

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

Anonymous Referee #2

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Major comments:

More caution is needed on these concentration-based "rate" measurements. Without isotope tracers, very little can be said about actual rates. Evidence for this is in the N₂O yields. The yields reported here are about 100X lower than ever reported from cultures or the field (see Ji et al. 2018 GBC).

The strength of the correlation between genes and rates absolutely cannot be used to apportion a relative importance of one group of ammonia oxidizers or the other to the total rates. Nothing can be concluded from the data presented about who the important nitrifiers are. One possibility would be to obtain to a range of cell-specific ammonia oxidation rates from the literature and then use those in combination with the qPCR data to calculate the relative contribution of each group to the observed

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"rates."

The literature review, both in the Introduction and Discussion, is severely lacking. There is a substantial literature about nitrification and N₂O production in estuaries, almost none of which are referenced here. Normally I would provide some specific suggestions, but the omissions are too vast to list. One place to start would be a review by Damashek and Francis 2018 Estuaries and Coasts, or a nice earlier paper with a summary of nitrification rates in estuaries, Damashek et al. 2016 Estuaries and Coasts.

The nirS data are not very useful to this manuscript in that there is essentially no relationship between nirS abundance and N₂O production from denitrification. nirS presence could just as easily be a marker for N₂O consumption.

All the physical dynamics in the system have been reduced to a very naive "water mass" identification. Basic concepts in estuarine biogeochemistry are absent—for example, using salinity as a conservative tracer in a two-end member mixing model to determine production and loss of the various biogeochemical parameters.

Specific comments:

p. 3 lines 15-16 Unclear to me what is meant by "runoff ranked 17th".

p. 5 lines 2-4 What N₂O standards were used? How was the GC calibrated?

p. 5 line 6 How was N₂O aquatic calculated?

p. 7 lines 3 How much did DO concentration change over the course of the 24 h incubations? What effect would this have on the measured N₂O production?

p. 7 lines 18-19 Were both N₂O yield equations used? Compared? Were they equal?

More details are needed about how you arrived at the Schmidt number for N₂O. Is this the Raymond and Cole reference?

Need additional details of the calibration of the isotopic values.

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p.7 Why is N₂O yield in units of permil? (line 18-19, and also in the Discussion). Also would be more conventional to list this as N₂O-N not N-N₂O

No discussion of particle attached versus free living amoA copies. Data is presented in multiple figures. Previous literature show no association. Did the filters clog?

P. 9 lines 4-6 “the entire PRE acts as a N₂O source” but negative air-sea fluxes are reported in the previous sentence?

p.11 lines 19-26: This paragraph confuses some important concepts. Some of these numbers are the isotopic composition of N₂O produced by ammonia oxidizers, but some of these numbers are the isotope effect (epsilon). Also, the isotopic composition of the N₂O being produced by nitrification is dependent on the isotopic composition of the NH₃ being oxidized, for which no measurements or even estimates are provided.

p.12 lines 15-17: Doesn't make sense to refer to 'water masses' in estuaries. There is a tremendous amount of mixing that leads to variation in these parameters. Just because something is a different salinity doesn't mean it's a different 'water mass.' These parameters are just 'hydrography.'

p. 12 lines 15-28 and p. 13 lines 1 – 18 A lot of results presented that should be moved to the results section.

p. 12 line 27 “ammonia oxidizer community” The use of the word “community” throughout the paper is confusing. More accurate to state the abundances of AOA and AOB?

p. 24 Fig 1 i,j It looks like two different slopes in the data upstream and Lingdingyang. This could be quantified using a break point analysis.

p. 32 I found this figure confusing. Perhaps it would be useful to have a table with the data presented in the figure? It is unclear using AOB and AOA% if the normalized N₂O production values are a result of the N₂O yield or low/high amoA abundance.

Technical corrections p. 2 lines 18-22 Needs citation

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“Denitrification by heterotrophic denitrifiers is another major pathway of N₂O production in marine environments. NO₂⁻ is reduced by a copper-containing (NirK) or cytochrome cd1-containing nitrite reductase (NirS) to nitric oxide (NO), and then by a heme-copper NO reductase (NOR) to N₂O.”

p.3 lines 3 citation should be after “soil”

“and arable (Clark et al., 2012; Jones et al., 2014) soils”

p. 3 lines 10-11 Needs citation

“Moreover, there is a potential niche overlap between nitrifiers and denitrifiers in low oxygen conditions.”

p. 4 lines 15-16 Should be moved to results section 2.2 discussing ammonia analysis

“Ammonia/ammonium concentrations were analyzed onboard.”

p. 4 line 25-26 What salinity, temperature and DO probes were used?

p. 5 lines 5-23 Not all variables in the equations are defined.

p. 31 Fig 6 Should axes be swapped?

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