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Substrate influences on archaeal and bacterial assemblages in constructed wetland microcosms

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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

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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-

Table 2
Archaeal 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	105	112	2.89	99.9
GM	112	137	2.37	99.9
GL	202	237	3.39	99.8
SU	147	180	3.68	99.8
SM	103	124	2.04	99.9
SL	209	230	3.64	99.8
ZU	121	153	3.25	99.9
ZM	116	149	2.9	99.8
ZL	175	206	3.53	99.8

tern of archaeal Chao1 richness. In wetland WG, archaeal Chao1 richness considerably increased with increasing wetland depth. In wetland WS, archaeal Chao1 richness illustrated a remarkable decrease followed by a considerable increase. However, in wetland ZS, archaeal Chao1 richness demonstrated a slight decrease followed by a remarkable increase. For the studied three CW systems, their archaeal Chao1 richness differed greatly in upper part, but slightly in both middle and lower parts. Moreover, a remarkable change of archaeal Shannon diversity index (2.04–3.64) was observed in the nine studied wetland samples. The three studied CW systems showed similar depth-related change pattern of archaeal Shannon diversity. Archaeal community diversity considerably decreased but then increased. For the three CW systems, their archaeal diversity differed greatly in upper and middle parts, but slightly in lower part. These results suggested that the substrate effect on archaeal community diversity was also dependent on wetland depth. In addition, wetland archaeal communities were found to have much lower Chao1 richness and Shannon diversity than bacterial communities.

3.2. UPGMA clustering analysis of microbial communities

The result of UPGMA clustering illustrated that bacterial communities in the nine studied wetland samples could be divided into three distinct groups (Fig. 1). Sample SU was distantly separated from other wetland samples. Samples GM, GU, SM and ZM formed a group, while Samples ZU, ZL, GL and SL were clustered together. In each CW system, the samples from three different parts were not grouped together, suggesting a considerable depth-related change of bacterial community structure. Moreover, for the three studied CW systems, their upper part samples were distantly separated, while the samples from either middle or lower parts were grouped together. This indicated that the substrate effect on bacterial community structure were also dependent on wetland depth.

The result of UPGMA clustering indicated that archaeal communities could be divided into two distinct clades (Fig. 2). Samples SM, GM, ZM and GU were clustered, while other samples formed another group. In each CW system, the samples from three different parts were not grouped together, indicating a considerable depth-related variation of archaeal community structure. In addition, for the three studied CW systems, their upper part samples were separated, while the samples from either middle or lower parts were clustered. This indicated that the substrate effect on archaeal community structure were also affected by wetland depth.

3.3. Microbial community composition

In the present study, a total of 12 bacterial phyla and one candidate division were usually identified among the nine studied wetland samples, including *Proteobacteria*, *Acidobacte-*

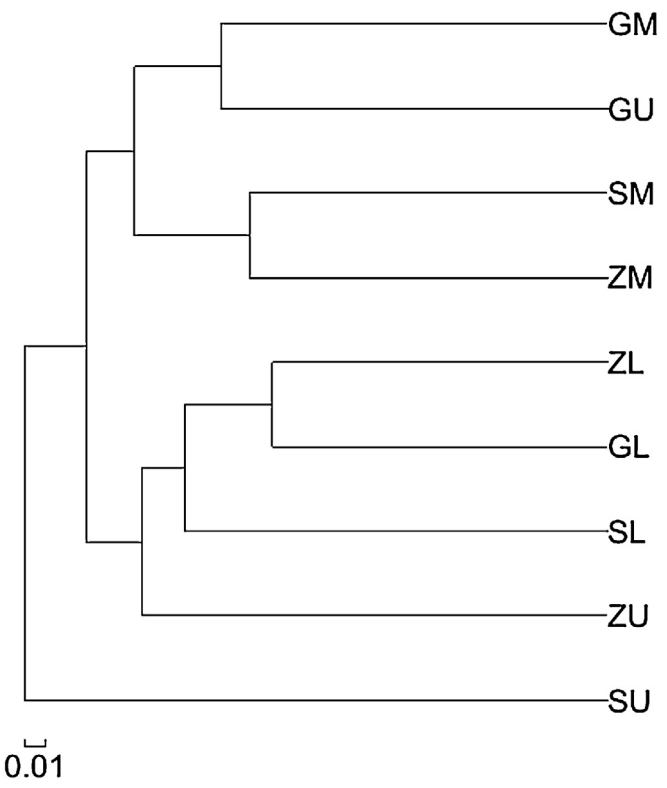

Fig. 1. UPGMA clustering of CW bacterial communities. Samples GU, GM and GL represent the particle samples from the upper, middle, and lower parts of wetland WG. Samples SU, SM and SL represent the particle samples from the upper, middle, and lower parts of wetland WS. Samples ZU, ZM and ZL represent the particle samples from the upper, middle, and lower parts of wetland WZ.

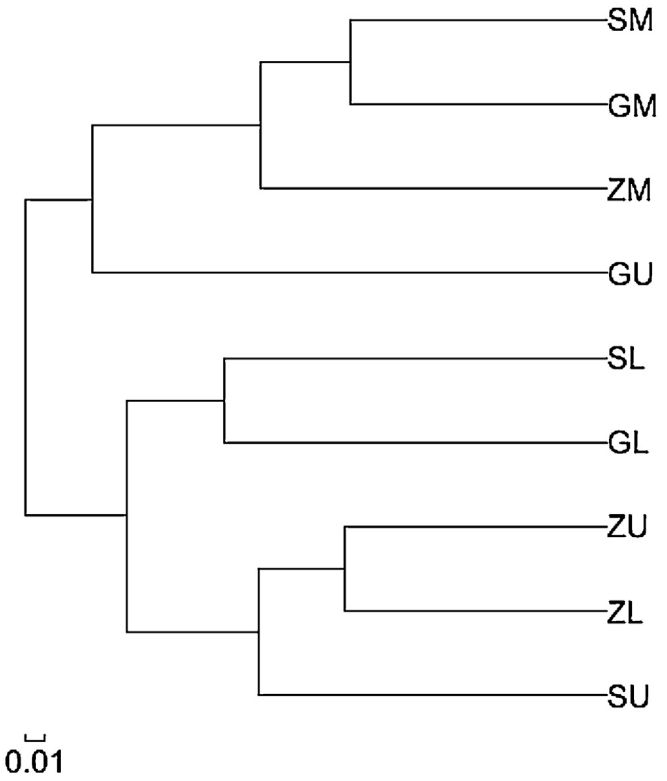

Fig. 2. UPGMA clustering of CW archaeal communities. Samples GU, GM and GL represent the particle samples from the upper, middle, and lower parts of wetland WG. Samples SU, SM and SL represent the particle samples from the upper, middle, and lower parts of wetland WS. Samples ZU, ZM and ZL represent the particle samples from the upper, middle, and lower parts of wetland WZ.

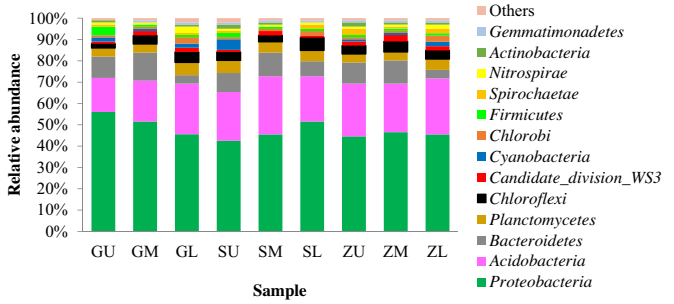

Fig. 3. Comparison of the quantitative contribution of the sequences affiliated with different bacterial phyla to the total number of bacterial sequences from wetland samples. Others include unclassified *Bacteria* and the bacterial phyla with the largest relative abundance less than 1% in each sample. 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.

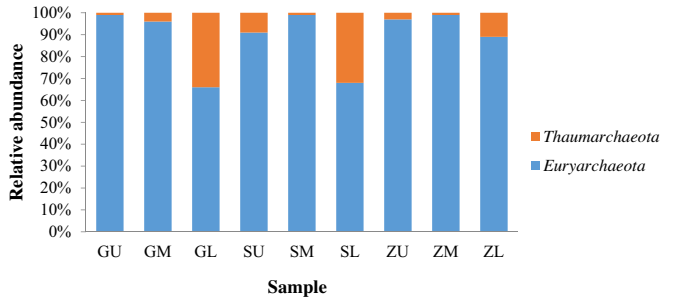

Fig. 4. Comparison of the quantitative contribution of the sequences affiliated with different archaeal phyla to the total number of archaeal sequences from wetland samples. Samples GU, GM and GL represent the particle samples from the upper, middle, and lower parts of wetland WG. Samples SU, SM and 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.

ria, *Bacteroidetes*, *Planctomycetes*, *Chloroflexi*, *WS3*, *Cyanobacteria*, *Cyanobacteria*, *Chlorobi*, *Firmicutes*, *Spirochaetae*, *Nitrospirae*, *Actinobacteria* and *Gemmatimonadetes* (Fig. 3). *Proteobacteria* (accounting for 43–56%) was the most abundant bacterial phylum group in each wetland sample. The three studied CW systems showed different depth-related change pattern of proteobacterial proportion. In wetland WG, the proportion of *Proteobacteria* decreased with increasing wetland depth. In wetland WS, the relative abundance of *Proteobacteria* increased with increasing wetland depth. Only a very slight depth-related change of proteobacterial proportion occurred in wetland WZ. *Acidobacteria* was the second largest bacterial phylum group in each wetland sample (16–27%). The three studied CW systems also showed different depth-related change pattern of *Acidobacteria* proportion. In wetland WG, the proportion of *Acidobacteria* increased with increasing wetland depth. In wetland WS, *Acidobacteria* proportion slightly increased but then decreased. However, in wetland WZ, *Acidobacteria* proportion showed a slight decrease followed by a slight increase. Moreover, for the three studied CW systems, at a given wetland depth, their *Proteobacteria* and *Acidobacteria* proportion showed an evident difference.

CW archaeal communities were composed of *Euryarchaeota* (66–99%) and *Thaumarchaeota* (1–34%) (Fig. 4). The three studied CW systems showed different depth-related change pattern of *Euryarchaeota* proportion. In wetland WG, the proportion of archaeal phylum *Euryarchaeota* decreased with increasing wetland depth. However, in either wetland WS or wetland WZ, *Euryarchaeota* proportion showed a slight increase followed by a considerable decrease. For the three CW systems, their *Euryarchaeota* proportion differed considerably in lower part, but slightly in upper and middle parts.

4. Discussion

4.1. Microbial community richness and diversity in surface water constructed wetland

Limited information exists on bacterial community richness and diversity in surface water CW system. In a large-scale horizontal subsurface flow CW (HSF-CW) system used for the purification of polluted river water, the inlet zone was found to have higher sediment bacterial richness and diversity than the outlet zone (Zhi et al., 2015). Tu et al. (2014) also indicated that the influent of a full-scale surface water HSF-CW had higher bacterial richness and diversity than the effluent. In contrast, the diversity of bacterial community attached on ceramic particles was found to increase with increasing wetland layer depth in a pilot-scale surface water VF-CW (He et al., 2016). So far, the substrate effect on CW bacterial richness and diversity remains unclear. Guan et al. (2015) reported a different s depth-related change pattern of bacterial diversity in three pilot-scale surface water VF-CW systems. In gravel VF-CW, a continuous increase of bacterial diversity was found with increasing wetland layer depth. Bacterial diversity illustrated a considerable increase followed by a slight decrease in sand VF-CW, but a considerable decrease followed by a remarkable increase in zeolite VF-CW (Guan et al., 2015). However, in the present study, the three lab-scale VF-CW systems showed similar depth-related change pattern of bacterial diversity. Bacterial diversity showed a decrease followed by an increase. Moreover, the present study suggested a slight substrate effect on bacterial diversity in VF-CW system, in agreement with the result found in VF-CW system treating piggery wastewater (Huang et al., 2013). In addition, the three studied lab-scale VF-CW systems showed much different depth-related change pattern of bacterial richness. A considerable substrate influence on bacterial richness was observed. These results were different from those found in bacterial diversity.

There is a paucity of knowledge on archaeal community richness and diversity in surface water CW system. Only He et al. (2016) reported a remarkable spatiotemporal change of archaeal richness and diversity in a pilot-scale surface water VF-CW. However, the change pattern of archaeal richness and diversity in surface water CW remains not understood. In this study, the three VF-CW systems showed similar depth-related change pattern of archaeal diversity. Archaeal diversity was subject to a decrease but then an increase. However, the three VF-CW systems showed different depth-related change pattern of archaeal richness. These results were consistent with those found for bacterial community. Moreover, the present study also provided the evidence for the first time that the substrate effects on CW archaeal richness and diversity were affected by wetland depth. In addition, the richness and diversity of archaeal community were found to be much lower than those of bacterial community. This was in agreement with the result found in a pilot-scale surface water VF-CW (He et al., 2016). Other previous studies also reported lower archaeal than bacterial diversity in natural wetland (Dorador et al., 2013) and CW system treating municipal wastewater (Bouali et al., 2013).

4.2. Microbial community structure in surface water constructed wetland

The remarkable spatial change of bacterial community structure have been found in waters and sediments of full-scale HSF-CW systems treating river water (Tu et al., 2014; Zhi et al., 2015). He

et al. (2016) also reported the remarkable depth-related change of bacterial community structure in pilot-scale surface water ceramic VF-CW. Moreover, Guan et al. (2015) found that bacterial community structure showed a considerable depth-related variation in both zeolite and sand VF-CW systems, but experienced a slight change in gravel VF-CW system. In the present study, the result of UPGMA clustering showed a considerable depth-related change of bacterial community structure in each VF-CW system. To date, the influential factors regulating bacterial community structure in surface water CW system remain unclear. Guan et al. (2015) suggested that VF-CW bacterial community structure could be significantly influenced by both substrate type and wetland depth. This was further sustained by the result obtained in the current study.

Proteobacteria might play important roles in biodegradation of organic compounds in CW system (Guan et al., 2015; He et al., 2016). Several previous studies have showed the dominance of proteobacterial organisms in surface water CW system (Guan et al., 2015; He et al., 2016; Zhi et al., 2015). In this study, *Proteobacteria* was found to be the largest bacterial phylum group in each VF-CW system, but its proportion showed different depth-related change pattern. Guan et al. (2015) also indicated that either sand or zeolite CW systems showed more evident depth-related shift in proteobacterial proportion than gravel CW system. Substrate type might have a strong effect on proteobacterial community structure in surface water CW system (Guan et al., 2015). The result obtained in this study further confirmed the influence of substrate type on CW proteobacterial community structure. Several previous studies showed the relative abundance of *Acidobacteria* in surface water CW system (Guan et al., 2015; He et al., 2016; Zhi et al., 2015), yet its influential factors remain elusive. In this study, different depth-related change patterns of *Acidobacteria* proportion were found in three surface water VF-CW systems. In addition, the present study provided the evidence for the first time that *Acidobacteria* proportion in surface water CW system could be influenced by substrate type.

So far, only one study reported the depth-related change of archaeal community structure in surface water CW system (He et al., 2016). Information on the influential factors driving archaeal community structure is still lacking. In this study, the result of UPGMA clustering showed a considerable depth-related shift in archaeal community structure in each VF-CW system. Archaeal community structure was found to be influenced by both substrate type and wetland depth. This was consistent with the result observed for bacterial community.

The composition of archaeal community in CW system remains poorly understood. *Thaumarchaeota* was found to be the predominant archaeal phylum in CW system treating domestic wastewater (Bouali et al., 2012, 2013), while Liu et al. (2015) showed the dominance of *Euryarchaeota* in CW system treating the mixture of domestic wastewater and reservoir water. He et al. (2016) reported that both *Thaumarchaeota* and *Euryarchaeota* were the major archaeal phylum groups in surface water CW system. However, in this study, *Euryarchaeota* was found to dominate the archaeal community in each surface water VF-CW system. Moreover, different depth-related change pattern of *Euryarchaeota* proportion was found in these VF-CW systems. *Euryarchaeota* proportion was found to be influenced by both substrate type and wetland depth.

5. Conclusions

Illumina high-throughput sequencing indicated the depth-related change of microbial community richness, diversity, structure in vertical-flow gravel, steel slag or zeolite VF-CW systems. Microbial community was considerably influenced by substrate type. Compared with bacterial community, archaeal community had relatively low richness and diversity. *Proteobacteria* and *Acidobacteria* were the largest two bacterial phyla, while *Euryarchaeota* predominated in archaeal community. Their relative abundance was also affected by substrate type.

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References

Adrados, B., Sanchez, O., Arias, C.A., Becares, E., Garrido, L., Mas, J., Brix, H., Morato, J., 2014. Microbial communities from different types of natural wastewater treatment systems: vertical and horizontal flow constructed wetlands and biofilters. Water Res. 55, 304–312.

Ansola, G., Arroyo, P., de Miera, L.E.S., 2014. Characterisation of the soil bacterial community structure and composition of natural and constructed wetlands. Sci. Total Environ. 473, 63–71.

Biswas, K., Taylor, M.W., Turner, S.J., 2014. Successional development of biofilms in moving bed biofilm reactor (MBBR) systems treating municipal wastewater. Appl. Microbiol. Biotechnol. 98, 1429–1440.

Bouali, M., Zrafi-Nouira, I., Bakhrouf, A., Le Paslier, D., Chaussonnerie, S., Ammar, E., Sghir, A., 2012. The structure and spatio-temporal distribution of the *Archaea* in a horizontal subsurface flow constructed wetland. Sci. Total Environ. 435, 465–47.

Bouali, M., Pelletier, E., Chaussonnerie, S., Le Paslier, D., Bakhrouf, A., Sghir, A., 2013. Characterization of rhizosphere prokaryotic diversity in a horizontal subsurface flow constructed wetland using a PCR cloning-sequencing based approach. Appl. Microbiol. Biotechnol. 97, 4221–4231.

Bouali, M., Zrafi, I., Bakhrouf, A., Chaussonnerie, S., Sghir, A., 2014. Bacterial structure and spatiotemporal distribution in a horizontal subsurface flow constructed wetland. Appl. Microbiol. Biotechnol. 98, 3191–3203.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336.

Chang, J.J., Wu, S.Q., Liang, K., Wu, Z.B., Liang, W., 2015. Comparative study of microbial community structure in integrated vertical-flow constructed wetlands for treatment of domestic and nitrified wastewaters. Environ. Sci. Pollut. Res. 22, 3518–3527.

Chen, D., Wang, H.Y., Ji, B., Yang, K., Wei, L., Jiang, Y., 2015. A high-throughput sequencing study of bacterial communities in an autohydrogenotrophic denitrifying bio-ceramsite reactor. Process Biochem. 50, 1904–1910.

Chu, L.B., Wang, J.L., Quan, F., Xing, X.H., Tang, L.M., Zhang, C., 2014. Modification of polyurethane foam carriers and application in a moving bed biofilm reactor. Process Biochem. 49, 1979–1982.

Cortes-Lorenzo, C., Sipkema, D., Rodriguez-Diaz, M., Fuentes, S., Juarez-Jimenez, B., Rodelas, B., Smidt, H., Gonzalez-Lopez, J., 2014. Microbial community dynamics in a submerged fixed bed bioreactor during biological treatment of saline urban wastewater. Ecol. Eng. 71, 126–132.

Dorador, C., Vila, I., Witzel, K.P., Imhoff, J.F., 2013. Bacterial and archaeal diversity in high altitude wetlands of the Chilean Altiplano. Fundam. Appl. Limnol. 182, 135–159.

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27, 2194–2200.

Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods 10, 996.

Ge, Y., Wang, X.C., Zheng, Y.C., Dzakpasu, M., Zhao, Y.Q., Xiong, J.Q., 2015. Functions of slags and gravels as substrates in large-scale demonstration constructed wetland systems for polluted river water treatment. Environ. Sci. Pollut. Res. 22, 12982–12991.

Ghannad, S.T., Boroomandnasab, S., Moazed, H., Jaafarzadeh, N., 2015. The effects of substrate type, HRT and reed on the lead removal in horizontal subsurface-flow constructed wetland. Desalin. Water Treat. 56, 3357–3367.

Guan, W., Yin, M., He, T., Xie, S.G., 2015. Influence of substrate type on microbial community structure in vertical-flow constructed wetland treating polluted river water. Environ. Sci. Pollut. Res. 22, 16202–16209.

Guo, Y.H., Gong, H.L., Guo, X.Y., 2015. Rhizosphere bacterial community of *Typha angustifolia* L. and water quality in a river wetland supplied with reclaimed water. Appl. Microbiol. Biotechnol. 99, 2883–2893.

He, T., Guan, W., Luan, Z.Y., Xie, S.G., 2016. Spatiotemporal variation of bacterial and archaeal communities in a pilot-scale constructed wetland for surface water treatment. Appl. Microbiol. Biotechnol. 100, 1479–1488.

Herfort, L., Kim, J.H., Coolen, M.J.L., Abbas, B., Schouten, S., Herndl, G.J., Damste, J.S.S., 2009. Diversity of *Archaea* and detection of crenarchaeotal *amoA* genes in the rivers Rhine and Tet. Aquat. Microb. Ecol. 55, 189–201.

Huang, X., Liu, C.X., Gao, C.F., Wang, Z., Zhu, G.F., Liu, L., Lin, G.X., 2013. Comparison of nutrient removal and bacterial communities between natural zeolite-based

and volcanic rock-based vertical flow constructed wetlands treating piggery wastewater. Desalin. Water Treat. 51, 4379–4389.
Li, J.B., Wen, Y., Zhou, Q., Zhao, X.J., Li, X., Yang, S.L., Lin, T., 2008. Influence of vegetation and substrate on the removal and transformation of dissolved organic matter in horizontal subsurface-flow constructed wetlands. Bioresour. Technol. 99, 4990–4996.
Liao, X.B., Chen, C., Wang, Z., Wan, R., Chang, C.H., Zhang, X.J., Xie, S.G., 2013. Pyrosequencing analysis of bacterial communities in drinking water biofilters receiving influents of different types. Process Biochem. 48, 703–707.
Liao, X.B., Chen, C., Zhang, J.X., Dai, Y., Zhang, X.J., Xie, S.G., 2015. Operational performance, biomass and microbial community structure: impacts of backwashing on drinking water biofilter. Environ. Sci. Pollut. Res. 22, 546–554.
Ligi, T., Oopkaup, K., Truu, M., Preem, J.K., Nolvak, H., Mitsch, W.J., Mander, U., Truu, J., 2014. Characterization of bacterial communities in soil and sediment of a created riverine wetland complex using high throughput 16S rRNA amplicon sequencing. Ecol. Eng. 72, 56–66.
Liu, Y., Li, H., Liu, Q.F., Li, Y.H., 2015. Archaeal communities associated with roots of the common reed (*Phragmites australis*) in Beijing Cuihu Wetland. World J. Microbiol. Biotechnol. 31, 823–832.
Shao, Y.Y., Pei, H.Y., Hu, W.R., Chanway, C.P., Meng, P.P., Ji, Y., Li, Z., 2014. Bioaugmentation in lab scale constructed wetland microcosms for treating polluted river water and domestic wastewater in northern China. Int. Biodeterior. Biodegrad. 95, 151–159.
Silyn-Roberts, G., Lewis, G., 2004. Substrata effects on bacterial biofilm development in a subsurface flow dairy waste treatment wetland. Water Sci. Technol. 48, 261–269.
Tu, Y.T., Chiang, P.C., Yang, J., Chen, S.H., Kao, C.M., 2014. Application of a constructed wetland system for polluted stream remediation. J. Hydrol. 510, 70–78.
Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73, 5261–5267.
Wang, Z., Yang, Y.Y., He, T., Xie, S.G., 2015. Change of microbial community structure and functional gene abundance in nonylphenol-degrading sediment. Appl. Microbiol. Biotechnol. 99, 3259–3268.
Zhang, J.X., Yang, Y.Y., Zhao, L., Li, Y.Z., Xie, S.G., Liu, Y., 2015. Distribution of sediment bacterial and archaeal communities in plateau freshwater lakes. Appl. Microbiol. Biotechnol. 99, 3291–3302.
Zheng, Y.C., Wang, X.C., Xiong, J.Q., Liu, Y.J., Zhao, Y.Q., 2014. Hybrid constructed wetlands for highly polluted river water treatment and comparison of surface- and subsurface-flow cells. J. Environ. Sci. 26, 749–756.
Zhi, E.Q., Song, Y.H., Duan, L., Yu, H.B., Peng, J.F., 2015. Spatial distribution and diversity of microbial community in large-scale constructed wetland of the Liao river conservation area. Environ. Earth. Sci. 73, 5085–5094.
Zhong, F., Wu, J., Dai, Y.R., Yang, L.H., Zhang, Z.H., Cheng, S.P., Zhang, Q., 2015. Bacterial community analysis by PCR-DGGE and 454-pyrosequencing of horizontal subsurface flow constructed wetlands with front aeration. Appl. Microbiol. Biotechnol. 99, 1499–1512.