

Anonymous Referee #3', received on 03 Sep 2013. We thank Anonymous Referee for their comments.

*General comments: This study investigated the nitrification process in turbid shelf water by measuring nitrification rate, dissolved oxygen (DO), community respiration rate (CR), and the abundance of relevant bacteria, etc. The results indicated that nitrification was a particle-associated process in Changjiang Plume, and the reactive  $Fe^{3+}/Mn^{4+}$  may play a role as oxidant in nitrification process which could provide some implications for further nitrogen studies. However, there are some tough spots in this study. The manuscript is too long and redundant, and the organization of the manuscript should be improved. The introduction does not clearly convey the originality or importance of the research. The introduction should state the importance of suspended sediment to nitrification, instead of describing the widespread of suspended sediment, and thereby provide a clear hypothesis for this study. The introduction should address the information which is relevant to the aim of this study. For example, the author state the nitrous oxide is a greenhouse gas which has not been discussed elsewhere. The author should distinguish the results obtained from this study and the results cited from the other references. In the introduction, the author should propose the hypothesis based on the results of other researches, instead of stating the results of this paper.*

Response:

As suggested, we rewrote the last paragraph of Introduction putting more emphasis on our hypothesis (P.8688 L.19-28) rather than results/findings we obtained. The refined paragraph was shown below.

“In the Changjiang Plume, the interplay of nitrogenous nutrient and suspended sediment in ammonia oxidation rate (AOR) has never been investigated before, particularly, during summer high flow with great particulate organic matter and nutrient input that may trigger seasonal hypoxia. Our cruise was set during flood season to explore the fractional contribution of nitrification in community oxygen consumption off the Chngjiang River estuary. We hypothesized that 1) enhanced supply of suspended particle during flood season may provide greater particle surface areas thus substrate (both adsorbed ammonium and ammonium sourced from remineralization) and micro-niche for nitrifier to exacerbate nitrification and 2) particle reactive redox sensitive elements, such as ferric iron and manganese might also act as electron acceptor aside oxygen for nitrification. This is the first observation for particle-associated nitrification in river plume in shallow water with high turbidity in the East China Sea by using stable isotope tracer method. The purpose is to explore

the biogeochemical relation among oxygen, nitrification and redox-sensitive elements in a stoichiometric way. “

*General comments:Material and method The author should explain why this paper select the sampling time at one week after typhoon Muifa passed. Line 21-26, the logic is very bad.*

Response:

We thank for this comment and rewrote this paragraph. Actually we did not plan to study the typhoon disturbance. Our original plan was simply investigating the hypoxia region at flood season. The typhoon event was not expected. However, typhoon is not uncommon in this region. We changed this paragraph as below.

“We examined the water discharge data collected at the upstream gauging station (Fig. 1c) to check whether the typhoon Muifa induced unusually high water discharge. However, the water discharge of the cruise period was in the range of historical discharge pattern of summer. Obviously, in such a large watershed, Typhoon Muifa was not strong enough to manipulate the water discharge. Therefore our cruise was still representative of the summer high flow condition.”

*General comments:The sampling sites described in Fig.1 are not clear, what is the meaning of N0-N5,what is the total numbers of sampling sites?*

Response:

We added the total number of sampling stations in the description. In the refined Table 2, we also listed the sample number used in the statistical calculation as well as other plots. The Qiantang River mouth (Sta. N0-N5) is famous for its magnified tidal surge (~8m) induced by pocket-lick coastline, thus, the water collected at Qiantang contained extremely high suspended sediment (1542~5415 mg L<sup>-1</sup> according to our observation) which fits our aim.

*General comments:The author should describe the detection method in detail; For example, “TSM sample were collected by filtering 1–4 L of water sample onto pre-combusted Whatman GF/F membrane.” what is the size of this membrane? In the incubation experiments section, I cannot get how these incubations are finished; what is a tank with continuous circulation of surface sea water; how are the bottles for experiment fixed in the tank?*

Response:

As suggested, we add more details for our experiment procedure. The incubation was terminated by filtering through 47mm polycarbonate membrane with pore size of 0.2  $\mu\text{m}$ . The 50 L tank fixed on the deck was fully covered by aluminum foil to block the light. A submersible water pump provided continuously flowing surface seawater on-deck to maintain the incubation temperature. The incubation bottles were fixed upside down in a stainless steel rack immersed in the tank. The refined descriptions of incubation experiment are shown below.

“Ammonia oxidation rate (AOR) was measured by stable isotopic tracer method (Lipschultz et al., 1986). For bulk ammonia oxidation rate ( $\text{AOR}_b$ ), six 250 mL narrow-necked gas tight glass bottles were overflowed for more than 2-fold volume and sealed without any headspace. Then  $^{15}\text{NH}_4\text{Cl}$  was injected to a final concentration of 50  $\text{nmol L}^{-1}$  in each bottle for 3, 6 and 12-hour dark incubation (duplicate) in a darkened 50 L tank fixed on deck. A submersible water pump provided continuously flowing surface seawater to maintain the incubation temperature. The incubation bottles were fixed upside down in a stainless steel rack immersed in the tank. The control sample was directly filtered by 47mm 0.22 $\mu\text{m}$  polycarbonate membrane and stored in freezer without incubation. The incubation was terminated by filtering through 0.22  $\mu\text{m}$  polycarbonate membrane, and the filtrate were stored frozen at -20  $^\circ\text{C}$  until laboratory analysis.

The change of  $^{15}\text{N}$  content in nitrate and nitrite pool was determined by denitrifier method (Casciotti et al., 2002; McIlvin and Casciotti, 2011; Sigman et al., 2001). The regression coefficient of the time course curves of  $^{15}\text{N}$  content in nitrate and nitrite pool for all measurements were better than 0.8 (not shown). The incubation of particle-free ammonia oxidation rate ( $\text{AOR}_{\text{pf}}$ ) was implemented after removing particle by using 3  $\mu\text{m}$  polycarbonate membrane (Berounsky and Nixon, 1993).  $\text{AOR}_{\text{pf}}$  was conducted only for selected Stas. Y0, Y3, 2Y3 and Y5.

The natural nitrification rate was calculated by the following equation:

$$R_{\text{NO}_x} = \frac{d[^{15}\text{N}]}{dt} \times \frac{[^{14}\text{NH}_4^+] + [^{15}\text{NH}_4^+]}{[^{15}\text{NH}_4^+]},$$

where  $R_{\text{NO}_x}$  is the ammonia oxidation rate,  $t$  is the incubation time,  $[^{15}\text{N}]$  is the concentration of  $^{15}\text{N}$  in nitrate plus nitrite pool in sample, and  $[^{14}\text{NH}_4^+]$  and  $[^{15}\text{NH}_4^+]$  are the observed natural ammonium concentration and artificial addition of stable isotopic tracer ( $^{15}\text{NH}_4^+$ ), respectively. We used the term  $\text{NO}_x$  since in our method both  $\text{NO}_3^-$  and  $\text{NO}_2^-$  were included.”

*General comments: For the incubation of particle-free nitrification, why use the water after removing particle by using 3  $\mu\text{m}$  membrane? It was not consistent with the later mentioned sediment fraction, for example, 0.22-3 $\mu\text{m}$ .*

Response:

For DNA samples, we used sequential filtration to separate  $>3\mu\text{m}$  and 0.2-3 $\mu\text{m}$ . The filter of pore size of 0.22 $\mu\text{m}$  will completely remove bacteria including nitrifier thus we cannot use 0.2  $\mu\text{m}$  for AOR. The revision about the DNA sample collection is as below now. And we update the footnotes for Table. 1 to illustrate the samples of AOR.

“1-2 L seawater samples were firstly filtered onto 47mm 3 $\mu\text{m}$  polycarbonate filter to collect the size fractions of  $>3\mu\text{m}$ . Then the filtrate was further filtered through 47mm 0.22 $\mu\text{m}$  polycarbonate filter to collect the size fractions of 0.22-3  $\mu\text{m}$ . The polycarbonate membranes were kept frozen in  $-80^{\circ}\text{C}$  until laboratory analysis.”

“The footnotes of Table 1:

Ammonia oxidation rate was presented as mean  $\pm$  standard deviation. BDL: below detection limit.

\* Bulk sample contained all nitrifier larger than 0.22  $\mu\text{m}$  in the incubation.

\*\* Filtered sample contained the nitrifier on 0.22~3  $\mu\text{m}$  particles.

\*\*\* Parti. stands for particulate sample and its size range. The percentage value in parentheses indicates the percentile contribution to total *amoA* abundance.”

*General comments: Results This section should be reorganized by dividing it into several subsections with titles. The results describe lots of data which is not so relevant to the aim of this study (e.g. the first four paragraphs of the Result section), maybe the author can shorten these descriptions and state the relevant results in a more logically method.*

Response:

In revised manuscript, we divided the Results into several sections. The table of content of Results is listed below. We also tried to shorten the four paragraphs. However, we do think to present these basic geochemical data is important for future estuarine studies for comparison.

### 3 Results

#### 3.1 Distribution of hydrographic and chemical parameters

- 3.1.1 Salinity and DO
- 3.1.2 Dissolved inorganic and organic nitrogen
- 3.1.3 Suspended particles
- 3.2 The correlation among AOR<sub>b</sub>, nitrogenous nutrient and TSM
- 3.3 Community respiration
- 3.4 The AOR<sub>pf</sub> and the *amoA* abundance on large (>3μm) and small (0.22-3μm) particle fraction.

*General comments: Page 8693 line 8-9, how can you deduce that the particulate organic matter was mainly marine sourced?*

Response:

We added one new reference by Lamb et al. (2006), in which C/N ratio is proved to be a good indicator to discern marine (C/N ~4 to 10) from terrestrial (C/N >12) organic matter.

*General comments: Page 8693 line 21, the intercept mentioned in the paper is different from that shown in figure 4, could the author explain where these differences come from?*

Response:

This is our mistake. The intercept in the text (P.8693 L.21) was wrong. We have corrected it.

*General comments: Page 8694 line 2-5, the statement is wrong and disagree with line 9-10 of page 8696, how can you get that aerobic degradation of organic matter was the major source of ammonium? The correlation is between the initial NH<sub>4</sub> concentration and CR.*

Response:

Since we measured both PON and DON, we can roughly discuss which is responsible for AOR. Though DON is not correlated to AOR, PON was significantly correlated to AOR in linear scale (river mouth:  $r=0.85$ ,  $p<0.01$ ; inner plume:  $r=0.51$ ,  $p<0.05$ ). Thus we speculate that PON was the major source that provided ammonia for ammonia oxidation. We changed the sentence in P.8694 L.2-5 to explain more detailed about the hypothesis shown below.

“Put all CR data together, positive linear correlation between ammonium and CR (Table 2-4) may imply that aerobic degradation of organic matter was the major source of ammonium which may fuel the AOR. Alternatively, high ammonium

content may also stimulate both CR and AOR.”

“Results showed strong affinity among nitrifier abundance, PON and AOR, which implies PON should play a role in determine AOR. However, no positive correlation was observed between DON and AOR implying that DON may not be as important as PON in supplying ammonia for nitrification in our study area. ”

*General comments: This Discussion The aim of this study the author present at the introduction is to investigate the interplay of nutrient and suspended sediment in nitrification rate, however, here in the discussion 4.1, the author discusses the interplay between nitrification and environmental parameters; this discrepancy leads the readers to be lost, and the readers cannot get what the author want to do in this study.*

Response:

We made corrections. The subtitle of Section 4.1 is now: The interplay of nitrogenous nutrient and TSM in AOR.

*General comments: Page 8695 line 26-28, the nitrification rate in the nitrite maximum layer was under detection limit indicating that nitrite was not sourced from ammonium, why? Then what is nitrite sourced from?*

This is definitely an interesting question. One popular hypothesis to support primary nitrite maximum is the incomplete nitrate reduction by phytoplankton (Lomas and Lipshultz, 2006). According to our observation, this nitrite maximum layer is shallow (10 m) below the Chl-a maximum(3m, data not shown). The *amoA* abundance was low there (Table. 1) compared to other region. In this layer, AOR is undetectable though ammonium is high. In contrary, the highest CR value was found. Such concurrence among Chl-a, CR, maximums of nitrite /ammonium reflects synchronously high activities in grazer, herterotrophic bacteria and phytoplankton. Since nitrifier is light-sensitive, we speculated that nitrifier photo-inhibition had happened in this shallow layer. The low abundance of AOA and AOB may be another reason caused the undetectable AOR. Thus nitrite is mainly sourced from incomplete nitrate reduction.

*General comments: 4.2 Reactive Fe as oxidant supply for nitrification in turbid river plume, why only Fe not Mn? Mn has also be analyzed in the following text The meaning of the first paragraph of 4.2 is not clear.*

Response:

The Fe(III)/Mn(IV) mediated ammonia oxidation are both thermodynamically favorable. Thus we added Mn into our statement. However, the amount of reactive Mn was too low to compensate the oxidant demand for ammonia oxidation in our study. The reactive Mn could only support up to 28% of ammonia oxidation according to our stoichiometric calculation in refined Table. 3. We change the third paragraph in 4.2 to clarify our statement shown below.

“Similar to the oxygen demand estimation presented above, stoichiometric calculation was performed to evaluate if the reactive Fe/Mn were sufficient to support the oxidant demand for ammonia oxidation or the whole nitrification. We used goethite (FeOOH) and manganese oxide (MnO<sub>2</sub>), which are both common in natural aerobic environment, in our estimation. According to thermodynamically favorable Equation 4, 5 and 6, six mole of goethite or three mole of manganese oxide are required for one mole of ammonia to oxidize to nitrite, 4 mole of manganese oxide mediated is required for the whole nitrification. Therefore, we can estimate Fe and Mn demand by using AOR<sub>b</sub> when assuming nitrification was entirely relied on Fe (III) and Mn (IV). In Table 3, we presented the observed reactive Fe/Mn in water column versus ammonia oxidation-associated Fe (III) and Mn (IV) demand. Obviously, observed reactive Fe is 10-fold higher than that to support measured AOR. Yet, the reactive Mn was insufficient and can only support up to 28% of ammonia oxidation. Our estimation only presented the upper bound of Fe (III) and Mn (IV) demand, and we clearly know O<sub>2</sub> must play a role. Further investigations on the speciation of Fe/Mn and the coupling process of oxygen, Fe/Mn reduction and nitrification are needed.”

*Specific comments: Page 8697 line 27, “The excess oxygen consumption may result from allochthonous ammonia”, what is allochthonous ammonia? Where it come from?*

Allochthonous ammonia represents the ammonia diffused or transported from other place; that is, non-*in situ* source. We described explicitly in revised version to make it clearer.

*Specific comments: Page 8700 line 1-6, how can “the different slopes of TSM against NRb from in inner shelf and river mouth” can imply that “ammonia can be supplied from in situ decomposition”. ? This conclusion was not obtained from the results of this manuscript.*

Response:

We have changed the sentence in P.8700 L.3-6 as shown below.

“First, ammonia can be supplied by not only ambient water but also *in situ* decomposition of particulate organic matter. The latter process was implied by significant positive correlation between TSM and AOR<sub>b</sub> distinctively in inner shelf and river mouth.”

*Specific comments: Page 8687 line 9-10, The sentence “One of the most affected processes is nitrification, in which ammonium is oxidized to nitrate and nitrous oxide, a greenhouse gas, is produced as a byproduct.” is awkward, rewrite it.*

Response:

Thanks for your suggestion. That sentence has been changed according to Referee 1’s suggestion.

“One of the most affected processes is nitrification, in which ammonium is oxidized to nitrate. Nitrification is composed by two steps, ammonia oxidation and nitrite oxidation. Ammonium oxidation is carried out by ammonia oxidizing archaea (AOA) and ammonia oxidizing bacteria (AOB), while nitrite oxidation is executed by nitrite oxidizing bacteria (NOB). Ammonia oxidation requires 3/4 of oxidants demand in nitrification and produce nitrous oxide, a greenhouse gas, as a byproduct.

*Specific comments: Some of data in this paper are not consistent, for example, the percentage of oxygen consumption by bulk nitrification reported at the discussion section and abstract section is different.*

Response:

Thanks for the notification by the reviewer. We have corrected this typo.

*Specific comments: In figure 4a and 4b, are these points fitted by linear regression? Do not they seem more like a curve instead of a straight line?*

Response:

We used linear regression in figure 4a and 4b. The curved shape is resulted from the semi-log scale in X-axis in 4a and Y-axis in 4b. We added notification for Figures 4 and 5.

Fig. 4 “Note that the X-axis in (a) and Y-axis in (b) are in log<sub>10</sub> scale.”

Fig. 5 “Note that the X-axis in (c) and (d) are in log<sub>10</sub> scale.”



According to Response above, we put additional reference below into our revision.

1. Lamb, Angela L., Graham P. Wilson, and Melanie J. Leng. "A review of coastal palaeoclimate and relative sea-level reconstructions using  $\delta^{13}\text{C}$  and C/N ratios in organic material." *Earth-Science Reviews* 75.1 (2006): 29-57.