We thank both reviewers for their constructive criticism of the manuscript. Both reviewers raise the point that the data may actually support the hypothesis that denitrification occurs within anoxic grains. Furthermore, reviewer 2 suggests additional stirred reactor experiments to test this hypothesis. We have now undertaken these and the results do indeed suggest denitrification occurs under bulk oxic conditions. On this basis, we have completely re-written the manuscript. Below is our detailed response to the reviewer comments (in bold).

Reviewer 1

The manuscript by Cook et al discusses, based on flow-through reactor experiments, the possibilities for denitrification in porous sand grains, which may possibly act as oxygen depleted microniches thus providing an environment for oxygen-sensitive den- itrification. The authors, however, conclude that there is no evidence for the existence of those microniches based on the oxygen sensitivity and the absence of diffusion lim- itation for nitrate. Given the current microniche-focus in N cycle research, Cook et al. add an interesting piece of work which should be available to the readers of Bio- geosciences. To me the manuscript is overall very interesting, nicely structured and focused to the point. I have several comments listed in the following which I believe have to be addressed to make it clearer to the reader:

General comments:

1. Denitrification is defined, in the beginning, however, other N cycle processes are not mentioned in this context (anammox, DNRA), although the methods part basically describes that at least anammox-derived N2 would have been measurable. Both of them would in principle be able to occur under similar conditions.

We did not quantify DNRA, so an exact measurement of anammox is not possible with our data, but we can make an estimate if we assume this process to be negligible. Using the 29/30 ratio from the 300 μM 15NO3- addition, we can conservatively assume that all the 29N2 production is due to anammox. Using equation 23 from Risgaard-Petersen et al, we calculate anammox comprised a maximum of 16% of N2 production.

We have now briefly mentioned this in the methods section, and we now state that anammox comprised <16% of N_2 production in the results

2. To me, the fact that denitrification occurs already at 10μM O2 is rather an indication that there is indeed a bit of an effect on the process. 10μM O2 is pretty high for mea- surable rates of N2 production. Dalsgaard et al (2014) actually showed that minimal changes in O2 can largely impact on denitrification rates

In response to both reviewers comments on this, we have undertaken addition stirred reactor experiments and now completely re-interpreted our results along the lines suggested here. Of particular relevance to this comment, we now start section 4.3 with the following sentences

'The experiments performed here showed denitrification was able to take place at oxygen concentrations oxygen concentrations below 20 μM at site 1 in the FTR and SR experiments and as high as 50 μM in the coarse fraction in the SR experiments (Figs 4 and 5). It has previously been shown that nanomolar concentrations of oxygen can inhibit

denitrification (Dalsgaard et al., 2014), suggesting that denitrification was taking places within anoxic niches within the grains.'

3. I would like to see more of a discussion of what this means globally, are there many sediments like this which were suspected to be sites of intense N loss? This basically requires to make a stronger statement on your results. In this context, those grains may not act as microniches for denitrification, however, there may be tipping points e.g. if the organic carbon source increases by eutrophication where indeed this changes. What I think of is that maybe they are just not microniches, yet.

As mentioned above, we have now completely re-interpreted our results. We believe there is evidence for microniches and the ecological implication for this are now discussed in the revised section 4.4. We have avoided any global sttements, as we believe further work is needed before this bigger picture implications are known.

T Dalsgaard et al., Oxygen at Nanomolar Levels Reversibly Suppresses Process Rates and Gene Expression in Anammox and Denitrification in the Oxygen Minimum Zone off Northern Chile mBio 5 (6), e01966-14, 2014.

Specific comments:

Throughout the text: Please check, whether abbreviations are spelled out when men- tioned the first time, please unify O2/ oxygen, please check units (sometimes it says uM instead of µM)

Checked and changed as suggested

p. 2, l18: Please clarify, which process rates.
l. 20: I disagree on that statement, 10µM are pretty high for full denitrification. L23, Please remove 'rates' after denitrification.
l. 23: I am missing a sentence on the meaning of the result, here.

Abstract now re-written taking these points into account

l. 31: This effect could be positive or negative, could you elaborate a bit more?

This gets complicated and is still under debate, we have just said it can both enhance and reduce denitrification.

l. 36 Insert 'under' after 'place'.

Changed

p. 3, l 57: Where does this number (50µM) come from?

We have now removed specific value and simply stated under low oxygen conditions

l. 70 ff: I would like to see a map with the sampling locations. Also, for all companies, a location should be added.

We believe a map is not necessary, there are many previous studies of Heron island with maps and with the advent of Google maps, and the coordinates, an interested reader can instantly look up the sites.

l.95: This also changes the CO2 content and with that the pH, what could be the impact?

This would increase the pH, which could change nitrification, however there are no studies that have shown an effect of pH on denitrification to our knowledge. We have been undertaking similar studies for many years now, and we typically see constant denitrification rates over hours of purging (Evrard et al., 2013)over which time, there would be the greatest pH change.

l. 117: This sentence is odd, please rephrase

Now rephrased to

Rates of denitrification were constant above NO_3^- concentrations of 18 μM at all three study sites, and were highest at site 3 which had the highest sediment oxygen consumption rates and lowest at site 2 which had the lowest oxygen consumption rates (Figure 2)

l. 163: This doesn't necessarily have to happen, it may be that denitrification occurs in a range where it wouldn't occur without porous grains.

This argument has been removed

l. 184: The fact that the anoxic zone is reduced doesn't necessarily translate into lower denitrification in a case where substrate supply is higher. It may actually well be that at the oxycline a zone of intense denitrification forms.

This part of the discussion has been removed

l. 190: remove 'rates'

We believe this wording is correct, left as is

l.201: Does this make sense in your sediments in terms of light penetration depths?

This argument is based not on light penetration, but on the continual mixing of permeable sediment leading to the burial of algae

l. 216: Could also be over-estimating- could you add a reference so that it gets clear what you are talking about, here?

This part of the discussion has been substantially revised so as to make this comment obsolete

I wish the conclusion could end with a stronger statement on the meaning of your results.

We have now re-written the final section. We have however avoided strong statements as further work is required to investigate the significance of this.

Reviewer 2

The authors test the hypothesis whether micro-sites in porous carbonate sands can become anoxic, thus providing important niches for denitrification under bulk oxic con- ditions. They use flow through reactors (FTRs) packed with carbonate sands from 3 station and measured the denitrification rates under various oxygen and nitrate con- centrations, postulating that any diffusion limitation of O2 or NO3- in the micro-niche should be observable in the bulk denitrification rates. The authors measured very dif- ferent O2 and NO3- consumption rates at the 3 stations, but they observed no change of denitrification rates at each site for decreasing NO3- concentrations down to 18 µM (the lowest inflow concentration tested). Under bulk oxic conditions, denitrification rates were only measured when the outflow O2 was below 10 µM. The authors suggested that anoxic micro-niches do not exist and that denitrification is not affected.

The authors address an important problem in sediment biogeochemistry which is still not resolved: whether denitrification is active in permeable sands under bulk oxic con- ditions. The manuscript is well organized and clearly written. I have, however, some major concerns about the proposed interpretation of the results.

The authors use flow through reactors (FTRs) to investigate the effect of diffusion lim- itation on oxic respiration and the formation of anoxic micro-niches, and subsequently on denitrification rates. In general, diffusive transport depends on concentration gradi- ents and such 'limitation experiments' should therefore have full control of the ambient O2 and NO3- concentrations. I doubt that FTRs are the right choice for such exper- iments, because they produce a considerable concentration gradient between inflow and outflow, which is actually necessary to determine the reaction rate. The differ- ences in O2 concentration at inflow and outflow are well documented in Fig. 3 where they are of the order of a 50-100µM.

This has some significant implications: when the authors state that (abstract) "denitrifi- cation was only observed to commence at substantial rates below 10 µM O2" they refer to outflow concentrations. This means that O2 concentrations at the inflow must have been between 55µM and 90µM (back calculated from O2 rates in table 1 and 10min retention time), so that spatially averaged concentrations in the FTRs are 30-50µM.

Now, from this perspective, the results actually do support the hypothesis of denitrifica- tion in anoxic micro-niches. This is also in line with the authors who conclude from their calculation of diffusion limitation: (equation 3, Line 171) ". . .we would expect denitrifi- cation to have commenced at O2 concentrations below 30-50 µM. . . (in case of anoxic micro niches)".

Another argument put forward was the non-limitation of denitrification rates at decreas- ing NO3- concentrations from 300µM down to 18µM (anoxic conditions). Of course, there is also a NO3- gradient in the FTR as described above for O2 which makes it complicated to study such concentration limitation. Further, when applying equation 3, the expected NO3- concentration gradient in a 3mm grain is only 0.3-4µM – a change which is probably too little to be reflected in decreased rates.

In summary, it is possible to interpret the results just as well in favor of micro-niche denitrification. In general, I feel that FTRs are not well suited to study concentration dependent rates, because they provide a large variety of different concentrations be- tween in- and outflow. The situation is even worse when considering the dispersion effects of a non-ideal plug flow, which was not discussed at all.

Because concentration differences between in- and outflow are necessary for the rate calculation in FTR studies they cannot be minimized without increasing the error of the rate calculations. A possible way out of this dilemma would be the use of stirred slurry incubations to study concentration dependent rates (as described for example in Gao et al. 2009, ISME doi:10.1038/ismej.2009.127).

We thank the reviewer for their thoughtful comments and suggestions. We have now undertaken the stirred reactor experiments as suggested. The results do indeed support the

presence of denitrification under oxic conditions and we have now re-written the manuscript to reflect this.

Minor comments:

Line 36: For denitrification to take place in (!) anoxic conditions

changed

Line 62: what units have ‘a’ and ‘J’ ? J is probably not a flux here. . ...

This part has now been removed

Line 76: please specify the dimensions of the FTRs.

These details now added. 4.6 cm diameter, 4 cm length

Line 80: “For denitrification to take place anoxic conditions. . .. . .” this sentence does not fit here. . .

Possible an error in refence to line number? I can’t see this here.

Line 87: please specify in this section if the measured rates are per volume porewater or per volume wet sediment

Per volume wet sediment now specified

References cited

Dalsgaard, T., Stewart, F. J., Thamdrup, B., De Brabandere, L., Revsbech, N. P., Ulloa, O., Canfield, D. E., and DeLong, E. F.: Oxygen at Nanomolar Levels Reversibly Suppresses Process Rates and Gene Expression in Anammox and Denitrification in the Oxygen Minimum Zone off Northern Chile, mBio, 5, e01966-01914, 2014.

Evrard, V., Glud, R. N., and Cook, P. L. M.: The kinetics of denitrification in permeable sediments, Biogeochemistry, 113, 563-572, 2013.