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Short communication

Influence of plantation on microbial community in porous concrete treating polluted surface water

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ABSTRACT

Porous concrete has found increasing applications in slope ecological protection and water purification. Indigenous microorganisms in porous concrete can play important roles in pollutant removal. However, the porous concrete microbial community and its associated influential factors remain essentially unclear. The current study investigated the influences of plantation and plant species on bacterial and archaeal communities in porous concrete unit treating polluted river water. Illumina MiSeq high-throughput sequencing revealed high microbial richness and diversity in both planted and unplanted porous concrete systems. Bacterial community had higher richness and diversity than archaeal community. The evident layer depth-related changes of microbial community richness, diversity and structure were observed in each porous concrete system. Microbial richness, diversity and structure in porous concrete unit were influenced by both plantation and plant species type. Moreover, *Proteobacteria* dominated in bacterial communities in both planted and unplanted porous concrete systems, while unplanted porous concrete system displayed much higher proteobacterial proportion than planted ones. *Thaumarchaeota* microorganisms accounted for a considerable proportion in archaeal communities in porous concrete units.

1. Introduction

Impermeable concrete was usually used to keep river bank from being destroyed by flood, which could bring about many ecological problems (etc., topographic and hydrological changes, vegetation destruction and soil erosion) (Chen et al., 2013). Recently, the application of pervious porous concrete for slope ecological protection has aroused increasing attention due to its functions of water permeability and grass plantation (Oh et al., 2014; Zhang et al., 2015). Vegetation porous concrete (VPC) can also effectively remove both organic and inorganic pollutants in waters (Park and Tia, 2004; Jiang et al., 2012; Song et al., 2012; Oh et al., 2014; Zhang et al., 2015). The indigenous microorganisms attached on aggregate particles in porous concrete are believed to play important roles in pollutant removal (Park and Tia, 2004; Song et al., 2012; Zhang et al., 2013; Nishimura et al., 2015). Hence, identification of microbial community structure can contribute to our knowledge of biological processes in porous concrete system. However, information on porous concrete microbial community structure is still very limited (Zhang et al., 2013). Moreover, although plant species was found to be a key determinant to the purification efficiency of porous concrete system (Jiang et al., 2012), there has been no report available on the influences of plantation and plant species on microbial community in porous concrete system.

High-throughput sequencing is theoretically able to profile the overall microbial diversity in complicated natural and manmade ecosystems. So far, high-throughput sequencing technologies have found increasing applications in characterizing microbial communities in biofilter or constructed wetland (CW) used to purify polluted surface water (Liao et al., 2013a; Ligi et al., 2014; Guan

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et al., 2015; Liao et al., 2015; He et al., 2016). Therefore, the major aim of the current study was to investigate the influences of plantation and plant species on microbial community in porous concrete system using high-throughput sequencing analysis.

2. Materials and methods

2.1. Description of porous concrete units

In this study, three sets of cylinder vertical-flow porous concrete units (diameter 20 cm, height 10 cm) were constructed (Fig. S1). The bottom layer (height 6 cm) of each porous concrete system was composed of natural zeolite (diameter 3–5 mm) and ordinary silicate cement with the water/binder ratio of 0.35:1 and the cement/zeolite ratio of 1:7. The porosity of the hardened concrete was about 25% and its water permeability coefficient was about 2.1 cm/s. Before this study, the concrete specimens had been put into deionized water for two months to lower pH to nearly 9.3. The top of the three porous concrete systems was filled with soil (height 4 cm). Two porous concrete units were planted with *Cynodon dactylon* L. (porous concrete unit A) and *Trifolium repens* (porous concrete unit B), respectively, while another one was unplanted (control) (porous concrete unit C). These three porous concrete units were continuously fed with the water of Dongjiang River at a flow rate of 20 L/h in down-flow mode. All these treatments were prepared in triplicate. Before the beginning of experiments, the three porous concrete units had been under continuous operation for nearly three months. During this period, the average influent water temperature and pH were 28 °C and 7.5, respectively. The average values of ammonia nitrogen (NH_4^+-N), total nitrogen (TN) and chemical oxygen demand (COD) in the influents of each porous concrete system were 5, 10.5 and 60 mg/L, respectively. The average NH_4^+-N and TN removal rates by porous concrete units A, B and C were 70.2% and 53.5%, 65.7% and 47.8%, and 47.6% and 36.5%, respectively, while the average COD removal rates by these three reactors were 67.3%, 42.8% and 29.3%, respectively.

2.2. High-throughput sequencing analysis

Soil or aggregate particles in triplicate were collected from 2 (upper part), 5 (middle part) and 9 cm (lower part) below the surface of each porous concrete system (in the concrete central zone). Before DNA extraction, the aggregate particles were placed into a 250 mL erlenmeyer flask containing 100 mL DNA-free water and were then subjected to ultrasonication (15 min; frequency 30 kHz) and vortexing (5 min). Microbial cell in the supernatant were retained using 0.22 μm pore-size membrane (diameter 50 mm; Millipore). Genomic DNA was extracted using Powersoil DNA extraction kit (Mobio Laboratories) and then amplified using bacterial primer sets 515F (5′-GTGCCAGCMGCCGCGG-3′)/907R (5′–CCGTCAATTCMTTTRAGTTT-3′) and archaeal primer sets Arch519F (5′-CAGCCGCCGCGGTAA-3′)/Arch915R (5′-GTGCTCCCCCGC CAATTCCT-3′) (Wang et al., 2015; He et al., 2016), respectively. The amplicons from triplicate samples were mixed in equal amounts and then were subject to Illumina MiSeq sequencing at Shanghai Majorbio Bio-pharm Technology Co., Ltd (China). The obtained raw Illumina reads were deposited in the NCBI short-read archive under accession numbers SRP072377 (*Bacteria*) and SRP072375 (*Archaea*). The paired-end reads were merged using FLASH (http://ccb.jhu.edu/software/FLASH/) and processed according to the literature (Caporaso et al., 2010). Chimeric reads were screened using UCHIME (Edgar et al., 2011). Chimeric-free sequences were assigned into the same operational taxonomic units (OTUs) with a maximum distance of 3%, and Chao1 richness estimator and Shannon diversity index were further generated using the UPARSE pipeline (Edgar, 2013). The taxonomic identities of the representative sequences from each OTU were assigned using the Silva 16S rRNA database (Quast et al., 2013). Moreover, to discriminate the difference in the overall microbial community composition between each pair of samples, the OTU-based beta diversity was calculated using UniFrac (Lozupone and Knight, 2005) and then unweighted pair group method with arithmetic mean (UPGMA) clustering was performed using unweighted UniFrac with the Quantitative Insights into Microbial Ecology (QIIME) program.

3. Results

3.1. Microbial community richness and diversity

In the present study, samples AU, AM, and AL are referred to the soil or aggregate samples in the upper, middle and lower parts of porous concrete unit A, respectively, while samples BU and CU, BM and CM, and BL and CL denote those in the upper, middle and lower parts of porous concrete units B and C, respectively. The number of bacterial sequences from each soil or aggregate sample was normalized to 21,235 for the comparison of bacterial richness and diversity. High Good's coverage (≥97.3%) indicated that the OTUs of each soil or aggregate bacterial library had been well captured (Table 1). Soil samples comprised 1168–1902 bacterial OTUs, while aggregate samples consisted of 1036–1868 bacterial OTUs. The Chao1 richness estimators of soil and aggregate bacterial communities were 1327–2293 and 1117–2,229, respectively. Moreover, soil and aggregate bacterial communities displayed the Shannon diversity indices of 5–6.52 and 4.92–6.2, respectively. In either of the two planted porous concrete units, the bacterial OTU number, Chao1 richness and Shannon diversity decreased with increasing layer depth. For the three samples from unplanted porous concrete unit, sample CL showed the highest bacterial OTU number, Chao1 richness and Shannon diversity, while sample CM had the lowest ones. At each sampling point, porous concrete unit B (planted with *Trifolium repens*) had more bacterial OTUs and higher Chao1 richness but lower Shannon diversity than porous concrete unit A (planted with *Cynodon dactylon* L.). In addition, compared with planted porous concrete units, unplanted porous concrete unit had much less bacterial OTUs and lower Shannon diversity in the upper and middle parts and much lower bacterial Chao1 richness at each sampling point.

In this study, the number of archaeal sequences from soil or aggregate samples was normalized to 17,624 for the comparison of archaeal richness and diversity. High Good's coverage (≥98.2%) illustrated that most of the archaeal OTUs in each sample had been captured (Table 2). Soil samples included 472–501 archaeal OTUs, while aggregate samples were composed of 230–578 archaeal OTUs. The Chao1 richness estimators and Shannon diversity indices of soil and aggregate archaeal communities were 492–866 and

Table 1
Bacterial community richness and diversity in porous concrete units.

Sample	OTUs	Chao1 estimator	Shannon index	Good's coverage (%)
AU	1894	2207	6.52	97.9
AM	1627	2036	6.2	98.1
AL	1563	2006	5.81	97.8
BU	1902	2258	6.46	97.7
BM	1868	2229	6.12	97.9
BL	1810	2223	5.39	97.3
CU	1168	1327	5	98.8
CM	1036	1117	4.92	99.2
CL	1627	1756	5.53	98.8