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Interactive comment on "Niche differentiation of ammonia and nitrite oxidizers along a salinity gradient from the Pearl River estuary to the South China Sea" *by* Lei Hou et al.

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The authors characterized the diversity, abundance and activity of nitrifiers associated with waters from the Pearl River estuary to the South China Sea. The data set provides novel insights into the niche separation and interactions of ammonia and nitrite oxidizers. However, I found technical issues in the quantification of archaeal ammonia oxidizers in the current version of manuscript as shown below.

In this study, archaeal amoA genes were used for a molecular marker for archaeal ammonia oxidizers, and there are technical issues in both diversity analysis and quantitative PCR. 1. The composition of archaeal amoA diversity is highly biased in PCR

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amplification comparing to the SSU rRNA gene analysis as reported previously (Meinhardt et al., 2015; Nunoura et al., 2015). In addition, it would lead inappropriate selection of sequences used to obtain standard curves in qPCR. 2. Thus, the authors should mention about the possibility that the interpretation of the niche separation among AOA subgroups may be influenced by PCR bias in this manuscript. 3. The authors obtained archaeal amoA gene sequences in the clone analysis and then used the selected sequences to obtain standard curves in the following qPCR. However, qPCR primer set for archaeal amoA genes was identical to a primer set used for the conventional clone analysis. This generally allows complete match between archaeal amoA clone sequences and primer sequences in PCR reaction for obtaining standard curves. In contrast, the presence of few mismatch residues is expected between environmental amoA gene sequences and primer sequences in qPCR. The gap would be a reason for underestimation of archaeal amoA genes in environmental samples. In qPCR of archaeal amoA, a primer set, Wuchter et al. 2006, or other primer set that does not overlap the annealing regions in the initial clone analysis is recommended.

Specific comments P2, L3: We analyzed diversity and abundance of ammonia-oxidizing archaea (AOA) and betaproteobacteria (AOB), nitrite-oxidizing bacteria (NOB), and nitrification rates to

P2, L6-7: AOA were generally more abundant than betaproteobacterial AOB, however,

P2, L12: What does “a coupling of ammonia and nitrite oxidizers” mean?

P4, L2-14: Please insert a sentence to present the close relationship between Nitrospina and “Ca. Nitromaritima”. Sequences belong to “Ca. Nitromaritima” had been reported as a group in Nitrospina until the definition of “Ca. Nitromaritima”. Thus, the discussion in Lucker et al. 2013 includes both Nitrospina and “Ca. Nitromaritima”.

P4, L6: Information from Pachiadaki et al. 2017 should be referred through the manuscript.

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P6, L5: Strains and/or genomic DNA from the public repositories used in this study should be summarized as the first paragraph in Materials and methods.

P8, L18-: Did the authors determine OTUs for each library or among the libraries obtained in this study?

P8, L18-: Did the authors conduct any chimera check programs? It has been reported that more than 10% of the archaeal amoA gene sequences in the public database are chimera sequences (Eloy Alves et al. 2018).

P9, L13: Names of the sequence used to obtain standard curves for the qPCR should be presented.

P9, L14: How did the authors obtain DNA fragments?

P11, L5: Pseudomonas chlororaphis subsp. aureofaciens (ATCC 13985)

P13, L3-: Did the authors obtain data of turbidity or light intensity during the sampling?

P15, L11-: Please clarify how many clone libraries constructed for each gene. Supplementary tables presenting distribution of OTUs will help readers to understand the results.

P18, L10: Please present the values of detection limits in each qPCR if possible.

P19, L6: As I know, the abundance of ammonia oxidizers is generally higher than nitrite oxidizers in aquatic environments. I am afraid that the result was influenced by the technical issues described above.

P21, L5: dominant NOB.

P21, L5-: Information from Hawley et al. 2014 should be integrated in this discussion.

P25, L17: “availability of ammonia” or “ammonia concentration/flux” would be better than “ammonia levels”.

P25, L17: Appropriate references should be provided.

P26, L12: Appropriate references for light inhibition on the growth of nitrifiers should be provided.

References: Proc. Natl. Acad. Sci. USA instead of P. Natl. Acad. Sci. USA

Fig. 5: Did the Nitromaritima sequence excluded in this phylogenetic analysis?

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