Author comments for manuscript bg-2022-135

„The highest methane concentrations in an Arctic river are linked to local terrestrial inputs"
Karel Castro-Morales, Anna Canning, Sophie Arzberger, Will A. Overholt, Kirsten Küsel, Olaf Kolle, Mathias Göckede, Nikita Zimov, and Arne Körtzinger

On behalf of all co-authors, we thank the support from the associate editor Alexey V. Eliseev and the useful comments from two anonymous reviewers for the improvement of our work. The answers to each of the referees' comments are provided below in blue font color.

Referee #2

Summary of the paper: *The highest methane concentrations in an Arctic river are linked to local terrestrial inputs*. Castro-Morales et al. studied methane concentrations on a 120 km portion of the Kolyma River during the late freshet (twice in June 2019). They observed a strong spatial disparity along the river bed with higher concentrations linked to warmer temperature, low conductivity and closeness to the river bank. This high spatial resolution study in the Kolyma River is the key start to better understand methane concentration pattern in (Arctic) rivers and their potential as methane source/sink. The correlation with temperature is interesting as it poses the question of increasing methane emission from Arctic rivers during the current global warming. It would be great to confirm if the microbes detected during this study are alive (active in the river itself) or dead (originating from the nearby permafrost surficial soils as stated by the authors) by sequencing RNA, although this type of sampling comes with logistical challenges in such remote environments. Overall this study is well done and brings key findings.

Thank you for your supportive comments. We do agree that measuring the activity of methanogens in the river water would be ideal to answer the question of the source of riverine CH_4. As the reviewer #2 pointed out, due to logistical constrains, we were not able to extract RNA from the samples for doing metatranscriptomics analyses.

Main comments:

- Why did the authors choose to only study dissolved organic carbon (DOC) as a food source of their methanogens while particulate organic carbon (POC) could a food source as well? Especially because the GF/F filter used to filter the water for DOC could be used to quantify POC. Eventually, DOC concentrations are not used at all in this study and could be removed.

The reviewer is correct that POC should have been included for this analysis, and referee #1 also made the same comment. We did not explicitly measure POC in the water samples but rather total organic carbon, TOC (i.e., unfiltered water aliquot). We will replace the results of DOC by those of TOC. For further details in this response, please see also our comments to reviewer #1.

- Were there any new OTU detected during the study? If so they should be deposited in GenBank.

All sequences have been deposited in GenBank with the reference BioProject No. PRJNA881395. The data is openly available. This information will be added in the revised manuscript in the data availability section.

Minor comments:

L364, 369: Could you add the instrumental error on the measurement of pCH_4?

The accuracy or measurement error of the CONTROS HydroC® TDLAS sensor for measuring pCH_4 is according to the manufacturer (-4H- JENA) in a range of ±2 µatm or 3 % of the reading (Canning et al., 2021a). This information will be added in the revised manuscript.

L437: Was the river anoxic where Methanobacterium, and Methanoregula were detected? If not, are they rather active or dead (originating from the nearby terrestrial environment)? Did you consider methane production within the river as this as been detected in other aquatic environments (see Bogard et al., 2014 (Oxic water column methanogenesis as a major component of aquatic CH4 fluxes, Nature).

Good point. Thanks for pointing to the Bogard et al., (2014) paper. The river water was oxic at all stations with an average O_2 saturation of 110 % (see figure added below), which should preclude methanogenesis in the river water, as it has traditionally been assumed to occur only in anoxic environments. Only based on our DNA analyses, we are not able to distinguish between active and dead cells.

There is indeed increasing evidence of CH_4 production in oxic marine and freshwater environments (Bogard et al., 2014; Grossart et al., 2011). Oversaturation of CH_4 in surface waters of lakes has been shown to result from two processes: CH_4 release from littoral sediments in combination with horizontal transport to the open water and *in situ* net production of CH_4 in oxic surface water. Their relative importance is under debate (Bogard et al., 2014; Encinas Fernández et al., 2016; Grossart et al., 2011; Peeters and Hofmann, 2021). As discussed in Bogard et al. (2014), the link between oxic *in situ* CH_4 production and algal dynamics would not explain the high spatial heterogeneous CH_4 concentrations in the Arctic River water with a maximum detected close to river banks and near tributaries during the late freshet. Release of CH_4-rich pore water and of soil-borne methanogens during permafrost melting and resuspension events, rather than *in situ* net production of CH_4 in oxic surface by active methanogens might be the main driver.

Similarly, CH_4 concentrations in shallow water zones of lakes can be explained by release of CH_4-rich pore water during resuspension events and by elevated anaerobic CH_4 production in warmer sediments at shallow water depths compared to deep water sediments (Peeters and Hofmann, 2021). We will discuss this in the revised version.

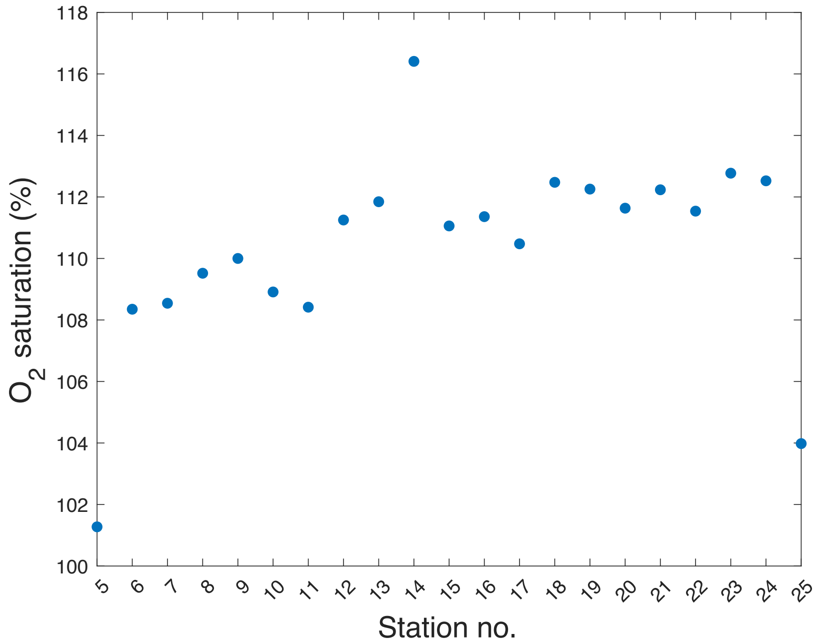

Fig. 1 – Oxygen saturation in the river water along the stations in Kolyma River.

L457: r^2 = 0.11 and 0.21 are rather low correlation coefficients, can you add p values? If p is not significant this needs to be indicated so as to not mislead the reader on the presence of correlation.

We have added the statistical significance of the mentioned correlations between water properties and the absolute abundances of methano-/methylotrophs at stations. The modified paragraph reads as follows:

"The correlations between the total absolute abundances of archaeal microbial communities against the water properties at stations (Fig. 7) show statistically significant ($p<0.05$) positive linear correlations between T and the abundance of methanogens ($r^2=0.35$, $p = 0.005$) and methano-/methylotrophs ($r^2=0.43$, $p=0.001$) (Figs. 7a and 7b). A statistically significant negative linear correlation was obtained against κ ($r^2=0.31$, $p=0.007$) for methanogens and for methano-/methylotrophs ($r^2=0.24$, $p=0.02$) (Figs. 7c and 7d). The pCH_4 at stations is also positively correlated to the abundance of methano-/methylotrophs ($r^2=0.22$, $p=0.04$) and methanogens ($r^2=0.11$, $p=0.15$) (Figs. 7e and 7f), however the latter is not statistically significant at $p<0.05$".

L459-470: The paragraph on DOC concentration comes a bit out of the blue after the microbial analysis, maybe add its separate paragraph? Especially because it is not used afterwards

As mentioned in the comment above, we will substitute DOC by TOC in the analysis of results. Given that there is no clear evidence that TOC (or DOC) played an important role as indicators for the CH4 distribution in the river, and that the TOC data is only available for 8 stations, this paragraph will be shortened and only the correlation results will be presented.

L585: Some methanogens have been detected in oxic environments before, see Bogard et al., 2014 (Oxic water column methanogenesis as a major component of aquatic CH4 fluxes, Nature).

Thanks again for this comment. We agree that it would strengthen the manuscript by adding in the revised manuscript a paragraph addressing the possibility of methanogenesis in oxic waters. Detailed information has been mentioned in the response above

Typographical corrections:

Since you choose to simplify methane as ”CH_4” please to do homogenously in the manuscript

Thanks for this comment, we will replace in all instances “methane” by “CH_4”.

References cited in this comment

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