Biogeosciences Discuss., 9, C3906–C3909, 2012
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Biogeosciences Discussions

Interactive comment on "Microbial methane oxidation at the redoxcline of the Gotland Deep (Central Baltic Sea)" *by* O. Schmale et al.

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Received and published: 11 September 2012

Schmale and co-workers present a comprehensive data set about the water column chemistry with respect to methane oxidation of the Gotland Deep in the Baltic Sea. They combine this approach with analysis of lipid biomarkers and pmoa gene expression from a single sample at ∼100 m water depth in order to narrow down the responsible microbial players. Whereas the water column chemistry is convincing the opposite is true for the microbial player analysis. Looking at the water column chemistry, the authors neglect zones of highest turnover (concentration changes) of oxygen and methane, which are in one case above (∼80 m water depth) and in the other below (∼120 m water depth) the zone of actual sampling. The former indicates biogeochemical processes driven by other electron donors than methane; the latter points to the fact

that there is likely a strong anaerobic oxidation component associated with methane turnover. Analyses of theses water depths would strongly affect the larger implications of this study. If, on the contrary, aerobic methane oxidation at low oxygen levels occurs, the authors would have missed the full representation of this process, eventually in terms of lipid biomarkers but most likely for the molecular biology work.

Generally, the study is a valuable piece of work but it suffers from a bad sample strategy. The authors should take care of adding the requested data, but depending on the availability of such samples this might not be possible, or they are left behind with insufficient information from current biomarker analysis and molecular work. Consequently, I would not recommend publishing the paper in its present form.

Specific comments:

Page 8784 Line 8: Exchange “mirrored” with “evident”. Line 9 and 10: The instrumental precision of the method is given with $\pm$ 1‰ (see methods). Therefore, I see no reason why the digit is justified here. Please check throughout the manuscript and change accordingly. Line 18: Delete “the idea”.

Page 8785 Line 3: Point out that oxygen (water column) and sulfate (sediments) are the most dominant electron acceptors, followed by all others. Please rephrase and also cite earlier studies. Line 5: Exchange “at” with “from”. Line 16: Add earlier publications by Schouten et al. (2001) and Wakeham et al. (2003, 2007) to the list. The addition of earlier studies is likewise recommended elsewhere in the paper. The authors tend to prefer the more recent literature.

Page 8788 Line 14 to 15: The polar fraction in this approach contains both, free and lipid bound FAs. After transesterification that mixes both pools. That is ok when active microbes are highly abundant but may get problematic when they are low in concentration as in this environment. Lines 25 to 27: Please give precision in d13C analysis of fatty acids.

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Page 8791 Line 17: The dominant pool of methane is already turned over below 135 m water depth (concentration decrease from 504 to 124 nM). Why is that the case? Mixing? Anaerobic oxidation processes? This should be discussed. Line 19: Isotope studies always refer to the isotopes with higher mass, i.e. 13C in this respect. Therefore, relative changes are always expressed as enrichment or depletion in 13C. So, please exchange "depletion in 12CH4" with "enrichment in 13CH4". Line 27: Exchange "of the" with "in".

Page 8792: Line 7: Exchange "12CH4 enrichment" with "13CH4 depletion". Line 17: Is "nothing" the correct expression? There definitely no other studies on that topic?

Page 8793 Line 13: Exchange "mirror" with "show". Line 15: If the fatty acids are purely derived from methanotrophs I would expect even more 13C-depleted isotope values. Assuming kinetic isotope fractionation of methanotrophs from cultures studies, isotopes of at least -40‰ and lower would have to be assumed for lipids solely derived from methane oxidation. Is this a valid assumption for C16:1w8 and C16:1w5? Moreover, co-elution (highly likely for C16:1w8 that elutes in the front of abundant C16:1w7) and production by other microbes can cause problems in accurate lipid biomarker isotope analysis. This should be considered.

Page 8794 Line 10: This is the weak point in the paper. Although there is a nice continuous record of the water column chemistry, fatty acid isotopes and pmoA genes are solely derived from one sampling depth which probably causes a strong bias in the outcome of the study. Especially the finding of only one phylotype (very low diversity!) of methanotrophic bacteria seems to be problematic. This is in contrast to earlier studies (see Refs given by the authors). Further tests of this low diversity should be performed. These could be the additional analysis of DNA. To overcome the problem, I strongly recommend the analysis of at least two more sampling depths, one coming from the upper oxygenated zone and one from the deeper suboxic part. With such an approach the authors would be able to narrow down the problem. The authors should integrate the zones of highest electron acceptor activity for this, namely the strong re-

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duction zone of oxygen at ∼80 m and likewise the strongest oxidation zone of methane at ∼120 m (Fig. 2). Since most of the methane is already oxidized below the zone of sampling (2/3 when looking at Fig. 2) the authors should consider an anaerobic process as well. So, what about adding an even deeper sample from the anoxic part at ∼200 m water depth as well? Line 14: To generalize the sampling site to be reduced in microbial diversity needs more explanation. For example, where/from which organisms do all the other fatty acids come from? The authors should enlighten that. Other chemoautotrophs such as. . .? Earlier studies from other anoxic basins made a much more comprehensive investigation in that respect (e.g., Wakeham et al., 2007, 2012). Line 21: See my comment above about the detection of a single methanotrophic bacterium. There is probably a strong bias coming from the analysis of one sampling depth. The addition of more analyses is essential in that respect.

Page 8795 Line 7: A periodically disturbed (how often actually? once a year?) water column may affect higher life forms but microbes adapt quickly. Line 9: Calling the theme "Fascinating" is very subjective. Please rephrase.

Interactive comment on Biogeosciences Discuss., 9, 8783, 2012.