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Effect of plant species compositions on performance of lab-scale constructed wetland through investigating photosynthesis and microbial communities

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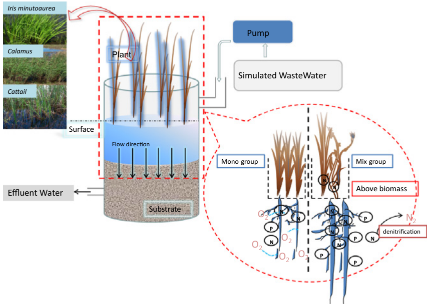
HIGHLIGHTS

- The mixed-culture groups improved TN and TP removal capacities significantly.
- Total biomass and N, P uptake were affected by species richness and type.
- Microorganisms displayed significant variances in the different plant compositions.
- Fungi mainly existed in SPA, SPAB, and SPABC to elevate the N, P accumulation.

GRAPHICAL ABSTRACT



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ABSTRACT

This study focused on the effects of plant compositions on removal rates of pollutants in microcosms through investigating rhizosphere microbial populations, photosynthetic efficiency and growth characteristics. Mixed-culture groups improved the removal efficiency of TN and TP significantly but exhibited lower COD removal rates. Total plant biomasses were improved as the species richness increased, but the N/P content in the plants was mainly affected by the type of species. The mixed-culture groups showed lower photosynthesis rates and oxygen supply generated from roots under high irradiation. Microbial communities of the cultured groups in the rhizosphere exhibited significant differences. According to principal component analysis (PCA), the fungi were the typical microbes of SPA, SPAB, and SPABC, resulted in improvement in nutrient accumulation. These results demonstrated that a mixed culture strategy can represent the overyielding of biomass, promote the photo-protection mechanism, and will further increase the removal rates of pollutants in a constructed wetland.

1. Introduction

Constructed wetlands (CWs) is a low-cost wastewater purification strategy that has been widely used around the world and includes the functions of plants, microorganisms, and substrates (Ojeda et al., 2008). In a CWs system, there are different pollutant removal mechanisms, such as plant uptake, microbial immobilization, and substrate absorption (Hu et al., 2016; Vymazal and Kröpfelová, 2011).

"Roots theory" first proposed in 1977, increased the importance of wetland plants in CWs (Vymazal, 2011). Plants can remove pollutants (*i.e.,* N, P and heavy metals) via uptake, absorption, retention and assimilation (Hu et al., 2016). In addition, plants have the capacity to accelerate the development of microbial communities and promote "living" environments around roots (*i.e.,* aerobic and anaerobic alternant environments) through root growth and

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oxygen production (Calhoun and King, 1997). Oxygen, produced through photosynthesis, transfers and diffuses into root surroundings (*i.e.,* microbial communities) (Armstrong et al., 1996), indicating that photosynthesis not only supplies oxygen to roots, but also helps plants uptake pollutants by means of assimilation. Previous studies have demonstrated that the photosynthesis of plants can affect the oxygen production and pollutant removal rates in different CWs (Huang et al., 2010).

To improve pollutant removal efficiency in CWs, it is essential to investigate the effect of plant biodiversity in CWs through monitoring their physiological functions. Previous studies have suggested that increasing plants species richness can improve biomass productivity, thus leading to higher uptake of nutrients (*i.e.,* N and P) and pollutants (*i.e.,* COD and heavy metals) (Cardinale et al., 2006). More importantly, different species and richness may change the utilization of nutrients or pollutants, which may consequently affect the ecological responses of CWs, such as photosynthesis, the respiration process and microbial communities (Wang et al., 2015). Although different plant species and richness are known to strongly influence microbial communities around roots, little is known regarding the interaction between a shift in microbial communities and pollutant removal rates; in addition, there have been very limited systematic studies on how plant species richness can affect the efficiency of photosynthesis. The PLFA technique and principal component analysis (PCA) are now powerful tools being used to identify differences in microbial communities in soil under different environmental factors, revealing similarities among the groups and soil microbial physiological traits (Yang and Zhang, 2014). However, to date, the PLFA and PCA techniques have yet to be widely applied to CWs. In particular, there have been no reports on the relation between pollutant removal rates in CWs and microbial communities in different plant compositions using a comprehensive analysis involving PCA and PLFA.

In this study, a new and suitable CWs planting allocation method was used as a candidate method by which to improve pollutant removal capacity because the applied aquatic plants are located in Northeast China and can survive in areas where they are under environmental stress (*i.e.,* low temperatures); moreover, the applied plants under consideration can co-exist very well. Therefore, in this study, their growth and physiological traits were initially explored by investigating their biomass, N and P accumulation and photosynthesis efficiency. In addition, the PLFA analysis combined with PCA was conducted to identify the intercorrelation between the plant compositions and microbial communities. Finally, the removal rates of the pollutant (*i.e.,* COD, TN, and TP) in the microcosms of different plant compositions were directly measured during the entire operational stage. The aim of this study was to improve the pollutant removal rates of constructed wetland microcosms via different plant compositions and to analyze the growth characteristics and microbial communities around roots. The information obtained from this work could be useful in terms of the different plant species located in Northeast China to improve the pollutant removal performance of CWs and to enhance the understanding of the role of plants in CWs.

2. Materials and methods

2.1. Operation of constructed wetland system

The subsurface constructed wetland microcosms used in this study were established at Harbin Institute of Technology, Harbin, Northeast China (126°40′E, 45°45′N). The outdoor microcosm system (diameter of 30 cm, height of 40 cm, working volume=5 L) was set up in April 2014 and filled with gravel (particle diameter ≈ 10 mm, a depth of 15 cm) and sediment (a depth of 10 cm). The simulated wastewater (depth ≈ 5 cm) was introduced into the system through inlet PVC pipes by pumping from a wastewater storage tank. *Acorus calamus* (A), *Iris minutoaurea* (B), *Cattail* (C) were selected as the experimental plant species (obtained from Ashi river, Heilongjiang, China), which are common aquatic plants in Northeast China. In our preliminary experiments, the growth performance of SPAC-(plant A + C, *Acorus calamus + Cattail*) group is extremely limited, resulted from their relatively thicker and longer roots which would cause a serious roots-competition between each other within the limited cultured space, and then both of their growth were significantly inhibited. Therefore, instead of SPAC, six cultured groups (SPA-(plant A, *Acorus calamus*), SPB-(plant B, *Iris minutoaurea*), SPC-(plant C, *Cattail*), SPAB-(plant A + B, *Acorus calamus + Iris minutoaurea*), SPBC-(plant B + C, *Iris minutoaurea + Cattail*), SPABC-(plant A + B + C, *Acorus calamus + Iris minutoaurea + Cattail*) were preformed in this study. The plant density was fixed in each microcosm under different cultured types, which contained 6 individual plants with even allocations (*i.e.,* 1:1 in SPAB/SPBC or 1:1:1 in SPABC). The microcosms were fed with simulated wastewater containing 60.12 mg/L COD, 30.33 mg/L total nitrogen (TN), 11.21 mg/L NO_3^--N, 20.51 mg/L NH_4^+-N, 5.01 mg/L total phosphorus (TP) (simulated to the primary effluent of a sewage treatment factory in Northeast China). The wastewater was pulsed into each microcosm per 24 h period intermittently from April to September, 2014 (172 days in total). The aquatic plants were cultured in tap water until they were transplanted into microcosms in April.

2.2. Sampling and analysis

Wastewater samples were collected once from inlet and outlet of each microcosm each day (from April to September, 2014). All samples were centrifuged at 1046g for 5 min and filtered through a 0.45 μm nylon filter for water quality analysis. The TN concentration was determined by Multi N/Celemental analyser (Analytik Jena AG 2100s, Germany), and TP concentrations were then determined using inductively coupled plasma atomic emissionspectroscopy (ICP-AES, PerkinElmer Optima 8300, USA). TheNO_3^--N concentrations were converted from NO_3^- determined using the ICS-2100 (Thermo Fisher, USA). COD and NH_4^+-N were analyzed according to the standard protocol of the National Environmental Protection Agency of China (2002). The removal rate was calculated as follows (Zhang et al., 2011b):

$$\text{Removal rate}\ (\%) = [1 - (V_{out} \times C_{out}/V_{in} \times C_{in})] \times 100\% \tag{1}$$

where V_{out} is the CW effluent volume (L); C_{out} is the CW effluent concentration (mg/L); V_{in} is the CW effluent volume (L), and C_{in} is the CW effluent concentration (mg/L).

After 6 months, the plants and substrate samples were harvested. The plant samples were washed with tap water and dried at 65°C until the weight was invariant for biomass determination (g $plant^{-1}$). The selected plants were separated into leaves, stems and roots, and then ground into powder (through a 0.2 mm mesh screen). The sample powder was digested using an acid solution ($HCl: HNO_3: HF = 5:2:2$) to analyze the N and P concentration in the plants' tissue uptake from the wastewater. The nutrient concentrations in the plant tissues were calculated as follows (Zheng et al., 2016):

$$C_i = \frac{(C_{i1} \times W_1) + (C_{i2} \times W_2) + (C_{i3} \times W_3)}{W_0}, \tag{2}$$

where C_i is the nutrient concentrations (i stands for N or P, w%); W_0 is the total biomass of one species (g); W_1 is the biomass of the leaves (g); W_2 is the biomass of the stems (g); W_3 is the biomass of the roots (g); C_{i1} is the concentration of nutrients (i stands for N or P, w%) in the leaves; C_{i2} is the concentration of nutrients