



Nitrifying activity and ammonia-oxidizing microorganisms in a constructed wetland treating polluted surface water

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HIGHLIGHTS

- Seasonality effects on ammonia-oxidizing organisms occurred in constructed wetland.
- AOB far outnumbered AOA in the FWSF-CW.
- Both *Nitrosospira* and *Nitrosomonas* were the dominant AOB in the FWSF-CW.

GRAPHICAL ABSTRACT



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ABSTRACT

Ammonia oxidation, performed by both ammonia oxidizing bacteria (AOB) and archaea (AOA), is an important step for nitrogen removal in constructed wetlands (CWs). However, little is known about the distribution of these ammonia oxidizing organisms in CWs and the associated wetland environmental variables. Their relative importance to nitrification in CWs remains still controversial. The present study investigated the seasonal dynamics of AOA and AOB communities in a free water surface flow CW (FWSF-CW) used to ameliorate the quality of polluted river water. Strong seasonality effects on potential nitrification rate (PNR) and the abundance, richness, diversity and structure of AOA and AOB communities were observed in the river water treatment FWSF-CW. PNR was positively correlated to AOB abundance. AOB (6.76×10^5 – 6.01×10^7 bacterial *amoA* gene copies per gram dry sediment/soil) tended to be much more abundant than AOA (from below quantitative PCR detection limit to 9.62×10^6 archaeal *amoA* gene copies per gram dry sediment/soil). Both AOA and AOB abundance were regulated by the levels of nitrogen, phosphorus and organic carbon. Different wetland environmental variables determined the diversity and structure of AOA and AOB communities. Wetland AOA communities were mainly composed of unknown species and *Nitrosopumilus*-like organisms, while AOB communities were mainly represented by both *Nitrosospira* and *Nitrosomonas*.

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1. Introduction

Constructed wetlands (CWs) have been applied worldwide to remove nitrogen pollutants in municipal and domestic wastewaters (Barco and Borin, 2017; Hua et al., 2017; Tan et al., 2017), industrial

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wastewaters (Bakhshoodeh et al., 2017a; Hussein and Scholz, 2017), leachate (Bakhshoodeh et al., 2017b; He et al., 2017a), and polluted surface water (Chyan et al., 2016; Zheng et al., 2016). In CWs, microorganism-mediated processes (especially nitrification and denitrification) are mainly responsible for nitrogen removal (Chen et al., 2017; Sims et al., 2012). Biotransformation of ammonia to nitrite, as the key step in nitrification process, can be performed by both ammonia oxidizing bacteria (AOB) and archaea (AOA). Both of these two ammonia oxidizing microorganisms harbor *amoA* gene encoding ammonia monoxygenase (Yang et al., 2016a; Zhang et al., 2016; Zhao et al., 2017). To date, numerous previous studies have revealed the distribution of AOA and AOB in various natural wetlands (Chen and Gu, 2017; He et al., 2017b; Hofferle et al., 2010; Lee et al., 2014; Moin et al., 2009; Zheng et al., 2013), however, little is known about their distribution in CWs. The relative importance of AOA and AOB to nitrification in CWs remains still in debate. Numerical advantage of AOA over AOB has been found in an integrated CW (ICW) for swine wastewater treatment (Zhang et al., 2017) and a free water surface flow CW (FWSF-CW) for advanced treatment of municipal wastewater (Liu et al., 2017). Fan et al. (2016) suggested that AOA was more critical than AOB for ammonia removal in FWSF-CWs treating river water. In contrast, AOB outnumbered AOA in horizontal subsurface flow CWs (HSF-CWs) treating raw domestic sewage (Chen et al., 2017), a vertical subsurface flow CW (VSF-CW) treating urban wastewater (Pelissari et al., 2017), and FWSF-CWs treating swine wastewater (Li et al., 2018). In a FWSF-CW treating river water, AOA was more abundant than AOB in plant-bed soils but less abundant in ditch sediments, however, AOB contributed more to ammonia oxidation than AOA in both plant-bed soils and ditch sediments (Wang et al., 2013a). In addition, the influential factors regulating the distribution of AOA and AOB in CWs remain elusive, although several previous studies suggested that AOA and AOB communities might be influenced by the presence and type of vegetation (Hu et al., 2016; Zhang et al., 2017), soil/sediment ammonia level (Wang et al., 2013a), soil/sediment oxidation–reduction potential (Wang et al., 2013a), sampling season (Sims et al., 2012; Yin et al., 2009), and substrate type (Huang et al., 2013). Fan et al. (2016) pointed out that a variety of environmental variables might collectively shape AOA and AOB communities in FWSF-CWs treating river water.

FWSF-CWs have found increasing applications to ameliorate the quality of polluted surface waters (Dzakpasu et al., 2015; Fan et al., 2016; Li et al., 2017; Zhi et al., 2015), yet there was few studies about the distribution of AOA and AOB communities and the associated environmental factors in FWSF-CWs (Fan et al., 2016; Wang et al., 2013a). Information about the seasonality effects on AOA and AOB communities in the FWSF-CW used for surface water treatment is still lacking. Therefore, the main objective of the present study was to study the seasonal dynamics of AOA and AOB communities in a FWSF-CW used for treatment of river water. The environmental factors regulating AOA and AOB communities in the FWSF-CW were also explored.

2. Materials and methods

2.1. Study sites and samples

The studied FWSF-CW (approximately 0.47 km²) was located in a subtropical Dali City (southwest China) and had been continuously operating to improve the water quality of an upstream river of the Erhai Lake for more than 8 years. The local area had annual mean precipitation and air temperature of 1000 mm and 15.7 °C, respectively. The average removal rates of NH₄⁺-N and total nitrogen (TN) by the FWSF-CW were 55.9% and 43.2%, respectively (Li et al., 2017). In consistence with our previous studies (Li et al., 2017; Xu et al., 2018), surface sediments or soils (0–10 cm) in triplicate were collected at eight different sites (A–H) (25°56′42″–25°57′11″ N, 100°6′0″–100°6′9″ E) during spring, summer and winter in the year 2016. The sediment sampling sites A–F were inundated throughout the year, while the soil sampling sites G

and H were inundated only during summer and autumn. These sites were vegetated with different dominant plant species. During winter, no sediment sample was obtained from site B due to inaccessibility. The collected wetland sediment/soil samples were labeled as A–H, in keeping with the sampling site, and SP, SU, or WI, in keeping with the season (spring, summer, or winter, respectively). These collected wetland samples had been also used for the studies on archaeal community and nitrite-dependent anaerobic methane oxidation (n-damo) organisms (Li et al., 2017; Xu et al., 2018). The information on sampling sites and sediment/soil physicochemical parameters (temperature, oxidation and reduction potential (ORP), pH, total organic carbon (TOC), NH₄⁺-N, TN, nitrate nitrogen (NO₃⁻-N), nitrite nitrogen (NO₂⁻-N), the ratio of TOC to TN (C/N), and total phosphorus (TP)) was described in detail in our previous studies (Li et al., 2017; Xu et al., 2018).

2.2. Potential nitrification rate

The potential nitrification rate (PNR) of each wetland sediment/soil was assessed by the chlorate inhibition method (Chen et al., 2013; Dai et al., 2015). Briefly, for each sediment/soil, fresh wetland sediment/soil (5 g, dry weight) was placed into a 50-mL centrifuge tube that contained 20 mL of 1 mM (NH₄)₂SO₄ and 1 mM phosphate buffer solution. Potassium chlorate (10 mg/L⁻¹) was added to the centrifuge tube to inhibit the nitrite oxidation, and then incubated at 25 °C for 24 h in the dark. NO₂⁻-N was extracted with 2 M KCl (5 mL) for further analysis.

2.3. Molecular analyses

The PowerSoil™ DNA extraction kit (MO BIO Laboratories, Carlsbad, CA, USA) was adopted to extract sediment/soil genomic DNA. The 1.2% (w/v) agarose gel electrophoresis was used to assess the quality of DNA, and the quantity of DNA was further measured using a NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The primer pairs Arch-amoAF/Arch-amoAR (Francis et al., 2005) and AmoA-1F/AmoA-2R (Rotthauwe et al., 1997) respectively were adopted for the quantitative PCR (q-PCR) assay of AOA and AOB abundance, and for the construction of AOA and AOB clone libraries, following the same conditions as previously described (Wang et al., 2014). Chimeras were discarded using UCHIME (Edgar et al., 2011). The obtained wetland *amoA* gene sequences were reported in the GenBank database under the accession numbers: MG658437–MG659290 for AOA and MG657380–MG658436 for AOB. *amoA* gene sequences having ≥95% similarity were defined as an operational taxonomic unit (OTU), and α-diversity indices (Chao1 richness and Shannon diversity) of AOA and AOB communities were further calculated using the software MOTHUR (Schloss et al., 2009). Phylogenetic analysis of the FWSF-CW *amoA* gene sequences was carried out using the software MEGA 6.0 (Tamura et al., 2013) with the neighbor-joining method, was further visualized with the software Interactive Tree Of Life (iTOL) v3 (<http://itol.embl.de>) (Letunic and Bork, 2016). In addition, weighted Unifrac was generated using R library GUniFrac, and then hierarchical clustering of wetland AOA or AOB communities was achieved using the R software (version i386, 3.3.2).

2.4. Statistical analysis

Differences in PNR or gene abundance among sites and seasons were checked at a 0.05 significance level with one-way analysis of variance. The links of environmental parameters with PNR and the richness, diversity and abundance of AOA or AOB were characterized using the software SPSS 20.0 (IBM, Armonk, NY, USA). Moreover, redundancy analysis (RDA) was applied to decode the correlations of community composition of FWSF-CW AOA and AOB communities with their surrounding environmental variables, using the software CANOCO 4.5.

3. Results

3.1. Potential nitrification rate

PNR is used to assess nitrifying activity in both constructed and natural wetlands (Li et al., 2018; Zheng et al., 2013). In this study, the PNR in the FWSF-CW ranged from 0.03 to 6.36 $\mu\text{g NO}_2\text{-N g}^{-1}$ dry sediment/soil h^{-1} (Fig. 1). Among different seasons, significant differences in PNR were detected at all sediment sites ($P < 0.05$). At sediment sites, the highest PNR occurred during winter, while the lowest PNR occurred during spring. The sediment PNR during spring, summer, and winter was 0.03–0.08, 0.10–0.15, and 0.16–0.21 $\mu\text{g NO}_2\text{-N g}^{-1}$ dry sediment h^{-1} , respectively. At soil sites, the lowest PNR occurred during summer. The PNR at soil site H ranged between 0.13 and 6.36 $\mu\text{g NO}_2\text{-N g}^{-1}$ dry soil h^{-1} , and a significant difference was detected among different seasons ($P < 0.05$). The PNR ranged between 0.1 and 0.33 $\mu\text{g NO}_2\text{-N g}^{-1}$ dry soil h^{-1} at site G, although no statistical difference was observed among seasons ($P > 0.05$). Moreover, during a given season, no significant difference in PNR was found among sediments ($P > 0.05$). Significant differences in PNR between soils could be observed during spring and winter ($P < 0.05$), instead of summer.

3.2. AOA and AOB abundance

In this study, the number of archaeal *amoA* gene in the sediments collected at sites A, C and F during winter was below q-PCR detection limit, while AOA abundance in other sediment/soil samples ranged between 8.54×10^3 and 9.62×10^6 archaeal *amoA* gene copies per gram dry sediment/soil (Fig. 2a). Among different seasons, significant differences in AOA abundance were found at both soil sites ($P < 0.05$). AOA abundance was 2.95×10^6 – 9.62×10^6 and 3.01×10^6 – 7.76×10^6 archaeal *amoA* gene copies per gram dry soil at sites G and H, respectively. Among (or between) different seasons, significant differences in AOA abundance were also found at sediment sites A, D, and E ($P < 0.05$). AOA abundance was 6.89×10^4 – 1.43×10^5 , 5.80×10^4 – 2.19×10^5 , and 8.54×10^3 – 5.31×10^4 archaeal *amoA* gene copies per gram dry sediment at sites A, D, and E, respectively. During spring and summer, significant differences in AOB abundance could be found among the six sediments (4.36×10^4 – 1.51×10^5 or 8.54×10^3 – 1.26×10^5 archaeal *amoA* gene copies per gram dry sediment) and between the two soils (3.01×10^6 – 9.62×10^6 or 2.95×10^6 – 7.76×10^6 archaeal *amoA* gene

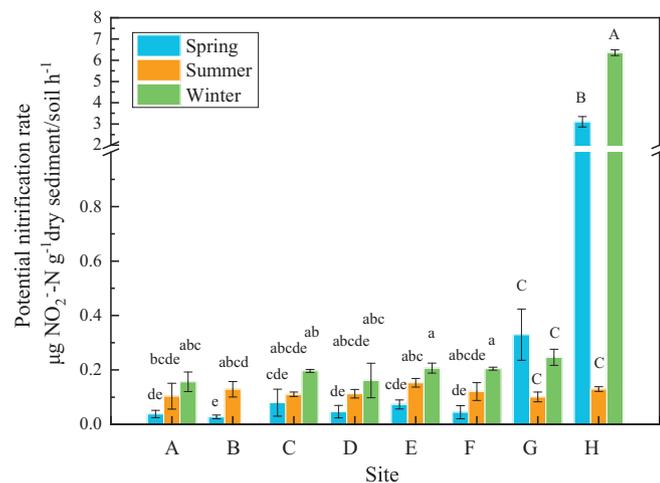


Fig. 1. Potential nitrification rates of different wetland sediment/soil samples. Values are the average of triplicate samples. Vertical bars indicate standard deviations. Different letters above the columns indicate significant differences ($P < 0.05$). The differences at a 0.05 significance level were separately checked among sediment samples or among soil samples.

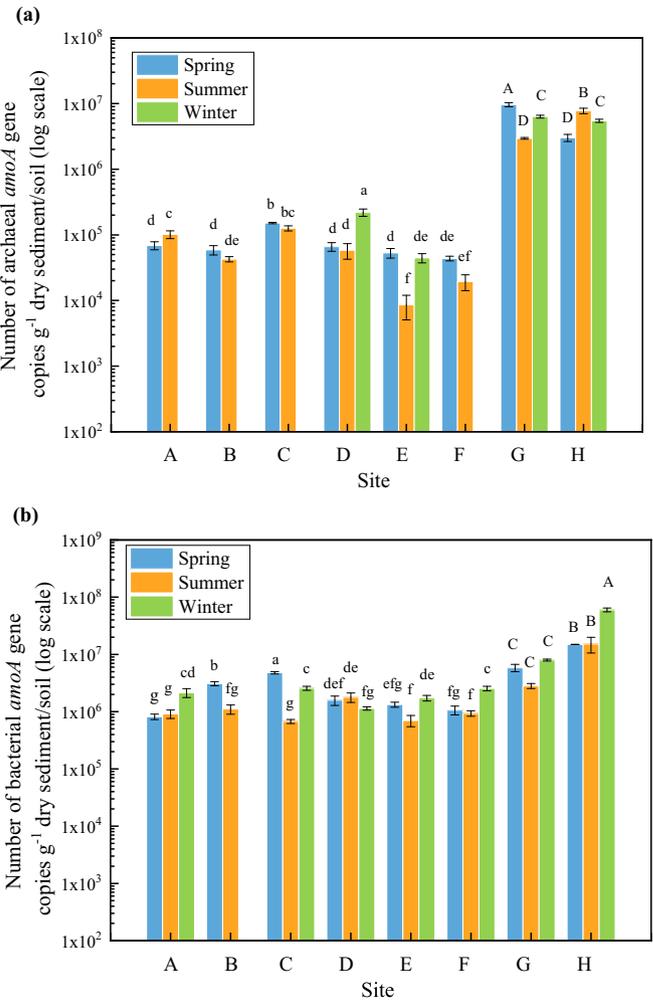


Fig. 2. Abundance of AOA (a) and AOB (b) communities in the FWSF-CW. Values are the average of triplicate samples. Vertical bars indicate standard deviations. Different letters above the columns indicate significant differences ($P < 0.05$). The differences at a 0.05 significance level were separately checked among sediment samples or among soil samples.

copies per gram dry soil) ($P < 0.05$). During winter, a significant difference in AOA abundance occurred between the sediments at sites D and E ($P < 0.05$), while AOA abundance was not statistically different between the soils at sites G and H ($P > 0.05$).

In the FWSF-CW, the number of bacterial *amoA* gene ranged between 6.76×10^5 and 6.01×10^7 copies per gram dry sediment/soil (Fig. 2b). AOB generally far outnumbered AOA, especially at wetland sediment sites. Among different seasons, significant differences in AOB abundance occurred at all sediment sites ($P < 0.05$). AOB abundance was 8.18×10^5 – 2.14×10^6 , 1.11×10^6 – 3.09×10^6 , 6.76×10^5 – 4.80×10^6 , 1.14×10^6 – 1.78×10^6 , 7.01×10^5 – 1.73×10^6 , and 9.34×10^5 – 2.54×10^6 bacterial *amoA* gene copies per gram dry sediment at sites A–F, respectively. Among different seasons, a significant difference in AOB abundance occurred at soil site H (1.49×10^7 – 6.01×10^7 bacterial *amoA* gene copies per gram dry soil) ($P < 0.05$), while no statistically significant difference was found at soil site G ($P > 0.05$), although AOB abundance varied from 2.82×10^6 to 7.99×10^6 bacterial *amoA* gene copies per gram dry soil). Moreover, during a given season, significant differences in AOB abundance were found among sediments and between soils ($P < 0.05$). In addition, a positive correlation was observed between PNR and AOB abundance ($R^2 = 0.8836$, $P < 0.05$) (Fig. S1b), while AOA abundance showed no significant correlation with PNR ($R^2 = 0.1109$, $P > 0.05$) (Fig. S1a).

3.3. AOA and AOB richness and diversity

In this study, the archaeal *amoA* gene of the sediments collected during winter at sites A, C and F was not successfully amplified for clone library construction. Each successfully constructed AOA library was composed of 24–59 archaeal *amoA* gene sequences and contained 1–13 OTUs (Table 1). Most of AOA rarefaction curves nearly leveled off (Fig. S2a), suggested that OTUs in these AOA libraries were well captured. The values of AOA Chao1 richness varied between 1 and 23.5 in sediments and between 3 and 12 in soils. The values of AOA Shannon diversity index were 0–2.1 and 0.59–2.02 in sediments and soils, respectively. Moreover, each AOB library had 21–58 bacterial *amoA* gene sequences and included 4–14 OTUs. A plateau appeared in the rarefaction curve for most of AOB clone libraries (Fig. S2b), suggested that the number of AOB clones in these libraries was satisfactory to represent AOB diversity and community structure. The values of AOB Chao1 richness and Shannon diversity index were 4–25 and 0.44–2.35, respectively. Considerable seasonal changes of AOA and AOB community richness and diversity occurred at FWSF-CW soil sites and most of sediment sites. In addition, considerable site-related changes of AOA and AOB community richness and diversity were also observed in the studied FWSF-CW.

3.4. Clustering analysis of AOA and AOB communities

Weighted UniFrac-based hierarchical clustering revealed a profound seasonality effect on AOA communities in the studied FWSF-CW (Fig. 3a). At a given site, the samples collected during different seasons were clearly separated. Moreover, the samples collected during a given season were not closely clustered together, suggesting a remarkable difference of AOA community structure among sites.

The strong seasonality effect on AOB communities also occurred in the studied FWSF-CW (Fig. 3b). At a given sediment site, the sediment collected during spring were clearly separated from those collected during other seasons, and the sediments collected during summer and winter were generally not closely grouped together. At a given soil site, the soils collected during three seasons were also clearly separated. Moreover, during a given season, the difference of AOB community structure among sites in the studied FWSF-CW was evident.

Table 1

Chao1 richness and Shannon diversity of wetland AOA and AOB communities.

Sample	AOA				AOB			
	Sequences	OTUs	Chao1	Shannon	Sequences	OTUs	Chao1	Shannon
ASP	44	13	23.5	2.1	44	14	19	2.35
ASU	42	7	7.5	1.35	45	6	7.5	0.73
AWI	–	–	–	–	58	10	17.5	1.13
BSP	42	8	8.5	1.65	43	9	15	1.57
BSU	45	11	12.2	1.85	47	9	15	1.75
CSP	33	2	2	0.66	49	6	6.5	0.91
CSU	37	11	21.5	1.65	47	4	5	0.44
CWI	–	–	–	–	33	7	17	1.18
DSP	40	9	12	1.7	46	11	18.5	1.82
DSU	45	9	19	1.47	44	5	5	0.9
DWI	52	8	18	1.06	47	5	5.3	0.55
ESP	42	6	6	1.36	48	8	8.3	1.51
ESU	37	5	5	1.12	44	10	25	1.62
EWI	24	1	1	0	21	6	7.5	1.23
FSP	41	10	11	1.94	42	13	15.5	2.21
FSU	44	10	11	1.75	45	7	8.5	1.02
FWI	–	–	–	–	51	7	7	1.24
GSP	59	6	6	1.26	49	7	8.5	1.25
GSU	43	7	8	1.65	48	6	6	1.48
GWI	47	3	3	0.59	52	4	4	0.97
HSP	46	5	6	1.06	48	7	8.5	1.25
HSU	44	12	19.5	2.02	51	5	5.5	0.9
HWI	47	4	5	0.74	55	9	24	1.32

“–” indicates unsuccessful amplification

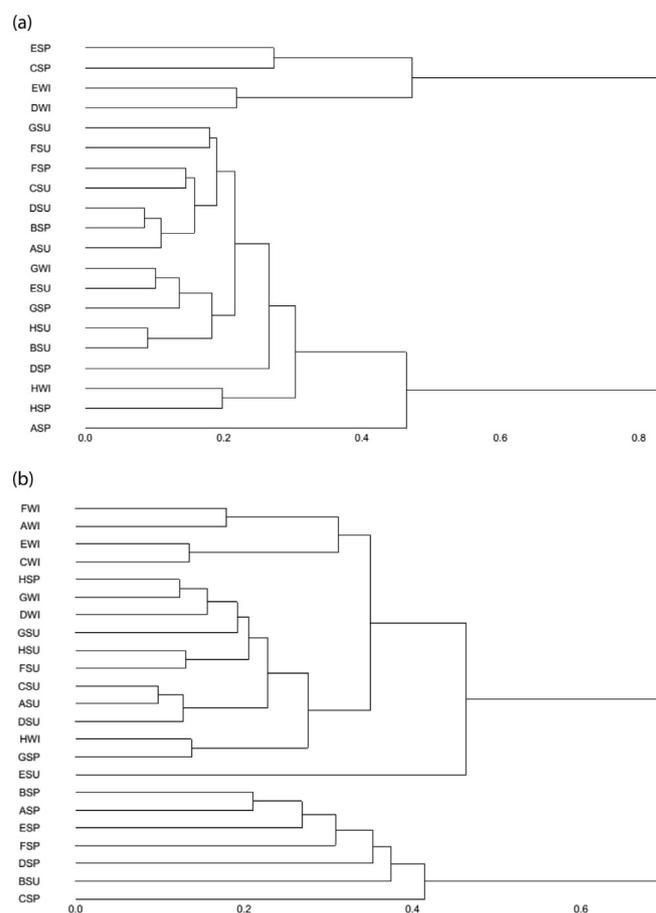
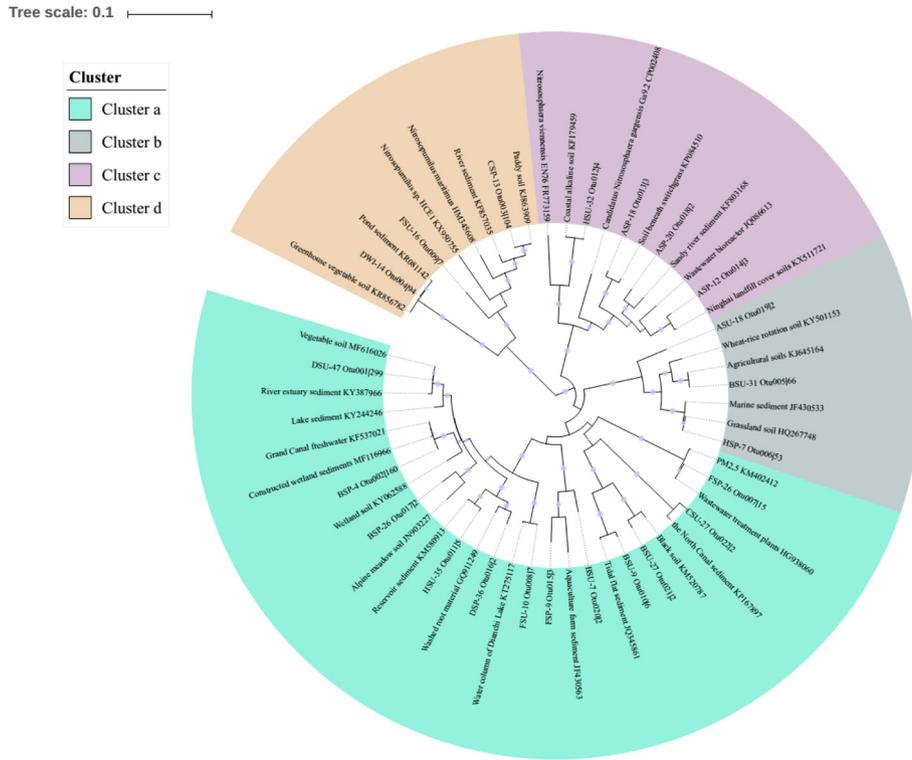


Fig. 3. Comparisons of wetland AOA (a) and AOB (b) communities based on weighted UniFrac.

3.5. Phylogenetic analysis of AOA and AOB communities

In this study, all *amoA* gene sequences could be grouped into 33 AOA and 49 AOB OTUs, respectively. The *amoA* OTUs contained at least two

(a)



(b)

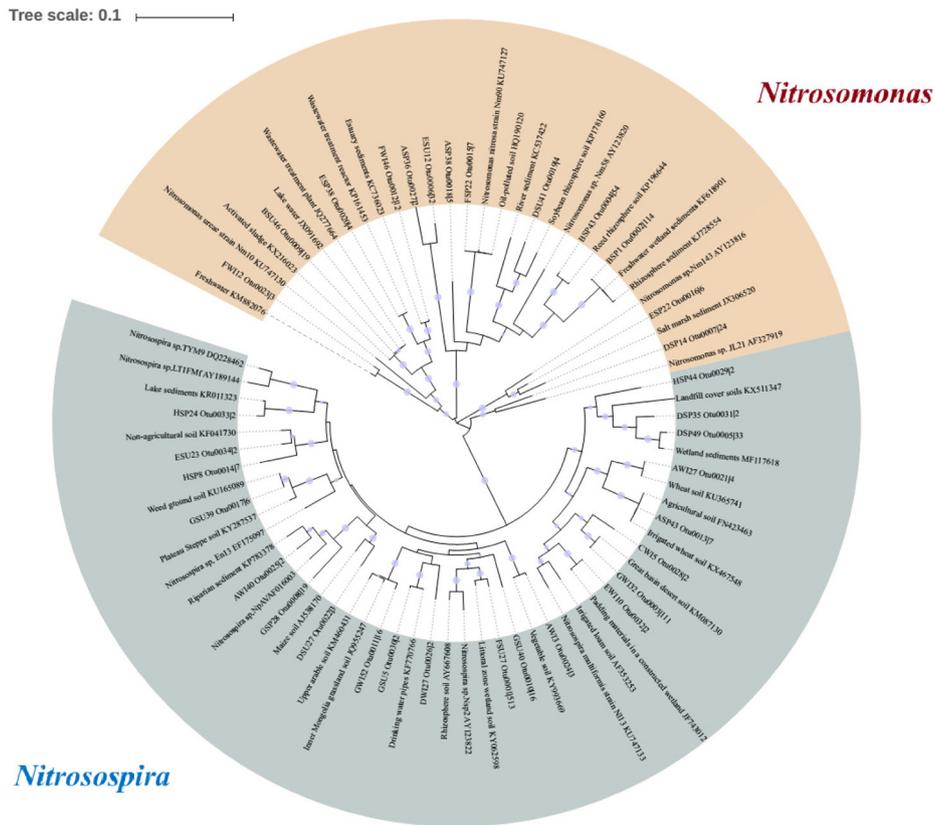


Fig. 4. Phylogenetic analysis of the representative archaeal (a) and bacterial (b) *amoA* gene sequences. The dots at branches indicate the support values from bootstrap test. Branch support values of not less than 50 were dotted. The digits after OTU name show the total *amoA* sequences from all wetland samples in the same OTU. The bar shows 10% sequence divergence based on neighbor-joining analysis.

sequences were used for further phylogenetic analysis. The obtained archaeal *amoA* gene sequences could be assigned into four AOA clusters (a, b, c and d) (Fig. 4a). Cluster a contained 13 AOA OTUs, and the 505 archaeal *amoA* gene sequences in cluster a could be grouped together with those obtained from diverse soil and sediment ecosystems, lake water, and wastewater treatment plants. Cluster a-like organisms usually dominated in the AOA communities in the studied FWSF-CW (Fig. S3a). Cluster b had only three AOA OTUs, and the 121 *amoA* gene sequences in this cluster were grouped together with those obtained from soil and sediment ecosystems. Cluster b-like organisms were usually a minor group in the studied FWSF-CW, but they were dominant in sample HSP. There were four AOA OTUs in cluster c. The 12 sequences in this cluster could be affiliated with some cultivated *Nitrososphaera* strains (Spang et al., 2012; Tourna et al., 2011). Cluster c-like organisms, with low proportions, were only detected in several samples. Moreover, there were three AOA OTUs including 205 archaeal *amoA* gene sequences in cluster d. Cluster d-like organisms were the second largest AOA group in the studied FWSF-CW, and they could be affiliated with cultivated *Nitrosopumilus* strains (Brown et al., 2013). Cluster d-like organisms were almost detected in all samples, and they even showed the dominance in sediment samples CSP, DWI, ESP and EWI.

The obtained bacterial *amoA* gene sequences could be assigned into two AOB clusters, namely *Nitrosospira* and *Nitrosomonas* (Fig. 4b). *Nitrosospira* cluster contained 21 AOB OTUs including a total of 756 bacterial *amoA* gene sequences. These bacterial *amoA* gene sequences could be affiliated with a number of *Nitrosospira* strains (Klotz and Norton, 1995; Mintie et al., 2003; Purkhold et al., 2003). *Nitrosomonas* cluster was composed of 13 AOB OTUs including a total of 286 *amoA* gene sequences that could be affiliated with a number of *Nitrosomonas* strains (Purkhold et al., 2003; Suwa et al., 1997). Both *Nitrosospira* and *Nitrosomonas* were the major AOB species in sediments of the studied FWSF-CW, while *Nitrosospira* predominated in soils (Fig. S3b). In addition, the results of phylogenetic analysis further confirmed the seasonal and spatial variations of AOA and AOB community structures in the studied FWSF-CW.

3.6. Influential factors regulating AOA and AOB communities

Spearman's rank correlation analysis indicated that PNR was positively correlated with the levels of TOC, NO_3^- -N and NO_2^- -N ($P < 0.05$ or $P < 0.01$), but negatively with the level of NH_4^+ -N ($P < 0.05$) (Table 2). Both AOA and AOB abundance showed positive correlations with the levels of NO_2^- -N, TN, TP and TOC ($P < 0.05$ or $P < 0.01$) but negative correlations with the level of NH_4^+ -N ($P < 0.05$ or $P < 0.01$). AOA diversity was significantly correlated with temperature, C/N and the level of TOC ($P < 0.05$), while AOB diversity showed a significant correlation with the level of TOC ($P < 0.05$). No significant correlations were found between the determined FWSF-CW environmental variables and AOA and AOB community richness ($P > 0.05$).

The environmental parameters in the first two principal RDA axes totally explained 73.5% and 58.1% of the cumulative variances for AOA and AOB OTU compositions, respectively (Fig. 5a,b). Sediment/soil temperature ($F = 3.804$, $P = 0.004$, 999 permutations) and NO_3^- -N ($F =$

4.309, $P = 0.002$, 999 permutations) significantly contributed to the FWSF-CW AOA OTU composition–environment relationship. In addition, sediment/soil temperature ($F = 2.045$, $P = 0.041$, 999 permutations) and TP ($F = 3.659$, $P = 0.003$, 999 permutations) also had significant contributions to the FWSF-CW AOB OTU composition–environment relationship.

4. Discussion

4.1. Variations of AOA and AOB abundance in wetlands

A few previous studies have revealed considerable changes of sediment/soil AOA and AOB abundance with both sampling site and sampling time (season) in natural wetlands (Hu et al., 2014; Wang et al., 2013b; Xia et al., 2015; Zheng et al., 2013). Considerable variations of sediment/soil AOA and AOB abundance with sampling site have also been documented in FWSF-CWs treating river water (Fan et al., 2016; Wang et al., 2013a). Moreover, several previous studies revealed a strong seasonality effect on AOB but a weak one on AOA abundance in FWSF-CWs used for tertiary wastewater treatment (Sims et al., 2012) and swine wastewater treatment (Li et al., 2018). So far, information on the seasonality effect on AOA and AOB abundance in FWSF-CW treating surface water is still lacking. In the studied FWSF-CW treating river water, both AOA and AOB abundance considerably varied with season and sampling site. This suggested a strong seasonality effect on both AOA and AOB abundance.

AOA organisms show relatively high specific affinity for ammonia nitrogen and have the preference for low levels of ammonium nitrogen (Erguder et al., 2009), while high levels of ammonium nitrogen favors AOB abundance (Di et al., 2009). However, the links of sediment/soil NH_4^+ -N with wetland AOA and AOB abundance remain elusive. Previous studies reported that the level of sediment/soil NH_4^+ -N was positively correlated with AOB abundance in natural wetlands (Hu et al., 2014; Kim et al., 2017; Wang et al., 2011), while increasing sediment NH_4^+ -N level might also promote AOA abundance in natural grove wetland (Li et al., 2011) and intertidal wetland (Hu et al., 2014). Wang et al. (2013a) suggested that a higher level of NH_4^+ -N increased AOB abundance but lowered AOA abundance in a FWSF-CW treating river water. In this study, the results of Spearman's rank correlation analysis suggested that a higher level of sediment/soil NH_4^+ -N might have negative influences on both AOA and AOB abundance in the FWSF-CW treating surface water. Moreover, the results of Spearman's rank correlation analysis further suggested that both AOA and AOB abundance were also collectively regulated by the levels of sediment/soil NO_2^- -N, TN, TP and TOC, as well as NH_4^+ -N. The results suggested that niche separation of AOA and AOB might not clearly exist in the studied FWSF-CW. TN, TP, TOC and NH_4^+ -N are the commonly known factors influencing the AOA and AOB abundance in freshwater lake sediment (Yang et al., 2016b). In a lake riparian freshwater wetland, TP was found to be one of the determinants of AOB abundance, while organic matter determined both AOA and AOB abundance (Zhang et al., 2015). To the authors' knowledge, this was the first report on the potential influences of sediment/soil NO_2^- -N and TN on wetland AOA and AOB abundance.

Table 2

Spearman's rank correlation analysis of environmental variables with PNR and the richness and diversity of AOA and AOB communities in the FWSF-CW.

	NH_4^+ -N	NO_3^- -N	NO_2^- -N	TN	TP	TOC	Temperature	ORP	pH	C/N
PNR	-0.496*	0.714**	0.548**	0.195	0.196	0.524*	-0.347	0.290	0.261	0.382
AOA abundance	-0.442*	0.382	0.524*	0.471*	0.720**	0.581**	-0.046	0.231	0.385	0.293
AOA richness	0.373	-0.264	-0.222	-0.126	0.142	-0.286	0.432	-0.267	0.027	-0.206
AOA diversity	0.394	-0.389	-0.426	-0.104	0.092	-0.471*	0.537*	-0.229	-0.092	-0.478*
AOB abundance	-0.673**	0.340	0.531**	0.529**	0.528**	0.643**	-0.294	0.281	0.271	0.267
AOB richness	0.208	0.132	-0.027	-0.062	-0.056	-0.321	-0.194	0.035	-0.027	-0.311
AOB diversity	-0.048	-0.195	-0.262	-0.065	0.012	-0.417*	-0.050	-0.002	-0.031	-0.385

* Correlation is significant at the 0.05 level.

** Correlation is significant at the 0.01 level.

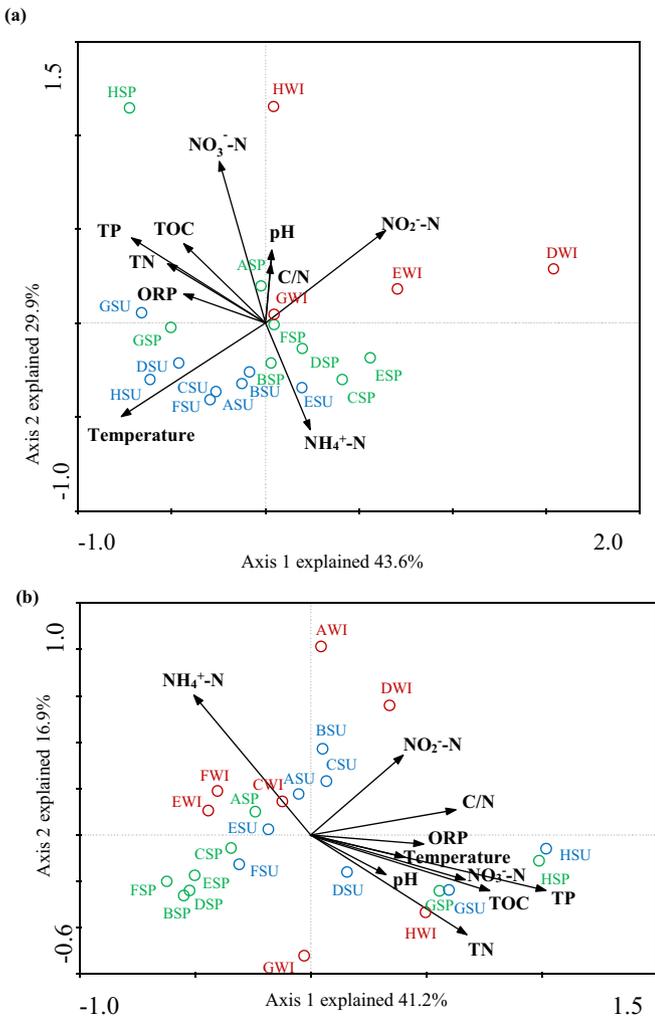


Fig. 5. RDA ordination plot for the first two principal dimensions of the links of AOA (a) and AOB (b) OTU compositions with sediment/soil physicochemical parameters in the FWSF-CW.

Wang et al. (2013a) suggested the more critical role of AOB in ammonia oxidation in a FWSF-CW treating river water. In contrast, Fan et al. (2016) suggested that AOA was the more important player in ammonia oxidation in FWSF-CWs treating river water. In this study, AOB tended to far outnumber AOA. This was in agreement with the results reported on natural freshwater wetlands (He et al., 2017b; Kim et al., 2017; Wu et al., 2013). Moreover, in this study, PNR showed a positive correlation with AOB abundance, instead of AOA abundance. However, further direct evidence would be necessary for the contribution of AOB on the total ammonia oxidation in the studied constructed wetlands.

4.2. Variations of AOA and AOB richness and diversity in wetlands

Although seasonal and spatial changes of sediment AOA and AOB richness and diversity have been documented in natural wetlands (Xia et al., 2015; Zheng et al., 2013), there was still a paucity of direct information about the variations of sediment or soil AOA and AOB richness and diversity in CW. In the studied FWSF-CW, AOA and AOB richness and diversity were found to differ considerably among sites and seasons. Moreover, so far, the links of AOA and AOB richness and diversity with wetland sediment/soil environmental variables remain largely unclear. In this study, Spearman's rank correlation analysis suggested that increasing TOC level could decrease both AOA and AOB diversity. However, the level of organic matter was found to be positively correlated

with AOB diversity in a lake riparian wetland (Zhang et al., 2015). In the studied FWSF-CW, the rise of sediment/soil temperature was found to favor AOA diversity, while the influences of temperature on AOA and AOB diversity were not considerable in salt marsh wetland (Xia et al., 2015) and mangrove wetland (Li et al., 2011). In addition, the present study further provided the evidence that AOA diversity might be influenced by wetland sediment/soil C/N.

4.3. Variations of AOA and AOB community structures in wetlands

A previous study indicated that *Nitrosopumilus* organisms were the dominant AOA species in a natural freshwater wetland (He et al., 2017b), while Zhang et al. (2015) reported that *Nitrososphaera* predominated in another natural freshwater wetland. Moreover, Liu et al. (2017) revealed that both *Nitrosopumilus* and *Nitrososphaera* were the dominant AOA species in a natural freshwater wetland. *Nitrososphaera*-like organisms were found to be the major AOA in FWSF-CWs treating river water (Fan et al., 2016) and municipal wastewater (Liu et al., 2017). In the present study, most of the retrieved archaeal *amoA* gene sequences were affiliated with uncultured AOA species. In addition, a considerable proportion of archaeal *amoA* gene sequences could be affiliated with cultivated *Nitrosopumilus* strains, while *Nitrososphaera*-like organisms were the minor AOA group. The results were different from those reported in other wetland ecosystems (Fan et al., 2016; He et al., 2017b; Liu et al., 2017; Zhang et al., 2015). Hence, the major AOA group might be wetland-specific.

Some previous studies indicated that *Nitrosomonas* was the dominant AOB species in natural freshwater wetlands (He et al., 2017b; Wu et al., 2013), while other studies reported the dominance of *Nitrosospira* in different natural freshwater wetlands (Lee et al., 2014; Liu et al., 2017; Zhang et al., 2015). Moreover, *Nitrosomonas* dominated in FWSF-CWs treating river water (Fan et al., 2016) and tertiary wastewater treatment (Sims et al., 2012), while *Nitrosospira* showed the dominance in a FWSF-CW for advanced treatment of municipal wastewater (Liu et al., 2017). In this study, although *Nitrosospira* predominated in the soils of the FWSF-CW treating river water, both *Nitrosospira* and *Nitrosomonas* were the major AOB species in sediments. This was not in harmony with the results reported in the previous studies (He et al., 2017b; Lee et al., 2014; Liu et al., 2017; Sims et al., 2012; Wu et al., 2013; Zhang et al., 2015). Therefore, the major AOB group might be wetland-related.

A few previous studies have reported evident seasonal and spatial changes of sediment/soil AOA and AOB community structures in natural wetlands (Hu et al., 2014; Wang et al., 2013b; Zheng et al., 2013). Evident spatial variations of AOA and AOB community structures have also been found in FWSF-CWs treating river water (Fan et al., 2016), and in an integrated constructed wetland for swine wastewater treatment (Zhang et al., 2017). However, the seasonality effects on AOA and AOB community structures in CWs remain unknown. In this study, the results of both weighted UniFrac-based hierarchical clustering and phylogenetic analysis indicated considerable season- and site-related variations of AOA and AOB community structures in the studied FWSF-CW. The result of RDA also suggested that sediment/soil temperature was a key factor influencing both AOA and AOB community structures in the studied FWSF-CW. This further confirmed the strong seasonality effects on AOA and AOB community structures. NO_3^- -N was a key factor shaping AOA community structure in freshwater sediment (Yang et al., 2016b). A strong correlation between AOA community structure and NO_3^- -N have also been found in intertidal wetland sediment/soil (Hu et al., 2014; Zheng et al., 2013). The result of RDA suggested that NO_3^- -N might also play an important role in shaping AOA community structure in the studied FWSF-CW. The present study provide the first evidence for the potential links of sediment/soil NO_3^- -N with AOA community structure in CW treating surface water. Moreover, although TP was a determinant of AOB community structure in freshwater sediment (Yang et al., 2016b), the links of TP with sediment/soil

AOB community structure remain unknown. In this study, TP was found to have an influence on AOB community structure in the FWSF-CW used for river water treatment.

5. Conclusions

Strong seasonality effects on PNR and the abundance, richness, diversity and structure of AOA and AOB communities occurred in a FWSF-CW treating river water. AOB generally far outnumbered AOA, and AOB abundance showed a positive correlation with PNR. A considerable proportion of archaeal *amoA* gene sequences could be affiliated with cultivated *Nitrosopumilus* strains. *Nitrosospira* and *Nitrosomonas* were the dominant AOB species. AOA and AOB abundance were collectively regulated by the same environmental variables, while the environmental variables determining the diversity and structure of AOA and AOB communities were different.

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Compliance with ethical standards

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest

The authors declare that they have no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2018.02.041>.

References

- Bakhshoodeh, R., Alavi, N., Paydary, P., 2017a. Composting plant leachate treatment by a pilot-scale, three-stage, horizontal flow constructed wetland in central Iran. *Environ. Sci. Pollut. Res.* 24, 23803–23814.
- Bakhshoodeh, R., Alavi, N., Majlesi, M., Paydary, P., 2017b. Compost leachate treatment by a pilot-scale subsurface horizontal flow constructed wetland. *Ecol. Eng.* 105, 7–14.
- Barco, A., Borin, M., 2017. Treatment performance and macrophytes growth in a restored hybrid constructed wetland for municipal wastewater treatment. *Ecol. Eng.* 107, 160–171.
- Brown, M.N., Briones, A., Diana, J., Raskin, L., 2013. Ammonia-oxidizing archaea and nitrite-oxidizing nitrospiras in the biofilter of a shrimp recirculating aquaculture system. *FEMS Microbiol. Ecol.* 83, 17–25.
- Chen, J., Gu, J.D., 2017. Faunal burrows alter the diversity, abundance, and structure of AOA, AOB, Anammox and n-damo communities in coastal mangrove sediments. *Microb. Ecol.* 74, 140–156.
- Chen, Y.L., Xu, Z.W., Hu, H.W., Hu, Y.J., Hao, Z.P., Jiang, Y., Chen, B.D., 2013. Responses of ammonia-oxidizing bacteria and archaea to nitrogen fertilization and precipitation increment in a typical temperate steppe in Inner Mongolia. *Appl. Soil Ecol.* 68, 36–45.
- Chen, J., Ying, G.G., Liu, Y.S., Wei, X.D., Liu, S.S., He, L.Y., Yang, Y.Q., Chen, F.R., 2017. Nitrogen removal and its relationship with the nitrogen-cycle genes and microorganisms in the horizontal subsurface flow constructed wetlands with different design parameters. *J. Environ. Sci. Health, Part A: Toxic/Hazard. Subst. Environ. Eng.* 52, 804–818.
- Chyan, J.M., Jhu, Y.X., Chen, I.M., Shiu, R.F., 2016. Improvement of nitrogen removal by external aeration and intermittent circulation in a subsurface flow constructed wetland of landscape garden ponds. *Process. Saf. Environ. Prot.* 103, 587–597.
- Dai, Y., Wu, Z., Zhou, Q.H., Zhao, Q., Li, N.N., Xie, S.G., Liu, Y., 2015. Activity, abundance and structure of ammonia-oxidizing microorganisms in plateau soils. *Res. Microbiol.* 166, 655–663.
- Di, H.J., Cameron, K.C., Shen, J.P., Winefield, C.S., O'Callaghan, M., Bowatte, S., He, J.Z., 2009. Nitrification driven by bacteria and not archaea in nitrogen-rich grassland soils. *Nat. Geosci.* 2, 621–624.
- Dzkapasu, M., Wang, X.C., Zheng, Y.C., Ge, Y., Xiong, J.Q., Zhao, Y.Q., 2015. Characteristics of nitrogen and phosphorus removal by a surface-flow constructed wetland for polluted river water treatment. *Water Sci. Technol.* 71, 904–912.
- Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 27, 2194–2200.
- Erguder, T.H., Boon, N., Wittebolle, L., Marzorati, M., Verstraete, W., 2009. Environmental actors shaping the ecological niches of ammonia oxidizing archaea. *FEMS Microbiol. Rev.* 33, 855–869.
- Fan, L.F., Chen, H.J., Hsieh, H.L., Lin, H.J., Tang, S.L., 2016. Comparing abundance, composition and environmental influences on prokaryotic ammonia oxidizers in two subtropical constructed wetlands. *Ecol. Eng.* 90, 336–346.
- Francis, C.A., Roberts, K.J., Beman, J.M., Santoro, A.E., Oakley, B.B., 2005. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. *Proc. Natl. Acad. Sci. U. S. A.* 102, 14683–14688.
- He, H.L., Duan, Z.W., Wang, Z.Q., Yue, B., 2017a. The removal efficiency of constructed wetlands filled with the zeolite-slag hybrid substrate for the rural landfill leachate treatment. *Environ. Sci. Pollut. Res.* 24, 17547–17555.
- He, Y., Hu, W.G., Ma, D.C., Lan, H.Z., Yang, Y., Gao, Y., 2017b. Abundance and diversity of ammonia-oxidizing archaea and bacteria in the rhizosphere soil of three plants in the Ebinur Lake wetland. *Can. J. Microbiol.* 63, 573–582.
- Hofferle, S., Nicol, G.W., Pal, L., Hacin, J., Prosser, J.I., Mandic-Mulec, I., 2010. Ammonium supply rate influences archaeal and bacterial ammonia oxidizers in a wetland soil vertical profile. *FEMS Microbiol. Ecol.* 74, 302–315.
- Hu, Z.Y., Meng, H., Shi, J.H., Bu, N.S., Fang, C.M., Quan, Z.X., 2014. Community size and composition of ammonia oxidizers and denitrifiers in an alluvial intertidal wetland ecosystem. *Front. Microbiol.* 5, 371.
- Hu, Y., He, F., Ma, L., Zhang, Y., Wu, Z.B., 2016. Microbial nitrogen removal pathways in integrated vertical-flow constructed wetland systems. *Bioresour. Technol.* 207, 339–345.
- Hua, Y.M., Peng, L., Zhang, S.H., Heal, K.V., Zhao, J.W., Zhu, D.W., 2017. Effects of plants and temperature on nitrogen removal and microbiology in pilot-scale horizontal subsurface flow constructed wetlands treating domestic wastewater. *Ecol. Eng.* 108, 70–77.
- Huang, X., Liu, C.X., Wang, Z., Gao, C.F., Zhu, G.F., Liu, L., 2013. The effects of different substrates on ammonium removal in constructed wetlands, a comparison of their physicochemical characteristics and ammonium-oxidizing prokaryotic communities. *Clean: Soil, Air, Water* 41, 283–290.
- Hussein, A., Scholz, M., 2017. Dye wastewater treatment by vertical-flow constructed wetlands. *Ecol. Eng.* 101, 28–38.
- Kim, H., Ogram, A., Bae, H.S., 2017. Nitrification, anammox and denitrification along a nutrient gradient in the Florida Everglades. *Wetlands* 37, 391–399.
- Klotz, M.G., Norton, J.M., 1995. Sequence of an ammonia monooxygenase subunit A-encoding gene from *Nitrosospira* sp. NpAV. *Gene* 163, 159–160.
- Lee, K.H., Wang, Y.F., Li, H., Gu, J.D., 2014. Niche specificity of ammonia-oxidizing archaeal and bacterial communities in a freshwater wetland receiving municipal wastewater in Daqing, Northeast China. *Ecotoxicology* 23, 2081–2091.
- Letunic, I., Bork, P., 2016. Interactive tree of life (ITOL) v3, an online tool for the display and annotation of phylogenetic and other trees. *Nucleic Acids Res.* 44, W242–W245.
- Li, M., Cao, H.L., Hong, Y.G., Gu, J.D., 2011. Spatial distribution and abundances of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) in mangrove sediments. *Appl. Microbiol. Biotechnol.* 89, 1243–1254.
- Li, B.X., Chen, H.L., Li, N.N., Wu, Z., Wen, Z.G., Xie, S.G., Liu, Y., 2017. Spatio-temporal shifts in the archaeal community of a constructed wetland treating river water. *Sci. Total Environ.* 605–606, 269–275.
- Li, X., Zhang, M.M., Liu, F., Chen, L., Li, Y.Y., Li, Y., Xiao, R.L., Wu, J.S., 2018. Seasonality distribution of the abundance and activity of nitrification and denitrification microorganisms in sediments of surface flow constructed wetlands planted with *Myriophyllum elatinoide* during swine wastewater treatment. *Bioresour. Technol.* 248, 89–97.
- Liu, Z.J., Xie, H.J., Hu, Z., Zhang, J., Zhang, J.D., Sun, H.M., Lan, W., 2017. Role of ammonia-oxidizing archaea in ammonia removal of wetland under low-temperature condition. *Water Air Soil Pollut.* 228, 356.
- Mintie, A.T., Heichen, R.S., Cromack Jr., K., Myrold, D.D., Bottomley, P.J., 2003. Ammonia-oxidizing bacteria along meadow-to-forest transects in the Oregon Cascade Mountains. *Appl. Environ. Microbiol.* 69, 3129–3136.
- Moin, N.S., Nelson, K.A., Bush, A., Bernhard, A.E., 2009. Distribution and diversity of archaeal and bacterial ammonia oxidizers in salt marsh sediments. *Appl. Environ. Microbiol.* 75, 7461–7468.
- Pelissari, C., Guivernau, M., Vinas, M., de Souza, S.S., Garcia, J., Sezerino, P.H., Avila, C., 2017. Unraveling the active microbial populations involved in nitrogen utilization in a vertical subsurface flow constructed wetland treating urban wastewater. *Sci. Total Environ.* 584, 642–650.
- Purkhold, U., Wagner, M., Timmermann, G., Pommerening-Roser, A., Koops, H.P., 2003. 16S rRNA and *amoA*-based phylogeny of 12 novel betaproteobacterial ammonia-oxidizing isolates, extension of the dataset and proposal of a new lineage within the nitrospiras. *Int. J. Syst. Evol. Microbiol.* 53, 1485–1494.
- Rotthauwe, J.H., Witzel, K.P., Liesack, W., 1997. The ammonia monooxygenase structural gene *amoA* as a functional marker, molecular fine-scale analysis of natural ammonia-oxidizing populations. *Appl. Environ. Microbiol.* 63, 4704–4712.
- Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing MOHUR, open-source, platform-independent, community supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* 75, 7537–7541.
- Sims, A., Gajaraj, S., Hu, Z.Q., 2012. Seasonal population changes of ammonia-oxidizing organisms and their relationship to water quality in a constructed wetland. *Ecol. Eng.* 40, 100–107.
- Spang, A., Poehlein, A., Offre, P., Zumbragel, S., Haider, S., Rychlik, N., Nowka, B., Schmeisser, C., Lebedeva, E.V., Rattai, T., Bohm, C., Schmid, M., Galushko, A., Hatzepichler, R., Weinmaier, T., Daniel, R., Schleper, C., Spieck, E., Streit, W.,

- Wagner, M., 2012. The genome of the ammonia-oxidizing *Candidatus Nitrososphaera gargensis*, insights into metabolic versatility and environmental adaptations. *Environ. Microbiol.* 14, 3122–3145.
- Suwa, Y., Sumino, T., Noto, K., 1997. Phylogenetic relationships of activated sludge isolates of ammonia oxidizers with different sensitivities to ammonium sulfate. *J. Gen. Appl. Microbiol.* 43, 373–379.
- Tamura, K., Stecher, G., Peterson, D., Filipiński, A., Kumar, S., 2013. MEGA6, Molecular Evolutionary Genetics Analysis version 6.0. *Mol. Biol. Evol.* 30, 2725–2729.
- Tan, E., Hsu, T.C., Huang, X.F., Lin, H.J., Kao, S.J., 2017. Nitrogen transformations and removal efficiency enhancement of a constructed wetland in subtropical Taiwan. *Sci. Total Environ.* 601, 1378–1388.
- Tourna, M., Stieglmeier, M., Spang, A., Könneke, M., Schintlmeister, A., Urich, T., Engel, M., Schloter, M., Wagner, M., Richter, A., 2011. *Nitrososphaera viennensis*, an ammonia oxidizing archaeon from soil. *Proc. Natl. Acad. Sci. U. S. A.* 108, 8420–8425.
- Wang, S.Y., Wang, Y., Feng, X.J., Zhai, L.M., Zhu, G.B., 2011. Quantitative analyses of ammonia-oxidizing Archaea and bacteria in the sediments of four nitrogen-rich wetlands in China. *Appl. Microbiol. Biotechnol.* 90, 779–787.
- Wang, C.X., Zhu, G.B., Wang, W.D., Yin, C.Q., 2013a. Preliminary study on the distribution of ammonia oxidizers and their contribution to potential ammonia oxidation in the plant-bed/ditch system of a constructed wetland. *J. Soils Sediments* 13, 1626–1635.
- Wang, Y.F., Feng, Y.Y., Ma, X.J., Gu, J.D., 2013b. Seasonal dynamics of ammonia/ammonium-oxidizing prokaryotes in oxic and anoxic wetland sediments of subtropical coastal mangrove. *Appl. Microbiol. Biotechnol.* 97, 7919–7934.
- Wang, X.Y., Wang, C., Bao, L.L., Xie, S.G., 2014. Abundance and community structure of ammonia-oxidizing microorganisms in reservoir sediment and adjacent soils. *Appl. Microbiol. Biotechnol.* 98, 1883–1892.
- Wu, L.H., Hui, L.H., Wang, X.Y., Li, J.Y., Yu, J.L., Zhao, J., 2013. Abundance and composition of ammonia-oxidizing bacteria and archaea of a degraded lake wetland, Inner Mongolian Plateau, China. *Soil Res.* 51, 554–560.
- Xia, F., Zeleke, J., Sheng, Q., Wu, J.H., Quan, Z.X., 2015. Communities of ammonia oxidizers at different stages of *Spartina alterniflora* invasion in salt marshes of Yangtze River estuary. *J. Microbiol.* 53, 311–320.
- Xu, Y.N., Tong, T.L., Chen, J.F., Li, B.X., Xie, S.G., 2018. Nitrite-dependent methane-oxidizing bacteria seasonally and spatially shift in a constructed wetland used for treating river water. *Ecol. Eng.* 110, 48–53.
- Yang, Y.Y., Li, N.N., Zhao, Q., Yang, M.X., Wu, Z., Xie, S.G., Liu, Y., 2016a. Ammonia-oxidizing archaea and bacteria in water columns and sediments of a highly eutrophic plateau freshwater lake. *Environ. Sci. Pollut. Res.* 23, 15358–15369.
- Yang, Y.Y., Zhang, J.X., Zhao, Q., Zhou, Q.H., Li, N.N., Wang, Y.L., Xie, S.G., Liu, Y., 2016b. Sediment ammonia-oxidizing microorganisms in two plateau freshwater lakes at different trophic states. *Microb. Ecol.* 71, 257–265.
- Yin, J., Jiang, L.Y., Wen, Y., Yao, Z.L., Zhou, Q., 2009. Treatment of polluted landscape lake water and community analysis of ammonia-oxidizing bacteria in constructed wetland. *J. Environ. Sci. Health, Part A: Toxic/Hazard. Subst. Environ. Eng.* 44, 722–731.
- Zhang, J.P., Liu, B., Zhou, X.H., Chu, J.Y., Li, Y.M., Wang, M.Y., 2015. Effects of emergent aquatic plants on abundance and community structure of ammonia-oxidizing microorganisms. *Ecol. Eng.* 81, 504–513.
- Zhang, F.Q., Pan, W., Gu, J.D., Xu, B., Zhang, W.H., Zhu, B.Z., Wang, Y.X., Wang, Y.F., 2016. Dominance of ammonia-oxidizing archaea community induced by land use change from Masson pine to eucalypt plantation in subtropical China. *Appl. Microbiol. Biotechnol.* 100, 6859–6869.
- Zhang, M.M., Luo, P., Liu, F., Li, H.F., Zhang, S.N., Xiao, R.L., Yin, L.M., Zhou, J., Wu, J.S., 2017. Nitrogen removal and distribution of ammonia-oxidizing and denitrifying genes in an integrated constructed wetland for swine wastewater treatment. *Ecol. Eng.* 104, 30–38.
- Zhao, K., Kong, W.D., Khan, A., Liu, J.B., Guo, G.X., Muhammad, S., Zhang, X.Z., Dong, X.B., 2017. Elevational diversity and distribution of ammonia-oxidizing archaea community in meadow soils on the Tibetan Plateau. *Appl. Microbiol. Biotechnol.* 101, 7065–7074.
- Zheng, Y.L., Hou, L.J., Liu, M., Lu, M., Zhao, H., Yin, G.Y., Zhou, J.L., 2013. Diversity, abundance, and activity of ammonia-oxidizing bacteria and archaea in Chongming eastern intertidal sediments. *Appl. Microbiol. Biotechnol.* 97, 8351–8363.
- Zheng, Y.C., Wang, X.C.C., Dzakpasu, M., Ge, Y., Zhao, Y.Q., Xiong, J.Q., 2016. Performance of a pilot demonstration-scale hybrid constructed wetland system for on-site treatment of polluted urban river water in Northwestern China. *Environ. Sci. Pollut. Res.* 23, 447–454.
- Zhi, E.Q., Song, Y.H., Duan, L., Yu, H.B., Peng, J.F., 2015. Spatial distribution and diversity of microbial community in large-scale constructed wetland of the Liao River conservation area. *Environ. Earth Sci.* 73, 5085–5094.