Submerged macrophyte Ceratophyllum demersum affects phosphorus exchange at the sediment–water interface
Yanran Dai, Shuiping Cheng, Wei Liang and Zhenbin Wu

ABSTRACT
Substantial research efforts were made to assess the effects of submerged macrophytes on water quality improvement, but information on the mechanism of submerged macrophytes relative to the exchange of phosphorus (P) at the sediment–water interface is very limited. To help fill the void, a popular species, Ceratophyllum demersum L., was chosen to address the effects and mechanisms of submerged macrophyte growth on the processes of P exchange across the sediment–water interface. In treatment mesocosms (planted), equilibrium phosphorus concentration (EPC0) value falls from 68.4 to 36.0 µg/L, with a mean value of 52.5 µg/L. Conversely, the distribution coefficient (Kd) value has a predominantly increasing trend. But they are both significantly higher than an unplanted control (p < 0.05). Also, in the planted mesocosm, maximum phosphate sorption capacity (Qmax) was significantly reduced (4,721–3,845 mg/kg), and most of the linear correlations between different forms of phosphorus and sediment P adsorption parameters were affected (p < 0.05). The percentage saturation parameters (EPCsat) in planted groups were 325% higher than that in control (p < 0.05). We conclude that C. demersum could promote the release of P from sediments, and soluble reactive phosphorus concentration in overlying water is probably the driving force for P exchange at the sediment–water interface.

Key words | Ceratophyllum demersum L., eutrophic water body, phosphorus release, phosphate sorption, sediment

INTRODUCTION
Eutrophication has resulted in a range of environmental, social and economic consequences at regional and global levels (Withers & Jarvie 2008). As a limiting nutrient to primary production, phosphorus (P) plays an important role in water eutrophication (Carpenter 2008; Huang & Zhang 2010). In most shallow lakes, although external P loading has gradually decreased in recent years (Jeppesen et al. 2007), internal loading of P from the sediments is becoming a potential problem (Spears et al. 2012). Internal P stored within the sediment has the potential to be remobilized and released back to the overlying water column by physical, geochemical and microbial processes (Palmer-Felgate et al. 2010). Therefore, understanding of P adsorption-release processes is very important for internal P loading control in water bodies. Several studies have been carried out on the influencing factors for the sediment P adsorption-release process. Some studies demonstrated the effect of pH and temperature on P adsorption (Zhou et al. 2005; Zhang & Huang 2011; Li et al. 2013). Wang et al. (2006) reported that particle size distribution in the sediments was an important factor affecting the phosphate sorption at the solid–liquid interface. As particle size increased, maximum phosphate sorption capacity (Qmax), equilibrium phosphorus concentration (EPC0), distribution coefficient (Kd) and phosphate sorption rate decreased. Goldberg & Sposito (1985) and Zhou et al. (2005) found that sediment composition was significant in affecting P sorption on the sediments. Meanwhile, Borovec & Hejzlar (2001) and Cyr et al. (2009) reported that EPC0 was not related to the chemical characteristics of sediments.

To enhance P retention capacity in lake sediments, many in-lake measures, including physical, chemical, biomanipulation and ecological engineering, have been studied (Schauer et al. 2003; Chen et al. 2009). Among them, revegetation of submerged macrophytes seems to be an economic and eco-friendly measure for controlling or
remediating lake eutrophication. It has been reported that submerged macrophytes can reduce different species of P by: (1) uptaking nutrients from overlying water, pore water and sediment; (2) inactivating the alkaline phosphatase activity in the overlying water and sediment; and (3) reducing sediment resuspension, reinforcing sedimentation and controlling the release of internal P loading, etc. (Horppila & Nurminen 2003; Palmer-Felgate et al. 2011). Although the role of macrophytes as water quality regulator has been given a considerable amount of attention, our knowledge about the effects and mechanisms that submerged macrophytes exert on the processes of P exchange across the sediment–water interface is far from comprehensive.

In this study, several laboratory mesocosms were set up and Ceratophyllum demersum L., which is a widespread species in China and often used for the ecosystem restoration of eutrophic shallow lakes, was chosen to evaluate the influence of submerged macrophytes C. demersum on the P sorption and release characteristics of the sediments, and to address the integrative effects and mechanisms of C. demersum on the P exchange at the sediment–water interface.

**METHODS**

**Sediments and overlying water**

Sediment samples used in the static experiments were collected from the top 0–10 cm of the sediment in Donghu Lake (30°33′N, 114°23′E), a typical eutrophic shallow lake in the central region of China (eutrophic water area accounts for 62.2% of its total water area), and then were thoroughly mixed prior to the experiments. The water used in the experiments was also taken from Donghu Lake.

**Experimental system**

The experimental system – four concrete ponds (1 × 1 × 1 m), was exposed to natural sunlight in a greenhouse, two as treatment (planted with C. demersum, 0.5 kg fresh weight per square metre) and two as control (unplanted). A 25 cm deep layer of sediment was placed in each pond. Initial water level was maintained at 30 cm for 12 h, then raised and maintained at 70 cm height throughout the experimental period by adding tap water periodically.

*Ceratophyllum demersum* collected from Donghu Lake in May 2010 were pre-incubated for 3 weeks prior to the experiment. The experiment was carried out from 28 June to 23 November 2010 when the plants were harvested. The overlying water (5 cm above sediment) and sediment (top 5 cm of sediment) in the ponds were sampled each month. At each pond, five sediment cores were collected, combined and thoroughly mixed into one sample. In the mesocosms with macrophytes growth, sediment samples were taken from the rhizome zone. All the sediment samples were divided into two portions and a major portion was used to extract pore water samples by centrifugation at 5,000g for 20 min; the remainder was naturally air-dried and sieved with a standard 100-mesh sieve. Detailed determination methods for phosphorus species in the water and sediment samples followed a previous study by Dai et al. (2012a, b).

**Sorption isotherm**

Dried sediment samples (0.2 g) were added to a series of 50 mL acid washed centrifuge tubes with 20 mL phosphate solution of 0 (0.01 mol/L KCl solution), 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 8.0, 10.0, 15.0, 20.0 and 25.0 mg P/L. The phosphate solutions were prepared by dissolving anhydrous KH₂PO₄ in distilled-deionized water. One millilitre of chloroform was added to the mixture to inhibit microbial activity. The centrifuge tubes were capped and placed at 25 ± 1 °C in an orbital shaker at 250 rpm for 24 h. After centrifugation at 5,000g for 10 min, the phosphate equilibrium concentration was analyzed in the suspension. The quantity of sorbed phosphate was calculated through the decrease of phosphate concentration in the solutions. Triplicate samples of each isotherm concentration point demonstrated the high repeatability with <5% relative standard deviation (r.s.d.)

Two models were used to fit the data. One is the modified Langmuir equation (Zhou et al. 2005)

\[
Q = Q_{\text{max}}K_L C/(1 + K_L C) - Q_{0L}
\]

where \( Q \) is sorbed phosphate (mg/kg), \( C \) is phosphate concentration in the solution after 24 h (mg/L), \( Q_{\text{max}} \) (mg/kg), \( K_L \) (L/mg) and \( Q_{0L} \) (mg/kg) are fitted coefficients. \( Q_{\text{max}} \) is the maximum phosphate binding capacity of the sediments, and \( K_L \) is a constant related to the sediment binding energy. \( Q_{0L} \) is the desorbed phosphate initially present in the sediments, also called native adsorbed phosphorus.

The second model was a modified Freundlich equation (Huang & Zhang 2010)

\[
Q = K_F C^n - Q_{0F}
\]

where \( Q \) and \( C \) are as defined above, \( Q_{0F} \) (mg/kg) has the same definition as \( Q_{0L} \). \( K_F \) (mg/kg) is the Freundlich coefficient, and
n (L/mg) is the exponential factor. $K_F$, $n$ and $Q_{OF}$ are fitted coefficients. A plot of $Q$ versus $C$ yields the phosphate adsorption isotherm. The value of $C$ at $Q = 0$ is EPC₀ for sediments, which represents a crossover concentration at which sediment is in equilibrium with overlying water with respect to phosphorus exchange. Sediment at EPC₀ displays a maximum capacity for buffering phosphorus in the overlying water.

The slope of the isotherm curve at $Q = 0$ is the distribution coefficient ($K_d$), which can be determined by taking the derivative of the modified Freundlich equation with respect to $C$ at the EPC₀

$$K_d = \frac{d[K_F(C)^n]}{d(C)_{EPC_0}} = nK_F(C)^{n-1}$$

**Sequential P extraction**

After sorption experiments, the centrifuge tubes with the addition of 25.0 mg P/L solution were decanted, then 20 mL 0.01 mol/L CaCl₂ solutions were added to start the successive P extractions. The centrifuge tubes were capped and placed at 25 ± 1 °C in an orbital shaker at 250 rpm for 24 h, followed by centrifugation at 5,000 g for 10 min, the supernatant was used for P concentration (equilibrium concentration) analysis. The procedure of decanting, filtering, adding CaCl₂ solution and shaking was repeated, until 18 sequential P extractions were performed. The r.s.d. of the triplicate experiments was set within 8%.

The released P data can be fitted by a simple power equation as follows (Wang et al. 2007a)

$$Q = a(x)^b$$

where $Q$ is the amount of released P (mg/kg), $x$ is the number of successive extractions, $a$ (mg/kg) and $b$ are fitted parameters. For the P release process, $b$ was negative.

**Data analysis**

The results were presented as mean values of duplicates. Origin Pro 8 was used for correlation and regression analysis. Correlations between the measures of sediment characteristics were tested with a Pearson correlation. Paired t-test was performed by SPSS 13.0 to compare the differences between variables. A probability level of 0.05 was used to establish significance ($p < 0.05$).

**RESULTS AND DISCUSSION**

**Effects of C. demersum on P adsorption**

The sorption isotherms of sediments from the two different groups had different sorption behavior, and both varied with time (Figure 1). The modified Langmuir and modified Freundlich models, taking the native adsorbed phosphorus...
into account, both fitted the P sorption data well ($R^2 > 0.99$). Olsen & Watanabe (1957) pointed out that the major advantage of the Langmuir equation was calculation of the adsorption maximum. Therefore, $Q_{\text{max}}$ was calculated by the modified Langmuir equation, while EPC$_{0}$ and $K_d$ were calculated by the modified Freundlich equation.

The fitting data to the two equations and other parameters are listed in Table 1. The $Q_{\text{max}}$, EPC$_{0}$ and $K_d$, which reflect the ability of phosphate sorption on sediments, were used to characterize the behavior of sediments in response to $C. \text{demersum}$ growth. During the experimental period, $Q_{\text{max}}$ ranged from 3.511 to 4.322 mg/kg (average 3.845 mg/kg) in the experimental group, and the highest and lowest value appeared in early August and at the end of the experiment, respectively. Meanwhile, the $Q_{\text{max}}$ in the control was lowest in early August, with an average value of 4.721 mg/kg, which was significantly higher than that of the experimental group ($p < 0.05$). Hence, it can be concluded that the $C. \text{demersum}$ revegetation could decrease the capacity of phosphate sorption on the sediments.

The EPC$_{0}$ values in the experimental group showed a decreasing trend, with the values ranging from 68.4 to 36.0 g/L (average 52.5 g/L). The controls showed no significant trends. There was a significantly higher than that of the experimental group ($p < 0.05$). Hence, it can be concluded that the $C. \text{demersum}$ revegetation could decrease the capacity of phosphate sorption on the sediments.

For $K_d$, which has been used to denote the attraction forces toward P, as opposed to EPC$_{0}$, an increasing trend was found in the treatment group and values ranging from 444 to 763 L/mg (average 612 L/mg). Significant difference was also detected between the two groups ($p < 0.05$). These indicated that sediments with $C. \text{demersum}$ might potentially hold higher attraction forces toward P.

Effects of $C. \text{demersum}$ on correlations between adsorption parameters and relevant sediment properties

As seen from Table 2, significant positive correlations between EPC$_{0}$ and most sedimentary phosphorus forms, including TP, IP, NaOH-rP (P exchangeable with OH$^-$, mainly Al-P), BD-P (P adsorbed to Fe and Mn) and NH$_4$Cl-P (loosely sorbed P), were observed in the experimental group. Among the slopes in the EPC$_{0}$ equations of linear regression, the slope of the equation of EPC$_{0}$ and NH$_4$Cl-P was the highest, followed by BD-P, NaOH-rP, TP and IP. It was noted that the slope value of the equation of EPC$_{0}$ and NH$_4$Cl-P was much higher than those of the others; meanwhile, the slope value for EPC$_{0}$ and TP was close to EPC$_{0}$ and IP. In contrast, no obvious correlation between EPC$_{0}$ and any forms of sedimentary phosphorus in the control was observed.

As for the distribution coefficient $K_d$, the situation was the reverse. First, all the significant correlation coefficients were negative, which might mean the buffer capacity of the sediment decreased with some specific forms of phosphorus content. Secondly, significantly linear correlations

### Table 1 | Sediment phosphorus sorption parameters of sediment samples at different dates of the experiment (mean ± SD)

<table>
<thead>
<tr>
<th>Items</th>
<th>Modified Freundlich equation</th>
<th>Modified Langmuir equation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_p$ mg/kg</td>
<td>EPC$_{0}$ μg/L</td>
</tr>
<tr>
<td>Original sediments</td>
<td>344 ± 7.3</td>
<td>60.2 ± 6.9</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-Aug</td>
<td>298 ± 14</td>
<td>68.4 ± 7.0</td>
</tr>
<tr>
<td>30-Aug</td>
<td>302 ± 13</td>
<td>60.7 ± 6.9</td>
</tr>
<tr>
<td>28-Sep</td>
<td>355 ± 16</td>
<td>50.1 ± 6.1</td>
</tr>
<tr>
<td>27-Oct</td>
<td>408 ± 15</td>
<td>47.5 ± 3.8</td>
</tr>
<tr>
<td>23-Nov</td>
<td>368 ± 14</td>
<td>36.0 ± 4.2</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-Aug</td>
<td>319 ± 13</td>
<td>53.3 ± 4.8</td>
</tr>
<tr>
<td>30-Aug</td>
<td>262 ± 6.7</td>
<td>64.9 ± 9.8</td>
</tr>
<tr>
<td>28-Sep</td>
<td>338 ± 9.0</td>
<td>30.8 ± 6.3</td>
</tr>
<tr>
<td>27-Oct</td>
<td>359 ± 10</td>
<td>52.0 ± 2.5</td>
</tr>
<tr>
<td>23-Nov</td>
<td>311 ± 5.8</td>
<td>60.3 ± 4.1</td>
</tr>
</tbody>
</table>
Table 2 | Effects of C. demersum on correlations between adsorption parameters and sedimentary phosphorus forms, OM in sediment and SRP in pore water. Bold: significant correlations and corresponding equations

<table>
<thead>
<tr>
<th>Properties</th>
<th>Treatment</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EPC0</td>
<td>Q&lt;sub&gt;max&lt;/sub&gt;</td>
</tr>
<tr>
<td>SRP</td>
<td>0.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.44</td>
</tr>
<tr>
<td>TP</td>
<td>0.97&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.55</td>
</tr>
<tr>
<td>IP</td>
<td>0.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.57</td>
</tr>
<tr>
<td>OP</td>
<td>−0.55</td>
<td>−0.45</td>
</tr>
<tr>
<td>OM</td>
<td>0.60</td>
<td>0.40</td>
</tr>
<tr>
<td>NaOH-rP</td>
<td>0.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.64</td>
</tr>
<tr>
<td>HCl-P</td>
<td>0.38</td>
<td>−0.27</td>
</tr>
<tr>
<td>BD-P</td>
<td>0.98&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.50</td>
</tr>
<tr>
<td>NH&lt;sub&gt;4&lt;/sub&gt;Cl-P</td>
<td>0.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.65</td>
</tr>
<tr>
<td>EPC&lt;sub&gt;0&lt;/sub&gt;</td>
<td>–</td>
<td>0.68</td>
</tr>
<tr>
<td>Q&lt;sub&gt;max&lt;/sub&gt;</td>
<td>0.68</td>
<td>–</td>
</tr>
<tr>
<td>K&lt;sub&gt;d&lt;/sub&gt;</td>
<td>−0.16</td>
<td>0.37</td>
</tr>
</tbody>
</table>

SRP, soluble reactive phosphorus in the pore water; OM, organic matter in sediment.

<sup>a</sup>p < 0.05.
<sup>b</sup>p < 0.01.

between K<sub>d</sub> and NaOH-rP, BD-P, NH<sub>4</sub>Cl-P were found in the control, while only significant correlation between K<sub>d</sub> and HCl-P was detectable in the experimental group. Beside this, the absolute slope in the K<sub>d</sub> equation in the experimental group was much lower than those in the control.

The significant correlations between EPC<sub>0</sub> and most of the sedimentary phosphorus forms were only found in the experimental group, which could be attributed to the restoration of *C. demersum* significantly changing the phosphorus forms in the sediments (reported in another paper, Dai et al. (2022b)), and implying that the sediment EPC<sub>0</sub> was governed by different forms of phosphorus in the sediments. Furthermore, the linear equations (Table 2) showed that NH<sub>4</sub>Cl-P content in the sediment with *C. demersum* had the strongest influence on EPC<sub>0</sub>. Meanwhile, the effects of TP and IP on EPC<sub>0</sub> were very near. The probable reasons were: (1) IP occupied 78–89% of TP throughout the experiment (Dai et al. 2022b); and (2) no significant linear correlation of organic phosphorus (OP) with EPC<sub>0</sub> was found.

As for Q<sub>max</sub>, no significant correlation was observed between Q<sub>max</sub> and the variables in both groups. Also, there was no significant correlation between sediment organic matter (OM) content and EPC<sub>0</sub>, Q<sub>max</sub> and K<sub>d</sub> in the two groups. It is commonly regarded that the content of OM plays a key role in the sorption of phosphate. Wang et al. (2006, 2007b) reported there was a positive correlation between OM and P adsorption. Zhou et al. (2011) reported there was positive correlation between OM content and phosphorus sorption by significantly enhancing the sorption energy. However, in our research, no direct correlation between sediment OM content and EPC<sub>0</sub>, Q<sub>max</sub> and K<sub>d</sub> for phosphate sorption was observed (Table 2). The different sediment composition may contribute to the various results. In the study of Pizzeghello et al. (2011), only the strong correlation between organic carbon and P<sub>Fe-Al</sub> (P bound to iron and aluminum) was found. In our sediment, P<sub>Fe-Al</sub> content is a small proportion of the sediment P (<35%), detailed data can be seen in Dai et al. (2022b)). Hence, the role of sediment OM was weakened, but the exact reason needs further research.

It is well known that pore water is the intermediate of sediment and overlying water. In our study, significant linear correlation of soluble reactive phosphorus (SRP) in pore water with EPC<sub>0</sub> was observed in the experimental group, but a negative correlation was found between SRP and K<sub>d</sub> in the control.

**Effects of *C. demersum* on P release from the sediments**

With subsequent extractions, the released P decreased, especially for the first three times, and P release was almost negligible after 18 extractions (Figure 2).
The simple power equation described P released from the different sediments well. The values of constant $a$ and $b$ in the experimental group were higher than those of control. In the experimental group, the constant $a$ decreased, while constant $b$ increased with the experiment, which indicated there was a reduction for P release rate from the sediments with single extraction and successive extractions. But no obvious change trend was detected in the control. The average values of $a$ and $b$ in the experimental group and control were 61.0 and 59.1 mg/kg, $/C_0 ^1.28$ and $/C_0 ^1.32$, respectively (Table 3). No significant difference was found ($p > 0.05$). This indicated that $C. demersum$ had no direct effect on the kinetics for P release from sediments.

**Effects of $C. demersum$ on P exchange at the sediment–water interface**

The EPC$_0$ percentage saturation (EPC$_{sat}$) is used to examine the role of bed sediment as a sink or source of SRP, and calculated as $EPC_{sat} = 100(\text{EPC}_0 - \text{SRP})/\text{EPC}_0\%$, where SRP is the soluble reactive phosphorus in the overlying water (Dai et al. 2012b). Negative and positive values of EPC$_{sat}$ indicate the potential for uptake of SRP from the water column and release of SRP to the water column, respectively. A value of zero corresponds to an equilibrium situation (Jarvie et al. 2005). Generally, due to the uncertainty of the environmental change, an EPC$_{sat}$ cutoff of less than or equal to ±20% is used to denote an approximate equilibrium between sediment and water, and EPC$_{sat}$ values are between $/C_0 ^20$ and 20% defined as the situation which is close to equilibrium with water SRP concentrations (Jarvie et al. 2005; Stutter & Lumsdon 2008).

In this study, EPC$_{sat}$ percentages in the control were far less than $/C_0 ^20$% most of the time, with a mean value of $/C_0 ^317$. For the experimental group, EPC$_{sat}$ percentages ranged from $/C_0 ^75.3$ to 96.9%, with a mean value of 8.38% (Figure 3). Paired $t$-test demonstrated that the EPC$_{sat}$ percentages in the control were significantly lower than that in the experimental group ($p < 0.05$). A change trend from uptake of SRP to release of SRP in the sediment was observed in the experimental group. These results further confirmed the conclusions of our previous studies, and the presence of submerged macrophytes $C. demersum$ was likely to promote the release of P in the sediment (Dai et al. 2012b).

To understand the effect of $C. demersum$ on the role of bed sediment, the EPC$_{sat}$ percentage was calculated. In the control, EPC$_{sat}$ percentages were far less than $/C_0 ^20$% for most of the time, which indicated significant potential of the sediment as a net sink for SRP. While in the experimental group, there was a trend from net sink to source of release.
Furthermore, the $EPC_{\text{sat}}$ percentages in control were significantly lower than that in the experimental group ($p < 0.05$). These results are accordant to that of our previous studies, sediment TP content decreased significantly due to the presence of $C. \text{demersum}$, especially OP, and further confirmed the conclusion that the presence of submerged macrophytes $C. \text{demersum}$ was likely to promote the release of P in the sediment (more details can be seen in Dai et al. (2012b)).

The effects and mechanisms of macrophyte growth on P exchange at the sediment–water interface are very complicated. It was reported that aquatic macrophytes can elevate pH values during intensive photosynthesis (Horppila & Nurminen 2003), influence the particle size of sediments (Madsen et al. 2001), introduce oxygen into the ambient sediment through radial oxygen loss and hence increase redox potential (Laanbroek 2010), and change the P form in the sediments (Palmer-Felgate et al. 2011). In addition, some aquatic macrophytes may excrete organic acids into the sediment to increase the chemical desorption of phosphorus (Bolan 1994). The effects of different experimental conditions and macrophyte species on P exchange at the sediment–water interface might also be different. Wang et al. (2007a) reported that $Hydrilla \text{verticillata}$ could increase the ability of phosphate sorption on the sediments, decrease the risk of P release from the sediments, and restrain the P release from the sediment. Similarly, Zhang et al. (2012) found that $Vallisneria \text{natans}$ increased the sediment ability of P sorption. But our results showed the opposite, the differences in morphological traits could be the main reason. Compared to $C. \text{demersum}$, $H. \text{verticillata}$ and $V. \text{natans}$ are both species which have relatively developed roots, therefore, further specific researches are in great need.

CONCLUSIONS

Submerged macrophyte $C. \text{demersum}$ increased sediment $EPC_0$ and $K_d$, but reduced $Q_{\text{max}}$ of the sediment significantly. The linear correlations between different forms of phosphorus and sediment P adsorption parameters were also affected by the presence of $C. \text{demersum}$. In closing, $C. \text{demersum}$ could promote the release of P from the
sediments and SRP concentration in the overlying water is the driving force for P exchange at the sediment-water interface.

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