

Interactive comment on “Major role of ammonia-oxidizing bacteria in N₂O production in the Pearl River Estuary” by L. Ma et al.

Anonymous Referee #1

Received and published: 5 June 2019

Ma et al. investigated the relationship between N₂O production and spatial distribution of AOA and AOB along a salinity gradient in the Pearl River Estuary, China by using qPCR, chemical analysis and in situ incubation experiment. Data are well analyzed and presented. However, the manuscript's structure should be modified because the some results were presented in the discussion section, and some conclusions needs to be re-phrased because the main findings in this study were mainly based on the correlation analysis OR statistical analysis (e.g., between N₂O production and the abundance of functional genes), which can't provide a solid support for a causal relationship between microbial contributors and N₂O production. More specific comments and suggestions are given below:

1. As mentioned by authors, both nirK and nirS genes are the key functional genes in

C1

the denitrification pathway, so why did not determine the abundance of nirK gene here?

2. Page 7, line 18-19, make subscript for some chemistry formulas (N₂O, NH₃ etc.);
3. Page 7, line 24, please correct the P value using the Bonferroni correction or other multiple-comparison methods;
4. Page 7, line 25, and Fig. 5. Please check the multicollinearity problems before perform the RDA analysis. Some environmental parameters are highly correlated with each other, some of them should be removed from the RDA analysis;
5. Page 8, line 5-8 and Fig. 6. I am not convinced with the usage of Mantel and partial Mantel tests here due to two following reasons: 1) for ammonia oxidizer community, actually there were only four variables based on qPCR analysis (PA AOA, FL AOA, PA AOB and FL AOB) but not community data based on sequencing, so I don't think the results of qPCR reflected the truly community composition of ammonia oxidizers; and 2) the authors divided the environmental into four groups, but the classification seems a bit confusing. For example, why classify silicate into water mass but not substrate parameters? And TSM, DO and pH were classify as water mass parameters by numerous previous studies;
6. Page 8, line 20, is the 63.0 $\mu\text{mol/L}$ the hypoxic threshold?
7. Page 9, line 11, please re-phrase this subtitle because only the transcripts of amoA and nirS genes from two freshwater stations were quantified here;
8. Page 12, line 12-13, too much speculation;
9. Page 12, line 15-26, please move this part into Results section, and again, I don't think the classification for environmental parameters is on the right way;
10. Page 12, line 23, “positive correlations between AOB amoA abundances and all N₂O parameters”, should be except for FL AOB;
11. Page 12, line 27, the results of RDA analysis also should be presented in Results

C2

section;

12. The most part of first paragraph of 4.3 subsection should be moved into Results section;

13. How about the potential role of comammox and nirK-type denitrifier for N₂O production in PRE, please discuss it in the 4.3 subsection.

14. Fig. 7. It is a little difficult to understand this figure. It seems like the AOA contributed more for N₂O production and yield in site P01, right?

15. Table 2, Spearman rank correlation analysis generate a rho () value rather than a R value.

Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2019-132>, 2019.