



## Effect of plant diversity on phosphorus removal in hydroponic microcosms simulating floating constructed wetlands



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### ABSTRACT

Phosphorus (P) removal is one of the major target services of constructed wetlands (CWs) treating wastewater. Previous studies have shown that plant diversity plays an important role in the functioning of CWs for wastewater treatment such as nitrogen removal, but the effect of plant diversity on P removal remains largely unexplored. In particular, less work has been done with free floating species in CW systems without substrate. In this study, we tested whether and how P removal is dependent on species richness and/or specific species composition in hydroponic microcosm stimulating floating CWs. Four common early-spring species (*Rumex japonicas*, *Oenanthe hookeri*, *Phalaris arundinacea* and *Reineckia carnea*) were transplanted into the microcosms, ranging from a richness gradient of 1–4 species. A total of 15 community treatments (4 monocultures, 6 two-species mixtures, 4 three-species mixtures and 1 four-species mixture) were set up. Our results showed that: (1) effluent total P concentration decreased with increasing species richness, primarily due to higher biomass production and larger plant P pool at higher species richness; (2) communities including *Oenanthe hookeri* outperformed other communities in removing P, and the interaction between *Oenanthe hookeri* and *Rumex japonicas* gave rise to an “under-depletion effect” on wastewater P; and (3) species composition exerted a stronger effect than species richness on P removal from wastewater. Assembling proper plants species composition in floating CWs might be more important than simply increasing species richness for treating wastewater nutrients.

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### 1. Introduction

Human activities such as agricultural practices, urbanization and industrialization have altered the biogeochemical cycles of phosphorus (P) (Bouwman et al., 2013; Penuelas et al., 2013). As a common constituent of agricultural fertilizers, manure, and organic wastes in municipal and industrial effluent, P concentration in aquatic ecosystems have increased during recent decades (Reckhow and Simpson, 1980; De-Bashan and Bashan 2004). Excessive P loading to receiving water bodies may contribute to nutrient enrichment, or eutrophication, of rivers and lakes, representing a widespread global environmental problem (Carpenter et al., 1998; Conley et al., 2009). The treatment of P containing wastewater remains a challenge around the world.

Conventional techniques for treating wastewater P, such as wastewater treatment plants, are often energy- and cost-intensive.

There is an urgent need to develop cost effective P removal methods. Floating beds are a type of constructed wetlands (CWs), an engineered system designed to take advantage of many processes occurring in natural wetlands (Tanner and Headley, 2011). Constructed wetlands have been widely used to treat a variety of wastewater (Vymazal, 2007; Zhang et al., 2009; Vymazal, 2014). Phosphorus removal is one of the major target services of CWs. With low operational and maintenance requirements, removal of P by CWs can be highly cost-effective. Rate of total P removal in various CW systems could be as high as 31–99% (Steer et al., 2002; Vymazal, 2007; Liu et al., 2009; Zhang et al., 2009).

Plants play an important role in P cycle of floating CWs (Vymazal, 2007). Direct uptake by plants and plant-mediated microbial processes account for major pathways of P removal in CWs (Stottmeister et al., 2003), in addition to the physical and chemical processes (e.g. sedimentation and sorption). While P incorporation into plant tissues is typically less than 1% (10 mg g<sup>-1</sup>), removal of P attributable to plants could be significant (Hunter et al., 2001; Hunter et al., 2001; Kyambade et al., 2004). By contrast, there are other studies showing that the amount of P sequestered in

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plant biomass was of minor importance (Kim and Geary, 2001). There is no generalization of the contribution of plants uptake to P removal in CWs, and species differ in their ability to support removal of P (reviewed in Vymazal, 2011). Plant diversity, which has been widely studied in grassland ecosystems worldwide (e.g. Tilman et al., 1996; Tilman et al., 2001; Cardinale et al., 2007; Abalos et al., 2014), is a major determinant of ecosystem dynamics and functions. Likewise, plant diversity also affects a variety of processes and functions of CWs, such as productivity (Zhu et al., 2010; Wang et al., 2013), nutrient retention (Silvan et al., 2004; Zhang et al., 2010), microbial activity (Ge et al., 2011; Zhang et al., 2011b), greenhouse gas emissions (Sun et al., 2013; Chang et al., 2014) and nitrogen removal (Fraser et al., 2004; Ge et al., 2015). Both species richness and community composition constitute a diversity effect on nutrient removal of CWs. High plant species richness often results in increased primary production (Naylor et al., 2003), which may reduce effluent nutrient contents due to increased uptake by vegetation (Wang et al., 2013; Han et al., 2016; Zhao et al., 2016). On the other hand, there could be a species-specific effect on the CW's potential to reduce pollutants (reviewed in Brisson and Chazarenc, 2009). Each plant species may have a distinct nutrient uptake rate and specific growth pattern in nature. Meanwhile, the activity and relative abundance of rhizosphere microbial communities are species-dependent (Garbeva et al., 2004; Hartmann et al., 2009). Moreover, the outcome of plant–plant interactions may vary depending on the competitive ability and stress tolerance of the coexisting species (Maestre et al., 2009), suggesting that plant community composition is another key component underlying nutrient status in wetland ecosystems (Engelhardt and Ritchie, 2002; Fraser et al., 2004; Khan and Shah, 2010; Zhao et al., 2016).

The role of species richness and composition as drivers of N removal has been widely studied in CWs filled with substrate (e.g. Zhu et al., 2010; Ge et al., 2015; Han et al., 2016; Zhao et al., 2016), however, there is a lack of information available on how plant diversity influences P removal, despite the fact that outflow total P is often measured to indicate a wetland's ability to treat potentially polluting nutrients (Carlson, 1977; Mitsch et al., 1995). Existing results vary for different plant species and in different CW systems and appear to be context-dependent. In a test of four species to reduce total P using subsurface wetland microcosms, Fraser et al. (2004) observed that plant species had a differential response to reducing total P in wastewater. Contrary to the above study, Picard et al. (2005) noted no clearly defined pattern of species-specific P removal found in another experiment using same species. Similarly, evidence as to whether species mixture is more effective than a single species at reducing P has proved inconclusive. Polycultures outperformed monoculture communities at certain levels of P loadings (Fraser et al., 2004) and only in certain months (Picard et al., 2005). In a microcosm experiment simulating CW, removal of inorganic P did not change with species richness, instead, plant type (monocot vs. dicot) impacted the removal of inorganic P (Zhang et al., 2011a). More specifically, accelerated degradation of organic P in a full-scale CW was found to result from significantly increased acid phosphatase activity induced by increasing species richness (Zhang et al., 2010). Alternatively, high plant diversity may not directly affect P retention in wetlands, but rather an indirect effect towards higher algal biomass which contributed to P loss (Engelhardt and Ritchie, 2002). Most of these past studies have focused on comparison between planted and unplanted systems, or between mono- and polyculture systems on P removal in CWs, with information restricted to observations describing the impact of the presence or absence of certain species on water quality. Most of the previously investigated species are emergent plants growing in soils of CWs, while free floating species in CW systems without substrate have been much less studied. The relative contribution of species richness and composition to the biodiver-

**Table 1**

Contents of nutrient components used in the simulated wastewater (modified Hoagland nutrient solution).

Macroelements	Contents (g L <sup>-1</sup> )	Microelements	Contents (mg L <sup>-1</sup> )
KNO <sub>3</sub>	1.46	H <sub>3</sub> BO <sub>3</sub>	2.86
Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O	1.13	MnCl <sub>2</sub> ·4H <sub>2</sub> O	1.81
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.50	ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.22
KH <sub>2</sub> PO <sub>4</sub>	0.14	CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.08
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.49	H <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O	0.09
KCl	0.09	FeSO <sub>4</sub> ·7H <sub>2</sub> O	5.56
		Na <sub>2</sub> EDTA	7.44

sity effect on P removal are seldom quantified and thus remains uncertain.

In this study, our objectives were to quantify whether and how P removal are dependent on species richness and/or specific species composition in microcosm stimulating floating CWs. Four common early spring species were chosen and transplanted. Due to their short productive lifespan (3–4 months), we ran the experiment for three months (May to March) and tested the biodiversity effect before senescence began. We asked three questions: (1) Does polyculture enhance the effectiveness of P removal compared with monoculture treatments (a test of richness effect)? (2) Are there differential responses among the community treatments in reducing P (a test of composition effect)? and (3) What are the relative importance of species richness vs. composition on P removal?

## 2. Materials and methods

### 2.1. Experiment design

The microcosm experiment was initiated at the campus of Zhejiang University in Hangzhou City (120°05'E, 30°18'N), Southeast China. This site has a humid subtropical climate with an average annual temperature of 18.7°C and rainfall of 1350 mm. Each microcosm consisted of a chinaware gutter (51 cm length × 38 cm width × 18 cm height) and a piece of polyethylene planting bed (43 cm length × 32 cm width × 7 cm height) with 12 evenly spaced planting holes.

In March, seedlings of four early-spring species, *R. japonicas*, *O. hookeri*, *P. arundinacea*, and *R. carnea* with similar size and vitality were transplanted into the raised beds of the microcosms, ranging from a richness gradient of 1–4 species. These species are common in subtropical wetlands in China and they are morphologically (broad and narrow leaved, tap and fibrous root) and functionally (monocot and dicot, grass and forb) different (Table S1). There were 15 species combination treatments (4 monocultures, 6 two-species mixtures, 4 three-species mixtures and 1 four-species mixture). The experiment was arranged in a complete block design with 6 blocks. Each treatment had 6 replicates and hence there were a total of 90 microcosms. Each microcosm had 12 individuals with an equal number of individuals assigned to each species.

The simulated wastewater was the Hoagland nutrient solution (Hoagland and Arnon, 1950) with a minor modification (Table 1). KH<sub>2</sub>PO<sub>4</sub> was the sole phosphorus source in the influent, and its concentration was 31.91 mg L<sup>-1</sup>. The simulated wastewater was supplied every 10 days (HRT = 10d) from early March to late May, for a total of eight times. At each time, 7 L wastewater (223 mg P) was supplied to each microcosm and then diluted to 21 L. During the experiment, the water level was kept consistent by adding tap water (municipal water) daily though multi-point dripping system to compensate for evaporation loss. Roots of the plants were totally immersed in the water. It should be noted that the amount of water evaporating from each microcosm may not be the same, however, compared with the high P loading in the simulated wastewater, P added by tap water (P concentration <0.02 mg L<sup>-1</sup>)

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