

Review of the manuscript 'Does denitrification occur within porous carbonate sand grains?' (bg-2016-530) by Perran Cook et al.

General comments:

The manuscript 'Does denitrification occur within porous carbonate sand grains?' by Cook et al. addresses the question whether porous carbonate sands provide anaerobic micro niches. The authors use a series of controlled incubation experiments with stirred and flow-through reactors in combination with isotopically labeled nitrate. This design enables to quantify and understand the kinetics of N-turnover in sediments. The appealing detail of the presented study is the use of porous carbonate sand, which potentially provides the disputed micro niches with a high probability. The comparison of N-turnover in sediment composed of either porous or non-porous grains might make an important point in the ongoing debate on micro niches.

The manuscript is well written and structured, the language is better than mine.

However, my main concern is that the authors missed good opportunities to add new insights to the ongoing discussion on the significance of micro niches.

1.) From my perspective, the question is not whether or not such anaerobic micro niches actually occur in natural sediment. There are always sheltered places within poorly connected pores and angles, and grooves and cracks occur even on quartz grains (a tracer break-through curve would reveal the connectedness of the pore network). In comparison with more exposed grain surfaces, these niches have a reduced exchange with the bulk pore water. It is thus inevitable that some spots within a complex pore network become earlier anoxic than the (averaged) oxygen concentration of the bulk pore. Vigorous stirring as performed in the experiments with the stirred reactor should eliminate or at least reduce such inter-granular micro niches caused by grain roughness or poorly connected intergranular pores. So one would expect that denitrification is more sensitive to the bulk oxygen concentration in the stirred reactor than in the packed flow-through reactor. But the presented data indicates the opposite effect: Denitrification in stirred reactor commenced at higher oxygen concentration (section 3), and the authors did not discuss this. It would be helpful here to see and to compare the volumetric denitrification rates (mol N per time and sediment volume) of both reactor types. Ultimately, the comparison of the results from the stirred reactor and porous grains with the results from Gao et al. (2010, doi:10.1038/ismej.2009.127) with non-porous sand could elucidate the effect of the internal porosity of the carbonate sand. Unfortunately, the authors missed this opportunity.

2.) The authors explain the significance of the anaerobic micro niches with the potentially tight coupling between nitrification and denitrification. In this context would it be interesting to see the in-situ concentrations of nitrate and ammonium (the substrate for nitrification). Additionally, the isotope pairing method employed by the authors enables to differentiate between the ambient nitrate (^{14}N) and the labeled nitrate (^{15}N). If the micro niches contribute to the overall N-cycling then I would expect that the contribution of ambient N (nitrate + ammonium) to total denitrification differs between the stirred and flow-through reactors. But unfortunately, the authors did not provide these results. A little more effort (e.g. labeling the ammonium in incubations) or measuring the

isotope ratio of the ambient nitrate (Deek et al. 2013, doi:10.3354/meps10514) would directly enable to quantify the coupled nitrification-denitrification.

3.) As the authors underlined in section 4.2 is the 'availability, and composition of organic matter [...] a key factor controlling potential denitrification rates'. However, the authors did not present any measurement on quantity or quality of the organic matter such as total organic carbon (TOC), amino-acid based reactivity index (RI) or degradation index (DI). Even a simple measurement of chlorophyll content would provide a proxy for the sediment reactivity, and would also enable to discuss the observed differences in the denitrification rates without having to speculate about phyto-detritus (p. 6, l. 190). Such organic matter proxies as part of a thorough description of the sampling site would also help other researchers to re-use the denitrification rates presented by the authors.

In summary, the presented results could make an important contribution to the understanding of N-cycling in sediment with complex structure and should be published. But I have the impression that the presentation of results and the discussion are incomplete, and that the presented manuscript does not tap its full potential. In its current form it is impossible to conclude whether or not the internal porosity of carbonate grains contributes to denitrification.

I suggest that the authors present and discuss the complete volumetric denitrification rates for both reactor types, preferably as a table. This summary should also give the detailed results of the isotope pairing incubation (D14 and D15 separately, estimates for anammox and nitrification). A more complete characterization of the sampled sites with an organic matter proxy (TOC, DI, RI, chlorophyll) and ambient concentrations of nutrients would increase the re-usability of the results for other topics.

Minor comments:

- Section 2.1, p. 2, l. 83: Reaction rates are were calculated ... -> delete 'are'
- Section 2.2, p. 4, l. 105: course -> coarse?
- Table 1: Are the denitrification rates from SR or FTR?
- Figure 2: Are the error bars of site 1 and 2 missing or just too small to show?
- Figure 6: From which type of experiment are the presented results- SR or FTR?
- Figure 6: Error bars (where applicable) are missing