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Interactive comment on "Does denitrification occur within porous carbonate sand grains?" *by* Perran L. M. Cook et al.

Anonymous Referee #2

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The authors test the hypothesis whether micro-sites in porous carbonate sands can become anoxic, thus providing important niches for denitrification under bulk oxic conditions. They use flow through reactors (FTRs) packed with carbonate sands from 3 station and measured the denitrification rates under various oxygen and nitrate concentrations, 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 different 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

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not resolved: whether denitrification is active in permeable sands under bulk oxic conditions. 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 limitation on oxic respiration and the formation of anoxic micro-niches, and subsequently on denitrification rates. In general, diffusive transport depends on concentration gradients 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 experiments, because they produce a considerable concentration gradient between inflow and outflow, which is actually necessary to determine the reaction rate. The differences 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) "denitrification 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 denitrification 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 denitrification 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 decreasing 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

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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 between 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).

Minor comments:

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

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

Line 76: please specify the dimensions of the FTRs.

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

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

Line 113: the permeability is very low for such coarse grain sizes (median 0.9 and 0.7mm). I would expect something in the range of X*10-10 m^2. Do you have an explanation?

Line 144: ". . .waited >14 hours before oxygen consumption measurements commenced. . ." Please specify if the cores were flushed and which volume flow you used.

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