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_4Cl and incubating the samples at 15 degrees at 15 degree, 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".

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 soil physiochemical properties 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.

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.

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.

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 "**oxic 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.

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".**

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

Author response: This has been corrected into **"A great majority"**

Line 346: typo in "reported"

Author response: This has been corrected in the revised manuscript.

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).

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.

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 moved to the section “Results”.