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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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Running Title: The Combined Effects of Macrophyte and Phoslock[®]

1 **Abstract**

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

21

22 *Keywords:* Restoration, Phosphorus, Eutrophication, Sediments

23

24 **1. INTRODUCTION**

25 Eutrophication, occurring mainly as a result of excessive loading of nutrients such as
26 nitrogen and phosphorus, is a severe problem worldwide (Downing, 2014; Smith and
27 Schindler, 2009). Due to the controllability and effectiveness, reducing phosphorus inputs has
28 generally been accepted as a key method to mitigate lake eutrophication (Schindler et al.,
29 2008, 2016). However, numerous studies have affirmed that lake recovery after control of the
30 external phosphorus loading is often delayed (Cooke et al., 2005; Jeppesen et al., 1991),
31 which is in part due to phosphorus release from the sediment (internal loading) maintaining
32 high water phosphorus concentrations and supporting continued algal growth (Søndergaard et
33 al., 2003; Spears et al., 2012). Accordingly, reduction of the internal loading has become a
34 key challenge in lake eutrophication reversal. In the past decades, various methods to reduce
35 internal loading have been tested in laboratory experiments, followed by full-scale field
36 applications. These include biomanipulation (“top-down” control) (Jeppesen et al., 1997,
37 2012), aquatic plant community restoration (Liu et al., 2018; Søndergaard et al., 2000),
38 sediment dredging (Van der Does et al., 1992), sediment oxidation (Ripl, 1976), and chemical
39 phosphorus inactivation (Hansen et al., 2003; Huser et al., 2016; Zamparas and Zacharias,
40 2014).

41 Submerged macrophytes play an important role in the phosphorus cycling process in
42 lakes. They can enhance the phosphorus cycling by mobilizing phosphorus from the sediment
43 through rhizosphere acidification (Long et al., 2008), but they can also reduce the phosphorus
44 release by oxidizing the rhizosphere and metals such as iron (Fe) and manganese (Mn)
45 (Christensen et al., 1997). (Hydr)oxides of these metals are coprecipitated with phosphorus
46 and deposited on the root surface in the form of plaques with high specific surfaces and
47 affinity for adsorbing phosphorus (St-Cyr et al., 1993; Wang et al., 2013). In addition,
48 submerged macrophytes can enhance sedimentation (Barko and James, 1998), deplete labile

49 phosphorus pools in the sediment (Barko and James, 1998), and take up nutrients from the
50 water column (Bole and Allan, 1978; Carignan and Kalff, 1980), thereby transferring the
51 bioavailable phosphorus from the environment into plant tissue. Phosphorus in healthy tissues
52 is rarely released until plant decomposition (Barko et al., 1991; Barko and Smart, 1980).

53 Given the overall favorable effects of submerged macrophytes on reducing the internal
54 loading and their structural role in shallow lake ecosystems, transplantation of submerged
55 macrophyte stands has been recognized as a possibly effective management tool (Barko and
56 James, 1998; Liu et al., 2018), but the effect may be hampered by continuous high internal
57 loading. Chemical intervention might, therefore, be considered as a supplementary tool.
58 Phoslock[®], a lanthanum-modified bentonite (LMB), developed by the Australian government
59 agency Commonwealth Scientific and Industrial Research Organisation (CSIRO) in the
60 1990s, is one of the most used techniques for chemical phosphorus immobilization (Bishop et
61 al., 2014; Douglas et al., 1999; Lüring and van Oosterhout, 2013; Meis et al., 2013).
62 Previous studies showed Phoslock[®] to be highly efficient at reducing phosphorus
63 concentrations in the water column (Crosa et al., 2013; Marquez-Pacheco et al., 2013) and
64 inactivating phosphorus in sediments (Bishop et al., 2014; Meis et al., 2012) over a wide
65 range of physico-chemical conditions in both lab experiments and natural water bodies.
66 However, confounding factors reducing the effect of phosphorus binding have also been
67 reported (e.g. dissolved organic carbon (DOC), high pH (>9)) (Lüring et al., 2014; Reitzel et
68 al., 2013a; Ross et al., 2008). Furthermore, several studies have combined Phoslock[®] with
69 other chemical capping agents (e.g. polyaluminiumchloride (PAC), iron (III) chloride)
70 (Lüring et al., 2016; Waajen et al., 2016b). However, studies combining Phoslock[®] with
71 submerged macrophytes are scarce (Waajen et al., 2016a).

72 The mechanisms of submerged macrophytes and Phoslock[®] in reducing internal

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

88

89 **2. MATERIALS AND METHODS**

90 **2.1. *Experimental set-up***

91 The mesocosm experiment was conducted from August to November 2018 at Dongshan
92 station located at Taihu Lake ecosystem research station near Taihu Lake, Suzhou City
93 (China), and the set-up involved four treatments: (1) control group without Phoslock[®] and
94 macrophyte, (2) Phoslock[®] added; (3) *V. d.* (*V. denseserrulata*) planted, (4) Phoslock[®] added
95 and *V. d.* planted. *V. denseserrulata* were procured from ponds of Belsun Aquatic Ecology
96 Science and Technology Ltd. Each treatment consisted of four replicate barrels (top diameter

97 84 cm, bottom diameter 66 cm, height 83 cm). All barrels were filled with a 20-cm mixed
98 sediment layer collected from the pond at Dongshan Town near Taihu Lake (Table 1) and
99 50-cm overlying water (water volume 227 L) pumped from the pond nearby. All the barrels
100 were situated in a pond statically. The treatments were randomly assigned to barrels. A week
101 after the initiation of the experiment, sediment cores and overlying water were sampled for
102 analysis of phosphorus speciation and water chemistry. Then, four *V. denseserrulata* shoots
103 with a wet weight of 6.40 ± 0.46 g and a length of 40.4 ± 2.9 cm were transplanted into each
104 barrel of the *V. d.* treatment and the Phoslock[®]+*V. d.* treatment. Three hundred and ninety
105 grams Phoslock[®] was mixed into slurry with 2 L overlying water and then added to the water
106 surface in each barrel of the Phoslock[®] treatment and the Phoslock[®] + *V. d.* treatment,
107 corresponding to a Phoslock[®]: P_{mob} (mobile phosphorus) mass ratio of 100:1. Subsequently,
108 the mesocosms were incubated for 12 weeks. The P_{mob} pool was calculated as the sum of
109 potentially mobile phosphorus consisting of porewater phosphorus, phosphorus bound to
110 reducible Fe/Mn, and labile organic phosphorus (i.e. H₂O-P, BD-P, NaOH-OP). NaOH-OP is
111 organic phosphorus in the extractant of sediment treated with NaOH (see 2.2.4).

112 **2.2. Sampling and measurements**

113 **2.2.1 Water samples**

114 The pH and temperature of the water column were measured by portable multiparameter
115 water monitoring probes (Aquaread AP-2000, UK) (Fig. S1, S2) every two weeks. Water
116 samples were collected every two weeks and analyzed for total phosphorus (TP), total
117 nitrogen (TN), and chlorophyll a (Chl.a). TP rather soluble reactive phosphorus (SRP) was
118 taken as a key parameter that reflecting the effects resulted from Phoslock[®] and *V.*
119 *denseserrulata* in reducing phosphorus concentrations. The changes in SRP concentrations in
120 the water column are the result of the dual effects of the uptake by algae and submerged

121 macrophytes and sediment release. The part absorbed by algae will occur as particulate
122 phosphorus. In addition, sediments may also release dissolved organic phosphorus forms that
123 are only measured after wet oxidation (Jensen et al., 2017). These forms also show up in TP
124 analyses but not in SRP analysis. However, we put SRP figure in the supplementary material
125 file to give a more complete understanding (Fig. S3). TP and TN concentrations were
126 spectrophotometrically determined after digestion with $K_2S_2O_8$ and H_2SO_4 at 120 °C for 30
127 min, as described in Jin and Tu (1990). Chl.a was measured spectrophotometrically from the
128 matter retained on a GF/C filter after extraction in a 90% (v/v) ethanol/water solution (Chen
129 and Gao, 2000).

130

131 **2.2.2 Light attenuation**

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

$$136 \quad K_d = \ln(I_0/I_z)/z \quad (1)$$

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

139

140 **2.2.3 Porewater soluble reactive phosphorus**

141 Porewater samples were gathered every four weeks with HR-Peeper probes (vertical
142 resolution of 5.0 mm, www.easysensor.net). The probes were randomly inserted into the
143 barrels and left for 48 h to equilibrate. After retrieval, the sediment solids adhering to the
144 surfaces of the probes was wiped off and the probes were rinsed with deionized water.

145 Samples were then immediately analyzed for SRP according to a miniaturized photometrical
146 method described in Laskov et al. (2007). Besides from presenting porewater SRP files the
147 total SRP content (mg m^{-2}) in the surface 10 cm sediment layers was calculated by the
148 equation (2):

$$149 \quad \text{SRP}_{\text{total}} = \sum_1^{20} C_i \cdot \left(\frac{M_i}{D}\right) / S \quad (2)$$

150 where i is the number of the layer and there are 20 layers in the 10 cm sediment; C_i is the
151 concentration of SRP in each layer (in mg mL^{-1}); M_i is the mass of porewater in each layer of
152 the sediment core and equals the wet weight of sediment minus the dry weight of sediment
153 (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
154 sediment core (in m^2).

155

156 **2.2.4 Sediment characteristics**

157 One sediment core from each barrel (16 cores in all) was sampled by a lucite tube
158 (internal diameter 36 mm) at both the beginning and at the end of the experiment. The initial
159 sediment cores were collected and the 0-5 cm sediment in each core was mixed to analyze
160 phosphorus fractions and calculate P_{mob} (Rydin, 2000), while the upper 8 cm of the terminal
161 cores were sliced at 1 cm intervals to investigate the changes of phosphorus forms with depth.
162 TP in the sediments (0.5 g DW) was determined following ignition of the sediment at $550 \text{ }^\circ\text{C}$
163 and subsequent digestion in 1 M HCl (50 ml) (Aspila et al., 1976). Identification of major
164 pools of phosphorus in the sediments was made following the sequential extraction scheme
165 modified by Paludan and Jensen (1995). Labile phosphorus was extracted from 1 g wet
166 sediment by H_2O ; reducible Fe and Mn hydroxide-bound phosphorus were extracted with BD
167 reagent (bicarbonate-dithionite); metal oxide-bound phosphorus (NaOH-IP) and labile
168 organic phosphorus (NaOH-OP) were extracted with 0.1 M NaOH; and inorganic phosphorus

169 pools, e.g., CaCO₃-bound phosphorus were extracted with 0.5 M HCl. Residual phosphorus
170 was calculated as TP minus the sum of the extracted phosphorus pools. The concentration of
171 each phosphorus fraction was converted to dry matter by the equation (3):

$$172 \quad C_{P(DW)} = \frac{C \cdot V}{m_w \cdot (1 - \text{water content})} \quad (3)$$

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

177

178 **2.2.5 Macrophyte traits**

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

$$187 \quad \text{RGR (d}^{-1}\text{)} = \ln \left(\frac{W_f}{W_i} \right) / \text{days} \quad (4)$$

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

190

191 **2.3. Statistical analysis**

192 The effects of Phoslock[®] and *V. denseserrulata* on the water chemistry and light

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

204

205 3. RESULTS

206 3.1. Water quality and light condition

207 Both Phoslock[®] and *V. denseserrulata* significantly reduced TP concentrations in the
208 water column (Table S1, $p < 0.001$ for both), and the most obvious reduction of TP was
209 observed in the Phoslock[®] + *V. d.* treatment (Fig. 1). However, Phoslock[®] significantly
210 increased TN concentrations while *V. denseserrulata* significantly reduced TN concentrations
211 relative to the control (Fig. 1; Table S1, $p = 0.002$ and $p = 0.009$, respectively). Demonstrating a
212 similar pattern as that of TP, the effects of Phoslock[®] and *V. denseserrulata* on the reduction
213 of Chl.a were statistically significant (Table S1, $p = 0.008$ and 0.012 , respectively), and the
214 most rapid and obvious reduction of Chl.a was observed in the Phoslock[®] + *V. d.* treatment
215 (Fig. 1). *V. denseserrulata* significantly reduced K_d while Phoslock[®] had no effect (Fig. 1;
216 Table S1, $p < 0.001$ and $p = 0.076$, respectively). Using mean TP and Chl.a concentrations

217 during the experiment period, reduction rates were calculated by comparing the different
218 treatments with the controls (Fig. S4). The reduction rates of TP and Chl.a concentrations in
219 the combined treatment was higher than that in the single treatments, however, the combined
220 effects were not additive (less than the sum of the two single effects).

221

222 3.2. *Phosphorus in the sediment*

223 During the experiment, both Phoslock[®] and *V. denseserrulata* significantly reduced the
224 porewater SRP concentrations (Fig. 2; Table S2, $p < 0.001$ at three time points), interaction
225 being observed only in week 4 (Table S2, $p < 0.001$). In the two treatments with Phoslock[®],
226 SRP concentrations in the mesocosms with *V. denseserrulata* were significantly lower than in
227 those without *V. denseserrulata* (Bonferroni test, $p < 0.001$). In the two treatments without
228 Phoslock[®], SRP concentrations did not differ significantly in either the with- or the without- *V.*
229 *denseserrulata* mesocosms ($p = 0.825$). At the end of the experiment, total SRP in the surface
230 10 cm sediment layers had decreased by 78% in the Phoslock[®] + *V. d.* treatment compared
231 with the control group without Phoslock[®] and macrophytes (Tukey test, $p < 0.001$), while in
232 the Phoslock[®] treatment and the *V. d.* treatment it had decreased by 35% and 33%,
233 respectively ($p = 0.033$, 0.046 , respectively). No significant difference appeared between the
234 two single treatments ($p = 0.996$) (Fig. 3).

235

236 In the Phoslock[®] treatment, the most obvious changes in phosphorus fractions were
237 observed in the 0-1 cm layer where HCl-P increased to 0.51 mg gDW^{-1} and became the major
238 pool (accounting for 68% of TP), while other potentially mobile phosphorus fractions (H₂O-P,
239 BD-P, NaOH-P) decreased compared with the control group without Phoslock[®] and
240 macrophyte (Fig. 4). In contrast to the Phoslock[®] treatment, metal (hydr)oxides-bound
241 phosphorus (i.e. BD-P and NaOH-IP) in the surface sediment layer in the *V. d.* treatment

242 increased by 50% and HCl-P decreased by 20% compared with the control group. BD-P and
243 NaOH-IP also increased in the deeper sediments compared with the control group. In the
244 treatment with both Phoslock[®] and *V. denseserrulata*, HCl-P increased to 0.53 mg gDW⁻¹ and
245 constituted 64% of TP in the 0-1 cm layer. However, BD-P in the sediments below 3 cm
246 exhibited an increase within the range of 17% to 28% compared with the control group (Fig.
247 4).

248

249 3.3. *Submerged macrophyte traits*

250 Compared with the *V. d.* treatment, the biomass and density of plants significantly
251 decreased by 35% and 27% (p=0.002, 0.009, respectively) in the Phoslock[®] + *V. d.* treatment,
252 respectively, whereas no significant changes occurred in individual weight (p=0.098) (Fig.
253 5a-c). RGR decreased markedly by 17% (p=0.002) (Fig. 5d). The biomass and total length of
254 stolons decreased significantly by 24% and 30% (p=0.010, 0.019, respectively) (Fig. 5e, 5f).

255

256 4. DISCUSSION

257 4.1. *Effects of Phoslock[®] and submerged macrophytes on phosphorus and nitrogen* 258 *concentrations*

259 The Phoslock[®]+*V. d.* treatment led to a much stronger improvement of water quality than
260 if the two measures were used alone, since the combined treatment had stronger effects on
261 phosphorus in both the water column and the sediment. However, water TP decreased and
262 clarity increased over time in all treatments and in the control, which can be explained by
263 both clam water conditions (no stirring) and decreasing water temperature over the course of
264 the experiment (Fig. S2).

265 In the two Phoslock[®] treatments, Phoslock[®] not only led to fast removal of phosphorus

266 from the water column during the addition, it also capped phosphorus on the surface of the
267 sediment, retarding the internal phosphorus loading. The capping layer depleted the SRP
268 diffused from deep sediment. At the end of the experiment, however, total SRP in the top 10
269 cm sediment of Phoslock[®] treatment had decreased only by 35% compared with the control
270 group without Phoslock[®] and macrophytes, while total SRP in the Phoslock[®]+*V. d.* treatment
271 had decreased by 78%, indicating that the combination of Phoslock[®] and macrophytes had a
272 stronger efficiency than if Phoslock[®] was used alone. This likely reflects that *V.*
273 *denseserrulata* enhanced the P-binding capacity by oxidizing metals in the deep sediment,
274 adsorbing more porewater SRP and thus increasing the content of metal (hydr)oxides-bound
275 phosphorus species as detected. Moreover, submerged macrophytes can also take up
276 porewater SRP by root for growth (Christiansen et al., 2016).

277 In the two treatments with Phoslock[®], the strong transformation of phosphorus forms in
278 the top layers was in line with previous studies on Phoslock[®] application (Bishop et al., 2014;
279 Meis et al., 2012; Reitzel et al., 2013b). However, BD-P in the deep sediment layers in the
280 combined treatment increased relative to the treatment with Phoslock[®] implemented alone. In
281 the upper sediment layer, the significant decrease of BD-P and NaOH-IP and the increase of
282 the HCl-P pool not only indicate a stronger binding capacity of Lanthanum (La) with
283 phosphorus compared with metal (hydr)oxides, but also phosphorus re-adsorption onto
284 available La during the sequential phosphorus extraction by the BD and NaOH solution
285 (Reitzel et al., 2013b). Furthermore, since BD-P is sensitive to redox and can be released
286 under anoxia or low redox conditions (Boström et al., 1988), the surface Phoslock[®] layer will
287 be capable of re-adsorbing the phosphorus released from BD-P in the deeper sediments when
288 reductive conditions occur (Reitzel et al., 2013b). Submerged macrophytes release oxygen
289 produced during photosynthesis into sediments through their roots (Santner et al., 2015),

290 which leads to oxidation of the metals and thus increase the phosphorus binding capacity.
291 Hence, influenced by both Phoslock[®] and macrophytes, the Phoslock[®]+*V. d.* treatment had
292 the lowest phosphorus concentration in the water column.

293 However, a side effect of Phoslock[®] in the form of increased nitrogen efflux appeared,
294 possibly reflecting the addition of ammonium with Phoslock[®] (Reitzel et al., 2013b; van
295 Oosterhout and Lürling, 2013). Moreover, the clonal growth of *V. denseserrulata* led to a
296 remarkable reduction in TN relative to the control treatment. This may result from absorption
297 of nitrogen by the plants or enhanced nitrification and denitrification (Barko and James, 1998;
298 Reddy et al., 1989). Nevertheless, the changes in nitrogen species are a topic warranting
299 further studies as we did not study the nitrogen-cycling in detail in present study.

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

305

306 **4.2. Effects of Phoslock[®] on submerged macrophyte growth**

307 Being one of the fundamental factors for photosynthesis, light plays an important role for
308 plant growth. In this study, however, the application of Phoslock[®] improved light conditions
309 only insignificantly, indicating that light was not the major influencing factor for macrophyte
310 growth. Therefore, the recorded negative effect on submerged macrophyte clonal growth
311 might be related to the reduction of bioavailable phosphorus in the surface sediment.
312 According to our observations, the clonal growth of *V. denseserrulata* was through elongation
313 of the stolon from the leaf sheath of the mother plant near the sediment-water interface,

314 followed by growth of leaves and roots from the apex of the stolon and formation of a new
315 ramet. Then the roots kept growing and penetrated into the deep sediment. In this study,
316 phosphorus fractions transformed mainly in the top 1 cm, and P_{mob} consisting of $\text{H}_2\text{O-P}$, BD-P
317 and NaOH-OP declined to 0.08 mg gDW^{-1} , accounting for only 10% of TP compared with
318 0.20 mg gDW^{-1} in the control group without Phoslock[®] and macrophytes. Since submerged
319 macrophytes can absorb phosphorus by roots and shoots (Gentner, 1977), and mainly through
320 roots (Christiansen et al., 2016), the low content of P_{mob} in the top sediment seems to be
321 unfavorable to the new ramets in their early life stage. With the slower growth of new ramets,
322 clonal growth was overall inhibited and, eventually, the shoot density and total biomass of *V.*
323 *denseserrulata* decreased. However, for the individual plant in the Phoslock[®] + *V. d.*
324 treatment, P_{mob} was not significantly different from the *V. d.* treatment when its roots
325 elongated into the sediments below 1 cm, and in its later life stages it can obtain a similar
326 level of phosphorus as in the *V. d.* treatment. However, the long-term (say >1 year) effects of
327 Phoslock[®] on submerged macrophytes require further studies.

328

329 5. Conclusion

330 In this study, the largest improvement in water quality was observed in the Phoslock[®] + *V.*
331 *d.* treatment; thus, using the methods in combination had a stronger effect than using them
332 individually. The combined treatment led to the most significant and dramatic decrease in
333 porewater SRP, and total SRP in the top 10 cm sediment layers decreased by 78% compared
334 with the control group without Phoslock[®] and macrophytes. In the 0-1 cm sediment layer,
335 HCl-P increased to 0.53 mg gDW^{-1} and constituted 64% of TP, and BD-P in the sediment
336 below 3 cm increased 17-28%. The phosphorus inactivation by La^{3+} in the surface layer as
337 well as the oxidization of metals by roots likely increased the P-binding capacity in the

338 sediment. Additionally, Phoslock[®] had a negative effect on *V. denseserrulata* growth, mainly
339 clonal growth with a decrease by 35% in biomass (dry weight) and 27% in plant density,
340 whereas the impact on individual weight was negligible, which likely can be ascribed to
341 phosphorus inactivation in the surface sediment. Hence, Phoslock[®] and submerged
342 macrophytes may complement each other in the early stage of lake restoration following
343 external nutrient loading reduction, potentially accelerating the restoration process in
344 eutrophic lakes, especially those where the internal phosphorus loading is high.

345

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355

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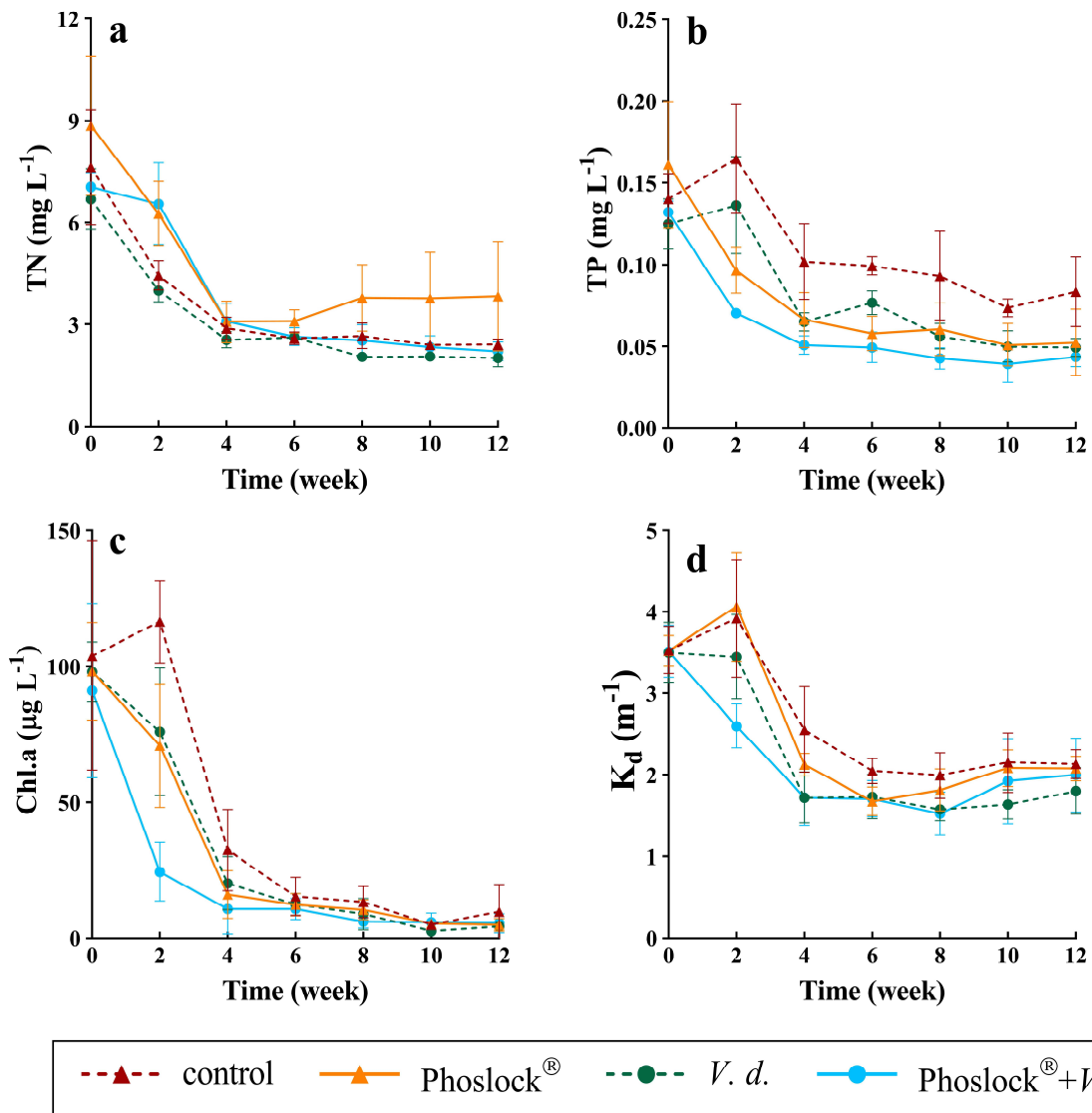
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- 542

543 **Table 1** Initial sediment properties (n=16).

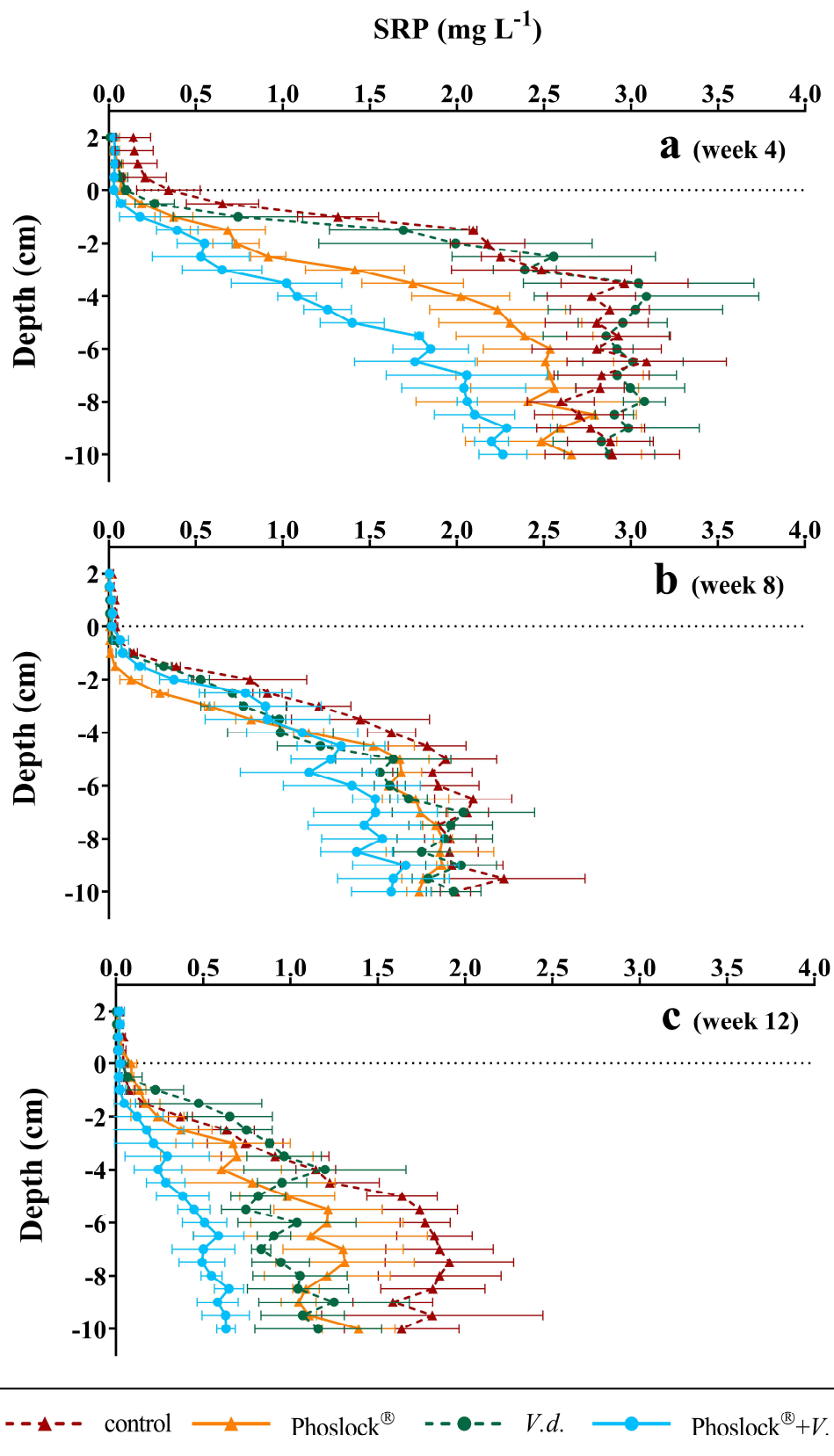
Properties	Mean \pm SD
Loss on ignition (LOI) (%)	4.31 \pm 0.87
Water content (%)	49.33 \pm 4.81
Dry bulk density (g cm ⁻³)	0.74 \pm 0.10
TP (mg gDW ⁻¹)	0.63 \pm 0.03
P _{mob} (mg gDW ⁻¹)	0.31 \pm 0.03

544



545

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



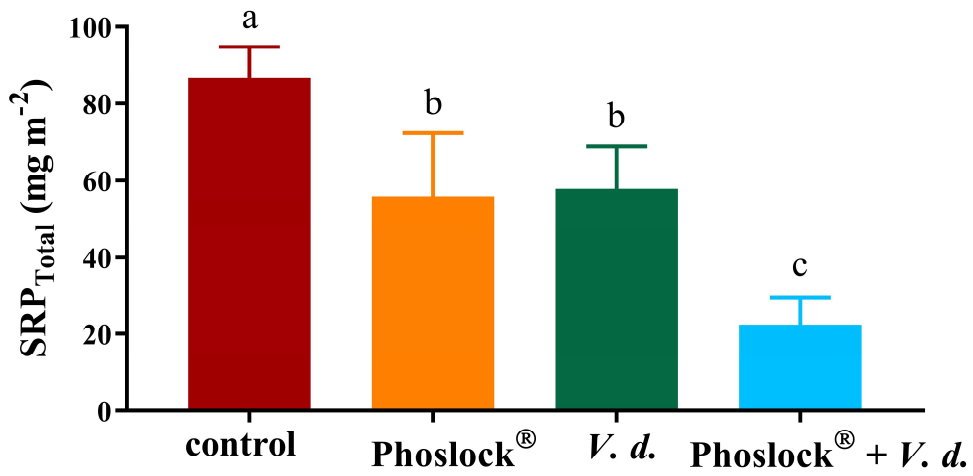
548

549 **Fig. 2.** Mean values (n=3) of SRP concentrations in depth profiles in near-bottom water and

550 porewater in the four different treatments at different times. Three replicates for each

551 treatment. Horizontal bars indicate standard deviation.

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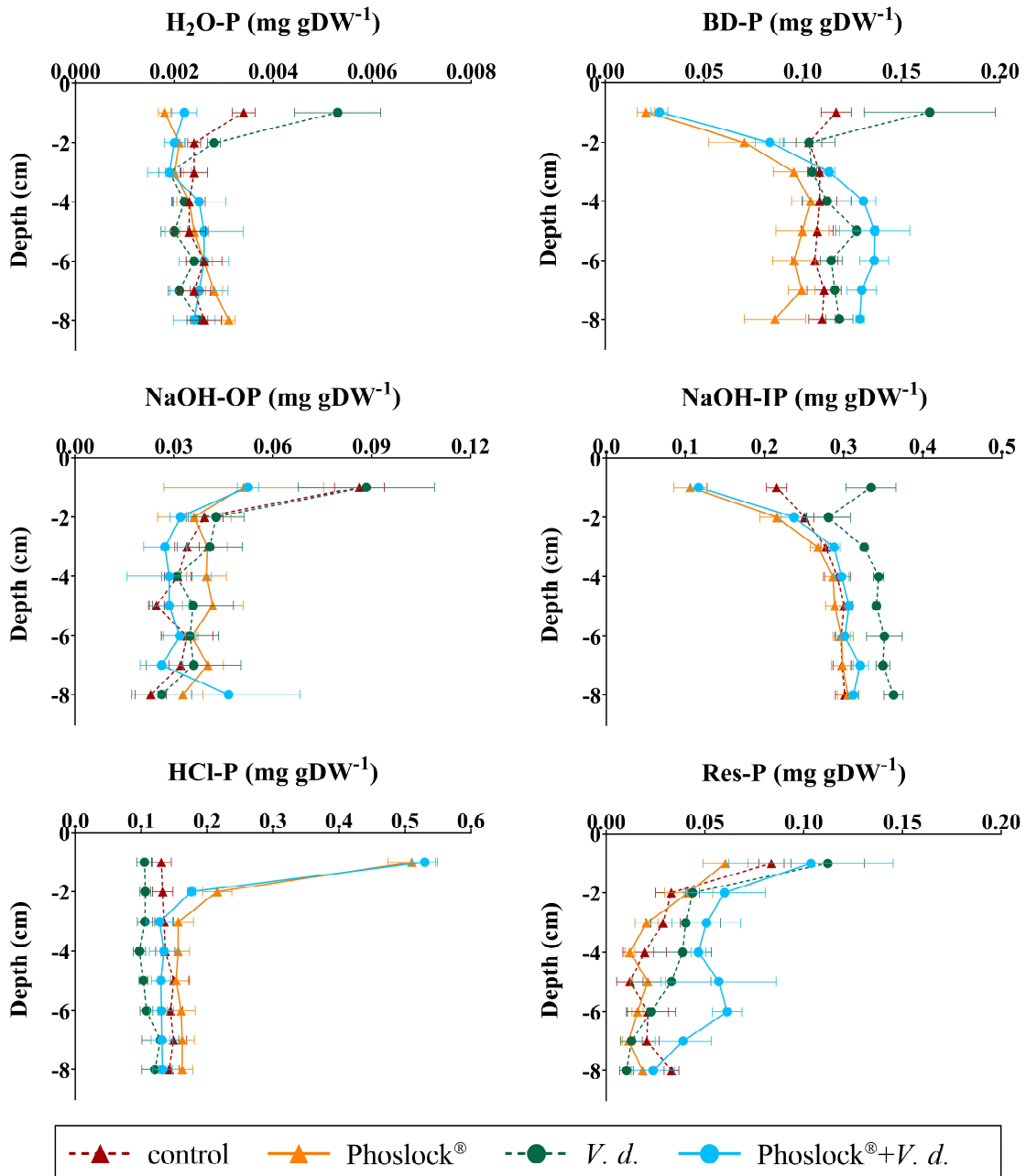


553

554 **Fig. 3.** Total SRP in the surface 10 cm sediment at the end of the experiment. (Different

555 letters indicate a significant difference among treatments, $p < 0.05$). Vertical bars indicate

556 standard deviation.



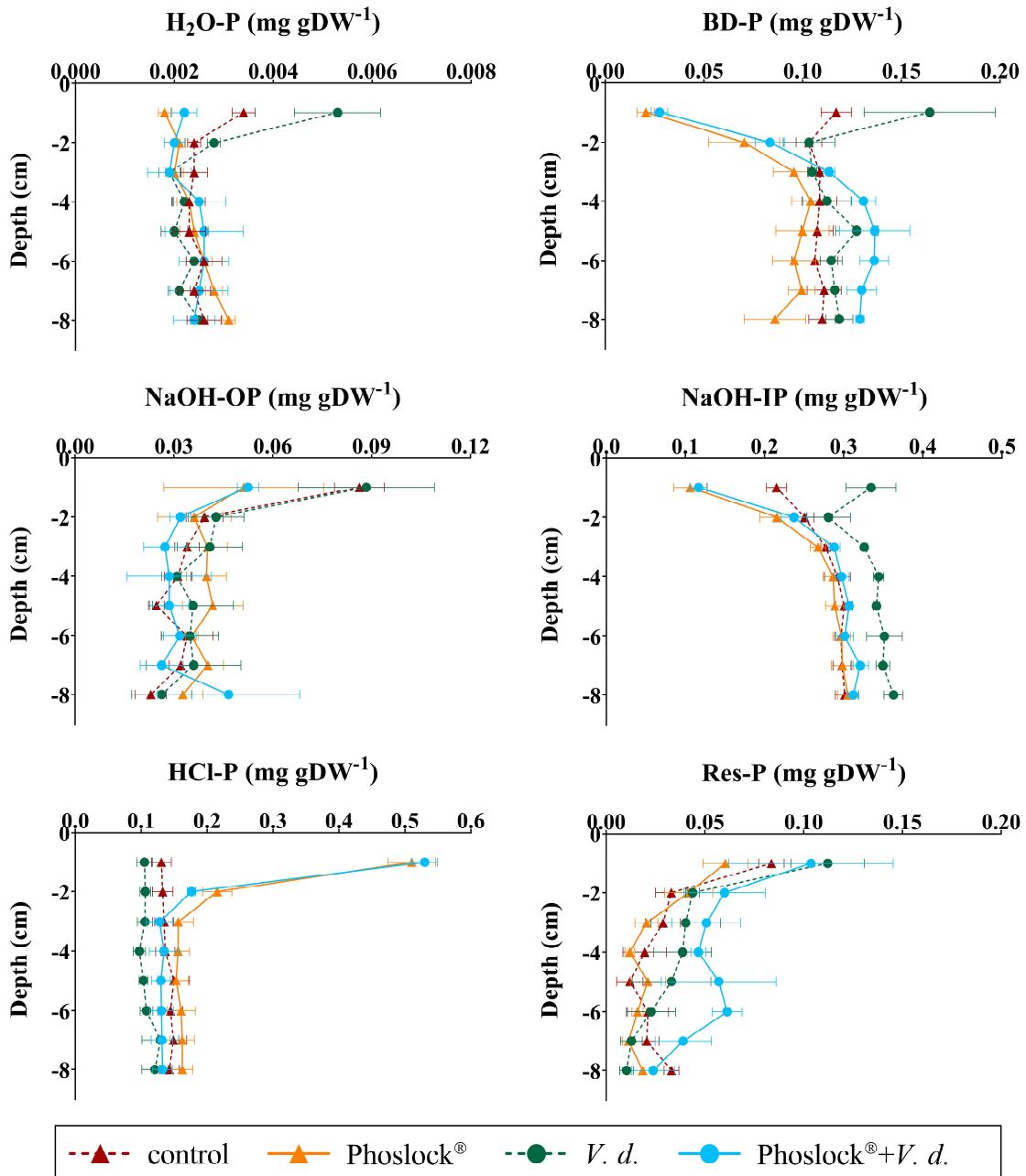
557

558 **Fig. 4.** Vertical distribution of different phosphorus fractions in the sediments of the different

559 treatments at the end of the experiment. Four replicates for each treatment. Horizontal bars

560 indicate standard deviation.

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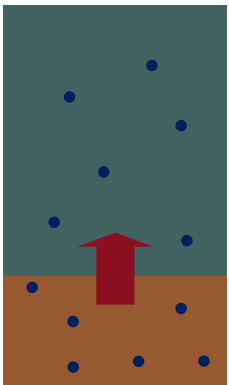
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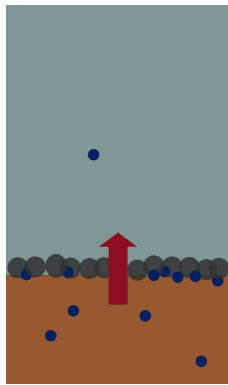
565

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.

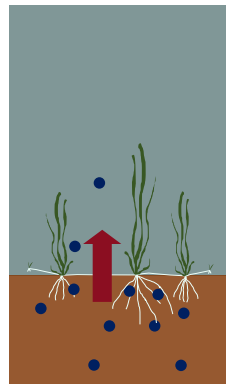
control



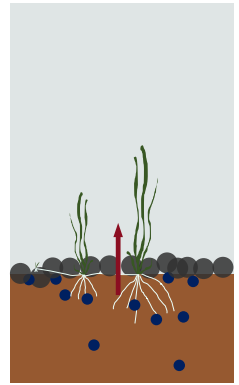
Phoslock®



V. d.




Phoslock® + *V. d.*



● Phosphorus

● Phoslock®

 *Vallisneria denseserrulata* (*V. d.*)

↑ Internal phosphorus loading