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Removal of metals and their pools in plant in response to plant diversity in microcosms of floating constructed wetlands

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A B S T R A C T

Plant species diversity could enhance nitrogen and phosphorus removal efficiencies in constructed wetlands (CWs). However, the effects of plant diversity on other pollutants removal efficiencies in CWs are still unknown. In this study, we conducted a microcosm experiment simulating floating CWs using four common early spring species to set up four species richness (1, 2, 3, and 4) and fifteen species compositions to explore the effects of plant diversity on the removal efficiencies of calcium (Ca), potassium (K), and magnesium (Mg) and their pools in plants. Results showed that (1) plant species richness had no effect on Ca, K, and Mg removal efficiencies; however, it increased plant K and Mg pools; (2) among the four monocultures, the Ca removal efficiency and plant Ca pool of the *Rumex japonicus* monoculture was the highest; the K removal efficiencies and plant K pools of *Oenanthe javanica* or *R. japonicus* monoculture were significantly higher than those of other two species monocultures; (3) the presence of *R. japonicas* increased the Ca removal efficiency and belowground plant Ca pool; however, the presence of *Phalaris arundinacea* decreased the Ca removal efficiency and belowground plant Ca pool; (4) the presence of *O. javanica* increased the K removal efficiency and plant (aboveground and belowground) K pool; however, the presence of *P. arundinacea* decreased the K removal efficiency and belowground plant K pool; and (5) no difference in Mg removal efficiency was found among species compositions. Assembling proper plant species composition in CWs might be more important than simply increasing species richness for increasing Ca and K removal efficiencies.

1. Introduction

With the continuing development of the global economy, the amount of wastewater has increased in recent decades (Galloway et al., 2008; Gu et al., 2013). Some industrial wastewater contains high levels of calcium (Ca), potassium (K), and magnesium (Mg), such as liner paper wastewater (Kim et al., 2004), mine wastewater (Tolonen et al., 2016), and landfill waste leachate (Tait et al., 2009). At present, most studies focus on the removal of nitrogen, phosphorus, and organic matter in wastewater (Vymazal, 2007; Saeed and Sun, 2012; Jesus et al., 2017), while other pollutants have only been assessed sporadically (Hawkins et al., 1997; Samecka-Cymerman et al., 2004; Vymazal and Šveha, 2012). In the traditional wastewater treatment process, high Ca and Mg concentrations in wastewater may result in operation problems, such as wire clogging and drainage rate reduction (Kim et al., 2004). Also, the presence of Ca, K, and Mg in wastewater affect the nitrogen and phosphorus removal process (Barat et al., 2005; Choi et al., 2011; Dou et al., 2017). Ion exchange processes are used commercially worldwide to treat wastewater with Ca, K, and Mg, but high-power consumption and periodical regeneration of saturated ion-exchanger resins are needed and may produce secondary pollution (Seo et al., 2010). It is a challenge to find an efficient and economical method to remove Ca, K, and Mg from wastewater.

Constructed wetlands (CWs) have been widely used as a cost-effective method to treat wastewater due to low operational and maintenance requirements (Liu et al., 2009; Vymazal, 2014). Plants are an important component of constructed wetlands. Ca is a component of calcium pectin in the intercellular layer of plant cell walls and plays an important regulatory role in many cellular responses (Lambers et al., 1998; Taiz and Zeigar, 2006). K is an activator of more than 40 enzymes and an important component of cell osmotic potential and charge balance (Kadlec and Wallace, 2008). Mg is a component of chlorophyll and chromosomes, and is also an activator of many enzymes in photosynthesis and respiration (Taiz and Zeigar, 2006). As essential elements for plant growth, Ca, K, and Mg account for 0.01% ~ 10% of the dry weight of a plant (Taiz and Zeigar, 2006). Plant uptake of Ca, K, and Mg may be an effective way to remove Ca, K, and Mg from wastewater in CWs.

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As plant species may have specific nutrient uptake ability and growth patterns, the activity and relative abundance of rhizosphere microbial communities are also species-dependent (Hartmann et al., 2009). Such a difference may affect the nutrient removal efficiency in CWs. Previous studies reported that Ca, K, and Mg removal efficiencies were 1%, 11%, and 6% in CWs planted with *Phalaris arundinacea* L. (reed canarygrass) and *Phragmites australis* (Cav.) Trin. (common reed) (Vymazal and Šveha, 2012), while the removal efficiencies of Ca, K, and Mg reached 42%, 30%, and 31% in CWs planted with *Salix viminalis* L. (basket willow) (Samecka-Cymerman et al., 2004). Plant species diversity is perhaps the major determinant of ecosystem functioning (Cardinale, 2011; Tilman et al., 2014). In CWs, many studies showed that increasing plant species richness increased plant productivity (Chang et al., 2014;Han et al., 2016), plant nitrogen and phosphate pools (Han et al., 2016; Geng et al., 2017) and the removal efficiencies of nitrogen and phosphate (Fraser et al., 2004; Ge et al., 2015; Geng et al., 2017). Species identity or composition can also enhance ecosystem functioning in CWs (Ge et al., 2015; Han et al., 2017), and this effect has even surpassed the species richness effect (Geng et al., 2017). However, further research is needed on the effects of plant species diversity on the removal of Ca, K, and Mg and their pools in plant tissues.

In this study, microcosms were established to simulate CWs and were fed with simulated wastewater with high concentrations of Ca, K, and Mg. Four common species were planted in a gradient of increasing richness (from 1 to 4 species) and assembled in 15 possible species combinations. The objectives of this study were to (1) test the effects of plant species diversity on Ca, K, and Mg removal efficiencies; (2) investigate the effects of species diversity on Ca, K, and Mg pool in plant tissues; and (3) determine the plant species composition with the highest net ecosystem service.

2. Materials and methods

2.1. Microcosm design, planting pattern and wastewater irrigation

We set up a microcosm experiment simulating floating CWs in an open field at Zhejiang University in Hangzhou City, in Southeast China. The microcosms were constructed using ceramic tubs (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 twelve evenly spaced planting holes in each microcosm.

Four common local early spring species, *Oenanthe javanica* (Blume) DC (water dropwort), *Rumex japonicas* Houtt. (Japanese dock), *P. arundinacea*, and *Reineckia carnea* (Andr.) Kunth (Jixiang cao), were selected based on their morphologic and functional differences. In March of 2013, seedlings of these four species with similar sizes were transplanted into the microcosms, with a density of twelve individuals per microcosm to establish four species richness levels (1, 2, 3, 4) and all possible species compositions: four monocultures, six two-species compositions, four three-species compositions, and one four-species composition. An equal number of individuals were assigned to each species and uniformly distributed in each microcosm. The experiment was conducted using a randomized complete block design with six blocks, and a total of 90 microcosms.

The simulated wastewater was the Hoagland nutrient solution (Hoagland and Arnon, 1950) with a minor modification (Table S1). The concentrations of Ca, K, and Mg in the wastewater were 327.58 mg L^{-1}, 651.02 mg L^{-1}, and 47.80 mg L^{-1}, respectively. The simulated wastewater was supplied once every ten days from late March to late May, simulating the intermittent water operation mode of CWs (Faulwetter et al., 2009). Each microcosm was supplied with 21 L of simulated wastewater. In total, each microcosm received 11271.13 mg of Ca, 21117.95 mg of K, and 1812.58 mg of Mg.

2.2. Sampling and measurements

Effluent samples (500 mL) were collected in each microcosm on the tenth day (May 20) after the last supply of simulated wastewater (the CWs operated 59 days) and were stored in a refrigerator at −18 °C. Prior to the analysis of the Ca, K, and Mg concentrations in the effluent, samples were filtered with the use of a membrane syringe filter (pore size = 0.45 μm). Concentrations of Ca, K, and Mg in the effluent were measured by inductively coupled plasma atomic emission spectroscopy (IRIS Intrepid II XSP; Thermo Fisher Scientific Inc., Waltham, MA). Once effluent sampling was completed (May 20), all plants were harvested by species, and were cleaned and divided into aboveground and belowground parts. Plants were oven dried at 65 °C to constant mass, weight to obtain aboveground and belowground biomasses. Dry plant tissue materials were ground into a fine powder, and then homogenized samples were digested using $HNO_3 + HClO_4$ under high temperature and pressure. Subsequently, plant tissues with Ca, K, and Mg concentrations were analyzed using inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Prodigy, Leeman Labs Inc., Hudson, NH). Plant nutrient pools were determined by multiplying the Ca, K, and Mg tissue concentrations per species by tissue biomass.

2.3. Parameter calculations

We used the deviation index (D_{max}) to compare metal removal efficiencies (Ca, K, and Mg) and their pools in plant tissues between the observed value in a species mixture with the highest value and with the monocultures included (Loreau, 1998), and was calculated, as follows:

$$D_{max} = (O_i - Max(M_i))/Max(M_i)$$

where O_i is the observed metal removal efficiencies or plant metal pools in a species mixture and $Max(M_i)$ is the highest metal removal efficiencies or plant metal pools of monocultures included. A D_{max} value > 0 means the mixture removed more metals or sequestered more metals than any of the monocultures included.

2.4. Statistical analysis

Linear regression analysis was performed to detect the effects of species richness on response variables (Ca, K, and Mg removal efficiencies and plant Ca, K, and Mg pools). One-way ANOVA was used to test plant species richness and composition treatment effects on the variables. If the treatment effects proved to be significant, the Fisher's Least Significant Difference (LSD) method was used to ascertain differences among treatment levels. An independent *t*-test was applied to find differences between the means of the response variables when a species was present and when it was absent in the system. One-sample *t*-test was used to examine whether the proportion indices (D_{max}) deviated significantly from zero. All data fulfilled the conditions of normality (Kolmogorov-Smirnow test) and equality of variance (Levene's test). All statistical analyses were conducted using the SPSS software (SPSS 20.0, SPSS Inc., Chicago, USA). We set the statistical significance level at $\alpha = 0.05$. All values were expressed as mean ± standard error.

3. Results

3.1. Metal removal efficiencies

Species richness had no significant effect on the Ca removal efficiency, but the lower bound (minimum) became higher in a system with high species richness (Fig. 1a). Species identity and composition had a significant effect among the four monocultures, and the Ca removal efficiency was the highest for the *R. japonicus* monoculture (Fig. 2a). The presence of *R. japonicus* increased the Ca removal efficiency by 18%, compared to the systems without this species, and the presence of *P. arundinacea* decreased the Ca removal efficiency by 9%, compared to