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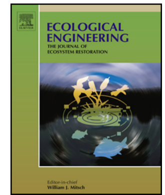

The uniqueness and biogeochemical cycling of plant root microbial communities in a floating treatment wetland

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ABSTRACT

Floating treatment wetlands (FTWs) are an innovative type of phytoremediation technique being used to reduce the impact of excess nutrient loading. Plants hydroponically grown on FTWs take up nutrients from water through their roots. In general microbial communities in the rhizosphere are important for healthy growth and nutrient uptake by plants. Despite most of previous studies focused on the nutrient removal processes, very little is known about microbial communities associated with FTW plant roots. The purpose of this study was to characterize the microbiomes revolving around the submerged roots of FTW in a manmade stormwater pond and to elucidate the source of FTW plant root microbiomes. The microbial communities collected from the plant roots *Canna flaccida* (golden canna) and *Juncus effusus* (soft rush), biofilms of plant pot (polyethylene) and floating mat foam (closed-cell urethane), and surrounding water were studied using 16S rRNA gene amplicon sequencing. The FTW plant root microbiomes were dominated by Alphaproteobacteria and Cyanobacteria at the class level, and *Anabaena*, *Rhizobium* and *Rhodobacter* at the genus level. Microbial communities of the FTW plant roots showed unique compositions resembling most closely the surrounding water samples while being quite different from the biofilm samples, leading the conclusion that the major source of microbial populations was the surrounding water. However, the dominance of *Rhizobium* species was only observed in the two plant roots and not recognized in the surrounding water samples, indicating that the FTW roots may selectively shape root microbiomes. Unexpectedly, quite a few groups of microbes were associated with the sulfur cycle. This finding indicates that the oxic-anoxic gradient is formed in the FTW rhizosphere, and this environmental gradient assists to extend the phylogenetic and functional diversities of microorganisms. We anticipate the presence of intrinsic rhizosphere microbiomes and the importance of complex biogeochemical processes that include carbon, sulfur and nitrogen driven by physical activity and chemical releases of FTW plant roots.

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1. Introduction

The process of eutrophication has become increasingly problematic across the world. The common problems associated with eutrophication include deprivation of drinking and recreational water, algal blooms and associated fish kills (Paerl, 2014; Paerl et al., 2016). To remediate eutrophication in limnetic systems, an excess nutrient control is essential. Floating treatment wetlands (FTWs) are a recent technology that has the ability to reduce nutrient concentrations in the water column and potentially reduce algal biomass (Bachand and Horne, 1999; Chang et al., 2012; Jones et al., 2017; White and Cousins, 2013). FTWs consist of aquatic vascular plants grown hydroponically on a floating mat in the limnetic water of the system (Hubbard et al., 2004; Vymazal, 2007). The plant roots are directly exposed to the water column, and take up nutrients hydroponically (White and Cousins, 2013). Once nutrients have become tied up in the mature plants, they can be removed from the system (White and Cousins, 2013; Wang et al., 2015; Zhou and Wang, 2010). Aside from the reduction of nutrient loading, it is anticipated there may be other playing factors that aid in the success of FTWs (Abed et al., 2017; Castro-Castellon et al., 2016; Stewart et al., 2008).

Rhizosphere microbiomes are important for the healthy growth of plants (Nihorimbere et al., 2011). Although the source of plant root microbiomes is considered as bulk soil, microbial communities between rhizosphere and bulk soils are generally distinguishable,

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suggesting the existence of selective force of microbial communities in the rhizosphere through plant-microbes interaction (e.g. excretion of rhizodeposits from plant roots) (Acosta-Martinez et al., 2008; Berg and Smalla, 2009; Berendsen et al., 2012; el Zahar Haichar et al., 2008, 2014). In the same fashion, we expect that FTW plant microbiomes can be distinguishable from the water column and the aquatic biofilm forming microbial communities. However, little is known about plant root microbial communities in FTWs although microbial functions such as denitrification are recognized as an essential component for the maintenance of healthy nutrient removal functions in FTWs (Stewart et al., 2008; Zhou and Wang, 2010).

The purpose of this study was to characterize the microbiomes surrounding the submerged roots of FTW in a manmade stormwater pond and to elucidate the source of FTW plant root microbiomes. Specifically, we attempted to identify the existence of microbial functional groups that are important for the biogeochemical cycling of FTWs and share similar phylogenetic traits (e.g. sulfate-reducing bacteria, cyanobacteria and nitrifying bacteria) through 16S rRNA gene amplicon sequencing.

2. Materials and methods

2.1. Description of a floating treatment wetland and physicochemical measurements

The floating treatment wetland ($3 \times 5\,m^2$ in size) used in this study was located in a manmade stormwater urban pond in Naples, Florida (26°09′18.5580" N and 81°48′08.3103" W) and was planted with *Juncus effusus* (soft rush) and *Canna flaccida* (golden canna) for two months (Fig. 1). The sampling occurred on 20 March 2014 around noon. Water depth was 1.4 m and the Secchi disk depth was 60 cm. A YSI 650 multiparameter display system coupled with a YSI data sonde 6600 V2 was used to record at approximately 0.25 m depth increment water column profiles (Fig. 1). The irradiance profile was assessed with a LICOR 1400 coupled with a LI-193 4π quantum type photosynthetically active radiation (PAR) sensor so that the light attenuation coefficient "k" in water was determined (e.g. Kirk, 1994). To access the water column underneath the center of the FTW, a plant pot was removed thus leaving enough clearance to lower the sonde, the irradiance sensor or the water sampler. Total nitrogen (TN) was determined using a standard test method (ASTM D 5176-91) for total chemically bound nitrogen in water by pyrolysis and chemiluminescence detection. Total phosphorus (TP) in water was determined by oxidizing and hydrolyzing all of the phosphorus-containing compounds in a sample to soluble reactive phosphate (Solorzano and Sharp, 1980). Both analyses were carried out at the Southeast Environmental Research Center (SERC) Nutrient Analysis Laboratory at Florida International University. In the limnetic water, TN and TP were $47.7 \pm 3.3\,\mu M$ (mean ± standard deviation) and $3.5 \pm 1.6\,\mu M$, respectively. Underneath the FTW, TN and TP were $51.2 \pm 0.9\,\mu M$ and $3.5 \pm 1.6\,\mu M$, respectively. Using the modified equations developed by Brezonik (1984), trophic state index (TSI) of the water column was 88 (i.e. hypereutrophic) and the mass TN/TP ratio was 14:1 (i.e. phosphorus limited water).

2.2. Plant root and artificial substratum sampling

Plant roots from the FTW were sampled for DNA analysis and measured from the base of the shoot to the root tip, approximately 60 cm (Fig. 1). The artificial substrata (i.e. potting pot and floating mat material) were cut out from their mother materials and conditioned similarly to the roots. A subsurface (0.5 m) pond water sample was collected in an autoclaved 1-l polycarbonate bottle from a meter away from the FTW. Upon return to the lab, 300 ml of water samples were passed through 0.2 μm polysulfone membrane filters (Millipore) to collect microbial cells (non-prefiltered sample). Additionally prior to collect microbial biomass, 450 ml of water sample was pre-filtered with P8 filter (>20 μm particle retention, Fisher Scientific) to eliminate eukaryotic phytoplankton then passed through 0.2 μm filters (prefiltered sample) to trap microbial cells. All samples were chilled in an icebox for transportation to the laboratory, and then frozen at −20 °C until further processing. A portion of plant root samples collected from three different locations of FTW (0, 80, 140 cm from the center) were proportionally mixed and two sets of duplicated samples were prepared for each plant root. The samples were sonicated in plastic bags for 40 min with 50 ml of 0.9% NaCl solution. The supernatant was transferred into a 50 ml centrifuge tube and 15 ml (canna1 and juncus1) or 5 ml (canna2 and juncus2) of solution was passed through a 0.2 μm polycarbonate filter. The DNA extraction was carried out using a Powerwater DNA isolation Kit (Mo Bio Laboratories) following manufacturer's instructions. Potting pot and floating mat samples were incubated with 5 ml of 0.9% NaCl solution containing proteinase K (final conc., 50 μg/ml) in a 50 ml centrifuge tube at 60 °C for 3 h. The tube was shaken by hand and centrifuged at $8000g \times 1$ min. The supernatant was finally transferred into a 2 ml plastic tube and the DNA extraction was carried out using a modified phenol-chloroform extraction method as described previously (Urakawa et al., 2010).

2.3. High-throughput sequencing and data analysis

The microbial communities collected from the plant roots, artificial substrata and water samples were analyzed using high-throughput sequencing to determine microbial diversity present in the samples. DNA was sequenced for the V1 to V3 region of 16S rRNA using the 28F (5′-GAGTTTGATCNTGGCTCAG) and 519R (5′-GTNTTACNGCGGCKGCTG) primer set and the Roche 454 GS/FLX + pyrosequencing platform (Research and Testing Laboratory, Lubbock, Texas). Denoising and chimera checking were performed on all the reads for each region of data using USEARCH and UCHIIME, respectively (Edgar 2010; Edgar et al., 2011). Individual 454 reads were further annotated using NCBI BLAST search, RDPipeline (Cole et al., 2014) and MG-RAST (Meyer et al., 2008). General statistics of sequence data were performed by MG-RAST. A one-way analysis of variance (ANOVA) was employed using SigmaPlot 13.0 to test for the statistical difference among samples. Further data analyses including Student's *t*-test, diversity index calculation (Shannon index and inversed Simpson index), richness-evenness plotting (Menhinick's richness and Pielou's evenness indexes) were implemented using Microsoft Excel. Heat map analysis and clustering analysis were performed using R (R Core Team, 2014) and PRIMER 6. The high-throughput 16S rRNA gene sequences were deposited into the GenBank Sequence Read Archive under accession number SRP110613.

3. Results and discussion

3.1. Physicochemical characteristics

The average water column temperature, conductivity, dissolved oxygen (DO) and pH were 16.5 ± 0.04 °C (mean ± standard deviation), 621.0 ± 0.9 μS/cm, 9.5 ± 0.4 mg/L and 8.1 ± 0.02, respectively, underneath the FTW and 16.6 ± 0.1 °C, 622.5 ± 1.6 μS/cm, 9.1 ± 0.3 mg/L and 7.9 ± 0.01, respectively, in the limnetic water. Temperature underneath the FTW was lower than the limnetic water. Conductivity and pH were higher underneath the FTW compared to the limnetic water. Irradiance profiles showed that there was minimal, if any, light penetrating the center of the FTW. Light

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