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Interactive comment on "Dissolved CH_4 coupled to Photosynthetic Picoeukaryotes in Oxic Waters and Cumulative Chlorophyll-a in Anoxia" *by* Elizabeth León-Palmero et al.

Anonymous Referee #3

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GENERAL COMMENTS:

Water column methane is commonly exclusively attributed to archaeal methanogenesis in anoxic sediments coupled with physical transport processes, especially in case of enclosed waterbodies. Throughout the last 2 decades evidence accumulated that methane can be also be produced under oxic conditions by archaeal and non-archaeal microbes. Some pathways have been identified, while others remain unknown. Withstanding, the relative contributions of the oxic and anoxic methane sources to whole-system budget are highly debated in the scientific community.

In their study, Leon-Palmero et al. investigate the origin of dissolved water column

methane in a series of reservoirs considering the commonly acknowledged sediment methanogenesis and the oxic methane source. The authors further resolve between different types of oxic methane sources: i) archaeal methanogenesis inside the water column, ii) microbial methylphosphonate degradation inside the water column, and iii) photosynthesis related methane production inside the water column. The authors apply a multi-method approach combining physicochemical analyses, gas analyses and molecular techniques to obtain a comprehensive dataset and proxy estimates for individual potential methane sources. The authors synthesize their results statistically to quantify the contribution of individual methane sources to dissolved water column methane. The following points summarize the major scientific advances of this study:

a) The oxic methane source(s) has not yet been investigated in reservoirs.

b) The study resolves the different methane sources throughout i) stratified, and ii) mixed period.

c) While the existences of the various methane sources have been reported in the literature, Leon-Palmero et al. present the first approach to resolve the contribution of anoxic sediment methanogenesis and various oxic sources simultaneously.

d) The general opinion in the scientific community is that either i) anoxic sediment methanogenesis, or ii) (oxic) methylphosphonate degradation are the dominant methane sources in whole basin budgets. In contrast, this study gives evidence that the photosynthetic methane source can be the dominant source in enclosed waterbodies what ties into the findings of several recent studies.

Accordingly, this study presents a series of new findings which is appropriate for Biogeosciences and will be interesting for a large readership. Applied methods are state of the art and clearly laid out, the authors are highly qualified. The authors present an appropriate introduction, however, the section about their research objectives falls a bit short (last introduction paragraph). As a result, the study appears less important to the reader than it actually is. I suggest, better connecting the introduction with the research objectives (e.g. recalling the scientific dispute of oxic versus anoxic methane sources and highlighting the major advances of the study – general advances will fit this paragraph whereas details may be stated in the conclusion section).

The authors should, further, include an overview of reservoir characteristics (carbon/phosphorus/nitrogen, trophic state, surface area, shoreline, mean depth, depth of the mixed layer). Also sampling locations should be characterized in a more detailed manner (location within the reservoir, shore distance, depth), for example, in tabular form. Towards the end of the Result&Discussion section I recommend recalling the general dispute between oxic and anoxic contribution and implementing the statistical results. Yet, there are not many literature information available, but please, place your overall finding into the context of DelSontro et al. 2018 and Günthel et al. 2020 (only studies so far presenting/summarizing contribution patterns for a series of lakes) who presented evidence for the importance of the oxic source increasing with basin size.

Does the dataset indicate reservoir conditions favoring individual oxic methane sources?

On some occasions the English should be improved throughout which will lead to a better understanding by the readership. Throughout the specific comments I acknowledged some of these occasions with rephrasing statements.

Statistical correlations make up a huge part of the study. I would find it practical to include a table summarizing the outcome of all significant and non-significant correlations (stating type of test, predictor variables and effect, p-value; covering not only the GAM model results). It will be helpful, to reference display items like this table in the method section (in this regard please check all the Supplementary Materials). Also, please include the correct units when statistical equations are stated throughout. Further, please check the wording when describing statistical results – on several occasions the statistical results are described with phrases like 'explains', 'determines' etc. Please use wording like 'points to explain', 'may determine', 'appears to be a main

driver' etc. instead. Although the correlations appear to partially explain a lot of variance, no actual (production) rates have been measured, and this should be reflected by the language.

The authors present a comprehensive dataset including many parameters. Please describe the obtained data in more detail throughout the method section or when recalled by display items. While many of the conditions can be read out of other display items, it complicates the reading flow. For example, in Fig. 7, data points are grouped into stratified and mixed data. The reader has no information about corresponding depths. But different conditions in epilimnic versus hypolimnic waters may lead to different contribution patterns of individual methane sources. Did the authors check if splitting the dataset into epilimnic/hypolimnic data improves the correlations? Another example are the PCR results presented in Figs. S11 and S12. What reservoirs (depths, time points, replicates?) are resembled by samples 1-12? Please clarify these details.

In summary, the authors present a valuable study for the scientific community. I recommend publication after addressing the general and specific comments.

SPECIFIC COMMENTS:

Abstract:

L 10-11. Please define CH4.

L 15-16. Methane supersaturation is a common observation in aquatic systems, including oxic waters (e.g. Tang et al. 2016 and references herein). Without differentiating between oxic and anoxic methane sources this sentence adds little content (e.g. close to sediments/in anoxia methane supersaturation is generally expected). Rephrase or combine with the following sentence. I recommend avoiding the percentage numbers, especially if they do not refer to significant correlations with picoeukaryotes or cumulative chlorophyll-a.

L 16-17. Here, it is important to state the size of the study reservoirs.

L 19. Replace 'determined' with a more appropriate wording, e.g. 'correlated with' etc.

L 20-21. Please rephrase this sentence.

Introduction:

L 77-83. Explain your research questions in more detail and state why they are of interest (link them better to the previous introductory part). - Picoeukaryotes are first mentioned in the (too short) section describing research questions, not all readers are familiar with this terminology. Accordingly, it should be defined. What organism does it include? Why are they of interest within your research agenda - Actual methane production rates have not been measured but were deduced from proxies!

L 28. Change 'much' to 'more'

L 29. Change 'attributed to' to 'determined by'

L 36-37. Add references, e.g. Tang et al. (2016) + references herein

L 41. Rephrase 'CH4 inputs may become from', e.g. 'CH4 may originate from'

L 44-47. Please incorporate the findings by Thalasso et al. (2020)

L 47-48. Please reference the findings by DelSontro et al. (2018)

L 52-53. Reference Hartmann et al. (2020), Bizic et al. (2020a) (isotopes); Khatun et al. (2020), Yao et al. (2016) (molecular approaches)

L 62. Change 'contrary', e.g. to 'in contrast'

L 78. Diverse in what sense? Please clarify.

Methods:

L 85-91. Please give an overview on the general reservoir characteristics (trophic state, size, location, temperature and oxygen conditions, epilimnion/hypolimnion depths etc). The reader requires this information to better place presented results into the study

context. Searching for all parameters in various figures hampers a direct comparison. In the following a series of different measurements are described. However, it is unclear if all these measurements were done simultaneously (at the same day or week) and at the same sites. Please clarify (maybe as a Supplementary Table). When measurements were done during mixed and stratified periods, have the exact same sites been re-sampled (location, depth, shore distance)?

L 88-90. Please rephrase this sentence.

L 91-93. Decide for either equation 1 or a definition in the running text to reduce redundancy.

L 102-104. Please state the units of each parameter as used throughout, and clarify what fluorescence relates to.

L 104-105. Do you mean measurement intervals in 6 or 9 m steps? Please clarify.

L 148-152. Please clarify the difference between integrated mean (integrated over depth? Consider defining it as total amount/content.), and cumulative chlorophyll a concentration. Consider labeling them with different Symbols.

L 168. Please state the equations used to compute GPP, NEP and R.

L. 180-181. Please clarify what you mean by 'specific primers from similar studies'. What pure cultures did you use (type, culture conditions, origin)?

L 170-196. Please lay out when (in the context of other parameters) and where (location in the reservoir and depth) you sampled. Also, specify which reservoirs have been sampled (you state 12 reservoirs throughout section 2.1, but Fig. S11 only shows 10 samples). Please clarify, what samples have been measured (depth, mixed/stratified period). Also, please add this information to Fig. S11 and S12 (define 'samples 1-12').

Sample 6 in Fig. S12 appears to have a very weak signal close to the phnJ bar. Did you verify that this is no positive signal (e.g. edit light/contrast properties to better resolve this area)? What was used as negative controls (in both assays: mcrA, phnJ)?

L 194-196. What was the DNA range you investigated?

Results&DISCUSSION:

L 218-219. Please avoid the large and unpractical percentage numbers (hard to read). Given, that the authors state the dissolved concentration, no content is lost when removing these numbers. For example, the authors could incorporate the average saturation concentrations in the following sentence.

L 223-225. The references are mixed up. E.g., Donis et al., Grossart et al., Tang et al. investigated temperate lakes, but Murase et al. researched a tropical lake etc. Please rephrase this sentence.

L 225. Please define the depth of 'surface waters'. Why is emphasize placed on Lake Kivu (not listed throughout the previous sentence)?

L 248. Please mention that the literature refers to studies in lakes. Note, a metalimnic methane maximum can be controlled by physical (e.g. differential gas solubility due to temperature change, emission) and biological factors (e.g. light inhibition of methane oxidation, variable phytoplankton methane production due to availability of nutrients/light/precursors). Also note, Kathun et al. (2019) presented a series of lakes with and without metalimnic methane maxima.

L 254. Referenced literature is not about boreal lakes.

L 256-263. Reference Tang et al. (2014) who also presented a distinction between oxic and anoxic methane concentrations in oxic and anoxic lake waters.

L 264-269. Please indicate what depths you analyzed.

L 272-273. Please rephrase the sentence. Also, please emphasize archaeal methane production is absent in the anoxic water column.

L 282. Please reword 'depend'. E.g. correlate with.

L 289-291. Please reference these considerations/results here.

L 296. Please define 'extreme' P limitation. Different Organisms require different amount of P. Accordingly, this is a relative terminology. Does it relate to the N:P ratio? Please clarify.

L 299-304. I appreciate the authors approach to evaluate the contribution of anoxic methane based on morphometrical parameters. However, lateral transport which is seen as major source of epilimnic methane is modulated by the shore-mid distance. Accordingly, when correlations are done this should be accounted for. Please clarify shore-mid distances in your dataset. I think, Hofmann et al. 2010 and DelSontro et al. 2018 should be referenced as these studies show major contributions by the littoral (especially in smaller lakes).

L 304-305. Please reference the lateral transport model by DelSontro et al. (2018) which agrees with the observation of exponential decay functions. A later discussion on this decay function should be warrant. E.g., close to the shore there should be a high content of dissolved methane that originated from the sediments; with increasing distance from the shore the relative contribution of the oxic methane source should increase.

L 306-307. Concentrations increased exponentially versus what parameter?

L 313-314. Please discuss why there was no significant correlation between dissolved methane and shallowness index. Potential reasons might be: variable sediment methane production rates among reservoirs (variable among temperature, trophic state, sediment porosity, soil type and community etc.) the ratio between oxic and anoxic methane production may lead to distortion. The authors may have other points. Did you try the ratio of 'mean depth : depth of surface mixed layer' as a proxy for lateral input (depth of the mixed layer relates to the amount of temperate sediments)?

L 315-317. I think the authors should remove the wind speed correlation throughout. Wind-forcing, or more generally, turbulence which drives surface emission and substantially affects surface water methane concentrations, is modulated by many environmental factors (basin geomorphometry, temperature, rain etc.). Also, internal production rates, lateral methane input and methane oxidation modulate surface concentrations beside emission. Accordingly, correlating wind speed with surface concentrations is an over-simplification and might be misleading.

L 317-318. Please rephrase.

L 318-319. Please define what you mean by 'extreme supersaturation' and where these concentrations were found.

L 323-327. This is an interesting observation. Consider highlighting this observation more. Is it possible that you filtered off the attachment partners of methanogenic Archaea (you filtered water samples before molecular analyses)?

L 341-342. Define 'extreme' limitation. According to my knowledge, it is unknown what are the P levels (N:P ratio) triggering MPN degradation and corresponding methane production in the field.

L 342-343. Are these correlations simple x-y regressions or do they include for multiple predictor variables?

L 345-350. Fig S12. Can you change the picture properties (light, contrast); it seems, there is a faint bluer in sample 6. Cyanobacteria have been detected (e.g. Fig. S13). What type of cyanobacteria were present? Do these result agree with findings by Yao et al. 2016 (some types possess the methane generating enzyme machinery)?

L 352-364. Many information listed belong to the result/method section. Please remove redundancy.

L 365-366. Given that many factors may affect the dissolved methane concentration (as discussed throughout) a p-value of 0.077 might still point to a connection. It has

been stated n=12; does that mean 1 value per reservoir during the stratified period? Same questions on other occasions. Please clarify.

383-384. Please add the reference Hartmann et al. 2020 who presented methane production by green algae, cyanobacteria, cryptophytes and diatoms.

L 396-405. Please mention methylated amines which can also serve as methane precursors (e.g. Bizic et al. 2018, Bizic et al. 2020b).

L 412-414. Please emphasize that this cyanobacterial methane 'production' does here not relate to MPN degradation (following the absence of phnJ).

L 435-436. Please rephrase and avoid the percentage number. Results by Bogard et al. (2014), Donis et al (2017), DelSontro et al. (2018) and Günthel et al. (2019) suggest that in larger waterbodies the majority of surface mid-water methane (/emission) might be explained by the oxic source.

L 437-438. Please rephrase.

L 458-460. I suggest moving the PPEs description to the result section or even earlier, when the terminology PPE is defined.

L 461-465. Please indicate to what extent the different methane sources might explain the dissolved methane concentrations (e.g. in percentage – relates to Fig. 8a).

L 467-468. Given other work on relationships between methane and chlorophyll/non-cyanobacteria phytoplankton (Tang et al. 2016, Hartmann et al. 2020), I think novel is not the right terminology here.

L 475-476. Please rephrase.

References:

Please check abbreviation punctuation (sometimes with and sometimes without dot).

L 582. Typo.

L 627. Capital letters

DISPLAY ITEMS:

L 760-761. Please state the regression statistics in the figure legend.

L 765-767. Please state the regression statistics in the figure legend.

L 769-771. Did you have data about reactive phosphorus? Using biologically accessible phosphorus instead of total phosphorus might improve the correlation statistics.

L 775-780. In case $R2<1.0$, the functions do not entirely explain the dissolved methane concentration data but only a certain fraction of data variance (e.g. 40% at $R2=0.40$ in a simple regression). Please rephrase accordingly.

What water depths do these readings (n=78 or 82) correspond to? In case some belong to the epilimnion and some to the hypolimnion, different nutrient availabilities may affect the correlation statistics and mask potential relationships.

L 784. (corresponding to Fig. 8a). I am not sure, 'significance' is the best terminology at this point. Consider rephrasing y-axes to explanatory power.

Could the author please comment on the partial effect of 'sediment methane production' (Fig. 8b)? Why is the trendline reversing the slope after ca. 27.5m mean depth? Does this parameter indicate bigger (deeper) reservoirs than 27.5 m mean depth have a higher sediment contribution to mid-water methane?

Supplementary Materials:

Table S1. Please discuss why the statistical correlation leads to substantial differences when applied to the combined data set of stratified and mixed period (e.g. no significant correlation listed with mean depth what is here used as a proxy for conventional sediment methanogenesis). Please discuss these details in the main document. âĂČ REFERENCES:

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Yao, M., Henny, C. and Maresca, J. A. Freshwater Bacteria Release Methane as a By-Product of Phosphorus Acquisition. Appl. Environ. Microbiol. 82(23): 6994-7003 (2016).

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