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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.

O. Schmale et al.

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General comment to the referees:

The present manuscript is presenting for the first time insights into the methane cycle within the pelagic redox-zone of the Gotland Deep. It is using a multidisciplinary approach to strengthen the statement that methane is oxidized under low-oxygen conditions by the combination of gas chemistry (incl. stable isotope analyses), molecular biology (method to detect in situ expression of pmoA), and biomarker studies (incl. stable isotope analyses) of cell membrane lipids that can be related to the process of aerobic oxidation of methane. Supported by references, we would like to emphasize that the redox-zone of the central Baltic differs from that of most other comparable systems because larger disturbance of the gradients by hydrographic processes (such as

intrusions, inflows, and breaking internal waves), which appears to have strong impact on the abundant microbial communities. Thus, we strongly believe that the results are of importance and offers new information for ongoing scientific studies in the Baltic Sea and elsewhere characterized by strong physical and chemical gradients (redox-zone, e.g. Black Sea). The main concern of both referees is about the number of samples analyzed to identify the organisms responsible for the turnover of methane within the redox-zone. During the time of creating the manuscript the authors wanted to focus on the fact that a combination of molecular biological and biomarker studies carried out on one sample in the centre of the redox-zone (100 m water depth) is pointing to methanotrophy, with only one methanotrophic phylotype detectable. We learned by the reaction of the referees and agree with the referees that additional results from genetic analyzes would be helpful to support our conclusion. Therefore, we have now integrated microbiological data of three additional samples (from 80 105, 119 m water depth), which strengthens our outcome.

The revised manuscript includes major revisions and is attached in the supplement of this reply.

Authors reply to Referee #1 (M. Elvert)

Referee: 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.

Comment: We have now included three more samples within the redox-zone for micro-

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bial analyses (water depth 80, 105, 119 m) to substantiate our results. This modification is now integrated in the manuscript (see changes below).

Referee: 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 these water depths would strongly affect the larger implications of this study.

Comment: Other biogeochemical process using other electron donors are not in the focus of the present manuscript. This manuscript is focused on processes in the redox-zone, which has been shown to be of major importance for methane oxidation in other stratified basins (e.g. Blumenberg et al., 2007; Schmale et al., 2011; Schmale et al., 2010a). The motivation was the question whether the different character of the less stable redox-zone in the central Baltic Sea has an impact on the role of methanotrophy in this layer. We recognize that the title might have been misleading in this regard. We therefore changed the title to "Aerobic methanotrophy within the pelagic redox-zone of the Gotland Deep (central Baltic Sea)". Preliminary data from the upper anoxic water layer, however, supports that the key-playing microorganisms indeed perform aerobic oxidation of methane. Results of this ongoing work will be subject of a future study, and cannot be added here because (a) this would change the focus of the paper and (b) these studies are part of an ongoing PhD thesis.

Referee: 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.

Comment: See response above and changes in the manuscript below.

Referee: 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.

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Comment: See response above and changes in the MS below

Specific referee comments: Page 8784 Line 8: Exchange “mirrored” with “evident”.

Comment: changed

Line 9 and 10: The instrumental precision of the method is given with ± 1‰ (see methods). Therefore, I see no reason why the digit is justified here. Please check throughout the manuscript and change accordingly.

Comment: We do not agree with the referee and kept the numbers with one digit. The precision gives a value for the likeliness the true value is in a certain “distance” from the measured value. If we shift the measured value (by rounding), we shift the center of the window of propability, which is scientifically wrong.

Line 18: Delete “the idea”.

Comment: We have deleted/changed the last two sentences of the abstract. It now reads: “An imprint of these organisms on the particular organic matter was revealed by distinctive lipid biomarkers showing bacteriohopanepolyols and lipid fatty acids characteristic for aerobic type I methanotrophs (e.g., 35-aminobacteriohopane-30,31,32,33,34-pentol), corroborating their role in aerobic methane oxidation in the redox-zone of the central Baltic Sea.”

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.

Comment: We slightly rephrased the paragraph according to the reviewer comment. It now reads (line 50): “Comprehensive studies on aquatic sediments in different settings show that methane is microbially oxidized by the use of different electron acceptors, with oxygen being most important for the water column and sulfate for sedimentary turnover (Barnes and Goldberg, 1976; Reeburgh, 1976; Hinrichs and Boetius, 2002; Reeburgh, 2007). Recently, anaerobic methane oxidation using iron, manganese and

nitrite has also been reported (Beal et al., 2009; Ettwig et al., 2010).

Line 5: Exchange “at” with “from”.

Comment: The referee most likely meant line 6. We have exchanged “at” with “from” there. It now reads: “Although these processes are efficient and consume the main part of dissolved methane before it escapes from the sediment/water interface, some parts of the ocean are characterized by strongly elevated methane concentrations in the water column.”

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.

Comment: We have added both publications in the revised manuscript.

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.

Comment: The reviewer is correct that our approach combines glycolipid-, phospholipid- (PLFA), and neutral lipid (free) fatty acids so that the PLFA signal might be slightly suppressed. However, the combination of these pools in environmental studies is not uncommon as the vast majority of fatty acids relates to the in situ biological community (particularly if using in situ pumps collecting mainly suspended material in contrast to sediment traps). For instance, in a current paper on the Gotland Deep water column the combined cellular fatty acids were successfully used to gain information about chemolithoautotrophy in the respective water column (Glaubitz et al., 2009).

Lines 25 to 27: Please give precision in δ13C analysis of fatty acids.

Comment: We added the respective phrase in the revised MS. The precision was generally better than 0.5 permill.

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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.

Comment: See comments above. This manuscript is focused on processes in the redox-zone, which has been shown to be of major importance for methane oxidation in other stratified basins (e.g. Blumenberg et al., 2007; Schmale et al., 2011, Schmale et al., 2010a). The motivation was the question whether the different character of the less stable suboxic layer in the central Baltic Sea has an impact on the role of methanotrophy in this layer.

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".

Comment: Done

Line 27: Exchange "of the" with "in".

Comment: Done

Page 8792: Line 7: Exchange "12CH4 enrichment" with "13CH4 depletion".

Comment: Done

Line 17: Is "nothing" the correct expression? There definitely no other studies on that topic?

Comment: We slightly modified this sentence and include now a reference, which proposed methane oxidation is a potential process in the redox-zone of the Gotland Deep (Schmale et al., 2010). It now reads (line 253ff): "Chemical gradients feature versatile environments and are known to harbour enhanced microbial abundance and activity. In the redox-zone of the central Baltic Sea, various biogeochemical processes have been identified, such as denitrification, ammonia oxidation, or dark CO2 fixation (Labrenz

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et al., 2005; Jost et al., 2008; Glaubitz et al., 2009; Labrenz et al., 2010) and also microbial consumption of methane was proposed as mechanism explaining the strong methane decrease in this water layer (Schmale et al., 2010b)."

Page 8793 Line 13: Exchange "mirror" with "show".

Comment: Done

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.

Comment: The referee is correct. Care must be taken when using ïĄď13C values of minor compounds, which is indeed the case for both cellular fatty acids used. And yes, there was partially no baseline separation from other fatty acids, which may have affected ïĄď13C values. As ïĄď13C values of the major fatty acids were less depleted in 13C than those for the 16:1w8c and 16:1w5c fatty acids, the values of the latter should be in practice even more depleted than -35.5 and -38.8 ‰Ṫhis theoretical shift towards stronger 13C-depletions would then also better fit to biomass, which is predominantly built on 13C-depleted methane carbon. This further supports our assumption that 16:1w8c and 16:1w5c were mostly sourced by methanotrophic bacteria of the type I cluster. We are not sure how we should consider this potential problem in the current MS as we think this is beyond the scope of the manuscript. However, we modified the respective paragraph slightly and added the standard deviation for stable isotope analyses in the method section.

Referee: 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

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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-duction zone of oxygen at _80 m and likewise the strongest oxidation zone of methane at _120 m (Fig. 2).

Comment: Three more samples (now 80, 100, 105 and 119 m water depth) for molecular biological analyzes are now integrated in that manuscript as stated above. See the revisions in the manuscript below.

Referee: 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?

Comment: The process of anaerobic oxidation of methane might be of interest for this basin but is not in the focus of that manuscript. As mentioned above, however, preliminary data suggest aerobic methanotrophy to be indeed the key process for the reduction of methane. This will be subject of a future publication.

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).

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Comment: We agree with the referee that a comprehensive discussion of lipid biomarkers (including cellular fatty acids shown here) can give interesting insights into multiple microbial turnovers at biogeochemical zones. However, our attempt, in contrast to Wakeham et al. (2007), is directed on the distinct process of microbial aerobic methanotrophy and not on the facets of microorganisms in that water depth. Unless we show all compounds in the table, we kept our discussion of only specific fatty acids relevant to the topic. However, we also mention that those are only of minor importance among the entire bacterial community. We therefore did not follow the referee's recommendation.

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.

Comment: Three more samples from the redox-zone are now integrated in the manuscript (see comment above). These results support our findings. We also made the statement less strong in the manuscript.

Page 8795 Line 7: A periodically disturbed (how often actually? once a year?) water column may affect higher life forms but microbes adapt quickly.

Comment: Additional information about "disturbances at the redox-zone" are given in Line 205ff To point out the strong variability at the redox-zone in the Gotland Deep caused by internal waves, intrusions or eddies we have revised the first paragraph of 3.1 Physical parameters and gas chemistry: The estuarine circulation in the Baltic Sea causes a strong vertical salinity gradient between the surface and deep water (Lass and Matthäus, 2008). This gradient is very pronounced in the deep basins of the central Baltic Sea (e.g. Gotland and Landsort Deep; Figure 2) and reflects a water column stratification that limits the vertical mixing and water renewal in the deep strata (Reissmann et al., 2009). Oceanographic investigations, carried out at the redox-zone of the Gotland Deep, show that this depth is periodically perturbed by intrusions, internal waves or eddies which can shift the amplitudes of isoclines up to 10 m within

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time-spans less than an hour (shown for temperature and salinity in Lass et al., 2003; Dellwig et al., 2012). Both citations are listed in our reference list of the manuscript. The publication of Dellwig et al. (2012) describes the influence of perturbations of the redox-zone on the manganese cycle. It also shows the variability at the redox-zone in August 2008 (Figure 9), which is very close to our time of sampling in June 2008. The publication of Lass et al. (2003) shows the variability of isoclines in temperature and salinity along the eastern Gotland Basin (e.g. Figure 5). The described variability within the redox-zone is influencing the selection of our POM sample for lipid biomarker studies (as described in section 2.3 Lipid biomarkers).

Line 9: Calling the theme "Fascinating" is very subjective. Please rephrase.

Comment: The last sentence was rephrased (line 333). It now reads:" How this variable environment is affecting the methane turnover in the water column and the microbial community responsible for this process is an interesting question that needs to be investigated in future studies."

In addition to these comments, we have revised the following parts of the manuscript to clarify the focus of the manuscript and the strategy of our work. We have also integrated the new results from the four samples taken along the redox-zone.

Line 1ff Title of the manuscript was changed to specify the focus of the present work that studies the: "Aerobic methanotrophy within the pelagic redox-zone of the Gotland Deep (central Baltic Sea)"

Line 33ff We have now included three more samples for microbial analyses (water depth 80, 105, 119 m) to substantiate our studies. This change is now integrated in the abstract: "Water column samples between 80 and 119 m were studied to identify the microorganisms responsible for the methane turnover in that depth interval."

Line 90ff To point out that our investigations are limited on the methane turnover at the pelagic redox-zone of the Gotland deep, we have changed the last sentences

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in our introduction (focus of the manuscript): “However, little is as yet known about the processes that regulate the methane flux in this environment. In this paper, we use a multidisciplinary approach that combines gas chemistry, molecular biology and lipid biomarker geochemistry and present data on a microbial methane sink within the pelagic redox-zone of the Gotland Deep. Thus, this study aims to investigate whether aerobic methane oxidation also plays a role in the more dynamic and turbulent redox-zone of the central Baltic Sea.”

Line 102ff To show that the focus of that manuscript is the aerobic oxidation of methane we have added the word “aerobic” in point (1) of the sampling strategy: “(1) identifying the depth interval of aerobic methane oxidation within the redox-zone based on physical parameters and on board gas chemistry”

Line 105ff To clarify the problem with chronologically staggered water column samplings due to the use of different sampling equipments, we have included the following sentences: “These samples were taken within a time frame of 3 days and with different sampling equipments (as described below).”

Method section The sampling strategy is better reflected by the new order within that section: 2.1 Physical parameters and gas chemistry Task: To gain information about the parameters which help to identify the depth interval of aerobic methane oxidation within the pelagic redox-zone. 2.2 pmoA gene expression analyses Sampling depths are based on results of 2.1 (CTD profile, methane and oxygen concentrations) Task: To gain information about the microbes involved in the process of aerobic methane oxidation within the redox-zone. 2.3 Lipid biomarkers Task: Within the center of the redox-zone a POM sample was analyzed to support the results of molecular biological work.

Line 136ff The first sentence was revised according the additional samples included in the manuscript. It now reads: “Within the identified redox-zone filter samples were taken in 80, 100, 105 and 119 m water depth using a rosette water sampler.”

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Line 139 We have added: “For each sample. . .”

Line 158 To point out that all water samples were excised and reamplified in a PCR reaction, we have changed the sentence: “All bands from each depth were excised and reamplified in a PCR reaction containing. . .”

Line 176ff To describe the sampling-strategy for the biomarker sample used in that manuscript in more detail we have extended the beginning of paragraph 2.3 Lipid biomarkers: “For lipid biomarker studies a sample was selected from the centre of the redox-zone at 100 m water depth. That depth was chosen to obtain a POM sample that reflects the in situ microbial turnover of methane under low-oxygen conditions and is not “contaminated” by external water masses (i.e. increased oxygen concentrations or anoxic conditions) which may also include other methane consuming microorganisms (e.g. consortia performing the anaerobic oxidation of methane). 214 l of water were filtered on glass microfiber filters (ø 30 cm; 0.7 μm pore size) over a time span of two hours using a PUMP-CTD system (Strady et al., 2008).”

Line 252ff According to the strategy of our studies and the new results from our molecular biological work, the section “3.2 Methanotrophic microorganisms within the redox-zone” was revised. See modification in the corrected MS.

Line 322 According to our strategy of our studies, we have exchanged “lipid geochemistry, and molecular biology”. It now reads:”molecular biology, and lipid geochemistry”

Line 325ff According to our strategy of our studies, we have changed “the occurrence of lipids specific for methanotrophic bacteria (e.g., aminopentol; 16:1ω8c fatty acid), and the detection of the key enzyme methane monooxygenase (pmoA)”. It now reads:”the detection of the key enzyme methane monooxygenase (pmoA), and the occurrence of lipids specific for methanotrophic bacteria (e.g., aminopentol; 16:1ω8c fatty acid).”

Line 327 Based on the new data added to the manuscript (3 more samples from our molecular biological work), we have deleted “retrieved from a 100 m water column

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sample".

References Six more references are listed: Dellwig, O., Schnetger, B., Brumsack, H.-J., Grossart, H.-P., and Umlauf, L.: Dissolved reactive manganese at pelagic redoxclines (part II): Hydrodynamic conditions for accumulation, Journal of Marine Systems, 90, 31-41, 2012. Lass, H. U., Prandke, H., and Liljebladh, B.: Dissipation in the Baltic proper during winter stratification, J. Geophys. Res., 108, 3187, 10.1029/2002jc001401, 2003. Wakeham, S. G., Amann, R., Freeman, K. H., Hopmans, E. C., Joergensen, B. B., Putnam, I. F., Schouten, S., Damste, J. S. S., Talbot, M., and Woebken, D.: Microbial ecology of the stratified water column of the Black Sea as revealed by a comprehensive biomarker study, Org. Geochem., 38, 2070-2097, 2007. Barnes, R.O., Goldberg, E.D.: Methane production and consumption in anaerobic marine sediments. Geology 4, 297-300, 1976. Reeburgh, W.S.: Methane consumption in Cariaco Trench waters and sediments. Earth and Planetary Science Letters 28, 337-344, 1976. Schouten, S., Wakeham, S.G., Sinninghe Damsté, J.S.: Evidence for anaerobic methane oxidation by archaea in euxinic waters of the Black Sea. Organic Geochemistry 32, 1277-1281, 2001.

Figure numbering According to the new structure of the manuscript, we have changed Fig. 3 and 4.

Figure 2 The sampling depth of our samples from our molecular biological work and lipid biomarker studies are indicated in Fig. 2. This is also mentioned in the figure capture of figure 2. We have also marked the redox-zone. It now reads:"Figure 2. Left: vertical distribution of salinity (black), temperature (red), and turbidity (gray). Right: vertical distribution of oxygen and hydrogen sulfide (expressed as negative oxygen equivalents, blue), methane (red), and ïĄď13C value of methane (green). The depth interval of the redox-zone is displayed in gray (oxygen concentration 0 0.8 ml L-1). The water depths for molecular biological and lipid biomarker studies are indicated with colored horizontal lines (black = molecular biology, red = molecular biology together with lipid biomarkers)."

Authors reply to anonymous Referee #2

Referee: From the data presented in Fig 2 and correctly discussed in the text it is obvious that CH4, produced in the deep layer, is consumed between 115 and 135 m water depth (see page 8791, lines 17-21). Moreover, it is correctly stated that a turbidity max. is visible in about 120 m and it is stated that it results from enhanced microbial activity (page 8791, line 4/5). So, I am wondering why the investigation of the methanotrophic microorganism has been performed with a sample from 100m (see page 8791, line 20). This water depth is clearly located outside the zone of the CH4 gradient (i.e. zone of CH4 oxidation). In other words I am not surprised by the finding of reduced diversity of methanothrophs, simply because of the fact that the water depth of 100m is not relevant for CH4 oxidation. Is there any reason why a water sample from 100m should be representative for the zone with the CH4 gradient (115-135 m), where the CH4 oxidation takes place? I would not be too surprised to find a higher diversity of methanotrophs at the depth of the turbidity max. in 120m.

Comment: We have now integrated three more samples (now 80, 100, 105 and 119 m water depth) from our molecular biological work to support our findings. One sample is taken within the turbidity maximum. Please find our replies and details about our changes in the revised MS in our replies to comments of referee #1.

Referee: The other aspect ignored is related to the question how representative are the results for the rest of the Baltic Proper? (results from only one station are presented!).

Comment: The regional examination of that process (e.g. in the Baltic Proper) is an interesting question and will maybe part of future studies. The present manuscript is focused on the pelagic redox-zone in the Gotland Deep (see the new title of the manuscript).

Referee: Thus I think that the main conclusion (‘ . . . support the idea that biogeochemical cycles in the Central Baltic Sea redoxclines are mainly driven by only a few microbial key species.’ see abstract and discussion on page 8794, lines 9-22)) is not

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justified by the presented results at all.

Comment: We agree with the referee. See our reply to referee's 1 comment and modifications made in the manuscript.

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/9/C4647/2012/bgd-9-C4647-2012-supplement.pdf

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