

Please see below the point-by-point responses to the reviewers and the actions taken regarding their concerns. In the text below, the suggestions and comments of the reviewers are in black and plain font, and our responses are in italics and blue font.

Anonymous Referee #1

COMMENTS TO THE AUTHOR (S)

Throughout the whole manuscript including abstract: Please only show 3 significant digits for numbers presented throughout the manuscript. For example, presenting a number of “7082234” might be confusing and implies an analytical precision which is much better than you actually obtain. Furthermore, when large changes were observed you should better use “increased by orders of magnitude or by a factor of x to y”.

We have checked the whole manuscript and followed the reviewer suggestion.

Specific comments

Introduction.

The literature review is fairly comprehensive. However, there are a few very recent studies (the authors might not be aware of) by Klintzsch et al. (2019) and Hartmann et al. (2020) dealing with formation of methane from picoeukaryotes and cyanobacteria in freshwater and seawater which the authors might include in the sections dealing with isotope studies and evidence for oxic methane production (this also applies to results and discussion section 3.2.2, “CH₄-production coupled to photosynthetic organisms”). Furthermore, the study by Günthel et al. (2019) dealing with methane emissions with respect to lake sizes and the contribution of vertical and lateral methane transport should be mentioned and further discussed/included in section 3.2.2. “Vertical and lateral CH₄-transport from anoxic environments”.

We agree with the reviewer that these articles are relevant for this manuscript, but we were not aware of the existence of these three recent studies at the moment of sending the manuscript. We thank reviewer advice and have included these references in the revised manuscript in the Introduction (lines 58, and 81) and Results and Discussion sections.

page 3, line 65-66: Only the study by Damm et al. (2010) considered bacteria as a potential methane source. The other three studies investigated archaea as likely sources.

We rephrased the sentence in the revised manuscript as follows:

“In the open ocean, archaea and bacteria appear to metabolize the algal osmolyte dimethylsulfoniopropionate producing methane as a byproduct (Damm et al., 2008, 2010, 2015; Zindler et al., 2013)” (Lines 72-73).

In some parts of the manuscript English writing could be improved (e.g. lines 26-31, 232-233, 277, 279-286, etc.). Please check carefully throughout the whole manuscript.

We have checked the English writing throughout the whole manuscript and correctly rewrote the parts suggested by the reviewer.

Material and Methods

(2.1): I suggest showing a geographical map including all 12 reservoirs studied. This would help the reader to better envisage the geographical locations of all 12 reservoirs.

We have included a new figure that shows the location of the study reservoirs (new Figure 1). We have also included geographical coordinates and ancillary information in the new Table 1 and Table 2.

page 4, lines 140-143: “NH₄⁺ and NO₂⁻ concentrations: : :” . These data were neither shown nor discussed in the manuscript. Why?

In the first version of this manuscript, we used the nitrate, nitrite and ammonia concentration to calculate the concentration of dissolved inorganic nitrogen (DIN). We used the DIN: TP ratio as an index for the phosphorus limitation, but the information was not explicitly explained. Now, in the revised manuscript, we have included the information how we obtain DIN in the method section (lines 170-171) and a table with basic information about the concentrations of carbon, nitrogen, phosphorus and the ratio DIN: TP in the water column of the study reservoirs (Table 2).

page 6, section 2.5 DNA analysis: Please mention which samples were investigated for DNA analysis.

We have clarified the details of the DNA analysis in the Method section “2.5 DNA analysis”. From the sampling of the water column, we selected 3 or 4 representative depths for the epilimnion, metalimnion (oxycline), and hypolimnion/bottom layers to determine the abundance of the functional genes during the stratification period. We also selected 3 or 4 equivalent depths during the mixing period. In total, we analyzed 77 samples: 41 samples from the stratification period, and 36 samples from the mixing period. We have included this information in the revised manuscript (Lines 234 – 237).

page 8, lines 220: Explain “V”!

We performed the Wilcoxon signed rank-sum test to compare the concentration and the saturation of CH₄ between the stratification and the mixing period. V is the statistical parameter obtained from this test and we have included this information in the method section (Line 272). We used the Wilcoxon test because our data were not normally distributed.

page 9, section 3.2. and section 3.2.1.: Improve the flow between the two sections.

We have rewritten the connection between the two sections (Line 339).

page 9, lines 269-272: Not very convincing argument. Please rewrite.

We have rewritten the argument in the revised manuscript (Lines 344-353).

page 10 section 3. 2. 2. and subsection “Vertical and lateral CH₄-transport from anoxic environments”: Improve the flow between the two sections.

We have slightly rewritten the connection between these two sections (lines 377-382).

page 10, section 3. 2. 2.: For discussion please include and discuss recent results by Günthel et al. (2019).

We have included the results of Günthel et al. (2019) in the discussion of the revised manuscript (Lines 540-542).

page 11, line 300: “methylphosphonates (MPn)”. If abbreviation was introduced before it is no more necessary to again use the full name and abbreviation. Please check throughout the whole manuscript for consistency.

We have checked and corrected it throughout the whole manuscript.

Page 12, section “CH₄-production coupled to photosynthetic organisms”: As mentioned above there are a two very recent studies by Klintzsch et al. (2019) and Hartmann et al. (2020) which unambiguously show using stable isotope labeling approaches that both picoeukaryotes/phytoplankton such as *Chrysochromulina* sp., and cyanobacteria in freshwater and seawater produce methane per se. Please include these results in this section as they do fully support the results of the presented study.

We agree with the reviewer, these two studies fully support our results. Therefore, we thank reviewer’s advice and we have included them in the revised manuscript (Lines 476- 479 and 486).

Figures 1 to 7: Please provide more information about the statistical values of the parameters presented (e.g. error bars, SDs, uncertainty range, number of replicates, etc.). Furthermore, little information about the analytical uncertainties for the measurement systems is available in the method section. This needs to be improved in the revised manuscript.

Throughout the methods section, we have included more information about the replicates, statistical values of the parameters presented and analytical uncertainties. Please, see the method section (lines 141, 143, 165, 166, 169, 171, 174, 181).

Figures 1 to 3 and S1 to S9: Please add the date of sampling (field campaign) next to stratification period/mixing period.

We have included the dates of sampling in the figure captions, and in the new Table 2.

Figure 2 legend, line 753: “The grey area represents the anoxic zone (DO < 7.5 _M)”. There is no grey area highlighted in Figure 2.

That sentence was included by mistake; therefore in the revised manuscript we have removed it.

Technical corrections

page 1, line 10, replace “CH4” by “methane”

page 1, line 28, include “more” after “much”

page 2, line 37: replace “called” by “described”

page 3, line 78: change to “: we considered the following CH4 sources:”

page 3, line 80: “León-Palmero et al. in review” has been nit listed in the reference section

page 4, line 103: spell out PAR (photo active radiation)

page 4, line 116: replace “concentration” by “mixing ratio”

page 9, line 268: delete “as free-living microorganisms”

We did most of the technical corrections suggested by the reviewer. We also replaced the reference "León-Palmero et al. in review" by "León-Palmero et al. (2020)" throughout the manuscript, and now this reference is listed in the reference section.

Concerning the last point, we have decided to keep "as free-living microorganism". We consider that it is necessary to clarify that the negative results in the analysis of the mcrA gene in the water column of the reservoirs mean that we did not find methanogenic Archaea in the water column as "as free-living microorganisms". They could have been present in anoxic micro-niches (i.e., in the guts of zooplankton or within sinking particles) but these other potential sources were not analyzed in this study. Previous works have demonstrated the occurrence of methanogenesis in micro-anoxic zones of zooplankton guts and within sinking particles (Angelis and Lee, 1994; Karl and Tilbrook, 1994). We have included this explanation in the main text (Lines 418-420).

References:

Angelis, M. A. de and Lee, C.: Methane production during zooplankton grazing on marine phytoplankton, *Limnol. Oceanogr.*, 39(6), 1298–1308, doi:10.4319/lo.1994.39.6.1298, 1994.

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Karl, D. M. and Tilbrook, B. D.: Production and transport of methane in oceanic particulate organic matter, *Nature*, 368(6473), 732–734, doi:10.1038/368732a0, 1994.

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Yvon-Durocher, G., Allen, A. P., Bastviken, D., Conrad, R., Gudas, C., St-Pierre, A., Thanh-Duc, N. and del Giorgio, P. A.: Methane fluxes show consistent temperature dependence across microbial to ecosystem scales, *Nature*, 507(7493), 488–491, doi:10.1038/nature13164, 2014.

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