Response to the Comments of Anonymous Referee #3 (RC C1034) 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 paper presents a study of the rates and relative importance of denitrification, anammox, and nitrate ammonification (DNRA) along a depth gradient in the East China Sea, based on slurry incubations with ^{15}N nitrate and ammonium. All three processes are found to be active and important. The presence of DNRA calls for modification of the standard approach for calculation of denitrification and anammox rates. Furthermore, a correction is made for the liberation of nitrate from an apparent intracellular nitrate pool.

The novelty of the study lies in it being the first to report this type of data from the East China Sea and in the detail of treatment of how to interpret data in the presence of DNRA and internal nitrate pools. In contrast to the impression left by this paper, it is, however, not the first to demonstrate experimentally the simultaneous activity of anammox, denitrification, and DNRA in sediments, nor the first to propose formula for resolving rates of the processes based on ^{15}N incubations (see below).

The paper is carefully conducted and clearly written, but the discussion of the methodological aspects is too long while the discussion of the environmentally relevant results could be expanded. Moreover, there are flaws in the interpretation of the data.

Reply: **Thank you very much for your positive evaluation of our work in the East China Sea. As suggested by Referee #3 we have toned down the statements about the novelty of our work in the revised manuscript. We shortened the discussion of the methodological aspects in section 4.1 and enhanced the discussion of the environmentally relevant results in section 4.2**

in the revised version of the MS.

Comment 2. The most serious issue is that rates determined in slurries from specific depth intervals in the sediment, amended with high concentrations of nitrate, are added together to obtain area-based rates. One issue here is that this relies on an accurate determination of the zone of nitrate consumption in situ, and it is not clearly specified how this was done. More importantly, however, the entire approach is not valid as clearly evident from loads of previous work on these processes in aquatic sediments. Sediment homogenization and slurrying generally stimulates activity, and at the high concentrations of nitrate the rates measured in the slurries are potential rates. Hence summing them up results in stark overestimation of the integrated in situ rate. This is easily seen in studies that determined rates in both slurries and intact sediment cores (e.g., see review by Trimmer and Engström 2011, in Ward et al. (eds.): Nitrification) and in the study by Sokoll et al. frequently cited in the present paper. It is correct, as suggested by Sokoll et al. that in situ rates may lie somewhere between whole-core and slurry rates when denitrification of intracellular nitrate plays a large role, since this is not captured with the isotope pairing technique. But the area-based rates obtained in the present study are 1-2 orders of magnitude higher than typical rates in continental sediments. Claiming such rates to be realistic is extraordinary and hence requires extraordinary evidence. Yet, the authors do not at all reflect on this matter. I strongly recommend that the depth integration exercise be abandoned altogether. It will be a serious setback if this type of rates makes it into the databases.

Reply: **We agree the reviewer's viewpoint that the depth integration of the potential rates in homogenized and high nitrate amended slurry incubation can overestimate *in situ* anammox, denitrification and DNRA rates. However, pore water nitrate penetrated to 8 cm in the sediment and our slurry incubations were performed with a resolution of 2 cm. In order to get a full understanding of the relative contribution of each nitrate reduction process, we integrated the potential rates down to nitrate penetration depth in slurry**

incubation.

Another question was that we did not describe how we defined the NO_x^- (NO_3^-+ NO_2^-) penetration depth in detail. We have improved the description on this matter in our revised MS. The NO_x^- penetration depth was defined down to the depth where the NO_x^- concentration did not decrease significantly with sediment depth increase. However, it should be noted that the low vertical resolution of nitrate profile might cause some over- or underestimation of nitrate penetration depth, and consequently the relative contribution of each process in total nitrate reduction.

We agree that the N-loss rates obtained in this study were the potential rates; they might not reflect the true N-loss rate. We followed your suggestion and removed the discussion about area-based N-loss in the revised MS. However, it should be noted that most of the previous studies on the N-loss rates were based on a steady state assumption and the nitrate supply in nitrate reduction was mainly controlled by molecular diffusion.

Comment 3. Another major issue has to do with the calculations and interpretations concerning intracellular nitrate pools. As presented now, there are several apparent inconsistencies, which need to be discussed (see comments below). The method used to determine 15N-labeling of ammonium to the best of my knowledge also converts organic N, which means that it is not possible to discriminate assimilatory and dissimilatory nitrate reduction to ammonium, ANRA and DNRA. This weakens the conclusion that DNRA is important in these sediments

4677, 15-17: The hypobromite method as applied by Preisler and here to whole slurries oxidizes organic amines as well as ammonium. This is why a distillation step was introduced by some workers. Thus, it is not possible to distinguish assimilatory and dissimilatory nitrate reduction to ammonium. This is particularly serious in a setting like this where it is argued that the sediment may contain algae that will reduce nitrate for assimilation.

Reply: **The interpretations on the effect of intracellular nitrate pools on**

calculations is discussed below (*see Comment 6*)

Concerning the Hypobromite conversion, we apologize for this confusion and have now described the hypobromite conversion more clearly in our Materials and methods section. We subsampled the water from the slurry and filtered it before hypobromite conversion. Thus, the $^{15}NH_4^+$ assimilated into organic matter would not have been converted into N_2 by hypobromite.

Consequently, we cannot make any statements about ANRA as this was not covered by our analysis.

Comment 4. Specific comments: 4674, 18-20: Novelty overstated. It is not correct that previous reports on the coexistence of denitrification, anammox, and DNRA are only from flux measurements. Dong et al. 2009, referenced later in the MS, did this in incubations. Also Trimmer and Nicholls 2009 (referenced) detected all three processes in experimental incubations, although DNRA rates were very low.

Reply: **We have revised this in the new version and the work of Dong et al. (2009) and Trimmer and Nicholls (2009) were cited in our revised MS.**

Comment 5. 4675, 1-2: Novelty overstated. It is not correct that there is currently no such model. Spott and Stange (2007) developed a model for this, and Jensen et al. 2011 (referenced and including authors of this paper!) present equations for situations where anammox and DNRA co-occur.

Reply: **We have rewritten these sentences in the new version and the work of Spott and Stange (2007) and Jensen et al. (2011) were discussed.**

Comment 6. 4678, 8 + 19 + 4680, 1: This is very confusing and seems self-contradicting. There are direct observations of nitrate accumulation, and nitrate release is assumed to happen only initially due to mixing, which is happens long before N-15 tracer is added, such that F_N does not change during incubation. In this case, it should be easy to correct for the release by simply using the nitrate concentration measured at the beginning. So why is there need for complicated

calculations to be able to “conclude” that nitrate release occurs? Surely, the best indication of this must be that it is directly measured? The only reason to do the complicated calculation must be that nitrate may be released gradually. What is the justification for the assumption that it is not? In any case, I find “conclude” to be too strong a word here, since one may think of other factors that could lead to $F_N^* < F_N$.

Reply: **Glud et al (2009) argued that nitrate should be released gradually during the incubation. However, results from both Sokoll et al (2012) and Dähnke et al (2012) indicated a rapid equilibration of the nitrate pools after $^{15}NO_3^-$ addition. From the current experiments and data in this study we cannot show a clear mechanism of nitrate release by the nitrate storing organisms. but this was not the main aim of our study. Therefore, we revised our discussion about nitrate release and removed the word “conclude”. Moreover, the evidence exhibited in our MS clearly indicated that nitrate release occurred in the slurry incubations and that this most likely was exchanged rapidly in accordance with Sokoll et al (2012) and Dähnke et al (2012), otherwise we would not have observed a linear relation between $^{29}N_2$ and $^{30}N_2$ production.**

Comment 7. 4681, 6-17: How is nitrate penetration depth defined? This is important for the integrated rates later. How does the depth integration deal with isolated subsurface peaks? And with the presence of nitrite?

Reply: **As we have discussed in *Comment 2*, the nitrate penetration depth was constrained to the depth where the NO_x^- concentration in the pore water did not decrease significantly with sediment depth increase. Undoubtedly, this could over- or underestimate actual NO_x^- penetration depth. The subsurface peaks of nitrate exceeding the bottom water NO_x^- concentration would clearly indicate active nitrification others may be a result of bioirrigation events. Thus, the peak layer was included when integrating the potential rates. Nitrite would be a part of the NO_x^- pool.**

Comment 8. 4683, 19-25: Some discussion of the depth distributions of these rates within the sediment is warranted, including the fact that they must be considered potential rates. The increase in DNRA with depth is consistent with the general finding of DNRA increasing in importance in more reducing sediments and that DNRA has often been observed when new nitrate is mixed into reducing, nitrate-free sediments (Nishio et al. 1982 etc.).

Reply: **We have stated more clearly that these are potential rates in the section of Materials and methods.**

Comment 9. 4686, 4-5: Again, the novelty is overestimated. Glud et al. (2009) did determine anammox in sediments with nitrate-storing forams and managed to discern the rates. Prokopenko et al. (2006) EPSL, suggested that anammox bacteria may receive nitrate/nitrite from nitrate-storing Thioploca. And in many other cases the good agreement between added and measured initial nitrate concentrations (e.g. early studies by Dalsgaard and others) indicate that there are no large hidden nitrate pools. There is no reason to suggest that earlier studies have all been ignorant about this, which is the impression given now.

Reply: **We discussed Glud et al (2009) in our revised version. However, as discussed above (*see Comment 6*), our result are more in line with the findings of Sokoll et al (2012) and Dähnke et al (2012).**

We also cited the work by Prokopenko et al. (2006) where they proposed a possible chemosymbiosis between *Thioploca* and anammox bacteria. However, there were no anammox rates in their work. Anyway, the possible chemosymbiosis between *Thioploca* and anammox bacteria implied that the prevalent Isotope Pairing Technique calculations are problematic if F_N is diluted when calculating denitrification and anammox rates.

It was not our intention to suggest that previous studies have been "ignorant" concerning "hidden" nitrate pools, however, there was little known on this topic until the last decade. Similar to the lack of consideration of anammox in these kind of experiments until 2002 (Thamdrup and Dalsgaard,

2002).

Comment 10. 4686, 11-13: Forams are present in most sediments. The important question is whether the species composition of the foram community hints at nitrate storage. And correct “similar TO areas”.

Reply: **Nitrate storage occurs widely in benthic foraminifera (Glud et al, 2009; Piña-Ochoa et al., 2010), but as mentioned above we have revised our discussion on this topic in the new version.**

Comment 11. 4686, 15: ”using conservatively”?

Reply: **This sentence is not included in the revised manuscript.**

Comment 12. 4686, 19-20: The fact that nitrate release only occurred in slurries with ^{15}N nitrate addition is an important, and highly confusing result and therefore should be mentioned in Results. According to p4682, 14-16, nitrate was already released during the preincubation, i.e. before ^{15}N-nitrate was added. So how did the organisms know which amendments were to come? And what about the incubations with ^{14}N-nitrate amendment? If, contrary to what I read on p4682 nitrate was released after amendment, what is the justification of assuming that F_N did not change during the incubation?

Reply: **As we discussed above (*see Comment 6*), we did not show a clear mechanism of nitrate release by the nitrate storing organisms in slurry incubation and it was not the main purpose of this MS. However, if the nitrate storing cells exchange their internal nitrate pool after addition of nitrate (Sokoll et al., 2012; Dähnke et al., 2012) they would react on the concentration change not on the amendment. In case of additions of ^{14}N-Nitrate this will not change the F_N and where there is no nitrate added there is no extra cellular pool to exchange with. If the F_N of the nitrate pool changed with time we would not expect the linear rates of $^{29}N_2$ and $^{30}N_2$ with time.**

Comment 13. 4687, 16: I suppose that "the effect of DNRA on denitrification and anammox" means its effect on the measurement of rates of these processes?

Reply: **Yes, it should be "the effect of DNRA on denitrification and anammox rates calculation when ^{15}N IPT was applied". In the revised version after condensing of the discussion on methodological aspects, this sentence was not included.**

Comment 14. 4687, 20-21 + Table 5: This is a useful table but it neglects a good number of studies that did not find DNRA to be of any significance (e.g., Binnerup et al. 1998, AEM; Rysgaard et al. 1993 AEM, Dalsgaard and Thamdrup 2002, Engstrom et al. 2009 L&O).

Reply: **We have revised Table 5 (now Table 4 in the revised manuscript) and added these studies mentioned by the reviewer.**

Comment 15. 4688, 5-8: The % DNRA depends critically on accurate determination of the zone of nitrate consumption and on the assumption that nitrate is not limiting for this process in situ (Km so low that potential and in situ rates are the same). These caveats should be discussed.

Reply: **There are contradicting results on Km values for DNRA vs Denitrification in marine sediments (i.e. *see* Dong et al 2011) and it is beyond the scope of this manuscript to determine them. Because the DNRA rates tend to increase with depth and did not correlate with the presence of measurable pore water nitrate like i.e. anammox rates, we have stated that these might be overestimated in the new version of the manuscript, furthermore all rates in the manuscript are considered as potential rates.**

Comment 16. 4688, 10 on: As discussed above, this discussion neglects the fact that slurrying generally stimulates rates. Also, a wide selection of half saturation constants can be found in the literature, often within the range of pore water concentrations. Why are those determined in permeable sediments particularly appropriate here? And

obviously nitrate concentrations MUST have been below the Km in part of the nitrate consumption zone. Otherwise nitrate would not be depleted. Rates exceeding 10 and up to 33 mmol/m^2/d are extreme, higher than most benthic N loss rates from marine settings. The loss rates reported here are NOT "a reasonable estimation of benthic N-loss on the ECS shelf"!

Reply: As we discussed above (*see* Comment 2 and Comment 15), we removed this discussion in the revised MS since it was not the main purpose of this study and we could not provide sufficient evidence to confirm the rates obtained from slurry incubation were close to *in situ* rate. However, please note that the *in situ* nitrate concentrations were up to 80 μM.

Comment 17. 4889, 25-26: The relative contribution of denitrification and anammox are interdependent variables. A correlation of the two makes no statistical sense.

Reply: **We have revised this in the new MS.**

Reference

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