

Anonymous Referee #2. Answers to referee are written in *italics* and improvements in corrected MS are **bolded**. Received and published: 3 January 2015

“(Aiming to give an unbiased review I did not read any other comments regarding this manuscript before making the below review.)

5 General comments: This study explores the dynamics of dissolved organic matter (DOM) and particularly the metabolic of degradation leading to production of inorganic carbon and methane (CH₄). This was done by whole lake additions of cane sugar having a distinctly different ¹³C signature compared to the original DOM. The study was conducted for three years with one year without sugar addition and two consecutive years with additions. Physicochemical variables, CH₄ and CO₂ concentrations and stable isotope ratios, and stable isotopes of some organic matter and biomass pools were monitored on a biweekly to monthly basis. Gas efflux to the atmosphere was largely estimated from concentrations and related models of fluxes and some kind of CH₄ bubble traps also were also deployed. The primary conclusions regard water column methane production and methane oxidation seemingly being inferred from mass balance calculations, and links to DIC and biomass cycling.

Fundamentally, this is a very valuable and rare ecosystem experiment that in some form should be published. However, based on the below comments I suggest a major revision totally reshaping the manuscript aiming to find a focus that is more robust to the data interpretation and to take a number of interesting opportunities to quantify contrasting carbon cycling pathways that I feel are presently missing. Critical methodological aspects are also presently unclear. As illustrated by my comments below I interpret the data in a fundamentally different way which change the conclusions completely in a way that is more in line with previous work in small stratified lakes surrounded by forests, and in a way it seems like the simple and more robust interpretations are absent while more speculative interpretations are highlighted.”

25 *Thanks for detailed and constructive comments and for approaching this review unbiased. Both reviewers found problems in clarity, methodology description and in use of excess of speculative interpretations. **We will attempt to address these issues in the revised version.***

30 *However, since we trust our methane oxidation measurements and our ebullition estimates and we have a fundamentally different view on lateral movement of CH₄ in this lake, **we will mostly keep our interpretations of the data. However, we will do our best to make this clearer and less speculative.***

“Specific comments

Abstract: Page 16448, Line 13-14 “Methane production and almost total consumption of CH₄ mostly in the anoxic water layers,” is difficult to read and understand. Please check if it can be clarified.

35 Page 16448, Line 18: Should it be “Even if gradient...”?

Page 16448, Line 21-22: The sentence “Increase in ¹³C-CH₄ was clear between the metalimnion and epilimnion where the concentration of dissolved CH₄ and the oxidation of CH₄ were small.” is also a bit unclear as the first part talks about a change between the metalimnion and the epilimnion and the latter part presumably refer to the situation in the epilimnion only...or do you mean that the

stable isotopes change markedly in spite of no differences in concentrations or independently measured oxidation rates? I guess this will become clear from the rest of the text but good to minimize the risk of misunderstandings in the abstract anyway. Introduction: Page 16451, Line 3-6: The sentences on the global CH₄ emissions are a bit unclear. There are estimates with more data of ebullition that suggest higher total emissions (eg. Bastvken et al. 2011)...so it may make a big difference if estimated emissions aim to include ebullition or not and this needs to be clarified when providing the numbers.

Page 16451, Page 16454, Line 20: Good that the O₂ measurements were supplemented with redox measurements! This is rare.”

10 ***These specific comments will be considered in the revised version and the possible contribution of ebullition will be clarified in total emission estimates.***

“Methods: Page 16456, Line 22: d¹³CH₄ from the bubble collectors: The sampling frequency of 7-20 days leave a lot of room for re-dissolution or potentially methane oxidation in the bubble traps. Was there any consistent pattern in d¹³CH₄ versus sampling time/sampling frequency? How about replication of the bubble traps? How many locations, what area and what depths were covered? If not potential hot spot areas were covered extensively most of the ebullition may have been missed.

Page 16456, Line 26 and below: It is stated that the efflux was calculated by boundary layer diffusion equations so presumable only diffusive efflux was accounted for. Why was not bubble flux considered? Was the bubble traps not suitable for quantitative bubbling estimates?”

20 *One reason for initially small role of ebullition measurements in this study was that in a previous study in a similar lake no ebullition was detected even though better coverage with subsurface funnels was used (Kankaala et al. 2006, LO, 51). We were always aware of risk that we lose methane due by ebullition when a new carbon source was added to lake. Ebullition was followed from autumn 2008 to autumn 2009. Three bubble-traps used were a small modification of that described in Huttunen et al. 2001, (Sci. Tot. Env.), rim of inverted funnel was located 60 cm below surface. Similar traps have detected ebullition in our earlier studies and also afterwards. Thus, they should have been technically OK. This data was just omitted from first MS since ebullition was considered to be insignificant, since only 8 times were tiny gas bubbles (diameter less than 2 mm) visible in the three bubble collectors during the nine measurement occasions. Dissolved CH₄ concentration from water in the bubble collectors was 2-3 times higher than that in water samples taken from the same depth as the bottom rim of the bubble collecting funnel. δ¹³C of CH₄ in the bubble collectors (-66 per mil) was lower than that of surface water δ¹³CH₄ (-62 per mil) indicating that the collectors indeed captured some bubbles with ¹³C-depleted CH₄ rising from the bottom. As the reviewer argues, some enrichment compared to bottom values may indicate methane oxidation and dissolution to prevailing dissolved methane in funnels. However enrichment of δ¹³C from bottom ~-70 per mil to -62 per mil would mean that small fraction of CH₄ was oxidized in traps or in the way there. So, we are confident with our method. **This data will be presented as supplement and small INCREASE of methane produced will be added to current values.***

Bastviken et al. 2011 has an estimate of global CH₄ emissions including ebullition (Bastviken et al., Science, 7, Table 1). For latitudes between 54 - 66 degrees, emission based on ebullition is 9.1 Tg (n= 9) annually and that due to diffusion 1.1 Tg (n=271), ie the majority comes from ebullition. If this same ratio (9.1/1.1) holds also in Alinen Mustajärvi, ebullition in our lake should have been nine times that of diffusional losses, thus 11 - 20 g m⁻² or 16-30 L m⁻² of methane during this same period. We are sure that we would have detected such a large (90 - 160 mL m⁻² day⁻¹) flux from the lake with method we used.

“Page 16457, Line 7: “Algae was sampled on 1 July 2009 straight from a surface scum, and represents photosynthetic material at the lake surface. Floating material from the bottom was taken from the Limnos tube sampler in early spring under ice 10 (6 April 2010).” I did not understand what the surface scum was and how algae could be separated from other types of organic material. Likewise “floating material from the bottom” is unclear to me. Please describe the sampling in greater detail so it is clear what the material represent and so that the sampling can be understood and repeated by others.”

*There was algal bloom consisting mostly of Gonyostomum semen, and there were floating green algae mats on lake surface and they were considered to represent algae. The algae found from bottom in Limnos sampler in next spring looked similar. **This explanation will be done in greater details.***

“Page 16457, Line 14-18: Section 2.3.1: The calculation of the water column methane oxidation seems critical for many of the main messages and therefor the description of it needs to be very clear. It appears that the estimation of the turbulent diffusion is critical for the obtained methane oxidation rates and thereby for the main results, but it is not explained how the eddy diffusion throughout the water column was calculated/estimated. The eddy diffusivity is a very complicated thing to estimate without extensive high frequency measurements of hydrodynamics along multiple profiles and accounting for both vertical and lateral water movements (for example, the occurrence of seiches or heterogenous deep currents makes conclusions from biweekly measurements at the deepest point only very uncertain. ...and if conditions were very stable making such large scale water movements negligible it also means that CH₄ transport may have been very slow. Given much slower vertical than horizontal transport an hyperbolic concentration gradient with depth may just be a function of volume and sediment area ration for each water layer (see also below) and may provide little information on processes in the water. I think this is a major weak point which may undermine the suggestion that anoxic methane oxidation was important. So, even if I think the overall study is very valuable, I think methodological unclarities in the assessment of methane oxidation and its distribution with depth undermine the present claims regarding anaerobic methane oxidation. My advice is to downplay this, to explain methods in greater details and stress the uncertainty and to properly address alternative interpretations of the data as well (see other comments).”

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The method used will be explained more detail in corrected version. The method is based on turbulent diffusion and it has been verified with syringe incubation method to be reliable (Kankaala et al. 2006, LO.51(2) 2006). Basically amount of CH₄ oxidized is difference between measured concentration gradient and from bottom concentration calculated theoretical concentration gradient in water column.

Since we believe that this method is reliable, we don't see any reason to go to alternative interpretations of the data as suggested by reviewer.

Referee #2 assumes that horizontal mixing being more important than vertical mixing and this was causing the form of measured gas concentration curves in lake. Here we have fundamental disagreement. We argue that there was no lateral movement of CH₄ from littoral zone to deeper water column. In Kankaala et al. (LO 2013, Fig. 2), Lake number (Ln) value in lake studied here Alinen Mustajärvi is >10, thus showing that internal wave mechanisms are suppressed and mixed layer deepening is the only mechanism causing vertical exchange in the water column. Also Read et al. 2012 (JGR 39, 2012) show that convection is more important causing turbulence than wind shear in lakes < 10 ha. There is also diurnal convection due to cooling during nighttime, furthermore oxycline gets deeper and gases are mixed in metalimnion during late summer and autumn (Huotari et al. Bor. Env. Res., 14, 2009).

Further evidence of lack of lateral movements comes from funnels used for bubble collection; concentration inside them was higher than that in the nearby open water and there also was not bigger CH₄ concentration in littoral zone than in the point of lake we did the sampling. This data will be added as supplementary material.

“Section 2.3.2: a) The estimated CH₄ production relies on accurate estimates of methane oxidation estimates, efflux by diffusion and bubbling. Efflux by diffusion is probably the most robust number here although boundary layer models have been shown to differ 2-fold or so. The methane oxidation estimates are probably uncertain (see above) and it is unclear how accurate the bubbling estimates (information about the type and number of bubble traps deployed, their distribution and the potential influence of the deployment time could not be found). If bubbling rates cannot be properly constrained on a whole lake basis I suggest to not write about total CH₄ production but rather diffusive CH₄ release from sediments (plus possible bottom water production if this is thought to be significant) and to make a proper uncertainty analysis.”

We still defend our methane oxidation values and believe our small methane ebullition estimates here as in other lakes of this same area. And since we are not calculating this on whole lake basis, we make our estimate for this one point only. Uncertainty analysis will be made.

“b) I wonder if the isotopes can provide robust evidence for separating hydrogenotrophic and acetotrophic methanogenesis the way described by Whiticar (1986). If I understood recent work by Martin Blaser, Ralf Conrad et al., the picture seems to be much more complicated than thought for methanogenesis. It may be beneficial to be careful with using simplistic ways to interpret the

methanogenic processes, to be open for other alternative explanations, and to check for more recent literature on this subject.”

5 ***Since separating hydrogenotrophic and acetotrophic methanogenesis (or methylotrophic as suggested in reference mentioned (Penger et al, AEM, 2012) is not vital to this manuscript and was doubted also by referee #1, this will be removed from the corrected version.***

“Section 2.3.3: While the stoichiometry that CH₂O mineralized under methanogenic conditions yield 50% CO₂ and 50% CH₄ seems correct I am not sure the explanation here is correct. I am not a biochemistry specialist but think that the described formation of acetate represents the specific process called acetogenesis (from H₂ and CO₂) only and if I
10 understood it correctly acetate may be formed in several different ways in fermentation processes. For example in the Embden-Mayerhof pathway 1 mol glucose can be fermented to 2 mol acetate, 2 mol CO₂ and 4 mol H₂. The simpler way is to just explain that CO₂ has to be formed along with CH₄ if the substrate is “CH₂O” for simple stoichiometric reasons rather than basing the argumentation on a few specific reaction formulas (not properly
15 representing the biochemical complexity). However, given that CH₄ production can probably not be assessed properly (if bubble flux was not carefully across the whole lake; see above) and I am not sure that CO₂ production can be estimated from this stoichiometric relationship. I would instead use the hypolimnetic accumulation over time for empirically assessing import of dissolved CH₄ and DIC separately to the water column from all possible
20 sources including production in sediment and water as well as hydrological input.”

We agree that use of chemical formulas was too speculative approach and it will be removed from results and moved to discussion as one possibility to explain relatively enriched $\delta^{13}\text{C}$ of CO₂ in very bottom. However, we will keep our CH₄ production estimates based on oxidation estimates by turbulent diffusion method.

25 ***Accumulation of CH₄ and CO₂ will be evaluated, since it shows something of the balance of substances in studied area of Alinen Mustajärvi.***

“Section 2.3.4. a) Why this choice of a single CCE value? Would it not be more appropriate to work with a range of likely values to express the uncertainty?2

Reviewer #2 has point here. This can be done. There is some newer values available.

30 “b) Given that I challenge the interpretation that AOM was important I also think this should be settled before going into details regarding the CCE of AOM and the d¹³C of ANME biomass. For example, the method description of the methane oxidation is much more important (as it is the foundation for much of the manuscript).”

35 ***This is how we shall proceed and probably keep our estimate of AOM and continue from that. Reviewer #1 argues that without proper measurements of oxygen probe capable to detect 1000 or 10000 smaller concentrations of oxygen that we have, we can't say that bottom was anaerobic, and we can't be totally sure was methane oxidation aerobic, microaerobic or anaerobic.***

*As written to answer to referee 1, we argue still that lake bottom was anaerobic. Even we could not measure absence of oxygen by device, we measured negative redox values, and found H₂S from the water column. Furthermore, studies from the same lake show existence of strictly anaerobic green sulphur bacteria in the lake hypolimnion peaking at depth of 4.5 m in summer 2009, and found also from depths of 2,5 and 3.5 (Karhunen et al. Aquatic Microbial Ecology 68: 267-272). **This will be added to corrected version.***

“Results: Section 3.1: It is stated that stratification depths different between years in spite of almost identical temperature profiles. Why? Were not the depths zones used based on temperature profiles? Was this accounted for in the mass balances? What were the implications for the results?”

10 *This resolution problem might have something to do with that that here we see averages over a season. **This will be modified to take account also the time of the year.***

Mass balances are calculated according to changed sampling scheme, thus masses are expressed to whole profile.

15 “Section 3.2: How did community respiration compare with hypolimnetic DIC accumulation? It seems that hypolimnetic accumulation of DIC and CH₄ could provide valuable alternative measures of lake metabolism. This would also be a way to put single negative dark respiration measurements in perspective.”

This will be tested.

“Section 3.3: a) Were the changes between years statistically significant?”

20 *There was some **statistical differences in study done from same experiment (Peura et al. Biogeochemistry 118, 177-194, 2014), these will be tested in next version.***

25 “b) The diffusive efflux is not necessarily strongly connected to the lake metabolism. Large quantities of CO₂ (and maybe CH₄) emitted may come from groundwater input in littoral zones (or with streams if present). Further the concentrations in the surface water is not only a consequence of the production or input but also of the output so during a year with more windy conditions yielding more turbulence and greater efflux rates there may very well be lower surface water concentrations as a consequence of the greater output. Was this accounted for in the analyses? It seems like most conclusions rest on concentrations and connects the concentrations to the water column metabolism, but this may be a minor part of the story behind the efflux in many systems. I suggest making a clear mass balance for the whole lake taking potential contributions of all these aspects into account by supplementing the concentration data with estimates of turbulence driven exchange over time (and between years). This could be done given information about wind, precipitation, and temperatures causing convection events. Presumably there is some information available for this seemingly well studied lake and catchment. If not, even crude estimates in order to make a full lake budget would be valuable.”

35 *This is right from the diffusive fluxes to capture rapid changes. Wind and precipitation will be considered more carefully in next version of MS. There was no input streams, littoral zone consisting of peatlands makes flow from there difficult to estimate. **We will estimate the***

possible inputs and estimate flow out from system based on evaporation, water level changes and rain events and few estimates of outflow of water from lake.

“Given that I challenge the importance of AOM I ask that large parts of this section are reconsidered in the light of my comments”

- 5 ***This will be modified according to how the MS develops, still having the AOM part included, thus mostly the changes will be in shortening and in clarity of the text.***

“Section 3.4: a) This significant shift in d13CH4 is very interesting. It indicates a link between addition of sugar in the epilimnion to the hypolimnetic CH4 in a short time and presumably via material reaching the sediments. Could this indicate that heterotrophic biomass from the water column can be
10 important for CH4 formation in DOC rich lakes? If so this is interesting and new information that deserves more attention and perhaps should be a main message while the AOM part is played down? As mentioned in the above comments the epilimnetic CH4 and DIC has a too short residence time and may have external sources so a clear long-term signal of the sugar addition in the epilimnion is not expected. Therefore I think the attention can be focused to the hypolimnion which
15 is where signals of the additions may be preserved and more clearly visible over time.”

The signal of cane sugar was seen in DOC and also in zooplankton, thus added sugar was used efficiently also in epilimnion (Peura et al. Biogeochemistry 118, 177-194 2014). But, since AOM part will not be played down, both these aspects will be discussed. And we agree on importance of hypolimnion and processes there.

20 “Page 16464, Line 18: It is a bit confusion to talk about differences that were not significantly different. Why not consistently say that numbers that were not significantly different were instead similar?”

OK.

“page 16464, Line 25: I do not think the fractionation factor can be calculated this way because the foundation for such a calculation is that there is a match between the concentrations of the depleted
25 and enriched CH4 so that the 13C enriched CH4 represents the leftover of the depleted CH4...but across a stratified water column CH4 in different layers may be disconnected due to lateral flux and concentrations may be affected not only by oxidation but also by transport controlled efflux. Having said this it is remarkable that numbers get relatively close to laboratory measurements at least 2009. So, I suggest to more clearly show awareness about these complicating factors and perhaps to present
30 this as a discussion point rather than as a result.”

We modify also this to discussion as referee suggests, but as there is no big role for lateral turbulence in water layers in Alinen Mustajärvi and other small lakes where oxidation happens determines how concentration and isotope gradients develop.

35 “Page 16465, Line 3-5: As commented below to Figure 2, methane oxidation can be extensive in spite of low apparent concentrations as rates may be limited by resupply rates which are faster the steeper the concentration gradient is. Rates should therefore not be estimated from concentrations alone.”

Molecular diffusion of gases in water is much slower than turbulent diffusion, thus this effect is not causing a big change in methane oxidation.

But in any case, if methanotrophy takes a same share of CH₄ from large amount and from small amount, then in later change in $\delta^{13}C$ of residual is big, even oxidation is small.

“Similar comments as for CH₄ above apply to the parts of Section 3.4 about DIC.”

If this means that DIC enrichment in bottom is also important, then why not.

5 “Discussion: Section 4.1: a) Page 16467 Line 21-23: The statements “In general, the addition of sugar carbon changed processes in the lake, but CH₄ oxidation and MDC formation were essentially similar to the reference year 2007, and typical of this kind of stratified lake.” are unclear to me. Please clarify what was changed by the sugar addition and what was not affected. Was the CH₄ release from sediments significantly increased while CH₄ oxidation remained unaffected? That is surprising as CH₄ oxidation is supposed to be substrate limited and thus transport patterns may have limited methane oxidation?...pointing at the importance of the uncertainty in the eddy diffusivity when estimating methane oxidation and interpreting the results. Why is this result typical for the type of lake studied? Are there many previous studied of year to year variability in methane oxidation in such systems so it is known what is typical?”

15 ***This may be too speculative wording and will be clarified, and also wording will be changed to better compare situation in studied lake to previous studies.***

“The evaluation of the efflux over time would benefit from being more rigorous. To what extent can the increasing fluxes be linked to the sugar addition versus to other possible factors...such as different weather (wind, hydrological input, temperature)? If the efflux can be clearly linked to the sugar addition there is an opportunity of an interesting discussion on what fraction of the labile DOC is emitted as CO₂ and CH₄ versus enters biomass over time...but the other factors affecting efflux has to be considered as well first.”

25 ***This will be done, however many things changed due to easily degradable carbon addition: nutrients, carbon source and oxygen status and it is bit demanding to cut it to pieces, but this will tested.***

c) “I still do not understand how the epilimnion could become much thinner without a corresponding change in temperature profiles (Figure 2).”

30 *One thing may be that here we see averages. Otherwise, if we accept that turbulent lateral movements are small here, then increase in oxygen consumption due to increased respiration demand will lead to bigger O₂ consumption, while temperature profile follows that of solar radiation and air temperature. In corrected version this is better described by graph having thermocline depth and oxycline depth at same figure with date.*

35 d) “Page 16468 Line 23-26: Most previous evidence from small stratified lakes surrounded by forest suggest that transport of dissolved compounds across the thermocline during stratification is very limited and that efflux to the atmosphere largely rely on gas production in the epilimnion and shallow sediment (including ground water intrusion). This is in clear contrast to the proposed contribution to efflux to the atmosphere from hypolimnetic CO₂ and CH₄. Can the

possible contribution of deep water gases to the efflux relative to other epilimnetic sources be quantified?"

5 ***This can be tried.*** Here littoral zone deepens fast, and there is also not much vegetation producing new easily degradable carbon to littoral zone, thus it is difficult to believe that input of CH₄ from littoral zone to whole lake is important, one possible source could be peat surrounding the lake. ***This was tested once and there was not bigger CH₄ concentrations of surface water at the lake shore area. This data will be added to supplementary material.***

10 "Page 16469: Many parts of the possible explanations for the observed stable isotope patters seem speculative. E.g. it is unclear why sugar added in solution should flocculate. The stable isotope discussion appear to neglect the most simple explanation: the sugar added to the epilimnion result in enriched CO₂ from respiration and enriched biomass. The CO₂ will be diluted in a lot of additional CO₂ from other sources and also have a short residence time before emitted to the atmosphere...and therefore no strong signal was seen. The labeled biomass however reached the hypolimnion and as the modest contribution to the 13C signal there (surprisingly large actually) could be detected as very little dissolved gas escaped the hypolimnion during the stratification period. This simple explanation seems more plausible to me than the present discussion and could be used as a framework for modelling of eg. possible partitioning of the added sugar into biomass versus CO₂ being emitted."

20 ***Have to admit that this flocculation was speculation, and thus can be removed as an explanation for change in bottom.*** What happens is really the case as reviewer writes and expressed in discussion in Peura et al. 2014 happening in epilimnion. CH₄ oxidation in hypolimnion also retains the traced carbon in hypolimnion.

25 "Section 4.2: a) Page 16473, Line 2-5 and elsewhere: The text "There was no, or only a minor, change in 13C-CH₄, so stable isotopic enrichment did not conclusively show that CH₄ was oxidized microbiologically in Alinen Mustajärvi, even though we could measure clear CH₄ oxidation." is not clear to me. First I challenge that clear methane oxidation could be measured in the anaerobic water because the methodology for the proposed methane oxidation estimates is unclear and this type of methodology is uncertain (see comments above). Second – is not the lack of change in stable isotopic composition in fact a strong argument that not much is happening with the CH₄ until it reaches zones where aerobic methane oxidation is possible. To me this argument is more robust than the methodology to estimate methane oxidation from eddy diffusivity based on scarce hydrodynamics data and the present conclusions are very sensitive for how this data is interpreted. I still think this study is extremely valuable as a rare and unique whole system labelling experiment but I think the focus should be chosen to be more robust for different ways to interpret the data."

35 ***We agree on importance of this study, but disagree on the methodology criticism.*** AOM has been found to be important in cited studies by Schubert et al, 2011, 2012 (p. 16472, r. 21-24). The problem is really in CH₄ isotopes showing only small fractionation in 2008 and any in 2009, thus their use as ultimate evidence for CH₄ oxidation was doubted in MS, (p. 16473, r 2 - 13). ***Possible reason for small or nonexistent fractionation will be discussed more carefully in corrected version.***

40

“b) I ask that much of the discussion in Section 4.2 is revised accounting for alternative interpretations of the data as explained above.”

We will revise discussion, and alternative approach will be discussed.

“Conclusions: As explained above I challenge the conclusions by interpreting the data differently.”

5 *We thank referee for this challenge making us to think this and other lake ecosystems more carefully and think about how differently things can be seen from same data. **Instead of changing point of view, we hope that in new version we can better show that our approach is right.***

10 “Supplementary Figure 1: This figure is interesting. The deviation in CH₄ and DIC concentrations from the bottom and up in the left panel is expected if CH₄ is contributed laterally from sediments only while DIC is contributed also from water column respiration. The hyperbolic shape of the methane curve is then simply a consequence of the water volume to sediment area ratio for each water layer in the hypolimnion and does not necessarily be caused by anaerobic methane oxidation. The right panel could indicate an interesting link between methane oxidation (maximum rates at
15 about 4 m depth) in the methanotrophic zone (indicated by the ¹³C shift of CH₄), and possibly by respiration of biomass with CH₄ carbon produced in and sinking to slightly below this zone.”

20 *Referee #2 turns also speculative here, but to get this hyperbolic shape we need a source of methane, continuous diffusion from bottom and oxidation of methane above this. Then mixing in the upper layer does the rest. And why CO₂ from methane oxidation would alone not lead to this decrease in DIC $\delta^{13}C$ values in water column?*

“Table 1: Which levels are really significantly different between the years? Can the large differences in water column O₂ content between the years be more dependent on weather and stratification patterns (shallow mixing means less of the water is oxygenated) rather than the sugar addition?”

25 *In some degree yes, there was such change also in control lake (Peura et al. 2014). Added carbon would consume oxygen, so it has to have some effect to oxygen amount.*

“Table 2: I assume the different rows represent different years?”

*Other mistake found by referee #2, thank you for careful reading. **This will be corrected.***

30 “Table 3: What are the uncertainty estimates of the calculated values presented in this table? I assume there is some uncertainty associated with fractionation factors as well as carbon conversion to biomass efficiency? Could the uncertainty be important for the comparisons?”

There is some uncertainty associated with fractionation factors and CCE values available, and this can be modified.

35 “Figure 2. Could not the fact that the strong “redox-cline” is much deeper than the oxycline also mean that microaerophilic conditions may prevail relatively deep and even though concentrations are very low this could be a result of relatively rapid transport based on strong concentration gradient combined with rapid consumption by methane oxidation. This interpretation of a rapid and

dynamic system driven by O₂ and CH₄ transport rates rather than actual concentrations, represents an alternative to the idea that AOM was important.”

5 *This zone between negative redox and limit set of our oxygen measurement sensitivity, could be the area where microaerophilic oxidation takes place. We used this area as such in calculations and it is considered to be zone of aerobic oxidation. Concentration gradient as driving force for CH₄ movement towards smaller concentration is slow and speed of it is only a small fraction of turbulent diffusion.*

10 “It is also difficult to imagine that relatively well oxygenated parts of the water column having negative redox potential in 2009. Could this not be primarily an offset in the measurements?”

This is a mistake. It will be corrected if possible or removed from the next version.

15 “Figure 6: a) I assume the concentration unit is $\mu\text{mol/L}$ or μM ? b) Why not indicating the O₂ profiles in this graph so it can easily be connected with the concentration and stable isotope profiles? It seems that at least micro aerophilic conditions suitable for aerobic MOB could have extended down to levels where interesting things happen with CH₄.”

We can do that with oxygen, but problem is still that detection limit is so high. Furthermore, H₂S causes interference to O₂ measurements (Bedard and Knowles, Can. J. Fish. Aquat. Sci. 54, 1997). Figures will be reconstructed to better fill the space given and oxygen and temperature can be added there.

20 “Figure 7: Interesting and nice presentation. As for Table 3, I am curious about the uncertainties. I also wonder why some of the bars are cut (if this is not just a file transfer problem).”

If we disagree with CH₄ AOM then there is no use of this figure, but it looks that we will have this also in next version! Scale is cut, because CH₄ oxidation is so small in the aerobic portion of water column.

25 “Figure 8: Interesting figure. The strong DIC depth profile with minimum levels in the central part of the water column is puzzling. I wonder if the hypolimnetic gradient in $\delta^{13}\text{C}$ could be maintained if the anaerobic methane oxidations is as great as suggested. (yes) Would not bottom water enrichment of ^{13}C of depleted respired carbon due to sediment methanogenesis (leaving ^{13}C -DIC behind) be a possible scenario? Some of the ^{12}C may be returned to the DIC in central water layers
30 by CH₄ oxidation (I would not rule out oxic MOB here) but the water column does perhaps not need to be at a balance as lots of CH₄ may be lost by bubbling.”

35 ***Concratulations to referee #2. There starts to be similar speculations as we have had here. As not much is lost in bubbling, then can it be methanogenesis there in bottom, anaerobic and aerobic oxidation in deep water layers returning CH₄ back and partly also leading to formation of sediment being lighter than other possible carbon sources?***

“Figure 9: I am not sure I understand this figure. Is the y axis showing cumulative fraction oxidized or not? Aslo, see comments to the text on the estimates of methane oxidation at various depths.”

*Figure shows fraction of total oxidized methane at different depths. Thus, in 2008 at depth of 5 m, there was fraction of oxidized methane from 0.3 to 0.8 and corresponding $\delta^{13}\text{C}$ from -80 to -75 per mil. **This figure will be modified or removed since it seems not to be clear.***