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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 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 system displayed much higher proteobacterial proportion than planted ones. *Thaumarchaeota* microorganisms accounted for a considerable proportion in archaeal communities in porous concrete units.

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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 (5'-CAGCCGCCGCGGTAA-3')/Arch915R Arch519F (5'-GTGCTCCCCGC 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 (\geq 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 (\geq 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 un	its.

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

 Table 2

 Archaeal community richness and diversity in porous concrete units.

Sample	OTUs	Chao1 estimator	Shannon index	Good's coverage (%)
AU	501	866	3.83	98.8
AM	578	676	3.58	99.2
AL	455	622	3.3	99.1
BU	493	763	3.36	98.9
BM	507	657	3.77	99
BL	373	520	3.58	98.2
CU	472	492	4.74	99.8
CM	230	248	4.77	99.9
CL	488	505	5.14	99.7

3.36-4.74, and 248-676 and 3.3-5.14, respectively. In both planted porous concrete units, the aggregate sample in the middle part had the most abundant archaeal OTUs, while in unplanted porous concrete unit, the least archaeal OTUs was observed in the middle part. In either of the two planted porous concrete units, the archaeal Chao1 richness decreased with increasing layer depth. For the three samples from unplanted porous concrete unit, sample CL had the highest archaeal Chao1 richness, but sample CM displayed the lowest one. Archaeal diversity decreased with increasing layer depth in Cynodon dactylon L. VPC unit, while an opposite trend was found in unplanted porous concrete unit. In Trifolium repens VPC unit, the middle part sample had the highest archaeal diversity followed by the lower part and then upper part samples. Moreover, at each sampling point, Cynodon dactylon L. VPC unit had the highest archaeal community richness followed by Trifolium repens VPC unit and then unplanted porous concrete unit. In contrast, at each sampling point, unplanted porous concrete unit showed the highest archaeal diversity. In both middle and lower parts, Trifolium repens VPC unit illustrated higher archaeal diversity than Cynodon dactylon L. VPC unit, but an opposite was observed in upper part.

3.2. UPGMA clustering analysis of microbial communities

The result of UPGMA clustering of bacterial communities illustrated that the samples from unplanted porous concrete unit were clearly separated from those from planted porous concrete units (Fig. 1), illustrating the distinct structure difference of bacterial communities between unplanted and planted porous concrete units. Sample AL was distantly separated from samples AU and AM,



Fig. 1. UPGMA clustering of bacterial communities in porous concrete units.

and sample BM was not clustered with samples BU and BL. These results indicated the remarkable change of bacterial community structure in either of the two VPC units. In addition, in either the upper or lower part, the sample from *Cynodon dactylon* L. VPC unit was distantly separated from that from *Trifolium repens* VPC unit.

The result of UPGMA clustering of archaeal communities displayed that the samples from unplanted porous concrete unit were not grouped with from those from planted porous concrete units (Fig. 2), showing that unplanted and planted porous concrete units had distinct archaeal community structures. The separation of sample CM from samples CU and CL illustrated the remarkable shift in archaeal community structure in unplanted porous concrete unit. Moreover, samples BU, BM, BL and AU were clustered but they were separated from samples AM and AL. Samples AM and AL were also separated. These results indicated that the variation of archaeal community structure in *Trifolium repens* VPC unit was greater than that in *Cynodon dactylon* L. VPC unit.

3.3. Microbial community composition

In the present study, a total of 9 major bacterial phyla (with relative abundance >2% in at least one sample) were identified, including Proteobacteria, Actinobacteria, Cyanobacteria, Acidobacteria, Chloroflexi, Bacteroidetes, Planctomycetes, Firmicutes and Gemmatimonadetes (Fig. 3). Proteobacteria (accounting for 32–73%) was the most abundant bacterial phylum group in each soil or aggregate sample. A considerable layer depth-related change of proteobacterial proportion occurred in each porous concrete unit. Unplanted porous concrete unit showed much higher proteobacterial proportion than planted ones. At each sampling point, the proteobacterial proportion evidently differed in two VPC units. Moreover, the proportions of Cyanobacteria, Acidobacteria, Chloroflexi, Bacteroidetes and Planctomycetes in VPC units tended to be higher than those in unplanted porous concrete unit. In addition, Trifolium repens VPC unit had much higher Cyanobacteria proportion but lower Acidobacteria proportion than Cynodon dactylon L. VPC unit.

In the present study, most of the retrieved archaeal sequences from soil or aggregate samples (accounting for 71–93%) could not be affiliated with any known archaeal phylum (Fig. 4). A total of 4 major archaeal phyla (with relative abundance $\geq 2\%$ in at least one sample) were identified, including *Thaumarchaeota*, *Euryarchaeota*,







Fig. 3. Comparison of the quantitative contribution of the sequences affiliated with different bacterial phyla to the total number of bacterial sequences from porous concrete units. Others include unclassified *Bacteria* and the bacterial phyla with the largest relative abundance less than 2% in each sample.



Fig. 4. Comparison of the quantitative contribution of the sequences affiliated with different archaeal phyla to the total number of archaeal sequences from porous concrete units.

Bathyarchaeota and Woesearchaeota. Thaumarchaeota was the largest archaeal phylum group (accounting for 5–27%). The *Thaumarchaeota* proportion illustrated a remarkable layer depth-related change in two VPC units but a slight one in unplanted porous concrete unit. In addition, unplanted porous concrete unit tended to have much lower *Thaumarchaeota* proportion than planted ones. *Euryarchaeota* organisms showed relatively high proportion in samples BL (12%) and CL (7%) but became less abundant in other samples (0-4%).

4. Discussion

4.1. Microbial community richness and diversity in porous concrete systems

So far, information on microbial community richness and diversity in porous concrete systems is still very limited. Only a recent study using denaturing gradient gel electrophoresis (DGGE) analysis showed that bacterial Chao1 richness estimator and Shannon diversity index in VPC system in river waterfront zone were 36–44 and 3.47–3.7, respectively (Zhang et al., 2013). In this study, Illumina MiSeq high-throughput sequencing was applied to reveal the richness and diversity of bacterial and archaeal communities in both planted and unplanted porous concrete units treating polluted river water. The bacterial Chao1 richness estimators and Shannon

diversity indices in porous concrete systems were 1117–2293 and 4.92–6.52, respectively, comparable to those observed in verticalflow CW (VF-CW) systems treating polluted surface water using Illumina MiSeq sequencing analysis (Guan et al., 2015; He et al., 2016). This suggested the presence of high bacterial community richness in porous concrete systems treating surface water. Compared to *Bacteria, Archaea* illustrated a relatively lower community richness in porous concrete systems. He et al. (2016) and Long et al. (2016a,b) also reported that VF-CW bacterial community had higher richness than archaeal community.

Several previous studies have reported the layer depth-related change of bacterial and archaeal community richness in surface water VF-CW (Guan et al., 2015; He et al., 2016; Long et al., 2016a). Zhang et al. (2013) also indicated the layer depth-related change of bacterial richness in VPC system in river waterfront zone. However, the influence of plantation on microbial community richness in surface water treatment bioreactors has not been addressed. In this study, the layer depth-related change of both bacterial and archaeal richness was also observed in porous concrete units. In *Cynodon dactylon L.* and *Trifolium repens* VPC units, both bacterial and archaeal community richness was found to continuously decrease with increasing layer depth, however, in unplanted porous concrete system, bacterial and archaeal community richness showed a considerable decrease followed by a considerable increase. Moreover, unplanted porous concrete unit had much lower microbial

richness than two VPC units. These results suggested the profound influence of plantation on microbial richness in porous concrete unit. In addition, *Trifolium repens* VPC unit tended to have higher bacterial but lower archaeal richness than *Cynodon dactylon* L. VPC unit. This suggested that microbial richness in porous concrete unit was also influenced by plant species type.

The layer depth-related changes of bacterial diversity in surface water VF-CW or biofilter have been well-documented (Liao et al., 2012; Feng et al., 2013; Guan et al., 2015; He et al., 2016; Long et al., 2016a). Zhang et al. (2013) reported the layer depth-related varition of bacterial diversity in VPC system in river waterfront zone. To date, little is known about the variation of archaeal diversity in surface water treatment bioreactor (He et al., 2016; Long et al., 2016a). Moreover, the influence of plantation on microbial diversity in surface water treatment bioreactor remains unknown. In the present study, the layer depth-related change patterns of bacterial and archaeal community diversity differed greatly in porous concrete units. With increasing layer depth, bacterial diversity showed a decrease followed by an increase in unplanted porous concrete unit, but a continuous decline in both VPC units. Archaeal diversity continuously increased in unplanted porous concrete system, while displayed an increase followed by a decrease in Trifolium repens VPC unit but a continuous decrease in Cynodon dactylon L. VPC unit. In addition, unplanted porous concrete unit tended to have higher archaeal but lower bacterial community diversity than VPC units. Trifolium repens VPC unit had lower bacterial diversity than Cynodon dactylon L. VPC unit. These results suggested that microbial diversity in porous concrete unit was influenced by both plantation and plant species type.

4.2. Microbial community structure in porous concrete systems

The remarkable layer depth-related changes of bacterial community structure have been found in surface water VF-CW (Guan et al., 2015; He et al., 2016; Long et al., 2016a) and biofilter (Feng et al., 2013; Liao et al., 2012, 2013b). In this study, the results of UPGMA clustering and phylogenetic analysis also illustrated the considerable layer depth-related change of bacterial community structure in all of the three studied porous concrete systems. To date, there has been no report available on the influence of plantation on microbial community structure in surface water treatment bioreactor. In this study, the result of UPGMA clustering indicated that both plantation and plant species type had profound impacts on bacterial community structure in porous concrete system. Proteobacteria usually dominated in bacterial community in surface water treatment bioreactors and is believed to play important roles in the biotransformation of organic pollutants (Liao et al., 2012, 2013b; Feng et al., 2013; Guan et al., 2015; He et al., 2016; Zhi et al., 2015), yet its influential factors remain elusive. In this study, Proteobacteria was the largest bacterial phylum group in both unplanted and planted porous concrete systems, which might contribute to the COD removal in these bioreactors. Proteobacterial organisms displayed a much higher proportion in unplanted porous concrete system than in two VPC units. This suggested the profound impact of plantation on proteobacterial proportion in porous concrete unit. In addition, the layer depth-related change pattern of proteobacterial proportion differed in two VPC units, which suggested that proteobacterial proportion in porous concrete unit was further influenced by plant species type.

Information on archaeal community structure in surface water treatment bioreactor is still very limited. Two recent studies revealed the layer depth-related change of archaeal community structure in surface water CW system (He et al., 2016; Long et al., 2016a). In this study, the results of UPGMA clustering and phylogenetic analysis illustrated the evident layer depth-related change of archaeal community structure in both unplanted and planted porous concrete systems. So far, the influential factor regulating archaeal community structure in surface water treatment bioreactor remains unclear. In this study, the results of UPGMA clustering illustrated the influences of both plantation and plant species type on archaeal community structure in porous concrete unit. Thaumarchaeota and Eurvarchaeota were found to dominate in archaeal community in surface water CW system (He et al., 2016). However, in this study, unclassified Archaea predominated in each porous concrete system. Microorganisms with phylum Thaumarchaeota might play important roles in ammonia oxidation in various CW systems (Bouali et al., 2012, 2013; He et al., 2016). In this study, Thaumarchaeota organisms comprised a considerable proportion in archaeal communities in porous concrete units, which might contribute to ammonia removal in these systems. In addition, the *Thaumarchaeota* proportion illustrated a remarkable layer depth-related change in two VPC units but a slight one in unplanted porous concrete unit. VPC units had much higher Thaumarchaeota proportion than unplanted porous concrete unit. To authors' knowledge, the present study provided the first evidence for the influence of plantation on Thaumarchaeota proportion in surface water treatment bioreactor.

5. Conclusions

The microbial richness, diversity and structure in porous concrete system treating river water were influenced by both plantation and plant species type. *Bacteria* had higher community richness and diversity than *Archaea*. *Proteobacteria* was the dominant bacterial group, while *Thaumarchaeota* consisted of a considerable proportion in archaeal community.

Ethical statement

No conflict of interest exits in this manuscript. The work has not been published previously, and not under consideration for publication elsewhere.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ibiod.2016.11.017.

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