

## ***Interactive comment on “New Insight to Niche Partitioning and Ecological Function of Ammonia Oxidizing Archaea in Subtropical Estuarine Ecosystem” by Yanhong Lu et al.***

**Anonymous Referee #2**

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I feel that this manuscript contains valuable information regarding ammonia oxidizing archaea in estuarine systems, particularly in that it focuses on processes occurring in the water column rather than the sediment, which, as the authors point out, is understudied. However, there are numerous issues with the manuscript in its current form.

First and foremost, there are serious issues throughout the manuscript with grammar and syntax. Sometimes these issues are so severe that they obscure the meaning of the text. This made it difficult to grasp the authors' meaning and to review the manuscript effectively.

In general, the description of the methods is unclear and lacking in detail. For example:

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line 78: "the 10-50m by 10m interval" What does this mean? lines 87-89: "Sea water was prefiltered... analysis (Liu et al. 2014)." Which analysis was this performed for? line 93: "Community respiration rates were measured" in what? Microcosms? Incubations are mentioned but no volume is given, whether a headspace was left in the bottle... line 94: "running seawater" Outside the (unmentioned) bottle? line 95: "less 10%" Does this mean "less than 10%"? line 96: "The del-15N in NO- x the product of nitrification" I have no idea what this means. line 97: "denitrifier method" What is that? The authors provide citations but for methods but do not explain what they are or how they are performed. Similarly the measurement of the nitrification rate is not described, only cited in an unpublished manuscript. lines 110-111: "Fast DNA SPIN Kit for Soil" Why would you use a soil kit for filter samples from seawater? line 117: "transpired" I assume you mean "transferred" line 136: "the DNA mixture" I don't know what is meant by this. DNA and cDNA?

Because the methods were so unclear in general, it is difficult for me to assess whether the claims made in the results and discussion sections are to be believed. For example, AOA and AOB copy numbers are referred frequently as evidence of dominance of one group over the other. Is this a rational claim, particularly without 16S data to support it? How many copy numbers of the amoA gene do AOA have vs AOB? And if archaeal amoA transcripts are more abundant than bacterial amoA transcripts, does that mean the archaea are more abundant or simply more active? Is the difference in gene/transcript number statistically significant?

As for the measurement of nitrification rates, so little detail is given regarding how these numbers were reached, as to render the data meaningless.

The sections on spatial distribution were in general unclear and difficult to follow.

More specific comments:

line 223: "B-proteobacteria amoA were under detection limit" Not in all your samples though, judging by Figure 5? line 257: "Besides" Besides what? What is meant by

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this? line 270: "heterotrophic bacteria abundance" How was this determined? It's not described in the methods. lines 271-272: "Nutrient concentration showed an opposite pattern comparing with salinity" I have no idea what this means. line 274: "which may be introduced by" Again, no idea. lines 295-296: "Intensive nitrification... oxygen consumption (Pakulski et al. 1995)." Was that observed in this study or in the study cited? lines 300-301: "It is well known... organic matter degradation (respiration)." Be that as it may, you still have to cite it- and it's hardly proof that ammonia is supplied to nitrification by this process. line 305: 229.21% oxygen consumption? How do you consume more than 100% of something in a closed microcosm? lines 328-329: "Though size-fractionated... were observed." I don't understand what is meant here. line 330: "higher substrate requirement" of what substrate?

In multiple locations in the document the authors mention previous DNA-based studies of AOA and how such studies may overlook active AOA populations. To begin with, those populations would not be overlooked, but perhaps underrepresented in the data. Additionally, several culture-independent studies of AOA activity utilizing stable isotope probing (in particular, the use of urea as a substrate, and heterotrophy) have been performed in both salt marsh sediment (Seyler et al., 2014, ISME J) and the open ocean (Seyler et al., 2018, FEMS Microbiol Ecol; Seyler et al., 2019, Frontiers Mar Sci), and none of these studies are cited in the text. AOA activity has also been previously described in an estuarine water column using similar techniques to this manuscript (Horak et al., 2013, ISME J; Happel et al., 2018, Env Microbiol)- these should be cited in the text.

As for the figures:

Figure 6 is impossible to read. Could it be separated into two figures by size fraction? Otherwise there's just too much going on.

Figure 7 has me completely puzzled. Firstly because the figure has no axes or scale. Secondly because there's no description of how NDMS analysis was performed in the

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text. But most importantly, how is it possible that there is absolutely no overlap between the DNA and RNA sequences? I find this incredibly difficult to believe. Are the DNA and RNA sequence data even capturing the same community?

Figure 8 I think is very interesting, but some of the pie charts are so small as to be illegible.

Figure 9 contains some of the most interesting data in the paper, but the figure needs improvement. I think you could combine this heatmap with your phylogenetic tree, and move Figure 6 to supplemental.

Overall I believe the findings presented in this manuscript are likely of interest to the community. The correlations of various AOA lineages to geochemical data and sampling location are very interesting, if difficult to parse in the manuscript's current format. But the issues with the methods in particular and the text in general made it difficult to understand the findings, and some of the claims lack sufficient evidence. I would very much like to see this manuscript again, after significant revisions.

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