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Manuscript title: Niche differentiation of ammonia and nitrite oxidizers along a salinity gradient from the Pearl River estuary to the South China Sea

Response to Reviewer #2

We greatly thank the reviewer for the valuable comments, useful suggestions and careful revisions, based on which we have revised the manuscript. And the point-by-point responses to the comments are in blue colour as follows.

Anonymous Referee #2

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Hou et al. investigated the distribution of Ammonia and Nitrite oxidizers in a subtropical estuary of China by using the functional gene-based clone library and qPCR analyses as well as the determination of nitrification rates. The main conclusion of this work is that substrate affinity/preference of Ammonia and Nitrite oxidizers may play an important role in determining their distribution patterns in estuarine-ocean gradient. Some small comments are provided for improving this manuscript.

1. page 2, Line 2, and page 4, Line 15, I think "between" should be changed into "of";

Response:

Revised as suggested.

2. page 4, Line 15-18, Please add some background information related to niche differentiation of ammonia and nitrite oxidizers. This may facilitate readers to get a quick view of the current research status.

Response:

Thanks for the reviewer's suggestion. We added some background information related to niche differentiation of ammonia and nitrite oxidizers. — "*For example, both AOA and AOB are frequently found together in estuarine and coastal regimes and share the same ecosystem function (Bernhard et al., 2010; Zhang et al., 2014a), but in many situations, only AOA or AOB are predominant (Cébron et al., 2003; Hollibaugh et al., 2011; Li et al., 2014) as their physiological responses to environmental stressors may be different. Similarly, Nitrospira, Nitrospina, Nitrococcus, and/or Nitrobacter are frequently found together in estuarine and marine regimes, but there is no a consistent distribution pattern between them*

(Cébron et al., 2005; Füssel et al., 2012; Nunoura et al., 2015; Pachiadaki et al., 2017), suggesting that niche partitioning and niche specialization support the coexistence of sympatric NOB. Moreover, between ammonia and nitrite oxidizers, there is a coupling in abundance and distribution in Monterey Bay and the North Pacific Subtropical Gyre (Mincer et al., 2007) or decoupling in Gulf of Mexico (Bristow et al., 2015)."

3. page 8, Line 9, could you provide a coverage information about this primer set you designed?

Response:

Thanks for the reviewer's suggestion. There were only two *nxr*B gene sequences (from *Nitrospina gracilis* 3/211) in the NCBI database when the primer pair of nxrBNF and nxrBNR was designed, and the coverage is 100%. We discussed the coverage of this primer pair in Discussion 4.1 section and added the coverage analysis. — "*Among 23 sequences of Nitrospina nxrB genes available in the databases, only seven sequence could not be targeted by the primers nxrBNF and nxrBNR due to >3 mismatching bases for either primer, indicating a ~70% coverage of the primers (100% if allowing 5 mismatching bases).*"

4. page 13, Line 6, reference citations? or based on your results?

Response:

Sorry for the unclear sentence. The reference citation on the characteristics of the upper, middle and lower reaches of the PRE is Wang et al. (2012). We revised this sentence as "*The upper reaches receive a small amount of freshwater, sewage, and industrial effluent discharge; the middle reaches receive about half of the freshwater from the North and West rivers, tributaries of the Pearl River, with little salinity stratification; the lower reaches are controlled mainly by estuarine mixing of freshwater and seawater (Wang et al., 2012).*"

5. page 16, Line 5, I think the group E of AOA belong to the typical Soil/Sediment cluster, while other groups you defined belong to the typical Water/Sediment cluster. Actually, the HAC and LAC clusters were defined on the basis of the later one, especially for the Marine cluster within the Water/Sediment cluster. If you want to

define the members within Soil/Sediment cluster, like group E, please provide more supporting evidence/cited references.

Response:

Many thanks for the reviewer's suggestion. Indeed, group E belongs to Soil/sediment cluster. We added the related statement in 3.3 section — "*According to the framework of Francis et al. (2005), groups A, Ba, and Bb were defined as Water column cluster, group D was defined as Sediments cluster, and group E was defined as Soil/sediment cluster.*" We also added the cluster information in Figure 3 and S3.

According to the framework of Sintes et al. (2013), there is a rough range of ammonia concentration for HAC (20 to 100 nM or even higher) and LAC (frequently below detection limit). Our field data on ammonia concentration confirmed the categorization of groups A (HAC), Ba and Bb (LAC), D (HAC), and E (HAC). We also added a reference citation to support that group E can be defined as HAC. — "*Tourna et al. (2011) and Hatzenpichler et al. (2008) have reported that two ammonia-oxidizing archaea Nitrososphaera viennensis and Nitrososphaera gargensis belonging to group E (crenarchaeal group I. 1b) tolerate high ammonia concentrations (1–15 mM and 0.14–3.08 mM, respectively).*"