



## Spatio-temporal shifts in the archaeal community of a constructed wetland treating river water



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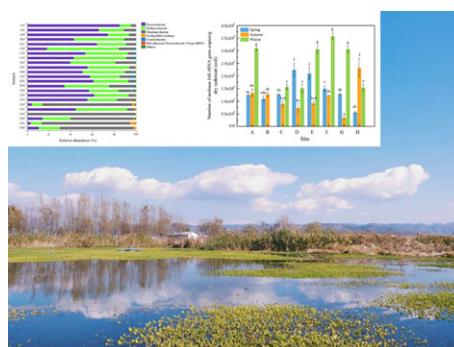
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### HIGHLIGHTS

- The spatio-temporal shifts in archaeal community in FWSF-CW was studied.
- Higher temperature adversely affected archaeal abundance in FWSF-CW.
- Wetland archaeal community was influenced by nutrients and vegetation type.
- *Euryarchaeota* and *Bathyarchaeota* dominated in sediments, but *Thaumarchaeota* dominated in soils.

### GRAPHICAL ABSTRACT



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### ABSTRACT

The distribution of archaeal community and the associated environmental variables in constructed wetland (CW), especially in free water surface flow CW (FWSF-CW), remain poorly understood. The present study explored the spatial and temporal dynamics of archaeal community in an FWSF-CW used for surface water treatment and evaluated the driving environmental variables. The archaeal density varied considerably among sites and seasons, ranging from  $3.37 \times 10^8$  to  $3.59 \times 10^9$  16S rRNA gene copies per gram dry sediment/soil. The archaeal population density was adversely affected by high temperatures and tended to be lower during summer than during spring and winter. Moreover, considerable spatio-temporal variations of archaeal richness, diversity and community structure also occurred in the FWSF-CW. Higher nutrient contents correlated with a lower archaeal richness and diversity. Nitrate and carbon/nitrogen ratio were found to play important roles in shaping the overall archaeal community structure. *Euryarchaeota* and *Bathyarchaeota* were the dominant archaeal phyla in wetland sediments, while *Thaumarchaeota* tended to be dominant in wetland soils. In addition, the wetland archaeal community was related to vegetation type.

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### 1. Introduction

Constructed wetlands (CWs), which are mainly represented by three configurations, namely free water surface flow CW (FWSF-CW), horizontal subsurface flow CW (HSF-CW) and vertical subsurface flow CW (VSF-CW), are engineered systems used to ameliorate water quality

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(Weber, 2016). CWs have been applied for the treatment of industrial, municipal and aquaculture wastewaters, as well as polluted surface water and groundwater (Bakhshoodeh et al., 2016; Guan et al., 2015; Jacome et al., 2016; Liu et al., 2016; Stefanakis et al., 2016; Xu et al., 2016). They can remove numerous types of pollutants (Sánchez, 2017). The removal of pollutants in CWs is achieved via a variety of physiochemical, plant related, and microorganism-mediated processes (Weber, 2016). The microbial community is considered to be the most important factor for in the dissipation of organic and inorganic contaminants in CWs (Chang et al., 2015; He et al., 2016; Ramond et al., 2013; Sánchez, 2017). Although the archaeal community has previously been linked with ammonia oxidation and methanogenesis in CWs (Bouali et al., 2012, 2013; Liu et al., 2015; Long et al., 2016a), information about the archaeal communities of CWs remains scarce. Archaeal diversity in a HSF-CW used for treating domestic wastewater was found to be related to plant and water chemistry (Bouali et al., 2012). In a VSF-CW used for treating surface water, archaeal diversity and community structure varied with layer depth and sampling time (He et al., 2016; Long et al., 2016b), and were influenced by plant type and substrate (Long et al., 2016a, b).

FWSF-CWs have been widely applied for surface water treatment (Dzakpasu et al., 2015; Hernandez-Crespo et al., 2016; Ibeke et al., 2007; Ligi et al., 2014; Zhi et al., 2015). To date, little is known about the archaeal community in FWSF-CWs. The spatio-temporal changes of the FWSF-CW archaeal community and the associated environmental factors remain unknown. Hence, the main aim of the current study was to explore the temporal and spatial dynamics of the archaeal community in an FWSF-CW used for surface water treatment and evaluate the associated environmental variables.

## 2. Materials and methods

### 2.1. Study site and sampling

The studied FWSF-CW system, which had a total surface area of approximately 0.47 km<sup>2</sup>, was designed to improve the quality of river water entering Lake Erhai in southwest China. Before this study, the FWSF-CW had been continuously operating for >8 years. The average hydraulic retention times in the FWSF-CW were about 3 and 10 days during wet and dry seasons, respectively. The local area had a subtropical monsoon climate, with an annual mean air temperature of 15.7 °C and an annual precipitation of 1000 mm. The FWSF-CW average influent total organic carbon (TOC), ammonia nitrogen (NH<sub>4</sub><sup>+</sup>-N), total nitrogen (TN) and total phosphorus (TP) were 15.4, 0.34, 3.33, and 0.11 mg/L, respectively. The average removal rates of TOC, NH<sub>4</sub><sup>+</sup>-N, TN and TP were 40.9%, 55.9%, 43.2%, and 45.4%, respectively.

Triplicate sediments at six sites (A–F) and soils at two sites (G and H) at the same depth (0–10 cm) were collected during March (spring), August (summer), and December (winter) in 2016 (Fig. S1). Soil site G (vegetated with alfalfa, located at the wetland center highland) and soil site H (vegetated with cattail, located in wetland edge) were inundated during wet season (summer and autumn), while the sediment sites were inundated during both the dry and wet seasons, and each site had a different dominant plant species (site A: water hyacinth; site B: reed; site C: watermilfoil; site D: penny grass; site E: duckweed; and site F: water-lily). In this study, the samples were coded as A–H, corresponding to the collection site, and SP, SU, or WI, corresponding to the collection period (spring, summer, or winter, respectively). The winter sediment sample at site B was not collected because the site was inaccessible during winter; thus, there was no sample coded “BWI.” The physicochemical properties of these sediments/soils are shown in Table S1.

### 2.2. Molecular analyses

The PowerSoil™ DNA extraction kit (MO BIO Laboratories, Carlsbad, CA, USA) was used to extract genomic DNA from FWSF-CW sediment/

soil samples. The quality and quantity of sediment/soil DNA were assessed by spectrophotometry (NanoDrop™ 2000 spectrophotometer, Thermo Fisher Scientific, Waltham, MA, USA). The number of archaeal organisms was assessed by quantitative polymerase chain reaction (PCR) using the primer pairs Arch349F (5'-GYGCASCAGKCGMGAAW-3')/Arch806R (5'-GGACTACVSGGGTATCTAAT-3') (Jung et al., 2011; Long et al., 2016a; Yang et al., 2016), with the following amplification conditions: 10 min at 95 °C followed by 40 cycles of 95 °C for 30 s, annealing for 30 s at 55 °C, and extension at 72 °C for 30 s. A standard curve was constructed using 10-fold serial dilutions of plasmids harboring the target 16S rRNA gene, yielding an amplification efficiency of 96% and a linear regression coefficient of 0.992.

For Illumina MiSeq sequencing, FWSF-CW sediment/soil DNA was amplified using the archaeal primer sets 524F-10-extF (5'-TGYCAGC CGCCGCGTAA-3')/Arch958-modR (5'-YCCGCGGTGAVTCCAATT-3') (Pires et al., 2012), with the following amplification conditions: 3 min at 95 °C followed by 35 cycles of 95 °C for 30 s, annealing for 30 s at 55 °C, and extension at 72 °C for 45 s, and a final extension step at 72 °C for 10 min. The purified amplicons from replicate samples were pooled in equal proportions before high-throughput sequencing using a HiSeq 2000 system (Illumina Inc., San Diego, CA, USA). The raw archaeal reads were deposited in the Sequence Read Archive database (accession number: SRP103586). Pairs of reads were combined into composite reads using FLASH and low quality archaeal sequences were deleted using QIIME (Caporaso et al., 2010). Chimeric wetland archaeal sequences were screened using UCHIME (Edgar et al., 2011). Archaeal sequences (with a 97% similarity cutoff) were assigned into operational taxonomic units (OTUs), and the Chao1 richness and Shannon diversity indices of the archaeal community were calculated using UPARSE (Edgar, 2013). For the comparison of wetland archaeal  $\alpha$ -diversity indices, the sequences of each sample were subsampled to the same sequencing depth (containing 23,170 sequences). Moreover, to compare the dissimilarity in archaeal community composition among samples, weighted UniFrac-based hierarchical clustering using the weighted pair group method with arithmetic mean (WPGMA) was conducted using R software (version i386, 3.3.0). In addition, the Silva rRNA database was used to classify the taxonomic identities of wetland archaeal sequences (Quast et al., 2013).

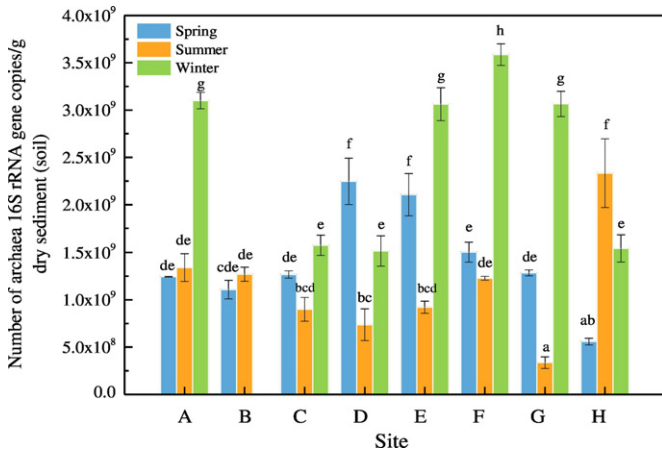
### 2.3. Statistical analysis

The differences (at a 0.05 significance level) in the densities of the FWSF-CW archaeal populations among sites were assessed by one-way analysis of variance. The correlations of FWSF-CW physicochemical variables with archaeal abundance, richness and diversity were determined by Spearman's rank correlation analysis, using SPSS 20.0 software (IBM, Armonk, NY, USA). Redundancy analysis (RDA) using Monte Carlo tests was carried out to uncover *Archaea*-environment relations with CANOCO 4.5 software (Microcomputer Power, Ithaca, NY, USA). The numbers of sequences classified into the major archaeal OTUs (with a minimum threshold of 50 sequences) were taken as species input, while FWSF-CW sediment/soil physicochemical parameters represented environmental input (Zhang et al., 2015).

## 3. Results

### 3.1. Population density of wetland archaeal communities

The archaeal population density in the FWSF-CW sediment/soil ranged between  $3.37 \times 10^8$  and  $3.59 \times 10^9$  16S rRNA gene copies per gram dry sediment/soil (Fig. 1). At each sampling site in the FWSF-CW, a significant difference in wetland archaeal population density was generally detected among the sediment/soil samples collected during different seasons ( $P < 0.05$ ), demonstrating a remarkable seasonal variability of archaeal population density in the FWSF-CW. Moreover, at sites C, D, E, F, and G, archaeal population density was lower during



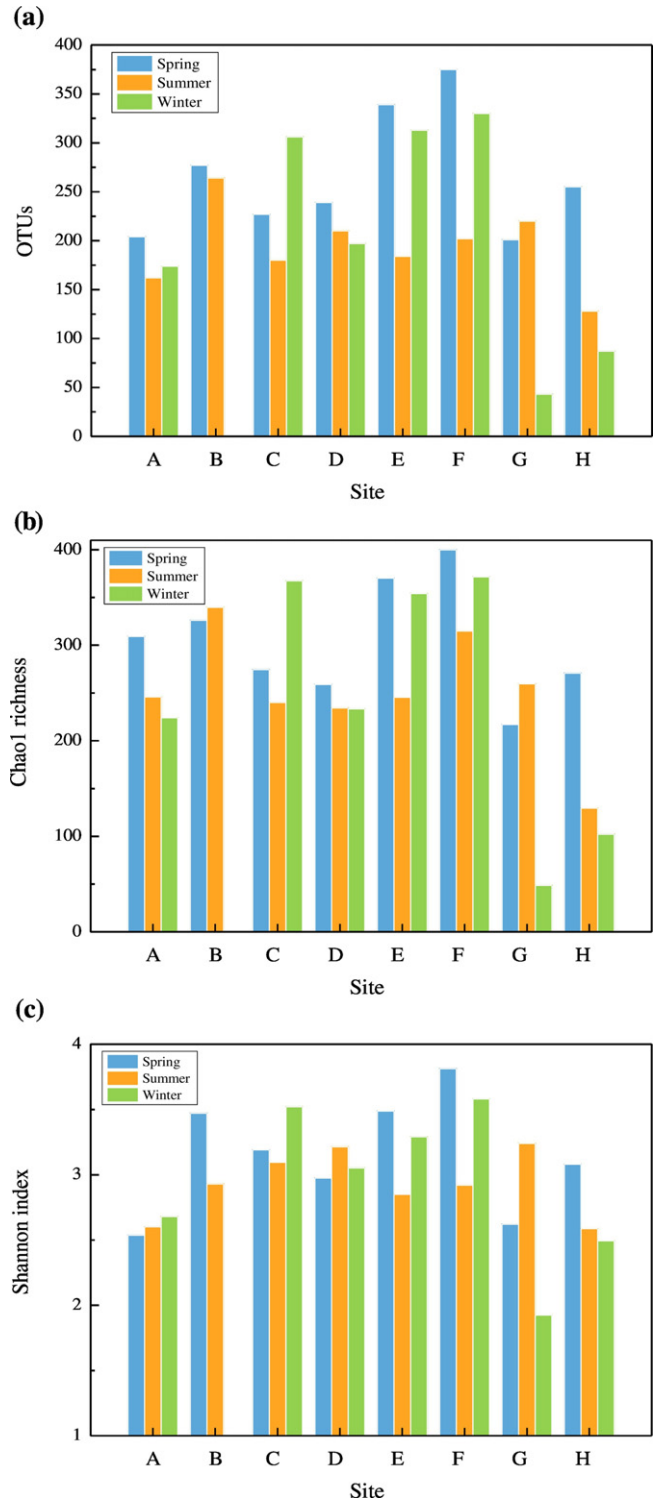
**Fig. 1.** Archaeal abundance of wetland sediments and soils. Values are the average of triplicate samples. Vertical bars indicate standard deviations. Different letters above the columns indicate the significant difference ( $P < 0.05$ ).

summer than during both spring and winter. However, at site H, the archaeal population density during summer far outnumbered that during spring and winter. At sites A and B, the archaeal population density during summer was higher than that during spring. Moreover, the archaeal population density was highest during winter at sites A, C, E, F and G, but while it was highest during spring at site D. In addition, during each season, a significant difference in the archaeal population density was detected between wetland soils or among wetland sediments ( $P < 0.05$ ), indicating a remarkable variation of archaeal population density among sampling sites in the surface water treatment FWSF-CW.

### 3.2. Wetland archaeal richness and diversity

Each wetland soil archaeal library was comprised of 43–255 OTUs, while each sediment archaeal library included 162–375 OTUs (Fig. 2a). At each site in the FWSF-CW, the number of archaeal OTUs showed an obvious seasonal change. The seasonal variation pattern of archaeal OTU number in the FWSF-CW was found to be site-specific. For examples, at sites A, C, E, and F, the sediment samples collected during summer contained fewer archaeal OTUs than those collected during spring and winter, while at site G, the sediment samples collected during summer contained more archaeal OTUs than those collected during spring and winter. Moreover, at sites D and H, the number of archaeal OTUs declined from spring to summer and then to winter. In addition, in each season, the number of archaeal OTUs differed considerably among sediments/soils collected at different sites in the FWSF-CW.

The archaeal Chao1 richness estimator varied between 224 and 400 in wetland sediment samples and between 49 and 271 in wetland soil samples (Fig. 2b). In the studied FWSF-CW, which was used for river water treatment, there were considerable seasonal changes in the archaeal richness of both sediments and soils. The seasonal variation patterns of FWSF-CW archaeal richness differed among the sampling sites. At sites C, E, and F, the sediment samples collected during summer had a lower archaeal richness than those collected during spring and winter. In contrast, at site G, the sediment sample collected during summer had a higher archaeal richness than those collected during spring and winter. At sites A and H, archaeal richness continuously decreased from spring to summer and then to winter. During each season, archaeal richness showed a considerable variability among sampling sites in the FWSF-CW. In addition, the archaeal Shannon diversity index of sediment/soil in the FWSF-CW varied between 1.93 and 3.81 (Fig. 2c). Both sediments and soils showed a seasonal shift in FWSF-CW archaeal diversity, and the pattern of seasonal change varied among the sampling sites. Evident spatial variations in archaeal diversity in the FWSF-CW could also be detected during all three seasons.



**Fig. 2.** Wetland sediment/soil archaeal OTUs (a), Chao1 estimator (b), and Shannon index (c).

### 3.3. Clustering analysis of wetland archaeal communities

The results of weighted UniFrac-based WPGMA clustering analysis demonstrated a remarkable variability of the archaeal community structure among sampling sites in the FWSF-CW (Fig. 3). Generally, wetland soil samples tended to be distantly separated from sediment samples. The samples DSU and GSU were clearly separated from the other wetland samples collected during summer. During both spring and winter, wetland sediment samples also displayed a clear separation.

For example, sample ASP was distantly separated from other sediments collected during spring, while samples ESP and FSP were not closely clustered with samples BSP, CSP and DSP. Sample AWI was distantly separated from the other sediments collected during winter, and CWI, DWI, EWI and FWI were not closely clustered together. Moreover, a remarkable seasonal variability in archaeal community structure could also be detected in the FWSF-CW system. At sites C, D, E, and F, the sediment samples collected during the three seasons were not clustered together, while at site A, sediment samples collected during the three seasons could be grouped together. Soil samples collected during summer were more distantly separated from those collected during spring and winter at site G than at site H. These results further suggested that the seasonal variability of the archaeal community structure was site-specific.

### 3.4. Wetland archaeal community composition

In the present study, most of the archaeal sequences (accounting for 91–100%) retrieved from the studied FWSF-CW were affiliated with known or candidate archaeal phyla including *Euryarchaeota*, *Bathyarchaeota*, *Thaumarchaeota*, *Aenigmarchaeota*, *Crenarchaeota*, *Diapherotrites*, *Parvarchaeota*, *Woesearchaeota*, *M1K20*, *Miscellaneous Euryarchaeotic Group (MEG)*, and *Marine Hydrothermal Vent Group (MHVG)* (Fig. 4). *Euryarchaeota* (17.8–85.1%) and *Bathyarchaeota* (10.4–66.5%) dominated in wetland sediment samples. The relative abundance of *Euryarchaeota* and *Bathyarchaeota* showed remarkable variations with both season and sediment sampling site. The seasonal variation patterns of *Euryarchaeota* and *Bathyarchaeota* proportions varied among the sediment sampling sites. *Euryarchaeota* (44.4%) and *Bathyarchaeota* (47.8%) were dominant in soil at site G during summer, whereas *Thaumarchaeota* dominated in other five wetland soils (57.8–99.1%). The relative abundance of *Thaumarchaeota* was related to both the season and the soil sampling site. The two wetland soil sampling sites displayed different patterns of seasonal change in the proportion of *Thaumarchaeota*.

In this study, 19 known or candidate archaeal genera were detected in the FWSF-CW, including *Methanobacterium* (0.1–60.5%), *Methanosaeta* (0–23.6%), *Methanosarcina* (0–11.4%), *Candidatus Nitrososphaera* (0–5.9%) and other less abundant archaeal groups (Fig. S2). Among all sites and seasons, *Methanobacterium* and *Methanosaeta*

were usually the two most abundant genera. Their relative abundance also showed remarkable variations among both seasons and sampling sites. Moreover, the relative abundance of *Candidatus Nitrososphaera* tended to be higher in soils than in sediments.

### 3.5. Influential factors regulating wetland archaeal community

Spearman's rank correlation analysis indicated that archaeal abundance in the wetland soil/sediment samples showed a positive correlation with the concentration of  $\text{NO}_2^-$ -N ( $P < 0.01$ ) but a negative correlation with temperature ( $P < 0.01$ ) (Table 1). Archaeal richness was negatively correlated with the concentrations of TN, TP and TOC ( $P < 0.05$  or  $P < 0.01$ ), while archaeal diversity was negatively correlated with the concentrations of TP and  $\text{NO}_3^-$ -N ( $P < 0.05$  or  $P < 0.01$ ). Moreover, the abundance of *Euryarchaeota* was significantly correlated with the concentrations of  $\text{NH}_4^+$ -N,  $\text{NO}_2^-$ -N, TN and TOC ( $P < 0.05$  or  $P < 0.01$ ). The abundance of *Bathyarchaeota* was negatively correlated with the concentrations of ( $P < 0.05$  or  $P < 0.01$ ). The abundance of *Thaumarchaeota* was negatively correlated with the concentration of  $\text{NH}_4^+$ -N ( $P < 0.05$ ), but positively correlated with that of TN ( $P < 0.01$ ) and TOC ( $P < 0.05$ ). In addition, the FWSF-CW environmental parameters in the first two RDA axes collectively explained 65.1% in the variance for archaeal OTU composition (Fig. 5). Sediment/soil  $\text{NO}_3^-$ -N concentration ( $F = 4.024$ ,  $P = 0.005$ , 999 permutations) and the C/N ratio ( $F = 2.303$ ,  $P = 0.013$ , 999 permutations) were found to significantly contribute to the Archaea–environment relationship.

## 4. Discussion

### 4.1. Spatial and temporal variations of wetland archaeal population density

Several previous studies have reported spatial and temporal variations in archaeal population density in natural wetland (Tian et al., 2012a; Yarwood et al., 2016; Ye et al., 2016), yet there is still a paucity of knowledge about changes in archaeal density among seasons and sampling sites in CWs. In this study of an FWSF-CW used for river water treatment, the archaeal population density was found to vary considerably among sampling sites and seasons. However, in this CW system, the observed bacterial population density ranged from  $1.71 \times 10^{10}$  to  $1.35 \times 10^{11}$  16S rRNA gene copies per gram dry sediment/soil (data not shown), which was much larger than the archaeal population density. Hence, *Archaea* only constituted a small fraction of the total prokaryotic community in the studied FWSF-CW. The numerical advantage of the bacterial community over the archaeal community has also been observed in a VSF-CW used for surface water treatment (Long et al., 2016a). Moreover, the seasonal changes in archaeal density in the studied FWSF-CW differed among sampling sites, which had different wetland vegetation types. These results suggested that the wetland vegetation type could have a considerable impact on the archaeal density in the studied FWSF-CW. The impact of vegetation type on archaeal density has also been reported in a VSF-CW used for surface water treatment (Long et al., 2016a) and natural wetlands (Tian et al., 2012a; Yarwood et al., 2016). Therefore, the vegetation type might play an important role in determining wetland archaeal population number density.

So far, little is known about the relationships between archaeal density and wetland environmental variables. Archaeal density in natural wetland was found to be positively correlated with the concentrations of soil  $\text{NH}_4^+$ -N, organic matter and moisture (Ye et al., 2016). In this study, it was found that archaeal density in a VSF-CW used for surface water treatment might have been negatively influenced by temperature. Archaeal density was usually lower during summer than during spring and winter. Tian et al. (2015) also indicated that warming could cause a significant decline in archaeal abundance in mesocosm wetland. Therefore, high temperatures may reduce archaeal density in wetland environments. Moreover, the relationship between archaeal

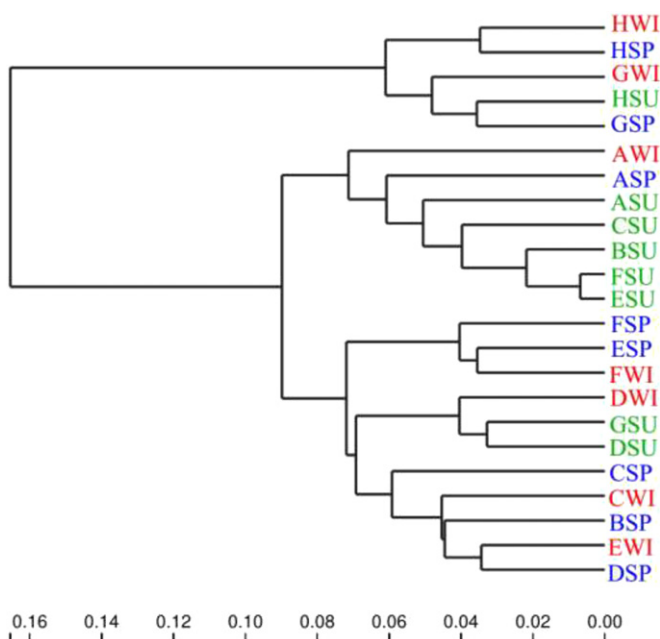
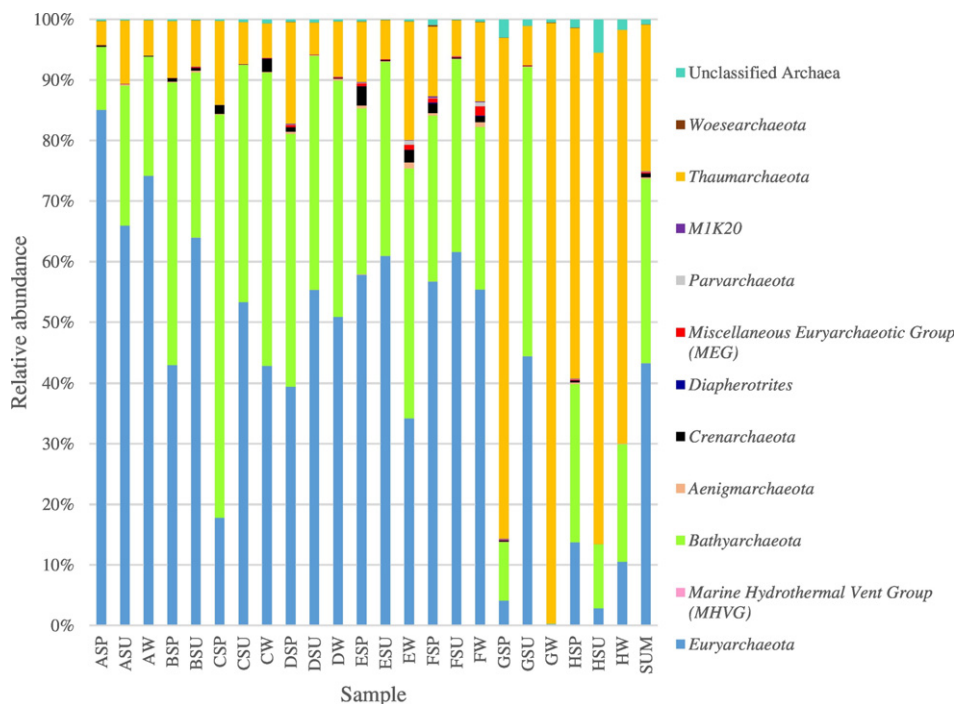


Fig. 3. WPGMA clustering of wetland archaeal communities based on weighted UniFrac.





**Fig. 4.** Comparison of the quantitative contribution of the sequences affiliated with different archaeal phyla to the total number of sequences from a given sediment/soil sample in the FWSF-CW used for river water treatment.

density and the concentration of  $\text{NO}_2^-$ -N in natural environments remains unclear. To the authors' knowledge, the present study provided the first evidence for the influence of  $\text{NO}_2^-$ -N on wetland archaeal density.

#### 4.2. Spatial and temporal variations of wetland archaeal richness and diversity

Spatial and temporal variations of archaeal richness and diversity have previously been observed in natural wetlands (Lliros et al., 2014; Ye et al., 2016), a HSF-CW used for treating domestic wastewater (Bouali et al., 2012), and VSF-CW treating surface water (He et al., 2016; Long et al., 2016b). The present study further revealed the spatial and temporal dynamics of archaeal richness and diversity in an FWSF-CW used for river water treatment. However, in this CW, the observed bacterial Chao1 richness and Shannon diversity index were 1794–3643 and 5.16–6.96, respectively (data not shown), which were much higher than those of the archaeal community. In a VSF-CW used for surface water treatment, the bacterial community also had much higher richness and diversity than the archaeal community (He et al., 2016; Long et al., 2016a, b). Moreover, archaeal richness and diversity in the FWSF-CW used for surface water treatment were related to the types of wetland plants present. Two previous studies also suggested that the plant types present have a profound impact on archaeal

richness and diversity in a natural wetland (Lliros et al., 2014) and a VSF-CW used for surface water treatment (Long et al., 2016a). Hence, the vegetation type could be a key determinant of wetland archaeal richness and diversity.

The environmental variables regulating wetland archaeal richness and diversity remain elusive. In the present study, both archaeal richness and diversity in an FWSF-CW used for surface water treatment were negatively correlated with nutrient levels. Higher nutrient content might lower archaeal richness and diversity. A previous study also indicated that the archaeal richness and diversity of freshwater lake sediment were negatively correlated with the concentrations of  $\text{NO}_3^-$ -N, TN, TP, and TOC (Yang et al., 2016).

#### 4.3. Spatial and temporal variations of wetland archaeal community structure

Several previous studies have documented spatial and temporal shifts in archaeal community structure in natural wetlands (Lliros et al., 2014; Rietl et al., 2016; Tian et al., 2012b; Ye et al., 2016), a HSF-CW used for domestic wastewater treatment (Bouali et al., 2012), and a VSF-CW used for surface water treatment (He et al., 2016; Long et al., 2016b). Moreover, the type of plants present was indicated to have a considerable impact on archaeal community structure in natural wetlands (Lliros et al., 2014; Rietl et al., 2016; Tian et al., 2012a, b;

**Table 1**

Spearman's rank correlation analysis of environmental variables with the abundance, richness and diversity of wetland archaeal communities and the proportions of *Euryarchaeota*, *Bathyarchaeota* and *Thaumarchaeota*.

	$\text{NH}_4^+$ -N	$\text{NO}_3^-$ -N	$\text{NO}_2^-$ -N	TN	TP	TOC	C/N	Temperature	ORP	pH
Abundance	-0.082	0.358	0.596 <sup>b</sup>	-0.095	-0.225	-0.076	-0.254	-0.590 <sup>b</sup>	-0.189	0.019
Chao1 richness	0.156	-0.401	-0.231	-0.454 <sup>a</sup>	-0.610 <sup>b</sup>	-0.453 <sup>a</sup>	-0.031	-0.087	-0.271	-0.106
Shannon diversity	-0.087	-0.542 <sup>b</sup>	-0.158	-0.342	-0.463 <sup>a</sup>	-0.194	0.271	-0.174	-0.236	-0.058
<i>Euryarchaeota</i>	0.677 <sup>b</sup>	-0.234	-0.417 <sup>a</sup>	-0.594 <sup>b</sup>	-0.356	-0.606 <sup>b</sup>	0.160	0.204	-0.213	-0.337
<i>Bathyarchaeota</i>	0.164	-0.592 <sup>b</sup>	-0.198	-0.325	-0.439 <sup>a</sup>	-0.252	0.208	-0.011	-0.276	-0.057
<i>Thaumarchaeota</i>	-0.488 <sup>a</sup>	0.375	0.275	0.530 <sup>b</sup>	0.210	0.452 <sup>a</sup>	-0.030	-0.176	0.400	0.062

<sup>a</sup> Correlation is significant at the 0.05 level.

<sup>b</sup> Correlation is significant at the 0.01 level.

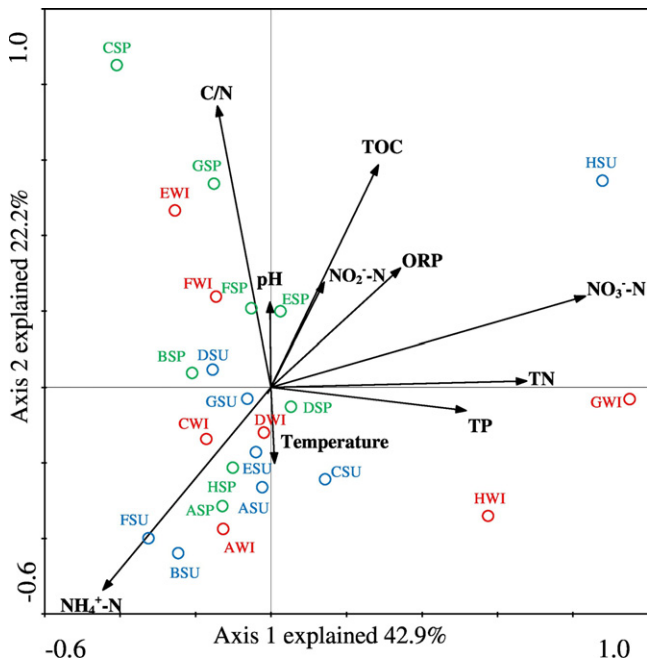


Fig. 5. RDA ordination plot for the first two principal dimensions of the links of archaeal OTU composition with wetland physicochemical parameters.

Yarwood et al., 2016) and a VSF-CW used for surface water treatment (Long et al., 2016a). In the present study, the results of both clustering and phylogenetic analyses provided evidence for spatial and temporal changes in archaeal community structure in an FWSF-CW used for surface water treatment. Moreover, vegetation type may also influence the structure of the archaeal communities in the studied FWSF-CW.

The environmental variables driving wetland archaeal community structure remain poorly known. Soil moisture and  $\text{NH}_4^+\text{-N}$  were found to be the two key determinants of archaeal community structure in natural wetland (Ye et al., 2016). In this study, wetland soils and sediments tended to have distinctly different archaeal community structures. These data suggested the importance of moisture content in shaping archaeal community structure. Moreover,  $\text{NO}_3^- \text{-N}$  might be a key factor driving the overall archaeal community structure in the studied FWSF-CW. A previous study also suggested the importance of  $\text{NO}_3^- \text{-N}$  in determining the archaeal community structure in black soils of northeast China (Ding et al., 2016).

So far, there has been no consensus on the major archaeal groups present in wetland systems. *Euryarchaeota* was found to be the dominant archaeal phylum in a high-altitude natural wetland (Dorador et al., 2013), an FWSF-CW used for treating a mixture of domestic wastewater and reservoir water (Liu et al., 2015), and a microcosm VSF-CW used for treating river water (Long et al., 2016b). In contrast, both *Euryarchaeota* and *Crenarchaeota* dominated in Louisiana saltmarshes (Rietl et al., 2016). Ye et al. (2016) indicated that *Thaumarchaeota* and *Euryarchaeota* were dominant in dry and wet wetland soils, respectively. Moreover, *Thaumarchaeota* was found to be dominant in a HSF-CW used for treating domestic wastewater (Bouali et al., 2012, 2013). Both *Euryarchaeota* and *Thaumarchaeota* dominated in a pilot-scale VSF-CW used for pond water treatment (He et al., 2016). However, Long et al. (2016a) reported that *Thaumarchaeota* dominated in a VSF-CW used for river water treatment that was planted with *Vetiveria zizanioides* or *Juncus effusus* L., while *Woesearchaeota* and *Euryarchaeota* dominated in a VSF-CW used for river water treatment that was planted with *Cyperus papyrus* or *Canna indica* L. In this study, in an FWSF-CW used for river water treatment, *Euryarchaeota* and *Bathyarchaeota* dominated in wetland sediments, while *Thaumarchaeota* usually dominated in wetland soils. To the authors' knowledge, this is the first report of the dominance of *Bathyarchaeota*

in a wetland system. In addition, the proportions of *Euryarchaeota*, *Thaumarchaeota*, and *Bathyarchaeota* were found to be influenced by nutrient levels.

#### 4.4. Potential functional role of archaeal community in wetland

The abundance of microorganisms belonging to methanogenic genera has been evaluated in a VSF-CW used for surface water treatment, and these methanogenic organisms were assumed to play important roles in degrading organic carbon (He et al., 2016). In this study, in an FWSF-CW used for surface water treatment, organisms within methanogenic genera (mainly *Methanobacterium*, *Methanosaeta* and *Methanosarcina*) usually accounted for a considerable proportion of wetland archaeal communities, suggesting their possible contribution to the reduction of organic matter. In addition, microorganisms belonging to genera *Candidatus Nitrososphaera* and *Candidatus Nitrosoarchaeum* have been linked with ammonia oxidation (Kim et al., 2011; Tourna et al., 2011). In the present study, both *Candidatus Nitrososphaera* and *Candidatus Nitrosoarchaeum* were detected in the FWSF-CW used for river water treatment, and *Candidatus Nitrososphaera* was abundant in wetland soils. Therefore, the presence of these two genera might contribute to ammonia oxidization.

## 5. Conclusions

The density, richness, diversity, and structure of archaeal communities in an FWSF-CW used for river water treatment were remarkably variable among seasons and sampling sites. The vegetation types present could have a considerable impact on wetland archaeal communities. In this study, archaeal density in a wetland environment appeared to be decreased by high temperatures but increased by high concentrations of  $\text{NO}_2^- \text{-N}$ . Higher nutrient contents were associated with a lower archaeal richness and diversity.  $\text{NO}_3^- \text{-N}$  concentration and C/N ratio might be two key factors driving the overall archaeal community structure. In addition, *Euryarchaeota* and *Bathyarchaeota* dominated in wetland sediments, while *Thaumarchaeota* tended to dominate in wetland soils.

## Conflict of interest

The authors declare that they have no competing interests.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2017.06.221>.

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