

Interactive comment on “Recycling and fluxes of carbon gases in a stratified boreal lake following experimental carbon addition” by H. Nykänen et al.

Anonymous Referee #2

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

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. Physico-chemical 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

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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.

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.

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.

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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.

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 presumably only diffusive efflux was accounted for. Why was not bubble flux considered? Was the bubble traps not suitable for quantitative bubbling estimates?

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.

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 therefore the description

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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 heterogeneous 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).

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.

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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.

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 understood it correctly acetate may be formed in several different ways in fermentation processes. For example in the Embden-Meyerhof 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 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 sources including production in sediment and water as well as hydrological input.

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?

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).

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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?

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.

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

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.

c) Given that I challenge the importance of AOM I ask that large parts of this section are reconsidered in the light of my comments.

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Section 3.4: a) This significant shift in $\delta^{13}\text{C}_{\text{CH}_4}$ is very interesting. It indicates a link between addition of sugar in the epilimnion to the hypolimnetic CH_4 in a short time and presumably via material reaching the sediments. Could this indicate that heterotrophic biomass from the water column can be important for CH_4 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 CH_4 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 is where signals of the additions may be preserved and more clearly visible over time.

b) 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?

c) 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 and enriched CH_4 so that the ^{13}C enriched CH_4 represents the leftover of the depleted CH_4 ...but across a stratified water column CH_4 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 this as a discussion point rather than as a result.

d) 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.

e) Similar comments as for CH_4 above apply to the parts of Section 3.4 about DIC.

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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 CH_4 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 CH_4 release from sediments significantly increased while CH_4 oxidation remained unaffected? That is surprising as CH_4 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?

b) 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 CO_2 and CH_4 versus enters biomass over time...but the other factors affecting efflux has to be considered as well first.

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

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 CO_2 and CH_4 . Can the possible contribution of deep water gases to the efflux relative to other epilimnetic sources be quantified?

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e) Page 16469: Many parts of the possible explanations for the observed stable isotope patterns seem speculative. E.g. it is unclear why sugar added in solution should flocculate. The stable isotope discussion appears to neglect the most simple explanation: the sugar added to the epilimnion results 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 e.g. possible partitioning of the added sugar into biomass versus CO₂ being emitted.

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.

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

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

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 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.

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?

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

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?

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

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that AOM was important. 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?

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.

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).

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 d¹³C could be maintained if the anaerobic methane oxidations is as great as suggested. Would not bottom water enrichment of ¹³C of depleted respired carbon due to sediment methanogenesis (leaving ¹³C-DIC behind) be a possible scenario? Some of the ¹²C may be returned to the DIC in central water layers 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.

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

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