Response to the Comments of Anonymous Referee #1 (RC C937) on Biogeosciences Discuss. 10, 4671–4710, 2013 (MS No.: bg-2013-61) “Anaerobic ammonium oxidation, denitrification and dissimilatory nitrate reduction to ammonium in the East China Sea sediment” (Authors: G. D. Song et al.)

Comment 1. The present study by Song et al. addresses multiple anaerobic processes of the nitrogen cycle in sea sediments. The authors did a good job in analyzing denitrification, DNRA and AnAmmOx by the ^{15}N-isotope pairing technique and correcting process rates for simultaneous occurring processes impairing the isotope pairing technique. Thus, the conclusion that AnAmmOx significantly contributes to N-losses in the ECS sediments is supported by the data. The manuscript is basically well written with some shortcomings outlined below. However, two major points need to be addressed to improve the manuscript: 1. Experimental design. AnAmmOx utilizes nitrite rather than nitrate to oxidize ammonium. Why was nitrate supplemented as a tracer in the E_Amox treatment designed to address AnAmmOx? In the current experimental setup, AnAmmOx would depend on denitrification to provide nitrite first. Please discuss.

Reply: **We thank Referee’1 for the constructive comments concerning the experimental design, and indeed, anammox is the reduction of nitrite coupled to ammonia oxidation (Mulder et al., 1995). However, nitrite can be produced from nitrate reduction, which is the first step of nitrate reduction in anaerobic sediment. Although this process is usually considered as a part of denitrification, it is also a stand-alone process that can be far higher than the denitrification (Kalvelage et al., 2013; Lam et al., 2009; Thamdrup and Dalsgaard, 2008). It has been demonstrated that anammox bacteria can perform nitrate reduction; in fact, they have even been shown to perform DNRA (Kartal et al., 2007). We used NO_3^- additions because this is more**

representative of the conditions in the sediments which have up to 80 µM of NO_3^-, but low NO_2^- concentrations, where anammox bacteria need to compete with denitrifiers and nitrite oxidizers for the NO_2^-.

Comment 2. Choice of data presented. Data presented in figures is inconsistent (Fig. 3 a and b, and Fig. 4 are from different samples, although Fig. 4 is related to the data in Fig. 3), a rational for the selection of data is not given. In case the authors would like to highlight some data, a rational should be given. In general, I would suggest to include the complete data (where a fraction is in Fig. 3 and 4) in a table. The authors might give rates and the F_As.

Reply: **We see the point of Referee#1, however, combining Figure 3 and Figure 4 would make a very large and un-over-viewable figure difficult to fit on one page. All N_2 data from all depths are shown in Supplementary Figures S1-5.**

Comment 3. Redundancies in data presentation. Figure 5 and Table 3 describe similar data, as well as Figure 6 and Table 4. Please avoid double presentation of data.

Reply: **Thank you for your constructive suggestion. We kept Fig. 5 and revised Table 3 in which the nitrate concentration after pre-incubation and F_N were added (*see Comment 13*). Table 4 was removed and Fig. 6 was kept in the revised MS.**

Minor points:

Comment 4. P4673 L26-27 AnAmmOx is described as a nitrate reduction pathway here. This is not fully correct, since AnAmmOx utilizes nitrite rather than nitrite. Please clarify.

Reply: **We have now changed this in the revised manuscript and as discussed above (*see Comment 1*), anammox is dependent on nitrate reduction to nitrite similar to full denitrification and DNRA.**

Comment 5. P4674 L7 Typo; "is" needed.

Reply: **This has been changed.**

Comment 6. P4674 L28 Please clarify: NH_4^+ combines with NO_2^- (nitrite) rather than nitrate (NO_3^-) during AnAmmOx!

Reply: **"$^{15}NO_3^-$" has been revised to "$^{15}NO_2^-$ (derived from $^{15}NO_3^-$ dissimilatory reduction)".**

Comment 7. P4675 L12 Please avoid first time claims. Only new data should be published anyway.

Reply: **This has been changed.**

Comment 8. P4676 L5 Please give final concentrations of Hg^{2+} in the samples utilized to preserve samples.

Reply: **The final concentration of Hg^{2+} is ~100 mg L^{-1} and has been added in the text (*see* Section 2.1).**

Comment 9. P4676 L22 What was the volume of such subsamples? Please clarify. Please give incubation temperatures.

Reply: **This has been changed to include a more detailed description. "After each tracer injection and mixing, subsamples were immediately filled into 6 ml Exetainer vials (Labco Ltd, High Wycombe, UK) with 0.1 ml pre-added saturated $HgCl_2$. The temperature of the incubations was between 18-24 °C at different sites (Table 1)." Thus, the actual volume of subsample is 5.9 ml.**

Comment 10. P4677 L4 "detected limitation". Do the authors refer to the "LOD (limit of detection)" or LOQ (limit of quantification)? Please clarify.

Reply: **It is "limit of detection". Thank you for your comment.**

Comment 11. P4677 L15 How can pure $^{15}NH_4^+$ be converted to $^{29}N_2$ via hypobromite?

In theory, all N_2 should be $^{30}N_2$. Please explain.

Reply: **$^{14}NH_4^+$ was inherently present in the sediment slurry samples (produced by organic matter mineralization and/or DNRA in natural sediment), when hypobromite was added; $^{28}N_2$, $^{29}N_2$ and $^{30}N_2$ were all produced according to isotope pairing principle when $^{15}NH_4^+$ and $^{14}NH_4^+$ were randomly oxidized by hypobromite.**

Comment 12. P4680 A description of the statistical methods utilized is lacking in the materials and methods section. Was data normally distributed? What statistical tests/ analyses were applied? Please explain.

Reply: **Thank you for your valuable comments. We agree that the statistical part is very important in a research paper and have added the description of the statistical methods in Section 2 Materials and methods in the revised MS.**

Comment 13. P4681 L20 Please add values of nitrate concentrations after pre-incubation in table 2.

Reply: **This has been added to the revised Table 3.**

Comment 14. P4681 L24 DH55 or DH15 as indicated in the legend to figure 3. Which is correct?

Reply: **Thank you for pointing out this mistake and Referee#1 is correct, it should be DH53 and DH15, and we have corrected them in the text.**

Comment 15. P4681 L26 What test was applied (see comment above)?

Reply: **The $^{29}N_2$ and $^{30}N_2$ production was derived from linear regression (*see Comment 12 and section 2.4 in the new MS*).**

Comment 16. P4682 L5 Why were only 2 depths and sites shown? Please provide the data of all treatments in a table (see above).

Reply: **As we discussed above (*see Comment 2*), the 2 depths and sites**

represented two cases after pre-incubation, i.e. with and without residual nitrate. Since the full data from all treatments would occupy a large page, we put them in the supplementary material 1 (Fig. S1-5).

Comment 17. P4682 L9 How can nitrate be limiting in E_Amox when nitrate was supplemented and AnAmmOx requires nitrite rather than nitrate? Please clarify.

Reply: **As we discussed above (*see Comment 1 & 4*), nitrate was the original electron acceptor providing nitrite for anammox, denitrification and DNRA processes. In P4682 L9 "Nitrate was never limiting in E_Amox and E_Denit." We want to express "Nitrate was not a limiting factor in E_Amox and E_Denit." We have revised the expression in the new MS.**

Comment 18. P4682 L10-12 Why was Fa shown in Fig. 4 for only one site (and not the same displayed in Fig. 3)? Please show complete data set (see above).

Reply: **We have added all the F_A data in the new Fig. 4 (*see Comment 2*).**

Comment 19. P4682 L20-22 Fn not documented in Table 3. Pleas show data.

Reply: **This has now been added.**

Comment 20. P4687 L20 Please avoid first time claims.

Reply: **We have removed the first time claim in the revised MS.**

Comment 21. P4688 L 16-25 Were the added nitrate concentrations in the range of saturation for the system? Arguing with half saturation constants runs short here, since it might not be excluded that rates increase with increased nitrate concentrations, thus still suggesting an overestimation of N-loss be the authors. Please clarify.

Reply: **We did not conduct the kinetics experiments for nitrate reduction in this study, so we did not know the exact half-saturation constants of nitrate reduction in the ECS sediments. However, the half saturation constants could range from 2 to 340 µM in marine environments (Oren and Blackburn, 1979;**

Joye et al., 1996). We could not exclude the possibility that our calculation of N-loss would be overestimated in the ECS. However, according to the suggestions of the Anonymous Referee #3, we removed the section about N-loss in our revised MS.

Comment 22. Table 1. Check header. Please report temperatures.

Reply: **We have checked the header and revised to "Table 1 Sampling locations and some general characteristics of bottom water and sediment. The porosity and organic matter content (expressed as LOI%) are the average of the top 8 cm, and data in parentheses represent the variation range." Incubation temperatures have been added.**

Comment 23. Table 4. Header: Nitrate reduction is not really performed by AnAmmOx. Please clarify.

Reply: **As we discussed above (*see Comment 1&4*), anammox was also a nitrate reduction process. And in the revised MS, Table 4 has been removed (*see Comment 3*).**

Reference

Joye, S. B., Smith, S. V., Hollibaugh, J. T., and Paerl, H. W.: Estimating denitrification rates in estuarine sediments: a comparison of stoichiometric and acetylene based methods, Biogeochemistry, 33, 197–215, 1996.

Kalvelage, T., Lavik, G., Lam, P., Contreras, S., Arteaga, L., Löscher, C. R., Oschlies, A., Paulmier, A., Stramma, L., and Kuypers, M. M. M.: Nitrogen cycling driven by organic matter export in the South Pacific oxygen minimum zone, Nat. Geosci., 6, 228-234, 2013.

Kartal, B., Kuypers, M., Lavik, G., Schalk, J., Op den Camp, H., Jetten, M., and Strous, M.: Anammox bacteria disguised as denitrifiers: nitrate reduction to dinitrogen gas via nitrite and ammonium, Environ. Microbiol., 9, 635–642, 2007.

Lam, P., Lavik, G., Jensen, M., van de Vossenberg, J., Schmid, M., Woebken, D., Gutiérrez, D., Amann, R., Jetten, M., and Kuypers, M.: Revising the nitrogen cycle in the Peruvian oxygen minimum zone, P. Nat. Acad. Sci.,, 106, 4752-4757, 2009.

Mulder, A., Graaf, A., Robertson, L., and Kuenen, J.: Anaerobic ammonium oxidation discovered in a denitrifying fluidized bed reactor, FEMS Microbiol. Ecol., 16, 177–184, 1995.

Oren, A. and Blackburn, T. H.: Estimation of sediment denitrification rates at in situ nitrate concentrations, Appl. Environ. Microb., 37, 174–176, 1979.

Thamdrup, B. and Dalsgaard, T.: Nitrogen cycling in sediments, in: Microbial ecology of the oceans 2nd Edn., edited by: Kirchman, D. L., John Wiley & Sons Inc., pp. 527–568, 2008.