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Performance and bacterial community structure of a 10-years old constructed mangrove wetland

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ABSTRACT

Constructed mangrove wetland has been used for wastewater treatment but its long-term performance has not been reported. One-year monitoring of a 10-years old horizontal subsurface-flow constructed mangrove wetland consisting of three belts, two with mangrove plants and one without, revealed that the system maintained high and stable removal percentages of organic matter and nutrients, and planted belts performed better than unplanted control. Substrates in belts planted with *Aegiceras corniculatum* or *Kandelia obovata* had higher abundance of ammonifiers, nitrifiers and denitrifiers but lower total heterotrophic bacteria than unplanted substrate. Denaturing gradient gel electrophoresis showed that microbial diversity in planted substrate was significantly lower than that in unplanted one. The bacteria in substrates, irrespective to belts, were phylogenetically related to Proteobacteria (most dominant), Acidobacteria, Firmicutes, Nitrospirae, Gemmatimonadetes, Chloroflexi and Cyanobacteria. The steady performance of this 10-year old constructed mangrove wetland was affected by the abundance and diversity of bacterial community in substrate.

1. Introduction

Constructed wetlands (CWs), compared with conventional wastewater treatment processes, have become favorite alternatives to treat different types of wastewater in recent decades, as they are effective and environmentally friendly, easy to manage and have low operation cost (Garcia et al., 2010). Constructed wetland, a mimic of natural sewage treatment system, improves water quality based on the combined actions among microorganisms, tolerant plants and soil/substrate/filter materials (Vymazal, 2011a). Microorganisms in substrates and roots play essential roles in the transformation and mineralization of nutrients and organic pollutants in CWs (Truu et al., 2009; Zhu et al., 2013). The purification of wastewater is mediated through different types of bacteria in roots and substrates under aerobic and anaerobic conditions (Truu et al., 2009; Saunders et al., 2013). For instance, nitrification and denitrification, catalyzed by nitrifying and denitrifying bacteria, respectively, are the controlling steps in the removal of nitrogen (Li et al., 2015). The removal of organic matter is the result of biodegradation carried out by various microorganisms, including heterotrophic and autotrophic bacteria, fungi and specific protozoa under aerobic and anaerobic conditions (Truu et al., 2009).

The quality and quantity of the microbial populations are affected by temperature and have significant seasonal variations (Jing and Lin, 2004; Chang et al., 2013). Faulwetter et al. (2013) considered that season has the greatest impact on the abundance and diversity of ammonia oxidizing bacteria in CWs. Low temperatures (lower than 15 °C) restrict the reproduction rates and metabolic activities of nitrifiers and denitrifiers, thus lowering nitrification and denitrification processes, respectively (Hsu et al., 2011; Huang et al., 2013). Toxic pollutants in wastewater, especially industrial discharges, can alter the microbial community structure, and different bacterial sub-populations have different responses to mixed pollutants (Wang et al., 2015). It is important to understand the microbial communities and the seasonal variations in microbial population size and structure in substrate when evaluating the purification and function of CWs (Adrados et al., 2014). However, research on microbial structure and function in CWs is still limited, and very few studies reported the relationship between removal performance and microorganisms in CWs.

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Mangroves are known to be one of the most productive ecosystems along the coastline of tropical and subtropical regions (De-León-Herrera et al., 2015). Mangrove plants can tolerate stressful environments, such as high salinity, water logging, alternating aerobic and anaerobic conditions, unstable substratum, high concentration of nutrients in wastewater, etc. (Zhang et al., 2010; Su et al., 2011). Due to special adaptations, mangroves have been suggested as effective macrophytes in CWs to removal pollutants (Yang et al., 2008; Moroyoqui-Rojo et al., 2012). It has been reported that constructed mangrove wetlands are more efficient to remove organic matter, nutrients and toxic pollutants from wastewater than conventional CWs (Moroyoqui-Rojo et al., 2012; Leung et al., 2016). The contaminants in wastewater, such as nitrogen and phosphorus, are absorbed directly by plants, and the efficiency is dependent on plant growth and nutrient uptake (Ong et al., 2010; Ko et al., 2011). Mangrove roots also provide excellent rhizosphere habitats for microorganisms and facilitate microbial activities, as roots form complex belowground 'nets' to trap sediment, provide aerobic condition by releasing oxygen and maintain the physical stability of an ecosystem (Gomes et al., 2011).

However, there is a lack of knowledge on the link between treatment efficiency and microbial communities in CWs, especially constructed mangrove wetlands. Although some successful paradigms for using mangrove wetlands for sewage treatment have been reported (Yang et al., 2008; Zhang et al., 2010), most of these published results were based on small-scale pot experiments within relatively short study period. The performance of constructed mangrove wetland after a long period of operation has not been reported. The present study therefore aims to (i) evaluate the purification performance of a horizontal subsurface-flow constructed mangrove wetland which has been operated for 10 years; (ii) analyze the population sizes of different bacterial groups and the bacterial community structure in substrates, particularly those associated with the removal of contaminants; (iii) relate the abundance of bacterial groups and bacterial diversity with purification efficiency; and (iv) compare the differences in bacterial community structure and function between planted and unplanted substrates.

2. Materials and methods

2.1. Constructed mangrove wetlands

The horizontal subsurface-flow (HSSF) constructed mangrove wetland system was located in the experimental zone of the Futian National Nature Reserve, Shenzhen Bay, Shenzhen, China (Latitude: 22°32′N; Longitude: 114°05′E), having mature mangrove trees at an average age of 50–80 years and height of 2–6 m. The system consisted of three independent belts, two were planted with true and dominant mangrove plant species, *Aegiceras corniculatum* (Ac) and *Kandelia obovata* (Ko), respectively, with the separation between two individual plants at 0.5 m in both belts, and the third one was the unplanted control (Ck) (Fig. S1). All belts were in the same size of 33 m × 3 m × 0.5 m (length × width × depth) and divided into five zones, inlet (1 m × 3 m × 0.5 m), $part_1$ (15 m × 3 m × 0.5 m), $outlet_1$ (1 m × 3 m × 0.5 m), $part_2$ (15 m × 3 m × 0.5 m) and $outlet_2$ (1 m × 3 m × 0.5 m). $Part_1$ and $part_2$ were filled with stones, gravels and mangrove sands as substrates. Stones of 3–5 cm in diameter were filled to a depth of 20 cm in the bottom, gravels of 0.5–1 m in diameter were placed in the middle with a depth of 20 cm, and mangrove sands of 10 cm deep was added at the top. Stones and gravels were purchased from a quarry nearby, while mangrove sands were collected from the foreshore of the Nature Reserve. Wastewater flowed from the $part_1$ to $part_2$ by gravity, as $part_1$ was 20 cm higher than $part_2$ (Fig. S1).

The treatment system began to operate in August 2005 and continued in the past ten years. Wastewater from surrounding premises, including residential, commercial and industrial quarters, was pumped to a sedimentation pond and settled for one hour prior to discharge into the inlet of the wetland system. The hydraulic loading rate (HLR) of each belt was 5 $m^3\ d^{-1}$ and the hydraulic retention time (HRT) was around 3 days. No changes were made to the system, including plants and substrates, during the 10-years operation.

2.2. Water quality analysis

Influent and effluent samples were collected once every two months at the inlet and $outlet_2$ of the three belts from August 2014 to June 2015 (Fig. S1). The chemical oxygen demand (COD), 5-day biological oxygen demand (BOD_5), total nitrogen (TN) and total phosphorus (TP) of samples were analyzed within 24 h after transported back to the laboratory. COD was determined using a HACH DR/3000 colorimeter after digestion in a HACH COD reactor (USA) according to its standard calibration and operation. BOD_5, TN and TP were measured according to the Standard Methods for Water and Wastewater Analyses (Environment Bureau of the State, 2002). Physico-chemical parameters of the water samples from the inlet and $outlet_2$, including pH, salinity (Sal), electrical conductivity (EC), dissolved oxygen (DO) were measured in situ using handheld pH meter (TPS WP-81, Brisbane, Australia), salinity meter (AZ 8371, Taiwan), conductivity meter (SQ-DDS-307A, YuAiQi, BeiJing) and dissolved oxygen meter (SevenGo pro™-SG6, Mettler Toledo, USA), respectively. The DO meter was also used to measure water temperature. The contaminant removal percentage of the system was calculated by the following formula:

$$\text{The removal percentages } (\%) = \frac{Ci - Co}{Ci} \times 100$$

where Ci and Co represents the influent and effluent concentrations (mg L^{-1}), respectively.

2.3. Substrate sampling

Substrate samples (the top 10 cm of mangrove sands) from the three belts were collected in December 2014 and June 2015 and the sampling method was the same for each belt. Four sampling points along the flow direction with an equal spacing, i.e., at 2.5 m, 12.5 m, 17.5 m and 27.5 m from the inlet and with the same dimension (1 m × 3 m) (Fig. S2). In each sampling point, three independent composite samples were collected. Each composite sample was made up of five random subsamples (100 g each) of substrates. These five subsamples after manually removed plant materials were mixed thoroughly in a polyethylene bag. The composite and homogenized sample was sealed and transported to the laboratory at 4 °C in an ice cooler. The composite samples were then divided into two portions, one portion was stored in a freezer at − 80 °C for bacterial community structure analysis and the other was stored at 4 °C for the enumeration of population size of specific bacterial groups.

2.4. Enumeration of bacterial populations

Substrate sample (10 g) was added to 100 mL of distilled water, and tenfold series of dilution (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}) were made using sterile distilled water, following the standard method described by Li et al. (2008). The total numbers of aerobic heterotrophic bacteria was determined by standard dilution plate method according to the colony forming units (CFUs) recorded on nutrient agar (NA) plates (Calheiros et al., 2009). In brief, serial dilutions of 10^{-3}, 10^{-4} and 10^{-5} from each sample were inoculated onto NA plates for total aerobic heterotrophic bacterial counts, with three replicates for each dilution. After incubated at 28 °C for 30 h, the number of positive growth on the plate at each dilution was recorded. The Most Probable Number (MPN) method was used to estimate the population size. For the three bacterial groups involved in nitrogen cycle, namely, ammonifiers, nitrifiers and denitrifiers, five levels of serial dilutions (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}) were inoculated into three different specific culture media, respectively (Li et al., 2008). Three replicates were made for each dilution and each bacterial group. The cultures for