

## general comments

This study by *Lu et al.* provides valuable new insights into the distribution of ammonia-oxidizing archaea (AOA) sublineages and AOA versus ammonia-oxidizing bacteria in the subtropical Pearl River estuary. The study shows a difference in the composition of AOA sublineages at the DNA and RNA level and correlation of nitrification rates with the relative abundance of only one AOA sublineage suggesting a niche partitioning between different AOA sublineages. Furthermore, the authors present data on the contribution of nitrification to oxygen consumption.

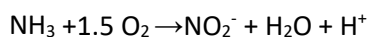
Parts of the data set are only superficially mentioned in the manuscript (e.g. fig 8) although they contain valuable information. Especially the comparison between particle attached vs free-living AOA community composition deserves more attention.

NOD/CRs ratios are a central focus of this manuscript. At the same time the NOD rates are part of different manuscript. In order to see the clear separation of focus and content, the other manuscript should be made accessible to the reviewers. This probably would also help to get important information on the method of NOD determination that are missing from this manuscript (e.g. how many time points were taken per rate measurement?).

A lot of emphasis is put on the relative importance of NOD in CR. It is stressed various times throughout the manuscript that NOD is high and at times amounts to more than 200%. However, at these stations NOD is not significantly higher compared to other stations, instead CR rates are VERY low. A critical discussion of the CR rates is absent and should be added to the discussion section. How can the observed patchiness of CR rates be explained?

Furthermore, this raises the question of how well constrained the CR data are. Are they based on two data points per rate measurement? How many replicates have been performed? No standard deviation is reported for NOD or CR. I ask the authors to add this information to the respective tables in the supplementary information and would like them include the number of replicates performed in the material and method section. According to the material and method section, triplicates were performed for the qPCR data. However, standard deviations are also missing in the respective data tables in the supplementary information. I ask the authors to add this.

For the calculation of the inferred nitrification oxygen demand, the authors use improperly balanced equations. This strongly influences the outcome: e.g. for ammonia oxidation, when using



instead of equation (1), the oxygen demand changes by 33%. During carbon fixation, some electrons are used to reduce CO<sub>2</sub> and not oxygen. However, the assumption that for every NH<sub>3</sub> molecule 1.98 HCO<sub>3</sub> gets fixed is hardly realistic. Furthermore, the authors assume 1:1 coupling between ammonia oxidation and nitrite oxidation. However, no data on the abundance of nitrite oxidizers is provided and the rate measurements provided do not distinguish between nitrite or nitrate production. I suggest that the estimate of oxygen demand should focus on the first step of nitrification only or at least a paragraph needs to be added to the discussion section.

The grammar and language need to be revised. There are too many issues throughout the manuscript to list here, which at times makes it hard to follow the authors line of thought.

## specific comments

l. 63 they would not have overlooked them, but rather underestimated their activity and relative contribution to ammonia oxidation.

II. 86-87 microbial instead of bacterial.

I. 96 clarify “running seawater”

I. 158 please provide an overview over the 76 samples (which stations and depths are they from) and refer to table S5. The 2523 reads per file does not match the data reported in table S5. The sample categories provided in table S5 need further explanations.

I. 162 Ion torrent is known for introducing homopolymers. Filtering reads with >8 homopolymers is quite a weak setting considering your aim of “performing fine-scale phylogenetic classification”. Please comment.

II. 170ff. What is the sampling depth of the samples you classified as “bottom”.

I. 330 substrate requirement: do the authors mean substrate concentration?

I. 355 “questionable” How so? Such a statement needs to be accompanied with an explanation.

Section 4.1 repeats results in great detail that are already described in the result section. Consider condensing this section.

Fig. 2: figure 2 consists of a selection of graphs to show the most interesting pattern among the environmental parameters measured. This is alright, but the rest of the graphs needs to be provided as well (e.g. supplementary info). For example, surface nitrate concentrations and bottom nitrite concentrations are shown, but bottom nitrate concentrations and bottom salinity are missing.

Fig. 3c: Data are only plotted for a fraction of the stations compared to 3a and b. Why is a part of the data missing?

Fig. 4: please provide the scale in the same number format for AOA and AOB. In order to compare abundances between surface layer and bottom layer please use the same range for the scale for 4a and c and b and d respectively.

Fig. 9: you include the temperature in the Spearman correlation in this table. Therefore, you should also provide the temperature data. Maybe add them to table S2.

Fig.9 and I. 391: How did you quantify heterotrophic bacteria? With the cell quantification method, you reported in the material and method section heterotrophic microbes cannot be distinguished from autotrophic non-phototrophic microbial cells (such as the nitrifiers that this study focuses on).

### **technical corrections**

As pointed out above, there are too many issues throughout the manuscript to address here. Some selected comments:

I. 42 “Based on the” instead of “as revealed by”

I. 47 The WCA, WCB, and SCM1-like groups correspond...

I. 102 introduce the abbreviation CR in line 93

Fig. 9: this is a table not a figure. Typos in the first column: Surface.