Interactive comment on “Vertical activity distribution of dissimilatory nitrate reduction in coastal marine sediments” by A. Behrendt et al.

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Received and published: 23 August 2013

We would like to thank the referee for his/her comments and suggestions on our manuscript. Below we provide a point-by-point response to the reviewer’ comments and indicate how and where we will modify the manuscript.

SPECIFIC COMMENTS
Anonymous Referee #1: 8066, 8: Indicate range (59-130% rather than >59%).
Authors: We will change this; see revised version of the manuscript (line 35).

Anonymous Referee #1: 8066, 11: It would be better to move the section between brackets to end of the sentence.
Authors: We first followed your suggestion, but then found the sentence more difficult to understand than before. Instead, we restructured the sentence and think that it is clearer now; see revised version of the manuscript (line 37-39).

Anonymous Referee #1: 8066, 18-20: Maybe include a sentence saying that you expect the real contribution of DNRA to have been in between that in the intact cores and that in the slurries. The current sentence in really method-focused.

Authors: We agree with you that the true DNRA rates may lie in between those measured with the slurry incubations and the gel probe method. We picked up your suggestion to include this statement in the Abstract; additionally, we moved the method-focused sentence to the Conclusions.

Anonymous Referee #1: 8066, 23: Not sure if “predisposition” is the correct term here.

Authors: We will change this to: The balance between retention and loss of fixed nitrogen, especially NO3-, in coastal marine ecosystems is crucial as it defines the degree of eutrophication in these environments (lines 50-52).

Anonymous Referee #1: 8067, 1-4: Here, anammox is addressed, but then it sort of disappears. It should be made clear that anammox was not included in the DEN measurement (assuming that the acetylene inhibition method is specific for canonical denitrification) and it should be explained why anammox was ignored.

Authors: In the Introduction (line 56-59), we now explain that the relative contribution of anammox to fixed nitrogen removal is particularly low in very shallow (< 1 m) coastal marine sediments and cite two important review papers in the field. From our own data, it can be seen that in 4 out of 5 sediments, N2 production (measured as N2O production) closely matched NO3- consumption, thus leaving no room for anammox activity related to NO3- consumption. Only at Janssand, more NO3- was consumed than N2 (N2O) and NH4+ was produced, leaving some room for anammox activity (but also for NO3- assimilation and NO3- storage). We now mention this possibility in the Discuss-
sion (line 461-465). In the Methods (line 204-211), we now explain that acetylene does also inhibit anammox and nitrification, but that this will not affect denitrification-derived N2O production in anoxic sediment layers.

Anonymous Referee #1: 8068, 15: No need to start new paragraph here (text is direct continuation of the preceding).

Authors: We will change this; see revised version of the manuscript (line 98).

Anonymous Referee #1: 8069, 18: Overnight incubation at 15 degrees is quite a strong deviation from in situ temperatures for Arhus Bay and the Mississipi delta. Potential implication?

Authors: You are right in stating that this temperature change would influence the gradients in the sediment cores due to changes in microbial activity. We forgot to mention that the storage over night at 15°C only occurred for the sediment from the Limfjord (due to logistic issues). Compared to the ambient temperature of 16.6°C, the storage at 15°C should not induce any changes in the chemical and the microbial community composition in this sediment. As overnight storage only occurred for Limfjord sediment that was anyway kept at 15°C throughout the experiment, we deleted this misleading sentence. See changes in revised manuscript version (line 128).

Anonymous Referee #1: 8070, 25-27: So the inhibitor is specific for denitrification, meaning that anammox is not included in these measurements? > this should be made clear (see other comments on this).

Authors: Please refer to your comment and our response above (8067, 1-4).

Anonymous Referee #1: 8071: The calculations of DEN from the microprofiles appears to be based on quite some assumptions and coefficients. It is difficult to get a feeling for how strongly the outcome of these calculations is influenced by these factors. Maybe there is a way to clarify this. This also applies to the DNRA calculation on page 8073.

Authors: We understand that the calculation of DEN and DNRA fluxes is difficult to
follow and we therefore streamlined the respective paragraphs (lines 226-232 and 268-273), with a special focus on removing phrases of minor importance that might distract the reader from the key points of the calculations. For instance, the offset correction of some N2O profiles does not affect the outcome of the flux calculations at all because the concentration gradients remain the same. The correction of 15NH4+ concentrations for the natural 15NH4+ abundance in coastal marine sediments only had a minor influence on the outcome of the flux calculations, which is now mentioned in the text. A few more rearrangements of sentences were made in 2.3 and 2.4 to improve the readability of this section. Aside from the maybe too complicated wording, the calculation procedures used are in general well established and not different from other rate or flux calculations based on microsensor measurements in diffusive sediments (diffusion-reaction modeling). Also the correction of the diffusion coefficient, based on the porosity of the respective sediment, is widely used in rate/flux calculations in sediments.

Anonymous Referee #1: 8072, 12-13: Explain why spikes of 14NH4 were added (used as carrier because ambient NH4 concentrations were too low for detection?) and that this was later corrected for (I assume).

Authors: The spike was added to the Mississippi and Janssand hypobromite assay to get a higher background and thereby higher peaks (including 14+15N) at the mass spectrometer. This improved the calculations and peak integration. We include a sentence stating that we corrected in these two sediments for the spike concentrations. See new manuscript version (line 259-261).

Anonymous Referee #1: 8072, 18-19: Just different concentrations or also different atom%15N

Authors: Standards were only set to different concentration, but the atom % was the same. We included now a better specification (Ammonium-15N Chloride 98 % 15N atom %, Cambridge Isotope Laboratories, Andover, MA, U.S.A.). See changes in re-
vised manuscript version (line 184-185 and 253-255).

Anonymous Referee #1: 8074, 18: Explain how NH4 adsorption was subsequently calculated.

Authors: The concentration of ammonium measured in the samples with the addition of solution A represents the ambient pore water concentration. The concentration of ammonium measured in the samples with the addition of solution B represents the ambient concentration plus the 50 \( \mu \text{mol L}^{-1} \) ammonium (which mimics newly produced ammonium) minus the ammonium lost due to adsorption to sediment particles. We added a short description of how we calculated the percentage of the added NH4+ that adsorbs to the sediment (see line 305-308).

Anonymous Referee #1: 8074: Write CNS in full.

Authors: Carbon-Nitrogen-Sulfur analysis. See changes in revised manuscript (line 309).

Anonymous Referee #1: 8075, section 2.7: It makes more sense to present this experiment before presenting all the analytical techniques.

Authors: We moved this section behind the paragraph of the experimental setup and sampling design beginning line 152. Additionally corrections due to the changed order have been made.

Anonymous Referee #1: 8076, 3-4: The KCl addition seems like a good method to overcome possible adsorption of 15NH4 to the sediment. Then why was the extra adsorption experiment performed? It seems like results from this experiment are not needed for calculation of the DNRA rates when results were not biased by adsorption.

Authors: We are sorry, but this is obviously a misunderstanding: The combination of ammonium desorption by KCl and the directly following hypobromite assay was only applied for the slurry experiments, in which at each time point the incubation was terminated in 1 exetainer by the addition of KCl. In fact, it did make a lot of sense to apply
KCl extraction in the slurry experiments because the Janssand sediment had proven before to bind significant amounts of NH4+. For the core experiments, KCl addition was not possible during the gel-probe measurements because the high KCl concentration would have strongly or even fatally affected microbial activities in the sediment. Therefore, the adsorption of ammonium to sediment particles and an underestimation of the newly produced ammonium via DNRA do play a role in the core experiments.

Anonymous Referee #1: 8077, 24-26: If I understand correctly, it is stated that there was only DNRA at Janssand because that was the only sediment with a usable shape of the sediment profile of 15NH4. However, when I look at Fig. 1, I also see clear production of 15NH4 for Arhus, Mississippi, and Limfjord, with most in the same range as for Janssand. It seems strange to me to interpret these results as no DNRA. If no fluxes can be calculated from these profiles, alternative ways to derived DNRA rates from these data should be investigated. Total 15NH4 production should provide a good indication of DNRA rates. If there is some reason not to do this, then this point should at least be addressed more explicitly in the discussion. (also see general comment).

Authors: The reviewer is right that significant 15NH4+ concentrations were also measured in sediments from sites other than Janssand. However, these data cannot be used for a proper quantitative comparison with the NO3-consumption fluxes and the N2O (N2)-production fluxes. For such comparisons, you either need steady-state concentration profiles with curvatures indicative of production/consumption of the three solutes, or you need time series of the production/consumption of the three solutes in the sediment. For Janssand, but not for any of the other sediments, the first condition is met. Unfortunately, the second condition is generally not met for the whole core incubations. If repeated samplings with the gel probes would exist, then the gradual build-up of 15NH4+ in the sediment could be used for rate calculations, but, as said, we don’t have such data. We added these explanations to the Results (line 378-384) and then pick them up again in the Discussion (line 441-446).

Anonymous Referee #1: 8078, 4-5: (“No N2O was detectable”) > There is N2O present...
in Dorum sediment without acetylene in Fig. 1f > explain.

Authors: We apologize that we forgot to mention that low N2O concentrations were measured in the sediment from Dorum without the inhibition with acetylene. We changed the sentence in the revised manuscript version (see line 375/376).

Anonymous Referee #1: 8078, 6-13: See previous comments to 15NH4 production. Moreover, this section includes some interpretation and speculations that would be more at place in the discussion.

Authors: Please refer to your comment and our response above (8077, 24-26).

Anonymous Referee #1: 8078, 11-13 and 20-22: Wasn’t adsorption of 15NH4 to the sediment prevented by addition of the KCl to the sediment samples for these analyses? This would mean that there is no bias from adsorption here. (see previous comments).

Authors: Please see previous answer (8076, 3-4).

Anonymous Referee #1: 8079, section 3.5: I think that results from this experiment deserve to be presented in a figure (stacked bar or similar).

Authors: We changed Table 4 into a Figure. See Figure 4 in revised manuscript version.

Anonymous Referee #1: 8080, 10-12: It should be better explained how these factors may have influenced these results. Moreover, the contribution by anammox would only apply when anammox was included in the DEN measurements, which appears not to have been the case (see other comments on this).

Authors: The influence of these factors on DEN and DNRA is addressed later in the discussion (see line 469-479). In this introductory part of the discussion, a short overview of the highlights to follow is given. The statement on anammox was indeed wrong and was therefore deleted here. However, we now mention the indirect contribution of anammox to NO3- removal in a later section where possible explanations for the not closed nitrogen budgets in the Janssand and Aarhus sediments are listed (line
Anonymous Referee #1: 8080, 18 (“In fact, in all four sediments, the measured 15NH4+ concentrations clearly exceeded the natural abundance of 15NH4+ usually found in the pore water of coastal marine sediment”): This means that DNRA did occur!? (see other comments on this).

Authors: Please refer to our response given above (8077, 24-26).

Anonymous Referee #1: 8080, 20-22: These mechanisms also involve DNRA, right? (see comment above and others).

Authors: Yes. Please refer to our response given above (8077, 24-26).

Anonymous Referee #1: Section 4.1: What I found missing in the discussion here is an evaluation of how the high added nitrate concentrations (compared to ambient concentrations) may have affected the outcome here. This seems particularly important according to this statement from section 4.2.1: “Supposedly, DNRA is the favored pathway under nitrate-limited conditions, while DEN is the favored pathway under nitrate-replete conditions”. (see general comment).

Authors: We added a whole paragraph to the Discussion (line 562-576) on this important issue. Additionally, we hint to the possible effect of NO3-enrichment of the overlying water on NO3-removal pathways in the M+M section (lines 229-232 and 271-273).

Anonymous Referee #1: 8081, 2-4: When KCl was added to the samples, then adsorption was not an issue here? See other comments on this.

Authors: Please see previous answer on this (8076, 3-4).

Anonymous Referee #1: 8081, 17-27: What about nitrate production by nitrification in the sediment as an additional source of nitrate? I was also wondering how this would affect the various depth profiles and their interpretation. If nitrification was relevant
indeed, this should be addressed.

Authors: Dorum was the only sediment where we could detect nitrification activity. We address this in the results part of the manuscript (see line 350-352). The increase of nitrate availability in the sediment of Dorum was nevertheless in the same order of magnitude as the daily methodical fluctuation of nitrate supply during the experiment. It might be that due to the slight nitrification activity in the sediment nitrate diffused deeper into the sediment, but the overall nitrogen budget was not affected. For the other sediments, no additional nitrate source through nitrification activity could be detected.

Anonymous Referee #1: 8082, 11-24: This includes quite some repetition of results (not necessary).

Authors: We agree that this paragraph contains some results, but these results provide the first synthesis of the outcome of the slurry vs. whole core incubations. For this reason, we would like to leave this paragraph as it is.


Authors: This is correct. We wanted to indicate that because of higher sulfide concentrations in the sediment, the N2O reductase in denitrifiers might be partially inhibited. That is why we expected to see higher N2O production in these samples. We added a sentence on this in the revised manuscript version. See new manuscript version (line 502-506).

Anonymous Referee #1: 8083, section 4.1.3: This section can be merged with the previous

Authors: The idea was to first discuss the two different methodical approaches separately and then lead over to a comparison of the methods with respect to their advantage and disadvantage in the different sediments. No changes have been made because we think that this structure supports our discussion. Additionally we corrected a wrong assignment in the discussion (see line 510-513). As this did not include any
changes in the conclusion we only rearranged the sentence.
Anonymous Referee #1: 8084, 9: “partitioning” can be removed.
Authors: We changed it. See new manuscript version (line 544).
Anonymous Referee #1: 8086, 20-25: See previous comments on this.
Authors: Please see previous answer.
Anonymous Referee #1: Figure 1. This figure is nice. However, as printed now it is too small. This should be a full page figure. In addition, some colors could be added for better distinction between the different profiles. pH profiles are shown in this figure but are not addressed in this manuscript. I would suggest to either address them (when relevant) or remove them completely from the figure.
Authors: Figure 1 was intended as a full page figure. We will get in contact with Biogeoscience to arrange this. Additionally we changed the figure and added colors. The pH profiles were used for the calculations of the total sulfide concentration in the sediments. This is addressed in the material and method part of the manuscript (line 233-236).

Interactive comment on Biogeosciences Discuss., 10, 8065, 2013.