Effects of Sea Animal Colonization on the Coupling between Dynamics and Activity of Soil Ammonia-oxidizing Bacteria and Archaea in Maritime Antarctica

Qing Wang [1], Renbin Zhu[1,*], Yanling Zheng[2], Tao Bao[1], Lijun Hou[2,*]

[1]Anhui Province Key Laboratory of Polar Environment and Global Change, School of Earth and Space Sciences, University of Science and Technology of China, Hefei 230026, P.R China

[2]State Key Laboratory of Estuarine and Coastal Research, East China Normal University, Shanghai 200062, P. R China

*Corresponding author: Renbin Zhu (zhurb@ustc.edu.cn) or Lijun Hou (ljhou@sklec.ecnu.edu.cn)

Abstract

The colonization by a large number of sea animals, including penguins and seals, plays an important role in the nitrogen cycle of the tundra ecosystem in coastal Antarctica. However, little is known about the effects of sea animal colonization on ammonia-oxidizing archaea (AOA) and bacteria (AOB) communities involved in nitrogen transformations. In this study, we chose active seal colony tundra soils (SS), penguin colony soils (PS), adjacent penguin-lacking tundra soils (PL), tundra marsh soils (MS), and background tundra soils (BS), to investigate the effects of sea animal colonization on the abundance, activity, and diversity of AOA and AOB in maritime Antarctica. Results indicated that AOB dominated over AOA in PS, SS, and PL; whereas AOB and AOA abundances were similar in MS and BS. Penguin or seal activities increased the abundance of soil AOB *amoA* genes, but reduced the abundance of AOA *amoA* genes, leading to very large ratios (1.5×10^2 to 3.2×10^4) of AOB to AOA *amoA* copy numbers. Potential ammonia oxidation rates (PAOR) were significantly higher ($P = 0.02$) in SS and PS than in PL, MS, and BS, and were significantly positively correlated ($P < 0.001$) with AOB *amoA* gene abundance. The predominance of AOB over AOA and their correlation with PAOR suggested that AOB play a more important role in the nitrification in animal colony soils. Sequence analysis for gene clones showed that AOA and AOB in tundra soils were from the *Nitrososphaera* and *Nitrosospira* lineages, respectively. Penguin or seal activities led to a predominance of AOA phylotypes related to *Nitrososphaera* cluster I and AOB phylotypes related to *Nitrosospira* clusters I and II, but very low relative abundances in AOA phylotypes related to cluster II, and AOB phylotypes related to cluster III and IV. The differences in AOB and AOA community structures were closely related

to soil biogeochemical processes under the disturbance of penguin or seal activities: soil C:N alteration and sufficient input of NH_4^+–N and phosphorus from animal excrements. The results significantly enhanced the understanding of ammonia-oxidizing microbial communities in tundra environment of maritime Antarctica.

Keywords: Antarctic soil, AOA, AOB, Sea animals, Nitrification, Microbial diversity

1 Introduction

Nitrification, the oxidation of ammonia into nitrate through nitrite, plays a pivotal role in the global biogeochemical nitrogen cycle (Nunes-Alves, 2016). As the first and rate-limiting step of nitrification, ammonia oxidation (the aerobic oxidation of ammonia into nitrite) is performed by phylogenetically and physiologically distinct groups of ammonia oxidizing archaea (AOA) and ammonia oxidizing bacteria (AOB) (Belser and Schmidt, 1978; Könneke et al., 2005). The AOA and AOB have been investigated using the *amoA* gene as a functional marker in a wide variety of environments, including soils (Di et al., 2009; Gubry-Rangin et al., 2017; Leininger et al., 2006; Ouyang et al., 2016; Shen et al., 2012), sediments (Li et al., 2015; Zheng et al., 2013), estuaries (Dang et al., 2008; Mosier et al., 2008; Santoro et al., 2011), oxic and suboxic marine water column (Baker et al., 2012; Bouskill et al., 2012), plateau permafrost (Zhang et al., 2009; Zhao et al., 2017), and in sub-arctic and arctic soils (Alves et al., 2013; Daebeler et al., 2017). Results indicated that the relative abundance and functional importance of AOA *vs*. AOB vary greatly in natural ecosystems. Environmental drivers, including substrate concentration, oxygen availability, pH, and salinity, might be responsible for the different AOA and AOB abundances and distribution (Alves et al., 2013; Bouskill et al., 2012; Le Roux et al., 2008; Wang et al., 2015). The abundance, diversity, and activity of ammonia-oxidizers have been explored in tundra soils of Antarctic Peninsula (Jung et al., 2011; Yergeau et al., 2007), the Antarctic Dry Valleys (Ayton et al., 2010; Magalhães et al., 2014; Richter et al., 2014), and in Antarctic coastal waters (Kalanetra et al., 2009; Tolar et al., 2016). However, there is still a large gap in our understanding of factors that

control AOA *versus* AOB prominence, and the relationships between nitrification rates and ammonia-oxidizer dynamics need to be explored in Antarctic.

In maritime Antarctica, a large number of sea animals, such as penguins or seals, settle on coastal ice-free tundra patches. Tundra vegetation including mosses, lichens, and algae, penguin colonies, and their interactions, form a special ornithogenic tundra ecosystem (Tatur et al., 1997). The soil biogeochemistry of an ornithogenic tundra ecosystem has become a research hotspot under the penguin-activity disturbance (Otero et al., 2018; Riddick et al., 2012; Simas et al., 2007; Zhu et al., 2013, 2014). Previous studies indicated that sea animals significantly affect the tundra N and P cycles (Lindeboom et al., 1984; Simas et al., 2007; Zhu et al., 2011), and the total N and P excreted by seabird breeders and chicks are 470 Gg N yr^{-1} and 79 Gg P yr^{-1} in Antarctica and the Southern Ocean, accounting for 80% of the N and P from total global seabird excreta (Otero et al., 2018). Uric acid is the dominant N compound in penguin guano, and during its mineralization, different N forms, such as NH_3, NH_4^+, and NO_3^-, can be produced via ammonification, nitrification, and deposition, following the changes in soil pH and the C:N ratio (Blackall et al., 2007; Otero et al., 2018; Riddick et al., 2012). The alteration of soil biogeochemistry under the sea animal-activity disturbance might have an impact on the abundance and diversity of the AOA and AOB involved in the nitrogen cycle. Increased bacterial abundance, diversity, and activity have been detected in penguin or seal colony soils (Ma et al., 2013; Zhu et al., 2015). Penguin or seal colonies have been confirmed as strong sources for greenhouse gas N_2O (Zhu et al., 2008, 2013), a by-product of microbial ammonia oxidation (Santoro et al., 2011).

However, the effects of sea animal colonization on AOA and AOB community structures have not been thoroughly investigated in the maritime Antarctic tundra.

In the present study, we investigated the abundance, potential activity, and diversity of soil AOA and AOB in five tundra patches, including a penguin colony, a seal colony, the adjacent animal-lacking tundra, tundra marsh, and background tundra, where soil biogeochemical properties were subjected to the differentiating effects of sea animal activities. Our objectives were (a) to examine the abundance, diversity, and community structure of soil AOA and AOB using the *amoA* gene as a functional marker; (b) to investigate potential links between *amoA* gene abundance, AOA and AOB community structures, potential activity, and environmental variables; and (c) to assess the relative contribution of these two distinct ammonia-oxidizing groups to nitrification.

2 Materials and methods

2.1. Study area

The study area is located on the Fildes Peninsula and Ardley Island in the southwest of King George Island (Fig. 1), having oceanic climate characteristics. Mean annual air temperature is about -2.5 °C, with the range of daily mean temperature from -26.6 to 11.7 °C, and mean annual precipitation is about 630 mm, mainly in the form of snow. The Fildes Peninsula (about 30 km^2 area) is a host to important sea animal colonies. Based on annual statistical data, the total of over 10,700 sea animals colonize this peninsula in the austral summer. On the western coast are established seal colonies including elephant seal (*Mirounga leonine*), weddell seal (*Leptonychotes*

weddellii), fur seal (*Arctocephalus gazella*) and leopard seal (*Hudrurga leptonyx*) (Sun et al., 2004). Ardley Island, with area of 2.0 km in length and 1.5 km in width, is connected with the Fildes Peninsula via a sand dam. This island belongs to an important Ecological Reserve for penguin populations in western Antarctica. A great majority of breeding penguins, including Adélie penguins (*Pygoscelis adeliae*), Gentoo penguins (*Pygoscelis papua*), and Chinstrap penguins (*Pygoscelis antarctica*), colonized on the east of this island in the austral summer. Seal excrements or penguin droppings rich in nitrogen and phosphorus were transported into local tundra soils by ice-snow melting water during the breeding period (Sun et al., 2000, 2004). Mosses and lichens dominate local vegetation. However, the vegetation is almost absent in penguin or seal colonies because of overmanuring and animal trampling. More detailed description about the study area can be found in Zhu et al. (2013).

2.2. Tundra soil collection

In the summer of 2014/2015, soil samples were collected from the following tundra patches, as illustrated in Fig. 1:

(i) Penguin colony and penguin-lacking tundra sites: The tundra on Ardley Island was categorized into three areas from the east to west according to the distance to the penguin nesting sites (i.e., the intensity of penguin activity): The eastern active penguin colony with nesting sites, PS (i.e., high penguin-activity area) where penguins have the highest density and high frequency presence during the breeding period; the adjacent penguin-lacking tundra areas, PL (i.e., low penguin-activity areas) in the middle of Ardley Island where penguins occasionally wander and

have a typically low density; and the western tundra marsh, MS, moderately far from penguin nesting sites (i.e., a slight penguin-activity area) where penguins rarely frequent the sites. In total, fourteen soil samples were collected from Ardley Island to study the effects of penguin colonization on the abundance, activity, and community structures of soil AOA and AOB. Specifically, samples PS1–PS5 were collected sequentially from the center of the colony in the PS. Samples PL1–PL4 and MS1–MS5 were randomly collected in the PL and MS. (ii) The seal colony and its adjacent tundra sites, SS: These sites are on the western coast of the Fildes Peninsula. According to the distance to seal wallows (i.e., the intensity of seal activity), samples SS1–SS5 were collected in sequence to investigate the effects of seal colonization. Site SS1 was closest to the seal colony (i.e., a high seal-activity site), whereas SS5 was the farthest from the seal colony (i.e., a low seal-activity site). (iii) Background tundra sites, BS: Three soil samples were collected from an upland tundra with about 40 m a.s.l. and the distribution of no sea animal around. The tundra surface is covered with mosses or lichens with a 10–15 cm organic clay layer (Zhu et al., 2013).

At each sampling site, soil was collected aseptically using a clean scoop from the top 5–10 cm at the four corners of a 1 m^2 subarea, and combined into one sample. Appropriate precautions were taken to avoid cross-site or human-made contamination. Immediately after collection, each sample was divided into two portions: one was stored in sterile plastic containers at −80 °C for the analysis of the microbial community structures, and the other portion was stored at close to the *in situ* temperature to determine the geochemical characteristics and potential ammonia oxidation rates. All of the analyses were conducted within one month.

2.3. General analysis of soil characteristics

Soil pH was determined by mixing the soil and 1 M KCl solution (1: 3 ratio). Soil moisture was measured by oven drying at 105 °C to a constant weight. Total carbon (TC), total nitrogen (TN) and total sulfur (TS) contents in the soils were determined through a CNS analyzer (vario MACRO, Elementar, Germany). The samples were digested in Teflon tubes using HNO_3-HCl-HF-$HClO_4$ digestion at 190 °C, and total phosphorus (TP) was determined using ICP-OES (Perkin Elmer 2100DV, Waltham, MA, USA). The NO_3^--N, NO_2^--N, and NH_4^+-N concentrations were determined through a continuous flow analyzer (Skalar, Netherlands) (Gao et al., 2018; Zhu et al., 2011).

2.4. Measurement of soil potential ammonia oxidation rate

Potential ammonia oxidation rate (PAOR) in tundra soil was determined using the chlorate inhibition method (Kurola et al., 2005; Xia, 2007). Sodium chlorate was used to inhibit NO_2^- from being oxidized into NO_3^-. Briefly, 5 g fresh tundra soil was incubated in 20 ml of 1 mM phosphate-buffered saline with 1 mM of $(NH_4)_2SO_4$ and $NaClO_3$ in the dark at 15 °C. After moderately shaking for 24 h, the 5 ml of 2 M KCl was used to extract the nitrite. The optical density for the supernatant after centrifugation was determined spectrophotometrically at 540 nm. The standard curve obtained from $NaNO_2$ (0–2.5 μmol l^{-1}) was used to calculate the PAOR in the tundra soils.

2.5. DNA extraction and gene amplification (PCR)

Genomic DNA was extracted from 0.25 g of homogenized tundra soils using PowerSoil™ DNA Isolation Kit (Mo Bio, Carlsbad, CA, USA) as described in manufacturer's protocol. The extracted DNA was eluted in 50 μl of elution buffer, quantified by a Nanodrop-2000 Spectrophotometer (Thermo Scientific, Waltham, MA, USA), and stored at −20 °C. AOA *amoA* gene fragments (635 bp) were amplified using the primers Arch-amoAF (5'-STAATGGTCTGGCTTAGACG-3') and Arch-amoAR (5'-GCGGCCATCCATCTGTATGT-3') (Francis et al., 2005). The *amoA* gene fragment (491 bp) of β-proteobacterial AOB, which represents known AOB in soil, was amplified using the primer set composed of amoA-1F (5'-GGGGTTTCTACTGGTGGT-3') and amoA-2R (5'-CCCCTCKGSAAAGCCTTCTTC-3') (Rotthauwe et al., 1997). All PCR reactions were performed using Taq PCR Master Mix (Sangon Biotech, Shanghai, China) in a total volume of 50 μl. PCR reactions were carried out with a thermal profile of 5 min at 95 °C; 35 cycles of 94 °C for 30 s, 56 °C for AOA or 55 °C for AOB for 45 s, 72 °C for 1 min; and a final 5-min extension cycle at 72 °C (Zheng et al., 2014). Subsequently, the amplification products were visualized by electrophoresis on 1.0 % agarose gels.

2.6. Sequencing and phylogenetic analysis

The amplification products were sent to Sangon Company (Shanghai, China) for purification, cloning and sequencing (Zheng et al., 2014). The sequences were edited using DNAstar (DNASTAR, Madison, WI, USA), and then aligned by muscle using the UPGMB clustering method with the ClustalX program. The sequences with 97% identity were grouped into one OTU

175 (operational taxonomic unit) using the mothur Program (version 1.23.0, Schloss et al., 2009) by
176 the furthest neighbor approach (Zheng et al., 2014). The closest reference sequences were
177 identified at NCBI (http://www.ncbi.nlm.nih.gov/BLAST/) using the BLASTn tool (Madden T,
178 2002), and phylogenetic trees were constructed by the neighbor-joining method using the
179 Molecular Evolutionary Genetics Analysis (MEGA) software (version 5.03,
180 https://www.megasoftware.net/). The sequences reported in this study have been deposited in
181 GenBank under accession unmbers MH318029 to MH318568 and MH301331 to MH302505.

182 **2.7. Quantitative real-time PCR**

183 The AOB and AOA *amo*A gene copy numbers for tundra soils were determined in triplicate
184 using quantitative real-time PCR (qPCR) on an ABI 7500 Sequence Detection System (Applied
185 Biosystems). The specific details were given by Zheng et al. (2014). The strong linear inverse
186 relationship confirmed the consistency of the qPCR assay between the threshold cycle and the log
187 value of gene copy numbers (R^2 = 0.997 for AOA; R^2 = 0.999 for AOB). The amplification
188 efficiencies for AOA and AOB were 99.8 % and 90.4 %, respectively. Melting curve analysis had
189 only one observable peak at a melting temperature (Tm) (84.9 °C for AOA and 89.6 °C for AOB)
190 (Fig. S1 in Supplementary Material). Negative controls were subjected to exclude any possible
191 carryover or contamination in all experiments.

192 **2.8. Statistical analysis**

193 The Shannon–Weiner Index, Simpson Index and the richness estimator Chao 1 were calculated
194 by the mothur program(version 1.23.0, Schloss et al., 2009). The coverage was the percentage of

the number of observed OTUs divided by the Chao 1 (Table S1). The Kruskal–Wallis test and Wilcoxon signed rank test were conducted for the comparison between *amoA* gene abundance and PAOR from five tundra patches using SPSS Statistics 17 (IBM Corp, Armonk, NY, USA). Correlations between ammonia-oxidizer gene abundance, PAOR and environmental variables were obtained by Spearman Correlation Analysis. The relationships between the ammonia-oxidizer community structure and environmental variables were explored using canonical correspondence analysis (CCA) in the software Canoco for windows (version 4.5; Microcomputer Power, Ithaca, NY, USA), because the maximum gradient length of both AOA and β-AOB was longer than four SD (AOA: 4.406; AOB: 18.326). All environmental parameter values were transformed into ln(x+1) before statistical analyses. The OTU richness (defined at 3% distance) served as the species input and several simulations of manual forward selection were performed with 499 Monte Carlo permutations to build the optimal models. The scaling in the final CCA biplots was focused on inter-sample relations.

3 Results

3.1. Soil chemistry and sea animal activities

Almost all the tundra soils were slightly acidic, and the mean pH ranged from 5.3 to 6.6 at each tundra patch (Table 1). In penguin or seal colony tundra soils, PS and SS, soil properties including TC, TN, TS, TP, NH_4^+-N and NO_3^--N levels showed high heterogeneity due to the deposition of penguin or seal excreta. In the seal colony tundra soils, the highest TC, TN, TP, TS, and NH_4^+-N levels occurred at the sites (SS1-2) close to the seal wallows. In the tundra soils on

215 Ardley Island, the highest TP, TS, and NH_4^+-N levels occurred in the soils close to the eastern
216 penguin nesting sites (PS1-5). PS and SS had generally lower C:N ratios than the penguin-lacking
217 tundra soils (PL), tundra marsh soils (MS), and background tundra soils (BS). Soil mean TN, TS
218 and NH_4^+–N levels were higher in PS, SS, PL, and MS than in BS. Soil NH_4^+–N contents were
219 1–2 orders of magnitude higher in PS and SS than in PL, MS, and BS, with the means of 176.9
220 and 137.6 mg NH_4^+-N kg^{-1}, respectively. The highest NO_3^--N contents occurred in SS.
221 Phosphorus levels were significantly greater ($p < 0.05$) in PS (10.6–32.9 mg g^{-1}) than in other
222 types of tundra soils (mean < 6.0 mg g^{-1}). Overall, penguin or seal activities altered the local soil
223 biogeochemical properties through the deposition of their excreta, leading to generally low C:N
224 ratios in tundra soils.

225 **3.2. Gene abundances under sea animal colonization**

226 AOB *amoA* gene abundances were significantly higher (by approximately 2–4 orders of
227 magnitude) than AOA *amoA* gene abundances (Wilcoxon test, n = 22, P = 0.002) in the penguin
228 and seal colony and the adjacent tundra soils, PS, SS, and PL. However, *amoA* gene abundances
229 were similar in the MS and BS soils (Fig. 2a). Overall, the abundances of AOB and AOA *amoA*
230 genes were significantly negatively correlated (r = -0.93, P = 0.002) across all the tundra patches
231 (Fig. S2). The AOA *amoA* gene abundances showed a heterogeneous distribution in the
232 abundances among the different tundra patches, and they were two orders of magnitude lower in
233 PS and SS relative to those in BS and MS. Maximum AOA *amoA* gene abundance appeared in
234 BS, followed by MS and PL, whereas the PS and SS soils had the lowest AOA *amoA* gene
235 abundances. The log values of soil AOA *amoA* gene abundances showed a significant positive

correlation (r=0.52, P<0.001) with C:N ratios (Fig. 3a), but their abundances showed a significant negative correlation with NH_4^+-N contents (r= -0.52, P = 0.013) (Table 2).

Unlike AOA *amoA* gene abundances, AOB *amoA* genes showed the opposite distribution pattern. AOB *amoA* gene abundances were significantly higher (by approximately 2–3 orders of magnitude) in PS and SS compared with those in MS and BS (Fig. 2a). The log values of soil AOB *amoA* gene abundances showed a significant negative correlation with C:N ratios (r= -0.71, $P < 0.001$) (Fig. 3b), but their abundances showed a significant positive correlation with NH_4^+-N (r=0.53 , $P < 0.05$) and TP (r=0.47 , $P < 0.05$) (Table 2). The ratios of AOB to AOA *amoA* copy numbers were strongly affected by animal activities, and were much higher in PS and SS than in PL, MS, and BS (Fig. 2b; Kruskal–Wallis test, $\chi^2 = 18.2$, $P = 0.01$). Their ratios showed significant positive correlation with NH_4^+-N contents (r=0.62; $P < 0.01$) and TP (r=0.43, $P < 0.05$) (Table 2), but significant negative correlation with the C:N ratios (r= -0.79; $P < 0.001$)(Fig. 3c). Overall, penguin or seal activities, which were indicated by soil C:N ratios, significantly increased the abundance of soil AOB *amoA* genes, but reduced the abundance of AOA *amoA* genes, leading to very large ratios (1.5×10^2 to 3.2×10^4) of AOB to AOA *amoA* copy numbers in PS and SS. However, the ratios varied only from 0.1 to 7.2 in BS and MS.

3.3. Potential ammonia oxidation rates under sea animal colonization

Potential ammonia oxidation rates (PAORs) ranged from 8.9 to 138.8 μg N kg^{-1} h^{-1} in all the soil samples (Table 1). The PAOR was slightly higher in SS (mean 76.1 μg N kg^{-1} h^{-1}) than in PS (mean 64.7 μg N kg^{-1} h^{-1}), but significantly higher than in PL, MS, and BS (mean 12.0–21.8 μg N kg^{-1} h^{-1}). Overall the PAOR was significantly higher in animal colony soils (mean 70.4 μg N

257 $kg^{-1} h^{-1}$ for SS and PS) than in non-animal colony soils (mean 15.7 μg N $kg^{-1} h^{-1}$ for PL, MS, and
258 BS; Kruskal–Wallis test, χ^2 =11.6, P = 0.02) (Fig. 2c). The greatest POAR occurred at the sites
259 PS1 nearest the penguin nests (88.8 ± 2.7 μg N $kg^{-1} h^{-1}$) and SS1 close to seal wallows (138.8 ±
260 0.8 μg N $kg^{-1} h^{-1}$). The PAOR followed the distribution changes of AOB *amoA* gene abundances,
261 but showed the opposite trend to the AOA *amoA* gene abundances. A significant positive
262 correlation (r^2 = 0.77, P < 0.001) was observed between the PAOR and the AOB *amoA* gene
263 abundance when the data from all the tundra patches were combined, whereas no correlation
264 occurred between PAOR and AOA *amoA* gene abundance (Fig. 4). The higher abundance of AOB
265 compared to AOA in PS, SS and PL and their correlation with the PAOR suggested that AOB
266 populations might contribute more to the PAOR than the AOA populations in penguin or seal
267 colony. In addition, PAOR significantly negatively correlated with soil C:N ratios (r= -0.73,
268 P<0.001)(Fig. 3d), but significantly positively correlated with TS contents (r=0.47, P<0.05) and
269 TP contents (r=0.43, P<0.05) (Table 2).

270 **3.4. Community structure of AOA and AOB under sea animal colonization**

271 The PCR products were insufficient to construct the clone libraries for the AOA *amoA* gene
272 from SS and PS because of the low AOA abundance in the soils, as was the case with the AOB
273 *amoA* gene from MS and BS. Overall, 10 AOA and 14 AOB *amoA* gene clone libraries were
274 successfully constructed. The 543 AOA sequences and 1175 AOB quality sequences were
275 generated from the respective sites. Within each individual site, 1–6 AOA OTUs and 6–15 AOB
276 OTUs were identified, as defined by < 3% divergence in nucleotides. The AOA and AOB OTU
277 numbers for each library are presented in Table S1. These numbers might be higher if more clones

were sequenced, based on the rarefaction curves (Fig. S3 and Fig. S4). AOB *amoA* gene diversity was generally higher compared to AOA, based on the indices of Shannon–Wiener and Simpson. Specifically, AOA *amoA* gene diversity was higher in PL and MS than in BS, whereas AOB *amoA* gene diversity was higher in SS and PS compared with that in adjacent animal-lacking tundra soils (Table S1).

The 543 AOA *amoA* gene sequences had 76–100% sequence similarity to each other, and 95–100% identity with the corresponding top hit *amoA* sequences deposited in GenBank. Phylogenetic analysis showed that the AOA *amoA* sequences were grouped into 16 unique OTUs, representing 100% of all the AOA *amoA* OTUs identified, and these sequences were affiliated with two *Nitrososphaera* clusters (Fig. 5a): Cluster I contained 11 OTUs and 264 clones, and 57.9% of AOA *amoA* sequences were from PL, 41.3% from SS, and only 0.8% from MS. In Cluster II, there are five unique OTUs and 279 clones, and 58.8% of them were from BS, 38.3% from MS, and only 2.9% from PL. Almost all the AOA phylotypes retrieved from PL and SS were related to *Nitrososphaera* cluster I, whereas the AOA phylotypes retrieved from MS and BS were distributed in cluster II (Fig. S5a). Seal or penguin activities led to the predominant existence of AOA phylotypes related to cluster I, but very low relative abundances in AOA phylotypes related to cluster II, which were almost completely excluded in SS and PL. Almost all AOA phylotypes in BS and MS were related to *Nitrososphaera* cluster II, whereas the relative abundances of AOA phylotypes related to cluster I were very low or undetectable.

The 1175 AOB *amoA* gene sequences shared 87–100% sequence identity to each other, and 93–100% identity with the closest matched GenBank sequences. Phylogenetic analysis showed

that the AOB *amoA* sequences could be grouped into 38 unique OTUs, representing 58.5% of all the AOB *amoA* OTUs identified, and they were grouped into four *Nitrosospira* clusters according to the evolutionary distance of the phylogenetic tree (Fig. 5b): Cluster I contained 11 OTUs and 226 clones, and 67.7% of AOB *amoA* sequences were from PS, 23.5 % from SS, 8.4% from PL, and only 0.4% from MS. Clusters II and III contained 17 unique OTUs and 521 clones. The sources of the OTUs in cluster II were similar to those of cluster I, with 69.8% from PS, 29.9% from SS, and 0.3% from PL. For cluster III, 79.2% of the sequences were from PL, 19.8% from SS, and 1.0% from MS. Cluster IV contained nine unique OTUs and 370 clones from PL (50.0%), SS (36.8%) and MS (13.2%), respectively. All the AOB phylotypes retrieved from PS were related to dominant *Nitrosospira* clusters I and II, whereas AOB phylotypes related to cluster III and IV were completely excluded because of penguin colonization (Fig. S5b). The AOB phylotypes retrieved from SS were distributed in clusters I, II, III, and IV (16–38% for each cluster). Almost all the AOB phylotypes retrieved from PL and MS were related to *Nitrosospira* clusters III and IV.

3.5. Relationships of the ammonia-oxidizer community structure with environmental variables

The relationships of the AOA and AOB communities with environmental variables were analyzed using CCA. The environmental variables explained 62.1% of the total variance in the AOA *amoA* genotype compositions, and 71.5% of the cumulative variance of the genotype-environment relationships in the first two CCA dimensions (Fig. 6a). Overall, the AOA community structures significantly correlated with C:N ($F=2.59$, $P=0.022$) and TC ($F=2.07$, $P=0.048$) in tundra soils (Table 3), and the combination of the two factors explained 39.6% of the

variation. High soil C:N and TC concentrations increased the AOA richness in MS and BS. Although other environmental parameters, including TP, pH, NH_4^+-N, and NO_3^--N were not statistically significant ($P > 0.05$), these variables additionally explained 47.3% of the variation. As illustrated in Fig. 6b, the first two dimensions explained 26.6% of the total variance in the AOB compositions, and 54.3% of the cumulative variance of the AOB genotype-environment relationships. The composition and distribution of AOB communities correlated significantly with C:N ratios (F=1.844, P=0.002) and NH_4^+-N (F=1.823, P=0.002), and the two factors combined yielded 21.9% of total CCA explanatory power. The others including TP, NO_3^--N and pH accounted for 27.1% of the variance. Penguin or seal activities significantly increased the AOB richness in SS and PS through higher NH_4^+-N and P input from sea animal excrement, whereas AOB richness was closely related to the soil C:N in PL and MS.

4 Discussion

4.1. Effects of sea animal colonization on AOA and AOB abundances

In this study, soil AOA *amoA* gene abundances were two orders of magnitude lower in PS and SS relative to BS and MS; however, AOB *amoA* gene abundances were approximately 2–3 orders of magnitude higher in PS and SS than in MS and BS, indicating that sea animal activities increased the AOB population size, but decreased AOA abundances in tundra soils (Fig. 2 and Fig. 3). Overall, the AOA *amoA* gene abundances obtained here were similar to the abundance range reported in the soils of the Antarctic Dry Valleys and arctic tundra soils; however, the AOB *amoA* gene abundances were two to three orders of magnitude higher in PS and SS than in Antarctic Dry Valleys (Alves et al., 2013; Magalhães et al., 2014). In contrast to previous studies

341 indicating that AOA were more abundant than AOB in some terrestrial or marine ecosystems
342 (Beman et al., 2008; Lam et al., 2007; Wuchter et al., 2006; Yao et al., 2011), and in soils from
343 Antarctic Peninsula (Jung et al., 2011), our qPCR estimates showed that the AOB *amoA* copy
344 numbers were much greater than those of AOA *amoA* in PS, SS and PL because of sea animal
345 activities. However, their abundances were very similar to each other in BS and MS. The ratios
346 of AOB to AOA abundance were strongly affected by sea animal activities, which were indicated
347 by soil C:N ratios (Fig. 2c). A shift in the relative abundance of AOA and AOB recorded
348 previously for the Antarctic Dry Valleys, with a greater abundance of AOB compared with that of
349 AOA for Battleship Promontory and Miers Valley, and the reverse for Upper Wright Valley and
350 Beacon Valley (Magalhães et al., 2014). The results for PS, SS, and PL are also in agreement with
351 those detected in subglacial soils (Boyd et al., 2011).

352 The ratios of AOB to AOA showed significant correlations with C:N, NH_4^+-N, and TP when
353 all the data were combined in the five tundra patches (Table 2). This suggested that C:N, NH_4^+-N,
354 and TP are key factors in determining a predominance of AOB over AOA. In Antarctica, the
355 productivity of terrestrial ecosystems is strongly limited because of the extremely low nitrogen
356 levels (Park et al., 2007). However, the physiochemical properties for tundra soils were strongly
357 influenced by the deposition of penguin or seal excreta under effects of local microbes (Tatur et
358 al., 1997). Sea animals provide considerable external N inputs for their colony soils and adjacent
359 tundra soils through direct input of their excreta and atmospheric deposition via ammonia
360 volatilization (Lindeboom, 1984; Sun et al., 2002; Blackall et al., 2007; Zhu et al., 2011; Riddick
361 et al., 2012). In addition to ammonium, phosphorus can typically be found in penguin guano (Sun

et al., 2000). Generally low C:N ratios and significantly elevated NH_4^+–N and TP concentrations occurred in PS and PL due to penguin or seal activities (Table 1). These conditions allow high abundance of AOB *amoA* genes, which explains the strong correlations between AOB abundances and C:N, NH_4^+-N, and TP in the sea animal colony soils (Table 2). This agreed with the high bacterial abundance previously documented in penguin or seal colony soils and ornithogenic sediments (Ma et al., 2013; Zhu et al., 2015).

The AOA abundance showed a significant negative correlation with NH_4^+-N levels in tundra patches (Table 2), indicating that AOA might better adapt to low NH_4^+ and oligotrophic environments (Martens-Habbena et al., 2009; Stieglmeier et al., 2014). High NH_4^+-N concentrations might partially inhibit AOA populations (Hatzenpichler et al., 2008). This result is similar to that reported for some agricultural soils with increased fertilization, and grassland soils with increased grazing (Fan et al., 2011; Prosser and Nicol, 2012; Pan et al., 2018), supporting the conclusion that AOA and AOB generally inhabit different niches in soil, distinguished by the NH_4^+ concentration and availability (Verhamme et al., 2011; Wessén et al., 2011).

4.2. Effects of sea animal colonization on soil potential ammonia oxidation rates

The PAOR ranged from 9 to 139 μg N kg^{-1} h^{-1}, lower than nitrification rates measured in most agricultural soils (83–1875 μg N Kg^{-1} h^{-1}) (Fan et al., 2011; Ouyang et al., 2016; Daebeler et al., 2017). One reason might be the selection of a 15 °C incubation temperature, which was lower than the incubation temperatures used in other studies. Generally, the gross nitrification rate and *amoA* abundance increased significantly when the incubation temperature was higher than 15 °C

382 (Daebeler et al., 2017; Zhao et al., 2014). Our measurements indicated that there were significant
383 differences ($P = 0.02$) in the PAOR across different tundra patches, and the PAORs in SS and PS
384 were about 10 times higher than those in BS and MS. A significant correlation was obtained
385 between the PAOR and C:N, TP, and TS (Table 2). Overall, ammonia oxidation activity was
386 modulated by soil biogeochemical processes under the disturbance of penguin or seal activities:
387 generally low C:N ratios, and sufficient input of the nutrients TP, TS, and NH_4^+–N from sea animal
388 excrements.

389 The higher AOB abundances (Fig. 2b) and significant negative correlation of AOA abundance
390 with NH_4^+–N levels (Table 2), indicated that AOB might play a more important role in nitrification
391 in tundra soils. In agreement with these results, AOB dominated nitrification in the areas where it
392 was easy to achieve nitrogen input, whereas the relative contribution of AOA to nitrification was
393 higher in the areas where the ammonium concentration remained low (Fan et al., 2011; Sterngren
394 et al., 2015). Moreover, the cell-specific activity for AOB was 10 times higher than that for AOA
395 due to the bigger cell size of AOB (Hatzenpichler et al., 2012; Prosser and Nicol, 2012). Therefore,
396 AOB might play a more important role in nitrification in SS, PS, and PL with the input of NH_4^+–
397 N from penguin or seal excrements.

398 In addition, AOA might play a role that cannot be ignored in MS and BS, just like the
399 prevalence of AOA among ammonia-oxidizers in Arctic soils (Alves et al., 2013; Daebeler et al.,
400 2017). AOB groups were mostly undetectable in the analysis of MS and BS. Although unknown
401 γ-AOB groups might not have been detected, the primer set used here covers the β-AOB groups
402 typically found in soils (Alves et al., 2013). The BS and MS were moderately far away from

403 penguin or seal colonies without the input of the nutrients from sea animal excrements, and their
404 substrates can be provided only through the mineralization of organic matter from local tundra
405 plants. The simple organic substrates and barren soil environment might favor AOA (Stopnišek et
406 al., 2010; Habteselassie et al., 2013). Therefore AOA showed relatively high abundance in MS
407 and BS compared with PS and SS.

408 **4.3. Effects of sea animal colonization on genotypic diversity of soil AOA and AOB**

409 In this study, distinct AOA communities appear to inhabit different types of tundra patches,
410 depending on sea animal activities (Fig. 5a). It was difficult to amplify the AOA *amoA* gene from
411 SS and PS, whereas a high diversity of AOA *amoA* genes was observed in PL, MS and BS.
412 Phylogenetic analysis indicated that the AOA *amoA* sequences in Cluster I were from PL and
413 tundra soils close to seal wallows, while the sequences in Cluster II were from BS and MS (Fig.
414 S5). AOA in most extreme environments have lower levels of microbial diversity than benign
415 ecosystems because of the requirement for specific physiological adaptations, which allow
416 organisms to exploit the combination of physical and biochemical stressors (Cowan et al., 2015).
417 Detected OTUs in Cluster I had their closest matches mainly from the hyper-arid soils of Antarctic
418 dry valleys (Magalhães et al., 2014), wetland soils (Zheng et al., 2014), alpine meadow soils (Zhao
419 et al., 2017), and some agricultural soils (Glaser et al., 2010). Cluster II were more prevalent in
420 BS and MS, probably because of their stronger adaptation to barren soil environments. In cluster
421 II, the sequences were affiliated with sequences recovered from cold environments, including the
422 soils of Tibetan Plateau (Xie et al., 2014) and Icelandic grassland soils (Daebeler et al., 2012).
423 The compositions of soil AOA populations are likely not to be explained by single

424 physicochemical properties, and their community structures significantly correlated with tundra
425 soil C:N, and TC, which was consistent with previous studies (Glaser et al., 2010; Wessén et al.,
426 2011).

427 AOB *amoA* gene diversity was higher than that of AOA, similar to results in the Antarctic Dry
428 Valley soils (Magalhães et al., 2014). A high diversity of AOB *amoA* genes occurred in SS, PS
429 and PL compared to BS, indicating that penguin or seal activities had important effects on AOB
430 genotypic diversity. Phylogenetic analysis indicated that the sequences in clusters I and II were
431 mainly from PS and SS (Fig. 5b), and the detected OTUs in Cluster I had their closest matches
432 from mixed community culture systems, meadow to forest transect in Oregon Cascade Mountains
433 (Mintie et al., 2003), and Dutch agricultural soils (Silva et al., 2012a) and reservoir sediments
434 (Silva et al., 2012b). For Clusters III and IV, the sequences were predominantly from PL and SS,
435 and they were affiliated with sequences recovered from high altitude wetland (Yang et al., 2014).
436 Previous studies have shown that multiple environmental factors affected the AOB communities
437 (Dang et al., 2008; Mosier and Francis, 2008). In this study, the C:N ratios and NH_4^+-N
438 concentrations seemed to be the most important factors influencing the AOB community structure,
439 which was in accordance with the results from different environments (Bouskill et al., 2012; Jung
440 et al., 2011; Li et al., 2015). Moreover, the TP also affected the AOB *amoA* community
441 compositions (Zheng et al., 2013). Therefore, the AOB community compositions were impacted
442 by the biogeochemical factors related to sea animal activities, such as low C:N ratios, and
443 sufficient supply of the nutrients NH_4^+–N and TP from sea animal excreta.

5 Conclusions

The findings of this study concerning the abundance, potential activity, and diversity of tundra soil AOA and AOB provide insights into microbial mechanisms driving nitrification in maritime Antarctica. We confirmed the presence of AOA and AOB *amoA* genes in five different tundra patches, and demonstrated that the spatial distribution heterogeneities of the tundra soil AOA and AOB communities were driven by penguin or seal activities. The soil AOB *amoA* copy numbers were generally higher than the AOA *amoA* copy numbers, following the higher PAOR in penguin or seal colonies and their adjacent tundra, compared with that in the background tundra and marsh tundra. Penguin or seal activities resulted in significant shift of soil AOA and AOB community compositions. AOB *amoA* gene diversity was higher in SS and PS than in PL and MS, and the majority of the AOB sequences were closely related to *Nitrosospira*-like sequences. The AOA *amoA* gene had higher diversity in PL and MS than in BS, and they were associated with *Nitrososphaera* sequences recovered from barren soils. Soil AOB and AOA abundances, and their community compositions, were related to soil biogeochemical processes under the sea animal-activity disturbance, such as soil C:N alteration, and a sufficient supply of the nutrients NH_4^+–N, N and P from animal excreta. This study significantly enhanced the understanding of ammonia-oxidizing microbial communities in tundra environment of maritime Antarctica.

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Table 1. Soil properties, potential ammonia oxidation rates, and ammonia oxidizer populations for the soil samples (n = 22) that span a penguin colony, a seal colony, and their adjacent animal-lacking tundra across Ardley Island and the Fildes Peninsula in maritime Antarctica.

Sampling No.	pH	Moisture (%)	TC (mg g^{-1})	TN (mg g^{-1})	C:N	TS (mg g^{-1})	TP (mg g^{-1})	NH_4^+-N (mg Kg^{-1})	NO_3^--N (mg Kg^{-1})	NO_2^--N (mg Kg^{-1})	PAOR (μgN $Kg^{-1}h^{-1}$)	AOA (copies g^{-1})	AOB (copies g^{-1})
Seal colony tundra soils (SS)													
SS1	4.8	31.3	48.7	12.1	4.0	3.4	3.6	650.9	4.6	0.1	138.8±0.8	1.79×10^{5}	9.22×10^{8}
SS2	8.2	32.5	70.1	16.9	4.1	4.8	5	17.7	19.1	0.7	115.3±15.5	3.99×10^{4}	5.92×10^{5}
SS3	4.6	19.6	5.6	0.9	6.2	ND	1.3	17.9	61.7	0.2	8.9±0.5	--	3.85×10^{8}
SS4	5.2	17.5	8.6	1.3	6.6	0.8	1.2	0.6	12.1	ND	38.4±5.1	5.53×10^{4}	2.57×10^{8}
SS5	5.4	26.6	11.5	1.3	8.8	0.7	0.8	1.1	13.9	ND	79.3±44.5	--	3.03×10^{7}
Mean±SE	5.6 ± 0.6^{ab}	22.5 ± 2.7^{ab}	28.9 ± 11.6^{a}	6.5 ± 3.0^{a}	6.0 ± 0.80^{a}	2.4 ± 0.8^{ab}	2.4 ± 0.7^{a}	137.6 ± 114.8^{a}	22.3 ± 9.1^{a}	0.3 ± 0.12^{a}	76.1 ± 21.4^{a}	$(9.1\pm2.7)\times10^{4a}$	$(4.0\pm1.4)\times10^{8ab}$
Active penguin colony tundra soils along the eastern coast on Ardley Island (PS)													
PS1	5.7	64.9	84.7	14.5	5.8	4.4	10.6	151.4	2.5	0.3	88.8±2.7	5.95×10^{4}	7.54×10^{8}
PS2	5.9	53.1	38.1	8.0	4.8	1.6	12.5	461	1.7	0.6	70.9±14.4	2.49×10^{4}	4.62×10^{8}
PS3	4.9	27.3	120.8	15.5	7.8	4.1	23.7	59.9	7.2	0.2	48.9±0.4	1.28×10^{4}	4.13×10^{8}
PS4	5.2	65.7	107.5	17.9	6.0	3.1	32.9	21.4	4.3	0.7	41.1±2.7	2.44×10^{4}	3.21×10^{8}
PS5	4.9	25.4	45.8	8.3	5.5	3.8	18.1	190.7	54.7	0.9	17.3±2.1	1.57×10^{4}	4.25×10^{8}
Mean±SE	5.3 ± 0.2^{a}	47.3 ± 7.9^{b}	79.4 ± 14.7^{a}	12.8 ± 1.8^{ab}	6.0 ± 0.45^{a}	3.4 ± 0.4^{b}	19.6 ± 3.6^{b}	176.9 ± 69.1^{a}	14.1 ± 9.1^{a}	0.5 ± 0.12^{a}	53.4 ± 11.0^{ac}	$(2.7\pm0.7)\times10^{4a}$	$(4.8\pm0.7)\times10^{8a}$
The middle penguin-lacking tundra soils on Ardley Island (PL)													
PL1	6.7	85.5	117.6	11.5	10.2	2.6	5.7	3.7	1.3	ND	19.8±1.2	2.58×10^{5}	7.94×10^{7}
PL2	6.6	41.9	38.1	3.9	9.8	0.7	8.1	5.7	1.2	ND	16.2±0.5	4.69×10^{5}	2.09×10^{7}
PL3	6.6	95.1	302.5	25.3	12.0	3.1	3.1	3.4	13.2	ND	33.1±0.9	1.75×10^{4}	5.03×10^{7}

PL4	6.5	85.1	71.9	7.2	10.0	1.8	5.4	1.2	2.5	ND	18.3±1.4	1.40×10^{5}	1.24×10^{8}
Mean±SE	6.6 ± 0.1^{b}	76.9 ± 10.3^{c}	132.5 ± 51.1^{ab}	12.0 ± 4.1^{ab}	10.5 ± 0.43^{b}	2.1 ± 0.5^{ab}	5.6 ± 0.9^{a}	3.5 ± 0.8^{b}	4.5 ± 2.5^{a}	-	21.8 ± 3.3^{bc}	$(5.4\pm2.6)\times10^{5b}$	$(6.9\pm0.2)\times10^{7b}$
The western tundra marsh soils on Ardley Island (MS)													
MS1	6.1	65.5	95.5	8.9	10.7	2.5	5.2	1.1	10.3	0.1	15.5±1.2	3.46×10^{6}	3.11×10^{5}
MS2	5.7	84.2	193.9	15.9	12.2	2.0	1.8	1.2	7.8	0.4	8.9±2.2	2.39×10^{6}	1.73×10^{7}
MS3	5.1	86.2	226.9	19.8	11.5	2.6	1.8	11.5	9.8	0.4	10.3±1.5	1.33×10^{5}	9.97×10^{4}
MS4	5	91.9	355.1	26.6	13.3	2.4	2.2	11.5	13.1	0.3	14.4±3.9	--	4.93×10^{4}
MS5	5.1	93.2	292.3	23.5	12.4	2.5	1.9	5.3	12	0.3	10.8±3.4	3.80×10^{5}	2.44×10^{5}
Mean±SE	5.4 ± 0.2^{ab}	84.0 ± 4.4^{c}	232.7 ± 39.4^{b}	18.9 ± 2.8^{b}	12.0 ± 0.40^{b}	2.4 ± 0.1^{ab}	2.6 ± 0.6^{a}	6.1 ± 2.1^{b}	10.6 ± 0.8^{a}	0.3 ± 0.1^{a}	12.0 ± 1.1^{b}	$(2.1\pm0.6)\times10^{6b}$	$(5.9\pm3.5)\times10^{6c}$
Background tundra soils on the upland of the Fildes Peninsula (BS)													
BS1	5.3	16.8	56.7	4.8	11.8	1.2	2.4	1.1	23.6	0.5	12.8±1.5	4.33×10^{6}	2.16×10^{7}
BS2	5.6	18.0	56.6	5.1	11.1	0.8	1.9	0.7	16.4	0.5	17.6±0.5	7.94×10^{6}	2.39×10^{6}
BS3	5.3	19.8	47.7	4.3	11.1	0.5	3	1.2	16.4	0.6	11.1±0.8	1.56×10^{7}	1.11×10^{7}
Mean±SE	5.4 ± 0.1^{ab}	18.2 ± 0.7^{a}	53.7 ± 2.4^{a}	4.7 ± 0.2^{a}	11.3 ± 0.20^{b}	0.8 ± 0.2^{a}	2.5 ± 0.3^{a}	2.3 ± 0.1^{b}	16.7 ± 2.0^{a}	0.5 ± 0.1^{a}	13.8 ± 1.6^{bc}	$(9.3\pm2.7)\times10^{6b}$	$(1.2\pm0.5)\times10^{7c}$

Note: ND indicated that the soil sample was not determined.

Table 2. Spearman correlations (n=22) among ammonia oxidizer populations, the ratios of AOA: AOB abundances, potential ammonia oxidation rates (PAOR), and environmental variables in the soils of maritime Antarctic tundra.

	pH	Moisture	TC	TN	C/N	TS	TP	NH_4^+-N	NO_3^--N	NO_2^--N
AOA (copies g^{-1})	0.331	-0.108	0.002	-0.243	0.373	-0.381	-0.195	**-0.523***	-0.112	0.027
AOB (copies g^{-1})	-0.191	-0.293	**-0.434***	-0.271	**-0.748****	0.232	**0.468***	**0.526***	-0.261	-0.108
AOB/AOA	-0.274	-0.206	-0.337	-0.108	**-0.720****	0.313	**0.425***	**0.622****	-0.117	-0.022
PAOR (μgN $Kg^{-1}h^{-1}$)	0.221	-0.104	-0.185	0.032	**-0.667****	**0.468***	**0.430***	0.307	-0.304	-0.138

Note: Significant correlations are indicated by * at the P=0.05 level, and ** at the P=0.01 level.

Table 3. Individual and combined contributions of soil biogeochemical properties to the AOA and AOB community structures in tundra patches.

	Soil properties	F	P	Individual contribution
AOA	**C:N**	**2.593**	**0.022**	**21.5%**
	TC	**2.068**	**0.048**	**18.0%**
	NO_3^--N	1.847	0.078	16.5%
	pH	1.458	0.144	13.5%
	TP	1.035	0.406	10.5%
	NH_4^+-N	0.731	0.622	7.3%
	Combined effect of all factors			86.9%
AOB	**C:N**	**1.844**	**0.002**	**11.6%**
	NH_4^+-N	**1.823**	**0.002**	**11.5%**
	TP	1.39	0.078	9.1%
	pH	1.383	0.066	9.0%
	NO_3^--N	1.161	0.258	7.7%
	Combined effect of all factors			48.9%

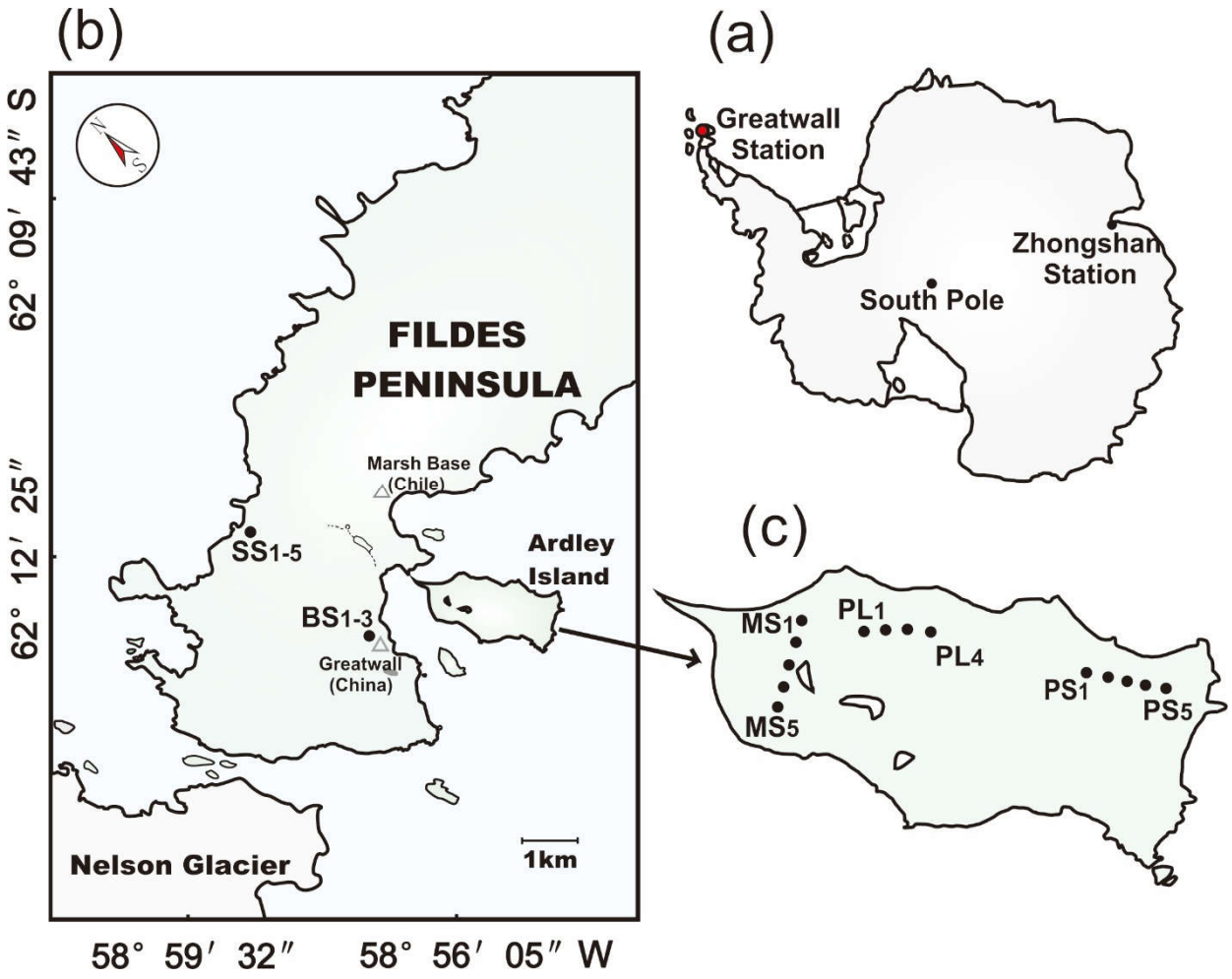


Figure 1. Study area and soil sampling sites. Panel (a), the red dot indicates the location of the investigation area in maritime Antarctica. Panel (b), location of the sampling sites on the Fildes Peninsula. The sampling soils from tundra patches included the active seal colony tundra soils SS (SS1–5) in the western coast of the Fildes Peninsula, and the background tundra soils on the upland areas (BS1–3). Panel (c), the location of the sampling sites on Ardley Island. The sampling soils from tundra patches included the western tundra marsh soils (MS1–5), the eastern active penguin colony tundra soils PS (PS1–5) and the adjacent penguin-lacking tundra soils PL (PL1–4). Note: The map was drawn using CorelDRAW X7 software (http://www.corel.com/cn/).

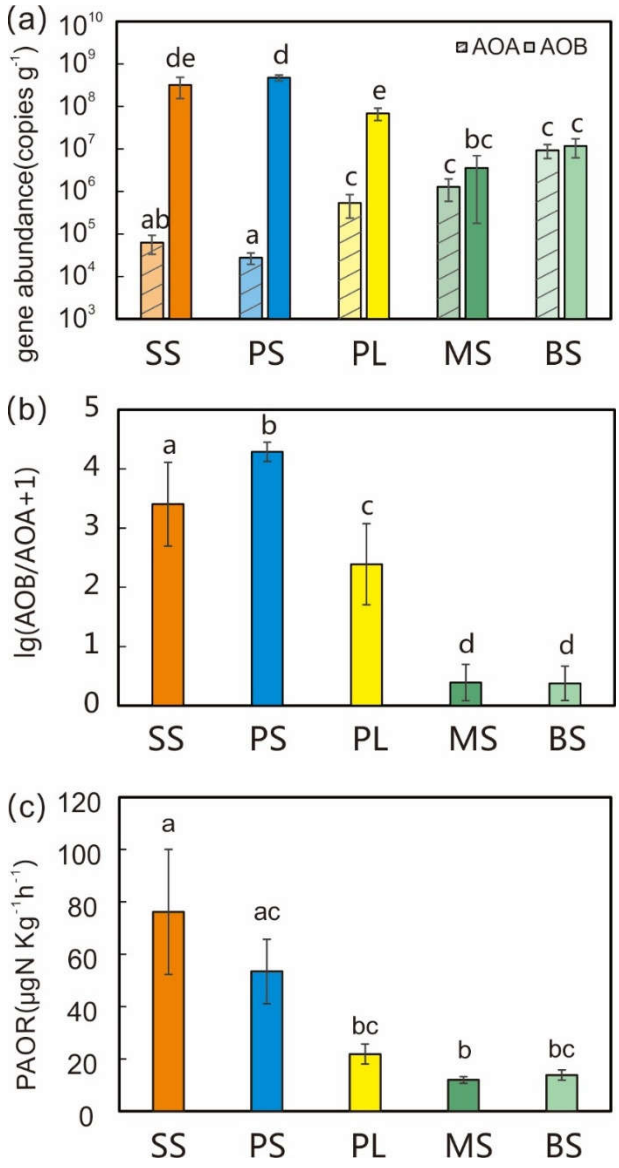


Figure 2. Comparisons of soil AOA and AOB *amoA* gene copy numbers (a), log ratio of AOB: AOA abundances (b), and potential ammonia oxidation rates (PAOR) (c) between five tundra patches. The error bars indicate standard deviations of the means.

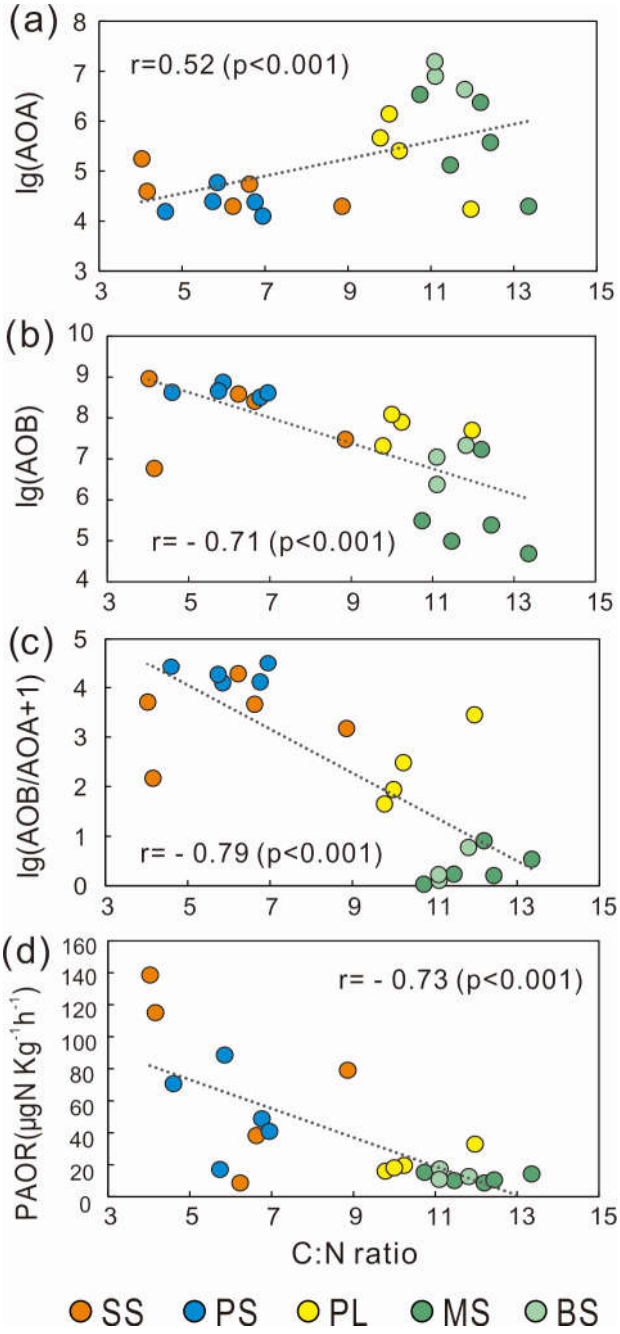


Figure 3. Effects of soil C:N alteration on AOA and AOB abundances, and potential ammonia oxidation rates (PAOR) at five tundra patches.

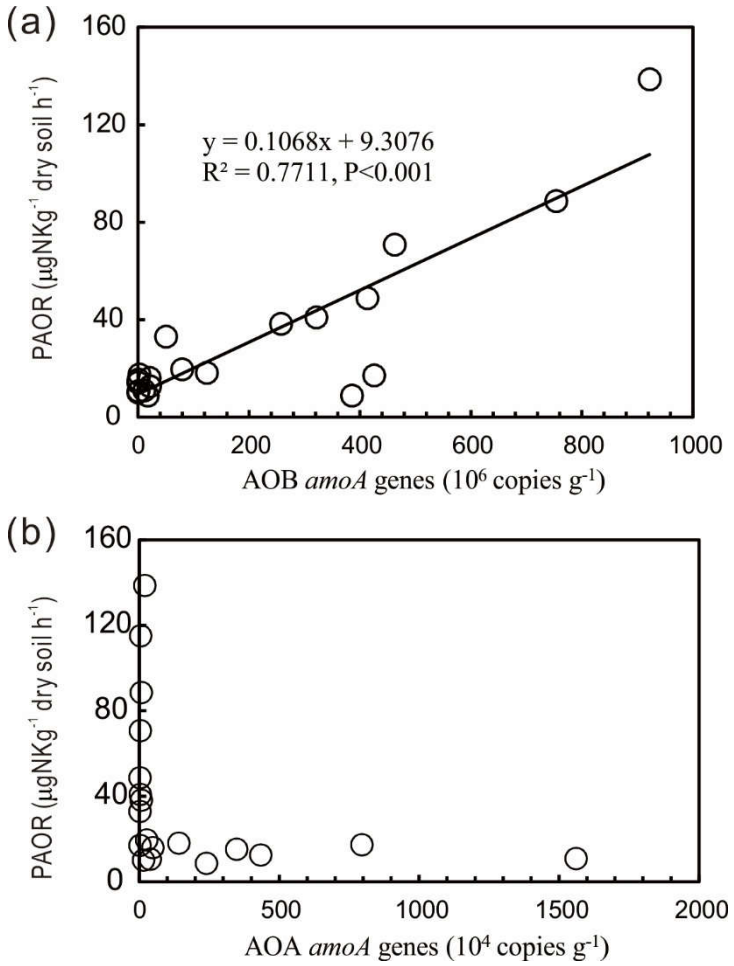
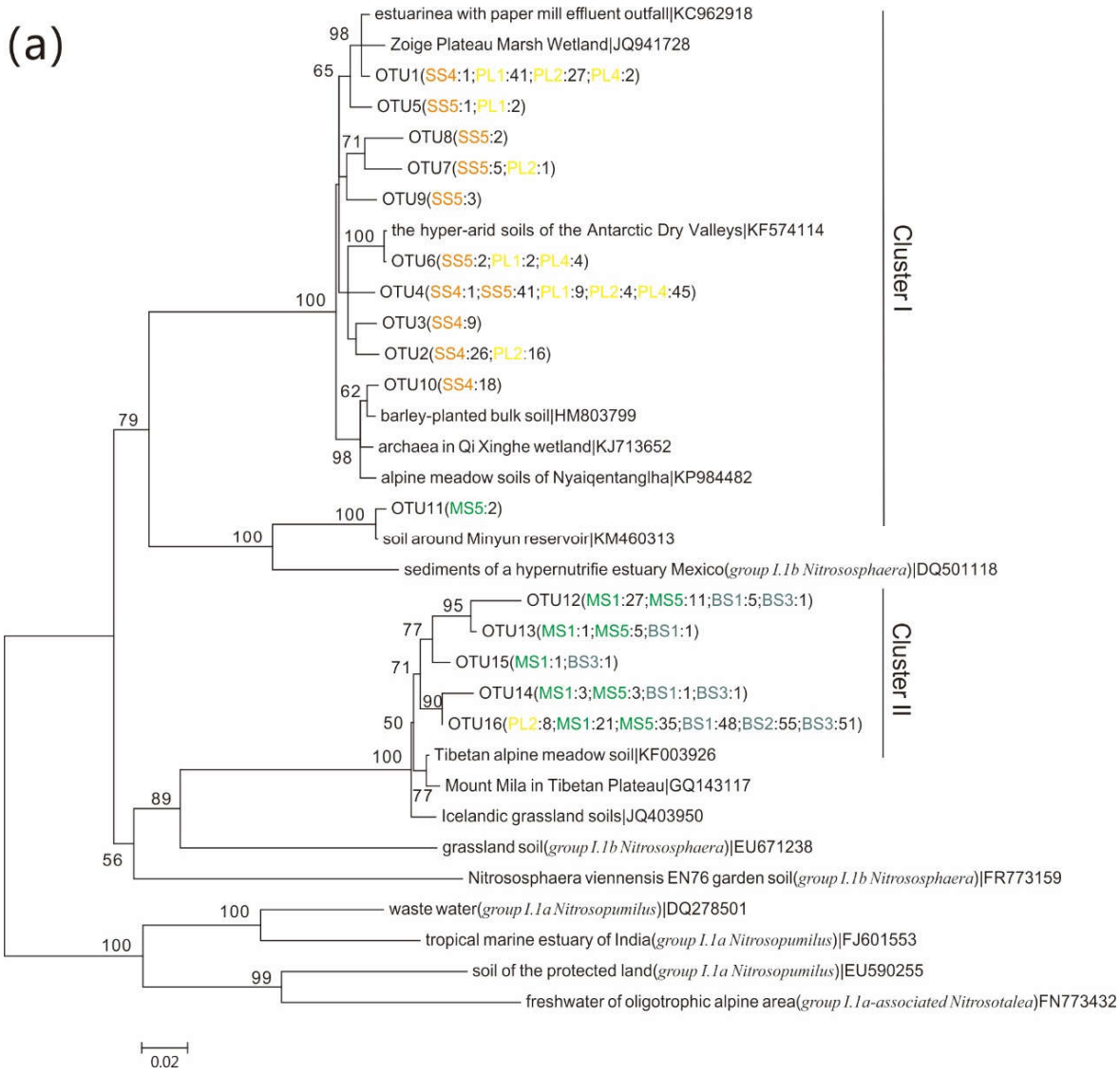
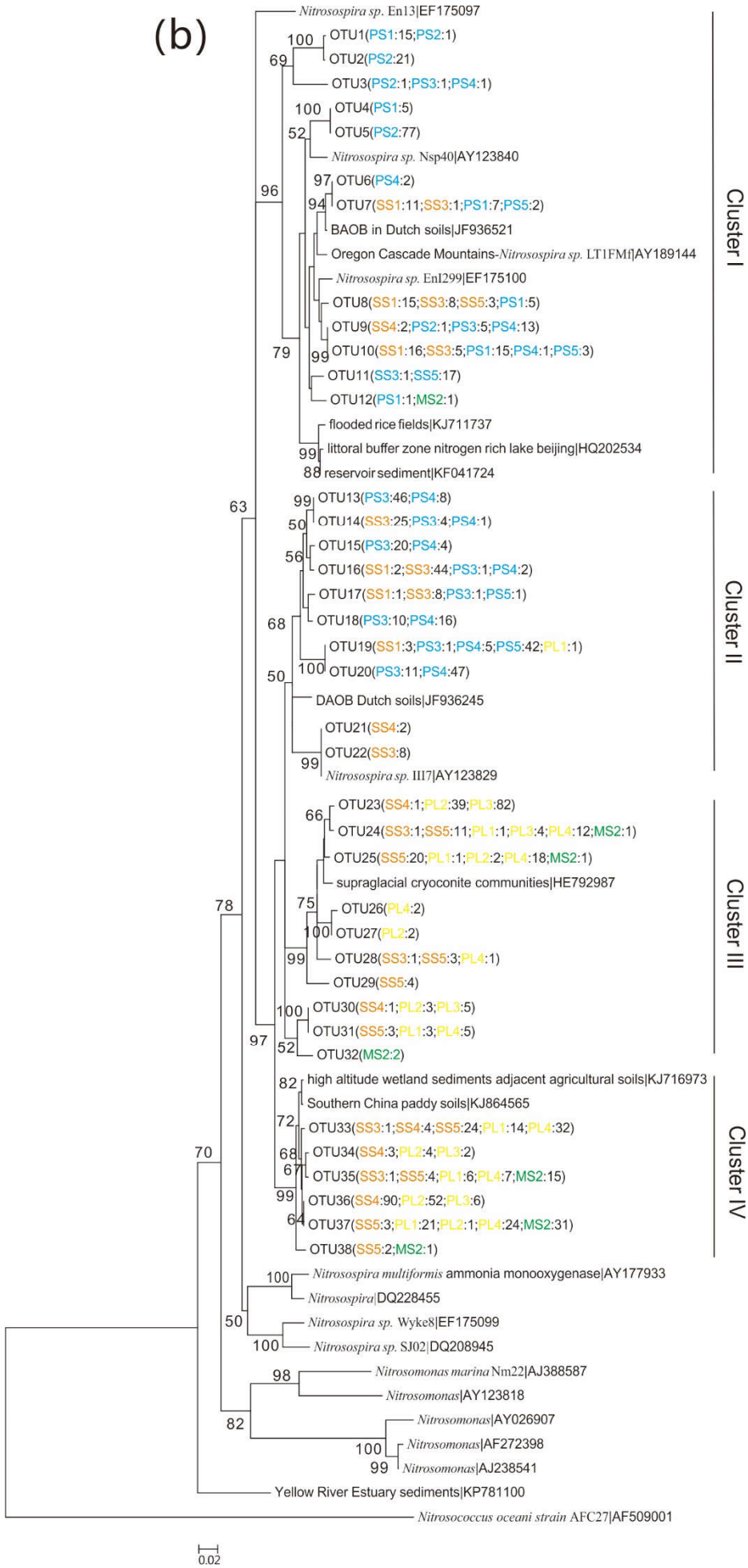


Figure 4. Correlation between potential ammonia oxidation rates (POARs) and AOA and AOB *amoA* gene copy numbers in tundra soils of maritime Antarctica.

Figure 5. Neighbor-joining phylogenetic tree of AOA *amoA* (a) and AOB *amoA* (b). The phylogeny is based on nucleotide sequences. Bootstrap values ≥ 50% (of 1000 iterations) are shown near the nodes. GenBank accession numbers are shown for sequences from other studies. OTUs were defined at 97% similarity. Numbers in parentheses following each OTU indicate the number of sequences recovered from each sampling site.




(b)
Nitrosospira sp. En13|EF175097
OTU1(PS1:15;PS2:1)
OTU2(PS2:21)
OTU3(PS2:1;PS3:1;PS4:1)
OTU4(PS1:5)
OTU5(PS2:77)
Nitrosospira sp. Nsp40|AY123840
OTU6(PS4:2)
OTU7(SS1:11;SS3:1;PS1:7;PS5:2)
BAOB in Dutch soils|JF936521
Oregon Cascade Mountains-Nitrosospira sp. LT1FMf|AY189144
Nitrosospira sp. EnI299|EF175100
OTU8(SS1:15;SS3:8;SS5:3;PS1:5)
OTU9(SS4:2;PS2:1;PS3:5;PS4:13)
OTU10(SS1:16;SS3:5;PS1:15;PS4:1;PS5:3)
OTU11(SS3:1;SS5:17)
OTU12(PS1:1;MS2:1)
flooded rice fields|KJ711737
littoral buffer zone nitrogen rich lake beijing|HQ202534
reservoir sediment|KF041724
OTU13(PS3:46;PS4:8)
OTU14(SS3:25;PS3:4;PS4:1)
OTU15(PS3:20;PS4:4)
OTU16(SS1:2;SS3:44;PS3:1;PS4:2)
OTU17(SS1:1;SS3:8;PS3:1;PS5:1)
OTU18(PS3:10;PS4:16)
OTU19(SS1:3;PS3:1;PS4:5;PS5:42;PL1:1)
OTU20(PS3:11;PS4:47)
DAOB Dutch soils|JF936245
OTU21(SS4:2)
OTU22(SS3:8)
Nitrosospira sp. II17|AY123829
OTU23(SS4:1;PL2:39;PL3:82)
OTU24(SS3:1;SS5:11;PL1:1;PL3:4;PL4:12;MS2:1)
OTU25(SS5:20;PL1:1;PL2:2;PL4:18;MS2:1)
supraglacial cryoconite communities|HE792987
OTU26(PL4:2)
OTU27(PL2:2)
OTU28(SS3:1;SS5:3;PL4:1)
OTU29(SS5:4)
OTU30(SS4:1;PL2:3;PL3:5)
OTU31(SS5:3;PL1:3;PL4:5)
OTU32(MS2:2)
high altitude wetland sediments adjacent agricultural soils|KJ716973
Southern China paddy soils|KJ864565
OTU33(SS3:1;SS4:4;SS5:24;PL1:14;PL4:32)
OTU34(SS4:3;PL2:4;PL3:2)
OTU35(SS3:1;SS5:4;PL1:6;PL4:7;MS2:15)
OTU36(SS4:90;PL2:52;PL3:6)
OTU37(SS5:3;PL1:21;PL2:1;PL4:24;MS2:31)
OTU38(SS5:2;MS2:1)
Nitrosospira multiformis ammonia monooxygenase|AY177933
Nitrosospira|DQ228455
Nitrosospira sp. Wyke8|EF175099
Nitrosospira sp. SJ02|DQ208945
Nitrosomonas marina Nm22|AJ388587
Nitrosomonas|AY123818
Nitrosomonas|AY026907
Nitrosomonas|AF272398
Nitrosomonas|AJ238541
Yellow River Estuary sediments|KP781100
Nitrosococcus oceani strain AFC27|AF509001
Cluster I
Cluster II
Cluster III
Cluster IV
0.02

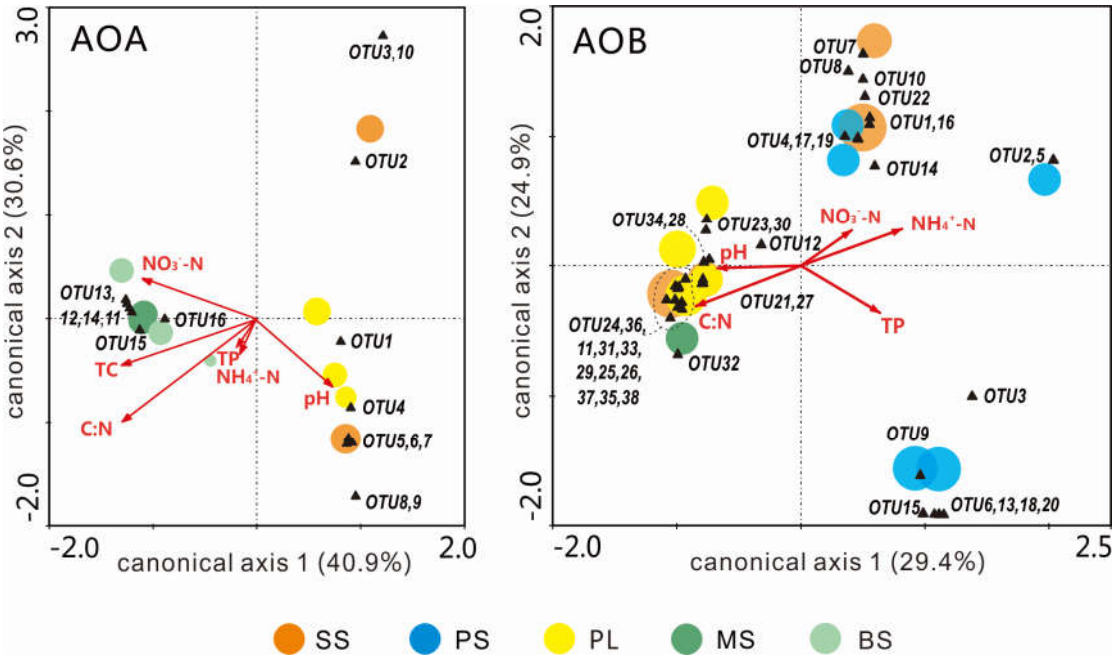


Figure 6. Canonical correspondence analysis (CCA) ordination plots for the relationship between the AOA and AOB community structures with environmental variables. The circles with different colors represent the various sampling sites. The size of the circles corresponds to the OTU richness in individual samples. The black triangles represent *amoA* phylotypes. Environmental variables are represented by red arrows. The percentage of species-environment relation variance explained by the two principal canonical axes is represented close to the axes.