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Substrate influences on archaeal and bacterial assemblages in constructed wetland microcosms

Yan Long[a], Zhengke Zhang[b], Xiaokang Pan[b], Bingxin Li[c], Shuguang Xie[c,*], Qingwei Guo[b,*]

[a] Key Laboratory of Water/Soil Toxic Pollutants Control and Bioremediation of Guangdong Higher Education Institutes, School of Environment, Jinan University, Guangzhou 510632, China
[b] South China Institute of Environmental Sciences (SCIES), Ministry of Environment Protection (MEP), Guangzhou 510655, China
[c] State Key Joint Laboratory of Environmental Simulation and Pollution Control, College of Environmental Sciences and Engineering, Peking University, Beijing 100871, China

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ABSTRACT

Microorganisms in surface water constructed wetland (CW) play crucial roles in pollutant removal. However, little is known about the diversity and structure of microbial community in surface water CW. The influential factors regulating microbial community diversity and structure remain poorly known. In the present study, Illumina high-throughput sequencing was used to characterize bacterial and archaeal communities in three lab-scale vertical-flow CW (VF-CW) systems with different substrate materials (fine gravel, steel slag or natural zeolite). The depth-related change of archaeal and bacterial community richness, diversity and structure occurred in these three VF-CW systems. Microbial diversity in three VF-CW systems showed the similar depth-related change pattern, but microbial richness illustrated the different change pattern. Substrate type had a profound effect on bacterial richness but only a slight effect on bacterial diversity. Archaeal richness and diversity were affected by substrate type and wetland depth. Moreover, archaeal community had much lower richness and diversity than bacterial community. Archaeal and bacterial community structure was regulated by substrate type and wetland depth. *Proteobacteria* and *Acidobacteria* were the most abundant bacterial phyla, while *Euryarchaeota* was the predominant archaeal phylum.

1. Introduction

Constructed wetland (CW) has become a favorite option for the purification of polluted surface water, due to its merits of cost-effectiveness and easy maintenance (Ge et al., 2015; Shao et al., 2014; Tu et al., 2014; Zheng et al., 2014). Microorganisms attached on the surfaces of substrate particles in CW system are responsible for biodegradation of organic matter and biogeochemical cycling of nutrients (Chang et al., 2015; Guan et al., 2015). Understanding microbial community structure can aid in our knowledge of the function of CW system and further contribute to the proper wetland design and maintenance (Adrados et al., 2014; Bouali et al., 2013, 2014). Although bacterial community diversity and structure in CW system treating domestic or industrial wastewaters has been well-documented (Bouali et al., 2014; Chang et al., 2015; Guo et al., 2015; Huang et al., 2013; Zhong et al., 2015), only several previous studies have investigated bacterial community in surface water CW (Guan et al., 2015; He et al., 2016; Ligi et al., 2014; Tu et al., 2014; Zhi et al., 2015). So far, the influential factors regulating the CW bacterial community remain unclear. Moreover, although substrate type is known to be a key factor influencing the performance of CW system on the pollutant removal (Ghannad et al., 2015; Huang et al., 2013; Li et al., 2008), the substrate effect on bacterial community in CW system remains under debate (Guan et al., 2015; Huang et al., 2013; Silyn-Roberts and Lewis, 2004).

Archaea can participate in various biogeochemical processes including methanogenesis, ammonia oxidation and sulfate reduction (Zhang et al., 2015), and it might participate in nitrogen removal in CW system treating domestic wastewater (Bouali et al., 2012, 2013). The presence of archaeal community in CW system has not been well studied (Adrados et al., 2014; Bouali et al., 2012, 2013). Only He et al. (2016) investigated archaeal community in surface water CW system. They also suggested its potential roles in methanogenesis and ammonia oxidation. However, information

* Corresponding authors.
E-mail addresses: xiesg@pku.edu.cn (S. Xie), guoqingwei@scies.org (Q. Guo).

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on the influential factors regulating CW archaeal community is still lacking.

High-throughput sequencing technologies are able to resolve the structure of complicated bacterial assemblage attached on substrate particles in water and wastewater treatment bioreactors (Biswas et al., 2014; Chen et al., 2015; Chu et al., 2014; Liao et al., 2013, 2015). They have also found increasing applications to characterize bacterial populations attached on substrate particles in CW system (Ansola et al., 2014; Guan et al., 2015; He et al., 2016; Ligi et al., 2014; Zhong et al., 2015). In contrast, high-throughput sequencing of archaeal community attached on substrate particles has not been addressed (Cortes-Lorenzo et al., 2014). Only a recent study applied Illumina sequencing to investigate archaeal community diversity and structure in a pilot-scale CW system (He et al., 2016). Therefore, the main aim of this study was to investigate the substrate effect on the diversity and structure of archaeal and bacterial communities in surface water CW system using Illumina high-throughput sequencing.

2. Materials and methods

2.1. Wetland description

Three cylinder vertical-flow CW (VF-CW) microcosms (diameter 20 cm, height 20 cm) were used to treat the polluted water of Dongjiang River (Guangdong Province). Wetlands WG, WS, and WZ were filled with fine gravels (3–5 mm), steel slags (4–6 mm), natural zeolites (3–5 mm), respectively. *Paspalum natatu*. (about 100 plants) was planted on the top of each wetland system. The hydraulic retention time of river water in each CW system was maintained for 7 days. Before this study, all of the three CW systems had been under continuous operation for nearly two months. During this period, the average values of ammonia nitrogen (NH_4^+-N), total phosphorus (TP) and total organic carbon (TOC) in the influents of each wetland system were 2.5, 0.5 and 30 mg/L, respectively. The average water temperature and pH were 25 °C and 7.8, respectively. The average ammonia removal rates by wetlands WG, WS, and WZ were 67%, 71%, and 88%, respectively, while the average TOC and TP removal rates by these three CW systems were 40% and 69%, 39% and 83%, and 35% and 61%, respectively.

2.2. Molecular analyses

In the present study, substrate particle samples in triplicate were collected from 3 cm (upper part), 10 cm (middle part), and 20 cm (lower part) below the wetland surface. Genomic DNA was extracted using Powersoil DNA extraction kit (Mobio Laboratories). PCR amplicon libraries for Illumina MiSeq high-throughput sequencing were constructed using archaeal and bacterial primers Arch519F (5′-CAGCCGCCGCGGTAA-3′)/Arch915R (5′-GTGCTCCCCCGC CAATTCCT-3′) and 515F (5′-GTGCCAGCMGCCGCGG-3′)/R907 (5′-CCGTCAATTCMTTTRAGTTT-3′) (Herfort et al., 2009; Wang et al., 2015).

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 raw Illumina reads have been deposited in the NCBI short-read archive as accession numbers SRP067426 (*Archaea*) and SRP067427 (*Bacteria*). The original DNA fragments were merged using FLASH and the quality filtering of archaeal and bacterial sequences was processed following the protocol (Caporaso et al., 2010). Chimera was discarded using UCHIME (Edgar et al., 2011), the resulting high-quality sequences were clustered into operational taxonomic units (OTUs) at a dissimilarity of 0.03 threshold using UPARSE pipeline (Edgar, 2013). Chao1 richness estimator and Shannon index were further calculated using UPARSE pipeline (Edgar, 2013). Taxonomic assignment of the representative sequence from each OTU was performed with the RDP classifier (Wang et al., 2007). To compare microbial communities among samples, unweighted unifrac using the software Quantitative Insights into Microbial Ecology (QIIME) was applied for unweighted pair group method with arithmetic mean (UPGMA) clustering.

Table 1
Bacterial richness and diversity of wetland samples. Samples GU, GM and GL represent the particle samples from the upper, middle, and lower parts of wetland WG. Samples SU, SM and SL represent the particle samples from the upper, middle, and lower parts of wetland WS. Samples ZU, ZM and ZL represent the particle samples from the upper, middle, and lower parts of wetland WZ.

Sample	OTUs	Chao1 estimator	Shannon index	Good's coverage (%)
GU	1262	1498	5.89	97.9
GM	1247	1552	5.85	97.7
GL	1436	1712	6.24	97.7
SU	1417	1674	6.2	97.8
SM	1232	1502	5.76	97.8
SL	1340	1642	6	97.8
ZU	1364	1626	6.12	97.7
ZM	1345	1650	6.04	97.6
ZL	1354	1659	6.13	97.7

3. Results

3.1. Microbial community richness and diversity

In this study, the valid bacterial reads retrieved from the particle samples in the upper, middle, and lower parts of wetlands WG (Samples GU, GM and GL), WS (Samples SU, SM and SL), and WZ (Samples ZU, ZM and ZL) ranged between 21,995 and 37,526, normalized to the minimum number for the comparison of bacterial community richness and diversity among samples. Good's coverage estimator ≥97.6% suggested that most of bacterial OTUs in each wetland sample has been captured (Table 1). Each bacterial library consisted of 1232–1436 OTUs. The bacterial Chao1 richness estimator illustrated a remarkable variation in the nine studied wetland samples, ranging from 1498 to 1712. The three studied CW systems showed much different depth-related change pattern of bacterial Chao1 richness. In wetland WG, bacterial Chao1 richness considerably increased with the increase of wetland depth, while only a slight increase occurred in wetland WZ. However, in wetland WS, bacterial Chao1 richness showed a remarkable decrease followed by a considerable increase. For the three CW systems, at a given wetland depth, their bacterial community richness differed greatly. Moreover, a slight change of bacterial Shannon diversity index was found in the nine studied wetland samples, ranging from 5.76 to 6.24. The three CW systems showed similar depth-related change pattern of bacterial Shannon diversity. Bacterial Shannon diversity decreased but then increased. Bacterial Shannon diversity differed slightly at a given wetland depth in the three CW systems.

The obtained high-quality archaeal sequences from the nine wetland samples ranged between 23,736 and 35,223, normalized to the minimum number in order to compare archaeal community richness and diversity among samples. Good's coverage estimator ≥99.8% suggested that archaeal OTUs in each wetland sample has been well captured (Table 2). Each archaeal library was composed of 103–209 OTUs. The samples from wetland lower part had much more archaeal OTUs than those from upper and middle parts. There was a remarkable variation of archaeal Chao1 richness (112–237) in the nine studied wetland samples. The samples from wetland lower part also showed much higher archaeal Chao1 richness than those from upper and middle parts. However, the three studied CW systems showed different depth-related change pat-