h, showed greater P retention capacity in sediment with benthic algae than in controls: 93% of ^{32}P being retained in the sediment with benthic algae compared with 84% in sediment without benthic algae. Our ^{32}P tracing experiment, conducted under similar light conditions as the previous ^{32}P lab study, concur with these results. Lower release means less phytoplankton and higher water clarity, which, in turn, stimulates benthic primary production further (Liboriussen & Jeppesen, 2003; Vadeboncoeur et al., 2003), and reduce P release from the sediment (Hansson, 1989).

Besides macrophytes and benthic algae, reduced disturbance by benthivorous fish and reduced sedimentation due to increased zooplankton grazing on

phytoplankton (which means less oxygen consumption in the sediment and thus less P-release) may potentially contribute to the lower concentration of the various P fractions found in the restored section, as judged from temperate lakes (Jeppesen et al., 2012). These factors may, however, be of less importance in subtropical and tropical lakes. In the restored section in our study, the fish community was still quantitatively dominated by omni-benthivorous fish (tilapia and crucian carp *Carassius auratus* Linnaeus). Moreover, no notable difference was traced in total fish biomass or fish size among the sections (Gao et al., 2014). Also, there was no clear change in the zooplankton community: the abundance of cladocerans remained low and small species such as *Chydorus* sp., *Alona* spp., and *Moina micrura* Kurz continued to dominate, with low potential grazing on phytoplankton in both sections as judged from a low zooplankton:phytoplankton biomass ratio, likely due to high abundance of juvenile fish (Liu et al., submitted), indicating that the role of these alternative factors in changing the P concentrations and the sediment P release were of less importance.

Although TP concentrations in overlying water were lower in the restored than unrestored section, no significant differences of TDP and SRP concentrations were recorded between the two sections, indicating that the main change in TP was in particulate phosphorus (PP). The lower release of injected ^{32}P from sediment cores in the restored section than unrestored section indicates that the restoration reduces P release from the sediment either by uptake by the more abundant benthic algae community or by complex binding to, for example, iron in the more oxidized sediment. It is important to notice, however, that accumulation of ^{32}P in overlying water does not necessarily represent a net release of P from the sediment as isotopic exchange (with ^{31}P) occurs after ^{32}P addition in the sediment (Jensen et al., 2009).

In conclusion, biomanipulation including adjustment of the fish assemblage and introduction of submerged macrophytes can decrease TDP concentrations in porewater and P release from the sediment, thereby reducing the P concentration in the lake water. These effects may contribute to creating an improved water quality after restoration. Oxidation by benthic algae and macrophytes, and perhaps lower sedimentation of algae were likely the most influential factors in the restored section of Huizhou West Lake, but

more detailed studies are needed to draw firm conclusions about the mechanisms involved and the contribution of the different factors.

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