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Impact of plant species on spatial distribution of metabolic potential and functional diversity of microbial communities in a constructed wetland treating aquaculture wastewater

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ABSTRACT

Two horizontal subsurface flow constructed wetlands, one planted with *Iris pseudacorus* (HF1) and the other with *Phragmites australis* (HF2), were built to treat aquaculture wastewater. Pollutants removal, especially the nitrogen removal efficiency, as well as the activities, metabolism, and functional diversities of the microbial communities involved were evaluated under different conditions. NO_3^--N was removed in HF1 in all seasons, while it accumulated during autumn and winter in HF2. HF2 was more efficient at removing NH_4^+-N than HF1 in spring and summer when HRT was above 2 d. Nitrification intensity was higher in HF2, while denitrification intensity was higher in HF1. Biolog-ECO indicated that microbial diversity was higher in HF1 than in HF2, and the structure of the microbial community was more different at the back end and in the lower layer than at other places. These results were further confirmed by qPCR and 454-pyrosequencing. The relative abundances of *Nitrospira* were highest in back end of the upper layer in HF2, while *Denitratisoma*, which involves in denitrification, was mainly found in the lower layer of HF1. The results illustrated that the radial oxygen loss from *P. australis* and root exudates of *I. pseudacorus* could have an impact on the nitrogen removal efficiency by influencing the development of microbial communities.

1. Introduction

Discharges of wastewater from aquaculture can cause severe environmental problems because of its low chemical oxygen demand (COD) level and high nitrogen and phosphorus contents. If not treated, large emissions will lead to eutrophication of surrounding waters (Cao et al., 2007). Nitrogen and phosphorus in the aquaculture water are mainly from residual food, pharmaceuticals, metabolites of aquatic products, and the fishpond sediments. Compared with traditional physical and chemical methods, constructed wetlands (CW) are very efficient at removing nitrogen pollutants (Hu et al. 2012; Zhu et al., 2014). They have benefits in terms of the low investment requirements, low implementation costs, and low energy consumption (Garcia et al., 2010; Meng et al., 2014), and have been widely used for treating municipal and aquaculture wastewater (Lin et al., 2002b; Shi et al., 2011).

Many studies have shown that the scientific basis for wastewater treatment is the cooperative growth of both the plants and the microorganisms associated with the plants (Bouali et al., 2014), especially in the removal of nitrogen pollutants. Nitrification and denitrification promoted by microorganisms are the main nitrogen removal processes in CWs (Gersberg et al., 1986), which were significantly influenced by oxygen and exudates from wetland plant roots (Faulwetter et al., 2009). Ammonia nitrogen is removed mainly through the nitrification of microorganisms (Sims et al., 2012), and is related to dissolved oxygen (DO). Radial oxygen loss (ROL) of plant roots is a main source of DO, and roots with high ROL cause increased DO in the rhizosphere. Nitrification occurs in wetlands when the oxygen concentrations are high, and may be inhibited when the DO concentrations are less than 2 mg/L (Zapater-Pereyra et al., 2014). NO_3^--N is mainly removed by denitrification (Lin et al., 2002a) and this process is influenced by the carbon source (Van Oostrom, 1995). Plant roots usually exude organic carbon, which could be used by microorganism as carbon source (Truu et al., 2009; Vymazal, 2011a). Emergent plants, such as *Phragmites australis*, *Phalaris arundinacea*, *Acorus calamus*, *Typha latifolia*, *Scirpus lacustris* and *Iris pseudacorus*, are commonly used

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for horizontal subsurface constructed wetlands (Vymazal, 2011b), and previous studies have shown that wetlands planted with *P. australis*, *P. arundinacea*, *T. latifolia*, or other species have a high nitrogen removal efficiency, usually between 50 and 90% (Gagnon et al., 2010; Salvato et al., 2012; Zhu et al., 2014). These results indicated that the microbial removal efficiency is related to the function of radial oxygen loss and root exudates (Fester et al., 2014; Vymazal, 2011b), which are significantly different among wetland plant species.

Aerobic, anoxic, and anaerobic areas appear in turn around the roots because of radial oxygen loss, which results in different microbial communities enriched to remove nitrogen. There are significant differences in the root porosity among plant species because of the difference in the structure of aerenchyma, which induced different transfer and release rates of oxygen (Lai et al., 2012). *P. australis*, *A. calamus* and *Cyperus flabelliformis*, with fibrous roots and porosity values of 40%, 26% and 32%, respectively, have relatively high ROL (Lai et al., 2011). Li et al. (2013) found that the roots of *I. pseudacorus* penetrated most deeply and showed the best nitrogen removal efficiency but did not have the strongest ROL. Plant roots can also release some exudates into the filter matrix to stimulate microbial activities (Maine et al., 2007; Maltais-Landry et al., 2007). These exudates include organic compounds such as sugar, amino acids, organic acids, small amounts of fatty acids and steroids, and trace amounts of growth substances (Gao et al., 2003), but differences exist in the quantity and composition among plant species. Zhai et al. (2013) found that the release of DOC from plant roots differed significantly between species and the release rates from *I. pseudacorus* and *P. australis* were significantly higher than from *J. effusus*. Plants may influence denitrification by supplying root exudates as an important source of organic carbon through rhizodeposition of substrates in subsurface flow constructed wetland systems (Faulwetter et al., 2009).

Recently, the role of plant–microbe interactions in the biodegradation of contaminants has attracted much attention (Fester et al., 2014). However, to date, few studies have focused on how plants influence the performance of microorganisms in nitrification and denitrification processes in constructed wetlands. In this study, two horizontal subsurface flow constructed wetlands planted with *Iris pseudacorus* and *Phragmites australis*, respectively, were built to treat aquaculture wastewater on field. The aim of the present study was to investigate the effect of common wetland plants on the removal efficiency and on the spatial distribution of microbial activities, metabolism, populations, and communities during the water treatment processes.

2. Materials and methods

2.1. Experimental design and operating mode

Two horizontal subsurface flow constructed wetland (HSSFCW) microcosms that were 150 cm long, 40 cm wide and 80 cm deep (effective depth of 60 cm) were built from PVC. These wetland microcosms were sited at an aquaculture farm in Changzhou city (31°35′N, 119°52′E), Jiangsu Province, China. The total area of the farm is approximately 150 ha, and *Pelteobagrus fulvidraco*, *Lateolabrax japonicas* and other kinds of fish are mainly cultured. The two ends of the microcosm were separated by perforated plates, forming a 15-cm water distribution area and a 15-cm water collection area, and they were filled to a depth of 40 cm with gravel (particle diameter of 30–50 mm). The treatment area was in turn filled with gravel (particle diameter of 5–10 mm) and zeolite (particle diameter of 5–15 mm), at a filling ratio of 3:1. All matrixes were fully washed before they were filled in the microcosms.

Table 1
Water quality of the influent to the system. Sampling and analyses were performed according to standard methods (State Environmental Protection Administration, 2002).

	Autumn–Winter		Spring–Summer	
	Range	Average	Range	Average
pH	7.57–8.54	8.16	7.25–8.53	7.95
Temperature (°C)	4.1–20.0	11.4	7.8–29.4	20.4
Conductivity (μS/cm)	508–609	548	414–618	507
DO (mg/L)	1.9–5.7	4.0	2.5–5.3	4.0
COD_{Cr} (mg/L)	28–51	39	40–92	62
TP (mg/L)	0.098–0.177	0.126	0.142–0.596	0.277
TN (mg/L)	1.923–3.102	2.600	2.315–4.683	3.204
NH_4^+-N (mg/L)	0.547–1.340	0.880	0.898–2.400	1.284
NO_3^--N (mg/L)	1.010–1.592	1.365	0.629–1.283	0.881
NO_2^--N (mg/L)	0.028–0.082	0.052	0.040–0.169	0.091

Our previous screening tests for wetland plants by sequencing batch culture showed that *Iris pseudacorus* and *Phragmites australis* were much different in root structure and nitrogen removal efficiency (data not shown). Therefore, the two common plants in the aquaculture farm were used in the experiments. The wetland plants were collected from the ecological purification system of the aquaculture farm. After collection, the plants were rinsed repeatedly with distilled water, trimmed, and then cultured with Hoagland for 10 days. Healthy plants demonstrating good growth and of similar size were chosen and planted in the microcosms. The two CWs, HF1 and HF2, were planted with *Iris pseudacorus* and *Phragmites australis,* respectively, and had a density of 50 plants m^{-2}.

The influent that fed the CWs was from a river in the aquaculture farm, and the amount of water that entered the CWs was controlled by peristaltic pump. The whole system was initiated in September 2013 and was run continuously. The hydraulic retention time (HRT) and the hydraulic load were controlled by the amount of influent. According to different operating conditions, the whole experiment was divided into six stages, with a stable period of 2 weeks between two stages. The operating parameters of each stage are presented in Table S1 in the Supporting information.

2.2. Sampling and analysis

Water samples were collected from the outflow of each microcosm and analyzed for temperature, conductivity, DO, pH, chemical oxygen demand (COD), total phosphorus (TP), total nitrogen (TN), ammonia nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), and nitrite nitrogen (NO_2^--N) following the Standard Methods for Monitoring Water and Wastewater Quality (State Environmental Protection Administration, 2002). The quality of the influent was also determined and is shown in Table 1. Besides, BOD_5 test was also performed in the initial stage of experiments, which showed that the average BOD_5 was 5.0 ± 0.2 mg/L and B/C was about 0.13, indicating that there were mainly refractory organics in the influent of the system. To maintain the equilibrium of the samples and to obtain a more reliable estimate of the removal efficiency of the system, the water quality parameters were measured immediately after sampling.

2.3. Microbial nitrification and denitrification intensity

Five sampling points were established in each microcosm in the substrates of the upper front (UF), the upper middle (UM), the upper back (UB), the middle (mm), and the lower middle (LM) (Fig. S1). The front sampling point was 30 cm from the water distribution area, the back sampling point was 30 cm from water collection area, and vertical sampling points were at the depth of 10 cm, 30 cm, and 50 cm, respectively. A portion (30 g) of the substrate from each sam-