Biogeosciences Discuss.,
doi:10.5194/bg-2015-533-RC2, 2016

Biogeosciences Discussions

Interactive comment on "Rates and pathways of CH_4 oxidation in ferruginous Lake Matano, Indonesia" *by* A. Sturm et al.

Anonymous Referee #2

Received and published: 13 April 2016

This is an interesting paper that contributes nicely to a growing body of work suggesting an important role for anaerobic methane oxidation in terrestrial systems. I think the conclusions should be more circumspect- the data to not add up to convincing proof or disproof for the role of any major electron acceptor.

p 2 line 22 "A significant fraction of this CH4 is then consumed through microbially catalyzed oxidation". This is a scientifically improper use of "significant". Consumption of 1% could be statistically significant, but would not be meaningful.

p 3 line 9 "Large intervals of diffusion limited marine sediments contain abundant sulfate and are therefore favorable to AOM". Interval implies a space or regular spaces between two delimiters. Perhaps "zone" would be better?

p 3 line 26 "However, natural systems rich in nitrate with rapid N-cycling, such as fresh-

water lakes, estuaries, and wetlands, should have the potential to oxidize immense amounts of CH4 through nitrate dependent AOM (Joye et al., 1999)." The adjectives "immense" and "rich" here are probably inappropriate. Nitrogen is limiting in most terrestrial environments, and the amounts of nitrate available will generally be stoichiometrically too low to account for methane oxidation. The implication that nitrate in freshwaters is an analog of sulfate in marine waters is unreasonable. Notably, the process has only been demonstrated so far in highly polluted waters and wastewater.

On the other hand, there are a growing number of studies demonstrating substantial AOM in freshwater wetlands, although (as in the present study) most lack clear evidence of what the main electron acceptors are. The authors list a few of these studies on p 4 line 1-5, but could give a broader perspective on recent environmental studies of AOM and the estimated potential magnitude of AOM in freshwater systems. See, e.g. Gupta et al Environ. Sci. Technol., 2013, 47 (15), pp 8273–8279; Segarra et al 2015 Nature Comm 6: 7477; and several others. The referencing in general in the manuscript is biased towards older studies. Only about 20 of the 75 references were written in the last decade. The Introduction and Discussion, and Table 1, could use some updated context.

Section 2.2.2 is a bit unclear: D is the O2 diffusivity in water (2.55386$\times$10$-$5 cm2 s$-$1) (at what T?), but the D along a tortuous path between the piston and the syringe wall would presumably be much less than this. Why did the authors not simply perform an experiment with sterilized anoxic water to control for this leakage? The conclusion about syringe leakage in Section 4.2 p 14 lines 2-5 is worrying: "Our calculations also suggest rates of O2 diffusion into the syringe of between 1.16$\times$10$-$6 and 4.21 $\times$ 10$-$7 μmol cm$-$2 s$-$1, could have supplied up to 19 % of the total O2 needed to match the observed CH4 oxidation." This is a large amount, and as we should assume that the calculation is only approximate, this is a very large potential source of error and uncertainty. Fortunately, the interesting part of the study is not the oxic water, but the anoxic waters discussed in section 4.3.

The units used to summarize the findings of sections 2.2.2 and 2.2.3 are different. It would be clearer if these were in the same units, e.g. if both were summarized in a similar way such as: "with the potential to oxidize CH4 at a maximum rate of xx nmol L^{-1} d^{-1}."

Figure 5 and p 15 line 12: "All electron acceptors considered provide more than this minimum amount of free energy, except for sulfate, which is close to the minimum energies (Fig. 5)." Firstly. I disagree with the interpretation given in the figure legend that sulfate is close to the -15 kJ mol^{-1} threshold. It is closer to -30, and a viable process. Secondly, this actually suggests to me that sulfate is the most likely electron acceptor for methane oxidation. If methane oxidation coupled to sulfate reduction is active and methane is in excess, then the bacterial community should grow to the point where it reduces sulfate close to an equilibrium point where a minimum energy is obtained. The excess available amounts of other electron acceptors suggest that these are not effectively being used to oxidise methane.

Another issue is that the rates are not measured in situ, they are estimated in closed incubation vessels (for a period of up to 18d) after sampling the waters. Therefore the rates do not account for diffusive fluxes of the electron acceptors in situ. In the absence of diffusive flux, sulfate, which is normally near an equilibrium level, could become depleted in the incubation syringes below the level where it can support methane oxidation. The methane oxidation rates and the sulfate reduction rates may therefore both be underestimated.

I also do not fully agree with the conclusion in the Abstract line 7: "Here, CH4 oxidation proceeds in the apparent absence of oxygen (O2) and instead appears to be coupled to nitrate ($NO^{-}3$), nitrite ($NO^{-}2$), iron (Fe), or manganese (Mn) reduction." This is repeated in the conclusion. It seems to be a selective interpretation of the data. I do not believe that sulfate can be discounted, not that there is clear evidence for nitrate, Fe or Mn. The data do not seem to add up to a coherent theory supporting any single oxidant. For example, the results presented on p 15 lines 17-22 ff contradict the au-

thors' conclusion. The calculations can account for only small amounts of measured methane oxidation via Fe, Mn or nitrate reduction, with the sole exception of nitrate at a single depth (130 m). On the other hand, sulfate concentrations could account for all the CH4 oxidation observed at these depths (except at 130 m). The argument against sulfate reduction is the low measured sufate reduction rates. However, the contradictory nature of the two lines of evidence, coupled with the absence of reported reduction rates for nitrate, Fe and Mn, does not add up to clear evidence for any single oxidant.

I think a better approach is to stress that the process of anaerobic methane oxidation occurs but to embrace the uncertainty (as on p 16 line 2) about the mechanism(s) in the abstract and conclusions.

Figure 6 should be cleaned up a bit. Please explain in the legend that the red dots indicate assimilation, and separate Fe2+ and O2 in the top x axis.

p 16 line 22 Some review of known assimilation efficiencies of aerobic and anaerobic methanotrophs would be useful here.

NOx is usually used to denote NO + NO2, and its use in this manustrict to denote nitrate + nitrate will be confusing to many.

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