Response to the Comments of Anonymous Referee #2 (RC C981) 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. In this study the authors investigated the co-occurrence of denitrification, anammox, and DNRA along a gradient reaching from coastal sampling sites to further off-shore in the East China Sea. The authors applied a new methodological approach for their rate calculations which also integrated effects of DNRA on calculations of denitrification and anammox as well as effects of nitrate release from organisms on all of these processes. The authors found that all these processes took place at their study site, and they could also show the errors introduced to the quantification of anammox and denitrification if processes such as DNRA or nitrate release were not considered. By proposing this new integrated approach, the authors make an important contribution to this field of research as their recommendations are likely to be relevant for a large number of similar studies. All the calculations are explained in detail, and the authors provide a sound mathematical model for the simultaneous investigation of denitrification, anammox, and DNRA. However, there are two different aspects that the authors should pay attention to: (1) An approach for measurements of denitrification and anammox based on calculations of isotope ratios that is not affected by the co-occurrence DNRA has been developed by Spott & Stange (2007, Rapid Communications in Mass Spectrometry) and should be mentioned here.

Reply: **We appreciate that you agree with our work on precisely quantifying anammox and denitrification when several nitrogen transformations co-occur. We have now included the mentioned reference (Spott & Stange, 2007), which is relevant to our study, and implemented their findings into our revised manuscript. Indeed, the approach of Spott & Stange (2007) gave a precise**

calculation of anammox and denitrification rates; however, their calculation approach could probably not be applied in closed system incubation like ours.

Comment 2. (2) The discussion is very long and should be shortened and be written more concisely. Although this is clearly a paper with a strong method focus, the discussion of the results within the ecological context of sediment N cycling should be further strengthened. Here, this reviewer would like to see more emphasis put on the combined discussion of the measured depth gradients of nitrogen compounds and the depth distribution of the different N cycling processes. In this context, it would also be helpful if data on the oxygen distribution in the sediments could be provided to evaluate which processes are likely to occur. If such data is not available, then the authors should at least take into consideration publications from similar environments. How likely is it that ammonium produced deeper in the sediment can reach the surface? What is the extent of natural sediment perturbation at the study site?

Reply: **We have shortened our discussion on the methodological aspects and enhanced the discussion on the measured nitrogen transformations and depth gradients of nitrate in the revised MS. In the revised MS, the original Sections 4.1 and 4.2 have been combined in a new Section 4.1. The more detailed discussion about the methodological aspects was moved to the Supplementary materials. In the new Section 4.2, the discussion on the distribution and regulation of anammox, denitrification and DNRA in the ECS sediments has been enhanced. In Section 4.3 the biogeochemical significance of anammox, denitrification and DNRA is now discussed.**

Unfortunately, we did not measure the oxygen distribution in sediment in this cruise. However, oxygen usually penetrates several millimeters in the cohesive continental shelves sediments (Glud, 2008; Lohse et al., 1996) and in non-cohesive sediments with deeper oxygen penetration denitrification has been shown to be oxygen tolerant (Gao et al., 2010). Consequently, we can only speculate about the effect of oxygen in our sediments. However, the main purpose of this study concerned the anoxic benthic nitrogen transformations,

so we carried our experiment in anoxic slurry incubation. As was shown in our result, even under the strict anoxic condition, different nitrogen transformations exhibited a very complicated interaction. Further complication by involvement of aerobic nitrogen cycling is beyond the scope of this manuscript, and the topic of future studies.

We have now added the pore water ammonium concentrations to the revised manuscript in Fig. 2. The ammonium in the pore waters generally increases with sediment depth, indicating the diffusive supply of ammonium to the sediment surface. Several µM of ammonium in the upper 0-1 cm of the sediment indicate that there is a flux of ammonium towards the bottom waters as well.

Investigation of the benthic fauna was not part of our study, thus the natural sediment perturbation can only be partly reflected by the pore water profiles of nitrate which indicated that bioturbation and bioirrigation were occurring at some sites. Isolated peaks of nitrate were observed at depth, these could represent intrusions of nitrate rich bottom water or local nitrification stimulated by intrusions of oxygenated bottom water (Henriksen et al., 1983).

Specific comments

Comment 3. p. 6: Experimental setup: although table 2 gives the complete overview of the experimental setup, I would suggest adding at least one sentence describing the setup also in the text in order to introduce the abbreviations used here to the reader and to briefly outline the different assays. p. 7, line 13: What does E. Denit and E. Amox mean? Please introduce the abbreviations also in the text.

Reply: **We have added a sentence describing the experimental setup and the abbreviation of each experiment in the revised MS.**

Comment 4. p. 11, l. 10 and 14: Is active nitrification likely to occur at this sediment depth? What is the distribution of oxygen in the sediment profile?

Reply: **Isolated peaks in the pore water nitrate to concentrations significantly higher than the bottom waters i.e. at sites DHa2 and DH55 indicate that nitrification occurred at this depth as a result of bioturbation and/or bioirrigation. As we mentioned above (*see Comment 2*), we did not measure O_2 profiles in this study.**

Comment 5. p. 13, l. 3: Please consider a modification of this sentence. The increase in denitrification rates you describe here could be mistaken for an increase across the coastal gradient as you just described the decrease in rates across this gradient one sentence before.

Reply: **We found some repetition with the discussion in the revised MS, so we removed this sentence from here.**

Comment 6. p. 14, l. 15-17: This is not exactly true, please see the method introduced by Spott & Stange (2007).

Reply: **Thank you for pointing this out and we have added the work of Spott & Stange (2007) in the revised MS.**

Comment 7. p. 16, l. 15: This sentence is not clear.

Reply: **The whole section has been rephrased to make it clearer.**

Comment 8. p. 16, l. 22-25: Would the addition of nitrate not rather have stimulated further uptake of nitrate? Please explain.

Reply: **We could not exclude the possibility that the addition of nitrate would stimulate nitrate uptake. As we could not give a clear interpretation of nitrate release from this study, we have changed the discussion about this and enhanced the discussion about the ecological context of sediment N cycling in the revised MS also following the suggestions of the Referee # 2 and 3.**

Comment 9. p. 17, l. 18: Please write "quantification of the extent of the effect of

DNRA on denitrification and anammox"

Reply: **Thank you very much for pointing out these errors and we have corrected it.**

Comment 10. Fig. 2: As far as I understand, sediment cores were investigated for pore water chemistry were investigated down to a depth of 60 cm. Why do the authors only provide the data for the first 10 cm in Fig. 2?

Reply: **Yes, the Plexiglas liner length for sediment cores was 60 cm and pore water chemistry data exist from 16 to 48 cm. As our slurry incubations were carried out down to the maximum NO_x^- penetration depths observed at 8 cm, we find the pore water chemistry below 10 cm less relevant for our interpretations. Similar ranges of data were also displayed in other studies regarding interpretation of incubations (Engström et al., 2009; Gao et al., 2012; Rysgaard et al., 2004).**

Reference

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Gao, H., Schreiber, F., Collins, G., Jensen, M. M., Kostka, J. E., Lavik, G., de Beer, D., Zhou, H., Kuypers, M. M. M.: Aerobic denitrification in permeable Wadden Sea sediments. The ISME Journal 4, 417–426, 2010.

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Lohse, L., Epping, E. H. G., Helder, W., and Van Raaphorst, W.: Oxygen pore water profiles in continental shelf sediments of the North Sea: turbulent versus molecular diffusion, Mar. Ecol-Prog. Ser., 145, 63–75, 1996.

Rysgaard, S., Glud, R., Risgaard-Petersen, N., and Dalsgaard, T.: Denitrification and anammox activity in Arctic marine sediments, Limnol. Oceanogr., 49, 1493–1502, 2004.

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