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Short communication

Comparisons of microbial abundance and community among different plant species in constructed wetlands in summer

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ABSTRACT

The performances of constructed wetlands (CWs) with different plant species have been extensively studied and compared, but no general conclusion has been found, especially in warm temperatures. Wetlands were planted with *Phragmites australis* (*PA*), *Typha orientalis* (*TO*) and *Arundo donax* (*AD*) were set up with unplanted ones as *control* (*CT*). The performances of wetlands in summer were measured. The microbial abundance and communities were analyzed with quantitative PCR (qPCR) and pyrosequencing. The summertime removal efficiency in CWs was not significantly different among plant species. Significant differences were found in the microbial community but not in the microbial abundance. Microbial diversities of *AD* and *PA* were higher than *TO*. Proteobacteria and Cyanobacteria were more prevalent than other bacteria in summer. The level of Proteobacteria was higher in *PA* (47.96%) and *AD* (45.58%) than in *TO* (33.54%). Photosynthetic bacteria accounted for 13.55% of the total bacteria in *AD*, followed by *PA* (13.07%) and *TO* (3.42%). However, more Cyanobacteria were in *TO* (26.09%) than in *AD* (12.62%) and *PA* (7.26%). Given that microbial abundances among the three plants had no significant differences, pollutant removal performances in summer were similar among plant species. Furthermore, a high level of Cyanobacteria can potentially be important in pollutant removal in summer.

1. Introduction

Constructed wetlands (CWs) are low-cost natural systems for various wastewater treatments (Vymazal, 2014). The pollutant removal performance of CWs is affected by the presence and activity of plants and microorganisms (Krasnits et al., 2009; Peng et al., 2014). Numerous studies have focused on comparing the effects of plant species on pollutant removal efficiency, but no general difference has been found. Interestingly, CW performances show no difference in any parameter among plant species at warmer temperatures (typically 24 °C) when plants are actively growing (Allen et al., 2002; Hook et al., 2003). Previous research attributed this phenomenon to the effect of pollutants, experimental design, type of wastewater, and other factors. Beyond empirical comparisons, differences between the effects of microbial mechanisms on pollutant removal efficiency have rarely been explained.

Microorganisms could contribute to the removal of pollutants in CWs (Hatano et al., 1994; Scholz and Lee, 2005). Plants and microorganisms in CWs are functionally linked because the plant roots provide large attachment surfaces, as well as sites of oxygen release and root exudation (Bürgmann et al., 2005; Bais et al., 2006; Faulwetter et al., 2012). However, some studies show that the potential for plants to enhance the microbial processes is more limited in summer climate when plants are actively growing. The reason is that microbial respiration must compete with plant root for the limited oxygen in wetlands, as most of the oxygen is consumed in the roots for supporting active growth (Moog and Brüggemann, 1998; Jackson and Armstrong, 1999). Therefore, understanding the variation of microbial assemblies in summer during plant active growth periods is critical.

In this study, CW microcosms planted with *Phragmites australis*, *Typha orientalis* and *Arundo donax* were set up with unplanted ones as control to characterize the microbial differences among plant species in summer. The CW performances were detected in

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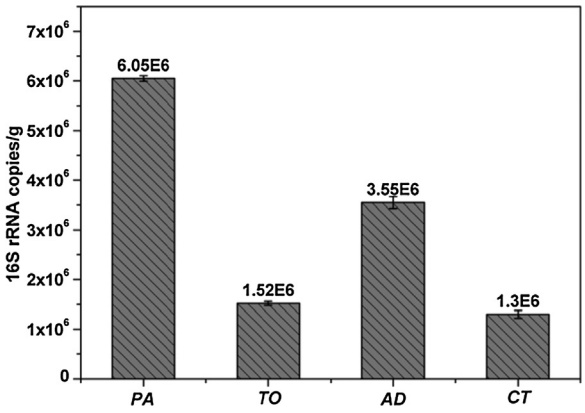

Fig. 1. DNA copies of the 16S rRNA genes in different wetland microcosms. The DNA copies was detected by quantitative polymerase chain reaction (qPCR) using a Roche LC-480 real-time PCR system.

summer. Microbial populations were determined by quantitative polymerase chain reaction (qPCR), and microbial communities were analyzed by pyrosequencing (454 platform) based on 16S rRNA gene (Fig. 1).

2. Materials and methods

2.1. Site description and design

The experiments were carried out in 11 independent microcosm wetland systems located in Shandong Normal University in Jinan, China, with a warm-temperature monsoonal climate. The average temperature in summer is 26.7 °C. The microcosm wetland systems were set up in a subsurface flow design on March 12, 2013 for treating domestic wastewater. The microcosms were polyvinyl chloride columns (depth = 48 cm; diameter = 30 cm) filled with a three-layer filter: 19 cm of gravel (diameter = 5–7 cm) at the bottom, 15 cm of gravel (diameter = 2–4 cm) in the middle, and 10 cm of washed sand (particle size < 2 mm) at the top. *P. australis, T. orientalis* and *A. donax* were transplanted from Nansi Lake, Shandong Province, China. Each plant species was set with three parallels, and two unplanted microcosms were set up as control. These CW microcosms were named *PA*, *TO*, *AD* and *CT*. The plant density was 15 rhizomes per cell. Microcosms were fed with synthetic wastewater simulating post-primary domestic wastewater effluent (ammonia nitrogen = 14.45 ± 0.24 mg/L; chemical oxygen demand = 59.38 ± 4.08 mg/L) (Taylor et al., 2011). The influent was pumped into cells to keep the water level below the sand surface. Each wetland operated in batches with a hydraulic retention time (HRT) of 7 days. Before the experiment, the wetlands were precultured for 4 months.

2.2. Sampling

Sand samples were obtained from each microcosm on July 23, 2013 for DNA extraction. First, each microcosm was completely drained. Sand samples were then collected from the top layer 5–10 cm deep at five different plots (Calheiros et al., 2010) in each parallel and then mixed into a composite sample. The sand samples were stored in 5 mL aseptic Eppendorf tubes, immediately placed on ice and stored at −80 °C before microbial analysis.

2.3. Microbial analysis

DNA was extracted using a MOBIO PowerSand™ DNA kit according to the manufacturer's instructions. qPCR was performed on a Roche LC-480 real-time PCR system (CH) according to the procedure described in our previous work (Wang et al., 2014). Pyrosequencing was performed on the 454 Genome Sequencer FLX Titanium platform in the Chinese National Human Genome Sequencing Center (Shanghai, China). The sequencing process and data analysis were obtained following the method described by Wang et al. (2015).

3. Results and discussion

3.1. Performance of wetlands

The chemical oxygen demand (COD) and NH_4^+-N removal efficiency of different CW microcosms during summer are described in Table 1. The result shows that the overall COD removal efficiencies were 77.29 ± 2.70% (*PA*), 71.9 ± 5.50% (*TO*), 69.30 ± 3.41% (*AD*), and 68.44 ± 5.32% (*CT*). The differences of COD removal efficiency among the CW microcosms were not significant during summer ($p > 0.05$). Table 1 also shows that high NH_4^+-N removals were obtained in all the wetlands during summer (94.96 ± 0.9% for *PA*, 94.42 ± 3.46% for *TO*, 93.72 ± 4.51% for *AD* and 93.54 ± 2.56% for *CT*). No significant difference in NH_4^+-N removal was detected among plant species. These results are in accordance with previous studies that showed CW performances are not different in any parameter among plant species at warmer temperatures (Allen et al., 2002; Hook et al., 2003).

3.2. Microbial population

The copy numbers of 16S rRNA gene were 6.05×10^6 copies/g, 3.55×10^6 copies/g, 1.52×10^6 copies/g, and 1.30×10^6 copies/g for *PA, AD, TO* and *CT*, respectively(Shown in Fig.1). The microbial population was highest in *PA*, followed by *AD* and *TO*, but the differences were not significant ($p > 0.05$). Moreover, the difference between planted and unplanted microcosms was also not significant, which corresponded with our previous work (Wang et al., 2015). The microbial population in CWs is critical for the decomposition of contaminants in the wastewater (Ahn et al., 2007; Iasur-Kruh et al., 2009). Our results show that the microbial populations in summer were not significantly different among plant species or between planted wetlands and the unplanted control. This finding could be one explanation why the CW performances were not different in any parameter between plant species at warmer temperatures. These results also mean that the effect of plants on microbial population is not apparent in summertime (Wang et al., 2015).

3.3. Microbial community

Pyrosequencing-based analysis of the 16S rRNA gene was used to detect the microbial community. Across all samples, 47,005 quality sequences were classified as bacteria with a read length of 250 bp. Sequences longer than 400 bp account for 94.36% (44,352) of the total sequences. The number of sequences per sample ranged from 9531 to 12,869 (average 11,751).

The coverage values of all 4 plots were higher than 93%, indicating that the sequencing capacity can reflect the composition

Table 1
The COD and NH_4^+-N removal efficiency (%) of different CWs microcosms during summer (mean ± SD, $n = 3$).

	PA	*TO*	*AD*	*CT*
COD	77.29 ± 2.70	71.9 ± 5.50	69.30 ± 3.41	68.44 ± 5.32
NH_4^+-N	94.96 ± 0.90	94.42 ± 3.46	93.72 ± 4.51	93.54 ± 2.56