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The combined effects of macrophytes (*Vallisneria denseserrulata*) and a lanthanum-modified bentonite on water quality of shallow eutrophic lakes: A mesocosm study☆

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Establishment of submerged macrophyte beds and application of chemical phosphorus inactivation are common lake restoration methods for reducing internal phosphorus loading. The two methods operate via different mechanisms and may potentially supplement each other, especially when internal phosphorous loading is continuously high. However, their combined effects have so far not been elucidated. Here, we investigated the combined impact of the submerged macrophyte *Vallisneria denseserrulata* and a lanthanum-modified bentonite (Phoslock®) on water quality in a 12-week mesocosm experiment. The combined treatment led to stronger improvement of water quality and a more pronounced reduction of porewater soluble reactive phosphorus than each of the two measures. In the combined treatment, total porewater soluble reactive phosphorus in the top 10 cm sediment layers decreased by 78% compared with the control group without Phoslock® and submerged macrophytes. Besides, in the upper 0–1 cm sediment layer, mobile phosphorus was transformed into recalcitrant forms (e.g. the proportion of HCl–P increased to 64%), while in the deeper layers, (hydr)oxides-bound phosphorus species increased 17–28%. Phoslock®, however, reduced the clonal growth of *V. denseserrulata* by 35% of biomass (dry weight) and 27% of plant density. Our study indicated that Phoslock® and submerged macrophytes may complement each other in the early stage of lake restoration following external nutrient loading reduction in eutrophic lakes, potentially accelerating the restoration process, especially in those lakes where the internal phosphorus loading is high.

1. Introduction

Eutrophication, occurring mainly as a result of excessive loading of nutrients such as nitrogen and phosphorus, is a severe problem worldwide (Downing, 2014; Smith and Schindler, 2009). Due to the controllability and effectiveness, reducing phosphorus inputs has generally been accepted as a key method to mitigate lake eutrophication (Schindler et al., 2008, 2016). However, numerous studies have affirmed that lake recovery after control of the external phosphorus loading is often delayed (Cooke et al., 2005; Jeppesen et al., 1991), which is in part due to phosphorus release from the sediment (internal loading) maintaining high water phosphorus

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concentrations and supporting continued algal growth (Søndergaard et al., 2003; Spears et al., 2012). Accordingly, reduction of the internal loading has become a key challenge in lake eutrophication reversal. In the past decades, various methods to reduce internal loading have been tested in laboratory experiments, followed by full-scale field applications. These include biomanipulation ("top-down" control) (Jeppesen et al., 1997, 2012), aquatic plant community restoration (Liu et al., 2018; Søndergaard et al., 2000), sediment dredging (Van der Does et al., 1992), sediment oxidation (Ripl, 1976), and chemical phosphorus inactivation (Hansen et al., 2003; Huser et al., 2016; Zamparas and Zacharias, 2014).

Submerged macrophytes play an important role in the phosphorus cycling process in lakes. They can enhance the phosphorus cycling by mobilizing phosphorus from the sediment through rhizosphere acidification (Long et al., 2008), but they can also reduce the phosphorus release by oxidizing the rhizosphere and metals such as iron (Fe) and manganese (Mn) (Christensen et al., 1997). (Hydr)oxides of these metals are coprecipitated with phosphorus and deposited on the root surface in the form of plaques with high specific surfaces and affinity for adsorbing phosphorus (St-Cyr et al., 1993; Wang et al., 2013). In addition, submerged macrophytes can enhance sedimentation (Barko and James, 1998), deplete labile phosphorus pools in the sediment (Barko and James, 1998), and take up nutrients from the water column (Bole and Allan, 1978; Carignan and Kalff, 1980), thereby transferring the bioavailable phosphorus from the environment into plant tissue. Phosphorus in healthy tissues is rarely released until plant decomposition (Barko et al., 1991; Barko and Smart, 1980).

Given the overall favorable effects of submerged macrophytes on reducing the internal loading and their structural role in shallow lake ecosystems, transplantation of submerged macrophyte stands has been recognized as a possibly effective management tool (Barko and James, 1998; Liu et al., 2018), but the effect may be hampered by continuous high internal loading. Chemical intervention might, therefore, be considered as a supplementary tool. Phoslock®, a lanthanum-modified bentonite (LMB), developed by the Australian government agency Commonwealth Scientific and Industrial Research Organisation (CSIRO) in the 1990s, is one of the most used techniques for chemical phosphorus immobilization (Bishop et al., 2014; Douglas et al., 1999; Lürling and van Oosterhout, 2013; Meis et al., 2013). Previous studies showed Phoslock® to be highly efficient at reducing phosphorus concentrations in the water column (Crosa et al., 2013; Marquez-Pacheco et al., 2013) and inactivating phosphorus in sediments (Bishop et al., 2014; Meis et al., 2012) over a wide range of physico-chemical conditions in both lab experiments and natural water bodies. However, confounding factors reducing the effect of phosphorus binding have also been reported (e.g. dissolved organic carbon (DOC), high pH (>9)) (Lürling et al., 2014; Reitzel et al., 2013a; Ross et al., 2008). Furthermore, several studies have combined Phoslock® with other chemical capping agents (e.g. polyaluminiumchloride (PAC), iron (III) chloride) (Lürling et al., 2016; Waajen et al., 2016b). However, studies combining Phoslock® with submerged macrophytes are scarce (Waajen et al., 2016a).

The mechanisms of submerged macrophytes and Phoslock® in reducing internal phosphorus loading differ, and their combined effects on water quality improvement are not clear. Since submerged macrophytes (e.g. *Myriophyllum spicatum*, *Hydrilla verticillata*, *Vallisneria spiralis*) take up phosphorus from both the sediment and the water column (Bole and Allan, 1978; Gentner, 1977), mainly from the sediment (Christiansen et al., 2016), the decreased phosphorus availability caused by Phoslock® may have negative effects on the growth of these plants. Conversely, the reduced algal biomass in the overlying water after Phoslock® addition may provide a better light environment for macrophyte growth (Gunn et al., 2013). To test the combined effects of submerged macrophytes and Phoslock® on the water quality and the influence of Phoslock® on submerged macrophyte growth, we conducted a 12-week mesocosm experiment. *Vallisneria denseserrulata*, a common perennial meadow-forming species in shallow lakes in China and often used in lake restoration (Liu et al., 2018; Zhou et al., 2016), was chosen for the experiment. We hypothesized that Phoslock® treatment in combination with transplantation of submerged macrophytes would complement each other via different mechanisms in reducing the internal phosphorus loading in the early stage of lake restoration.

2. Materials and methods

2.1. Experimental set-up

The mesocosm experiment was conducted from August to November 2018 at Dongshan station located at Taihu Lake ecosystem research station near Taihu Lake, Suzhou City (China), and the set-up involved four treatments: (1) control group without Phoslock® and macrophyte, (2) Phoslock® added; (3) *V. d.* (*V. denseserrulata*) planted, (4) Phoslock® added and *V. d.* planted. *V. denseserrulata* were procured from ponds of Belsun Aquatic Ecology Science and Technology Ltd. Each treatment consisted of four replicate barrels (top diameter 84 cm, bottom diameter 66 cm, height 83 cm). All barrels were filled with a 20-cm mixed sediment layer collected from the pond at Dongshan Town near Taihu Lake (Table 1) and 50-cm overlying water (water volume 227 L) pumped from the pond nearby. All the barrels were situated in a pond statically. The treatments were randomly assigned to barrels. A week after the initiation of the experiment, sediment cores and overlying water were sampled for analysis of phosphorus speciation and water chemistry. Then, four *V. denseserrulata* shoots with a wet weight of 6.40 ± 0.46 g and a length of 40.4 ± 2.9 cm were transplanted into each barrel of the *V. d.* treatment and the Phoslock®+*V. d.* treatment. Three hundred and 90 g Phoslock® was mixed into slurry with 2 L overlying water and then added to the water surface in each barrel of the Phoslock® treatment and the Phoslock® + *V. d.* treatment, corresponding to a Phoslock®: P_{mob} (mobile phosphorus) mass ratio of 100:1. Subsequently, the mesocosms were incubated for 12 weeks. The P_{mob} pool was calculated as the sum of potentially mobile phosphorus consisting of porewater phosphorus, phosphorus bound to reducible Fe/Mn, and labile organic phosphorus (i.e. H_2O−P, BD-P, NaOH-OP). NaOH-OP is organic phosphorus in the extractant of sediment treated with NaOH (see 2.2.4).

2.2. Sampling and measurements

2.2.1. Water samples

The pH and temperature of the water column were measured by portable multiparameter water monitoring probes (Aquread AP-2000, UK) (Fig. S1, S2) every two weeks. Water samples were collected every two weeks and analyzed for total phosphorus (TP),

Table 1
Initial sediment properties (n = 16).

Properties	Mean ± SD
Loss on ignition (LOI) (%)	4.31 ± 0.87
Water content (%)	49.33 ± 4.81
Dry bulk density (g cm^{-3})	0.74 ± 0.10
TP (mg gDW^{-1})	0.63 ± 0.03
P_{mob} (mg gDW^{-1})	0.31 ± 0.03

total nitrogen (TN), and chlorophyll *a* (Chl.a). TP rather soluble reactive phosphorus (SRP) was taken as a key parameter that reflecting the effects resulted from Phoslock® and *V. denseserrulata* in reducing phosphorus concentrations. The changes in SRP concentrations in the water column are the result of the dual effects of the uptake by algae and submerged macrophytes and sediment release. The part absorbed by algae will occur as particulate phosphorus. In addition, sediments may also release dissolved organic phosphorus forms that are only measured after wet oxidation (Jensen et al., 2017). These forms also show up in TP analyses but not in SRP analysis. However, we put SRP figure in the supplementary material file to give a more complete understanding (Fig. S3). TP and TN concentrations were spectrophotometrically determined after digestion with $K_2S_2O_8$ and H_2SO_4 at 120 °C for 30 min, as described in Jin and Tu (1990). Chl.a was measured spectrophotometrically from the matter retained on a GF/C filter after extraction in a 90% (v/v) ethanol/water solution (Chen and Gao, 2000).

2.2.2. Light attenuation

Light intensity (in μmol quanta m^{-2} s^{-1}) was measured using an underwater photosynthetically active radiation meter (Apogee MQ-510, USA) at a depth of 0.3 m (near the top of the plant shoots) every two weeks, and the vertical light attenuation coefficient (K_d) (in m^{-1}) was calculated by equation (1) (McPherson and Miller, 1987):

$$K_d = \ln(I_0 / I_z)/z \tag{1}$$

where I_0 is light intensity at the water surface, I_z is light intensity at depth z, and z is the depth where measurements were made (in m).

2.2.3. Porewater soluble reactive phosphorus

Porewater samples were gathered every four weeks with HR-Peeper probes (vertical resolution of 5.0 mm, www.easysensor.net). The probes were randomly inserted into the barrels and left for 48 h to equilibrate. After retrieval, the sediment solids adhering to the surfaces of the probes was wiped off and the probes were rinsed with deionized water. Samples were then immediately analyzed for SRP according to a miniaturized photometrical method described in Laskov et al. (2007). Besides from presenting porewater SRP files the total SRP content (mg m^{-2}) in the surface 10 cm sediment layers was calculated by equation (2):

$$SRP_{total} = \sum_{1}^{20} C_i \cdot \left(\frac{M_i}{D}\right) \Big/ S \tag{2}$$

where i is the number of the layer and there are 20 layers in the 10 cm sediment; C_i is the concentration of SRP in each layer (in mg mL^{-1}); M_i is the mass of porewater in each layer of the sediment core and equals the wet weight of sediment minus the dry weight of sediment (in g); D is the density of porewater, i.e. 1 g ml^{-1}; and S is the area of the cross section of the sediment core (in m^2).

2.2.4. Sediment characteristics

One sediment core from each barrel (16 cores in all) was sampled by a lucite tube (internal diameter 36 mm) at both the beginning and at the end of the experiment. The initial sediment cores were collected and the 0–5 cm sediment in each core was mixed to analyze phosphorus fractions and calculate P_{mob} (Rydin, 2000), while the upper 8 cm of the terminal cores were sliced at 1 cm intervals to investigate the changes of phosphorus forms with depth. TP in the sediments (0.5 g DW) was determined following ignition of the sediment at 550 °C and subsequent digestion in 1 M HCl (50 ml) (Aspila et al., 1976). Identification of major pools of phosphorus in the sediments was made following the sequential extraction scheme modified by Paludan and Jensen (1995). Labile phosphorus was extracted from 1 g wet sediment by H_2O; reducible Fe and Mn hydroxide-bound phosphorus were extracted with BD reagent (bicarbonate-dithionite); metal oxide-bound phosphorus (NaOH-IP) and labile organic phosphorus (NaOH-OP) were extracted with 0.1 M NaOH; and inorganic phosphorus pools, e.g., $CaCO_3$-bound phosphorus were extracted with 0.5 M HCl. Residual phosphorus was calculated as TP minus the sum of the extracted phosphorus pools. The concentration of each phosphorus fraction was converted to dry matter by equation (3):

$$C_{P(DW)} = \frac{C \cdot V}{m_w \cdot (1 - \text{water content})} \tag{3}$$

where $C_{P(DW)}$ is the concentration of phosphorus fractions in dry matter (in mg g DW^{-1}); C is the concentration of phosphorus in the extractant (in mg L^{-1}); V is the volume of extractant (in L); m_w is the wet weight of sediment (in g); water content = (wet weight − dry weight)/wet weight. Dry weight was measured after drying the sediment at 105 °C for 24 h.

2.2.5. Macrophyte traits

Macrophyte (*V. denseserrulata*) traits (i.e. biomass, length, shoot number) were determined at the start and at the end of the experiment. Also, at the start, an additional 10 shoots were chosen to measure the water content, which was used to calculate the initial dry weight. At the end of the experiment, all plants were uprooted by hand and rinsed carefully to remove attached material on leaves and roots. Dry weight (biomass dried at 45 °C) and physical dimensions were estimated using an electronic balance (to the nearest 0.01 g) and ruler (to the nearest 1 mm), respectively. The relative growth rate (RGR) of the plant in each barrel was calculated using equation (4) (Hunt, 1982):

$$RGR\ (d^{-1}) = \ln\left(\frac{W_f}{W_i}\right) \Big/ \text{days} \tag{4}$$

where W_f (g) and W_i(g) are the final and initial total biomass (DW) in each barrel, respectively.

2.3. Statistical analysis

The effects of Phoslock® and *V. denseserrulata* on the water chemistry and light attenuation coefficient versus time were analyzed by repeated measures analysis of variance (rm-ANOVA) in SPSS 20.0. If the assumption of sphericity was violated, we used the Greenhouse-Geissler correction of the degrees of freedom when the epsilon was <0.75 and the Huynh-Feldt correction of the degrees of freedom when the epsilon was >0.75 (Lürling and Faassen, 2012). Two-way ANOVA was used to identify the effects of Phoslock® and *V. denseserrulata* on SRP concentrations, and depth was set as a random factor. If a significant interaction was observed, a simple effects test (Bonferroni method) was conducted to identify where the difference occurred. One-way ANOVA followed by post hoc test (Tukey method) was conducted to analyze the difference in SRP_{total} in the surface 10 cm sediment between each of the two treatments. *t*-test was applied to elucidate the effects of Phoslock® on *V. denseserrulata* traits. The level of significance was set to $p < 0.05$ for all tests.

3. Results

3.1. Water quality and light condition

Both Phoslock® and *V. denseserrulata* significantly reduced TP concentrations in the water column (Table S1, $p < 0.001$ for both), and the most obvious reduction of TP was observed in the Phoslock® + *V. d.* treatment (Fig. 1). However, Phoslock® significantly increased TN concentrations while *V. denseserrulata* significantly reduced TN concentrations relative to the control (Fig. 1; Table S1, $p = 0.002$ and $p = 0.009$, respectively). Demonstrating a similar pattern as that of TP, the effects of Phoslock® and *V. denseserrulata* on the reduction of Chl.a were statistically significant (Table S1, $p = 0.008$ and 0.012, respectively), and the most rapid and obvious reduction of Chl.a was observed in the Phoslock® + *V. d.* treatment (Fig. 1). *V. denseserrulata* significantly reduced K_d while Phoslock® had no effect (Fig. 1; Table S1, $p < 0.001$ and $p = 0.076$, respectively). Using mean TP and Chl.a concentrations during the experiment period, reduction rates were calculated by comparing the different treatments with the controls (Fig. S4). The reduction rates of TP and Chl.a concentrations in the combined treatment was higher than that in the single treatments, however, the combined effects were not additive (less than the sum of the two single effects).

3.2. Phosphorus in the sediment

During the experiment, both Phoslock® and *V. denseserrulata* significantly reduced the porewater SRP concentrations (Fig. 2; Table S2, $p < 0.001$ at three time points), interaction being observed only in week 4 (Table S2, $p < 0.001$). In the two treatments with Phoslock®, SRP concentrations in the mesocosms with *V. denseserrulata* were significantly lower than in those without *V. denseserrulata* (Bonferroni test, $p < 0.001$). In the two treatments without Phoslock®, SRP concentrations did not differ significantly in either the with- or the without- *V. denseserrulata* mesocosms ($p = 0.825$). At the end of the experiment, total SRP in the surface 10 cm sediment layers had decreased by 78% in the Phoslock® + *V. d.* treatment compared with the control group without Phoslock® and macrophytes (Tukey test, $p < 0.001$), while in the Phoslock® treatment and the *V. d.* treatment it had decreased by 35% and 33%, respectively ($p = 0.033$, 0.046, respectively). No significant difference appeared between the two single treatments ($p = 0.996$) (Fig. 3).

In the Phoslock® treatment, the most obvious changes in phosphorus fractions were observed in the 0–1 cm layer where HCl–P increased to 0.51 mg gDW^{-1} and became the major pool (accounting for 68% of TP), while other potentially mobile phosphorus fractions (H_2O–P, BD-P, NaOH–P) decreased compared with the control group without Phoslock® and macrophyte (Fig. 4). In contrast to the Phoslock® treatment, metal (hydr)oxides-bound phosphorus (i.e. BD-P and NaOH-IP) in the surface sediment layer in the *V. d.* treatment increased by 50% and HCl–P decreased by 20% compared with the control group. BD-P and NaOH-IP also increased in the deeper sediments compared with the control group. In the treatment with both Phoslock® and *V. denseserrulata*, HCl–P increased to 0.53 mg gDW^{-1} and constituted 64% of TP in the 0–1 cm layer. However, BD-P in the sediments below 3 cm exhibited an increase within the range of 17%–28% compared with the control group (Fig. 4).

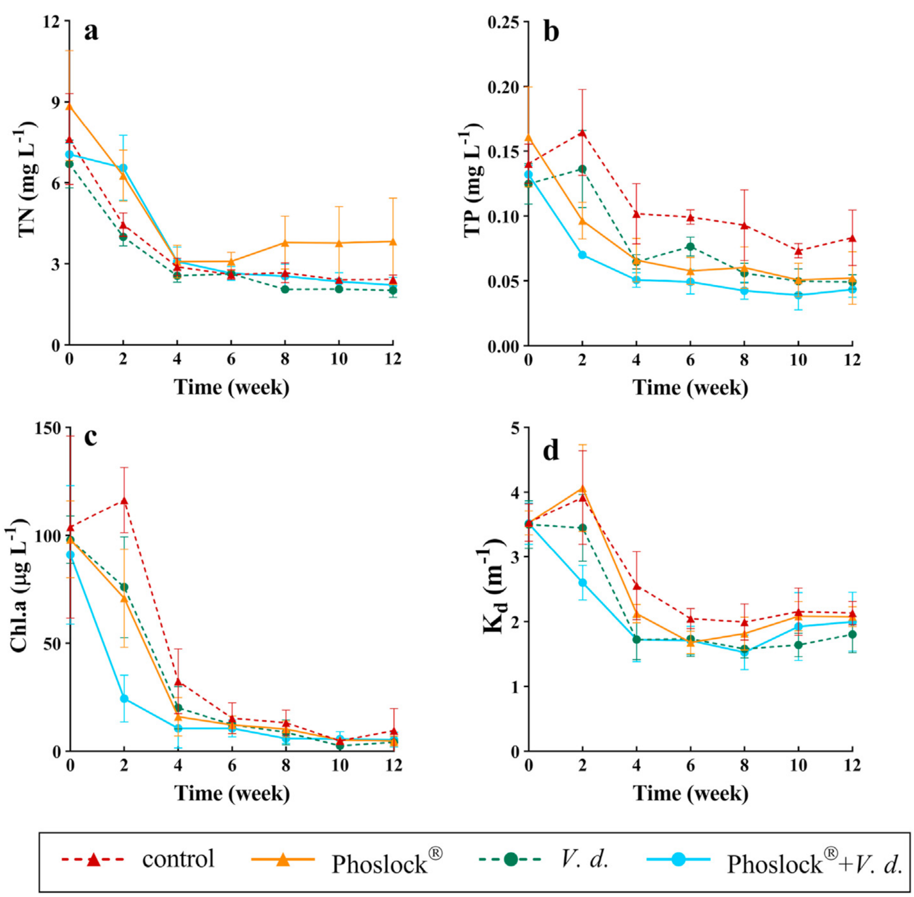

Fig. 1. Water chemistry and light attenuation coefficient (K_d) in the four treatments during the experiment. Vertical bars indicate standard deviation.

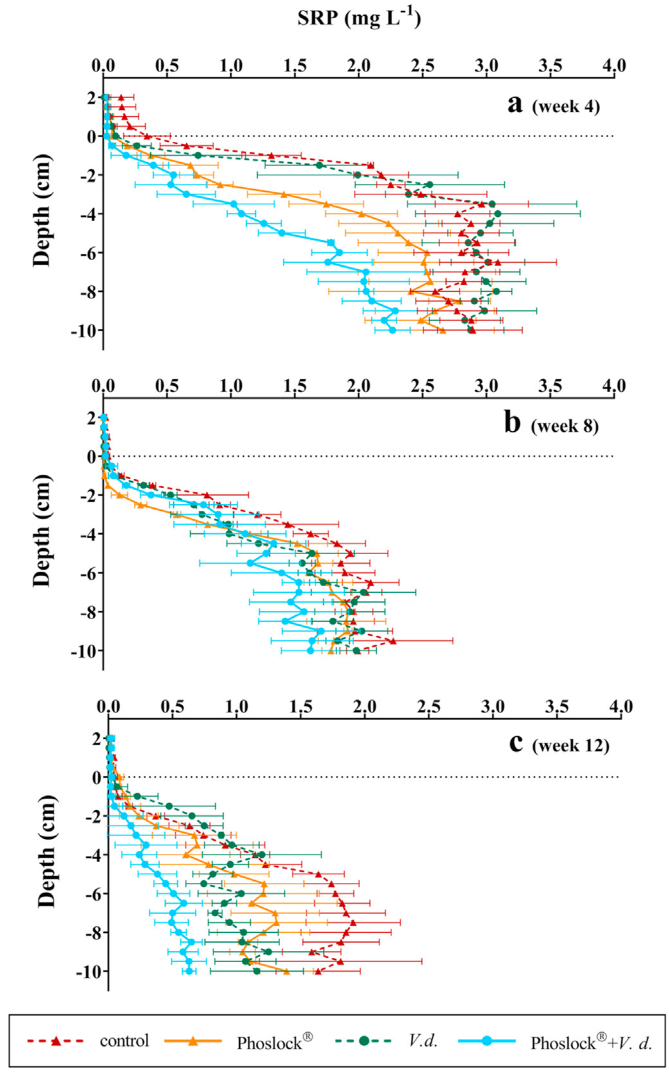

Fig. 2. Mean values (n = 3) of SRP concentrations in depth profiles in near-bottom water and porewater in the four different treatments at different times. Three replicates for each treatment. Horizontal bars indicate standard deviation.

3.3. Submerged macrophyte traits

Compared with the *V. d.* treatment, the biomass and density of plants significantly decreased by 35% and 27% ($p = 0.002$, 0.009, respectively) in the Phoslock® + *V. d.* treatment, respectively, whereas no significant changes occurred in individual weight ($p = 0.098$) (Fig. 5a–c). RGR decreased markedly by 17% ($p = 0.002$) (Fig. 5d). The biomass and total length of stolons decreased significantly by 24% and 30% ($p = 0.010$, 0.019, respectively) (Fig. 5e and f).

4. Discussion

4.1. Effects of Phoslock® and submerged macrophytes on phosphorus and nitrogen concentrations

The Phoslock®+*V. d.* treatment led to a much stronger improvement of water quality than if the two measures were used alone, since the combined treatment had stronger effects on phosphorus in both the water column and the sediment. However, water TP decreased and clarity increased over time in all treatments

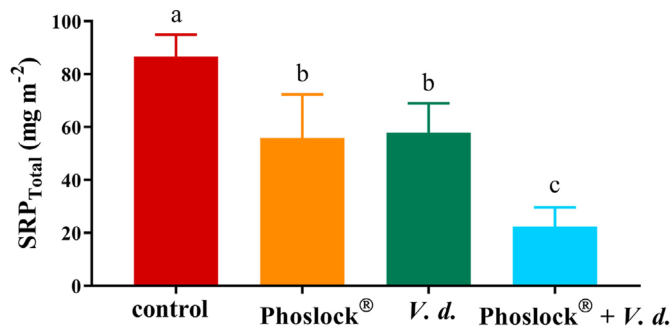

Fig. 3. Total SRP in the surface 10 cm sediment at the end of the experiment. (Different letters indicate a significant difference among treatments, $p < 0.05$). Vertical bars indicate standard deviation.

and in the control, which can be explained by both clam water conditions (no stirring) and decreasing water temperature over the course of the experiment (Fig. S2).

In the two Phoslock® treatments, Phoslock® not only led to fast removal of phosphorus from the water column during the addition, it also capped phosphorus on the surface of the sediment, retarding the internal phosphorus loading. The capping layer depleted the SRP diffused from deep sediment. At the end of the experiment, however, total SRP in the top 10 cm sediment of Phoslock® treatment had decreased only by 35% compared with the control group without Phoslock® and macrophytes, while total SRP in the Phoslock®+*V. d.* treatment had decreased by 78%, indicating that the combination of Phoslock® and macrophytes had a stronger efficiency than if Phoslock® was used alone. This likely reflects that *V. denseserrulata* enhanced the P-binding capacity by oxidizing metals in the deep sediment, adsorbing more porewater SRP and thus increasing the content of metal (hydr)oxides-bound phosphorus species as detected. Moreover, submerged macrophytes can also take up porewater SRP by root for growth (Christiansen et al., 2016).

In the two treatments with Phoslock®, the strong transformation of phosphorus forms in the top layers was in line with previous studies on Phoslock® application (Bishop et al., 2014; Meis et al., 2012; Reitzel et al., 2013b). However, BD-P in the deep sediment layers in the combined treatment increased relative to the treatment with Phoslock® implemented alone. In the upper sediment layer, the significant decrease of BD-P and NaOH-IP and the increase of the HCl–P pool not only indicate a stronger binding capacity of Lanthanum (La) with phosphorus compared with metal (hydr)oxides, but also phosphorus re-adsorption onto available La during the sequential phosphorus extraction by the BD and NaOH solution (Reitzel et al., 2013b). Furthermore, since BD-P is sensitive to redox and can be released under anoxia or low redox conditions (Boström et al., 1988), the surface Phoslock® layer will be capable of re-adsorbing the phosphorus released from BD-P in the deeper sediments when reductive conditions occur (Reitzel et al., 2013b). Submerged macrophytes release oxygen produced during photosynthesis into sediments through their roots (Santner et al., 2015), which leads to oxidation of the metals and thus increase the phosphorus binding capacity. Hence, influenced by both Phoslock® and macrophytes, the Phoslock®+*V. d.* treatment had the lowest phosphorus concentration in the water column.

However, a side effect of Phoslock® in the form of increased nitrogen efflux appeared, possibly reflecting the addition of ammonium with Phoslock® (Reitzel et al., 2013b; van Oosterhout and Lürling, 2013). Moreover, the clonal growth of *V. denseserrulata* led to a remarkable reduction in TN relative to the control treatment. This may result from absorption of nitrogen by

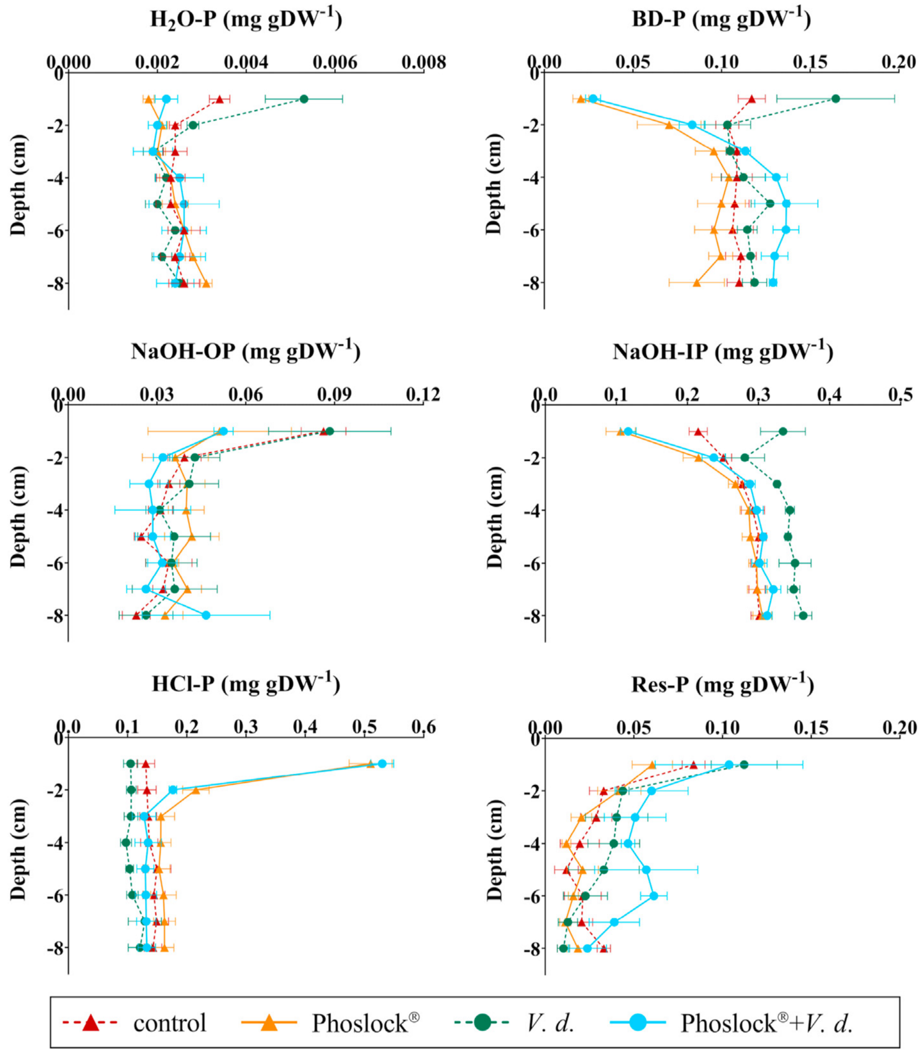

Fig. 4. Vertical distribution of different phosphorus fractions in the sediments of the different treatments at the end of the experiment. Four replicates for each treatment. Horizontal bars indicate standard deviation.

the plants or enhanced nitrification and denitrification (Barko and James, 1998; Reddy et al., 1989). Nevertheless, the changes in nitrogen species are a topic warranting further studies as we did not study the nitrogen-cycling in detail in present study.

Thus, compared with the single treatment, the combined treatment had two ways of binding phosphorus and retarding the phosphorus release into the water column, which is more conducive to reducing internal phosphorus loading. In addition, *V. denseserrulata* can offset the effect of Phoslock® on the nitrogen increase, as shown in the Phoslock® + *V. d.* treatment.

4.2. Effects of Phoslock® on submerged macrophyte growth

Being one of the fundamental factors for photosynthesis, light plays an important role for plant growth. In this study, however, the application of Phoslock® improved light conditions only insignificantly, indicating that light was not the major influencing factor for macrophyte growth. Therefore, the recorded negative effect on submerged macrophyte clonal growth might be related to the reduction of bioavailable phosphorus in the surface sediment. According to our observations, the clonal growth of *V. denseserrulata* was through elongation of the stolon from the leaf sheath of the mother plant near the sediment-water interface, followed by growth of leaves and roots from the apex of the stolon and formation of a new ramet. Then the roots kept growing and penetrated into the deep sediment. In this study, phosphorus fractions transformed mainly in the top 1 cm, and P_{mob} consisting of H_2O–P, BD-P and NaOH-OP declined to 0.08 mg gDW^{-1}, accounting for only 10% of TP compared with 0.20 mg gDW^{-1} in the control group without Phoslock® and macrophytes. Since submerged macrophytes can absorb phosphorus by roots and shoots (Gentner, 1977), and mainly through roots (Christiansen et al., 2016), the low content of P_{mob} in the top sediment seems to be unfavorable to the new ramets in their early life stage. With the slower growth of new ramets, clonal growth was overall inhibited and, eventually, the shoot density and total biomass of *V. denseserrulata* decreased. However, for the individual plant in the Phoslock® + *V. d.* treatment, P_{mob} was not significantly different from the *V. d.* treatment when its roots elongated into the sediments below 1 cm, and in its later life stages it can obtain a similar level of phosphorus as in the *V. d.* treatment. However, the long-term (say >1 year) effects of Phoslock® on submerged macrophytes require further studies.

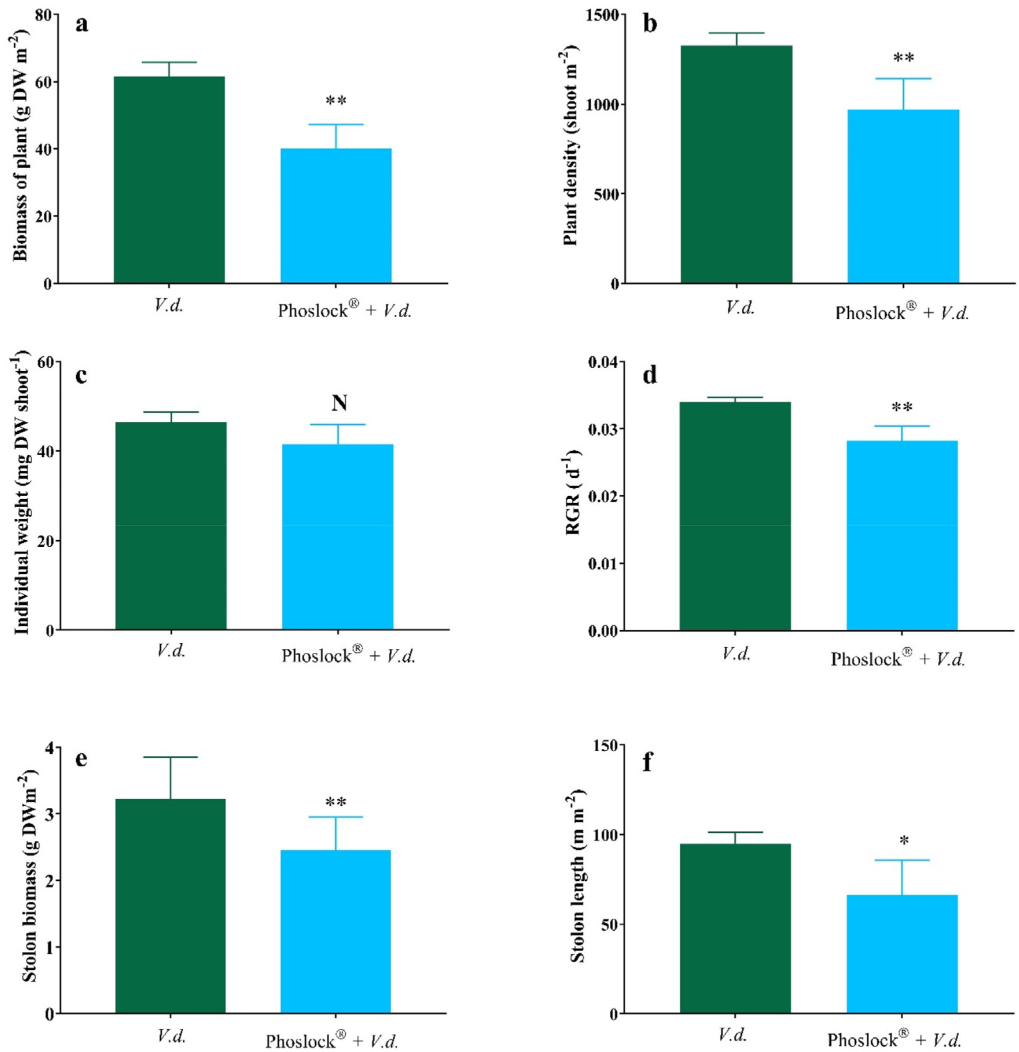

Fig. 5. Macrophyte traits at the end of the experiment. Significance results of *t*-test relative to the Phoslock® treatments and controls indicated by N ($p > 0.05$); * ($p < 0.05$); ** ($p < 0.01$). Vertical bars indicate standard deviation.

5. Conclusion

In this study, the largest improvement in water quality was observed in the Phoslock® + *V. d.* treatment; thus, using the methods in combination had a stronger effect than using them individually. The combined treatment led to the most significant and dramatic decrease in porewater SRP, and total SRP in the top 10 cm sediment layers decreased by 78% compared with the control group without Phoslock® and macrophytes. In the 0–1 cm sediment layer, HCl–P increased to 0.53 mg gDW^{-1} and constituted 64% of TP, and BD-P in the sediment below 3 cm increased 17–28%. The phosphorus inactivation by La^{3+} in the surface layer as well as the oxidization of metals by roots likely increased the P-binding capacity in the sediment. Additionally, Phoslock® had a negative effect on *V. denseserrulata* growth, mainly clonal growth with a decrease by 35% in biomass (dry weight) and 27% in plant density, whereas the impact on individual weight was negligible, which likely can be ascribed to phosphorus inactivation in the surface sediment. Hence, Phoslock® and submerged macrophytes may complement each other in the early stage of lake restoration following external nutrient loading reduction, potentially accelerating the restoration process in eutrophic lakes, especially those where the internal phosphorus loading is high.

Credit author statement

Xiumei Zhang: Conceptualization; Methodology; Investigation; Formal analysis; Writing – original draft. Wei Zhen: Conceptualization; Methodology; Investigation; Formal analysis; Writing - review &editing. Henning S. Jensen: Writing - review &editing. Kasper Reitzel: Writing - review &editing., Erik Jeppesen: Conceptualization; Writing - review &editing. Zhengwen Liu: Conceptualization; Funding acquisition; Project administration; Supervision; Writing - review &editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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