1 # Effects of Sea Animal Colonization on the Coupling between Dynamics and

2 ## Activity of Soil Ammonia-oxidizing Bacteria and Archaea in Maritime Antarctica

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Abstract

The colonization by a large number of sea animal, 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 increases 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 were more important 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

31 to soil biogeochemical processes under the disturbance of penguin or seal activities: soil C:N

32 alteration and sufficient input of NH_4^+–N and phosphorus from animal excrements. The results

33 significantly enhanced the understanding of ammonia-oxidizing microbial communities in tundra

34 environment of maritime Antarctica.

35 **Keywords:** Antarctic tundra, AOA, AOB, Marine 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 soil (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 the Antarctic coastal waters (Kalanetra et al., 2009; Tolar et al., 2016). However, there is still a large gap in our understanding of factors that

56 control AOA *versus* AOB prominence, and the relationships between nitrification rates and

57 ammonia-oxidizer dynamics need to be explored in the Antarctic.

58   In maritime Antarctica, a large number of sea animals, such as penguins or seals, settle on

59 coastal ice-free tundra patches. Tundra vegetation including mosses, lichens, and algae, penguin

60 colonies, and their interactions, form a special ornithogenic tundra ecosystem (Tatur et al., 1997).

61 The soil biogeochemistry of an ornithogenic tundra ecosystem has become a research hotspot

62 under the penguin-activity disturbance (Otero et al., 2018; Riddick et al., 2012; Simas et al., 2007;

63 Zhu et al., 2013, 2014). Previous studies indicated that sea animals significantly affect the tundra

64 N and P cycles (Lindeboom et al., 1984; Simas et al., 2007; Zhu et al., 2011), and the total N and

65 P excreted by seabird breeders and chicks are 470 Gg N yr^{-1} and 79 Gg P yr^{-1} in Antarctica and

66 the Southern Ocean, accounting for 80% of the N and P from total global seabird excreta (Otero

67 et al., 2018). Uric acid is the dominant N compound in penguin guano, and during its

68 mineralization, different N forms, such as NH_3, NH_4^+, and NO_3^-, can be produced via

69 ammonification, nitrification, and deposition, following the changes in soil pH and the C:N ratio

70 (Blackall et al., 2007; Otero et al., 2018; Riddick et al., 2012). The alteration of soil

71 biogeochemistry under the sea animal -activity disturbance might have an impact on the

72 abundance and diversity of the AOA and AOB involved in the nitrogen cycle. Increased bacterial

73 abundance, diversity, and activity have been detected in penguin or seal colony soils (Ma et al.,

74 2013; Zhu et al., 2015). Penguin or seal colonies have been confirmed as strong sources for

75 greenhouse gas N_2O (Zhu et al., 2008, 2013), a by-product of microbial ammonia oxidation

76 (Santoro et al., 2011). However, the effects of sea animal colonization on AOA and AOB

77 community structures have not been thoroughly investigated in the maritime Antarctic tundra.

78   In the present study, we investigated the abundance, activity, and diversity of soil AOA and

79 AOB in five tundra patches, including a penguin colony, a seal colony, the adjacent animal-lacking

80 tundra, tundra marsh, and background tundra, where soil biogeochemical properties were

81 subjected to the differentiating effects of sea animal activities. Our objectives were (a) to examine

82 the abundance, diversity, and community structure of soil AOA and AOB using the *amoA* gene as

83 a functional marker; (b) to investigate potential links between *amoA* gene abundance, AOA and

84 AOB community structures, activity, and environmental variables; and (c) to assess the relative

85 contribution of these two distinct ammonia-oxidizing groups to nitrification.

86 **2 Materials and methods**

87 **2.1 Study area**

88   The study area is located on the Fildes Peninsula and Ardley Island in the southwest of King

89 George Island (Fig. 1), having an oceanic climate characteristics. Mean annual air temperature is

90 about $-2.5\ ^\circ C$, with the range of daily mean temperature from -26.6 to $11.7\ ^\circ C$, and mean annual

91 precipitation is about 630 mm, mainly in the form of snow. The Fildes Peninsula (about 30 km^2

92 area) is a host to important sea animal colonies. Based on annual statistical data, the total of over

93 10,700 sea animals colonize this peninsula in the austral summer. On the western coast are

94 established seal colonies including elephant seal (*Mirounga leonine*), weddell seal (*Leptonychotes*

95 *weddellii*), fur seal (*Arctocephalus gazella*) and leopard seal (*Hudrurga leptonyx*) (Sun et al.,

96   2004). Ardley Island, with an area of 2.0 km in length and 1.5 km in width, is connected with the

97   Fildes Peninsula via a sand dam. This island belongs to an important Ecological Reserve for

98   penguin populations in western Antarctica. A great majority of breeding penguins, including

99   Adélie penguins (*Pygoscelis adeliae*), Gentoo penguins (*Pygoscelis papua*), and Chinstrap

100 penguins (*Pygoscelis antarctica*), colonized on the east of this island in the austral summer. Seal

101 excrements or penguin droppings rich in nitrogen and phosphorus were transported into local

102 tundra soils by ice-snow melting water during the breeding period (Sun et al., 2000, 2004). Mosses

103 and lichens dominate local vegetation. However, the vegetation is almost absent in penguin or

104 seal colonies because of overmanuring and animal trampling. More detailed description about the

105 study area can be found in Zhu et al. (2013).

106 **2.2. Tundra soil collection**

107 In the summer of 2014/2015, soil samples were collected from the following tundra patches,

108 as illustrated in Fig. 1:

109 (i) Penguin colony and penguin-lacking tundra sites: The tundra on Ardley Island was

110 categorized into three areas from the east to west according to the distance to the penguin nesting

111 sites (i.e., the intensity of penguin activity): The eastern active penguin colony with nesting sites,

112 PS (i.e., high penguin-activity area) where penguins have the highest density and high frequency

113 presence during the breeding period; the adjacent penguin-lacking tundra areas, PL (i.e., low

114 penguin-activity areas) in the middle of Ardley Island where penguins occasionally wander and

115 have a typically low density; and the western tundra marsh, MS, moderately far from penguin

116 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 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 (operational taxonomic unit) using the mothur program (version 1.23.0) by the furthest neighbor

175   approach (Schloss et al., 2009). The closest reference sequences were identified at NCBI

176   (http://www.ncbi.nlm.nih.gov/BLAST/) using the BLASTn tool (Madden, 2002), and

177   phylogenetic trees were constructed by the neighbor-joining method using the Molecular

178   Evolutionary Genetics Analysis (MEGA) software (version 5.03, https://www.megasoftware.net/).

179   The sequences reported in this study have been deposited in GenBank under accession unmbers

180   MH318029 to MH318568 and MH301331 to MH302505.

2.7. Quantitative real-time PCR

182   The AOB and AOA *amo*A gene copy numbers for tundra soils were determined in triplicate

183   using quantitative real-time PCR (qPCR) on an ABI 7500 Sequence Detection System (Applied

184   Biosystems). The specific details were given by Zheng et al. (2014). The strong linear inverse

185   relationship confirmed the consistency of the qPCR assay between the threshold cycle and the log

186   value of gene copy numbers (R^2 = 0.997 for AOA; R^2 = 0.999 for AOB). The amplification

187   efficiencies for AOA and AOB were 99.8 % and 90.4 %, respectively. Melting curve analysis had

188   only one observable peak at a melting temperature (Tm) (84.9 °C for AOA and 89.6 °C for AOB)

189   (Fig. S1 in Supplementary Material). Negative controls were subjected to exclude any possible

190   carryover or contamination in all experments.

2.8. Statistical analysis

192   The Shannon–Weiner Index, Simpson Index and the richness estimator Chao 1 were calculated

193   by the mothur program (version 1.23.0, Schloss et al., 2009). The coverage was the percentage of

194   the number of observed OTUs divided by the Chao 1 (Table S1). The Kruskal–Wallis test and

195   Wilcoxon signed rank test were conducted for the comparison between *amoA* gene abundance and

196   PAOR from five tundra patches using SPSS Statistics 17 (IBM Corp, Armonk, NY, USA).

197   Correlations between ammonia-oxidizer gene abundance, PAOR and environmental variables

198   were obtained by Spearman Correlation Analysis. The relationships between the ammonia-

199   oxidizer community structure and environmental variables were explored using canonical

200   correspondence analysis (CCA) in the software Canoco for windows (version 4.5; Microcomputer

201   Power, Ithaca, NY, USA), because the maximum gradient length of both AOA and β-AOB was

202   longer than four SD (AOA: 4.406; AOB: 18.326). All environmental parameter values were

203   transformed into ln(x+1) before statistical analyses. The OTU richness (defined at 3% distance)

204   served as the species input and several simulations of manual forward selection were performed

205   with 499 Monte Carlo permutations to build the optimal models. The scaling in the final CCA

206   biplots was focused on inter-sample relations.

207   **3 Results**

208   **3.1. Soil chemistry and sea animal activities**

209   Almost all the tundra soils were slightly acidic, and the mean pH ranged from 5.3 to 6.6 at

210   each tundra patch (Table 1). In penguin or seal colony tundra soils, PS and SS, soil properties

211   including TC, TN, TS, TP, NH_4^+-N and NO_3^--N levels showed high heterogeneity due to the

212   deposition of penguin or seal excreta. In the seal colony tundra soils on Fildes Peninsula, the

213   highest TC, TN, TP, TS, and NH_4^+-N levels occurred at the sites (SS1-2) close to the seal wallows.

214   In the tundra soils on Ardley Island, the highest TP, TS, and NH_4^+-N levels occurred in the soils

215 close to the eastern penguin nesting sites (PS1-5). PS and SS had generally lower C:N ratios than

216 the penguin-lacking tundra soils (PL), tundra marsh soils (MS), and background tundra soils (BS).

217 Soil mean TN, TS and NH_4^+–N levels were higher in PS, SS, PL, and MS than in BS. Soil NH_4^+–

218 N contents were 1–2 orders of magnitude higher in PS and SS than in PL, MS, and BS, with the

219 means of 176.9 and 137.6 mg NH_4^+-N kg^{-1}, respectively. The highest NO_3^--N contents occurred

220 in SS. Phosphorus levels were significantly greater (P < 0.05) in PS (10.6–32.9 mg g^{-1}) than in

221 other types of tundra soils (mean < 6.0 mg g^{-1}). Overall, penguin or seal activities altered the local

222 soil biogeochemical properties through the deposition of their excreta, leading to generally low

223 C:N ratios in tundra soils.

224 ### 3.2. Gene abundances under sea animal colonization

225 The abundance of the AOB *amoA* gene was significantly higher (by approximately 2–4 orders

226 of magnitude) than that of the AOA *amoA* gene (Wilcoxon test, n = 22, P = 0.002) in the penguin

227 and seal colony and the adjacent tundra soils, PS, SS, and PL. However, the abundances of the

228 *amoA* gene were similar in the MS and BS soils (Fig. 2a). Overall, the abundances of AOB and

229 AOA *amoA* genes were significantly negatively correlated (r = -0.93, P = 0.002) across all the

230 tundra sites (Fig. S2). The AOA *amoA* gene abundances showed a heterogeneous distribution

231 among the different tundra patches, and they were two orders of magnitude lower in PS and SS

232 relative to those in BS and MS. The maximal AOA *amoA* gene abundance appeared in BS,

233 followed by MS and PL, whereas the PS and SS soils had the lowest AOA *amoA* gene abundances.

234 The log values of soil AOA *amoA* gene abundances showed a significant positive correlation

235   (r=0.52, P < 0.001) with C:N ratios (Fig. 3a), but their abundances showed a significant negative

236   correlation with NH_4^+-N contents (r= -0.52, P = 0.013) (Table 2).

237   Unlike the AOA *amoA* genes, AOB *amoA* gene abundances showed the opposite distribution

238   pattern. The AOB *amoA* gene abundances were significantly higher (by approximately 2–3 orders

239   of magnitude) in PS and SS compared with those in MS and BS (Fig. 2a). The log values of soil

240   AOB *amoA* gene abundances showed a significant negative correlation with C:N ratios (r= -0.71,

241   P < 0.001) (Fig. 3b), but their abundances showed a significant positive correlation with NH_4^+-N

242   (r=0.53 , P < 0.05) and TP (r=0.47 , P < 0.05) (Table 2). The ratios of AOB to AOA *amoA* copy

243   numbers were strongly affected by animal activities, and were much higher in PS and SS than in

244   PL, MS, and BS (Fig. 2b; Kruskal–Wallis test, χ^2 = 18.2, P = 0.01). Their ratios showed significant

245   positive correlation with NH_4^+-N contents (r=0.62; P < 0.01) and TP (r=0.43, P < 0.05) (Table 2),

246   but significant negative correlation with the C:N ratios (r= -0.79; P < 0.001)(Fig. 3c). Overall,

247   penguin or seal activities, which were indicated by soil C:N ratios, significantly increases the

248   abundance of soil AOB *amoA* genes, but reduced the abundance of AOA *amoA* genes, leading to

249   very large ratios (1.5×10^2 to 3.2×10^4) of AOB to AOA *amoA* copy numbers in PS and SS.

250   However, the ratios varied only from 0.1 to 7.2 in BS and MS.

251   **3.3 Potential ammonia oxidation rates under sea animal colonization**

252   Potential ammonia oxidation rates (PAORs) ranged from 8.9 to 138.8 µg N kg^{-1} h^{-1} in all the

253   soil samples (Table 1). The PAOR was slightly higher in SS (mean 76.1 µg N kg^{-1} h^{-1}) than in

254   PS (mean 64.7 µg N kg^{-1} h^{-1}), but significantly higher than in PL, MS, and BS (mean 12.0–21.8

255   µg N kg^{-1} h^{-1}). Overall, the PAOR was significantly higher in animal colony soils (mean 70.4 µg

256 N $kg^{-1} h^{-1}$ for SS and PS) than in non-animal colony soils (mean 15.7 μg N $kg^{-1} h^{-1}$ for PL, MS,

257 and BS; Kruskal–Wallis test, $\chi^2 = 11.6$, P = 0.02) (Fig. 2c). The greatest POAR occurred at the

258 sites PS1 nearest the penguin nests (88.8 ± 2.7 μg N $kg^{-1} h^{-1}$) and SS1 close to seal wallows (138.8

259 ± 0.8 μg N $kg^{-1} h^{-1}$). The PAOR followed the distribution changes of AOB *amoA* gene abundances,

260 but showed the opposite trend to the AOA *amoA* gene abundances. A significant positive

261 correlation ($r^2 = 0.77$, P < 0.001) was observed between the PAOR and the AOB *amoA* gene

262 abundance when the data from all the tundra patches were combined, whereas no correlation

263 occurred between PAOR and AOA *amoA* gene abundance (Fig. 4). The AOB dominance over

264 AOA in the abundance in PS, SS and PL and their correlation with the PAOR suggested that AOB

265 populations might contribute more to the PAOR than the AOA populations in penguin or seal

266 colony. In addition, PAOR significantly negatively correlated with soil C:N ratios (r= -0.73,

267 P<0.001)(Fig. 3d), but significantly positively correlated with TS contents (r=0.47, P<0.05) and

268 TP contents (r=0.43, P<0.05) (Table 2).

269 **3.4. Community structure of AOA and AOB under sea animal colonization**

270 The PCR products were insufficient to construct the clone libraries for the AOA *amoA* gene

271 from SS and PS because of the low AOA abundance in the soils, as was the case with the AOB

272 *amoA* gene from MS and BS. Overall, 10 AOA and 14 AOB *amoA* gene clone libraries were

273 successfully constructed. The 543 AOA sequences and 1175 AOB quality sequences were

274 generated from the respective sites. Within each individual site, 1–6 AOA OTUs and 6–15 AOB

275 OTUs were identified, as defined by < 3% divergence in nucleotides. The AOA and AOB OTU

276 numbers for each library are presented in Table S1.These numbers might be higher if more clones

277 were sequenced, based on the rarefaction curves (Fig. S3 and Fig. S4). The diversity of the AOB

278 *amoA* was generally higher than that of AOA *amoA*, based on the indices of Shannon–Wiener and

279 Simpson. Specifically, the AOA *amoA* gene had higher diversity in PL and MS than in BS,

280 whereas the AOB *amoA* gene showed higher diversity in SS and PS compared with that in adjacent

281 animal-lacking tundra soils (Table S1).

282   The 543 AOA *amoA* gene sequences had 76–100% sequence similarity to each other, and 95–

283 100% identity with the corresponding top hit *amoA* sequences deposited in GenBank.

284 Phylogenetic tree showed that the AOA *amoA* sequences were grouped into 16 unique OTUs,

285 representing 100% of all the AOA *amoA* OTUs identified, and these sequences were affiliated

286 with two *Nitrososphaera* clusters (Fig. 5a): Cluster I had 11 OTUs and 264 clones, and 57.9% of

287 AOA *amoA* sequences were from PL, 41.3% from SS, and only 0.8% from MS. In Cluster II,

288 there are five unique OTUs and 279 clones, and 58.8% of them were from BS, 38.3% from MS,

289 and only 2.9% from PL. Almost all the AOA phylotypes retrieved from PL and SS were related

290 to *Nitrososphaera* cluster I, whereas the AOA phylotypes retrieved from MS and BS were

291 distributed in cluster II (Fig. S5a in Supplementary Material). Seal or penguin activities led to the

292 predominant existence of AOA phylotypes related to cluster I, but very low relative abundances

293 in AOA phylotypes related to cluster II, which were almost completely excluded in SS and PL.

294 Almost all AOA phylotypes in BS and MS were related to *Nitrososphaera* cluster II, whereas the

295 relative abundances of AOA phylotypes related to cluster I were very low or undetectable.

296   The 1175 AOB *amoA* gene sequences shared 87–100% sequence identity to each other, and

297 93–100% identity with the closest matched GenBank sequences. AOB phylogenetic tree showed

298 38 unique OTUs, representing 58.5% of all the AOB *amoA* OTUs identified, and they were

299 grouped into four *Nitrosospira* clusters according to the evolutionary distance of the phylogenetic

300 tree (Fig. 5b). Cluster I had 11 OTUs and 226 clones, and 67.7% of AOB *amoA* sequences were

301 from PS, 23.5 % from SS, 8.4% from PL, and only 0.4% from MS. There are 17 unique OTUs

302 and 521 clones in clusters II and III. The sources of the OTUs in cluster II were similar to those

303 of cluster I, with 69.8% from PS, 29.9% from SS, and 0.3% from PL. For cluster III, 79.2% of

304 the sequences were from PL, 19.8% from SS, and 1.0% from MS. Cluster IV had nine unique

305 OTUs and 370 clones from PL (50.0%), SS (36.8%) and MS (13.2%), respectively. Of all the

306 AOB phylotypes retrieved from PS were related to dominant *Nitrosospira* clusters I and II,

307 whereas AOB phylotypes related to cluster III and IV were completely excluded because of

308 penguin colonization (Fig. S5b). The AOB phylotypes retrieved from SS were distributed in

309 clusters I, II, III, and IV (16–38% for each cluster). Almost all the AOB phylotypes retrieved from

310 PL and MS were related to *Nitrosospira* clusters III and IV.

311 **3.5. Relationships of the ammonia-oxidizer community structure with environmental variables**

312 The relationships of the AOA and AOB communities with environmental variables were

313 analyzed using CCA. The environmental variables explained 62.1% of the total variance in the

314 AOA *amoA* genotype compositions, and 71.5% of the cumulative variance of the genotype-

315 environment relationships in the first two CCA dimensions (Fig. 6a). Overall, the AOA

316 community structures significantly correlated with C:N (F=2.59, P=0.022) and TC (F=2.07,

317 P=0.048) in tundra soils (Table 3), and the combination of the two factors explained 39.6% of the

318 variation. High soil C:N and TC concentrations increased the AOA richness in MS and BS.

319 Although other environmental parameters, including TP, pH, NH_4^+-N and NO_3^--N were not

320 statistically significant ($P > 0.05$), these variables additionally explained 47.3% of the variation..

321 As illustrated in Fig. 6b, the first two dimensions explained 26.6% of the total variance in the

322 AOB compositions, and 54.3% of the cumulative variance of the AOB genotype-environment

323 relationships. The composition and distribution of AOB communities correlated significantly with

324 C:N ratios (F=1.844, P=0.002) and NH_4^+-N (F=1.823, P=0.002), and the two factors combined

325 yielded 21.9% of total CCA explanatory power. The others including TP, NO_3^--N and pH

326 accounted for 27.1% of the variance. Penguin or seal activities significantly increased the AOB

327 richness in SS and PS through higher NH_4^+-N and P input from sea animal excrements, whereas

328 AOB richness was closely related to the soil C:N in PL and MS.

329 **4 Discussion**

330 **4.1. Effects of sea animal colonization on AOA and AOB abundances**

331 In this study, soil AOA *amoA* gene abundances were two orders of magnitude lower in PS and

332 SS relative to BS and MS; however, AOB *amoA* gene abundances were approximately 2–3 orders

333 of magnitude higher in PS and SS than in MS and BS, indicating that sea animal activities

334 increased the AOB population size, but decreased AOA abundances in tundra soils (Fig. 2 and

335 Fig. 3). Overall, the AOA *amoA* gene abundances obtained here were similar to the abundance

336 range reported in the soils of the Antarctic Dry Valleys and arctic tundra soils; however, the AOB

337 *amoA* gene abundances were two to three orders of magnitude higher in PS and SS than in

338 Antarctic Dry Valleys (Alves et al., 2013; Magalhães et al., 2014). In contrast to previous studies

339   indicating that AOA were more abundant than AOB in some terrestrial or marine ecosystems

340   (Beman et al., 2008; Lam et al., 2007; Wuchter et al., 2006; Yao et al., 2011), and in soils from

341   Antarctic Peninsula (Jung et al., 2011), our qPCR estimates showed that the AOB *amoA* copy

342   numbers were much greater than those of AOA *amoA* in PS, SS and PL because of sea animal

343   activities. However, their abundances were very close to each other in BS and MS. The ratios of

344   AOB to AOA abundance were strongly affected by sea animal activities, which were indicated by

345   soil C:N ratios (Fig. 2c). A shift in the relative abundance of AOA and AOB recorded previously

346   for the Antarctic Dry Valleys, with a greater abundance of AOB compared with that of AOA for

347   Battleship Promontory and Miers Valley, and the reverse for Upper Wright Valley and Beacon

348   Valley (Magalhães et al., 2014). The results for PS, SS, and PL are also in agreement with those

349   detected in subglacial soils (Boyd et al., 2011).

350   The ratios of AOB to AOA showed significant correlations with C:N, NH_4^+-N, and TP when

351   all the data were combined in the five tundra patches (Table 2). This suggested that C:N, NH_4^+-N,

352   and TP are key factors when AOB *amoA* genes are much more abundant than AOA *amoA* genes.

353   In Antarctica, the productivity of terrestrial ecosystems is strongly limited because of the

354   extremely low nitrogen levels (Park et al., 2007). However, the physiochemical properties for

355   tundra soils were strongly influenced by the deposition of penguin or seal excreta under effects of

356   local microbes (Tatur et al., 1997). Sea animals provide considerable external N inputs for their

357   colony soils and adjacent tundra soils through direct input of their excreta and atmospheric

358   deposition via ammonia volatilization (Lindeboom, 1984; Sun et al., 2002; Blackall et al., 2007;

359   Zhu et al., 2011; Riddick et al., 2012). Like ammonium, P is typical elements in penguin guano

360 (Sun et al., 2000). Generally low C:N ratios and significantly elevated NH_4^+–N and TP

361 concentrations occurred in PS and PL due to penguin or seal activities (Table 1). These conditions

362 might be more favorable for AOB, allowing high abundance of AOB *amoA*, which explains the

363 strong correlations between AOB abundances and C:N, NH_4^+–N, and TP in the sea animal colony

364 soils (Table 2). This agreed with the high bacterial abundance previously documented in penguin

365 or seal colony soils and ornithogenic sediments (Ma et al., 2013; Zhu et al., 2015).

366   The AOA abundance showed a significant negative correlation with NH_4^+-N levels in tundra

367 patches (Table 2), indicating that AOA might better adapt to low NH_4^+ and oligotrophic

368 environments (Martens-Habbena et al., 2009; Stieglmeier et al., 2014). High NH_4^+-N

369 concentrations might partially inhibit AOA populations (Hatzenpichler et al., 2008). This result is

370 similar to that reported for some agricultural soils with increased fertilization, and grassland soils

371 with increased grazing (Fan et al., 2011; Prosser and Nicol, 2012; Pan et al., 2018), supporting the

372 conclusion that AOA and AOB generally inhabit different niches in soil, distinguished by the

373 NH_4^+ concentration and availability (Verhamme et al., 2011; Wessén et al., 2011).

374 ### 4.2. Effects of sea animal colonization on soil ammonia oxidation rates

375   The PAOR ranged from 9 to 139 $\mu g \ N \ kg^{-1} \ h^{-1}$, lower than nitrification rates measured in most

376 agricultural soils (83–1875 $\mu g \ N \ Kg^{-1} \ h^{-1}$) (Fan et al., 2011; Ouyang et al., 2016; Daebeler et al.,

377 2017). One reason might be the selection of a 15 °C incubation temperature, which was lower

378 than the incubation temperatures used in other studies. Generally, the gross nitrification rate and

379 *amoA* abundance increased significantly when the incubation temperature was higher than 15 °C

380 (Daebeler et al., 2017; Zhao et al., 2014). Our measurements indicated that there were significant

381 differences (P = 0.02) in the PAOR across different tundra patches, and the PAORs in SS and PS

382 were about 10 times higher than those in BS and MS. A significant correlation was observed

383 between the PAOR and C:N, TP, and TS (Table 2). Overall, ammonia oxidation activity was

384 modulated by soil biogeochemical processes under the disturbance of penguin or seal activities:

385 generally low C:N ratios, and sufficient input of the nutrients TP, TS, and NH_4^+–N from sea animal

386 excrements.

387   The AOB dominance over AOA in the abundance (Fig. 2b) and significant negative

388 correlation of AOA abundance with NH_4^+–N levels (Table 2), indicated that AOB might play a

389 more important role in nitrification in tundra soils. In agreement with these results, AOB

390 dominated nitrification in the areas where it was easy to achieve nitrogen input, whereas the

391 relative contribution of AOA to nitrification was higher in the areas where the ammonium

392 concentration remained low (Fan et al., 2011; Sterngren et al., 2015). Moreover, the cell-specific

393 activity for AOB was 10 times higher than that for AOA due to the bigger cell size of AOB

394 (Hatzenpichler et al., 2012; Prosser and Nicol, 2012). Therefore, AOB might play a more

395 important role in nitrification in SS, PS, and PL with the input of NH_4^+–N from penguin or seal

396 excrements.

397   In addition, AOA might play a role that cannot be ignored in MS and BS, just like the

398 prevalence of AOA among ammonia-oxidizers in Arctic soils (Alves et al., 2013; Daebeler et al.,

399 2017). AOB groups were mostly undetectable in the analysis of MS and BS. Although unknown

400 γ-AOB groups might not have been detected, the primer set used here covers the β-AOB groups

401 typically found in soils (Alves et al., 2013). The BS and MS were moderately far away from

penguin or seal colonies without the input of the nutrients from sea animal excrements, and their

substrates can be provided only through the mineralization of organic matter from local tundra

plants. The simple organic substrates and barren soil environment might favor AOA (Stopnišeket

al., 2010; Habteselassie et al., 2013). Therefore AOA showed relatively high abundance in MS

and BS compared with PS and SS.

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

In this study, distinct AOA communities appear to inhabit different types of tundra patches,

depending on sea animal activities (Fig. 5a). It was difficult to amplify the AOA *amoA* gene from

SS and PS, whereas a high diversity of AOA *amoA* genes was observed in PL, MS and BS.

Phylogenetic analysis indicated that the AOA *amoA* sequences in Cluster I were from PL and

tundra soils close to seal wallows, while the sequences in Cluster II were from BS and MS (Fig.

S5). AOA in most extreme environments have lower levels of microbial diversity than benign

ecosystems because of the requirement for specific physiological adaptations, which allow

organisms to exploit the combination of physical and biochemical stressors (Cowan et al., 2015).

Detected OTUs in Cluster I had their closest matches mainly from the hyper-arid soils of Antarctic

dry valleys (Magalhães et al., 2014), wetland soils (Zheng et al., 2014), alpine meadow soils (Zhao

et al., 2017), and some agricultural soils (Glaser et al., 2010). Cluster II were more prevalent in

BS and MS, probably because of their stronger adaptation to barren soil environments. In cluster

II, the sequences were affiliated with sequences recovered from cold environments, including the

soils of Tibetan Plateau (Xie et al., 2014) and Icelandic grassland soils (Daebeler et al., 2012).

The compositions of soil AOA populations are likely not to be explained by single

423 physicochemical properties, and their community structures significantly correlated with tundra

424 soil C:N, and TC, which was consistent with previous studies (Glaser et al., 2010; Wessén et al.,

425 2011).

426   The AOB *amoA* gene generally had a higher diversity than AOA, similar to results in the

427 Antarctic Dry Valley soils (Magalhães et al., 2014). A high diversity of AOB *amoA* gene occurred

428 in SS, PS and PL compared to BS, indicating that penguin or seal activities had important effects

429 on AOB genotypic diversity. Phylogenetic analysis indicated that the sequences in clusters I and

430 II were mainly from PS and SS (Fig. 5b), and the detected OTUs in Cluster I had their closest

431 matches from mixed community culture systems, meadow to forest transect in Oregon Cascade

432 Mountains (Mintie et al., 2003), and Dutch agricultural soils (Silva et al., 2012a) and reservoir

433 sediments (Silva et al., 2012b). For Clusters III and IV, the sequences were predominantly from

434 PL and SS, and they were affiliated with sequences recovered from high altitude wetland (Yang

435 et al., 2014). Previous studies have shown that multiple environmental factors affected the AOB

436 communities (Dang et al., 2008; Mosier and Francis, 2008). In this study, the C:N ratios and NH_4^+-

437 N concentrations seemed to be the most important factors influencing the AOB community

438 structure, which was in accordance with the results from different environments (Bouskill et al.,

439 2012; Jung et al., 2011; Li et al., 2015). Moreover, the TP also affected the AOB *amoA* community

440 compositions (Zheng et al., 2013). Therefore, the AOB community compositions were impacted

441 by the biogeochemical factors related to sea animal activities, such as low C:N ratios, and

442 sufficient supply of the nutrients NH_4^+–N and TP from sea animal excreta.

5 Conclusions

The findings of this study concerning the abundance, 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. The diversity of the AOB *amoA* gene was greater 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.

Acknowledgments

This work was supported by the NSFC (Grant No. 41576181; 41776190). We are particularly grateful to the members of Chinese Antarctic Research Expedition and Polar Office of National

463   Ocean Bureau of China for their support and timely help. The final derived data presented in this

464   study are available at https://doi.org/10.5281/zenodo.1260292.

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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±2.7)×10^{4a}	(4.0±1.4)×10^{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±0.7)×10^{4a}	(4.8±0.7)×10^{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±2.6)×10^{5b}	(6.9±0.2)×10^{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}	$(2.1\pm0.6)\times10^{6\ b}$	$(5.9\pm3.5)\times10^{6\ c}$
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}	$(9.3\pm2.7)\times10^{6\ b}$	$(1.2\pm0.5)\times10^{7\ c}$

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 ($\mu gN\ Kg^{-1}h^{-1}$)	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%
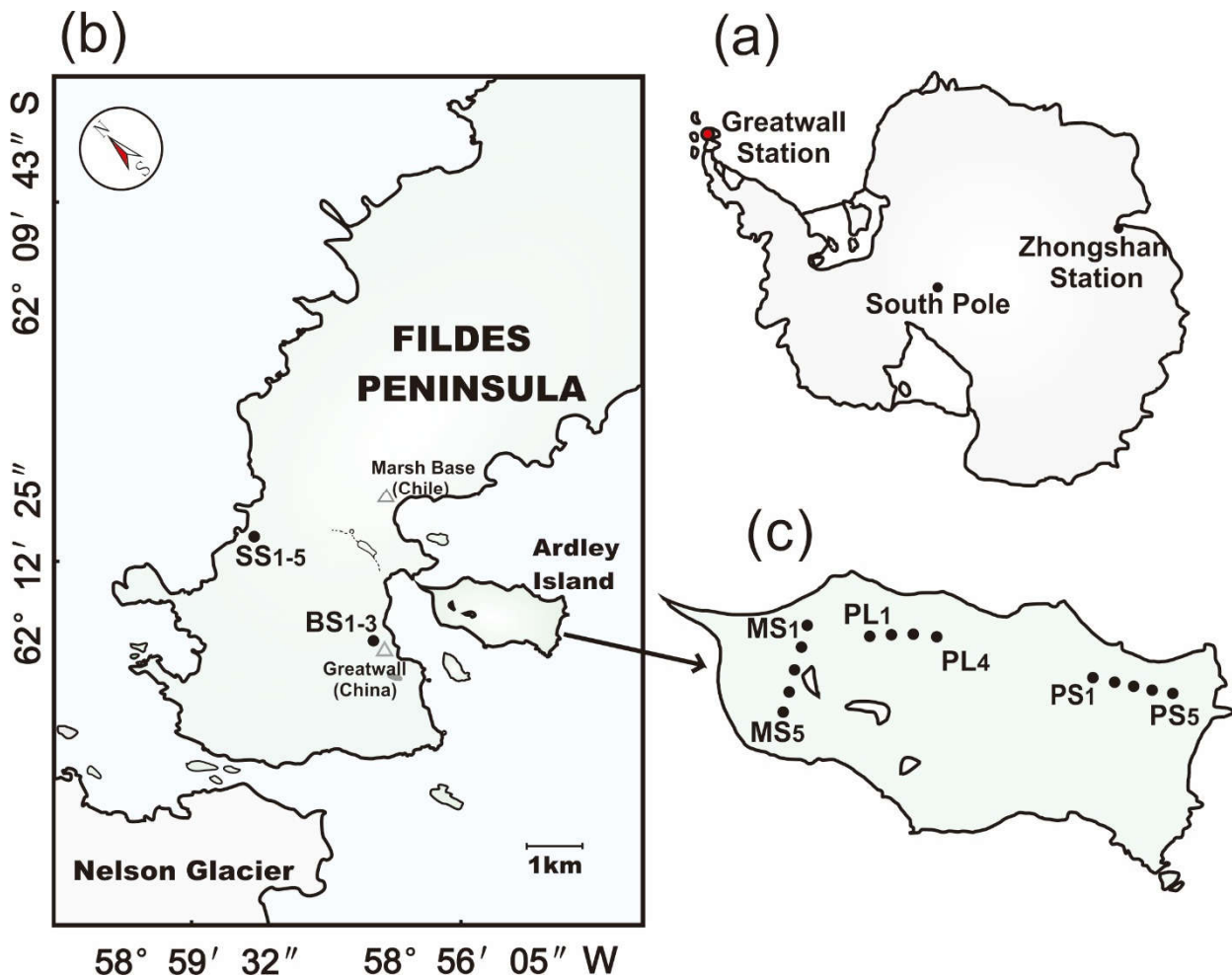
Figure 1. Study area and soil sampling sites. Panel (a), the red dot indicates the location of the investigation area in maritime Antarctica. Panel (b), location of the sampling sites on the Fildes Peninsula. The sampling soils from tundra patches included the active seal colony tundra soils SS (SS1–5) 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/).
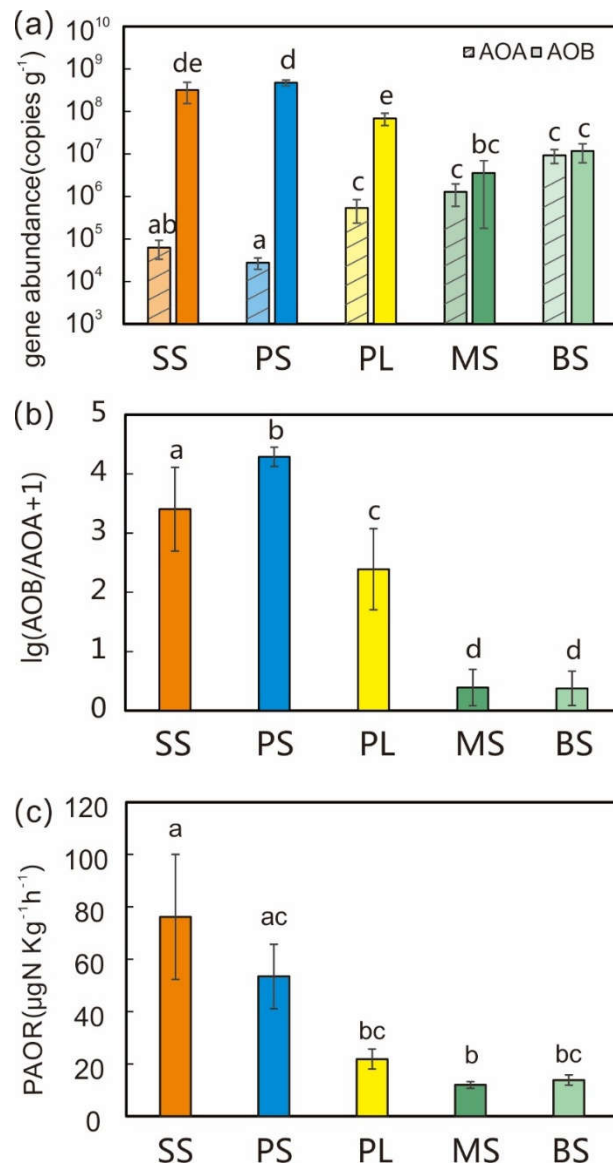
Figure 2. Comparisons of soil AOA and AOB *amoA* gene copy numbers (a), log ratio of AOB: AOA abundances (b), and potential ammonia oxidation rates (PAOR) (c) between five tundra patches. The error bars indicate standard deviations of the means.
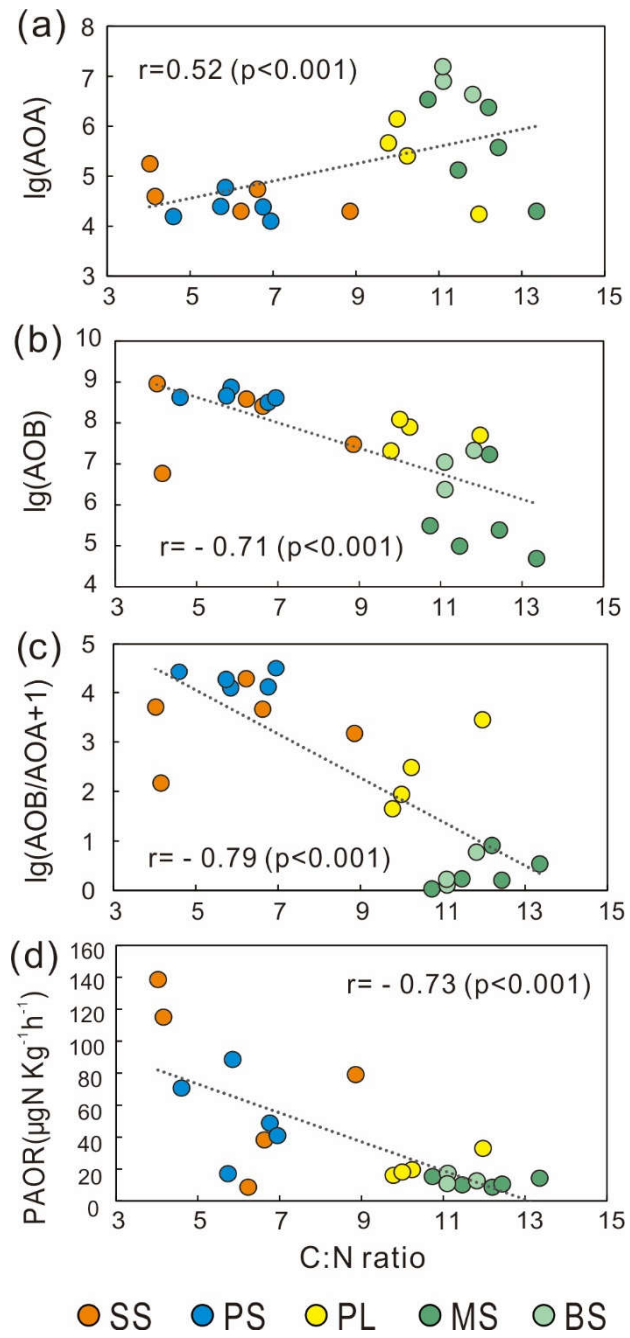
Figure 3. Effects of soil C:N alteration on AOA and AOB abundances, and potential ammonia oxidation rates (PAOR) at five tundra patches.
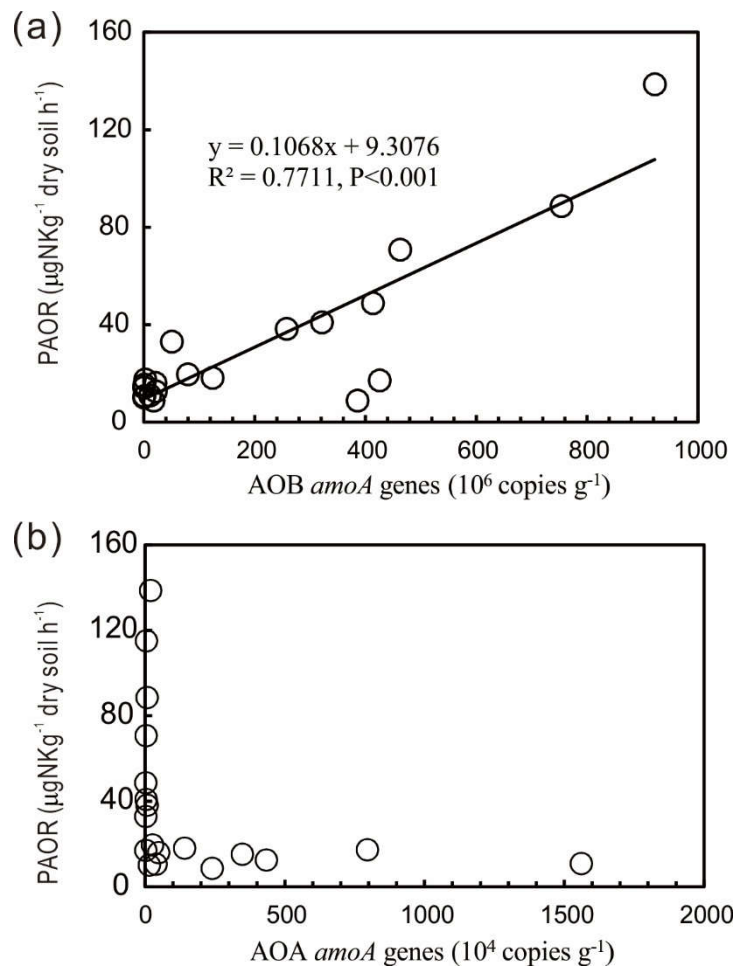
Figure 4. Correlation between potential ammonia oxidation rates (POARs) and AOA and AOB *amoA* gene copy numbers in tundra soils of maritime Antarctica.

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.
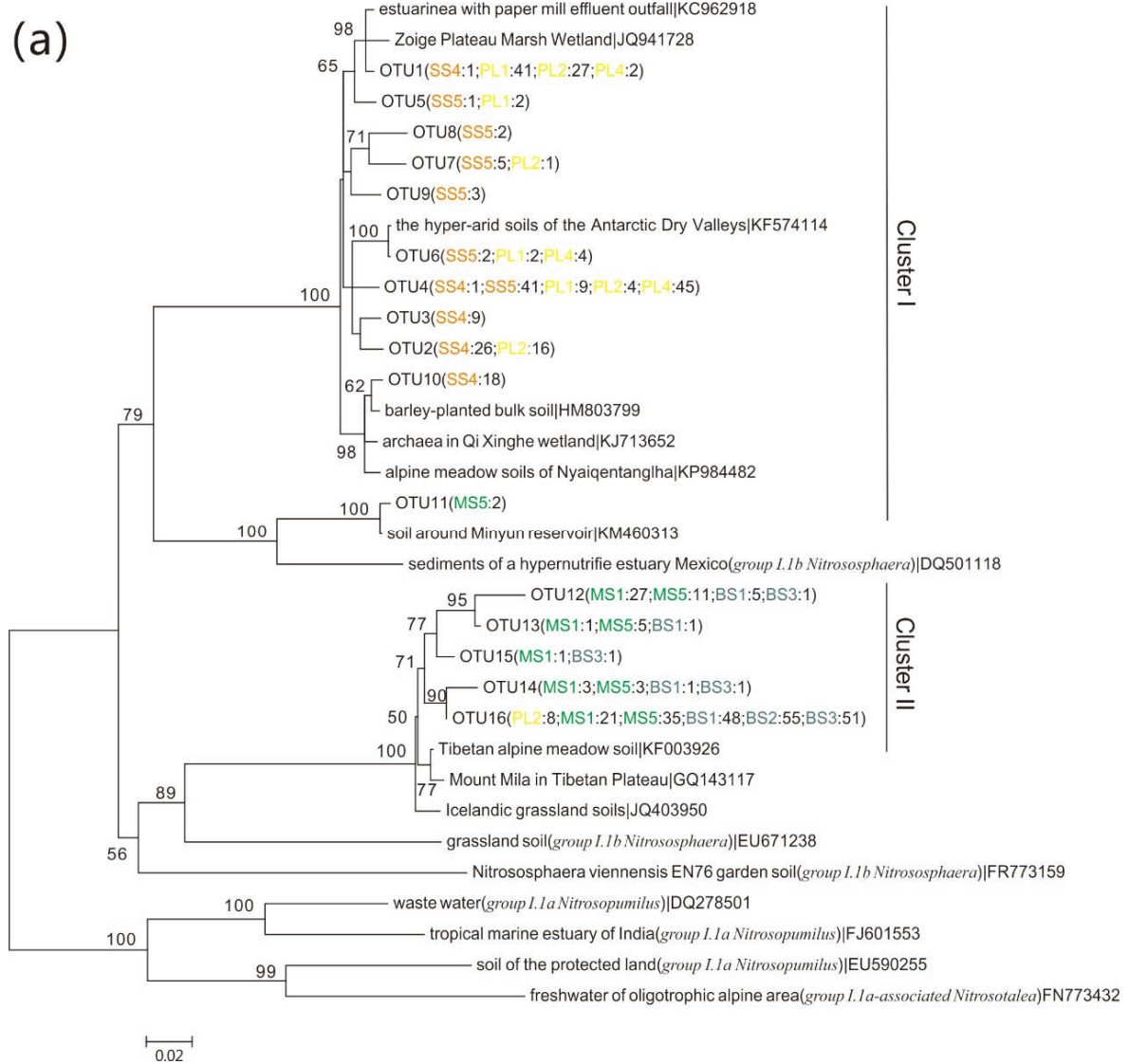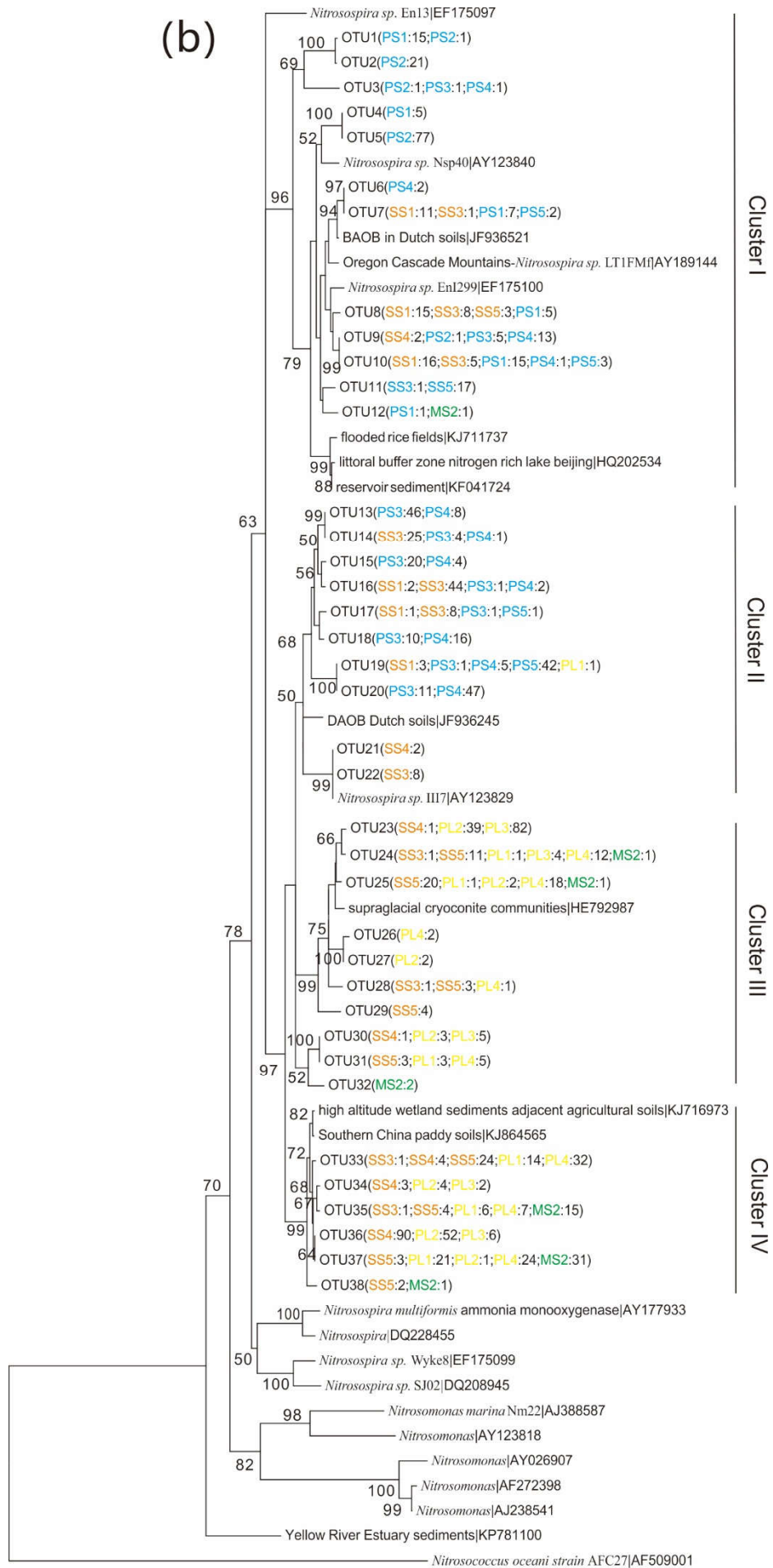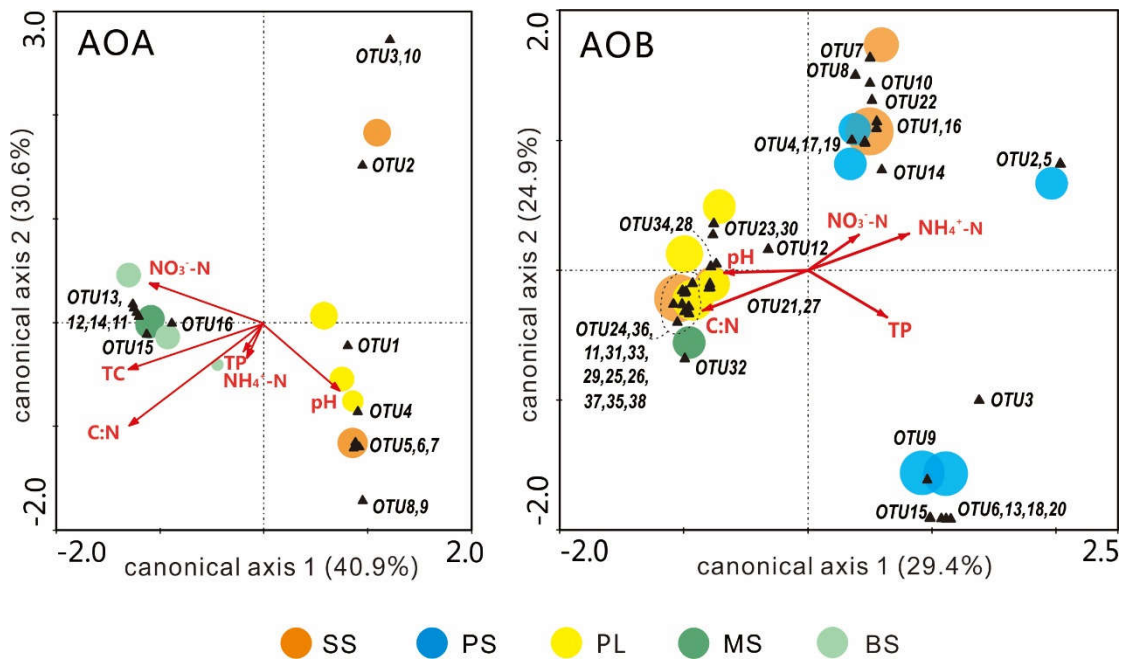
Figure 6. Canonical correspondence analysis (CCA) ordination plots for the relationship between the AOA and AOB community structures with environmental variables. The circles with different colors represent the various sampling sites. The size of the circles corresponds to the OTU richness in individual 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.