

Elevational characteristics of the archaeal community in full-scale activated sludge wastewater treatment plants at a 3,660-meter elevational scale

Lihua Niu, Xue Zhang, Yi Li, Peifang Wang, Wenlong Zhang, Chao Wang and Qing Wang

ABSTRACT

Due to the important roles of archaea in wastewater treatment processes, archaeal communities have been studied extensively in various anaerobic reactors, but the knowledge of archaeal communities in full-scale activated sludge wastewater treatment plants (WWTPs) remains quite poor. In this study, 454-pyrosequencing was for the first time employed to investigate archaeal communities from 20 full-scale activated sludge WWTPs distributed at a 3,660-meter elevational scale in China. Results showed that archaeal communities from WWTPs were dominated by *Methanosarcinales* (84.6%). A core archaeal population (94.5%) composed of *Methanosaeta*, *Methanosarcina*, *Methanogenium* and *Methanobrevibacter* was shared among WWTPs. The elevational pattern of archaeal communities was observed in WWTPs, with an elevational threshold associated with archaeal community richness and structures at approximately 1,500 meters above sea level (masl). A declining trend in community richness with increasing elevation was observed at higher elevations, whereas no trend was presented at lower elevations. Spearman correlation analysis indicated that the archaeal community richness at higher elevations was associated with more environmental variables than that at lower elevations. Redundancy analysis indicated that wastewater variables were the dominant contributors to the variation of community structures at higher elevations, followed by operational variables and elevation.

Key words | archaeal community, elevational gradient, environmental variable, full-scale wastewater treatment plant, pyrosequencing

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INTRODUCTION

Activated sludge is composed of a highly complex system, including eukaryotes (protozoa and fungi), bacteria, archaea, and viruses (You *et al.* 2009). The complex microbial community plays a crucial role in the removal of various pollutants in biological wastewater treatment processes. A complete understanding of the microbial community in activated sludge is important to the adjustment and optimization of wastewater treatment systems. Most previous studies focused on the ecological functions and characteristics of bacterial communities in activated sludge wastewater treatment plants (WWTPs) (Hashimoto *et al.* 2014), but overlooked the parallel characterization of archaeal communities.

Aerobic phase coupled to anoxic and anaerobic phases is the most widely used configuration process for carbon

and nitrogen removal in activated sludge WWTPs. Aerobic ammonia-oxidizing archaea (AOA) was commonly indicated as one of the important components in the nitrogen removal process in WWTPs (Wei *et al.* 2011). Fredriksson *et al.* (2012) quantified the higher abundance of methanogenic archaea than bacteria in the anaerobic phase of activated sludge WWTPs. The observation implied the important activity of archaea in carbon removal in the anaerobic phase and the potential role of methanogenic archaea in the structure of activated sludge flocs, according to its crucial contribution to the formation and integrity of granular sludge in anaerobic bioreactors (Angenent *et al.* 2004). In addition, the production of methane may promote the growth of methanotrophic bacteria, which are involved

in the nitrification and denitrification in WWTPs (Bodelier & Frenzel 1999). Due to these potentially important roles of archaea, it is necessary to understand the characteristics of archaeal community completely in activated sludge WWTPs.

In recent years, a few studies have paid attention to AOA in activated sludge (You *et al.* 2009; Wei *et al.* 2011), whereas much less focus has been put on the total archaeal community. To date, there are only three reported studies on the activated sludge archaeal communities in full-scale WWTPs. One study reported a simple archaeal community in two small WWTPs using denaturing gradient gel electrophoresis (DGGE) (Gray *et al.* 2002). The other two studies investigated the diversity and abundance of archaea in full-scale WWTPs using fluorescence *in situ* hybridization (FISH) and clone library methods (Sanchez *et al.* 2011; Fredriksson *et al.* 2012). However, the information on archaeal communities in activated sludge WWTPs remains quite poor. First, the low sequencing depth of these traditional methods applied in previous studies hindered the complete characterization of archaeal communities in activated sludge. Second, the previous studies on activated sludge archaeal communities were limited to one or two WWTPs. Third, no knowledge of the geographical characterization of archaeal communities in WWTPs has been described.

Besides conventional operational and wastewater variables, geographic distribution of WWTPs is also supported to be an influential variable of the microbial communities in WWTPs (Wang *et al.* 2012; Zhang *et al.* 2012). However, there has been no report on the association between elevation and microbial communities from WWTPs. In recent years, multiple WWTPs were constructed at high elevations, such as the Bolivia and Tibetan plateau above 3,000 meters above sea level (masl). However, many exhibit lower efficiency for the removal of contaminants than those at low elevations (Zabalaga *et al.* 2007). The function and performance of WWTPs are directly associated with the microbial communities in activated sludge. Thus, it is necessary to reveal the characteristics of microbial communities on the elevation scale. Elevational gradient is characterized by distinct changes in climate and biotic turnover over short elevation distances. It is indicated that natural archaea respond significantly in the community richness to elevational gradients (Singh *et al.* 2012). Yang *et al.* (2014) reported that the archaea in the Tibetan plateau show a larger variation in endemic genes than bacteria to adapt to the elevation-dependent environmental conditions. Therefore, we hypothesized that the activated sludge

archaeal community in man-made WWTPs also varies with elevational gradients.

The objectives of this study were (1) to systematically characterize the archaeal diversity in activated sludge WWTPs via 454-pyrosequencing, (2) to test the archaeal community variability with elevation according to the above hypothesis, and (3) to evaluate the linkage of environmental variables and archaeal community.

MATERIALS AND METHODS

Activated sludge sampling and DNA extraction

Twenty municipal WWTPs scattered along a 3,660-masl altitude gradient were selected in China (Figure 1). Details of the geographic locations, wastewater and operational variables of 20 WWTPs are listed in Tables S1 and S2 (available with the online version of this paper). Effluent concentrations and removal efficiencies of pollutants from wastewater can characterize WWTP performance to some extent (Table S2). Activated sludge samples were collected from the anaerobic, anoxic and aerobic phases separately in May, August and December 2014. In order to minimize the influence of spatial variance on activated sludge archaeal communities at different sites within each phase, a composite sample was collected, which consisted of five replicates of activated sludge within a 5 meter diameter circle in each phase. All samples were stored in a dry ice box immediately after collection and stored at -80°C in the laboratory until DNA extraction. Genomic DNA was extracted from samples using an Ultraclean DNA Extraction Kit (Mo-Bio, Carlsbad, CA). DNA quality was analyzed by the 260/280 nm and 260/230 nm absorption ratios using an ND-2000 spectrophotometer (NanoDrop Inc., Wilmington, DE) and 1% agarose gel electrophoresis.

Polymerase chain reaction (PCR) amplification and pyrosequencing

The V1 and V2 regions of the archaeal 16S rRNA gene were amplified using two forward primers A2Fa and N3F, coupled with one reverse primer A571R (Baker *et al.* 2003; Kan *et al.* 2011). Barcodes that allow sample multiplexing during pyrosequencing were incorporated between the 454 adapter and the forward primers. Pyrosequencing was performed using a Roche 454 FLX Titanium sequencer (Roche, Nutley, USA) at the Chinese National Human Genome Center (SinoGenoMax). In this study, 454-pyrosequencing of archaeal 16S rRNA genes generated 730,854 quality-filtered sequence



Figure 1 | Geographical locations of 20 WWTPs in China. Elevation of WWTPs is indicated by the size of symbols.

reads, with an average of 4,350 sequences for each sample. The sequence library of each sample was normalized to the size of 3,627 sequences, which was the smallest sample, to conduct the statistical analyses for different samples at the same sequence depth (Figure S1, available with the online version of this paper). The obtained raw reads were deposited in the NCBI short-reads archive database (Accession number: SRX982227 and SRR3316543).

Data analysis

The raw reads were processed using Mothur. Raw reads were excluded if they were shorter than 200 bp or if there was a single base bias. PCR chimeras were removed using the *chimerera.uchime* command implemented in Mothur. Similar sequences were clustered into operational taxonomic units (OTUs) with a 3% sequence dissimilarity cut-off. The phylogenetic taxonomy of remaining sequences was determined using the RDP classifier database with a confidence threshold of 80%. The comparison of the relative abundances of the most abundant OTUs among samples was conducted using the heatmap package in R (v. 3.12, <http://www.r-project.org/>).

The relationships between environmental variables and archaeal community, which were characterized as community richness, evenness and structure, were performed

separately. Alpha-diversity indices, including Shannon-H index and evenness index, were calculated using R software. Correlation coefficients between environmental variables and archaeal community richness were measured using the Spearman correlation method by SPSS 20.0 software. The similarity analysis of the OTU structures of samples was performed via clustering analysis based on the Bray-Curtis similarity using PAST 3.0 software. Redundancy analysis (RDA) was performed to evaluate the relationship between archaeal community structures and environmental variables with 999 permutations. The contributions of individual variables to the variance of archaeal communities were assessed via variance partitioning analysis. Environmental parameters were log-transformed for the analysis. A *p*-value <0.01 was considered significant.

RESULTS AND DISCUSSION

Archaeal diversity in activated sludge WWTPs

The systematic investigation of archaeal diversity in activated sludge was performed for the first time via 454 pyrosequencing. The 575,736 normalized archaeal sequences were grouped into 176 OTUs in total. The OTU richness, Shannon-H index, and evenness index are

summarized in Table S3 (available with the online version of this paper). The results show that the archaeal diversity was much less than that of bacteria reported for municipal WWTPs at the same pyrosequencing depth, for the latter had at least 1,000 bacterial OTUs per sample (Zhang et al. 2012). The result was consistent with the finding that archaea commonly tend to have lower diversity than bacteria in the majority of environments (Cardinali-Rezende et al. 2012). We speculated that archaea might make use of the activated sludge environment in ways that are more restrictive than for bacteria. The speculation was supported by a previous study (Fredriksson et al. 2012), which confirmed that methanogenic archaea constantly existed in anoxic microenvironments of aerobic activated sludge by FISH, while bacteria occurred broadly in activated sludge.

Archaeal sequences were assigned to 3 phyla, 10 orders and 22 genera using the RDP classifier. The unclassified sequence portions in the total community increased from the phylum level (0.8%) to the genus level (26.5%). In general, methanogenic archaeal orders observed in this study were close to those from Althorpe (England) and Rya (Sweden) WWTPs investigated using cloning libraries and DGGE. However, at the genus level, the archaeal diversity observed here was higher than that in any other previous study on activated sludge WWTPs (Gray et al. 2002; Fredriksson et al. 2012).

As shown in Figure 2, *Euryarchaeota* was the most abundant phylum in WWTPs, accounting for 94.71–97.97% of the classified sequences. This was similar to the results of archaeal communities in a variety of anaerobic reactors (Tabatabaei et al. 2010). *Crenarchaeote* was the second abundant phylum in all samples (0.45–1.89%). The low abundance of *Crenarchaeote* across the 60 samples was

in agreement with previous studies (Gray et al. 2002; Cortes-Lorenzo et al. 2014). This suggested that although *Crenarchaeote* accounted for a small proportion of the total archaeal microbiota, the phylum was a constant component in aerobic wastewater treatment bioreactors.

Within *Euryarchaeota*, eight archaeal orders were revealed in activated sludge WWTPs. Acetoclastic methanogen *Methanosarcinales*, accounting for 84.6% of the total classified sequences, was the most dominant *Euryarchaeota*, followed by hydrogenotrophic methanogens *Methanomicrobiales* (6.2%), *Methanobacteriales* (4.2%), and *Methanococcales* (1.3%). The four orders were present in all samples, except LS, which did not contain *Methanococcales*. The other four orders only occurred in 2 to 11 WWTPs with low abundances (<1%). It was reported that hydrogenotrophic methanogen was predominant at high ammonia and chemical oxygen demand (COD) levels (>2.8 g NH₄⁺ L⁻¹ and >1 g COD L⁻¹); otherwise, acetoclastic methanogen dominated in activated sludge (Regueiro et al. 2012; Fotidis et al. 2014). The low influent levels of ammonia and COD might contribute to the greater abundance of *Methanosarcinales* than that of hydrogenotrophic methanogens in this study (Table S2). Although *Methanococcales* was usually obtained from marine environments and wastewater-fed anaerobic digesters by previous investigations, the minor but stable proportion of *Methanococcales* among geographically different WWTPs suggests that it is a basic component of archaeal community in activated sludge. As a novel hydrogenotrophic methanogen, *Methanocellales* has only been found in anaerobic rice paddy soil (Sakai et al. 2008). In this study, *Methanocellales* was for the first time reported from activated sludge.

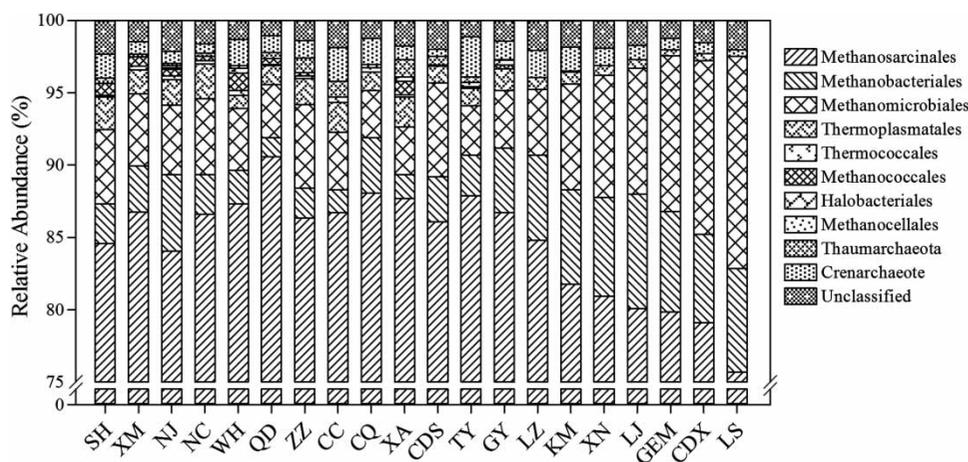


Figure 2 | Relative abundances of archaeal orders of *Euryarchaeota* and other phyla from 20 WWTPs. The phylogenetic assignment of the sequences was determined using the RDP classifier database with a confidence threshold of 80%.

In total, 22 genera were observed from all activated sludge WWTPs in this study. Four genera were shared by all samples, accounting for 94.5% of the classified sequences. A total of 14 genera were commonly shared by more than 10 WWTPs, accounting for 98.6% of all classified sequences. Six genera appeared in no more than four WWTPs, accounting for only 0.2% of the classified sequences. As shown in Figure 3, *Methanosaeta*, dominating approximately 65.4–84.6% of the classified sequences, was the most dominant genus, followed by *Methanosarcina*, *Methanogenium* and *Methanobrevibacter*. The four genera composed a core archaeal population in activated sludge from 20 WWTPs. The high proportion of the core archaeal population across 20 WWTPs suggests that activated sludge systems were more favorable to these core populations than other archaeal genera.

Methanosaeta and *Methanosarcina* are the only known acetoclastic methanogens. *Methanosaeta* was shown to greatly outcompete *Methanosarcina* in all samples. As

reported in anaerobic reactors, the low acetate concentration and long solids retention time (SRT) in activated sludge yielded *Methanosaeta* dominance (McMahon *et al.* 2001; Conklin *et al.* 2006). All of the investigated WWTPs were operated with SRTs of at least 10 days, which were longer than the archaea doubling time of 6.5 days described in Althorpe WWTPs (Gray *et al.* 2002). In activated sludge bioreactors, low prevailing residual acetate concentrations, which were limited by the metabolic pathway of tricarboxylic acid cycle of aerobic micro-organisms (Zhang & Bryant 2011) in activated sludge, contributed to the suppression of *Methanosarcina* by *Methanosaeta*.

Filamentous *Methanosaeta* has been demonstrated to play important roles in the formation and maintenance of granular sludge in upflow anaerobic sludge blanket reactors (UASBR) (Zheng *et al.* 2006). However, the function of *Methanosaeta* in activated sludge remains poorly understood. *Methanosarcina* was usually observed in systems experiencing poor performance, indicating operational instability and acetate

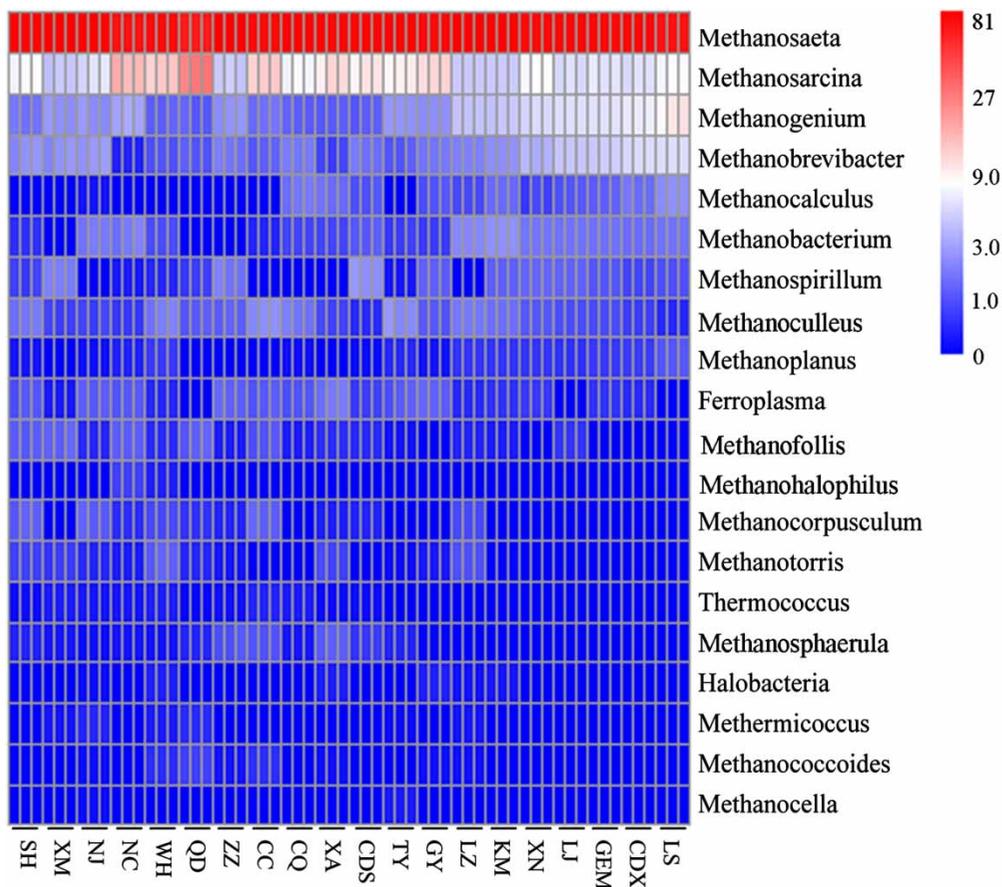


Figure 3 | Heatmap of the top 10 abundant genera from different wastewater treatment phases of each WWTP. The colour intensity in each box indicates the relative percentage of each genus within each WWTP. Composite data of the three samples collected from the same wastewater treatment phase of each WWTP are shown here. Samples from anaerobic, anoxic and aerobic phases displayed very similar community structures. Please refer to the online version of this paper to see this figure in colour: <http://dx.doi.org/10.2166/wst.2017.215>.

accumulation (McMahon *et al.* 2004). Thus, the low abundance of *Methanosarcina* might indicate the good performance of the WWTPs investigated in this study. *Methanogenium*, which uses cold shock proteins to help itself to adapt to cold environments, is usually detected from cold marine sediments (Kendall *et al.* 2007). This study for the first time reported that *Methanogenium* was also present in activated sludge. *Methanobrevibacter*, commonly detected in the rumen and faeces, plays an important role in the efficient digestion of polysaccharides (Federici *et al.* 2015; Nkamga *et al.* 2015). The constant presence of *Methanobrevibacter* in municipal WWTPs implied the potential influence of influent wastewater on activated sludge archaeal communities, due to the great input of human faeces into municipal wastewater.

It is noteworthy that AOA was only detected in SH and CQ WWTPs, represented by 16 raw reads in total. One possible reason was that the sequencing depth in this study was insufficient to detect it. The other possible reason was that AOA was really absent from the samples. The latter possibility was supported by the results of multiple other studies that the specific amplification of AOA ammonia monooxygenase genes usually failed in activated sludge from many municipal WWTPs (Zhang *et al.* 2011).

Variance in archaeal community richness with elevation

As shown in Figure 4, a threshold of elevation associated with archaeal community richness was observed at approximately 1,500 masl. Both OTU richness and Shannon-H index demonstrated that the archaeal community richness varied by approximately two to three times between samples below and above 1,500 masl. The archaeal OTU richness varied stochastically between 29 and 45 at elevations below 1,500 masl, whereas a significant change was

observed at elevation approximately 1,500 masl with a linear decline towards the summit ($R^2 = 0.8473$, $P < 0.01$). The archaeal OTU richness declined from 45 to 14 with elevations from 1,500 to 3,660 masl. In all, a linear declining trend in archaeal community richness was observed in activated sludge WWTPs along the elevational gradient from 1,500 to 3,660 masl. It indicated that the archaeal community richness in activated sludge WWTPs varied significantly with elevation, similarly to the archaeal community of mountain soil (Zhang *et al.* 2009; Singh *et al.* 2012). In the downstream analysis, the lower and higher elevation gradients were distinguished by the threshold of 1,500 masl.

The relative abundance of individual archaeal orders and genera exhibited different associations with elevations above 1,500 masl (Figure S2, available with the online version of this paper). Among the most abundant orders, *Methanosarcinales* presented a significant and negative correlation in relative abundance with elevation ($P < 0.001$), while the relative abundance of *Methanomicrobiales* and *Methanobacteriales* was significantly and positively associated with elevations ($P < 0.001$). At the genus level, both *Methanosaeta* and *Methanobacterium* declined in their relative abundances with increasing elevations ($P < 0.001$). In contrast, the relative abundance of both *Methanogenium* and *Methanobrevibacter* was positively correlated with elevations ($P < 0.001$). Multiple studies reported the adaptation of *Methanogenium* to cold based on its cold shock proteins in other environments (Saunders *et al.* 2003). Thus, the extreme temperature fluctuation in high elevations might contribute to the increase of *Methanogenium* in relative abundance with increased elevation. In all, the changes in the proportions of individual archaea taxa should partly result from the selection of elevation-dependent environmental variables to archaea in WWTPs.

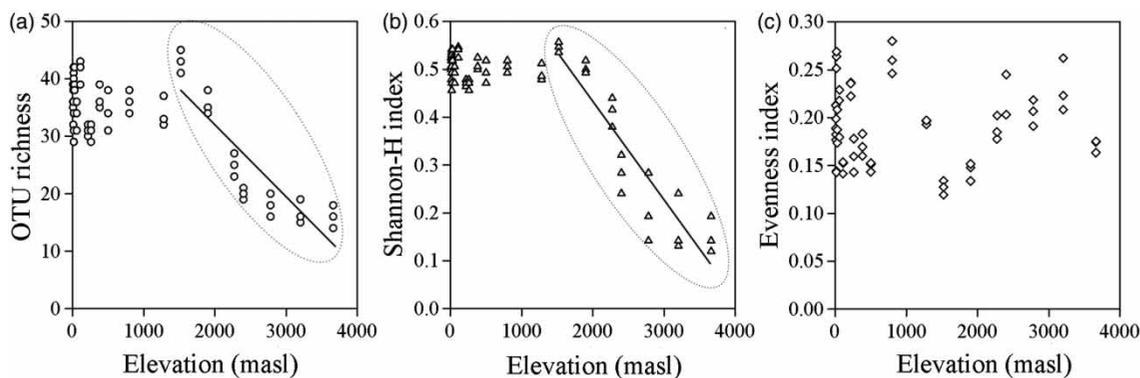


Figure 4 | Elevational patterns in the OTU richness (a), Shannon-H index (b), and evenness index (c) of archaeal communities. A composite dataset of the two or three samples collected from different wastewater treatment phases is shown here. Samples collected in different seasons displayed some minor differences in community richness and evenness.

The linear declining trend in archaeal community richness with increased elevation observed in this study was in agreement with the pattern observed from Mount Fuji, which displayed a mid-elevation ‘peak’ of archaeal community richness at 1,500 masl (Singh *et al.* 2012). This might suggest that the elevational threshold of 1,500 masl is shared in natural environments and human-controlled activated sludge systems. Increasing elevation is characterized by lower air pressure, stronger UV, and more extreme temperature fluctuations (Yang *et al.* 2014). These factors might put greater stress on the archaeal community above 1,500 masl than that below 1,500 masl. Thus, consistent with natural archaeal community, archaeal community richness in WWTPs might be partly affected by elevation-dependent environments. On the other hand, the influent wastewater from outside higher-elevation environment might also contribute to the variation of WWTP archaeal communities. Although some researchers reported that a majority of the archaea were from the recycled mature activated sludge (Fredriksson *et al.* 2012), there is no denying that the great variance among archaeal communities from geographically different WWTPs might originate from the long-term selective accumulation of partial archaea from influent archaea. This assumption needs to be demonstrated in future research.

Spearman correlation analysis indicated that at lower elevations, none of the assessed variables, including elevation and other 30 variables, was significantly and strongly associated with archaeal community richness (absolute values of correlation coefficients (avcc) < 0.5, $P < 0.001$). However, at higher elevations, archaeal community richness was most strongly correlated with elevation, followed by multiple

wastewater variables including effluent concentration of ammonia nitrogen (efNH₄-N), influent concentration of COD (inCOD), influent concentration of biological oxygen demand (inBOD), etc. (avcc > 0.8, $P < 0.001$, Figure 5). Furthermore, Spearman correlation analysis showed that multiple variables, such as inCOD (cc = -0.964) and inBOD (cc = -0.952), were significantly associated with elevation. The result meets with the sharply declining population density and greatly changed lifestyle with increased elevation in western China. The results suggest two possible reasons. On the one hand, regardless of the potential association between altitude and wastewater variables, the observed changes in archaeal community richness should be greatly and directly driven by wastewater variables. On the other hand, altitude is also one of the important variables influencing the quantitative relationships of nitrogen-removal bacteria in WWTPs particularly above 1,500 masl. Although the mechanism of how altitude-dependent natural variables, such as temperature, UV radiation and biogeochemical elements, affect archaea in WWTPs remains unknown, the challenges posed by altitude to archaea in natural habitats have been demonstrated at the metagenome level (Yang *et al.* 2014). In summary, the archaeal community richness in activated sludge WWTPs at higher elevations turned out to be much more closely associated with environmental factors than that at lower elevations.

Linkage between environmental variables and archaeal community structures from activated sludge

Significant elevational differences in archaeal community structure among WWTPs were also observed via clustering

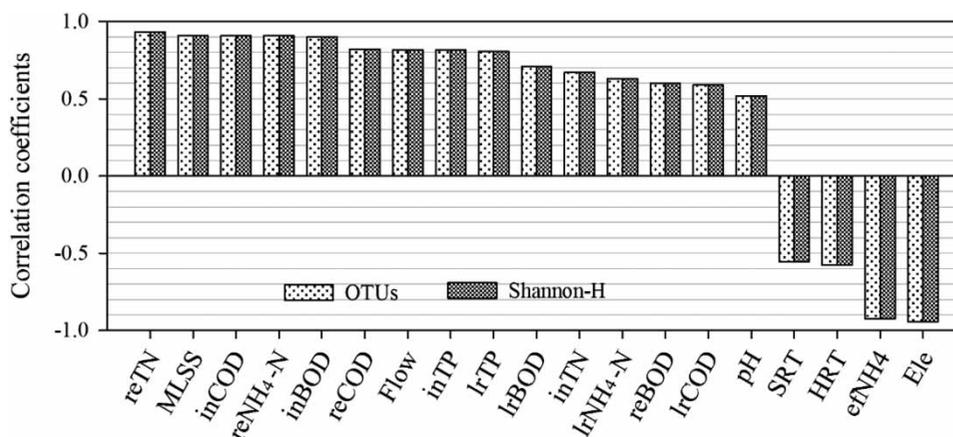


Figure 5 | Spearman correlation analysis for the associations between archaeal taxonomic richness and environmental variables above 1,500 masl (two-sided $P < 0.01$). The taxonomic richness was characterized by OTU richness and Shannon-H index separately. Ele, elevation; SRT, solids retention time; HRT, hydraulic retention time; Flow, flow rate; TN, total nitrogen; NH₄-N, ammonia nitrogen; BOD, biological oxygen demand; COD, chemical oxygen demand; TP, total phosphorus; MLSS, mixed liquor suspended solids; reTN, removal efficiency of TN; inCOD, influent concentration of COD; IrBOD, loading rate of BOD.

analysis. Figure S3 (available with the online version of this paper) shows that the archaeal communities from 20 WWTPs could be clustered into eight groups: Group I contains the sampled WWTPs located at 1,500–3,660 masl; Groups II to V contain the WWTPs located at 110–1,280 masl in western China; Groups VI to VIII contain all WWTPs below 60 masl. The grouping pattern of these WWTPs was significantly characterized by the three elevational gradients. A threshold of elevation associated with archaeal community structures was also obtained at 1,500 masl. The clusters of WWTPs below 1,500 masl did not agree with their elevational distances. For example, XA samples (386 masl) were more close to GY samples (1,280 masl) than TY samples (800 masl) on the clustering tree. Group II (110 masl) displayed a larger phylogenetic distance to group V (218 masl) than to groups III and IV (261–1,280 masl). However, the clustering relationships of WWTPs within group I almost corresponded to the elevational distances among WWTPs above 1,500 masl.

RDA showed that the explanatory percentages of elevation to the variance of archaeal community structures were 2% and 6% at lower and higher elevations, respectively. The explanatory percentage of variables to community structures corresponds to the influencing power of variables to community structures (Wang *et al.* 2012). Thus, the result suggested that the influence of elevation on archaeal community structures in WWTPs at higher elevations was much more than that at lower elevations. In addition, it is noteworthy that the explanatory power of operational and wastewater variables to archaeal

community structures was much higher than that of elevation (averaging 4%) at both lower and higher elevations. This result indicates that although the archaeal community structure in activated sludge was significantly associated with elevation, it remains more influenced by operational and wastewater variables.

Archaeal community structures were correlated with different environment variables at lower and higher elevations separately (Figure 6). At lower elevations, the community structures were significantly associated with 12 variables among the 31 assessed variables (Figure 6(a)). SRT (12%), inBOD (8%), food to microorganism ratio (F/M, 8%), and treatment process (7%) were the dominant contributors to the variance of community structures. At higher elevations, only six variables significantly contributed to the variance of community structures (Figure 6(b)). Loading rate of BOD (lrBOD, 22%), effluent concentration of BOD (efBOD, 18%), dissolved oxygen (DO, 9%), and hydraulic retention time (HRT, 9%) were the four dominant contributors to the variance of community structures. The results indicate that the archaeal community structure was most associated with wastewater variables at higher elevations, followed by operational variables and elevation.

Scarce information on the relationships between environment variables and archaeal community structures in activated sludge was described in previous studies. However, multiple studies have demonstrated the important influences of environment variables on archaeal community structures in anaerobic reactors. It was reported that the fluctuations in the organic loading rate and F/M ratio affected the

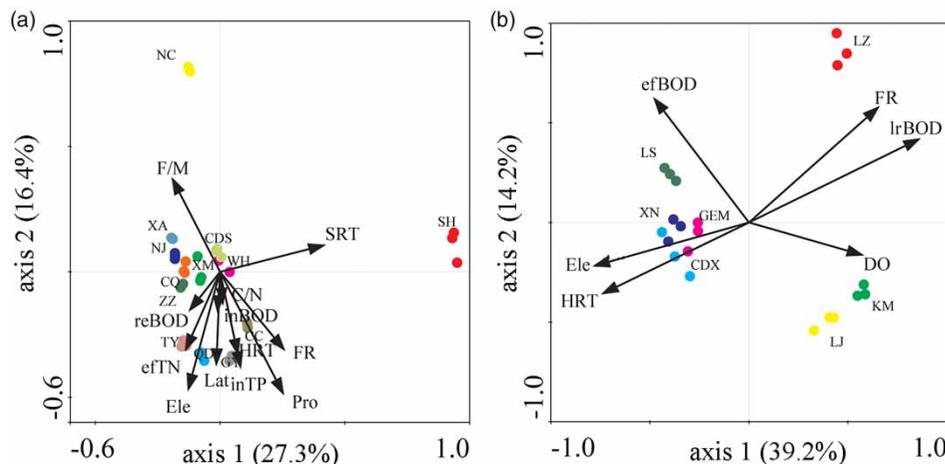


Figure 6 | RDA of archaeal community structures and environmental variables at the elevational gradients below 1,500 masl (a) and above 1,500 masl (b) separately. Composite data of the two or three samples collected from different wastewater treatment phases in each season are shown here. Samples collected in different seasons displayed some minor difference in community structure. Pro, treatment process; FR, flow rate; F/M, food to microorganism ratio; inBOD, influent concentration of BOD; reBOD, removal efficiency of BOD; DO, dissolved oxygen.

abundance and structure of archaeal communities at the species level in UASBR (Cardinali-Rezende *et al.* 2013). Jing *et al.* (2013) demonstrated the significant influence of HRT on the function and community of UASBR. HRT was determined to be the third influencing variable on the performance and community of UASBR reactors via the method of partitioning connection weights in the study of Shi *et al.* (2009). Conklin *et al.* (2006) reported that *Methanosaeta* was more abundant than *Methanosarcina* in anaerobic reactors with longer SRT. All WWTPs assessed in this study were performed with SRTs more than 10 days, which were longer than the archaea doubling time of 6.5 days as described in other studies (Gray *et al.* 2002). Therefore, the long SRT in activated sludge might contribute to the competition of individual methanogens.

The variance of archaeal community structure in activated sludge presented a weak association with removal efficiency of BOD (reBOD, 1%) at lower elevations, but significant and strong association with efBOD (18%) at higher elevations (Figure 6). On the other hand, the archaeal community richness also showed significantly positive association with multiple variables of WWTP performance, such as reCOD and reBOD, at higher elevations (Figure 5). These results implied that the archaeal community structure and richness in activated sludge might potentially influence the WWTP performance at higher elevations. Fredriksson *et al.* (2012) also reported that the relative abundance of *Methanosaeta* in activated sludge was significantly and negatively correlated with effluent water parameters. However, it cannot be concluded that archaea have an important and direct influence on WWTP performance. As the community structure of methanogens in bioreactors was greatly shaped by the metabolic products (e.g. acetate) of bacterial community (Zhang & Bryant 2011), the archaeal community structures might be interfered with by the variance of bacterial communities in activated sludge. Alternatively, since bacteria have been thought to be the important contributor to WWTP performance, the functional and taxonomic linkages of bacteria and archaea in wastewater treatment processes must be explored completely before determining their relevant contributions to WWTP performance.

CONCLUSIONS

This study for the first time investigated the archaeal diversity in full-scale activated sludge WWTPs at the elevation scale via 454-pyrosequencing. A core archaeal group composed of four genera dominated the archaeal community in

activated sludge. Archaeal community richness and structure presented significant elevational patterns, with a threshold of elevation at approximately 1,500 masl. At higher elevations, a declining trend in community richness with increased elevation was observed. The community structure was more associated with wastewater variables at higher elevations than at lower elevations. This study provides new insights into the relationship between elevation and archaeal communities in activated sludge WWTPs. The results observed in this study would fundamentally aid in further researches on the manipulation and optimization of archaeal mediated processes in WWTPs at higher elevations.

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