Response to Associate Editor

Dear Dr. Denise M. Akob,

We are very grateful to you for your valuable comments about the revision of this manuscript (MS No.bg-2019-114). We have revised the manuscript carefully according to your comments, and provided a version of track changes in the re-submitted files.

The detailed responses inserted into associate editor's comments are attached as follows:

1. In the response to reviewers you provide a table that compares your soil physiococchemical parameters (e.g., TOC, TN) to previous work by your group. The earlier studies have different samples names. Can you make the naming consistent over the different papers so that the readers can compare?

Author response: In the response to reviewers, we re-measured soil TC and TN concentrations based on the same soil samples collected in 2015, and provided a table that compared the measurement results, which are similar to those in this paper. In our previous papers, the soil samples were collected by different people in different years, thus it is very difficult to make the naming consistent over the different papers although these soils were collected in the same study area. However, **the readers can still clearly compare the concentration differences of soil physiochemical parameters in different tundra areas according to our previous published papers and this paper.**

2. Define OTU at first usage

Author response: OUT has been defined as "operational taxonomic unit" on **line 182** in the revised manuscript with track changes.

3. mothur is actually lower case and this reference to the program needs to be included: Schloss PD, Westcott S, Ryabin T, Hall J, Hartmann M, Hollister E, Lesniewski R, Oakley B, Parks D, Robinson C, Sahl J, Stres B, Thallinger G, Van Horn D, Weber C. 2009. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75(23):7537-7541, doi: http://dx.doi.org/10.1128/AEM. 01541-09.

Author response: We have revised the error of initial, and added the reference. Please

see line 183, 201 in the revised manuscript.

4. Please include the reference for BLAST (Madden T. 2002. The BLAST Sequence Analysis Tool. In McEntyre J, Ostell J (ed), The NCBI Handbook. National Center for Biotechnology Information, Bethesda, Maryland USA.)

Author response: The reference for BLAST has been added. Please see line 184 in revised manuscript.

5. Is Figure 6 really needed for the main paper? I feel like it could move to supplemental information. Up to you. Or is there a way to combine the information in Figure 5 and 6?

Author response: Considering that some of the information provided in Figure 6 has been shown in Figure 5, therefore Figure 6 has been moved to Supplementary Material in the revised manuscript.

6. Other minor revisions

Author response: The incorrect words and phrases have been corrected in the revised manuscript.

Response to Referee #1

At first, we would like to express our appreciations to you for your kind help and valuable comments about the revision of this manuscript (MS No.bg-2019-114). We have considered your valuable suggestions and carefully revised this manuscript.

The detailed responses inserted into reviewer #1 comments are attached as follows:

Anonymous Referee #1

Received and published: 19 April 2019

The manuscript "Effects of Sea Animal Colonization on the Coupling between Dynamics and Activity of Soil Ammonia-oxidizing Bacteria and Archaea in Maritime Antarctica" investigated the abundance, diversity, community structure and bioactivity of ammonia oxidising bacteria and archaea in Antarctic maritime soils colonized by sea animals. The results found that soils colonized by seals and peguines exhibited higher AOB than AOA, as well as higher ammonia oxidizing rates than the control tundra soils. These findings suggest that AOB may play a more important role than AOA in driving ammonia oxidizing in penguine and seal colonized soils, while AOA more important in control tundra soils. The research provided very interesting findings, which contributes to understand the nitrogen cycling in Antarctic coastal soils. Here are my concerns:

Author response: Thanks for your positive comments and valuable suggestions.

1. Too many abbreviations for samples and sites, authors got PS, PL, MS, SS and BS for sample IDs and PTS, PLS, MS, STS and BS for sites. This is just too confusing to read.

Author response: Thanks for your good suggestions. In the revised manuscript, we have used SS, PS, PL, MS, and BS for the samples and sites consistently to escape the ambiguity.

2. Line 25, Nitrosospira is an AOB, Nitrososphaera is an AOA, need to change their order in the sentence.

Author response: The order has been changed in the sentence. Please see **line 26-27** in revised manuscript with track changes.

3. Line 41, Comammox (COMplete AMMonia OXidiser) is an abbreviation, please provide its full name. 4. line 40-41, "Only recently…", this sentence seems to be out of

picture, I would suggest to remove it.

Author response: Thanks for your good suggestions. This sentence and comammox has been removed in the revised manuscript.

5. Line 56, "However, there has been limited…", I don't think this sentence is correct, especially after the authors listed so many studies on ammonia-oxidisers in line 52-56.

Author response: This sentences has been removed in the revised manuscript.

6. Line 210 "mean pH range of 5.3-6.6", The word mean and range seems contradict to each other, I guess the word "mean" here represents the mean of each sampling site. This is better to be clarified.

Author response: This has been corrected into "Almost all the tundra soils were slightly acidic, and the mean pH ranged from 5.3 to 6.6 at each tundra patch". Please see **line 220-221** in revised manuscript.

7. Line 211, "Penguin and seal colony tundra soils, PTS and STS, had lower TOC…" Firstly I couldn't find the C:N ratio in Table 1;

Author response: The data about C:N ratios have been added in **Table 1**.

secondly the table 1 used id SS, PS, PL etc, but main text used PTS, STS etc, therefore lacking consistency,

Author response: In the revised manuscript, we have used id SS, PS, PL, MS and BS in **both Table 1 and the main text** for their consistency.

lastly, the TOC level of PS (PTS) site was not significantly different from the PLS, MS and BS sites. I think the lack of significance was due to large variations?

Author response: Yes. The lack of significance might be due to large variations of TC contents caused by high soil heterogeneity in each tundra patch. Generally, penguin or seal colonies and the active areas are devoid of vegetation due to toxic overmanuring and their trampling. Penguin and seal colony tundra soils, PS and SS, had lower TC contents and C:N ratios than the animal-lacking tundra soils (PL), tundra marsh soils (MS), and background tundra soils (BS).

8. Line 213, "as expected, soil nutrient levels…", why is this expected? I could understand that TN may be higher with penguin guano and seal faeces input, but why

TP and TS? Furthermore, there was no significant TN difference in BS with SS, PS and PL, similarly for TS, TP, and even ammonia. This greatly reduces the reliability of authors' claim.

Author response: (1) According to food chains, krill, as main food for penguins, is rich in N, P and S, whereas penguin is one of main foods for seals. The N, P, and S are highly enriched in penguin guano, and they are typical elements for penguin guano (**Sun et al., 2000; Sun et al., 2004; Zhu et al., 2013; Zhu et al., 2014**). Therefore soil nutrients N, P and S are higher in penguin or seal colony soils due to the deposition of penguin guano or seal excrements in maritime Antarctica; **(2)** Generally, penguin or seal colonies and the active areas are devoid of vegetation due to toxic overmanuring and trampling (**Tatur et al., 1997; Sun et al., 2004**), whereas the animal-lacking tundra areas adjacent to penguin or seal colonies, with moderate amount of nutrients, is favorable for vegetation, such as mosses and algae, due to the volatilization and deposition of ammonia and sulfur-containing compounds from penguin guano or seal excreta. **The growth and nitrogen fixation of the vegetation, and the volatilization and deposition of ammonia and sulfur-containing compounds** increased soil TC, TN and TS contents in animal-lacking tundra soils (**Zhu et al., 2011; Zhu et al., 2013**). In penguin or seal colonies, the penguin or seal populations showed high inhomogeneous distribution, and this led to the large differences in soil TC, TN, TS, NH_4^+-N contents. **Therefore, overall mean TC, TN and TS contents showed no significant differences between SS, PS, PL and BS (Table 1).** We have revised the corresponding part in the manuscript, please see the **line 221-229**.

The related references are as follows:

Tatur, A., Myrcha, A., and Niegodzisz, J.: Formation of abandoned penguin rookery ecosystems in the maritime Antarctic, Polar Biology, 17, 405–417, https://doi.org/10.1007/s003000050135, 1997.

Sun, L. G., Xie, Z. Q., and Zhao, J. L.: Palaeoecology: A 3,000-year record of penguin populations, Nature, 407, 858, https://doi.org/10.1038/35038163, 2000.

Sun, L. G., Liu, X. D., Yin, X. B., Zhu, R. B., Xie, Z. Q., and Wang, Y. H.: A 1,500-year record of Antarctic seal populations in response to climate change, Polar Biology, 27, 495–501, https://doi.org/10.1007/s00300-004-0608-2, 2004.

Zhu, R. B., Liu, Y. S., Xu, H., Ma, D. W., and Jiang, S.: Marine animals significantly increase tundra N_2O and CH_4 emissions in maritime Antarctica, Journal of Geophysical Research: Biogeosciences, 118(4), 1773–1792, https://doi.org/10.1002/2013JG002398, 2013.

Zhu, R. B., Sun, J. J., Liu, Y. S., Gong, Z. J., and Sun, L. G.: Potential ammonia emissions from

penguin guano, ornithogenic soils and seal colony soils in coastal Antarctica: effects of freezing-thawing cycles and selected environmental variables, Antarctic Science, 23(1), 78–92, https://doi.org/10.1017/S0954102010000623, 2011.

Zhu, R. B., Wang, Q., Ding, W., Wang, C., Hou, L. J., and Ma, D. W.: Penguins significantly increased phosphine formation and phosphorus contribution in maritime Antarctic soils, Scientific Reports, 4, 7055, https://doi.org/10.1038/srep07055, 2014.

After a close inspection on the numbers provided in the table 1, it seems that the large ammonia in SS and PS was due to a single sample in each site, I don't know how far SS1 and SS2 are to generate such large differences. Furthermore, this may not make much sense, the SS1 has ammonia concentration of 650 mg/kg, the highest among all other SS samples and 35 times higher than SS2, but its total nitrogen was only 1.2%, even 0.4% lower than SS2. Similar unusual pattern was also in the ammonia concentration in PS2 sample I would strongly suggest the authors to recheck their measurements. As these environmental factors are the basis of many statistical analysis performed later, this would completely make authors conclusion invalid.

Author response: We have rechecked the measurement results, and confirm that our data are right and valid. The reasons are as follows:

(1) We measured soil TC and TN concentrations again, **which are provided in the following Table**, and the results are similar to those in this study, and their concentrations still showed large differences at the each sites within penguin or seal colony; **(2)** We have measured soil physiochemical properties several times which were given in our previous published papers:

Zhu RB, Liu YS, Xu H, Ma DW, Jiang S. Marine animals significantly increase tundra N_2O and CH_4 emissions in maritime Antarctica. Journal of Geophysical Research: Biogeosciences, 2013, 118: 1773–1792, doi:10.1002/2013JG002398.

Zhu RB, Liu YS, Ma ED, Sun JJ, Xu H, Sun LG. Nutrient compositions and potential greenhouse gas production in penguin guano, ornithogenic soils and seal colony soils in coastal Antarctica. Antarctic Science, 2009, doi:10.1017/S0954102009990204.

Soil chemical properties, especially NH_4^+-N, NO_3^--N, P and S concentrations, also showed large differences due to effects of penguin or seal activities according to the two papers above.

Therefore we think that TC, TN, TS, TP, NH_4^+-N and NO_3^--N levels showed high heterogeneity in penguin or seal colony tundra soils, PS and SS **due to the deposition**

of penguin or seal excreta, and the differences of tundra vegetation and soil texture caused by animal tramp.

	Original No.	No. in the paper	Detection in 2015			Re-detection in 2019		
			N(mg/g)	C(mg/g)	C/N	N(mg/g)	C(mg/g)	C/N
Seal colony soils in western coast on Fildes Peninsula	SK1	SS1	12.12	48.67	4.02	9.99	54.52	5.51
	SK4	SS2	16.94	70.06	4.13	13.38	81.81	6.15
	SK6	SS3	0.87	5.56	6.37	1.51	10.34	6.85
	SK7		2.40	13.64	5.69	The sample is used up.		
	SK8	SS4	1.28	8.59	6.71	The sample is used up.		
	SK9		2.63	18.88	7.19	2.51	18.98	7.56
	SK10	SS5	1.30	11.54	8.87	**The sample is used up, the same as below**		
Penguin colony soils on Ardley Island	E1		10.54	50.58	4.8	8.68	55.83	6.43
	E2	PS1	14.55	84.65	5.82			
	E3		7.73	51.64	6.68	7.92	55.84	7.05
	E4	PS2	8.34	38.08	4.56			
	E5		15.07	89.71	5.95	13.48	92.33	6.85
	E6	PS3	17.90	120.76	6.75			
	E7		27.33	156.78	5.74	26.34	162.93	6.19
	E8	PS4	15.45	107.47	6.96			
	E9		9.99	73.10	7.31	8.87	79.72	8.99
	E10	PS5	7.97	45.82	**5.75**			
The middle tundra soils on Ardley Island	M1	PL1	11.53	117.64	10.2	9.88	124.91	12.64
	M2		13.61	138.41	10.17			
	M3	PL2	3.93	38.05	9.68	4.51	50.41	11.18
	M4		8.09	82.40	10.18			
	M5	PL3	25.30	302.52	11.96	23.94	301.93	12.61
	M6		20.19	222.45	11.02			
	M7	PL4	7.17	71.85	10.02	6.37	74.82	11.75
	M8		9.84	114.99	11.69			
	M9		11.47	110.65	9.65			
	M10		15.84	177.48	11.21	15.69	190.83	12.16
	M11		11.61	119.29	10.27			
	M12		4.34	44.40	10.23			
	M13		9.65	116.36	12.05			

	M14		3.33	30.13	9.04	2.77	30.49	11.01
	M15		12.95	147.59	11.39			
The tundra marsh soils in west of Ardley Island (almost no animals)	W1	MS1	8.93	95.54	10.7	9.65	111.82	11.59
	W2		11.92	148.81	12.49			
	W3	MS2	15.89	193.95	12.2	14.35	191.57	13.35
	W4		17.83	217.76	12.21			
	W5		12.93	141.64	10.95	10.79	136.73	12.67
	W6	MS3	19.79	226.90	11.46			
	W7		10.81	122.84	11.37	9.37	122.43	13.07
	W8	MS4	26.57	355.02	13.36			
	W9		21.88	254.01	11.61	20.87	257.11	12.32
	W10	MS5	23.51	292.00	12.42			
	adw-A		20.67	260.05	12.58	19.98	265.81	13.30
	adw-B		14.74	188.68	12.8			
	adw-C		17.29	235.79	13.63	17.76	252.1	14.19
The background tundra soils On Fildes Peninsula	GW1	BS1	4.76	56.72	11.91	4.81	56.89	11.83
	GW2	BS2	5.05	56.63	11.21	5.2	63	12.12
	GW3	BS3	4.30	47.69	11.09			
	gwc1		3.29	31.78	9.66	3.1	35.4	11.42
	gwc2		3.09	29.65	9.6			
	gwc3		2.41	24.03	9.96	2.5	28.3	11.32
	gwc4		2.37	24.39	10.29			

9. Line 219, "likewise, soil…", which site is author referring here? PTS or PLS? Or stating a generally pattern from PTS, PLS to MS? Please clarify. As PTS is clearly not showing this pattern.

Author response: This only stated a general pattern from PS, PL sites to MS sites. Considering that PS sites do not show this pattern due to large spatial variations, this sentence was removed in the revised manuscript. The related description about soil chemical properties has been reorganized as follows:

PS and SS had generally lower C:N ratios than the penguin-lacking tundra soils (PL), tundra marsh soils (MS), and background tundra soils (BS). Soil mean TN, TS and NH_4^+–N levels were higher in PS, SS, PL, and MS than in BS. Soil NH_4^+–N contents were 1–2 orders of magnitude higher in PS and SS than in PL, MS, and BS, with the means of 176.9 and 137.6 mg NH_4^+-N kg^{-1}, respectively. The highest NO_3^--N contents occurred in SS. Phosphorus levels were significantly greater ($p < 0.05$) in PS (10.6–32.9 mg g^{-1}) than in other types of tundra soils (mean < 6.0 mg g^{-1}). Overall,

penguin or seal activities altered the local soil biogeochemical properties through the deposition of their excreta, leading to generally low C:N ratios. Please see the **line 226-236** in the revised manuscript.

10. Line 222, "therefore, the soil TP and NH4…" this is a very bold statement, and lacking proof. Something like linear regression would be required.

Author response: It is difficult to quantify **animal activity intensity,** therefore we do not use the phrase **"animal activity intensity"** to avoid ambiguity in the revised manuscript. This statement has been corrected as follows: **Overall, penguin or seal activities altered the local soil biogeochemical properties through the deposition of their excreta, leading to generally low C:N ratios in tundra soils.** Please see the **line 234-236** in the revised manuscript.

11. Line 229, (fig. 2), figure 2 has 3 parts (a, b and c), please specify which part of the figure 2 is referred to.

Author response: It is Fig. 2**a**. This has been added in the revised manuscript (**line 247**).

12. Line 229, "overall…" please provide a scatter plot to visualise this (can be put in supplementary)

Author response: A scatter plot (**Fig. S2**) has been provided to visualize this in Supplementary Material.

13. Line 231, "the archaeal amoA gene showed a heterogeneous distribution" what does heterogeneous distribution mean?

Author response: It means that the AOA amoA gene showed a heterogeneous distribution **in the abundance** among the different tundra patches. i.e. The AOA amoA gene abundances were two orders of magnitude lower in PS and SS relative to those in BS and MS. The maximal AOA amoA gene abundance appeared in BS, followed by MS and PL, whereas the PS and SS soils had the lowest AOA amoA gene abundances. This sentence has been reorganized in the revised manuscript (**lines 249-253**).

14. Line 232, there was a mixed usage of AOA amoA and archaeal amoA in the manuscript, please make them consistent.

Author response: For consistency, we have used AOA *amoA* instead of archaeal

amoA in the revised manuscript.

15. Line 237, "fig 3", similar to a previous comment, there are 6 parts of figure 3, please specify which part does this refer to.

Author response: This figure related to sea animal activity intensity has been removed in the revised manuscript. We added **Fig. 3**: Effects of soil C:N alteration on AOA and AOB abundances, and potential ammonia oxidation rates (PAOR) at five tundra patches, to show effects of sea animal activities on AOA and AOB abundances and PAOR.

16. Line 235, "Soil AOA amoA gene abundances were significant…" This statement is inappropriate, I would agree that animal activity reduces archaeal amoA gene abundance, but the statement of increasing archaeal gene abundance with reduced animal activity need a better proof. A correlation analysis between the activity intensity index and archaeal amoA gene abundance would be required.

Author response: Thanks for your good suggestion. This figure related to sea animal activity intensity has been removed in the revised manuscript, and the corresponding statement of increasing archaeal gene abundance with reduced animal activity has also been deleted. We added **Fig. 3**: Effects of soil C:N alteration on AOA and AOB abundances, and potential ammonia oxidation rates (PAOR) at five tundra patches, to show effects of sea animal activities on AOA and AOB abundances and PAOR. The related statements are reorganized as follows:

The log values of soil AOA *amoA* gene abundance showed a significant positive correlation ($r=0.52$, $p<0.001$) with C:N ratio (Fig. 3a), but a significant negative correlation with NH_4^+-N contents ($r= -0.52$, $P = 0.013$) (Table 2). 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. Please see **line 253-256, 270-274** in the revised manuscript.

17. Line 240, "The soil AOB amoA gene abundances increased…" this is incorrect, author stated that the order of sampling reflected the intensity of seal activity (highest in SS1 and lowest in SS5) (line 123-127), but clearly the abundance of bacterial AOB gene reduced with reduced penguin or seal activity.

Author response: The related statements about animal activity intensity has been deleted. The related statements are reorganized as follows: **The log values of soil AOB**

amoA gene abundances showed a significant negative correlation with C:N ratio (r=-0.71, P < 0.001) (Fig. 3b), but 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 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. Please see line 261-273 in the revised manuscript.

18. line 242 "The ratios of AOB to AOA amoA…" please cite figure 2c for this sentence.

Author response: Fig. 2b has been cited for this sentence (line 267).

19. line 250, " The PAOR was significantly higher in STS…", this is not fully correct, the PAOR of PS samples was not significantly different from the BS site.

Author response: This sentence has been reorganized as follows: 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 kg^{-1} h^{-1} for SS and PS) than in non-animal colony soils (15.7 μg N kg^{-1} h^{-1} for PL, MS, and BS; Kruskal–Wallis test, $\chi^2 = 11.6$, P = 0.02). Please see line 277-281 in the revised manuscript.

20. Figure 3, again, archaeal results appeared before bacterial results, thus their figure should appear before bacterial figures.

Author response: The figures for AOA results have been moved before the figures for AOB in the revised manuscript.

21. Line 258, "Interestingly, the PAOR…" Please confirm this statement with a statistical analysis, as PAOR increased from SS3 to SS5.

Author response: This statement has been corrected and reorganized as follows: PAOR significantly negatively correlated with soil C:N ratios (r=0.73, P<0.001)(Fig. 3d), but significantly positively correlated with TS contents (r=0.47, P<0.05) and TP

contents (r=0.43, P<0.05) (Table 2). Please see **line 290-293** in the revised manuscript.

22. Line 271, "Specifically, the AOA amoA gene…" please present these results as a table or a figure.

Author response: These results have been provided in Table S1.

23. Line 276 "Phylogenetic analysis showed that the AOA…" Why and how phylogenetic analysis was used to group sequences into OTUs? In addition, the entire sentence is confusing, please revise.

Author response: (1) The sequences with 97% identity were grouped into one OTU using the Mothur Program by the furthest neighbor approach (Zheng et al., 2014); **(2)** The entire sentence has been reorganized as: **Phylogenetic tree 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).** Please see **line 311-313** in the revised manuscript.

24. Line 289, "Phylogenetic analysis showed that AOB amoA…"Why and how phylogenetic analysis was used to group sequences into OTUs? In addition, the entire sentence is confusing, please revise.

Author response: (1) Phylogenetic analysis was used to find the evolutionary ties between species. 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 using the Mothur Program by the furthest neighbor approach. AOB *amoA* sequences were grouped into 65 unique OTUs in total, but the OTUs containing only one sequence were not displayed in the AOB phylogenetic tree. **(2)** The entire sentence has been revised as follows: **AOB phylogenetic tree showed 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).** Please see **line 345-347** in the revised manuscript.

25. Line 312, "The AOA richness and phylotypes were evidently inhibited: : :" what does this mean? The richness of AOA is indeed lower in STS and PLS, but this result has already been presented in line 269.

Author response: It means the AOA richness was lower in SS and PL because of

seal or penguin activities. This results has been presented on line 304-308, **therefore here this sentences was deleted in the revised manuscript.**

26. Line 323 why RDA was used to investigate the correlation among amoA gene abundance, diversity and etc? I would think RDA is used to deal with matrix dataset, but all these variables are vector variable. If only correlations were required, Pearson or partial Pearson correlation would be sufficient. If the contribution of each variable is required, I would think VPA analysis would be a better option.

Author response: Thanks for your good suggestions. According to your suggestions, we deleted the description about the RDA analysis and results. Our data about environmental variables did not show normal distribution, therefore **we used Spearman correlation analysis to show their relationships between *amoA* gene abundance, the ratios of AOB to AOA, PAOR and environmental variables, and the results were given in Table 2.** Please see **line 255-256, line 263-264, line 268-269 and line 291-293** in the revised manuscript.

27. Line 325, "The AOA amoA gene abundance: : :", which type of correlation is this? Please report the r value, and may be also scatter plots in the supplementary. Furthermore, authors stated that both AOA amoA gene abundance and diversity were related to C:N ratio, but only one P-value was reported.

Author response: (1) The description about the RDA analysis and results has been deleted in the revised manuscript, and Spearman correlation coefficients and P-values were given in the text and Table 2; **(2)** The scatter plots about *amoA* gene abundance, the ratios of AOB to AOA, PAOR and C:N ratios have been provided in Fig. 3.

28. Table 1 need to provide full name of site, also the site codes do not match those in the main text.

Author response: The full name for the site has been given in Table 1, and all the site codes have been corrected for the consistency with the main text.

29. Figure 2. The order of figure need to change, Figure 2b appeared first in the manuscript, and they should appear first in figure 2.

Author response: The order of this figure has been changed in the revised manuscript.

Response to Referee #2

At first, we would like to express our appreciation to the reviewer for your kind help and valuable comments about the revision of this manuscript (MS No.: bg-2019-114). We have considered your valuable suggestions and carefully revised this manuscript.

The detailed responses inserted into reviewer #2 comments are attached as follows:

Anonymous Referee #2

Received and published: 7 June 2019

The manuscript entitled "Effects of sea animal colonization on the coupling between dynamics and activity of soil ammonia-oxidizing bacteria and archaea in maritime Antarctica" by Wang et al. describes the effect of sea animal colonization on the community composition of ammonia oxidizers. The subject matter is interesting and the work in general is technically sound, however, my main concern is that the authors make claims about the relationship between nitrification rates and ammonia-oxidizer dynamics. Furthermore, there are some inconsistencies within the environmental parameter data, as well as very speculative parts in the discussion which need to be addressed.

Author response: Thanks for your positive comments and valuable suggestions. We concentrated on *nitrification rates*, *some inconsistencies within the environmental parameter data, and speculative parts in the discussion,* and revised this manuscript carefully.

General comments: The authors measured potential ammonia oxidation rates by adding 1mM NH_4Cl and incubating the samples at 15 degrees, which seems to be very artificial and far from in situ rates. It is highly speculative to comment on in-situ ammonia oxidation rates based in these measurements. Hence, assessing the relative contribution of AOA and AOB to nitrification rates based on the presented measurements is highly speculative and can only be suggested based on the differences in abundance between those two groups. Further, the authors talk about "inhibition" of AOA due to seal and penguin activities (e.g., lines 312-313, line 344), however, the presented data simply suggests a higher abundance of AOB over AOA. While the environmental conditions might be more favorable for AOB, it is highly speculative to assume that this is caused by inhibition and should be phrased more carefully.

Author response: Thanks for your good comments. **(1)** Indeed we measured ammonia oxidation rates by adding 1mM $(NH_4)_2SO_4$ and incubating the samples at 15 °C, and they are different from in-situ ammonia oxidation rates. Therefore we used the word "**Potential** ammonia oxidation rates (PAOR)" to discriminate from "in-situ ammonia oxidation rates". **We concentrated the comparisons and analyses of POAR differences between the soils in tundra patches and their affecting factors.** The substrate concentration and incubation temperature in this study referred to several previous studies listed below.

Sample	substrate concentration	incubation temperature	references
Antarctic soils	1 mM $(NH_4)_2SO_4$	room temperature	(Jung et al., 2011)
cold climate Soils	1.25mM $(NH_4)_2SO_4$	25°C	(Fan et al., 2011)
Arctic soils	1.7-2.5 mM NH_4Cl	15°C.	(Alves et al., 2013)
Antarctic soils	1 mM $(NH_4)_2SO_4$	15°C.	This study

Jung, J., Yeom, J., Kim, J., Han, J., Lim, H. S., Park, H., et al.: Change in gene abundance in the nitrogen biogeochemical cycle with temperature and nitrogen addition in Antarctic soils, *Research in Microbiology*, 162, 1018–1026, https://doi.org/10.1016/j.resmic.2011.07.007, 2011.

Fan, F., Yang, Q., Li, Z., Wei, D., Cui, X. A., and Liang, Y.: Impacts of organic and inorganic fertilizers on nitrification in a cold climate soil are linked to the bacterial ammonia oxidizer community, *Microbial Ecology*, 62, 982–990, https://doi.org/10.1007/s00248-011-9897-5, 2011.

Alves, R. J. E., Wanek, W., Zappe, A., Richter, A., Svenning, M. M., Schleper, C., and Urich, T.: Nitrification rates in Arctic soils are associated with functionally distinct populations of ammonia-oxidizing archaea, The *ISME* Journal, 7(8), 1620–1631, https://doi.org/10.1038/ ismej.2013.35, 2013.

(2) According to your comments, the relative contribution of AOA and AOB to nitrification rates was assessed based on the differences in abundance between the AOA and AOB groups and the correlation between their abundances and POAR; **(3)** The statement about "inhibition" has been removed, we phrased more carefully, and just say "the environmental conditions might be more favorable for AOB". Please see **line 382-385** and **line 414-417** in the revised manuscript with track changes.

The ammonia concentrations of the 5 samples within the same site are sometimes extremely variable (e.g. 650 vs 0.1 in the STS site). How far were the different sampling points apart? Some of the data in Table 1 seems surprising or/and might be not well represented, e.g. the sum of the percentage of total carbon, nitrogen and sulfur makes up e.g. only 0.5%. What are the other 99.5%? Reporting total carbon, nitrogen and sulfur in mg/kg might be more useful as well. Additionally, the abbreviations of the sites are not very intuitive and easy to confuse.

Author response: (1) In penguin or seal colonies, the penguin or seal populations showed high inhomogeneous distribution, and the deposition of penguin guano or seal excreta into the soil led to the large variations in soil TC, TN, TS, NH_4^+-N contents, even within very small tundra areas; **(2)** Our sampling points were 50-100 m apart. Soil nutrients N, P and S are higher in penguin or seal colony soils due to the deposition of penguin guano or seal excrements in maritime Antarctica. However, they are relatively lower in tundra areas moderately far away from animal colonies, and most of the soil are primary minerals, such as SiO_2, feldspar, mica and metallic oxides; **(3)** We used mg g^{-1} to report total carbon, nitrogen and sulfur contents; **(4)** In the revised manuscript, we have used SS, PS, PL, MS, and BS for the samples and sites consistently to escape the ambiguity. **(5)** We have **rechecked** the measurement results of Table 1, and confirm that our data are right and valid. The reasons are as follows:

a. We measured soil TC and TN concentrations again, **which are provided in the following Table**, and the results are similar to those in this study, and their concentrations still showed large differences at the each sites within penguin or seal colony; **b.** We have measured the physiochemical properties of other soil samples in this area several times which were given in our previous published papers:

Zhu, R. B., Liu, Y. S., Xu, H., Ma, D. W., and Jiang, S.: Marine animals significantly increase tundra N2O and CH4 emissions in maritime Antarctica, *Journal of Geophysical Research: Biogeosciences*, 118(4), 1773–1792, https://doi.org/10.1002/2013JG002398, 2013.

Zhu RB, Liu YS, Ma ED, Sun JJ, Xu H, Sun LG. Nutrient compositions and potential greenhouse gas production in penguin guano, ornithogenic soils and seal colony soils in coastal Antarctica. *Antarctic Science*, doi:10.1017/S0954102009990204, 2009.

Soil chemical properties, especially NH_4^+-N, NO_3^--N, P and S concentrations, also showed large differences due to effects of penguin or seal activities according to the two papers above.

Therefore we think that TC, TN, TS, TP, NH_4^+-N and NO_3^--N levels showed high heterogeneity in penguin or seal colony tundra soils, PS and SS **due to the deposition of penguin or seal excreta, and the differences of tundra vegetation and soil texture caused by animal tramp.**

	Original No.	No. in the paper	Detection in 2015			Re-detection in 2019		
			N(mg/g)	C(mg/g)	C/N	N(mg/g)	C(mg/g)	C/N
Seal colony soils in western coast on Fildes Peninsula	SK1	SS1	12.12	48.67	4.02	9.99	54.52	5.51
	SK4	SS2	16.94	70.06	4.13	13.38	81.81	6.15
	SK6	SS3	0.87	5.56	6.37	1.51	10.34	6.85
	SK7		2.40	13.64	5.69	The sample is used up.		
	SK8	SS4	1.28	8.59	6.71	The sample is used up.		
	SK9		2.63	18.88	7.19	2.51	18.98	7.56
	SK10	SS5	1.30	11.54	8.87	**The sample is used up, the same as below**		
Penguin colony soils on Ardley Island	E1		10.54	50.58	4.8	8.68	55.83	6.43
	E2	PS1	14.55	84.65	5.82			
	E3		7.73	51.64	6.68	7.92	55.84	7.05
	E4	PS2	8.34	38.08	4.56			
	E5		15.07	89.71	5.95	13.48	92.33	6.85
	E6	PS3	17.90	120.76	6.75			
	E7		27.33	156.78	5.74	26.34	162.93	6.19
	E8	PS4	15.45	107.47	6.96			
	E9		9.99	73.10	7.31	8.87	79.72	8.99
	E10	PS5	7.97	45.82	**5.75**			
The middle tundra soils on Ardley Island	M1	PL1	11.53	117.64	10.2	9.88	124.91	12.64
	M2		13.61	138.41	10.17			
	M3	PL2	3.93	38.05	9.68	4.51	50.41	11.18
	M4		8.09	82.40	10.18			
	M5	PL3	25.30	302.52	11.96	23.94	301.93	12.61
	M6		20.19	222.45	11.02			
	M7	PL4	7.17	71.85	10.02	6.37	74.82	11.75
	M8		9.84	114.99	11.69			
	M9		11.47	110.65	9.65			
	M10		15.84	177.48	11.21	15.69	190.83	12.16
	M11		11.61	119.29	10.27			
	M12		4.34	44.40	10.23			

	M13		9.65	116.36	12.05			
	M14		3.33	30.13	9.04	2.77	30.49	11.01
	M15		12.95	147.59	11.39			
The tundra marsh soils in west of Ardley Island (almost no animals)	W1	MS1	8.93	95.54	10.7	9.65	111.82	11.59
	W2		11.92	148.81	12.49			
	W3	MS2	15.89	193.95	12.2	14.35	191.57	13.35
	W4		17.83	217.76	12.21			
	W5		12.93	141.64	10.95	10.79	136.73	12.67
	W6	MS3	19.79	226.90	11.46			
	W7		10.81	122.84	11.37	9.37	122.43	13.07
	W8	MS4	26.57	355.02	13.36			
	W9		21.88	254.01	11.61	20.87	257.11	12.32
	W10	MS5	23.51	292.00	12.42			
	adw-A		20.67	260.05	12.58	19.98	265.81	13.30
	adw-B		14.74	188.68	12.8			
	adw-C		17.29	235.79	13.63	17.76	252.1	14.19
The background tundra soils On Fildes Peninsula	GW1	BS1	4.76	56.72	11.91	4.81	56.89	11.83
	GW2	BS2	5.05	56.63	11.21	5.2	63	12.12
	GW3	BS3	4.30	47.69	11.09			
	gwc1		3.29	31.78	9.66	3.1	35.4	11.42
	gwc2		3.09	29.65	9.6			
	gwc3		2.41	24.03	9.96	2.5	28.3	11.32
	gwc4		2.37	24.39	10.29			

Specific comments: Line 25: Nitrosospira are AOB and Nitrososphaera are AOA, needs to be switched.

Author response: The order has been switched in this sentence. Please see **line 26-27** in revised manuscript.

Line 32-33: "The results provide insights into the mechanism how microbes drive nitrification in maritime Antarctica", here again the authors make claims that are not supported by the presented data. The mechanisms of nitrification are not studied.

Author response: According to your suggestion, this sentence has been removed in the revised manuscript.

Line 37: "biogeochemical nitrogen cycle" instead of "biogeochemical cycle for nitrogen"

Author response: This has been corrected in the revised manuscript. Please see **line 40** in revised manuscript.

Line 40: AOB were discovered much earlier than 2015, please chose a different reference

Author response: The reference has been changed as follows:

Belser, L. W., and Schmidt, E. L. Diversity in the ammonia-oxidizing nitrifier population of a soil. Applied and Environmental Microbiology, 36, 584–588, 1978.

Please see **line 43** in revised manuscript.

Line 41: comammox should be spelled out

Author response: According to another reviewer's comments, this sentence and *comammox* is out of picture. Therefore this sentence and *comammox* have been removed in the revised manuscript.

Line 46: Are you referring to the marine water column or sediments? Please specify (instead of mentioning "marine layers") and add the appropriate references.

Author response: The research object of Baker et al (2012) and Bouskill et al (2012) was marine water column. "Oxic and suboxic marine layers" has been replaced by "o**xic and suboxic marine water column**" for more accurate expression. The references "Baker et al (2012) and Bouskill et al (2012)" are still used in the revised manuscript. Please see **line 49-50** in revised manuscript.

Line 93: "daily mean range" is contradictory, please correct.

Author response: This sentence is to explain that the minimum daily mean temperature is -22.6℃ in winter, and the highest daily mean temperature is 11.7 ℃, which occurs in summer. This has been corrected into **"the range of daily mean temperature".** Please see **line 97** in revised manuscript.

Line 101: "A great many" should probably read "A great majority"

Author response: This has been corrected into **"A great majority".** Please see **line 105** in revised manuscript.

Line 346: typo in "reported"

Author response: This has been corrected in the revised manuscript (line 386).

Lines 378-380: This statement is not necessarily correct. There might be more diversity within Km's of AOA that differ from that of N. maritimus. Making such a claim based on a single organism is very speculative.

Author response: I agree with your comments, AOA group I.1b **might** exhibit a broader range of metabolism and adaptation and making such a claim **based on a single organism** is very speculative. We have removed this statement "……because the half-saturation constant for ammonia oxidation by *Thaumarchaeota* is lower than that by AOB", and only discussed the effects of NH_4^+-N levels on the AOA abundance and diversity, based on the correlation between NH_4^+-N levels on the AOA abundance and cited more references (Stieglmeier et al., 2014): This statement is reorganized as follows:

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). Please see **line 420-431** in revised manuscript.

Lines 393-397: The connection with comammox is not very intuitive. Did you detect comammox? Also, the reference of Santoro 2016 does not fit here because it measures actual rates (instead of potential rates) using stable isotopes in marine environments where no comammox has been found thus far.

Author response: According to your comments, this statement and the reference have been removed in the revised manuscript.

Lines 417-420: Why does a high organic carbon favor AOA over AOB? So far most studies have shown that AOA are inhibited by complex organic substrates (Stieglmeier et al 2011, Qin et al 2017, etc).

Author response: This statement has been corrected and reorganized as follows: **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.** Please see **line 467-474** in revised manuscript.

Lines 430-433: This statement is highly speculative and likely wrong. Why would the presence of an amoA gene be an ancestral remnant that is not active? There is no data presented supporting such claims.

Author response: Thanks. I agree with you. According to your comments, we have removed this statement.

Lines 446-455: this section does not discuss the data and should be moved to results

Author response: This section has been delete, considering that the information was already provided in Figure 5b.

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 ~~of~~ 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 (S~~T~~S), penguin colony soils (P~~T~~S), adjacent penguin-lacking tundra soils (PL~~S~~), 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 P~~T~~S, S~~T~~S, and PL~~S~~; 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 a~~A~~mmonia oxidation rates (PAOR) were significantly higher ($P = 0.02$) in S~~T~~S and P~~T~~S than in PL~~S~~, 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 ~~suggesting~~ suggested that AOB ~~are~~ 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* ~~*Nitrosospira*~~ and *Nitrosospira* ~~*Nitrososphaera*~~ lineages, respectively. Penguin or seal activities led to ~~the~~ a ~~predominant~~ predominance ~~existence~~ 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 ~~provide insights into the mechanisms how microbes drive nitrification in~~ maritime Antarctica.

Keywords: Antarctic tundra, AOA, AOB, Marine animals, Nitrification, Microbial diversity ~~Nitrogen deposition~~

1 Introduction

Nitrification, the oxidation of ammonia into nitrate through nitrite, plays a pivotal role in the global biogeochemical nitrogen cycle ~~for nitrogen~~ (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~~; Wang et al., 2015~~). ~~Only recently were the comammox, which directly oxidize ammonia to nitrate on their own, identified in the bacterial genus *Nitrospira* (Daims et al., 2015; Kessel et al., 2015).~~ 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 ~~layers~~ (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 ~~has been limited research about the abundance and diversity of microbes and genes involved in the nitrogen cycle in the remote Antarctic terrestrial ecosystems. 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 the Antarctic.

In maritime Antarctica, a large number of sea animals, such as penguins or seals, settle on ~~some~~ 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 ~~disturbance from~~ sea animal ~~activities~~ 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, 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, 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 an oceanic climate characteristics. Mean annual air temperature is about −2.5 °C, with the range of ~~a~~ daily mean temperature~~range~~ 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 ~~some~~ 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 an 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 ~~many~~ 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, PTS (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, PLS (i.e., low

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

136 At each sampling site, soil was collected aseptically using a clean scoop from the top 5–10 cm
137 at the four corners of a 1 m^2 subarea, and combined into one sample. Appropriate precautions
138 were taken to avoid cross-site or human-made contamination. Immediately after collection, each
139 sample was divided into two portions: one was stored in sterile plastic containers at −80 °C for
140 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 chemical volumetric method was used to measure soil total organic carbon (TOC).~~ 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~~Yue~~, 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.

160 The standard curve obtained from $NaNO_2$ (0–2.5 μmol l^{-1}) was used to calculate the PAOR in the
161 tundra soils.

162 **2.5. DNA extraction and gene amplification (PCR)**

163 Genomic DNA was extracted from 0.25 g of homogenized tundra soils using PowerSoil™
164 DNA Isolation Kit (Mo Bio, Carlsbad, CA, USA) as described in manufacturer's protocol. The
165 extracted DNA was eluted in 50 μl of elution buffer, quantified by a Nanodrop-2000
166 Spectrophotometer (Thermo Scientific, Waltham, MA, USA), and stored at −20 °C. AOA *amoA*
167 gene fragments (635 bp) were amplified using the primers Arch-amoAF (5'-
168 STAATGGTCTGGCTTAGACG-3') and Arch-amoAR (5'-GCGGCCATCCATCTGTATGT-3')
169 (Francis et al., 2005). The *amoA* gene fragment (491 bp) of β-proteobacterial AOB, which
170 represents known AOB in soil, was amplified using the primer set composed of amoA-1F (5'-
171 GGGGTTTCTACTGGTGGT-3') and amoA-2R (5'-CCCCTCKGSAAAGCCTTCTTC-3')
172 (Rotthauwe et al., 1997). All PCR reactions were performed using Taq PCR Master Mix (Sangon
173 Biotech, Shanghai, China) in a total volume of 50 μl. PCR reactions were carried out with a
174 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
175 for 45 s, 72 °C for 1 min; and a final 5-min extension cycle at 72 °C (Zheng et al., 2014).
176 Subsequently, the amplification products were visualized by electrophoresis on 1.0 % agarose gels.

177 **2.6. Sequencing and phylogenetic analysis**

178 The amplification products were sent to Sangon Company (Shanghai, China) for purification,
179 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~~ mothur program (version 1.23.0) by the furthest neighbor approach (Schloss et al., 2009). The closest reference sequences were identified at NCBI (http://www.ncbi.nlm.nih.gov/BLAST/) using the BLASTn tool (Madden, 2002), and phylogenetic trees were constructed by the neighbor-joining method using the Molecular Evolutionary Genetics Analysis (MEGA) software (version 5.03, https://www.megasoftware.net/). The sequences reported in this study have been deposited in GenBank under accession unmbers MH318029 to MH318568 and MH301331 to MH302505.

2.7. Quantitative real-time PCR

The AOB and AOA *amo*A gene copy numbers for tundra soils were determined in triplicate using quantitative real-time PCR (qPCR) on an ABI 7500 Sequence Detection System (Applied Biosystems). The specific details were given by ~~zheng~~ Zheng et al. (2014). The strong linear inverse relationship confirmed the consistency of the qPCR assay between the threshold cycle and the log value of gene copy numbers ($R^2 = 0.997$ for AOA; $R^2 = 0.999$ for AOB). The amplification efficiencies for AOA and AOB were 99.8 % and 90.4 %, respectively. Melting curve analysis had only one observable peak at a melting temperature (Tm) (84.9 °C for AOA and 89.6 °C for AOB) (Fig. S1 in Supplementary Material~~Supplementary Fig. S3~~). Negative controls were subjected to exclude any possible carryover or contamination in all experments.

2.8. Statistical analysis

The Shannon–Weiner Index, Simpson Index and the richness estimator Chao 1 were calculated by the ~~Mothur~~ 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 (~~Supplementary~~ Table ~~S2~~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. ~~Correlations between ammonia-oxidizer gene abundance, diversity, PAOR, and the AOB/AOA ratio with environmental variables were explored using redundancy analysis (RDA), because the maximum gradient length was shorter than three SD (AOA: 0.09; AOB: 0.088; PAOR and AOB/AOA: 1.105).~~

3 Results

3.1. Soil chemistry and sea animal activities

~~verall, a~~Almost all the tundra soils were slightly acidic, ~~with~~ and the mean pH ranged ~~of~~ from 5.3~~–~~ to 6.6 at each tundra patch (Table 1). In ~~Penguin~~ penguin ~~and~~ or seal colony tundra soils, P~~T~~S and S~~T~~S, 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 on Fildes Peninsula, 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 Ardley Island, the highest TP, TS, and NH_4^+-N levels occurred in the soils close to the eastern penguin nesting sites (PS1-5). PS and SS had generally lower C:N ratios than the penguin~~animal~~-lacking tundra soils (PL~~S~~), tundra marsh soils (MS), and background tundra soils (BS). Soil mean TN, TS and NH_4^+–N levels were higher in PS, SS, PL, and MS than in BS. ~~As expected, soil nutrient levels (TN, TP, TS, and NH_4^+–N) were higher in PTS, STS, PLS, and MS than in BS (Table 1).~~ Soil NH_4^+–N contents were 1–2 orders of magnitude higher in P~~T~~S and S~~T~~S than in PL~~S~~, MS, and BS, with the means of 176.9 and 137.6 mg NH_4^+-N kg^{-1}, respectively. The highest NO_3^--N contents occurred in S~~T~~S. Phosphorus levels were significantly greater ($P < 0.05$) in P~~T~~S (10.6–32.9 mg g^{-1}) than in ~~the~~ other types of tundra soils (mean < 6.0 mg g^{-1}). Overall, penguin or seal activities altered the local soil biogeochemical properties through the deposition of their excreta, leading to generally low C:N ratios in tundra soils. ~~In the seal colony tundra, soil TOC, TN, TP, TS, and NH_4^+-N levels decreased with the distance from the seal wallow. Likewise, soil TP, TS, and NH_4^+-N levels decreased from the eastern penguin nesting sites to the western tundra marsh. Sea animal activities altered the local~~

~~soil biogeochemical properties through the deposition of their excreta, leading to generally low C:N ratios and a marked increase in soil NH_4^+-N and TP contents. Therefore, the soil TP and NH_4^+-N levels and the distance from seal wallows and penguin nesting sites could be used to assess the intensity of seal or penguin activities.~~

3.2. Gene abundances under sea animal colonization

The abundance of the AOB *amoA* gene was significantly higher (by approximately 2–4 orders of magnitude) than that of the AOA *amoA* gene (Wilcoxon test, n = 22, P = 0.002) in the penguin and seal colony and the~~ir~~ adjacent tundra soils, P~~T~~S, S~~T~~S, and PL~~S~~. However, the abundances of the *amoA* gene were similar in the MS and BS soils (Fig. 2a). Overall, the abundances of AOB and AOA *amoA* genes were significantly negatively correlated (r = -0.~~90~~93, P = 0.~~037~~002) across all the tundra sites (Fig. S2). The ~~archaeal~~ AOA *amoA* gene abundances showed a heterogeneous distribution among the different tundra patches~~. AOA *amoA* gene~~, and they were two orders of magnitude lower in P~~T~~S and S~~T~~S relative to those in BS and MS. The maximal AOA *amoA* gene abundance appeared in BS, followed by MS and PL~~S~~, whereas the P~~T~~S and S~~T~~S soils had the lowest ~~archaeal~~AOA *amoA* gene abundances. The log values of s~~S~~oil AOA *amoA* gene abundances ~~were~~ showed a significant~~ly~~ 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).~~increased with decreasing animal activity intensity (i.e., the distance from eastern penguin nesting sites PS1–PS5 to western tundra marsh MS1–MS5, and from seal wallow site SS1 to the background tundra sites) (Fig. 3).~~

Unlike the AOA *amoA* genes, AOB *amoA* gene abundances showed the opposite distribution pattern. The AOB *amoA* gene abundances were significantly higher (by approximately 2–3 orders of magnitude) in PTS and STS 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).~~increased significantly with increasing animal activities (i.e. the distance from eastern penguin nesting sites and from the seal wallow) (Fig. 3).~~ The ratios of AOB to AOA *amoA* copy numbers were strongly affected by animal activities, and were much higher in PTS and STS than in PLS, 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 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 in PTS and STS. 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~~significantly~~ higher in STS (mean 76.1 μg N kg^{-1} h^{-1}) than in ~~and~~ PTS (mean 64.7 μg N kg^{-1} h^{-1}), but significantly higher than in PLS, 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 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 BS; Kruskal–Wallis test, χ^2 =11.6, P = 0.02) (Fig. 2c). The greatest POAR occurred at the sites PS1 nearest the penguin nests (88.8 ± 2.7 μg N kg^{-1} h^{-1}) and SS1 close to seal wallows (138.8 ± 0.8 μg N kg^{-1} h^{-1}).~~Kruskal–Wallis test, χ^2 = 11.6, P = 0.02).~~ The PAOR followed the distribution changes of AOB *amoA* gene abundances, but showed the opposite trend to the AOA *amoA* gene abundances ~~(Fig. 2)~~. A significant positive correlation (r^2 = 0.77, P < 0.001) was observed between the PAOR and the AOB *amoA* gene abundance when the data from all the tundra patches were combined, whereas no correlation occurred between PAOR and AOA *amoA* gene abundance (Fig. 4). The AOB dominance over AOA in the abundance in PS, SS and PL and their correlation with the PAOR suggested that ~~Therefore, the~~ AOB populations might contribute more to the PAOR than the AOA populations in penguin or seal colony. In addition, PAOR significantly negatively correlated with soil C:N ratios (r= -0.73, P<0.001)(Fig. 3d), but significantly positively correlated with TS contents (r=0.47, P<0.05) and TP contents (r=0.43, P<0.05) (Table 2). ~~the study area. Interestingly, the PAOR greatly increased with penguin or seal activity intensity, and the greatest rates occurred at the sites nearest the penguin nests (88.8 ± 2.7 μg N kg^{-1} h^{-1}) and seal wallows (138.8 ± 0.8 μg N kg^{-1} h^{-1}) (Fig. 3).~~

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

The PCR products were insufficient to construct the clone libraries for the AOA *amoA* gene from S~~T~~S and P~~T~~S because of the low AOA abundance in the soils, as was the case with the AOB *amoA* gene from MS and BS. Overall, 10 AOA and 14 AOB *amoA* gene clone libraries were successfully constructed. The 543 AOA sequences and 1175 AOB quality sequences were

generated from the respective sites. Within each individual site, 1–6 AOA OTUs and 6–15 AOB OTUs were identified, as defined by < 3% divergence in nucleotides. The AOA and AOB OTU 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. ~~S1~~ S3 and Fig. ~~S2~~S4). The diversity of the AOB *amoA* was generally higher than that of AOA *amoA*, based on the indices of Shannon–Wiener and Simpson. Specifically, the AOA *amoA* gene had higher diversity in PL~~S~~ and MS than in BS~~.~~, ~~The~~ whereas the AOB *amoA* gene showed higher diversity in S~~T~~S and P~~T~~S 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~~ tree showed that the AOA *amoA* sequences ~~could~~ 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 had 11 OTUs and 264 clones, and 57.9% of AOA *amoA* sequences were from PL~~S~~, 41.3% from S~~T~~S, 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~~S~~. Almost all the AOA phylotypes retrieved from PL~~S~~ and S~~T~~S were related to *Nitrososphaera* cluster I, whereas the AOA phylotypes retrieved from MS and BS were distributed in cluster II (Fig. ~~6~~S5a in Supplementary Material). 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 S~~T~~S and PL~~S~~. 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. AOB phylogenetic tree ~~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 these~~ *amoA* sequencesy~~ were grouped into four *Nitrosospira* clusters according to the evolutionary distance of the phylogenetic tree ~~with known sequences from AOBs in the *Nitrosospira* genera~~ (Fig. 5b). Cluster I had 11 OTUs and 226 clones, and 67.7% of AOB *amoA* sequences were from P~~T~~S, 23.5 % from S~~T~~S, 8.4% from PL~~S~~, and only 0.4% from MS. There are 17 unique OTUs and 521 clones in clusters II and III. The sources of the OTUs in cluster II were similar to those of cluster I, with 69.8% from P~~T~~S, 29.9% from S~~T~~S, and 0.3% from PL~~S~~. For cluster III, 79.2% of the sequences were from PL~~S~~, 19.8% from S~~T~~S, and 1.0% from MS. Cluster IV had nine unique OTUs and 370 clones from PL~~S~~ (50.0%), S~~T~~S (36.8%) and MS (13.2%), respectively. Of all the AOB phylotypes retrieved from P~~T~~S were related to dominant *Nitrosospira* clusters I and II, whereas AOB ~~phylotypes~~ phylotypes related to cluster III and IV were completely excluded because of ~~strong~~ penguin colonization (Fig. S5b)~~activity (Fig. 6)~~. The AOB phylotypes retrieved from S~~T~~S were distributed in clusters I, II, III, and IV (16–38% for each cluster). Almost all the AOB phylotypes retrieved from PL~~S~~ 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 ~~58.4~~62.1% of the total variance in the AOA *amoA* genotype compositions, and ~~66.8~~71.5% of the cumulative variance of the genotype-environment relationships in the first two CCA dimensions (Fig. ~~7a~~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) ~~, TOC, and NO_3^--N~~ in tundra soils (Table ~~2~~3), and the combination of the ~~three~~ two factors explained ~~60.3~~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, ~~and~~ NH_4^+-N and NO_3^--N were not statistically significant ($P > 0.05$), these variables additionally explained ~~26.5~~47.3% of the variation. ~~The AOA richness and phylotypes were evidently inhibited in STS and PLS because seal or penguin activities. However, high soil C:N and TOC concentrations increased the AOA richness and phylotypes in MS and BS~~. As illustrated in Fig. ~~7b~~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 C:N ratios~~, 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 ~~and phylotypes~~ in S~~T~~S and P~~T~~S through higher NH_4^+-N and P input from sea animal excrements, whereas AOB richness ~~and phylotypes were~~ was closely related to the soil C:N in PL~~S~~ and MS.

~~Correlations among *amoA* gene abundance, diversity, PAOR, and the ratios of AOB:AOA abundance with environmental variables were examined via Redundancy Analysis (RDA) (Fig. 8). The AOA *amoA* gene abundance and diversity were positively related to the C:N ratio (P = 0.002), and negatively correlated with NH_4^+-N (P = 0.004). Two factors combine yielded 63.5% of the total RDA explanatory power (Table S2). Higher soil C:N increased the AOA abundance and diversity in BS and MS, but higher NH_4^+-N input inhibited their abundance and diversity in PLS and STS because of penguin or seal activities. Significant correlations were obtained between AOB *amoA* gene abundance, diversity, and environmental factors including the C:N ratio (P = 0.004), TOC (P = 0.012), and NH_4^+-N (P = 0.05). These three factors combined yielded 73.2% of the total explanatory power (Table S3). The ratios of AOB to AOA and PAOR showed positive correlations with NH_4^+-N (P = 0.002), TP (P = 0.046), and TS (P = 0.030), but negative correlations with the C:N ratio (P = 0.002) and TOC (P = 0.048). These factors explained 87.5% of the variation (Table S4). Compared with those in BS and MS, penguin or seal activities significantly increased the AOB *amoA* gene abundance, diversity, PAOR, and the ratios of AOB to AOA in STS, PTS, and PLS because of the increase in NH_4^+-N and TP input from animal excrement.~~

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 P~~T~~S and S~~T~~S relative to BS and MS; however, AOB *amoA* gene abundances were approximately 2–3

orders of magnitude higher in P~~T~~S and S~~T~~S than in MS and BS, indicating that sea animal activities increased the AOB population size, but ~~inhibited~~ decreased AOA abundances in tundra soils (Fig. 2 and Fig. 3). Overall, the ~~archeal~~ 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 ~~bacterial~~ AOB *amoA* gene abundances were two to three orders of magnitude higher in P~~T~~S and S~~T~~S than in Antarctic Dry Valleys (Alves et al., 2013; Magalhães et al., 2014). In contrast to previous studies indicating that AOA were more abundant than AOB in some terrestrial or marine ecosystems (Beman et al., 2008; Lam et al., 2007; Wuchter et al., 2006; Yao et al., 2011), and in soils from Antarctic Peninsula (Jung et al., 2011), our qPCR estimates showed that the ~~bacterial~~ AOB *amoA* copy numbers were much greater than those of ~~archeal~~ AOA *amoA* in P~~T~~S, S~~T~~S and PL~~S~~ because of sea animal activities. However, their abundances were very close to each other in BS and MS. The ratios of AOB to AOA abundance were strongly affected by sea animal activities, which were indicated by soil C:N ratios (Fig. 2c). A shift in the relative abundance of AOA and AOB recorded previously for the Antarctic Dry Valleys, with a greater abundance of AOB compared with that of AOA for Battleship Promontory and Miers Valley, and the reverse for Upper Wright Valley and Beacon Valley (Magalhães et al., 2014). The results for P~~T~~S, S~~T~~S, and PL~~S~~ are also in agreement with those detected in subglacial soils (Boyd et al., 2011).

The ratios of AOB to AOA showed significant ~~positive~~ correlations with C:N, NH_4^+-N, and TP~~, and TS~~ when all the data were combined in the five tundra patches (Table 2~~Fig. 8~~). This suggested that C:N, NH_4^+-N, and TP~~, and TS~~ are key factors when ~~bacterial~~ AOB *amoA* genes are

much more abundant than ~~archeal~~ AOA *amoA* genes. In Antarctica, the productivity of terrestrial ecosystems is strongly limited because of the extremely low nitrogen levels (Park et al., 2007). However, the physiochemical properties for tundra soils were strongly influenced by the deposition of penguin or seal excreta under effects of local microbes (Tatur et al., 1997). Sea animals provide considerable external N inputs for their colony soils and adjacent tundra soils through direct input of their excreta and atmospheric deposition via ammonia volatilization (Lindeboom, 1984; Sun et al., 2002; Blackall et al., 2007; Zhu et al., 2011; Riddick et al., 2012). Like ammonium, P ~~and S are~~is typical elements in penguin guano ~~, and they have been used to indicate penguin activity intensity~~ (Sun et al., 2000). Generally low C:N ratios and s~~S~~ignificantly elevated NH_4^+–N and TP concentrations occurred in P~~T~~S and PL~~S~~ due to penguin or seal activities~~compared with those in BS~~ (Table 1). These conditions might be more favorable for AOB~~may be beneficial for nitrification~~, allowing high abundance ~~and diversity~~ of ~~bacterial~~ AOB *amoA*, which explains the strong correlations between AOB abundances and C:N, NH_4^+–N, ~~TP,~~ and ~~TS~~ TP in the sea animal colony soils (Table 2~~Fig. 8~~). This ~~is~~ agreed with the high bacterial ~~diversity and~~ abundance previously documented in penguin or seal colony soils and ornithogenic sediments (Ma et al., 2013; Zhu et al., 2015).

The AOA abundance ~~and diversity~~ showed a significant negative correlation with NH_4^+-N levels ~~a positive correlation with C:N~~ 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)~~, but a significant negative correlation with NH_4^+ N levels (Fig. 8). AOA might better adapt to low NH_4^+ and oligotrophic environments because the half-saturation~~

~~constant for ammonia oxidation by *Thaumarchaeota* is lower than that by AOB (Martens-Habbena et al., 2009).~~ 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 ammonia oxidation rates

~~In this study,~~ The PAOR ranged from 9 to 139 μg N kg^{-1} h^{-1}, ~~which was~~ 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 ~~is~~ 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 (Daebeler et al., 2017; Zhao et al., 2014). ~~Notably, comammox *Nitrospira* may actually compete with ammonia oxidizers for ammonium, after which comammox oxidize ammonia to nitrate on their own via a one-step process (Daims et al., 2015; van Kessel et al., 2015). In this study, the method of measuring nitrification rates did not include the activity of these organisms because sodium chlorate was used to prevent NO_2^- from being oxidized to NO_3^-, whereas other methods likely capture the comammox activity (Santoro, 2016).~~ Our measurements indicated that there were significant differences (P = 0.02) in the PAOR across different tundra patches ~~(P = 0.02)~~, and the PAORs in S~~T~~S and P~~T~~S were about 10 times higher than those in BS

and MS. A significant correlation was observed between the PAOR and C:N, TP, and TS (Table 2)~~NH_4^+ N, TP, and sulfur (Fig. 8)~~. Overall, ammonia oxidation activity was modulated by soil biogeochemical processes under the disturbance of penguin or seal~~sea animal~~ activities: generally low C:N ratios, and sufficient input of the nutrients TP, TS, and NH_4^+–N from sea animal excrements.~~sufficient input of the nutrients NH_4^+ N, TP, and TS from sea animal excreta.~~

The AOB dominance over AOA in the abundance (Fig. 2b) and significant negative correlation of AOA abundance with NH_4^+–N levels (Table 2)~~The gene abundance of AOB *amoA* was markedly higher that of AOA *amoA*, and AOA found it difficult to tolerate the high ammonium environment in PTS, STS, and PLS~~, ~~indicating~~ indicated that AOB might play a more important role in nitrification in tundra soils. In agreement with these results, AOB dominated nitrification in the areas where it was easy to achieve nitrogen input, whereas the relative contribution of AOA to nitrification was higher in the areas where the ammonium concentration remained low (Fan et al., 2011; Sterngren et al., 2015). Moreover, the cell-specific activity for AOB was 10 times higher than that for AOA due to the bigger cell size of AOB (Hatzenpichler et al., 2012; Prosser and Nicol, 2012). Therefore, AOB might play a more important role in nitrification in S~~T~~S, P~~T~~S, and PL with the input of NH_4^+–N from penguin or seal excrements.~~S compared with that in BS and MS.~~

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

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~~covered with lush tundra plants and were rich in organic carbon (Table 1), which has been shown to favor AOA because their substrates can be provided through the mineralization of soil organic matter~~ (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 S~~T~~S and P~~T~~S, whereas a high diversity of AOA *amoA* genes was observed in PL~~S~~, MS and BS. Phylogenetic analysis indicated that the AOA *amoA* sequences in Cluster I were from PL~~S~~ and tundra soils close to seal wallows, while the sequences in Cluster II were from BS and MS (Fig. ~~6~~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). ~~Cluster I found in the PLS might represent AOA adapting to survival in the presence of relatively high soil nutrients, for which the presence of the *amoA* gene represents either secondary metabolism or an ancestral remnant no longer active because of the high AOB abundance in these areas.~~ 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~~Zhang et al., 2017~~) and Icelandic grassland soils (Daebeler et al., 2012~~Lam et al., 2009~~). The compositions of soil AOA populations are likely not to be explained by single physicochemical properties, and their community structures significantly correlated with tundra soil C:N, and T~~O~~C, ~~and NO_3^- N,~~ which was consistent with previous studies (Glaser et al., 2010; Wessén et al., 2011).

The AOB *amoA* gene generally had a higher diversity than AOA, similar to results in the Antarctic Dry Valley soils (Magalhães et al., 2014). A high diversity of AOB *amoA* gene occurred in S~~T~~S, P~~T~~S and PL~~S~~ compared to BS, indicating that penguin or seal activities had important effects on AOB genotypic diversity. ~~According to the evolutionary distance of the p~~Phylogenetic analysis indicated that the~~tree, AOB *amoA* sequences were grouped into four clusters with known sequences from the *Nitrosospira* genera, and they are in the lineages of *Nitrosospira sp.* En13 (EF175097), *Nitrosospira sp.* LT1FMf (AY189144), *Nitrosospira sp.* EnI299 (EF175100), *Nitrosospira sp.* III7 (AY123829), and *Nitrosospira sp.* Wyke8 (EF175099) (Fig. 5b). The~~ sequences in clusters I and II were mainly from P~~T~~S and S~~T~~S (Fig. 5b), and the detected OTUs in Cluster I had their closest matches from mixed community culture systems, meadow to forest transect in Oregon Cascade Mountains (Mintie et al., 2003), and Dutch agricultural soils (Silva et al., 2012a) and reservoir sediments (Silva et al., 2012b). For Clusters III and IV, the sequences

were predominantly from PL~~S~~ and S~~T~~S, and they were affiliated with sequences recovered from high altitude wetland (~~Shan~~ Yang et al., 2014). Previous studies have shown that multiple environmental factors affected the AOB communities (Dang et al., 2008; Mosier and Francis, 2008). In this study, the C:N ratios and NH_4^+-N concentrations seemed to be the most important factors influencing the AOB community structure, which was in accordance with the results from different environments (Bouskill et al., 2012; Jung et al., 2011; Li et al., 2015). Moreover, the ~~C:N ratio and~~ TP also affected the AOB *amoA* community compositions (Zheng et al., 2013). Therefore, the AOB community compositions were impacted by the biogeochemical factors related to sea animal activities, such as low C:N ratios, and 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~~, which are moderately far away from the animal colonies~~. 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 S~~T~~S and P~~T~~S than in PL~~S~~ and MS, and the majority of the AOB

sequences were closely related to *Nitrosospira*-like sequences. The ~~archaeal~~ AOA *amoA* gene had higher diversity in ~~STS,~~ PL~~S,~~ 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 ~~of sea animal activities~~, such as soil C:N alteration, and a sufficient supply of the nutrients NH_4^+–N, N and P from animal excreta. ~~Overall, t~~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 Ocean Bureau of China for their support and timely help. The final derived data presented in this study are available at https://doi.org/10.5281/zenodo.1260292.

References

Alves, R. J. E., Wanek, W., Zappe, A., Richter, A., Svenning, M. M., Schleper, C., and Urich, T.: Nitrification rates in Arctic soils are associated with functionally distinct populations of ammonia-oxidizing archaea, *The ISME Journal*, *7*(8), 1620–1631, https://doi.org/10.1038/ ismej.2013.35, 2013.

548 Ayton, J., Aislabie, J., Barker, G. M., Saul, D., and Turner, S.: Crenarchaeota affiliated with group
549 1.1 b are prevalent in coastal mineral soils of the Ross Sea region of Antarctica, *Environmental*
550 *Microbiology*, *12*(3), 689–703, https://doi.org/10.1111/j.14622920. 2009.02111.x, 2010.
551 Baker, B. J., Lesniewski, R. A., and Dick, G. J.: Genome-enabled transcriptomics reveals archaeal
552 populations that drive nitrification in a deep-sea hydrothermal plume, *The ISME Journal*, *6*(12),
553 2269–2279, https://doi.org/10.1038/ismej.2012.64, 2012.
554 Belser, L. W., and Schmidt, E. L.: Diversity in the ammonia-oxidizing nitrifier population of a soil,
555 *Applied and Environmental Microbiology*, 36, 584–588, 1978.
556 Beman, J. M., Popp, B. N., & Francis, C. A.: Molecular and biogeochemical evidence for ammonia
557 oxidation by marine Crenarchaeota in the Gulf of California, *The ISME Journal*, *2*(4), 429–441,
558 2008.
559 Blackall, T. D., Wilson, L. J., Theobald, M. R., Milford, C., Nemitz, E., Bull, J., Sutton, M., et al.:
560 Ammonia emissions from seabird colonies, *Geophysical Research Letters*, 34(10),
561 https://doi.org/10.1029/2006GL028928, 2007.
562 Bouskill, N. J., Eveillard, D., Chien, D., Jayakumar, A., and Ward, B. B.: Environmental factors
563 determining ammonia‐oxidizing organism distribution and diversity in marine
564 environments, *Environmental Microbiology*, *14*(3), 714–729, https://doi.org/10.1111/j.1462-
565 2920.2011.02623.x, 2012.
566 Boyd, E. S., Lange, R. K., Mitchell, A. C., Havig, J. R., Hamilton, T. L., Lafrenière, M. J., et al.:
567 Diversity, abundance, and potential activity of nitrifying and nitrate-reducing microbial

assemblages in a subglacial ecosystem, *Applied and Environmental Microbiology*, *77*(14), 4778–4787, https://doi.org/10.1128/AEM.00376-11, 2011.

Cowan, D. A., Ramond, J. B., Makhalanyane, T. P., and De Maayer, P.: Metagenomics of extreme environments, *Current Opinion in Microbiology*, *25*, 97–102, https://doi.org/10.1016/j.mib.2015.05.005, 2015.

Daebeler, A., Abell, G. C., Bodelier, P. L., Bodrossy, L., Frampton, D. M., Hefting, M. M., and Laanbroek, H. J.: Archaeal dominated ammonia-oxidizing communities in Icelandic grassland soils are moderately affected by long-term N fertilization and geothermal heating, *Frontiers in microbiology*, 3, 352, https://doi.org/10.3389/fmicb.2012.00352, 2012.

Daebeler, A., Bodelier, P. L., Hefting, M. M., Rütting, T., Jia, Z., and Laanbroek, H. J.: Soil warming and fertilization altered rates of nitrogen transformation processes and selected for adapted ammonia-oxidizing archaea in sub-arctic grassland soil, *Soil Biology and Biochemistry*, *107*, 114–124, https://doi.org/10.1016/j.soilbio.2016.12.013, 2017.

Dang, H., Zhang, X., Sun, J., Li, T., Zhang, Z., and Yang, G.: Diversity and spatial distribution of sediment ammonia-oxidizing crenarchaeota in response to estuarine and environmental gradients in the Changjiang Estuary and East China Sea, *Microbiology*, *154*, 2084–2095. https://doi.org/10.1099/mic.0.2007/013581-0, 2008.

Di, H. J., Cameron, K. C., Shen, J. P., Winefield, C. S., O'callaghan, M., Bowatte, S., and He, J. Z.: Nitrification driven by bacteria and not archaea in nitrogen-rich grassland soils, *Nature Geoscience*, *2*, 621–624, https://doi.org/10.1038/ngeo613, 2009.

588 Fan, F., Yang, Q., Li, Z., Wei, D., Cui, X. A., and Liang, Y.: Impacts of organic and inorganic
589 fertilizers on nitrification in a cold climate soil are linked to the bacterial ammonia oxidizer
590 community, *Microbial Ecology*, *62*, 982–990, https://doi.org/10.1007/s00248-011-9897-5, 2011.
591 Francis, C. A., Roberts, K. J., Beman, J. M., Santoro, A. E., and Oakley, B. B.: Ubiquity and
592 diversity of ammonia-oxidizing archaea in water columns and sediments of the
593 ocean, *Proceedings of the National Academy of Sciences of the United States of America*, *102*(41),
594 14683–14688, https://doi.org/10.1073/pnas.0506625102, 2005.
595 Gao, Y. S., Yang, L. J., Wang, J. J., Xie, Z. Q., Wang, Y. H., and Sun, L. G.: Penguin colonization
596 following the last glacial-interglacial transition in the Vestfold Hills, East
597 Antarctica, *Palaeogeography, Palaeoclimatology, Palaeoecology*, *490*, 629–639, https://doi.org/
598 10.1016/j.palaeo.2017.11.053, 2018.
599 Glaser, K., Hackl, E., Inselsbacher, E., Strauss, J., Wanek, W., Zechmeister-Boltenstern, S., and
600 Sessitsch, A.: Dynamics of ammonia-oxidizing communities in barley-planted bulk soil and
601 rhizosphere following nitrate and ammonium fertilizer amendment, *FEMS Microbiology*
602 *Ecology*, *74*, 575–591, https://doi.org/10.1111/j.1574-6941.2010.00970.x, 2010.
603 Gubry-Rangin, C., Novotnik, B., Mandič-Mulec, I., Nicol, G. W., and Prosser, J. I.: Temperature
604 responses of soil ammonia-oxidising archaea depend on pH, *Soil Biology and Biochemistry*, *106*,
605 61–68, https://doi.org/10.1016/j.soilbio.2016.12.007, 2017.
606 Habteselassie, M. Y., Xu, L., and Norton, J. M.: Ammonia-oxidizer communities in an agricultural
607 soil treated with contrasting nitrogen sources, *Frontiers in Microbiology*, *4*, 326,
608 https://doi.org/10.3389/fmicb.2013.00326, 2013.

609 Hatzenpichler, R., Lebedeva, E. V., Spieck, E., Stoecker, K., Richter, A., Daims, H., and Wagner,
610 M.: A moderately thermophilic ammonia-oxidizing crenarchaeote from a hot spring, *Proceedings*
611 *of the National Academy of Sciences*, *105*, 2134–2139, https://doi.org/10.1073/pnas.0708857105,
612 2008.
613 Hatzenpichler, R.: Diversity, physiology, and niche differentiation of ammonia-oxidizing
614 archaea, *Applied and Environmental Microbiology*, *78*, 7501–7510, https://doi.org/10.1128/
615 AEM.01960-12, 2012.
616 Jung, J., Yeom, J., Kim, J., Han, J., Lim, H. S., Park, H., et al.: Change in gene abundance in the
617 nitrogen biogeochemical cycle with temperature and nitrogen addition in Antarctic
618 soils, *Research in Microbiology*, *162*, 1018–1026, https://doi.org/10.1016/j.resmic.2011.07.007,
619 2011.
620 Kalanetra, K. M., Bano, N., and Hollibaugh, J. T.: Ammonia-oxidizing Archaea in the Arctic Ocean
621 and Antarctic coastal waters, *Environmental Microbiology*, 11, 2434-2445, https://doi.
622 org/10.1111/j.1462-2920.2009.01974.x, 2009.
623 Könneke, M., Bernhard, A. E., José, R., Walker, C. B., Waterbury, J. B., and Stahl, D. A.: Isolation
624 of an autotrophic ammonia-oxidizing marine archaeon, *Nature*, *437*, 543–546,
625 https://doi.org/10.1038/nature03911, 2005.
626 Kurola, J., Salkinoja‐Salonen, M., Aarnio, T., Hultman, J., & Romantschuk, M.: Activity,
627 diversity and population size of ammonia‐oxidising bacteria in oil‐contaminated landfarming
628 soil, *FEMS Microbiology Letters*, *250*, 33–38, https://doi.org/10.1016/j.femsle.2005.06.057, 2005.

Lam, P., Jensen, M. M., Lavik, G., McGinnis, D. F., Müller, B., Schubert, et al.: Linking crenarchaeal and bacterial nitrification to anammox in the Black Sea, *Proceedings of the National Academy of Sciences*, *104*, 7104–7109, https://doi.org/10.1073/pnas.0611081104, 2007.

Le Roux, X., Poly, F., Currey, P., Commeaux, C., Hai, B., Nicol, G. W., et al.: Effects of aboveground grazing on coupling among nitrifier activity, abundance and community structure, *The ISME Journal*, *2*(2), 221–232, https://doi.org/10.1038/ismej.2007.109, 2008

Leininger, S., Urich, T., Schloter, M., Schwark, L., Qi, J., Nicol, G. W., et al.: Archaea predominate among ammonia-oxidizing prokaryotes in soils, *Nature*, *442*, 806–809, https://doi.org/10.1038/nature04983, 2006.

Li, J., Nedwell, D. B., Beddow, J., Dumbrell, A. J., McKew, B. A., Thorpe, E. L., and Whitby, C.: amoA gene abundances and nitrification potential rates suggest that benthic ammonia-oxidizing bacteria and not archaea dominate N cycling in the Colne Estuary, United Kingdom, *Applied and Environmental Microbiology*, *81*, 159–165, https://doi.org/10.1128/ AEM.02654-14, 2015.

Lindeboom, H. J.: The nitrogen pathway in a penguin rookery, *Ecology*, *65*, 269–277, https://doi.org/10.2307/1939479, 1984.

Madden, T.: The BLAST Sequence Analysis Tool. In McEntyre J, Ostell J (ed), *The NCBI Handbook*. National Center for Biotechnology Information, Bethesda, Maryland USA, 2002.

Ma, D. W., Zhu, R. B., Ding, W., Shen, C. C., Chu, H. Y., and Lin, X. G.: Ex-situ enzyme activity and bacterial community diversity through soil depth profiles in penguin and seal colonies on Vestfold Hills, East Antarctica, *Polar Biology*, *36*, 1347–1361, https://doi.org/10.1007/ s00300-013-1355-z, 2013.

Magalhães, C. M., Machado, A., Frank-Fahle, B., Lee, C. K., and Cary, S. C.: The ecological dichotomy of ammonia-oxidizing archaea and bacteria in the hyper-arid soils of the Antarctic Dry Valleys, *Frontiers in Microbiology*, *5*, 515, https://doi.org/10.3389/fmicb.2014.00515, 2014.

Magalhães, C., Stevens, M. I., Cary, S. C., Ball, B. A., Storey, B. C., Wall, D. H., and Ruprecht, U.: At limits of life: multidisciplinary insights reveal environmental constraints on biotic diversity in continental Antarctica, *PLoS One*, *7*(9), e44578, https://doi.org/10.1371/journal.pone.0044578, 2012.

Martens-Habbena, W., Berube, P. M., Urakawa, H., José, R., and Stahl, D. A.: Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria, *Nature*, *461*, 976–979, https://doi.org/10.1038/nature08465, 2009.

Mintie, A. T., Heichen, R. S., Cromack Jr, K., Myrold, D. D., & Bottomley, P. J.: Ammonia-oxidizing bacteria along meadow-to-forest transects in the Oregon Cascade Mountains, *Applied and Environmental Microbiology*, *69*(6), 3129–3136, https://doi.org/10.1128/AEM.69.6.3129-3136.2003, 2003.

Mosier, A. C., and Francis, C. A.: Relative abundance and diversity of ammonia‐oxidizing archaea and bacteria in the San Francisco Bay estuary, *Environmental Microbiology*, *10*(11), 3002–3016, https://doi.org/10.1111/j.1462-2920.2008.01764.x, 2008.

Otero, X. L., Peña-Lastra, S., Pérez-Alberti, A., Ferreira, T. O., and Huerta-Diaz, M. A.: Seabird colonies as important global drivers in the nitrogen and phosphorus cycles, *Nature Communications*, *9*(1), 246, https://doi.org/0.1038/s41467-017-02446-8, 2018.

670 Ouyang, Y., Norton, J. M., Stark, J. M., Reeve, J. R., and Habteselassie, M. Y.: Ammonia-oxidizing
671 bacteria are more responsive than archaea to nitrogen source in an agricultural soil, *Soil Biology*
672 *and Biochemistry*, *96*, 4–15, https://doi.org/10.1016/j.soilbio.2016.01.012, 2016.
673 Pan, H., Xie, K. X., Zhang, Q. C., Jia, Z. J., Xu, J. M., Di, H. J., and Li, Y.: Archaea and bacteria
674 respectively dominate nitrification in lightly and heavily grazed soil in a grassland
675 system, *Biology and Fertility of Soils*, *54*(1), 41–54, https://doi.org/10.1007/s00374-017-1236-7,
676 2018.
677 Park, J. H., Day, T. A., Strauss, S., and Ruhland, C. T.: Biogeochemical pools and fluxes of carbon
678 and nitrogen in a maritime tundra near penguin colonies along the Antarctic Peninsula, *Polar*
679 *Biology*, *30*, 199–207, https://doi.org/10.1007/s00300-006-0173-y, 2007.
680 Prosser, J. I., and Nicol, G. W.: Archaeal and bacterial ammonia-oxidisers in soil: the quest for
681 niche specialisation and differentiation, *Trends in Microbiology*, *20*, 523–531, https://doi.org/
682 10.1016/ j.tim.2012.08.001, 2012.
683 Richter, I., Herbold, C. W., Lee, C. K., McDonald, I. R., Barrett, J. E., and Cary, S. C.: Influence of
684 soil properties on archaeal diversity and distribution in the McMurdo Dry Valleys,
685 Antarctica, *FEMS Microbiology Ecology*, *89*, 347–359, https://doi.org/10.1111/1574-6941. 12322,
686 2014.
687 Riddick, S. N., Dragosits, U., Blackall, T. D., Daunt, F., Wanless, S., and Sutton, M. A.: The global
688 distribution of ammonia emissions from seabird colonies, *Atmospheric Environment*, *55*, 319–327,
689 https://doi.org/10.1016/j.atmosenv.2012.02.052, 2012.

690 Rotthauwe, J. H., Witzel, K. P., and Liesack, W.: The ammonia monooxygenase structural gene
691 amoA as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing
692 populations, *Applied and Environmental Microbiology*, *63*, 4704–4712, 1997.
693 Santoro, A. E., Buchwald, C., McIlvin, M. R., and Casciotti, K. L.: Isotopic signature of N_2O
694 produced by marine ammonia-oxidizing archaea, *Science*, *333*, 1282–1285, https://doi.org/
695 10.1126/science.1208239, 2011.
696 Schloss, P. D., Westcott, S., Ryabin, T., Hall, J., Hartmann, M., Hollister, E., et al.: Introducing
697 mothur: Open-source, platform-independent, community-supported software for describing and
698 comparing microbial communities, *Applied and Environmental Microbiology*, 75(23):7537-7541,
699 doi: http://dx.doi.org/10.1128/AEM.01541-09, 2009.
700 Shen, J. P., Zhang, L. M., Di, H. J., and He, J. Z.: A review of ammonia-oxidizing bacteria and
701 archaea in Chinese soils, *Frontiers in Microbiology*, *3*, 296,
702 https://doi.org/10.3389/fmicb.2012.00296, 2012.
703 Simas, F. N. B., Schaefer, C. E. G. R. , Melo, V. F., Albuquerque-Filho, M. R. A., Michel, R. F. M.,
704 Pereira, V. V., et al.: Ornithogenic cryosols from Maritime Antarctica: Phosphatization as a soil
705 forming process, *Geoderma*, *138*(3–4), 191–203, https://doi.org/10.1016/j.geoderma.2006.
706 11.011, 2007.
707 Silva, A. F., Carvalho, G., Oehmen, A., Lousada-Ferreira, M., van Nieuwenhuijzen, A., Reis, M.
708 A., and Crespo, M. T. B.: Microbial population analysis of nutrient removal-related organisms in
709 membrane bioreactors, Applied Microbiology and Biotechnology, 93(5), 2171-2180,
710 https://doi.org/10.1007/s00253-011-3499-5, 2012b.

711 Silva, M. C., Poly, F., Guillaumaud, N., van Elsas, J. D., and Falcão Salles, J.: Fluctuations in
712 ammonia oxidizing communities across agricultural soils are driven by soil structure and pH,
713 *Frontiers in Microbiology*, 3, 77, https://doi.org/10.3389/fmicb.2012.00077, 2012a.
714 Sterngren, A. E., Hallin, S., & Bengtson, P.: Archaeal ammonia oxidizers dominate in numbers, but
715 bacteria drive gross nitrification in N-amended grassland soil, *Frontiers in Microbiology*, *6*, 1350,
716 https://doi.org/10.3389/fmicb.2015.01350, 2015.
717 Stieglmeier, M., Mooshammer, M., Kitzler, B., Wanek, W., Zechmeister‐Boltenstern, S., Richter,
718 A., and Schleper, C.: Aerobic nitrous oxide production through N-nitrosating hybrid formation in
719 ammonia-oxidizing archaea, *The ISME Journal*, 8, 1135–1146, 2014.
720 Stopnišek, N., Gubry-Rangin, C., Höfferle, Š., Nicol, G. W., Mandič-Mulec, I., and Prosser, J. I. :
721 Thaumarchaeal ammonia oxidation in an acidic forest peat soil is not influenced by ammonium
722 amendment, *Applied and Environmental Microbiology*, 76(22), 7626-7634,
723 https://doi.org/10.1128/AEM.00595-10, 2010.
724 Sun, L. G., Liu, X. D., Yin, X. B., Zhu, R. B., Xie, Z. Q., and Wang, Y. H.: A 1,500-year record of
725 Antarctic seal populations in response to climate change, *Polar Biology*, *27*, 495–501,
726 https://doi.org/10.1007/s00300-004-0608-2, 2004.
727 Sun, L. G., Xie, Z. Q., and Zhao, J. L.: Palaeoecology: A 3,000-year record of penguin
728 populations, *Nature*, *407*, 858, https://doi.org/10.1038/35038163, 2000.
729 Sun, L. G., Zhu, R. B., Xie, Z. Q., and Xing, G. X.: Emissions of nitrous oxide and methane from
730 Antarctic tundra: role of penguin dropping deposition, *Atmospheric Environment*, *36*(31), 4977-
731 4982, https://doi.org/10.1016/S1352-2310(02)00340-0, 2002.

Tatur, A., Myrcha, A., and Niegodzisz, J.: Formation of abandoned penguin rookery ecosystems in the maritime Antarctic, *Polar Biology*, *17*, 405–417, https://doi.org/10.1007/s003000050135, 1997.

Tolar, B. B., Ross, M. J., Wallsgrove, N. J., Liu, Q., Aluwihare, L. I., Popp, B. N., and Hollibaugh, J. T.: Contribution of ammonia oxidation to chemoautotrophy in Antarctic coastal waters, *The ISME Journal*, *10*, 2605, https://doi.org/10.1038/ismej.2016.61, 2016.

Verhamme, D. T., Prosser, J. I., and Nicol, G. W.: Ammonia concentration determines differential growth of ammonia-oxidising archaea and bacteria in soil microcosms, *The ISME Journal*, *5*(6), 1067–1071, https://doi.org/10.1038/ismej.2010.191, 2011.

Wang, B. Z., Zhao, J., Guo, Z. Y., Ma, J., Xu, H., and Jia, Z. J.: Differential contributions of ammonia oxidizers and nitrite oxidizers to nitrification in four paddy soils, *The ISME Journal*, *9*, 1062–1075, https://doi.org/10.1038/ismej.2014.194, 2015.

Wessén, E., Söderström, M., Stenberg, M., Bru, D., Hellman, M., Welsh, A., et al.: Spatial distribution of ammonia-oxidizing bacteria and archaea across a 44-hectare farm related to ecosystem functioning, *The ISME Journal*, *5*, 1213–1225, https://doi.org/10.1038/ismej.2010.206, 2011.

Wuchter, C., Abbas, B., Coolen, M. J. L., Herfort, L., van Bleijswijk, J., Timmers, P., et al.: Archaeal nitrification in the ocean, *Proceedings of the National Academy of Sciences*, *103*(33), 12317–12322, https://doi.org/10.1073/pnas.0600756103, 2006.

751 Xia, Y., Zhu, Y. G., Gu, Q., He, J. Z.: Does long-term fertilization treatment affect the response of
752 soil ammonia-oxidizing bacterial communities to Zn contamination?, *Plant and Soil*, *301*(1-2),
753 245-254, https://doi.org/10. 1007/s11104-007-9441-z, 2007.
754 Xie, Z., Le Roux, X., Wang, C., Gu, Z., An, M., Nan, H., et al.: Identifying response groups of soil
755 nitrifiers and denitrifiers to grazing and associated soil environmental drivers in Tibetan alpine
756 meadows, *Soil Biology and Biochemistry*, 77, 89-99, https://doi.org/10.1016/j.soilbio.2014.06.
757 024, 2014.
758 Yang, Y., Shan, J., Zhang, J., Zhang, X., Xie, S., and Liu, Y. : Ammonia-and methane-oxidizing
759 microorganisms in high-altitude wetland sediments and adjacent agricultural soils, *Applied*
760 *Microbiology and Biotechnology*, 98(24), 10197-10209, https://doi.org/10.1007/s00253-014-
761 5942-x, 2014.
762 Yao, H. Y., Gao, Y. M., Nicol, G. W., Campbell, C. D., Prosser, J. I., Zhang, L. M., et al.: Links
763 between ammonia oxidizer community structure, abundance, and nitrification potential in acidic
764 soils, *Applied and Environmental Microbiology*, *77*, 4618–4625, https://doi.org/
765 10.1128/AEM.00136-11, 2011.
766 Yergeau, E., Kang, S., He, Z., Zhou, J., and Kowalchuk, G. A.: Functional microarray analysis of
767 nitrogen and carbon cycling genes across an Antarctic latitudinal transect, *The ISME Journal*, *1*(2),
768 163–179, https://doi.org/10.1038/ismej.2007.24, 2007.
769 Zhang, L. M., Wang, M., Prosser, J. I., Zheng, Y. M., and He, J. Z.: Altitude ammonia-oxidizing
770 bacteria and archaea in soils of Mount Everest, *FEMS Microbiology Ecology*, *70*(2), 208–217,
771 https://doi.org/10.1111/j.1574-6941.2009.00775.x, 2009.

772 Zhao, K., Kong, W. D., Khan, A., Liu, J. B., Guo, G. X., Muhanmmad, S., et al.: Elevational
773 diversity and distribution of ammonia-oxidizing archaea community in meadow soils on the
774 Tibetan Plateau, *Applied Microbiology and Biotechnology*, *101*(18), 7065–7074, https://doi.org/
775 10.1007/s00253-017-8435-x, 2017.
776 Zhao, X., Wang, S., and Xing, G.: Nitrification, acidification, and nitrogen leaching from
777 subtropical cropland soils as affected by rice straw-based biochar: laboratory incubation and
778 column leaching studies, *Journal of soils and sediments*, *14*(3), 471-482, 2014.
779 Zheng, Y. K., Wang, X. B., Gu, Y. F., and Zhang, X. P.: Diversity of ammonia-oxidizing archaea in
780 Tibetan Zoige plateau wetland, *Acta Microbiologica Sinica*, *54*(9), 1090–1096, 2014.
781 Zheng, Y. L., Hou, L. J., Liu, M., Lu, M., Zhao, H., Yin, G. Y., and Zhou, J. L.: Diversity, abundance,
782 and activity of ammonia-oxidizing bacteria and archaea in Chongming eastern intertidal
783 sediments, *Applied Microbiology and Biotechnology*, *97*, 8351–8363, https://doi.
784 org/10.1007/s00253-012-4512-3, 2013.
785 Zheng, Y. L., Hou, L. J., Newell, S., Liu, M., Zhou, J. L., Zhao, H., You, L. L., and Cheng, X. L.:.
786 Community dynamics and activity of ammonia-oxidizing prokaryotes in intertidal sediments of
787 the Yangtze estuary, *Applied and Environmental Microbiology, 80*, 408,
788 https://doi.org/10.1128/AEM.03035-13, 2014.
789 Zhu, R. B., Liu, Y. S., Xu, H., Ma, D. W., and Jiang, S.: Marine animals significantly increase tundra
790 N_2O and CH_4 emissions in maritime Antarctica, *Journal of Geophysical Research:*
791 *Biogeosciences*, *118*(4), 1773–1792, https://doi.org/10.1002/2013JG002398, 2013.

792 Zhu, R. B., Liu, Y. S., Xu, H., Ma, J., Zhao, S. P., and Sun, L. G.: Nitrous oxide emissions from sea
793 animal colonies in the maritime Antarctic, *Geophysical Research Letters*, *35*(9), L09807,
794 https://doi.org/10.1029/2007GL032541, 2008.
795 Zhu, R. B., Shi, Y., Ma, D. W., Wang, C., Xu, H., and Chu, H. Y.: Bacterial diversity is strongly
796 associated with historical penguin activity in an Antarctic lake sediment profile. *Scientific*
797 *Reports*, *5*, 17231., https://doi.org/10.1038/srep17231, 2015.
798 Zhu, R. B., Sun, J. J., Liu, Y. S., Gong, Z. J., and Sun, L. G.: Potential ammonia emissions from
799 penguin guano, ornithogenic soils and seal colony soils in coastal Antarctica: effects of freezing-
800 thawing cycles and selected environmental variables, *Antarctic Science*, *23*(1), 78–92,
801 https://doi.org/10.1017/S0954102010000623, 2011.
802 Zhu, R. B., Wang, Q., Ding, W., Wang, C., Hou, L. J., and Ma, D. W.: Penguins significantly
803 increased phosphine formation and phosphorus contribution in maritime Antarctic soils, *Scientific*
804 *Reports*, *4*, 7055, https://doi.org/10.1038/srep07055, 2014.

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±0.6)$\times10^{6b}$	(5.9±3.5)$\times10^{6c}$
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±2.7)$\times10^{6b}$	(1.2±0.5)$\times10^{7c}$

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%

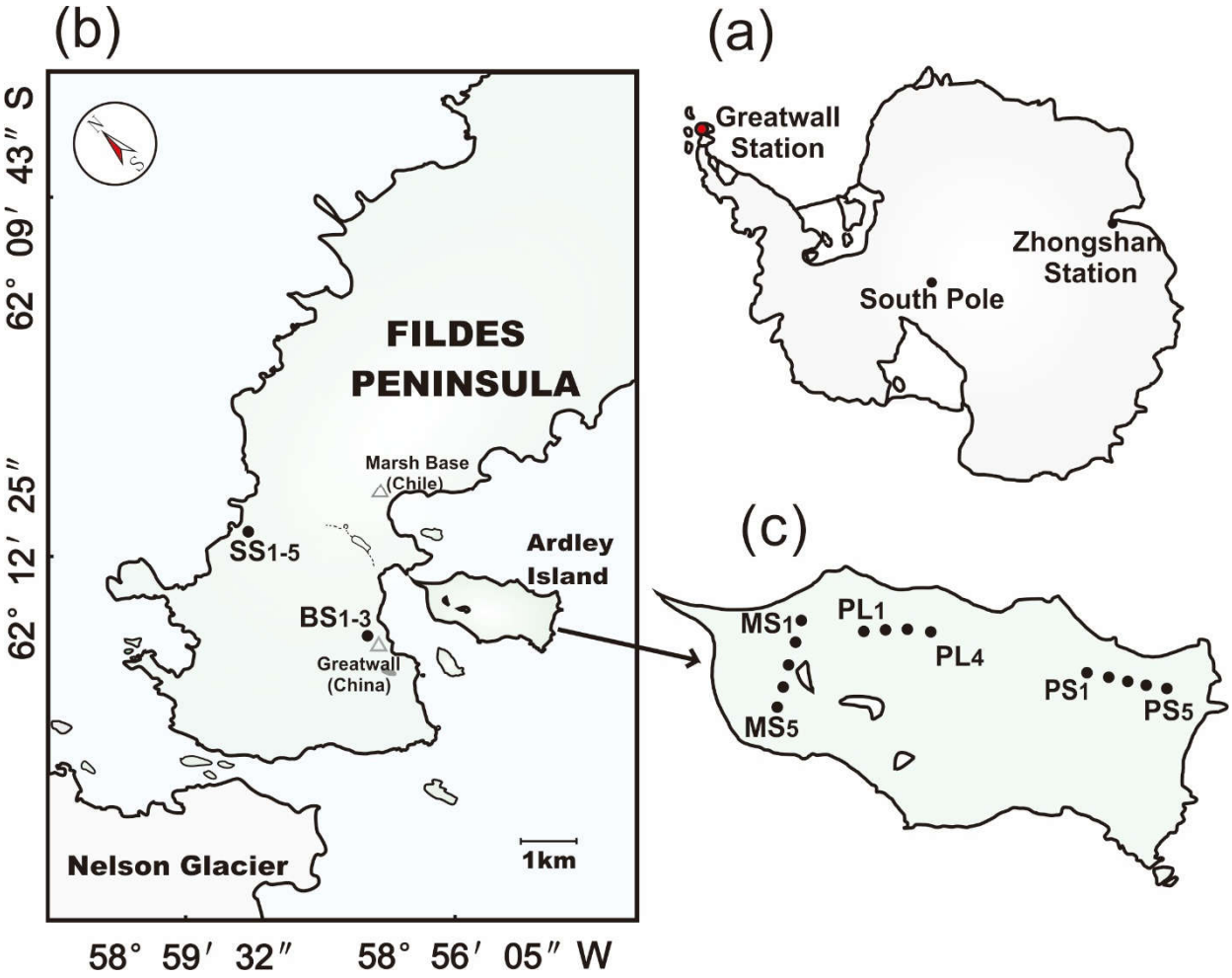


Figure 1. Study area and soil sampling sites. Panel (a), the red dot indicates the location of the investigation area in maritime Antarctica. Panel (b), location of the sampling sites on the Fildes Peninsula. The sampling soils from tundra patches included the active seal colony tundra soils SS (SS1–5) 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/).

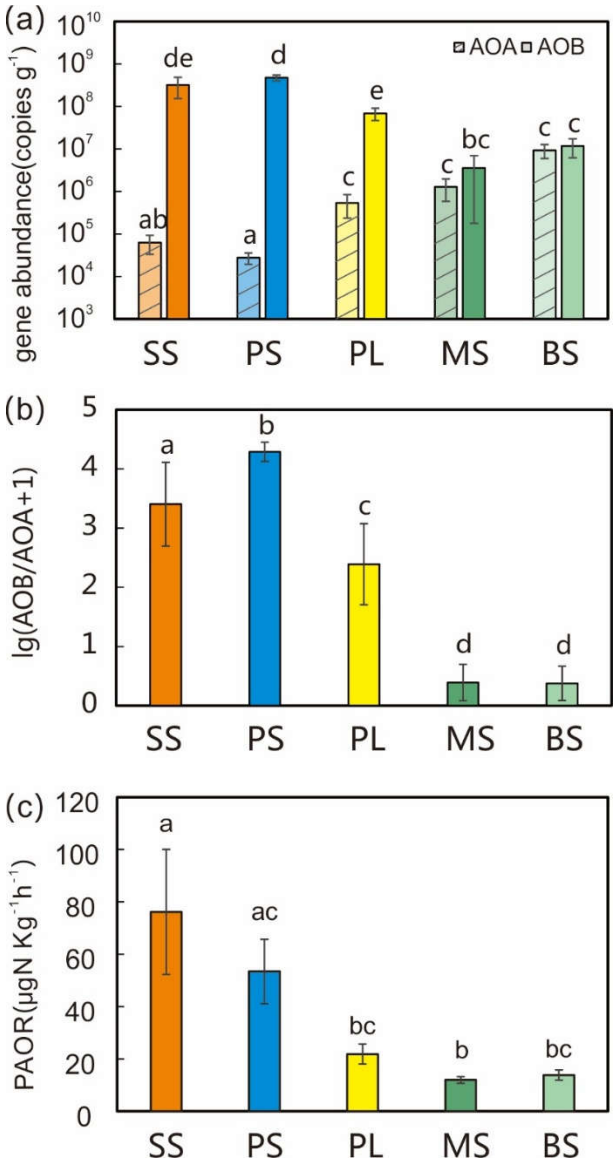


Figure 2. Comparisons of soil AOA and AOB *amoA* gene copy numbers (a), log ratio of AOB: AOA abundances (b), and potential ammonia oxidation rates (PAOR) (c) between five tundra patches. The error bars indicate standard deviations of the means.

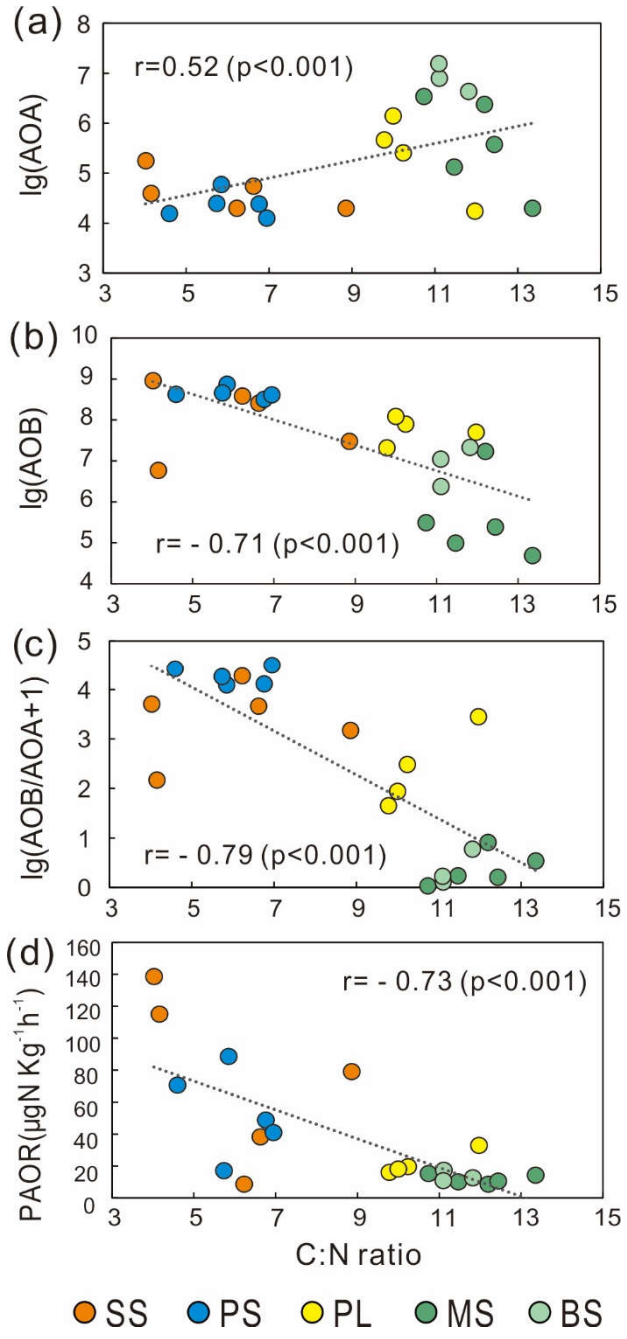


Figure 3. Effects of soil C:N alteration on AOA and AOB abundances, and potential ammonia oxidation rates (PAOR) at five tundra patches.

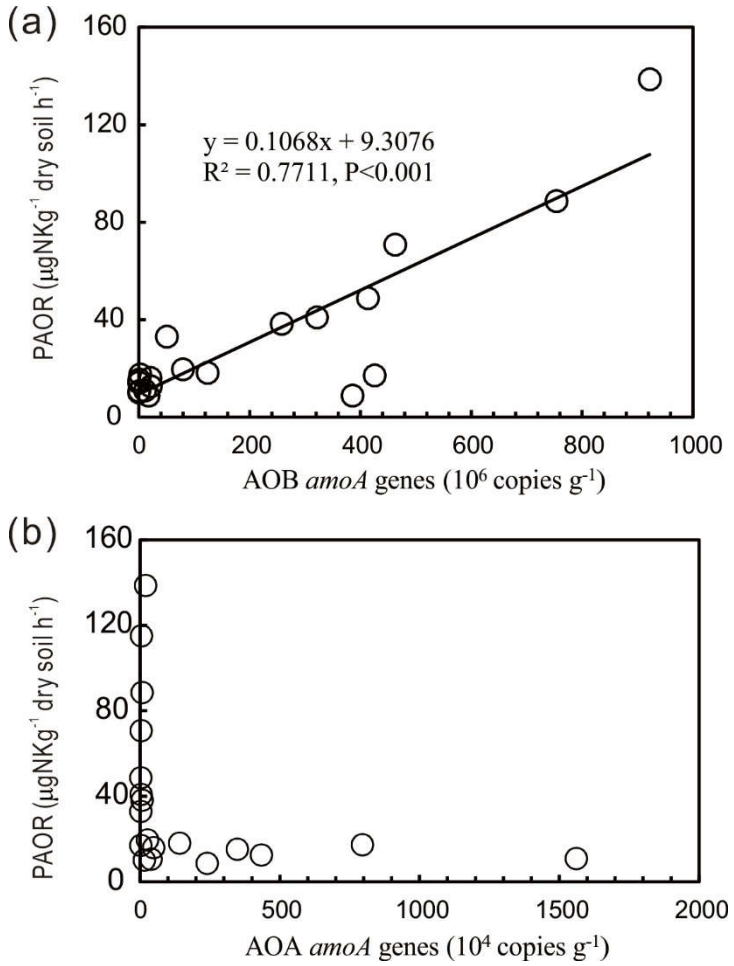
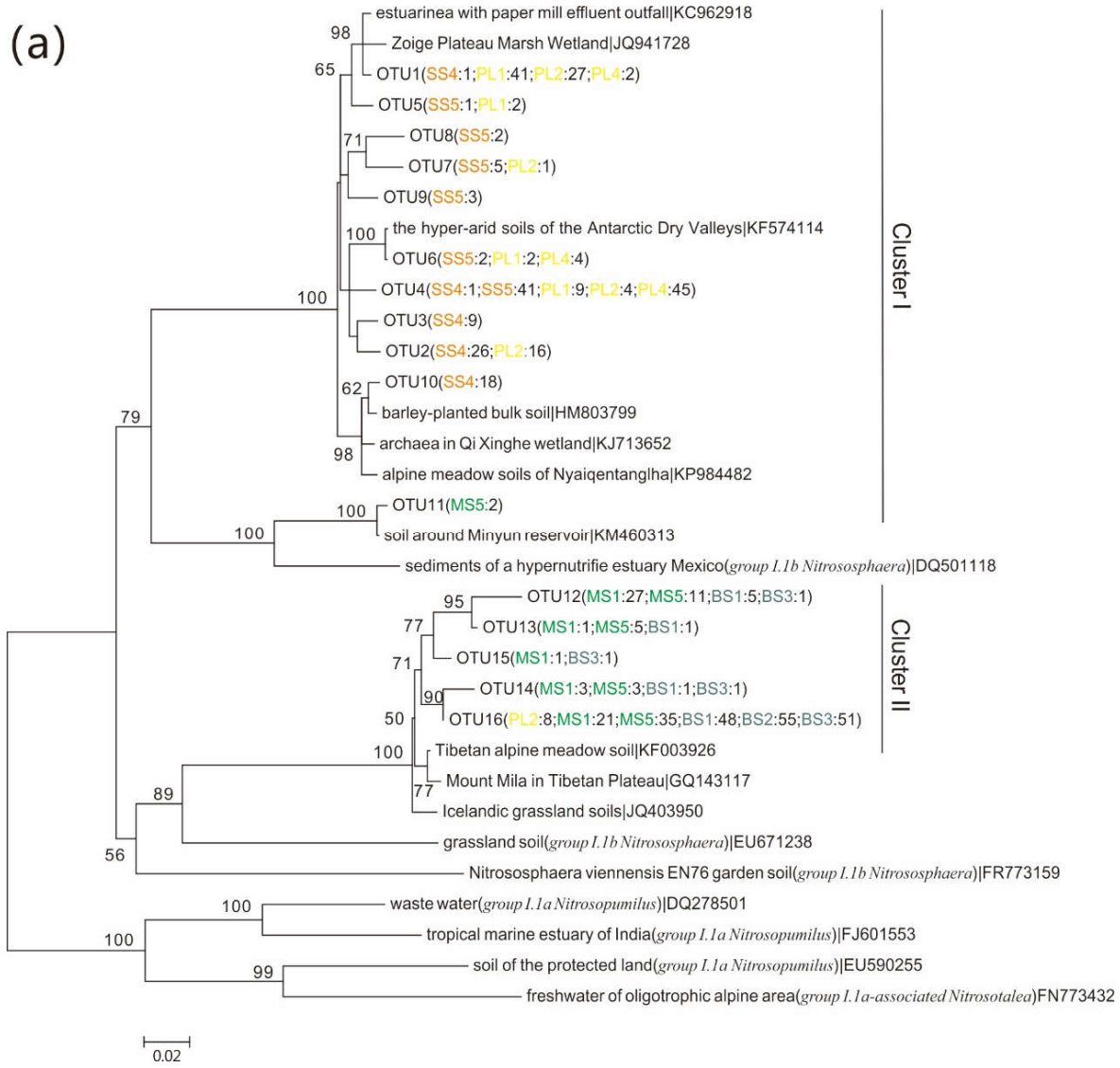
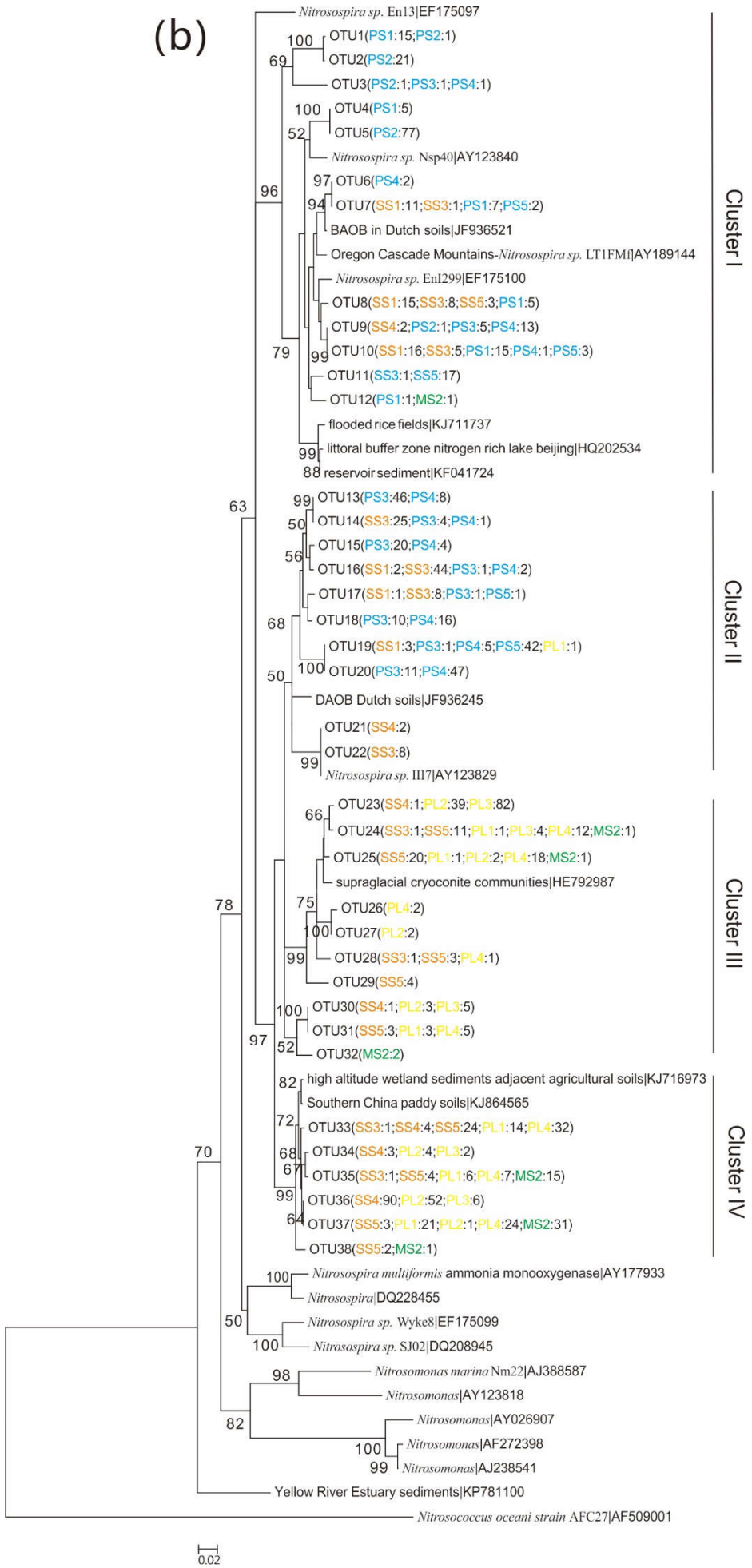


Figure 4. Correlation between potential ammonia oxidation rates (POARs) and AOA and AOB *amoA* gene copy numbers in tundra soils of maritime Antarctica.

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. III7|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

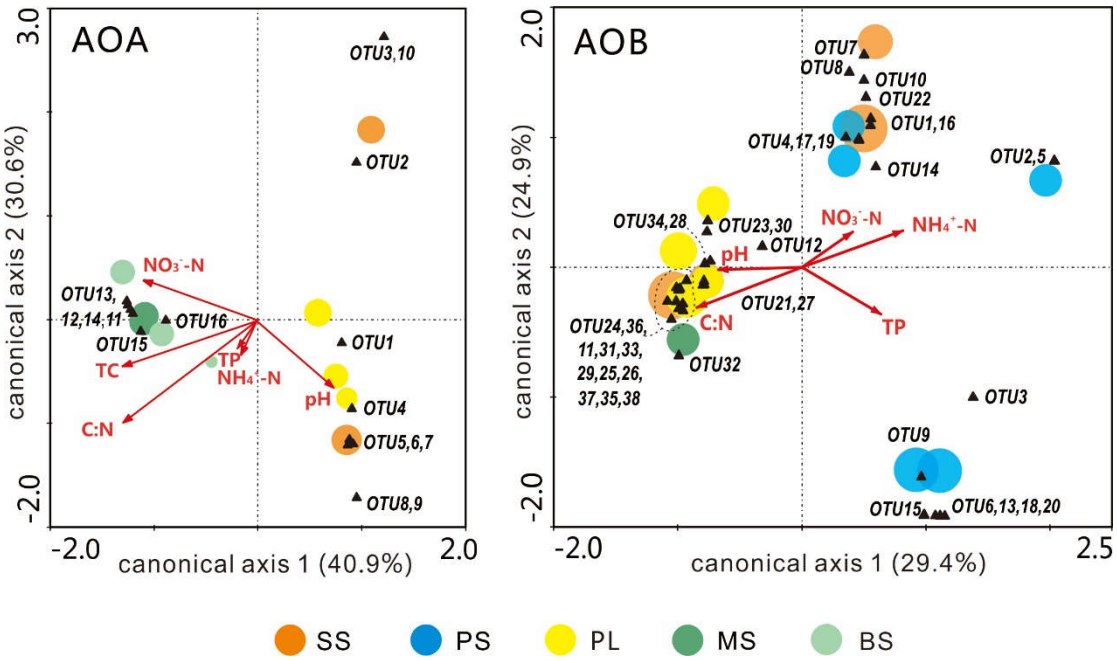


Figure 6. Canonical correspondence analysis (CCA) ordination plots for the relationship between the AOA and AOB community structures with environmental variables. The circles with different colors represent the various sampling sites. The size of the circles corresponds to the OTU richness in individual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

Supplementary Material for

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: Email: zhurb@ustc.edu.cn; or ljhou@sklec.ecnu.edu.cn; Tel: 0086-551-3606010; Fax: 0086-551-360758

Contents of this file

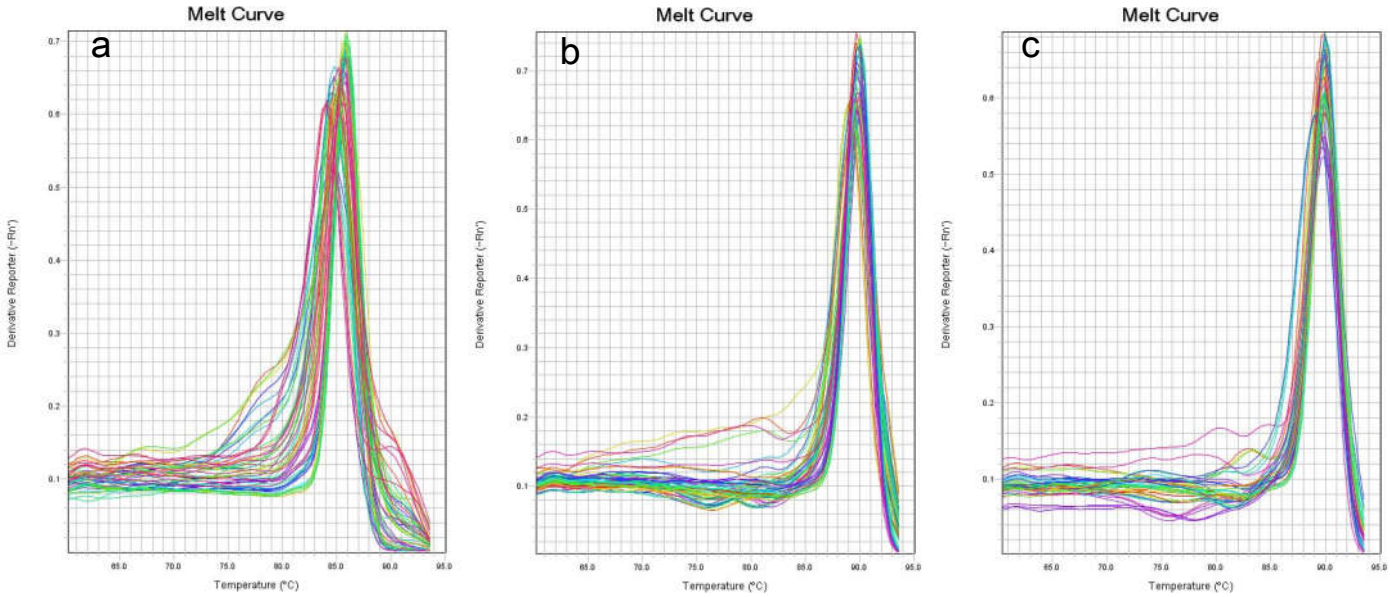


Fig. S1. Melting curve analysis had only one observable peak at a melting temperature (Tm=84.9 °C for AOA (a), Tm=89.6 °C for β-AOB (b, c)), no detectable peaks associated with primer-dimer artifacts or other non-specific PCR amplification products were observed.

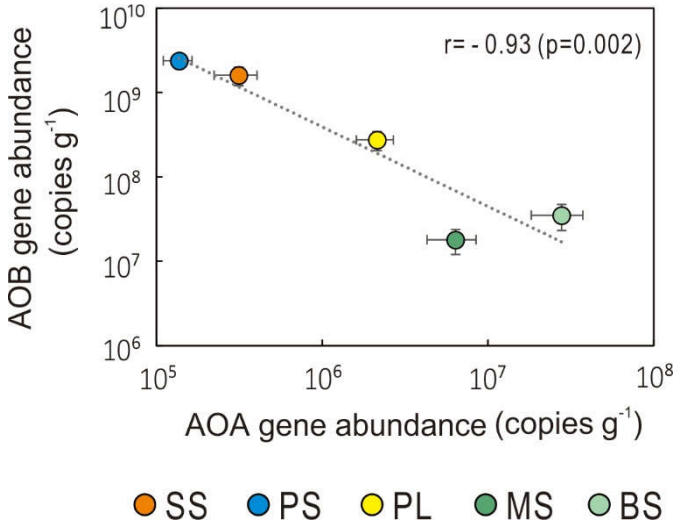


Fig. S2. The correlation between the abundances of AOB and AOA amoA genes across all the tundra patches.

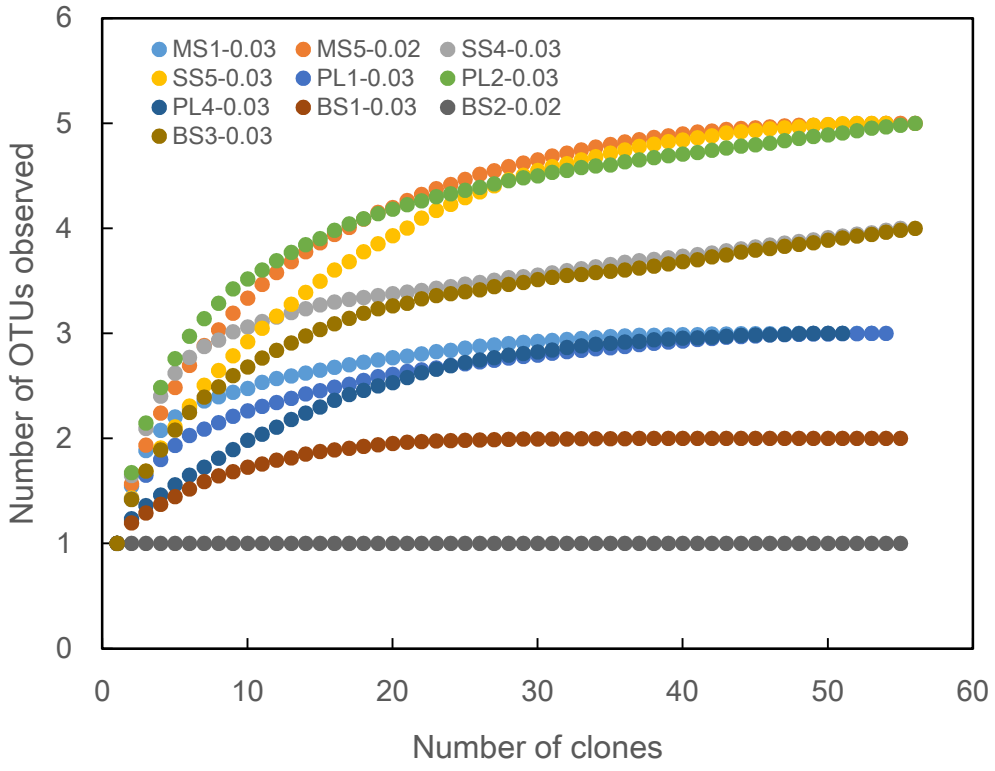


Fig. S3. Rarefaction curves of the ammonia oxidizing archaeal (AOA) clone libraries. OTUs are defined at 3 % divergence in nucleotides.

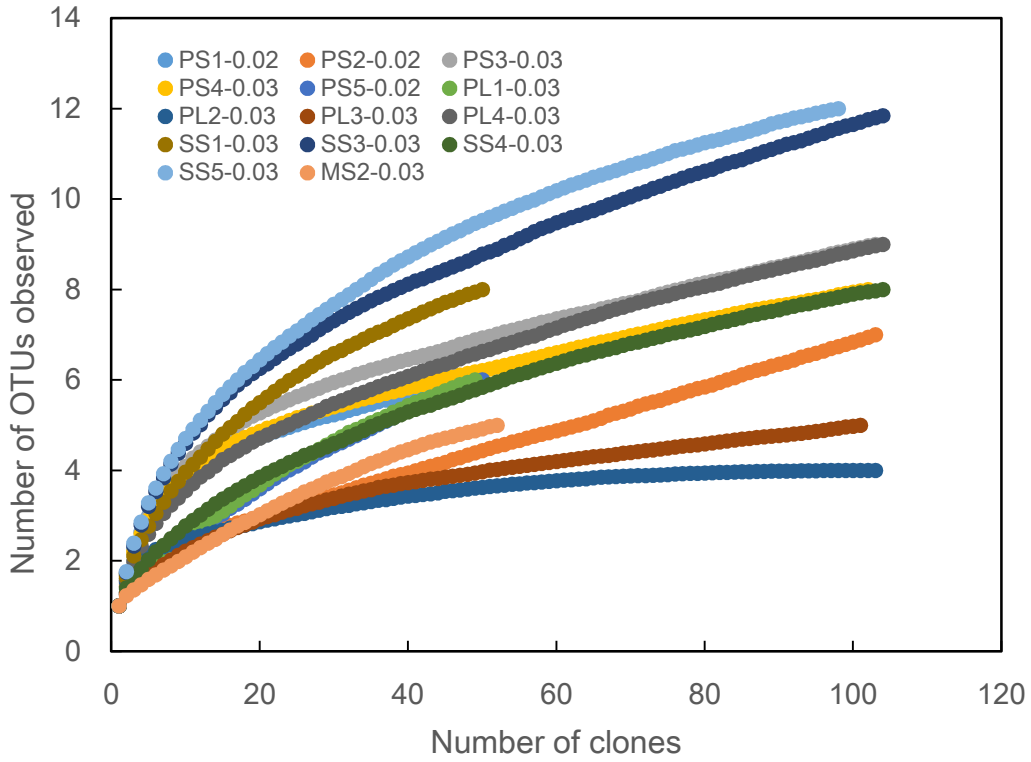


Fig. S4. Rarefaction curves of the ammonia oxidizing bacteria (AOB) clone libraries. OTUs are defined at 3 % divergence in nucleotides.

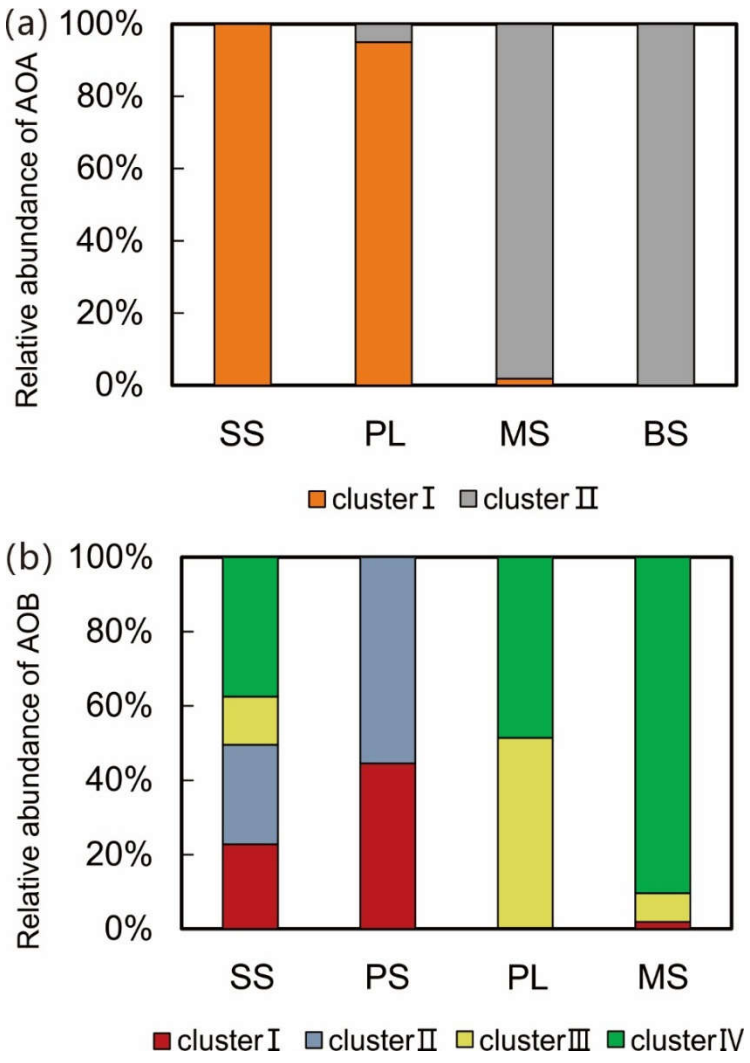


Fig. S5. Relative abundance of partial AOA (a) and AOB (b) sequences retrieved from five tundra patch soils subjected to different effects of sea animal activities, as related to different *Nitrososphaera* or *Nitrosospira* clusters.

Table S1. Diversity characteristics of clone libraries of AOB and AOA.

Sample	No. of clones	OTUs[a]	Chao1[b]	Shannon-Wiener[c]	1/Simpson[d]	Coverage (%)[e]
AOA						
SS4	55	5	6	1.16	2.89	83.3%
SS5	54	6	6	0.91	1.71	100.0%
PL1	54	4	4	0.75	1.67	100.0%
PL2	57	5	5	1.25	3.05	100.0%
PL4	51	3	3	0.44	1.28	100.0%
MS1	53	5	6	1.02	2.44	83.3%
MS5	56	5	5	1.10	2.32	100.0%
BS1	55	4	5	0.48	1.30	80.0%
BS2	55	1	1	0.00	1.00	100.0%
BS3	54	4	5	0.28	1.12	80.0%
AOB						
SS1	50	8	9.5	1.59	4.31	84.2%
SS3	107	15	25	1.82	4.23	60.0%
SS4	104	8	9	0.64	1.33	88.9%
SS5	98	15	18	2.17	6.97	83.3%
PS1	49	7	8	1.10	4.69	87.5%
PS2	103	7	9	0.77	1.68	77.8%
PS3	103	13	18	1.73	3.92	72.2%
PS4	102	13	16.3	1.77	3.89	79.6%
PS5	50	6	7.5	0.68	1.42	80.0%
PL1	49	9	11	1.55	3.69	81.8%
PL2	103	7	7	1.14	2.52	100.0%
PL3	101	7	7.5	0.78	1.51	93.3%
PL4	104	11	14	1.84	5.24	78.6%
MS2	52	7	10	1.10	2.32	70.0%

a. OTUs are defined at 3% nucleotide acid divergence.

b. Nonparametric statistical predictions of total richness of OTUs based on distribution of singletons and doubles.

c. Shannon diversity index. A higher number represents more diversity.

d. Reciprocal of Simpson's diversity index. A higher number represents more diversity.

e. Percentage of coverage: percentage of observed number of OTUs divided by Chao1 estimate.