

Interactive comment on “Dissolved CH₄ coupled to Photosynthetic Picoeukaryotes in Oxic Waters and Cumulative Chlorophyll-a in Anoxia” by Elizabeth León-Palmero et al.

Anonymous Referee #1

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General comments

Several recent studies provided strong evidence of methane production in oxygenated freshwaters and seawater challenging the long-standing paradigm that microbial methane production occurs only under anoxic conditions and forces us to rethink the environmental dynamics of this greenhouse gas. Thus the manuscript by León-Palmero et al. certainly deals with one of the ‘hot’ scientific topics under current debate. The authors clearly show the occurrence of methane supersaturation in oxic surface waters (with seasonal dependence) of 12 reservoirs and discuss these results in the context of other environmental parameters such as abundance of photosynthetic

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picoeukaryotes and cyanobacteria, and chlorophyll-a concentration. They found that dissolved methane was coupled to the abundance of photosynthetic picoeukaryotes during periods of summer stratification and the winter mixing, and to chlorophyll-a concentration and the abundance of cyanobacteria during the stratification period. Overall, this is an interesting and straightforward manuscript including novel results, but several issues need to be addressed before it is ready for publication.

Please note that I will not comment on the experimental set up of the DNA analysis and the respective results as this is not my particular field of expertise.

Specific comments

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”.

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”.

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.

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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.

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.

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

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

page 8, lines 220: Explain "V"!

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

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

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

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

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.

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.

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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.

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

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

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"

Mentioned additional references:

Günthel, M., Donis, D., Kirillin, G., Ionescu, D., Bizic, M., McGinnis, D.F., Grossart, H.-P. and Tang, K.W. (2019) Contribution of oxic methane production to surface methane emission in lakes and its global importance. *Nature Communications* 10, 5497.

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Hartmann, J.F., Günthel, M., Klintzsch, T., Kirillin, G., Grossart, H.-P., Keppler, F. and Isenbeck-Schröter, M. (2020) High Spatiotemporal Dynamics of Methane Production and Emission in Oxic Surface Water. *Environ. Sci. Technol.* 54, 1451-1463.

Klintzsch, T., Langer, G., Nehrke, G., Wieland, A., Lenhart, K. and Keppler, F. (2019) Methane production by three widespread marine phytoplankton species: release rates, precursor compounds, and potential relevance for the environment. *Biogeosciences* 16, 4129-4144.

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