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Abundance and diversity of ammonia-oxidizing archaea and bacteria in the rhizosphere soil of three plants in the Ebinur Lake wetland

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**Abundance and diversity of ammonia-oxidizing archaea and bacteria in the rhizosphere soil of
three plants in the Ebinur Lake wetland**

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Abstract

Ammonia oxidation is carried out by ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA). The Ebinur Lake wetland is the best example of a temperate arid zone wetland ecosystem in China. Soil samples were collected from rhizosphere and non-rhizosphere soil containing *Halocnemum strobilaceum* (H and H'), *Phragmites australis* (R and R'), and *Kareliniaca sp.* (K and K') to study the relationship between environmental factors and the community structure of AOB and AOA. Phylogenetic analysis showed that the AOA sequences belonged to the *Nitrosopumilus* and *Nitrososphaera* clusters. AOB were grouped into *Nitrospira* sp. and *Nitrosomonas* sp. Quantitative polymerase chain reaction (qPCR) results showed that the AOA abundance ranged from 2.09×10^4 to 2.94×10^5 gene copies/g soil. The highest number of AOA was detected in sample K, followed by samples R and H. AOB abundance varied between 2.91×10^5 to 1.05×10^6 gene copies/g soil, which was higher than that of AOA. Redundancy analysis (RDA) indicated that electrical conductivity (EC), pH, and $\text{NH}_4^+\text{-N}$ might influence the community structure of AOA and AOB. AOB might play a more crucial role in ammonia oxidation based on its higher diversity and abundance compared to AOA in the Ebinur Lake wetland in Xinjiang.

Key words: Ammonia-oxidizing bacteria (AOB); Ammonia oxidizing archaea (AOA); q-PCR; clone library; rhizosphere soil

Introduction

Ammonia oxidation, the first and rate-limiting step in the nitrification process, is traditionally assumed to be performed mainly by ammonia-oxidizing bacteria (AOB) in a variety of environments [Chen et al. 2014; Gao et al. 2014; Zhang et al. 2015]. AOB commonly belong to the classes β -proteobacteria and γ -proteobacteria, and include three primary genera: *Nitrosomonas*, *Nitrospira*, and *Nitrosococcus*

[Prosser 2008; Purkhold et al. 2000]. However, this view has changed over time due to the detection of ammonia monooxygenase (AMO) genes in archaea [Könneke et al. 2005], as well as the successful isolation of two autotrophic ammonia-oxidizing archaea (AOA): *Nitrosopumilus maritimus* from a marine aquarium [Könneke et al. 2005] and *Candidatus Nitrosoarchaeum koreensis* from agricultural soil [Jung et al. 2011]. Pester found that the *amoA* gene sequences of *Thaumarchaeota* are grouped into the five major clusters: the *Nitrosopumilus* cluster, the *Nitrosotalea* cluster, the *Nitrososphaera* cluster, the *Nitrososphaera* sister cluster and the *Nitrosocaldus* cluster [Pester et al. 2012].

By detecting the functional gene *amoA*, previous studies have investigated the distribution and abundance of AOA and AOB in soil [Ding et al. 2015], sediments [Jin et al. 2011], water columns [Francis et al. 2005], and wastewater [Gao et al. 2014]. A number of studies have demonstrated that AOA typically outnumber AOB by several orders of magnitude in soil environments based on quantitative PCR (qPCR) analysis of *amoA* genes [Zhang et al. 2015]. However, Sher et al. [2013] found that the number of AOB outnumber AOA in some soil regions, suggesting a possible shift in competitive dominance of ammonia oxidizers under certain conditions. Ebinur Lake wetland national nature reserve is the center of the evolution of oasis and desertification in the northern slope of Tianshan mountains [Ren et al. 2011]. There is high species diversity, but research on species in the Ebinur Lake wetland is still limited. While AOB and AOA play a critical role in the nitrogen (N) biogeochemical cycle, the dominant plants species, *Halocnemum strobilaceum*, *Phragmites australis* and *Kareliniacaspia*, play a key role in regulating the climate and maintaining regional ecological balance in the Ebinur Lake wetland [Liu 2004]. However, studies of AOB in the Ebinur Lake wetland are limited to Chen et al. (2012), Wang et al. (2015), and Hu et al. (2016); these only investigated the diversity and number of AOB and not AOA.

This study aims to evaluate the diversity and abundance of ammonia-oxidizing microorganisms in three dominant plants using a clone library, quantitative polymerase chain reaction (q-PCR) and redundancy analysis (RDA) to study the relationship between soil environment factors and the diversity and abundance of AOB and AOA in the rhizosphere and non-rhizosphere soil of *Halocnemum strobilaceum*, *Phragmites australis*, and *Kareliniaca sp.* in the Ebinur Lake wetland. We hope to provide a foundation for future restoration and reconstruction of the wetland ecological environment.

Materials and methods

Ethics statements

This research was carried out with the permission of the director (Gao Xiang) and chief (Xu Wei) of the Ebinur Lake wetland bird island station in Xinjiang, where samples were collected.

Site description, sample collection, and physicochemical characteristics analysis

The Ebinur Lake wetland, which is the center of the lowest depression and salt-water [Ren et al. 2011], is located in northwestern Jinghe County, Xingjiang (82°36'–83°50'E, 44°30'–45°09'N), southwest of the Junggar Basin. In this study, three types of plants were chosen: *Halocnemum strobilaceum*, *Phragmites australis*, and *Kareliniaca sp.* Then, three sample plots (sample size: 10 m × 10 m) were selected for sampling of each species. In each plot, three plants situated in a triangle were sampled. So, the soil attached to the root in 1-5 cm sphere with monospecies stands of *Halocnemum strobilaceum* / *Phragmites australis* / *Kareliniaca sp.* (H / R / K) (totally collected from 9 plants for each vegetation) were mixed and named as rhizosphere soil. Finally, non-rhizosphere soil from *Halocnemum strobilaceum* / *Phragmites australis* / *Kareliniaca sp.* (H' / R' / K') was collected from unvegetated areas, where distance from plants was > 5 m. Sampling points for the non-rhizosphere soil and the rhizosphere soil were in the same plot for each species. More detailed descriptions of the sampling sites

in the Ebinur Lake wetland are listed in Table 1. The soil samples were put into sterile plastic bags and transported to the laboratory on ice. All samples were immediately stored at -20°C after being collected until later use for molecular analysis. Soil physicochemical characteristics were assayed as described by Lu [1999]. The details of collected samples and their physiochemical parameters are summarized in Table S1.

Table 1 near

DNA extraction, PCR amplification, clone library construction, and Phylogenetic analyses

Genomic DNA was extracted from 0.3 g soil using PowerSoil DNA Isolation Kit (MoBio Company). Archaeal and bacterial *amoA* genes were amplified using primers Arch-*amoA*F / Arch-*amoA*R and *amoA*-1F / *amoA*-2R [Jin et al. 2011]. The PCR products were purified using EasyPure PCR Purification Kit (TransGen Biotech, China) and ligated into pMD18-T vector (Takara, Japan), and the vector primers M13F (−47) / M13R (−48) [Jin et al. 2010] were used to reamplify the cloned DNA fragments. PCR products were screened for correct size and grouped by restricted fragment length polymorphisms (RFLP) with restriction enzymes *HaeIII* and *MspI*. For each RFLP group, representative clones were selected and sequenced by Beijing Genomics Institute (BGI, Beijing, China). Firstly, operational taxonomic units (OTUs) based on 97% DNA sequence identity, were calculated using the DOTUR program [Schloss 2005]. Then, nucleotide sequences were aligned using BLASTn. The top-hit *amoA* gene sequences from were retrieved from GenBank and all sequences were aligned using the Clustal X program [Thompson et al. 1997]. Finally, the phylogenetic tree was constructed using the neighbor-joining technique [Saitou 1987] using Mega 6 software [Tamura 2007] with 1,000 bootstrap replicates.

Quantitative PCR analysis

AOA and AOB bacteria and archaea population densities were analyzed by qPCR with the LightCycler480 qPCR analyzer using SYBR Green Real-Time PCR Master Mixes (Beijing ComWin Biotech, Beijing, China). In order to set up standard curves, a plasmid carrying the AOA and AOB *amoA* gene and bacterial and archaeal 16S rDNA fragments was extracted from *Escherichia coli* hosts and the concentration of plasmid was measured by Thermo NanoDrop 2000 (Thermo, USA). The AOA and AOB *amoA* genes were quantified with primer Arch-amoAF/R and amoA-1F/2R. Archaeal and bacterial genes were quantified with primers ARC344F/ARC915R [Nakaya et al. 2009] and 515F/927R [Ducey et al. 2011]. The 10 µl qPCR mixture contained the following reagents: 5 µL FastStart Universal SYBR Green Master (Rox) (Roche Diagnostics, Mannheim, Germany), 0.2 µL of each primer for the *amoA* gene and the 16S rDNA of bacteria and archaea, 1 µL plasmid DNA, 3.6 µL ddH₂O. The qPCR thermocycling parameters were as follows: an initial denaturation at 95 °C for 20 s, followed by 35 cycles of denaturing at 95 °C for 3 s, and annealing at 60 °C for 30 s. Melting curve analysis was routinely performed to confirm the specificity of the qPCR. In all experiments, negative controls that contained no template DNA were subjected to the same qPCR procedure to detect and exclude any possible contamination or carryover.

Statistical analysis

The estimated coverage of the *amoA* gene libraries was calculated using the formula $C = (1 - n_1/N) \times 100\%$, where C is the homologous coverage, n_1 is the number of OTUs containing only one sequence, and N is the total number of clones that were analyzed in the library [Singleton et al. 2001]. The diversity indices for each sample, including Shannon, Chao1, ACE and Simpson indices, were generated by DOTUR. Correlations between the *amoA* gene communities and environmental factors were analyzed by the redundancy analysis (RDA) using the software CANOCO for Windows (version

4.5) [ter Braak 2002].

Nucleotide sequence accession numbers

The sequences in this study were deposited in GenBank under accession numbers: KX390237-KX390476 for AOA, KP126525-KP126604, KP196614-KP196711, KX390477-KX390517 for AOB.

Results

Diversity and phylogenetic analysis of AOA and AOB in the rhizosphere soil

In this study, library coverage (C) ranged from 98.39 to 100% (Table 2); this was confirmed by rarefaction analysis (Fig S1a and b). Within each individual sampling site, 2 - 10 AOA OTUs and 8 - 15 AOB OTUs were found. The highest AOA and AOB richness occurred at R, and the lowest occurred in K, which was in accordance with values from the Shannon, Chao1, ACE, and Simpson indices. In addition, AOB showed a higher richness and diversity than AOA in all sample sites.

Table 2 near

Based on 98, 93, and 49 AOA *amoA* gene sequences in the H, R, and K clone libraries, 7, 10 and 2 OTUs were obtained, respectively. Fig. S2a shows the AOA distribution of OTU relative abundance in the different samples. Among the seven OTUs, H-AOA-OTU4 was the most dominant (60%). There were 10 OTUs in R, R-AOA-OTU1 (77%) was dominant; however, there were only 2 OTUs in K, K-AOA-OTU1 (57%) and K-AOA-OTU2 (43%), which indicated that the diversity of AOA in K was the lowest, but the highest was in sample R. Figure 1 shows the AOA N-J tree; AOA *amoA* sequences fell into two clusters: *Nitrosopumilus* and *Nitrososphaera*. About 92.92% of AOA *amoA* sequences were affiliated to the *Nitrosopumilus* cluster, which was the largest branch in the phylogenetic tree and was recovered in every sample, whereas the *Nitrososphaera* cluster accounted for 7.08%. In the K sampling site, all of AOA *amoA* sequences were affiliated to the *Nitrosopumilus* cluster.

Insert Fig. 1 here.

In the H, R, and K AOB clone library, 80, 98, and 41 AOB *amoA* gene sequences were divided into 9, 15, and 8 OTUs respectively. This indicated that the diversity of AOB in sample R was highest. Fig. S2b showed that the H-AOB-OTU3 (41.25%), H-AOB-OTU6 (20%), R-AOB-OTU10 (18.38%) and K-AOB-OTU4 (26.83%) were relatively dominant in H, R and K. The N-J phylogenetic tree (Fig. 2) of AOB based on *amoA* gene sequences showed that AOB *amoA* gene OTUs fell into two genera: *Nitrosomonas* and *Nitrospira*. However, only about 9.76% AOB *amoA* sequences were affiliated to the *Nitrospira* genus, and 90.24% AOB *amoA* sequences were assigned to the *Nitrosomonas* genus, which originated from sample K. All of the AOB *amoA* sequences in H and R fell into the *Nitrosomonas* genus, including four clusters: the *Nitrosomonas oligotropha* cluster, the *Nitrosomonas*-like cluster, the *Nitrosomonas communis* cluster and the *Nitrosomonas* sp. Nm143 cluster. Most of the AOB *amoA* OTUs in H and R belonged to the *N. oligotropha* cluster (11 OTUs) and the *N. communis* cluster (9 OTUs).

Insert Fig. 2 here

Abundance of the *amoA* gene, bacterial and archaeal 16S rRNA genes

Fig. 3 shows qPCR results of AOA and AOB *amoA* genes, bacterial and archaeal 16S rRNA genes of the six samples, including rhizosphere soil (H, R, K) (Fig. 3a) and non-rhizosphere soil (H', R', K') (Fig. 3b). In this study, the amplification efficiency was greater than 94.3% for AOA and 93.2% for AOB and the error value was less than 0.2. In addition, the melting curve analysis presented a single peak, demonstrating specificity of amplification. The abundance of AOA *amoA* genes ranged from 2.09×10^4 to 2.94×10^5 copies/g soil, and the highest abundance occurred in sample K, which was almost fourteen fold greater than the lowest (sample H) (Fig. 3a). The abundance of AOB was higher in all

samples, varying between 2.91×10^5 to 1.05×10^6 copies/g soil. Similar to AOA, the lowest abundance occurred at H, but the highest abundance occurred in sample R. The abundance of AOA and AOB was higher in sample R than that in non-rhizosphere soil (sample R'), while the abundance of AOA and AOB was lower in sample H than that in non-rhizosphere soil (sample H'). In addition, the abundance of AOA was higher in sample K, but the abundance of AOB was higher in sample K'. In each investigated sample, the ratios of AOB/AOA *amoA* gene copy numbers varied from 2.05 at sample K to 29.91 at sample R'. Furthermore, the amplification efficiency was greater than 88% for archaea and 90.5% for bacteria and the error value was less than 0.1. In sample H, the abundance of both bacteria and archaea were highest, being 4.8×10^8 copies/g soil and 5.15×10^7 copies/g soil, respectively. The abundance of bacteria and archaea were lowest in sample K (3.51×10^6 copies/g) and sample K' (8.78×10^5 copies/g).

Insert Fig. 3 here

Relationships of ammonia-oxidizer and environmental factors

The correlations between AOA and AOB community distributions with environmental parameters were analyzed by RDA. Total N, EC, OM, AP and NO_3^- -N were the main contributors to the diversity and distribution of AOA (Fig. 4a), while AN, total P, total K, and NH_4^+ -N were the most important factors influencing the AOB community structure (Fig. 4b). In addition, environmental factors also influenced the abundance of ammonia oxidizers and bacteria and archaea (Fig. 4c). The ratio of AOB/AOA *amoA* gene copy numbers was correlated positively with NH_4^+ -N, and negatively with OM. Most of the environmental factors had an influence on the abundance of bacteria and archaea, such as SM, total P, total K, total N, AN, EC and so on.

Insert Fig. 4 here

Discussion

This study revealed the presence of both AOA and AOB *amoA* genes in the Ebinur Lake wetland, which reinforces the idea that the AOA and AOB can be found in various natural environments [Francis et al. 2005; Jin et al. 2011; Pester et al. 2012]. Some studies such as those by Herrmann [2008] and Alves [2013], showed that the abundance of AOA outnumbered that of AOB, whereas others studies revealed that AOB communities were more abundant than AOA [Sun et al. 2013; Zheng et al. 2013]. In the present study, AOA and AOB copy numbers varied from 2.09×10^4 to 2.94×10^5 gene copies/g soil and from 2.91×10^5 to 1.05×10^6 gene copies/g soil in the rhizosphere and non-rhizosphere soil of *Halocnemum strobilaceum*, *Phragmites australis* and *Kareliniacaspia* (Fig. 3). These values were three orders of magnitude lower for AOA and one order lower for AOB than those found in rice rhizospheres [Zhang et al. 2016], and two orders of magnitude lower than those found in paddy rhizosphere soil [Chen et al. 2008]. Therefore, this study showed lower abundance of AOA and AOB in the Ebinur Lake wetland, which is related to the saline environment [Ren et al. 2011]. The highest number of AOA was detected in the sample K plot, but the highest diversity of AOA and the highest number and diversity of AOB were detected in sample R. In addition, the abundance of AOA and AOB was higher in sample R than that in sample R', whereas it was lower in sample H than that in sample H'. The abundance of AOA was higher in sample K, but the abundance of AOB was higher in sample K', which indicated that sample R vegetation had a higher AOA and AOB abundance than sample K or sample H. In sample K, it might be adaptable for AOA not AOB, the rhizosphere environment of sample H might be unsuitable for the growth of AOA and AOB, which showed that the abundance and diversity of AOA and AOB might be related to plant community and plant root secretion, these results showed a good agreement with previous reports [Herrmann et al. 2008, Santoro et al. 2008]. In addition, a large

number of studies had shown that rhizosphere effects had a strong effect on soil microbial activity, including microbial processes such as nitrogen cycling [Toberman et al. 2011; Concilio et al. 2015]. The positive effect of rhizospheres on nitrification was confirmed in Penton's study [Penton et al. 2013]. However, there are some potential rhizosphere nitrification inhibitors that could slightly inhibit the rhizosphere microbial nitrification activity (Liu et al. 2007). Therefore, some inhibitors or microorganism might have had an influence on the growth of AOA and AOB in the rhizosphere soil of sample H. Furthermore, the abundance and diversity of AOB were higher than those of AOA in all samples, which indicated AOB might dominate the ammonia oxidation progress in the Ebinur Lake wetland, as suggested by Katrin [2010].

Phylogenetic tree analysis showed that AOA *amoA* sequences from rhizosphere soil fell into two clusters: the *Nitrosopumilus* (group I.1 a) and *Nitrososphaera* clusters (group I.1b). In addition, the *Nitrosopumilus* cluster was distributed in each sample. While most studies found that the *Nitrososphaera* cluster was the dominant species in soil, the *Nitrosopumilus* cluster was dominant in the ocean [Massana et al. 2000; Ochsenreiter et al. 2003; Peste et al. 2012]; these results are at odds with the present study. This phenomenon may be related to the salinity level, which fluctuates to a great degree in this area [Qu et al. 2008; Wu et al. 2004], and provides a more suitable environment for the *Nitrosopumilus* cluster. The *Nitrosopumilus* cluster was dominant in rhizosphere soil of three plants. Compared to AOA, the AOB *amoA* gene were grouped into *Nitrospira* sp. and *Nitrosomonas* sp., and *Nitrosomonas* was the most dominant AOB species. Previous studies about AOB from soil also found similar results [Wang et al. 2015]. Most of the AOB *amoA* OTUs belonged to the *N. oligotropha* cluster and the *N. communis* cluster, and no AOB sequences belonged to the *N. europaea* cluster, which was consistent with the findings of Kayee [Kayee et al. 2011].

The abundance and diversity of AOA and AOB vary in different environments with different environmental factors [Zhang et al. 2015]. In this study, total N, EC, NO_3^- -N, PH and NH_4^+ -N were proposed to be the main environmental factors affecting the AOA and AOB. Total N has been reported to be a potential environmental factor influencing the structure of AOA and AOB communities in the soil [Wessén et al. 2011]. NH_4^+ -N and NO_3^- -N might have influence on the diversity and abundance of AOA and AOB. In recent years, the content of ammonia and nitrogen increased significantly because of the amount of fertilizer and pesticide used on farmland upstream of the Ebinur Lake wetland [Wu et al. 2004]. The higher concentration of ammonia better suited the growth of AOB, and a lower concentration of ammonia was better for suited for AOA growth [Erguder et al. 2009]. Therefore, the abundance and diversity of AOB was higher than that of AOA. pH is another important factor for AOA and AOB. Some studies showed that the abundance of AOA in acidic soil was higher than that in alkaline soil [Chen et al. 2011]. The pH in an alkaline sandy loam (pH 7.21 - 8.64) of the Ebinur Lake wetland was not suitable for the growth of AOA. In addition, salinity is a factor that shapes the structure and abundance of ammonia-oxidizing microbial community. Mosier [Mosier et al. 2012] pointed out that the low salinity can enrich AOA, but Zhang Yan [2015] stated that AOA is better equipped than AOB to grow in moderate and high salinity environments. Until now, the effect of salinity on abundance of AOA and AOB remained ambiguous [2015]. In general, higher ammonia concentration, more alkaline soils and high salinity (as well as other environment factors) result in greater abundance and diversity of AOB as compared to AOA. AOB, not AOA might dominate the ammonia oxidation process in the Ebinur Lake wetland.

Conclusion

This study provides evidence of the presence of both AOA and AOB in the Ebinur Lake wetland.

Phylogenetic analysis showed that the AOA *amoA* sequences fell into *Nitrosopumilus* and *Nitrososphaera* clusters; the AOB *amoA* gene fragments were grouped into *Nitrospira* sp. and *Nitrosomonas* sp. and most of them fell within the *Nitrosomonas oligotropha* and *Nitrosomonas communis* clusters. The qPCR results showed that there was a higher abundance of AOA and AOB in sample R than that in samples K and H, so the *Phragmites australis* (R) vegetated terrain might promote the ammonia oxidation to some extent by a plant root secretion in the rhizosphere environment. In sample H, there was a lower AOA and AOB abundance than that in sample H', showing that the rhizosphere environment of sample H was not suitable for growth of AOA and AOB; whereas rhizosphere environment of sample K was suitable for AOA, the non-rhizosphere environment of sample K was suitable for AOB. Meanwhile, AOB were more abundant than AOA in all investigated samples, suggesting that AOB might play a more significant role in ammonia oxidation. Furthermore, Total N, EC, NO_3^- -N, pH, and NH_4^+ -N were proposed to be the main environmental factors affecting the community structure and abundance of AOA and AOB in the Ebinur Lake wetland in Xinjiang.

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Figure legends:

Fig. 1 Neighbor-joining phylogenetic tree based on AOA *amoA* gene sequences

Note: every number behind of OTU represents the number of OTU, and every bracket's number represents the number of sequence in this OTU. ▲: AOA clone library of *Halocnemum strobilaceum* rhizosphere soil, ●: AOA clone library of *Phragmites australis* rhizosphere soil, ■: AOA clone library of

Kareliniascopia rhizosphere soil. AOA: ammonia oxidizing archaea

Fig. 2 Neighbor-joining phylogenetic tree based on AOB *amoA* gene sequences

Note: every number behind of OTU represents the number of OTU, and every bracket's number represents the number of sequence in this OTU. ▲: AOB clone library of *Halocnemum strobilaceum* rhizosphere soil, ●: AOB clone library of *Phragmites australis* rhizosphere soil, ■: AOB clone library of *Kareliniascopia* rhizosphere soil. AOB: ammonia-oxidizing bacteria

Fig. 3 Abundance of the *amoA* gene, bacterial and archaeal 16S rRNA genes in the rhizosphere (a) and non-rhizosphere (b) soil

Note: H, *Halocnemum strobilaceum* rhizosphere soil; R, *Phragmites australis* rhizosphere soil; K, *Kareliniascopia* rhizosphere soil. H', *Halocnemum strobilaceum* non-rhizosphere soil; R', *Phragmites australis* non-rhizosphere soil; K', *Kareliniascopia* non-rhizosphere soil.

Fig. 4 Redundancy analysis (RDA) ordination plots for the environmental factors and community composition(a and b) and abundance (c) of AOA and AOB

Note: H, *Halocnemum strobilaceum* rhizosphere soil; R, *Phragmites australis* rhizosphere soil; K, *Kareliniascopia* rhizosphere soil. H', *Halocnemum strobilaceum* non-rhizosphere soil; R', *Phragmites australis* non-rhizosphere soil; K', *Kareliniascopia* non-rhizosphere soil.

Supplementary data

Fig. S1 Rarefaction curves of *amoA* gene sequences from three rhizosphere soils in Ebinur Lake wetland

Note: H, *Halocnemum strobilaceum* rhizosphere soil; R, *Phragmites australis* rhizosphere soil; K, *Kareliniascopia* rhizosphere soil. AOA: ammonia oxidizing archaea; AOB: ammonia-oxidizing

bacteria.

Fig. S2 Distribution and relative abundance of AOA (a) and AOB (b) amoA gene OTUs in three rhizosphere soils in the Ebinur Lake wetland.

Note: H, *Halocnemum strobilaceum* rhizosphere soil; R, *Phragmites australis* rhizosphere soil; K, *Kareliniacaspia* rhizosphere soil. AOA: ammonia oxidizing archaea; AOB: ammonia-oxidizing bacteria.

Draft

Table 1 Basic description of sampling sites in the Ebinur Lake Wetland

Site	Longitude	Latitude	Elevation (m)	Vegetation types	Soil types
H/H'	82°48'49.8"E	44°50'51.6"N	186	<i>Halocnemum strobilaceum</i>	Clay, Sandy loam soil
R/R'	82°49'50.0"E	44°49'58.7"N	189	<i>Phragmites australis</i>	Clay, Sandy loam soil
K/K'	82°52'11.37"E	44°44'6.792"N	184	<i>Kareliniacaspia</i>	Sandy loam soil

H/H' = Rhizosphere/Non-rhizosphere soil of *Halocnemum strobilaceum*; R/R'= Rhizosphere/Non-rhizosphere soil of *Phragmites australis*; K/K'= Rhizosphere/Non-rhizosphere soil of *Kareliniacaspia*

Draft

Table 2 Diversity characteristics of AOA and AOB clone library in rhizosphere of *Halocnemum strobilaceum*, *Phragmites australis* and *Kareliniacaspia*

Sample	Clone number	Sequences number	OTU _S	Shannon–Wiener index	Ace	Simpson index	Chao1 index	Coverage (%)
AOA								
H	307	98	7	1.08	7.79	0.53	7	99.67%
R	311	93	10	1.13	16.02	0.63	15	98.39%
K	165	49	2	0.68	0	0.5	2	100%
AOB								
H	270	80	9	1.93	9.99	0.23	10	99.26%
R	281	98	15	2.44	16.43	0.26	16.5	98.93%
K	280	41	8	1.91	8	0.13	8	100%

H: Rhizosphere soil of *Halocnemum strobilaceum*; R: Rhizosphere soil of *Phragmites australis*; K: Rhizosphere soil of *Kareliniacaspia*

383x301mm (300 x 300 DPI)

217x356mm (300 x 300 DPI)

378x197mm (300 x 300 DPI)

404x382mm (300 x 300 DPI)

Table S1 The result of physical and chemical properties

Sample plot	Soil physiochemical factors											
	pH	SM(%)	EC(ms/cm)	OM(g/kg)	TN(g/kg)	AN(mg/kg)	NH ₄ ⁺ -N(mg/kg)	NO ₃ ⁻ -N(mg/kg)	TP(g/kg)	AP(mg/kg)	TK(g/kg)	AK(mg/kg)
H	8.64	14.39	10.65	5.06	0.72	33.63	7.33	3.11	0.88	32.23	6.51	296.95
H'	8.56	17.60	5.21	4.05	0.65	29.51	6.52	0.92	0.61	29.06	5.90	279.38
R	8.20	18.64	7.38	3.57	0.56	30.20	9.13	1.59	0.83	25.85	6.12	281.14
R'	8.11	20.25	4.26	3.39	0.46	27.45	8.42	1.28	0.70	25.01	5.47	263.57
K	8.61	16.09	3.42	6.20	0.32	18.53	8.11	1.18	0.67	24.87	5.01	249.51
K'	7.21	1.38	2.18	3.15	0.54	65.78	6.96	1.12	0.62	30.32	4.36	228.76

H=Rhizosphere of *Halocnemum strobilaceum*, H'= Non-rhizosphere of *Halocnemum strobilaceum*, R= Rhizosphere of *Phragmites australis*, R'= Non-rhizosphere of *Phragmites australis*, K= Rhizosphere of *Kareliniaca sp.* Less, K'= Non-rhizosphere of *Kareliniaca sp.* Less. pH pH, SM soil moisture, EC electrical conductivity, OM organic matter, TN total Nitrogen, AN available Nitrogen, TP total Phosphorus, AP available Phosphorus P, TK total Potassium, AK available Potassium, NH₄⁺-N ammonium content, NO₃⁻-N nitric nitroge

390x171mm (300 x 300 DPI)

220x340mm (300 x 300 DPI)