Spatiotemporal succession of ammonia-oxidizing archaea abundance and composition in a created riparian wetland

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Cascaded performance and compartments of constructed wetlands may harbor the internal eco-environmental gradients and further differentiate the niche and community structure of ammonia-oxidizing archaea (AOA). Here, we used quantitative PCR and 454 pyrosequencing of AOA *amo*A gene to investigate the abundance, diversity, composition, and driving factors of sedimentary AOA community in a created riparian wetland---Shijiuyang constructed wetland (SJY-CW) in China. Results showed that the heterogeneous wetland with serial-connected combinations of pretreatment pond, plant-bed/ditch system, and post-treatment pond as well as the third through fifth year succession (2011--2013, corresponding to the third through fifth year of actual operation after the initial two-year trial operation) had exerted significant spatiotemporal niche selection effects on AOA abundance and composition. AOA abundance in the pre- and post-treatment ponds was almost invariant in 2011--2012 and decreased in 2013, while the abundance in the plant-bed/ditch system decreased gradually with the succession of SJY-CW. The AOA abundance ended up with one order of magnitude lower and tended to be consistent in the whole wetland. In contrast to abundance, the temporal niche differentiation of AOA diversity was insignificant. Nonetheless, a rough decrease trend was detected for the average community richness index Chao1 and diversity index Shannon *H*' in 2011--2013. Community structure analysis showed that *Nitrososphaera* was dominant genus, while *Nitrosopumilus* cluster and *Nitrososphaera* sister cluster increased with the succession of SJY-CW. Hierarchical clustering analysis and redundancy analysis both verified the horizontal shifts of AOA communities and these shifts inclined to occur in the most heterogeneous plant-bed/ditch system. Operational duration of the wetland which comprehensively reflected the wetland hydrology, water quality, and associated functions became the key factor driving the AOA abundance and community shift in SJY-CW sediments. In a nutshell, with the third through fifth year succession of wetland, the

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sedimentary ammonia-oxidizing archaea tended to be characterized by temporally decreased and spatially consistent abundance, while spatially variable and temporally decreased richness and diversity; regarding the AOA community structure, temporally from local species and relatively uniform distribution to mixing with allochthonous species and highly diversified distribution, while spatially occurring significant horizontal shifts of AOA *amo*A genes. These results showed the considerable if not significant niche selection effect of AOA abundance, diversity, and community structure with the operation of SJY-CW, and this will have some implications on the microbial ecology of archaeal ammonia-oxidization in a man-made wetland ecosystem.

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ABSTRACT

Cascaded performance and compartments of constructed wetlands may harbor the internal eco-environmental gradients and further differentiate the niche and community structure of ammonia-oxidizing archaea (AOA). Here, we used quantitative PCR and 454 pyrosequencing of AOA *amo*A gene to investigate the abundance, diversity, composition, and driving factors of sedimentary AOA community in a created riparian wetland—Shijiuyang constructed wetland (SJY-CW) in China. Results showed that the heterogeneous wetland with serial-connected combinations of pretreatment pond, plant-bed/ditch system, and post-treatment pond as well as the third through fifth year succession (2011–2013, corresponding to the third through fifth year of actual operation after the initial two-year trial operation) had exerted significant spatiotemporal niche selection effects on AOA abundance and composition. AOA abundance in the pre- and post-treatment ponds was almost invariant in 2011–2012 and decreased in 2013, while the abundance in the plant-bed/ditch system decreased gradually with the succession of SJY-CW. The AOA abundance ended up with one order of magnitude lower and tended to be consistent in the whole wetland. In contrast to abundance, the temporal niche differentiation of AOA diversity was insignificant. Nonetheless, a rough decrease trend was detected for the average community richness index Chao1 and diversity index Shannon *H'* in 2011–2013. Community structure analysis showed that *Nitrososphaera* was dominant genus, while *Nitrosopumilus* cluster and *Nitrososphaera* sister cluster increased with the succession of SJY-CW. Hierarchical clustering analysis and redundancy analysis both verified the horizontal shifts of AOA communities and these shifts inclined to occur in the most heterogeneous plant-bed/ditch system. Operational duration of the wetland which comprehensively reflected the wetland hydrology, water quality, and associated functions became the key factor driving the AOA abundance and community shift in SJY-CW sediments. In a nutshell, with the third through fifth year succession of wetland, the sedimentary ammonia-oxidizing archaea tended to be characterized by temporally decreased and spatially consistent abundance, while spatially variable and temporally decreased richness and diversity; regarding the AOA community structure, temporally from local species and relatively uniform distribution to mixing with allochthonous species and highly diversified distribution, while spatially occurring significant horizontal shifts of AOA *amo*A genes. These results showed the considerable if not significant niche selection effect of AOA abundance, diversity, and community structure with the operation of SJY-CW, and this will have some implications on the microbial ecology of archaeal ammonia-oxidization in a man-made wetland ecosystem.

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INTRODUCTION

Ammonia oxidation, the first and rate-limiting step of nitrification, has been known to be mediated by certain groups of chemolithoautotrophic proteobacteria—ammonia-oxidizing bacteria (AOB) for more than a hundred years (Prosser, 1989; Schleper, 2010) which are confined to Beta- and Gammaproteobacteria (Teske et al., 1994). The recent discovery of homologs of ammonia monooxygenase genes in archaea by metagenomic analysis (Treusch et al., 2005) as well as the isolation and cultivation of ammonia-oxidizing Crenarchaeota *Nitrosopumilus maritimus*—a marine archaeon (Könneke et al., 2005) have radically changed this view and proved the existence of an additional, quite predominant group of ammonia-oxidizing archaea (AOA) and their critical role in catalyzing the ammonia oxidation process (Treusch et al., 2005; Schleper, 2010). Archaea differ from eukarya and bacteria in terms of genetic, biochemical, and structural features. All currently known AOA belong to the phylum Thaumarchaeota. They can be classified into five monophyletic clusters: *Nitrosopumilus*, *Nitrososphaera*, *Nitrosocaldus*, *Nitrosotalea*, and *Nitrososphaera* sister clusters, and then were hierarchically subdivided into a second and the third phylogenetic level (Pester et al., 2012). Leininger et al. (2006) first reported that AOA was dominant among ammonia oxidizers in soils. Subsequent studies have confirmed the widespread distribution of AOA and their numerical predominance over AOB in various environments, such as ocean system (Wuchter et al., 2006), agricultural acidic soil (Leininger et al., 2006), drinking water plant (Kasuga et al., 2010), and alluvial intertidal wetland ecosystem (Hu et al., 2014). The significant roles of AOA in ammonia oxidization of various ecosystems and environments has attracted intense attention in recent years (Shen et al., 2012). Numerous studies provide strong support of the dominance of archaea in regulating the ammonia oxidation in both soil and aquatic systems (Leininger et al., 2006; Zhang et al., 2010; Pratscher et al., 2011).

The physiological and biochemical characteristics of AOA determine their evolution and adaption to the particular sets of environmental features (Prosser and Nicol, 2012), and the diversity and community structure of AOA can be in turn significantly influenced and altered by environmental conditions, including ammonia nitrogen level, temperature (Tourna et al., 2008), pH (Nicol et al., 2008), plant species (David et al., 2013), and salinity (Bernhard et al., 2010) and so on. For example, AOA from marine water column and those from soil environments were clustered separately, indicating different AOA species had different requirements for environmental conditions (Francis et al., 2005). Meta-analysis and high-throughput sequencing analysis by Gubryrangin et al. (2011) demonstrated that soil pH significantly determined the community structure of AOA. In particular, AOA had a preferential adaptation to much lower ammonia content (Verhamme et al., 2011), and could survive in extreme low ammonia concentrations ($\leq$ 10 nM) with a half-saturation constant (K_m) of 133 nM total ammonium (Martenshabbena et al., 2009), so the substrate availability was an essential element for AOA growth. Although most AOA species are mesophilic (Brochierarmanet et al., 2008), *Nitrosocaldus yellowstonii* was found to grow in hot springs at temperatures as high as 74 °C (de la Torre et al., 2008), indicating that temperature was an important factor which affected the AOA distribution. All above mentioned researches revealed that the environmental factors might dramatically change the behavior, distribution, and evolution of AOA.

Constructed wetlands (CWs), also known as treatment wetlands, are found to be generally effective in removing nutrients, chemicals, and microorganisms, and such effects may lead to some specific gradients of environmental variables (Vymazal, 2011), such as soil features, nutrient level, soil temperature, organic matter condition, wetland hydrology and water quality. Consequently, it was assumed that the operation and succession of CW can potentially affect the distribution and community structure of AOA because of the altered environmental factors. Fan et al. (2016) has already reported the changes of AOA abundance and community composition along the series treatment cells in two CWs due to the different removal efficiency of the wetlands, and inferred that AOA may be more critical than AOB for depleting nutrients in the CWs. Although approaches have been developed to study microbial diversity in natural wetlands, knowledge of the microbial diversity of constructed wetlands is scarce. Although the spatial variation of AOA communities in CWs had been documented, the information on AOA's temporal change is still limited, especially in a continually operated CW and for a relatively long period of time.

Of particular ecological significance, and being unclear about the spatiotemporal patterns of AOA communities with the preliminary operation of a created riparian wetland, it is urgent to get a thorough understanding of the distribution and drivers of ammonia-oxidizing archaea diversity and composition with the succession of a constructed wetland. In the present study, we chose a created riverine drinking water source treatment wetland—Shijiuyang constructed wetland (SJY-CW) in Zhejiang

Province, China to explore the distribution and differentiation of AOA abundance, diversity, and community structure. The wetland is composed of the serial-connected combinations of pretreatment pond, plant-bed/ditch system, and post-treatment pond. The constructed root channel technology and enhanced plant-bed/ditch system (shortly as Plant-bed system) in SJY-CW were adopted to enhance the nutrient interception, filtration, and removal efficacy (Wang et al., 2012; Zheng et al., 2012), and the gradient nutrient concentrations with the operation time and the distinctive functions of individual compartments may contribute to specific AOA populations. In this study, we aim to examine the temporal and spatial distribution of AOA abundance, diversity, and community structure in SJY-CW. The whole study was designed to test the hypothesis that the horizontal shifts of AOA diversity and community structure would occur with the three-year (the third through fifth year of actual operation time) primary succession of SJY-CW on temporal scale and mainly in the heterogeneous plant-bed/ditch system on spatial scale. We also examined the environmental drivers which significantly affected the distribution and structure of AOA communities.

MATERIALS AND METHODS

Ethics statement

Field experiments were approved by the Jiaxing Science and Technology Bureau (project number: 2011AZ2019, 2015AY23008). Our research work did not involve capture or any direct manipulation or disturbance of animals. We collected samples of sediments and water for molecular and physicochemical analyses. We obtained access to the wetland under the permission of the Jiaxing Water Conservancy Investment Company Ltd. (Jiaxing, Zhejiang, China), which is responsible for the operation and management of the wetland.

Site description and sampling

Shijiuyang constructed wetland (SJY-CW), a created riverine drinking water source treatment wetland, is located in Jiaxing City, Zhejiang Province, China, and is the final defensive barrier before the source river water enters the intake of a local drinking water plant (Wang et al., 2013a, 2016). This region belongs to a subtropical monsoon zone with a clear distinction between four seasons. The SJY-CW is composed of a pretreatment zone (Inlet region), Plant-bed system I (West region), a water-level rising and aeration zone, Plant-bed system II (South region), Plant-bed system III (North region), and a post-treatment zone or deep purification zone (Outlet region), covering a total surface area of about 110 ha and a watered area of about 60 ha. On the top of Plant-bed system, southern reed (*Phragmites australis* Trin.) was transplanted as the dominant species because its rhizospheric environment showed a strong water treatment and the associated ammonia-oxidizing process (Wang et al., 2002; Wang and Yin, 2008; Wang et al., 2013a). The detailed structure, composition, design parameters, and water quality performance assessment of SJY-CW refer to the publication (Wang et al., 2016).

To detect the temporal succession trend of AOA communities, we collected sediment samples from the third through fifth operational year of SJY-CW after experiencing the initial two years of trial operation and systematic debugging, i.e., October 2011, December 2012, and August 2013, respectively. Considering the specific purification process of each functional zone (separate compartment), sampling was carried out in five stations corresponding to the key nodes of hydraulic pathways, namely the inflow or outflow of each functional zone (Figure 1). Two or three replicate surface (0–10 cm) sediment subsamples were collected at each station and mixed evenly to form one composite sample. The homogenized samples were sealed and transported to the laboratory on ice while kept cold at 0–4°C for chemical component analysis. Simultaneously, subsamples were stored at -80°C for later DNA extraction and molecular analysis.

Chemical analyses of the samples

Sedimentary pH and redox potential (E_h) were measured in situ using an IQ150 probe (HACH, USA). Ammonia (NH_4^+-N) and nitrate (NO_3^--N) of the sediments were extracted with 2 $mol\,L^{-1}$ KCl in 1:5 ratio (fresh sediment to solution) and measured colorimetrically (Strickland and Parsons, 1972). Organic matter (OM), total carbon (TC), total nitrogen (TN), and total sulfur (TS) of sediment samples were determined according to standard methods (Bao, 2000). Each measurement was carried out in triplicate for QA/QC. Results were presented in the supplementary Table S1.

DNA extraction and PCR amplification

In order to avoid DNA extraction bias, DNA was extracted in triplicate for each sample using a FastDNA SPIN kit for soil (MP Biomedicals, Solo, OH, USA) according to the manufacturer's protocol. The extracted DNA was checked on 1% agarose gel and the concentration was determined with NanoDrop® ND-1000 ultraviolet-visible spectrophotometry (Thermo Fisher Scientific Inc., USA). PCR was performed in a C1000™ thermal cycler (BioRad, USA). Each replicate DNA extract was used to amplify an approximately 629 bp long fragment of the archaeal *amo*A using the primers Cren*amo*AF (5'-GCARGTMGGWAARTTCTAYAA) and Cren*amo*AR (5'-AAGCGGCCATCCATCTGTA) (Mincer et al., 2007). To distinguish AOA *amo*A amplicons originating from different sediment samples the barcode of 7 bp in length were used (Binladen et al., 2007). The following PCR procedure was applied following the related PCR protocol (Francis et al., 2005): an initial denaturation at 95 °C for 5 min, followed by 39 cycles of denaturation at 94 °C for 50 s, annealing at 53 °C for 70 s, and extension at 72 °C for 60 s followed by a final extension step at 72 °C for 10 min. The PCR products were checked by 2% (w:v) agarose gel electrophoresis and gel-purified following the manufacturer's instructions. The quality and quantity of purified PCR products were determined by NanoDrop® ND-1000 ultraviolet-visible spectrophotometry (Thermo Fisher Scientific Inc., USA). Finally, the purified PCR products were performed on the GS FLX Titanium platform (454 Life Sciences, Branford, CT, USA) at the Personalbio Company in Shanghai.

Real-time quantitative PCR

SYBR Green I based real-time quantitative PCR assays were carried out in a volume of 20 μL, containing 10 μL SYBR® Premix Ex Taq™ (TAKARA, Dalian, China), 4 pmol of each primer, and 2 μL of 10-fold diluted DNA template. The primer pairs for real-time PCR assays were described as above (Mincer et al., 2007). Amplification and detection were carried out with an ABI Prism 7300 Sequence Detection System (Applied Biosystems, USA) with the following procedure: 95 °C for 30 s; the major cycling program: 30 cycles of denaturation at 95 °C for 30 s, annealing at 53 °C for 30 s, and extension at 72 °C for 45 s; followed by 95 °C for 15 s, 60 °C for 1 min, 95 °C for 15 s, and 60 °C for 15 s for dissociation. Three no-template controls (NTCs) were run for each quantitative PCR assay. Tenfold serial dilutions of a known copy number of the plasmid DNA were subjected to real-time PCR in triplicate to generate an external standard curve. Melting curves were generated after each assay to check the specificity of amplification. PCR efficiencies were 90–103% (average 92%) for the ammonia-oxidizing archaea *amo*A gene. Only the results with correlation coefficients above 0.98 were used. Data were analyzed using ABI 7500 System SDS Software (Applied Biosystems).

Pyrosequencing and data analysis

The pyrosequencing was performed to analyze the AOA *amo*A community structure at the Personalbio Company in Shanghai. Bioinformatic analysis was performed mainly using Mothur software (Schloss et al., 2009). The obtained raw sequences were assigned to each samples according to their specific barcodes and primer sequences, and low-quality sequences (quality score, <25; length, <350 bp; ambiguous bases, >1; homopolymer >6) were removed. Muscle was applied to screen for chimeras (Edgar, 2004). Total of 44111 high-quality raw sequence reads were obtained for AOA after this process.

The 454 sequencing errors were further minimized in the next step. High-quality sequences by the above selection procedures were pre-clustered using pre.cluster function in Mothur (http://www.mothur.org). Pre.cluster function assigns less abundant sequences to more abundant sequences by assuming that the possibility of a 454 sequencing error to occur is higher in less abundant sequences (Pester et al., 2012). Thereafter, representative sequences of pre.cluster were screened for frame shifts to reduce error when translating DNA sequence to protein sequence (caused by 454 sequencing error) using reference-based approach with Framebot (Wang et al., 2013b). Total 42159 high quality-screened sequences were obtained for AOA community analysis.

The obtained high-quality sequences were clustered into operational taxonomic units (OTUs) based on 97% sequence similarity identity via the Uclust (Edgar, 2010). Phylogenetic assignment of AOA was made by aligning representative sequence of each OTU with the reference database using QIIME (Caporaso et al., 2010). What's more, the reference database was deduced by ARB software package (Ludwig et al., 1998).

Statistical analysis and drawing

OTU-based community diversity indicators (Shannon *H'* and Chao1) and rarefaction curves were obtained at 97% cutoff using Mothur program (Schloss et al., 2009) after normalizing the AOA sequence to the smallest library sequence. Coverage was calculated as the ratio of the observed OTU to Chao1 (Hou et al., 2013), and this index was used to evaluate the sequencing depth.

Linear relationships between environmental and operational variables and AOA *amo*A gene abundance, diversity indexes were measured using Spearman Correlation under SAS for Windows 9.2 software (SAS Institute Inc., Cary, NC, USA), and the statistical significance was generally set at the $\alpha = 0.05$ level (He et al., 2010). Parametric methods are preferably selected for data following normal distribution as tested by PROC UNIVARIATE procedure, and reverse nonparametric methods are well suited for situations where little is known about the distribution under study. Mantel test was adopted to assess the correlation between AOA community structure and environmental variables (He et al., 2012) and the analysis was performed by functions in the vegan package (v.2.2-0) in the R software (The R Foundation) (Dixon, 2003). Multiple regression by stepwise selection was used to determine the contribution of the predicator variable to AOA abundance and diversity under SAS for Windows 9.2 software (SAS Institute Inc., Cary, NC, USA). The criteria for entry into the model and for remaining in the model were set as the 0.1500 significance level.

UPGMA (unweighted pair-group method with arithmetic means) hierarchical clustering was implemented to compare the similarity of AOA communities among the samples based on the Bray-Curtis matrix. Analysis of similarity (ANOSIM) was executed to further confirm significant similarity in community composition between the sediment samples as identified by the UPGMA clustering. The relative abundance of *amo*A AOA subclusters was produced by the Circos software online (http://circos.ca). Redundancy analysis (RDA) was employed to link AOA communities with the sediment features using the vegan package (v.2.2-0) in the R software version 3.1.2 (The R Foundation) (Dixon, 2003).

Figures were generally drawn by OriginPro 8.5.1 SR2 (OriginLab Corporation, MA, USA) and Adobe® Illustrator® CS3 13.0.0 (Adobe Systems Incorporated, CA, USA).

Deposited 454 sequence read accession numbers

Sequences were submitted to the Sequence Read Archive (SRA) at the National Center for Biotechnology Information (NCBI) under the accession number range from SRR4244883 to SRR4244897 in a single BioProject SRP089950.

RESULTS

Abundance of AOA in SJY-CW sediments

For any complex system, the number and relative abundance of parts is fundamental to a quantitative description of the system (Gans et al., 2005). The AOA *amo*A gene copy numbers in SJY-CW sediments were shown in Figure 2. In general, the AOA *amo*A gene copy numbers decreased significantly from 2011 to 2013, with median numbers of 7.0×10^5 copies g^{-1} dry soil in 2011, 3.4×10^5 copies g^{-1} dry soil in 2012, and 0.8×10^5 copies g^{-1} dry soil in 2013. However, the AOA *amo*A gene copies had different variation patterns in compartments. The abundance levels in the Inlet and Outlet regions (ponds) were close in every year of the studied three years; the abundance in both regions maintained a nearly same level in 2011 and 2012, and then sharply decreased in 2013. Compared with the Inlet and Outlet regions, the Plant-bed system (West, South, and North regions) generally had a higher AOA *amo*A gene copies. Furthermore, the abundance levels in three regions of the Plant-bed system differed considerably and decreased gradually with the succession of SJY-CW (Figure 2). South region, the central pivotal purification zone, always had the highest AOA *amo*A gene abundance in contrast to the rest regions of SJY-CW in the studied three years. These variation patterns suggested that the differentiation of AOA abundance first occurred in the Plant-bed system.

In order to investigate the reasons of AOA abundance variation, the environmental and operational factors were analyzed linking with the AOA *amo*A gene copy numbers (Table S2). Since the Plant-bed system (West, South, and North regions) as well as the Inlet and Outlet regions had different selection effect on AOA abundance, the Spearman correlation analyses were carried out among all samples ($n = 15$) from five regions, samples ($n = 6$) from the Inlet and Outlet regions, and samples ($n = 9$) from the Plant-bed system (West, South, and North regions), respectively. When all samples were examined, the AOA *amo*A functional gene abundance was significantly correlated with Days ($r = -0.7422$, $p =$

0.0015), Temperature ($r = -0.6047$, $p = 0.0169$), and NO_3^--N ($r = 0.5536$, $p = 0.0323$); stepwise regression analysis revealed that the operation time (Days) could explain the variation of AOA abundance by 48.03%, and no other variable met the 0.1500 significance level for entry into the model. The AOA abundance from the Plant-bed system (West, South, and North regions) samples was correlated with Days ($r = -0.9115$, $p = 0.0006$), TC ($r = 0.7500$, $p = 0.0199$), and TN ($r = 0.7167$, $p = 0.0298$); stepwise regression analysis revealed that Days could explain the variation of AOA abundance in the Plant-bed system by 76.18%, and no other variable met the 0.1500 significance level for entry into the model. Correlation analysis did not detect the significant correlation at the $\alpha = 0.05$ level between the AOA abundance from the Inlet and Outlet regions and environmental and operational factors; nonetheless, stepwise regression analysis revealed that Temperature and ORP could explain the variation of AOA abundance in the Inlet and Outlet regions by 78.94% and 13.91% respectively which together explained an accumulative proportion of 92.85%, and no other variable met the 0.1500 significance level for entry into the model.

Diversity of AOA in SJY-CW sediments

The 454-amplicon sequencing approach was used to investigate the AOA *amo*A gene diversity in SJY-CW sediments. In total, 42159 high quality sequences with an average length of 519 bp (ranging between 359–675 bp) were obtained from 15 sediment samples after completing the quality control process. Rarefaction analysis (Figure S1) and Good's coverage index (Good, 1953) (Table 1) all revealed that the sequences in current study could cover the diversity of AOA communities in SJY-CW sediments. Normalizing sequences to an equal sampling depth of 1500 reads per sample (the smallest library sequences), all sequences were clustered into 79 to 224 observed OTUs at 97% cutoff (Table 1). Meanwhile, two biodiversity indexes including Chao1 estimator and Shannon *H'* index were calculated as given in Table 1.

Table 1. AOA *amo*A gene diversity in the sediments of SJY-CW.

Year	Functional zone	Number of OTUs	Coverage	Chao1	Shannon *H'*
2011	Inlet region	141	0.964	219	3.75
	West region	139	0.961	229	3.70
	South region	123	0.974	148	3.54
	North region	145	0.956	268	3.59
	Outlet region	224	0.937	492	4.44
2012	Inlet region	151	0.958	353	3.99
	West region	195	0.944	289	4.03
	South region	117	0.976	149	3.49
	North region	114	0.969	174	3.21
	Outlet region	116	0.982	141	4.07
2013	Inlet region	79	0.981	120	2.98
	West region	192	0.958	246	4.39
	South region	161	0.956	272	4.04
	North region	126	0.977	163	3.63
	Outlet region	87	0.978	143	3.30

Results indicated that the AOA microdiversity had a rough decrease trend with the succession of SJY-CW. The average Shannon *H'* indexes for the studied three years were 3.80±0.37 (mean±S.D.) in 2011, 3.76±0.39 in 2012, and 3.67±0.56 in 2013. Shannon *H'* indexes had a spatially variable state in separate compartments. The AOA diversity of sediment samples in the Outlet region was higher than that in the Inlet region in the studied three years (Table 1). Among the Plant-bed system, West region had the highest AOA community diversity than the other two regions. In general, the AOA diversity kept a relatively stable state (Shannon *H'* index: 2.98–4.44, coefficient of variation: 11.17%) and there were no apparent variation trends from the third through fifth year succession of SJY-CW.

Community structure of AOA in SJY-CW sediments

Microbial microdiversity demonstrates shifts in the dominance of functional microorganisms in ecosystems (Brazelton et al., 2010). The relative abundance of AOA species in SJY-CW was shown in Figure

3. Results showed that compared with the database (Pester et al., 2012), the SJY-CW sediments contained a wide range of AOA phylotypes. To be specific, 87.26% of all sequences were affiliated with the *Nitrososphaera* cluster. Only 7.88%, 4.22%, and 0.64% of AOA *amo*A sequences were clustered into *Nitrosopumilus* cluster, *Nitrososphaera* sister cluster, and *Nitrosotalea* cluster respectively. However, there was no sequence affiliated with *Nitrosocaldus* cluster (Thermophilic AOA) which was reported more suitable at 60–74 °C (Hatzenpichler et al., 2008). Although the individual samples were all dominated by *Nitrososphaera* cluster, the other three AOA clusters gradually increased in the second and third studied year of SJY-CW (Figure 3), which indicated that the AOA community structure changed from relatively uniform distribution to highly diversified ones with the succession of SJY-CW.

In detail, *Nitrososphaera* cluster could be further classified into 7 major subclusters (with sequences $>2\%$, subcluster 1, 3, 4, 6, 8–10); *Nitrosopumilus* cluster could be classified into subclusters 1, 5 (Figure 4 and Figure S2). The *Nitrososphaera* subclusters 9, 3, 10, 4 were dominated in most samples except for 2013-N and 2013-Out, while the samples in 2013-N and 2013-Out were dominated by *Nitrososphaera* subcluster 9, *Nitrosopumilus* subcluster 5, and *Nitrososphaera* subclusters 10, 1 respectively. *Nitrososphaera* subcluster 1 and *Nitrosopumilus* subclusters 1, 5 represented specific implantation position in the studied wetland. There was an obvious increase trend for *Nitrososphaera* subcluster 1 in sample 2013-Out. *Nitrosopumilus* subclusters 1, 5 showed the similar trend in SJY-CW sediments: at first, *Nitrosopumilus* species were rare in every functional zone in 2011; these two subclusters increased in the front parts of functional zones in 2012; then *Nitrosopumilus* species were disappearing along the water flow pathways with the operation of wetland, and their abundance ended up with an increase in the rear parts of SJY-CW.

Community shifts of AOA in SJY-CW sediments

Based on the Bray-Curtis similarity matrix, the AOA *amo*A gene libraries in SJY-CW were classified into eight different groups ($p < 0.05$). These groups were not clustered only according to sampling time or functional zones; they were influenced by an interaction of both factors (Figure 5). In 2011, the AOA libraries were clustered into two groups, one group for Outlet region and another for the rest four regions. In 2012, the AOA libraries were clustered into four groups: Inlet region and North region shared one group, and the other three functional zones were distributed in three different groups. In 2013, the AOA libraries were clustered into five entirely separate groups. It strongly suggested that the community structure of AOA in SJY-CW sediments was developing from spatial similarity to more and more diversified phylogeny. In spatial dimension, the samples from Inlet region during the studied three years shared the same group; those from the Plant-bed system harbored more diversified communities compared with the Inlet and Outlet regions indicating that the community structure shifts preferred to occur in the complex and heterogeneous Plant-bed system; while the samples from Outlet region in the first and second studied years had a close relationship but not homogeneous with the sample from the third studied year (the red hot block in Figure 5 represents the succession of AOA communities). AOA community structure was going on differentiation with the succession of SJY-CW, and the community shifts mainly occurred in the Plant-bed system.

The redundancy analysis (RDA) results (Figure 6) showed that the samples from the studied three years were only partly overlapped with each other, which suggested to some extent that the AOA community structure was changed by the primary succession of SJY-CW. In spatial dimension, five compartments samples from 2011 showed relative close distance, and different compartments samples from 2012 and 2013 had higher variations; it further demonstrated that the AOA communities changed from relative uniform to diversified. The RDA analysis also showed the relationship between AOA community structure and environmental, operational variables, and the environmental, operational factors accounted for 50% (the first two RDA axes, 30.15% and 19.85%, respectively) of total variance for sedimentary AOA OTU composition (Figure 6). The Days (namely the operation time, $F = 1.5145$, $p = 0.055$) which comprehensively reflected the wetland hydrology, water quality, and associated functions significantly contributed to the sedimentary AOA assemblage-environment relationship in SJY-CW sediments; temperature and nutrients (NH_4^+-N, TN, TC, TS, and C/N ratio) also modified the AOA community composition additionally.

DISCUSSION

Constructed wetlands (CWs) have the positive characteristics of a natural wetland and can also be controlled to eliminate the negative aspects of natural wetlands (USEPA, 1988; Gopal, 1999; Kivaisi, 2001). CWs should be compartmentalized with several cells arranged in series or in parallel (USEPA, 1988; Vymazal, 2006). The well-designed CWs with multiple cells are generally effective in removing nutrients such as nitrogen and exhibit the cascading treatment performance which consequently harbored some internal environmental gradients and hot spots among the functional zones and land/water interface (Wang et al., 2013a, 2016). Shijiuyang constructed wetland (SJY-CW) was designed as typical pond-wetland complexes with serial-connected combinations of pretreatment pond, plant-bed/ditch system, and post-treatment pond. The key land/water interfacial reactions mainly occurred in the plant-bed/ditch systems (Wang et al., 2013a). A series of water quality attenuation gradients had been already formed during its initial two-year operation period (2008–2010) (Wang et al., 2016) and maintained the similar gradients from the third through fifth (2011–2013) operational years (Figure S3). The treatment efficacy of CWs was also seasonally variable with the succession in respect of operation time (Hsu et al., 2011; Wang et al., 2016), which promoted the formation of internal eco-environmental gradients and interfacial hot spots, and further differentiated the micro-niche of ammonia-oxidizing archaea (AOA) (Erguder et al., 2009).

The declines of AOA *amo*A gene abundance in SJY-CW from 2011 to 2013 (Figure 2) reflected the comprehensive effects of operational duration of SJY-CW on the members of AOA community. Operation time (Days) of the wetland could explain 48.03% and 76.18% of the variation of AOA abundance on the scale of holistic wetland systems and individual compartments of Plant-bed system respectively. However, operation time had no statistically significant contribution to the variation of AOA abundance in the Inlet and Outlet regions (both pond systems). This strongly suggested that the niche differentiation of AOA abundance preferentially and primarily occurred in the complex and heterogeneous Plant-bed system. It can be said that the “hot spots” harboring the high AOA abundance were located in the central Plant-bed system of SJY-CW. Conversely, the relatively homogeneous pond systems regardless of their locations did not significantly differentiate the AOA abundance. Mozhayskiy and Tagkopoulos (2012) reported that the rate of evolution can be accelerated by evolving cell populations in sequential combinations of environments that are increasingly more complex; strong positive and negative correlations between the intermediate and final environments lead to the increase of evolutionary rates, when the environmental complexity increases. Spatial and temporal environmental heterogeneity is inextricably linked to successional changes in community composition and diversity (Rosenzweig, 1995; Kassen, 2002; Hornerdevine et al., 2004). Superficially, operation times were just a series of simple linear counting numbers by constant increase intervals; in fact, the time of wetlands continual operation under artificially controlled steady state was the most comprehensive and accumulative integration which reflected the extremely complicated and numerous indefinable variables exerting on the systems. In other words, the separate and individual environmental variables such as ammonia nitrogen and oxygen level played relatively minor, fluctuating, and labile roles accompanying with complex biochemical processes and interactions, which nevertheless did not overwhelm the impact of wetlands succession. The present study suggested that the operation time of wetland might be a simple and practical indicator for predicting the microbial community ecology.

In addition to operation time, the temporal niche differentiation on AOA *amo*A gene abundance was closely related with some environmental and soil physicochemical factors. On the scale of holistic wetland systems, Temperature (range 12.1–33.6 °C) was negatively correlated with AOA abundance that means the abundance was instead high when temperature was relatively low, which was in agreement with the finding in previous study (Sahan and Muyzer, 2008). This reflected the fact that the conditions of SJY-CW sediments would be in favor of harboring the nonthermophilic rather than thermophilic members of the ammonia-oxidizing crenarchaeota (Erguder et al., 2009). Temperature effect on AOA abundance was even more intense (contributing 78.94%) in the Inlet and Outlet regions than in the Plant-bed system. Another potential reason for the decrease of AOA *amo*A gene abundance may be attributed to phylogenetic shifts in AOA communities (Figure 3 and Figure 4). The diversified AOA and actually coexistent ammonia-oxidizing bacteria (AOB) as well as anaerobic ammonium oxidation (anammox) bacteria may compete for the same substrates thus causing the low AOA abundance (Li et al., 2011; Li and Gu, 2013; Pan et al., 2016) with the succession of CW. In spatial dimension, the niche selection effect on AOA *amo*A gene copy numbers was different in diversified functional zones. The AOA abundance presented distinctive patterns among the Inlet, Outlet regions and Plant-bed system respectively and the decrease of AOA abundance first occurred in the Plant-bed system (Figure 2). The higher AOA abundance in the Plant-bed system than in the pretreatment pond and post-treatment pond was attributed to the massive plants, where the hydrophytes in the Plant-bed system formed the oxygenated and nutrient micro-habitat (Prosser and Nicol, 2012) facilitating the AOA's growth. Understanding the coexistence and reciprocal symbiosis of microbes and plants in the plant-microbe-soil feedback system would be essential in explaining the microbial diversity (Miki et al., 2010). However, it is likely that there is sufficient specificity in microbial-plant interactions and negative feedback between the abundance of a plant and the abundance of its microbial partner (Bever et al., 1997; Silvertown, 2004). Consequently, variable functional zones and compartments should be included as an important parameter when studying the nitrogen biogeochemical cycle in wetlands.

Different from the variation of abundance, the temporal niche differentiation of AOA biodiversity was insignificant with a spatially variable state in separate compartments of SJY-CW. In the Plant-bed system, individual regions such as South region with high abundance did not necessarily have the high diversity, which indirectly implied that some specific and possibly local AOA species were inhabited and adapted there. With the succession of wetland, the AOA diversity in the Plant-bed system was gradually rising up and even overtook that in the pretreatment pond and post-treatment pond systems, which illustrated that the AOA diversity was step-by-step penetrating and migrating into the Plant-bed system. However, the West region adjacent to the Inlet region had always the highest AOA diversity in the Plant-bed system during the studied three years, which clearly indicated that the increase of AOA diversity in the SJY-CW was mainly due to the exogenous species input as transported by the source river water. The coexistent many-species of AOA in the overlying water column of SJY-CW may drift down and migrate horizontally along the hydraulic flow pathways. The AOA diversity in the rear post-treatment pond was always or generally higher than that in the front pretreatment pond and in the middle Plant-bed system respectively, which suggested that in comparison with the crisscrossed and meandering Plant-bed system, the pelagic zone of post-treatment pond was more easily to be influenced and invaded by biodiversity. The slow and lag response of AOA biodiversity in the Plant-bed system reflected the stability and resistance of microbial species in this system.

Further analysis on the habitat adaptation revealed that the higher AOA diversity in the Outlet region than that in the Inlet region might partly be attributed to the water quality purification effect in SJY-CW. The water quality in the Outlet region was ameliorated than that in the Inlet region, for example, the dissolved oxygen (DO) was higher (mean±S.D., from Inlet $3.32\pm0.92\ \mathrm{mg\,L^{-1}}$ to Outlet $5.06\pm1.34\ \mathrm{mg\,L^{-1}}$) and ammonia nitrogen was lower (from Inlet $1.75\pm1.52\ \mathrm{mg\,L^{-1}}$ to Outlet $1.23\pm1.59\ \mathrm{mg\,L^{-1}}$) (Figure S3). As we know, the AOA participating in aerobic ammonia oxidation belongs to aerobe, so the oxygen-rich environment in the Outlet region benefited various AOA species (Trias et al., 2012). From the angle of substrate, AOA preferred to grow in a lower substrate concentrations since the value of archaeal K_s was lower, and the K_s values in soil for AOA were estimated as 2–20 nM NH_3 (Martenshabbena et al., 2009). In the sediments of SJY-CW, the total ammonium ($NH_3+NH_4^+$) ranged between 0.33–5.90 mM under temperature 12.1–33.6 °C and pH 6.09–7.47 during the study period (Table S1), and the un-ionized ammonia (UIA, NH_3) was calculated as 0.40–30.70 μM which means the ammonium was far more than the extreme nutrient limitation for AOA while total ammonium concentrations as high as ≥ 2 mM would cause a decline of activity (Martenshabbena et al., 2009). The similar results were also reported in other studies that DO was positively related with AOA community diversity and lower ammonia concentration was in favor of higher AOA diversity (Lian et al., 2014). However, the results differed from some other studies which indicated that the AOA diversity was negatively correlated with DO level in the eastern South Pacific (Molina et al., 2010) or different DO levels did not affect AOA diversity in estuarine wetland (Luo et al., 2014). This inconsistency may reflect the different effects of habitats, gradients of DO, nutrients on the community diversity of AOA. In all, the oxygen and ammonia demand of AOA determined its spatial diversity shifts in the studied wetland.

The AOA in the sediments of SJY-CW were clustered into four clusters, *Nitrososphaera*, *Nitrososphaera* sister, *Nitrosopumilus* cluster, and *Nitrosotalea* cluster according to phylogenetic analysis (Figure 4). The sequences of *Nitrososphaera* cluster were partly from soil environments and some were from river and estuarine sediments, which was usually detected to be dominant in agricultural soils (Jiang et al., 2014) and freshwater wetland sediment (Wang and Gu, 2013). The detection of *Nitrososphaera* sister might be attributed to that the *Nitrososphaera* sister cluster shared a common ancestor with the *Nitrososphaera* cluster (Pester et al., 2012), and it was often detected from soil environment. However, *Nitrosopumilus* cluster was often detected from estuarine and marine environments (Zhang et al., 2016), for example, the strain *Nitrosopumilus maritimus* SCM1 was isolated from a marine tropical fish tank at an aquarium (Könneke et al., 2005). Jiaxing is bounded by the East China Sea to the east in the coastal Southeastern China. The huge wave of almost nine meters high by the tidal bore of Qiantang River, a natural event, occurs upriver from its mouth. At that time, it reached 40 km/h and was heard 22 kilometers away. The influence of tidal flow at the estuary of Qiantang River on the plain stream networks may be the reason that 7.88% of AOA *amo*A sequences (the second highest) were affiliated with *Nitrosopumilus* cluster. The rare occurrence of *Nitrosotalea* cluster was because the strain *Nitrosotalea devanaterra* was enriched from an acidic agricultural soil (Lehtovirta-Morley et al., 2011) and it was dominant in acidic soil (Pester et al., 2012), while the studied wetland was at approximately neutral pH (6.09–7.47). The AOA group was determined by niche selection in different environments.

In this study, all samples were dominated by *Nitrososphaera* cluster, which was consistent with other agricultural soils and constructed freshwater wetland sediments. Although the dominant AOA group did not change with the operation of CW, *Nitrososphaera* sister cluster and *Nitrosopumilus* cluster increased with the succession of wetland. What's more, the *Nitrososphaera* subcluster 1 and *Nitrosopumilus* subcluster 5 began to be dominant in some samples instead of the local dominant *Nitrososphaera* subclusters 9, 3, 10, 4. The horizontal migration of AOA clusters had occurred during the studied three years (Figure 2 and Figure S2). Raymond and Alsop (2015) pointed out that, more importantly, both mechanisms—horizontal transfer or microbial migration—underscore that genetic/genomic mobility and habitat ranges of microbial species are substantially greater that previously known even in the greater Yellowstone hydrothermal systems. In all, it indicated that the SJY-CW showed considerable niche selection effects on AOA community structure and cultured diversified AOA population.

In combination with genomic information, the assessment of environmental conditions that contribute to spatial or temporal heterogeneity in species composition might enable the identification of traits that are important to microbial adaptation in the community (Allen and Banfield, 2005). The UPGMA cluster and RDA results indicated the AOA community shifts with the succession of CW and this selection effect preferred to occur in the heterogeneous plant-bed/ditch system. So, we speculated that the micro-habitat in the Plant-bed system would facilitate the AOA community niche selection effect. Based on previous research, the effective nutrient removal in the Plant-bed system of SJY-CW led to the gradients of DO, redox potential, and nutrients (Wang et al., 2016), thus promoting the culture of different AOA species; the presence of plants also generated niche selection effect on AOA community structure (Ruizrueda et al., 2009). The RDA results (Figure 6) showed that the AOA community structure was distinguished by the wetland operation time, which is a comprehensive reflection of the change of wetland succession conditions. What's more, temperature and nutrients (NH_4^+, TC, TN, C/N) were the main factors driving the AOA community shifts. Peng et al. (2013) and Biller et al. (2012) confirmed that temperature had influences on the distinct distribution of AOA communities because AOA species had the optimal temperature range for growth; the nutrient concentration was also reported to influence the habitat of AOA community structure (Hatzenpichler et al., 2008; Zheng et al., 2014). In this study, the first two axes of RDA ordination together explained just only 50.0% of the variation of AOA community shifts with the succession of SJY-CW; it needs to further explore the unincluded factors controlling AOA composition, just as Strom (2008) proposed that community interactions warranted more consideration and such interactions require experimental verification.

Overall, the abundance and composition of AOA *amo*A gene in the SJY-CW sediments were significantly driven and determined by Operation time (Days) in a great extent and by Temperature (seasonal trend) in a considerable extent, while nutrients (such as NO_3^--N, TC, TN) played only additional, secondary roles in explaining the AOA abundance and composition. It seemed that the non-resource factors contributed predominantly to the abundance and composition of AOA community in SJY-CW. Schoolmaster Jr (2013) presented a model of species competition under spatial heterogeneity and resource factors to probe into the resource competition and coexistence in heterogeneous metacommunities, and concluded that many-species coexistence is unlikely to be facilitated by spatial variation in resources. Under simple models of resource competition, in which all species grow best at high resource levels, the specialization on different parts of the gradient of the heterogeneous factor is impossible. This analysis suggests that, in the absence of additional mechanisms other than the spatial storage effect, spatial heterogeneity in a single resource is unlikely to facilitate many-species coexistence and, more generally, that when evaluating the relationship between heterogeneity and diversity, a distinction should be made between resource and non-resource factors (Schoolmaster Jr, 2013). Our present study disclosed general trends about the spatiotemporal succession of ammonia-oxidizing archaea abundance and composition in a created riparian wetland, but did not include the actually coexistent ammonia-oxidizing bacteria as well as anaerobic ammonium oxidation bacteria. Integrating these functional group of microorganisms responsible for ammonia oxidation processes into the metacommunities and discovering the mechanisms of their coexistence, competition, and reciprocal symbiosis as well as with plants would conspicuously deepen the comprehensive understanding of microbial nitrogen cycling in a created wetland ecosystem.

Based on the results and discussion, we attempted to present a generalized conceptual framework to depict the spatiotemporal succession of AOA abundance, composition, and diversity (Figure S4). Here we assumed that the plant-bed/ditch system was more complex than the pond system (pretreatment pond, post-treatment pond). In the plant-bed/ditch system, the abundance, composition, and diversity of AOA were generally higher, more complex or more diversified than the corresponding values in the pond system. With the succession of wetland, the preferential niche differentiation or evolutionary rate as well as strength for the attributes of AOA communities were in the following sequence: Abundance $>$ Composition $\gg$ Diversity. The diversity was relatively robust or resistant to external conditions than abundance and composition. Horizontal shift of AOA *amo*A gene had occurred in the different compartments of SJY-CW during the studied three years. Operation times of wetland (Days) may explain most of the variations of AOA abundance and composition. In addition, the environmental factors such as Temperature or nutrients may partly modify the succession mode of AOA communities either positively or negatively. More clear characterization of AOA community ecology in a created riverine wetland may need more systematic researches in a longer period of time.

CONCLUSIONS

Ammonia-oxidizing archaea abundance and composition in the sediments of a created riparian wetland—SJY-CW had showed some considerable spatiotemporal succession characteristics during its third through fifth operational year. The AOA abundance in the heterogeneous plant-bed/ditch system was significantly higher than that in the homogeneous pond system, which both decreased with a tendency of being uniformly distributed with the succession of wetland. The AOA diversity in the plant-bed/ditch system decreased gradually along the hydraulic flow pathways, while the diversity in the post-treatment pond was higher than that in the pretreatment pond. AOA communities in the sediments of SJY-CW were dominated by *Nitrososphaera* cluster during the studied three years, however, *Nitrosopumilus* cluster and *Nitrososphaera* sister cluster increased with the succession of wetland. The horizontal shifts and rearrangement of AOA *amo*A genes had occurred mainly in the plant-bed/ditch system and the AOA community structure was changing from evenly distributed to highly diversified. Operation/succession time of the wetland, which comprehensively reflected the wetland hydrology, water quality, and associated functions, was the most conspicuous determining factor which significantly influenced the spatiotemporal differentiation of AOA abundance and composition. Temperature and nutrients played minor and auxiliary roles in explaining the temporal and niche partitioning effect on AOA abundance and composition. Comparatively, the AOA community diversity did not exhibit apparent spatiotemporal succession trends, and no significant operational and environmental factors could explain the diversity succession of AOA. It suggested that the community diversity succession of wetland ecosystem need a longer period of time to be stabilized. This study provided a snapshot on the microbial community ecology of archaeal ammonia oxidization occurring in a created riparian wetland during its initial several years. Integrating bacterial ammonia oxidization into the nitrogen cycling framework and distinguishing their niche selection and relative contribution would be of great significance in the future researches.

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Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Yu Su performed the experiments, analyzed the data, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.
- Guibing Zhu conceived the experiments, contributed reagents/materials/analysis tools, reviewed drafts of the paper.
- Baoling Wang conducted field sampling, performed the experiments.
- Yu Wang conducted field sampling, performed the experiments.
- Linjie Zhuang analyzed the data.
- Weidong Wang conceived and designed the experiments, conducted field sampling, analyzed the data, prepared figures and/or tables, constructed the paper, reviewed drafts of the paper.

Field Study Permissions

The following information was supplied relating to field study approvals (i.e., approving field sampling and experiments):

Field experiments were approved by the Jiaxing Science and Technology Bureau (project number: 2011AZ2019, 2015AY23008). Our research work did not involve capture or any direct manipulation or disturbance of animals. We collected samples of sediments and water for molecular and physicochemical analyses. We obtained access to the wetland under the permission of the Jiaxing Water Conservancy Investment Company Ltd. (Jiaxing, Zhejiang, China), which is responsible for the operation and management of the wetland.

DNA Deposition

The following information was supplied regarding the deposition of DNA sequences:
NCBI: Sequence Read Archive (SRA) database
Accession number: SRR4244883 through SRR4244897 in a single BioProject SRP089950.

Data Availability

The following information was supplied regarding data availability:

The raw data has been supplied as a Supplemental Dataset.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.xxxx #supplemental-information.

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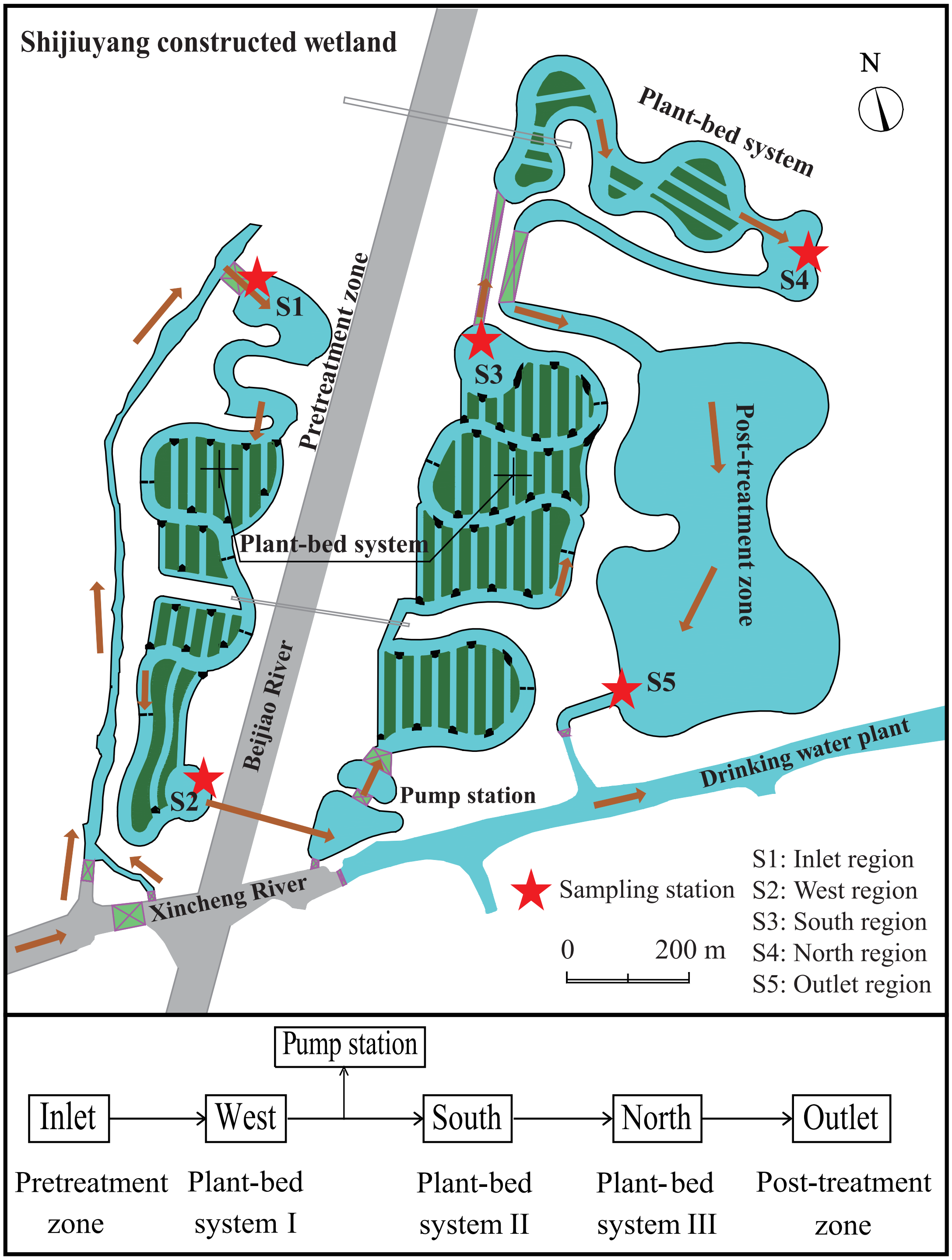

Figure 1. Plan view and configurations of Shijiuyang constructed wetland (SJY-CW) showing the sampling stations.

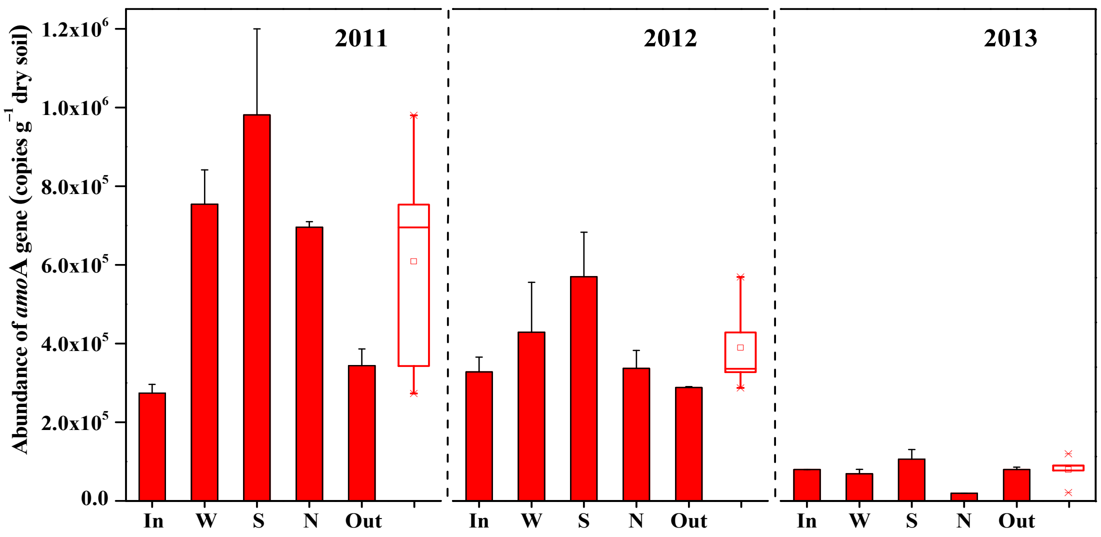

Figure 2. Abundance of AOA *amo*A gene copies in the sediments during the third through fifth year succession of SJY-CW. The error bar denotes the standard deviation from three analytical replications. In: Inlet region; W: West region; S: South region; N: North region; Out: Outlet region.

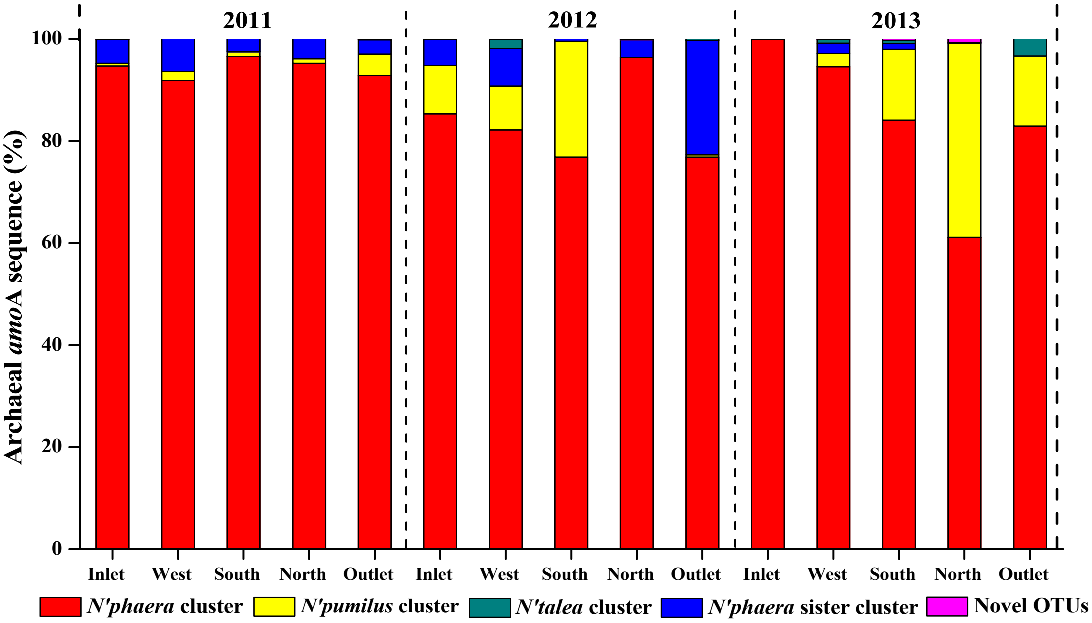

Figure 3. Relative abundance of sequences affiliated with five major AOA *amo*A clusters in SJY-CW sediments.

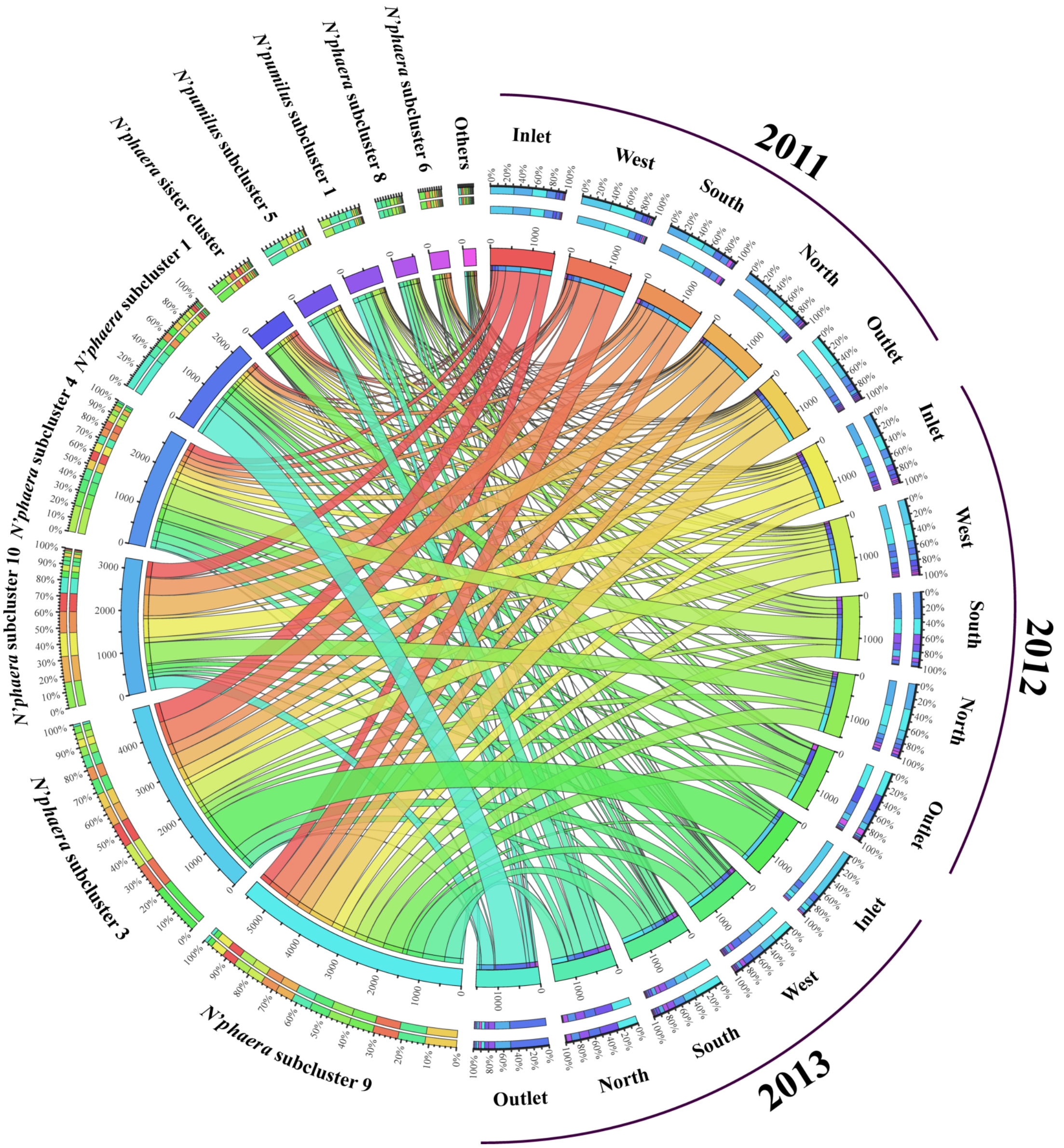

Figure 4. Evolution of AOA communities during the third through fifth year succession of SJY-CW at the AOA subcluster level. The width of bars from each AOA subcluster indicates the relative abundance of subcluster in the samples.

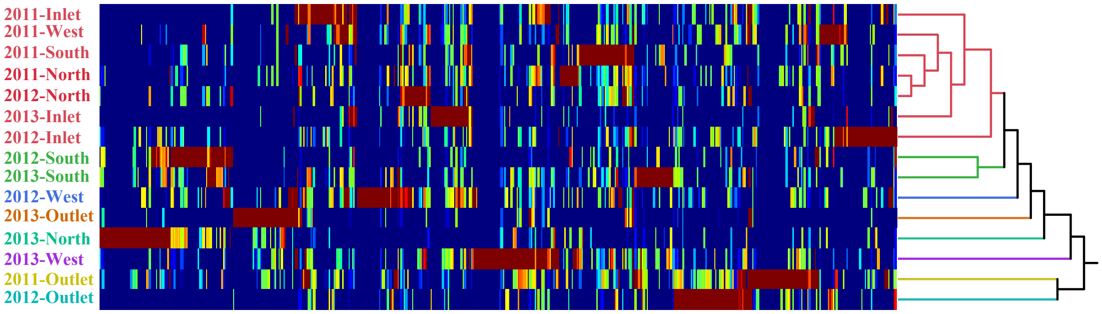

Figure 5. UPGMA clustering dendrogram constructed based on the 97% cutoff level-based unweighted Unifrac matrix (at OTU level).

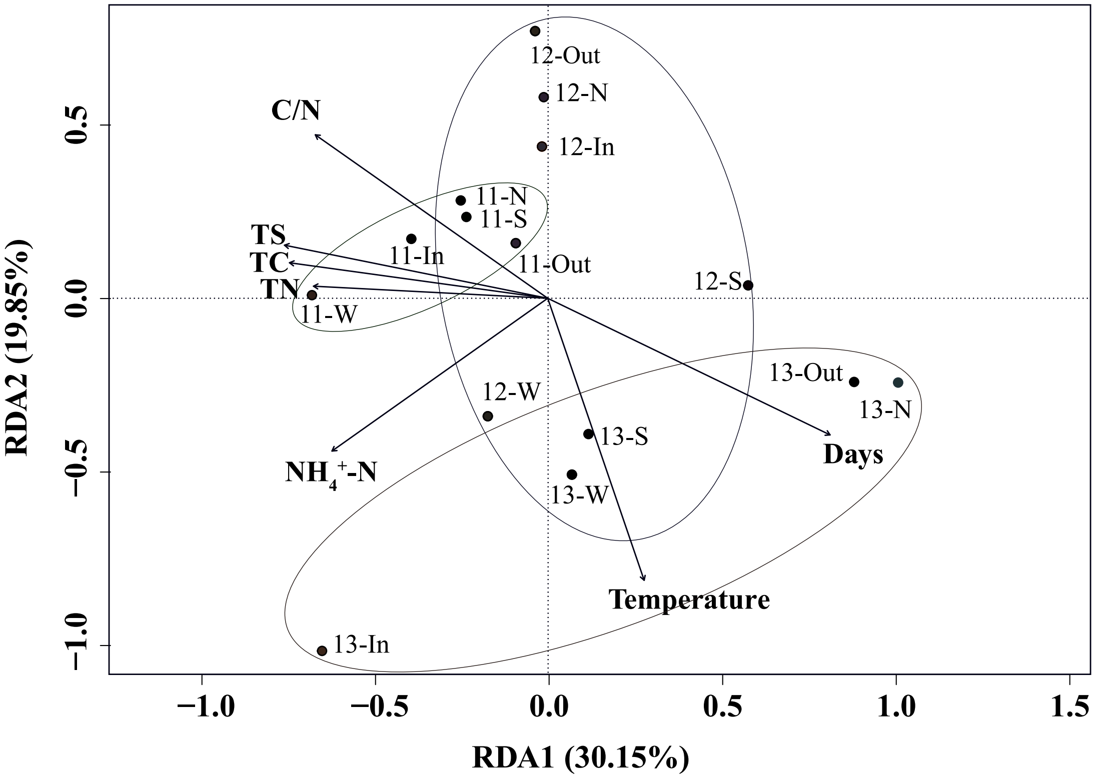

Figure 6. Redundancy analysis (RDA) of AOA communities (at OTU level) and the environmental and operational factors.

Supplemental Information

Journal: PeerJ

Title: Spatiotemporal succession of ammonia-oxidizing archaea abundance and composition in a created riparian wetland

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Supplemental Information contains Tables S1 to S2 and Figures S1 to S4.

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Table S1 **Physicochemical indexes of sediments in SJY-CW from 2011 to 2013.**

Year	Functioning zone	Operation time (Days)	Temperature (°C)	pH	ORP (mV)	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	TN (g kg^{-1})	TC (g kg^{-1})	TS (g kg^{-1})	C/N
2011	Inlet	1003	21.0	6.85	−176.8	18.52	10.48	1.38	13.52	0.95	11.43
	West	1003	21.0	6.89	−157.7	28.21	13.01	2.56	27.17	0.24	12.38
	South	1205	21.0	7.08	−120.5	12.09	2.97	0.90	8.68	0.59	11.25
	North	1205	21.0	7.47	−131.9	13.16	10.9	1.00	10.78	1.09	12.57
	Outlet	1205	21.0	7.27	−124.3	18.78	7.47	0.87	8.42	0.88	11.29
2012	Inlet	1414	12.1	7.28	−16.7	31.41	7.18	1.57	15.64	0.89	11.62
	West	1414	12.1	6.32	41.6	16.30	3.21	0.55	3.47	0.20	7.36
	South	1616	12.1	6.09	53.7	21.24	4.28	0.91	6.70	0.50	8.59
	North	1616	12.1	6.49	30.4	18.28	3.92	0.74	7.91	0.88	12.47
	Outlet	1616	12.1	6.35	38.6	24.00	5.03	1.07	10.65	0.63	11.61
2013	Inlet	1653	33.6	6.69	18.3	82.62	0.85	1.58	16.04	0.88	11.84
	West	1653	33.6	6.24	44.4	7.50	0.46	0.65	5.59	0.55	10.03
	South	1855	33.6	7.01	−1.5	22.22	0.95	0.84	6.96	1.00	9.66
	North	1855	33.6	7.23	−14.3	8.69	3.83	0.59	5.13	0.19	10.14
	Outlet	1855	33.6	7.27	−15.9	4.57	1.39	0.95	6.67	0.27	8.19

Table S2 **Spearman correlation matrix between physicochemical indexes and AOA *amo*A functional gene abundance as per all samples (*n*=15) and Plant-bed system (*n*=9).**

		Days	Temperature	pH	ORP	NH_4^+-N	NO_3^--N	TN	TC	TS	C/N
Abundance	*r*	−0.7422	−0.6047	ns	ns	ns	0.5536	ns	ns	ns	Ns
(All samples)	*p*	0.0015	0.0169	/	/	/	0.0323	/	/	/	/
Abundance	*r*	−0.9115	ns	ns	ns	ns	ns	0.7167	0.7500	ns	ns
(Plant-bed system)	*p*	0.0006	/	/	/	/	/	0.0298	0.0199	/	/

Notes.

ns: not significant.

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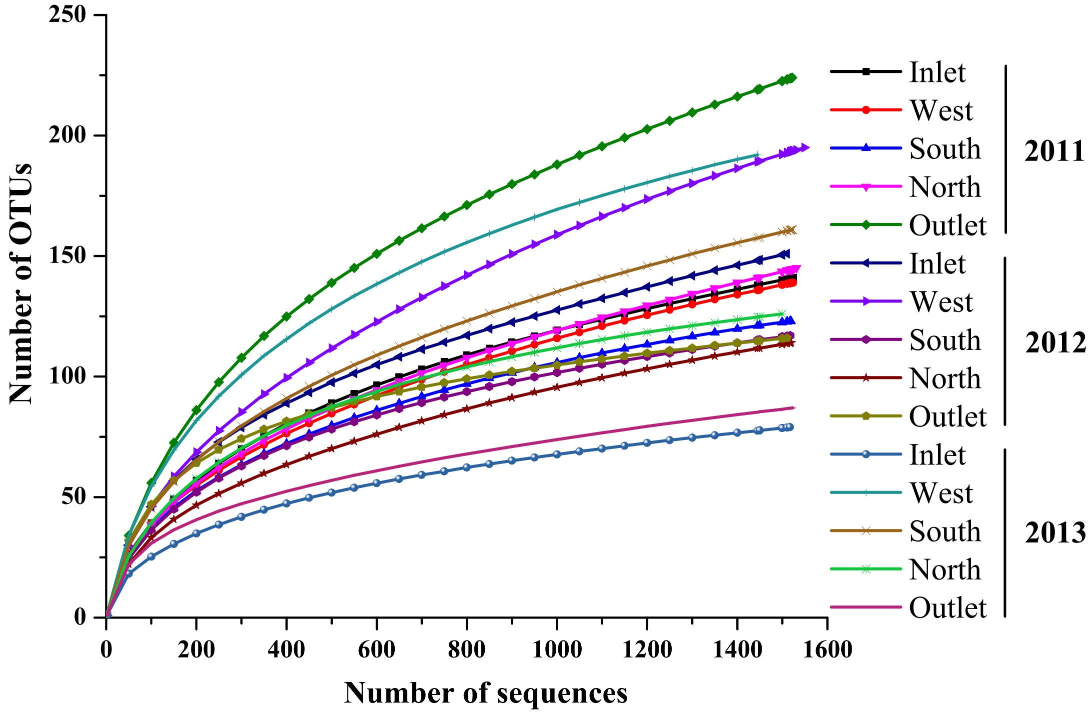


Figure S1 Rarefaction curves of OTUs in AOA libraries.

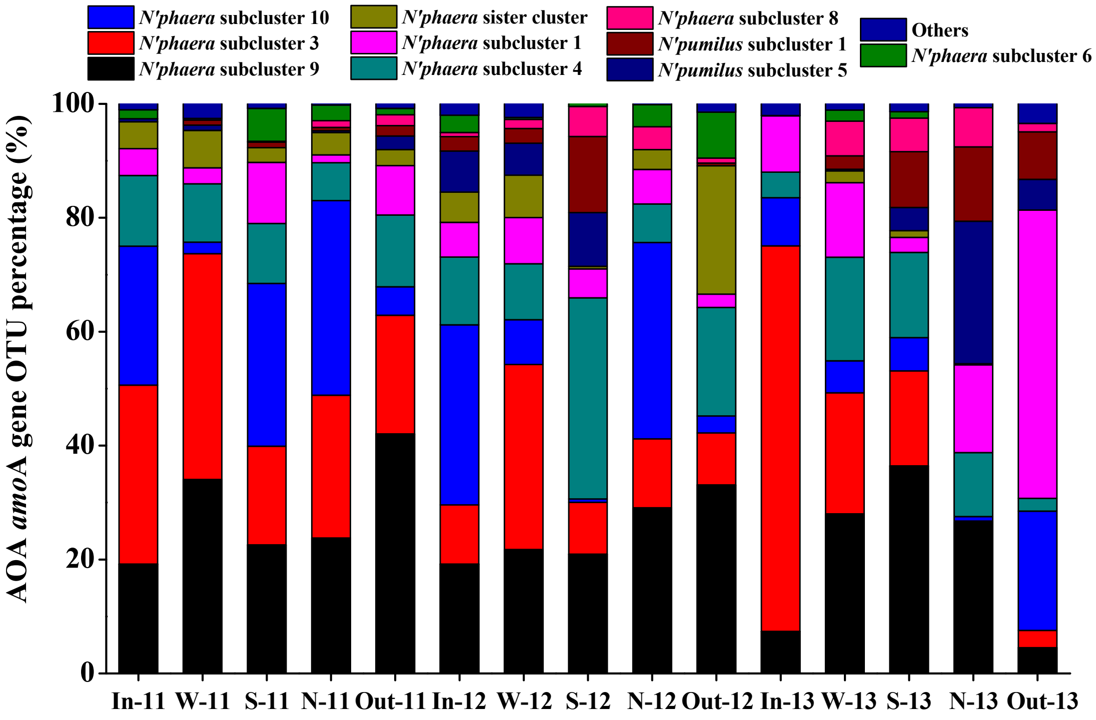
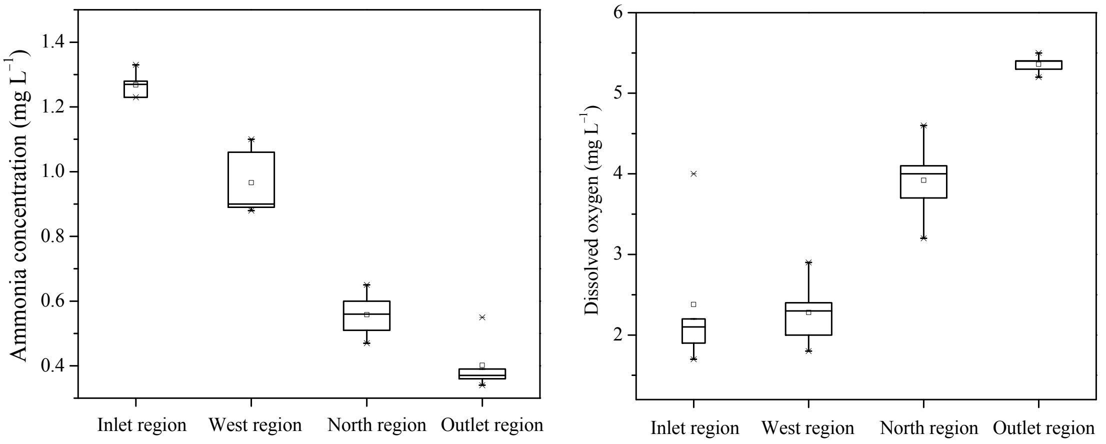
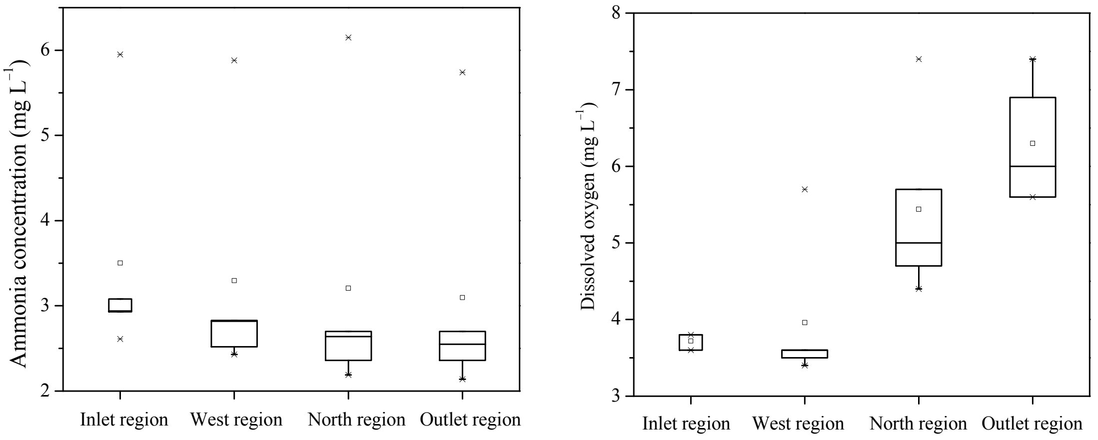


Figure S2 Relative abundance of sequences affiliated with AOA subclusters in SJY-CW sediments.



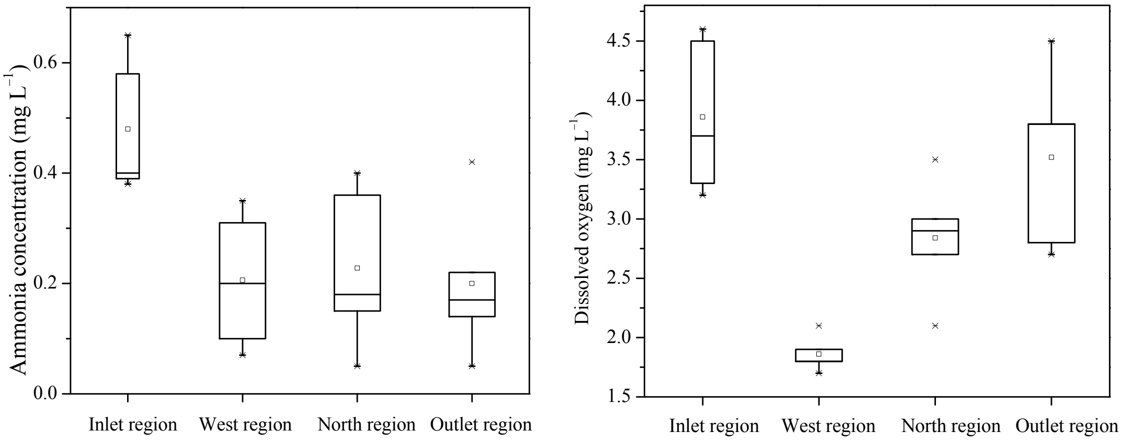
(a) Variation of ammonia concentration in 2011

(d) Variation of dissolved oxygen in 2011



(b) Variation of ammonia concentration in 2012

(e) Variation of dissolved oxygen in 2012



(c) Variation of ammonia concentration in 2013

(f) Variation of dissolved oxygen in 2013

Figure S3 Spatial variation of ammonia nitrogen and dissolved oxygen in SJY-CW water. In the box and whisker plot (box plots), the central line indicates the median of the data, while the bottom and top edges of the box indicate the first and third quartiles (that is, the 25th and 75th percentiles). The center square symbol is the average value (mean) of the data. The vertical lines, or whiskers, extend from the box as far as the data extend, to a distance of at most 1.5 interquartile ranges. The upper and lower line symbols illustrate the maximum and minimum of the data. The upper and lower cross symbols show the 99% and 1% percentiles of the data.

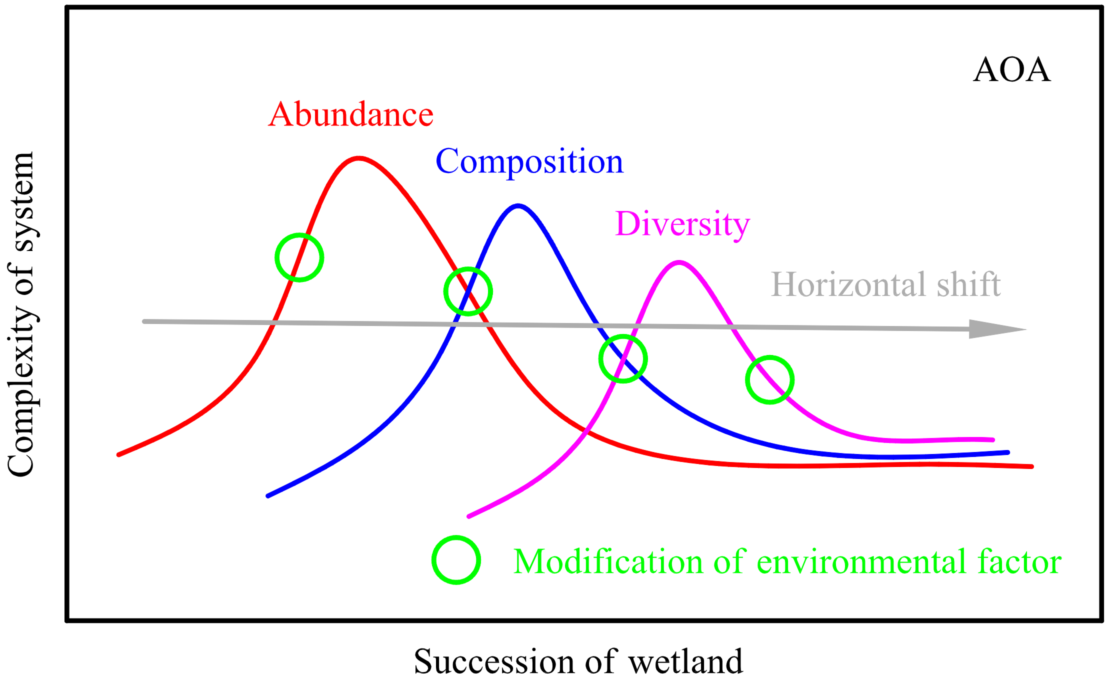


Figure S4 **Conceptual framework of AOA community succession in SJY-CW.**

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