

Methane oxidation in African lakes increases with phytoplankton and outweighs photosynthesis-driven oxic methane production

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Attachments originally included by the reviewers as part of their assessment can be found at the end of this file.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

This study by Borges and colleagues investigated methane oxidation (MOX) patterns across 81 African lakes spanning all trophic states from oligotrophic to hypereutrophic conditions, testing drivers like trophic state and nutrient limitation. The researchers quantified MOX rates alongside CH₄ concentrations, chlorophyll-a (Chl-a), nutrients, and phytoplankton composition, comparing CCC (Cuvette Centrale Congolaise) vs. non-CCC lakes.

MOX rates increased with CH₄ and Chl-a, peaking in productive lakes due to (presumably) methanotrophs attached to phytoplankton, and in CCC lakes from wetland soil inputs. MOX dominated CH₄ removal (>70% median) over diffusive emissions, with phosphate limitation evident. Oxic methane production (OMP), estimated from literature Chl-a relationships, contributed minimally (<12%) declining in high-CH₄ systems sustained by sedimentary sources.

The authors conclude that eutrophication boosts MOX more than it boost OMP/sedimentary production, attenuating diffusive CH₄ emissions despite rising phytoplankton biomass.

Overall the provided information that MOX, presumably attached to phytoplankton attenuates a significant portion of the methane produced in lakes is important and rather reassuring,

I have made specific comments in the attached annotated PDF files of the manuscript and the supplementary material. Hence the text here addresses general issues

General assessment

The paper provides a broad scale assessment of methane concentration in a large number of African lakes and thus provides a significant contribution to our knowledge on methane emissions from lakes worldwide. The authors also provide information on controls of methane emissions from African lakes at different trophic states. This is also very important for bottom-up modelling efforts assessing the contribution of freshwater lakes to the global methane budget.

In their work, the authors also try to assess the contribution of methane produced in oxic environments (OMP) and specifically that produced as a byproduct of photosynthesis, to the total methane flux. Several aspects make this part of the paper less convincing. I generally have the feeling that the authors confuse OMP with photosynthesis associated methane production. The latter is part of OMP with varying significance that was not determined in this paper.

First, the authors did not experimentally determine general OMP derived from multiple processes. The OMP rates taken from literature for 3 out of the 81 lakes are driven from ¹³CO₃ uptake in the light – i.e photosynthesis and not the complete OMP (ref 19 Morana et al...Borges). Additionally, do we know if these rates measured by Morana and colleagues reflect OMP rate all year-round? Also here (like below) some number conversions seem to be wrong. Morana et al also report a 4th lake Karinda, why was this lake not included in the OMPcom?

There are at least two studies by now, the latest I know of is: <https://pubs.acs.org/doi/abs/10.1021/acs.est.2c01730> that reveal a morphometric relationship between lakes and contribution of OMP to the methane flux. How do these lakes fall on that curve? Or, can this curve be used to model the contribution of OMP to the observed flux? Looking at table S1 there are lakes like lake Besako with a large area and are very shallow but also lakes like Kyamwinda or Katinda which are deeper and with a smaller surface area. The contribution of OMP in these lakes to the total CH₄ flux is expected to be different.

Second, while estimating the potential of phytoplankton to produce methane based on published data, it seems there is a mistake that underestimates these rates by 4 orders of magnitude (see calculations under specific comments). Even if these rates derived from cultures are an overestimate when it comes to environmental conditions, they could easily account for the mentioned "OMP" rates.

Overall the author's conclusion could be that Photosynthesis associated methane production makes up ca. 12 % of the methane flux with the general OMP likely more. In addition they show the significance of MOX in attenuating the CH₄ flux, specifically OMP and phytoplankton derived.

As stated in my specific comments the authors made what appears to be some arbitrary decision that are not so transparent to the reader. These can be numerical values like the 6.2 m MLB or 18 µg Chl-a. Why not 7 m or 20 µg. There are also some comparisons of African lakes and rivers with European rivers, why not also European Lakes and why European and not Global? It feels like the authors chose values and data that would support their ideas.

I also wonder, as the MOB are thought to be attached to phytoplankton, is the increase in MOX an effect of more MOB attached to the increased phytoplankton biomass rather than an increase in MOX activity?

Last Could characterization of methanotrophic community help elucidate the differences in MOX? Perhaps MOX increases not only a result of an increase in MOX activity or MOB, but also because of the difference in methanotrophic communities present in each areas/lakes.

Reviewer #2

(Remarks to the Author)

The Manuscript "Methane oxidation in African lakes increases with phytoplankton and outweighs aerobic methane production" addresses a topic that has been hotly debated for >10 years, as can be assessed looking at the long and comprehensive list of papers cited, including ground-breaking publications that appeared in Nature Communications by Beaulieu, DelSontro, & Downing in 2019, Günthel et al. in 2019 and in 2021, and by Focardi et al. in 2025. Afrotropical inland waters have been characterised as hotspots of methane production and release to the atmosphere. Drivers governing both of these processes are imperfectly understood as they involve a balance between: anoxic (termed "sedimentary") and oxic methane production, methane oxidation, and methane diffusion into the atmosphere. The authors attempted to quantify these processes and relate them to classical limnological parameters including nutrient concentrations, Chl "a", oxygen availability, total suspended matter. The research strategy included: 1. a large sampling campaign, with 503 samples in 81 tropical African lakes, 2. controlled measurements carried out in the laboratory on pure phytoplankton cultures, and 3. in situ oxic methane production measurements conducted in lakes Edward, George, and Nyamusingere. At the end of the Introduction the authors explicitly state their objectives: characterise relationships between methane oxidation and limnological parameters and quantify it in relation to methane release to the atmosphere, and to oxic methane production. The H0 being set ("MOX changes with the trophic state of lakes and is limited by nutrients") does not sound to be particularly ambitious, as it rephrases results obtained in previous research cited in the Manuscript, for example: Nijman et al. 2021 (as well as others).

As a strong point, this research usefully corroborated previous findings indicating the attenuation of diffusive CH₄ emissions via enhanced methane oxidation (MOX), a process that reduced early expectations about contributions of tropical inland waters to the atmospheric methane budget. To do this, the Manuscript assembles a useful comparative database of methane concentration in African inland waters including lakes, wetlands and rivers (however a competent overview was recently offered by Cuvert et al. 2025 in Nature Water), and it discusses the relevance of methane oxidation in the overall methane budget. Drivers controlling methane oxidation were investigated through direct measurement and statistical inter-comparisons between different waterbodies.

The Results highlight a striking difference between "lakes" comprised within the Congolese Cuvette Centrale and lakes situated elsewhere. This pattern was explained by a connection between "wetland soils" and CCC lakes that drives their limnology, as opposed to no-CCC lakes in which phytoplankton-driven processes predominate. This observation does not read as ground-breaking evidence, it does not fulfil the objectives of this research and it should be explained more explicitly that the contrast between these two groups of lakes serves as an argument that tries to identify what drivers determine methane dynamics.

The manuscript offers a description of phytoplankton community patterns, citing main phytoplankton groups potentially associated to methane metabolism, as reported in published literature. Lines 135-140 propose mechanisms that could explain the potential association between phytoplankton and bacteria. These observations are not directly supported by the Results presented but cite adequate literature. Further on, in Lines 156-157, it is stated that this attachment does not seem to be specific to Cyanobacteria. This last observation could be given higher emphasis and be added to the Conclusions.

Apart for Cyanobacteria, no reference is being made to other bacterial groups (in particular Archaea) that have been related to methane metabolism in African lakes (see publications by Seymour et al. 2017 in Nature Microbiology, Fazi et al. 2020 in Communications Biology, Nijman et al. 2021 in ISME Communications, Li et al. 2021 in Environmental Microbiology, Gontijo et al. 2024 in Environmental Microbiome, Focardi et al. 2025 in Nature Communications).

The manuscript does not include salinity as a relevant factor involved in the establishment of high microorganism biomass and high methane concentration, as shown by Fazi et al. 2020 in Communications Biology, who reported high methane release rates in a hypersaline lake, higher than the range reported in the studied lakes (see Line 95).

Trophic state (nutrient) dependency of bacterial growth is well supported in the literature, so are the nutrient dependencies of methane production and methane oxidation. The significant relationships illustrated in Figure 2 between methane oxidation and methane concentration, and between K(MOX:CH₄) and Chl "a" do not come as a surprise but corroborate expectations. Oxygen availability is often referred to in this manuscript as it represented a conservative value, while it is well-known that it changes widely according to the hour of the day, especially in standing or slow flowing waters rich in organic matter such as the CCC lakes. For this reason, it is difficult to interpret dissolved oxygen values (reported as O₂) as characteristic of the limnology of different lakes. An O₂ max could be more indicative, or a daily range O₂ min-O₂ max.

Reference to "electron acceptors" in Lines 44-46 is rather surprising, given that O₂ will always come first in the chain regardless of the availability of other acceptors. It is not evident that MOX could be limited by the scarcity of alternative electron acceptors in tropical lakes (convincing evidence of this is not presented). Low O₂ abundance can be caused by high organic matter loads, but the same would then presumably provide available SO₄²⁻, NO₃⁻, Mn and Fe oxides.

The first paragraph of the Conclusions that should emphasise the main achievements obtained during this research, highlights as main result the distinction between CCC and no-CCC lakes, attributed to the connection of CCC lakes to "wetland soils". However, distinction between CCC and no-CCC was not the main objective presented in the Introduction. Secondly, "wetland soils" is a rather vague term. Several of the "lakes" sampled during this research would be classified as wetlands, being shallower than 3 m (see Line 71); "wetland soils" may indicate anything between "wetland aquatic sediments" and more or less wet pastures, or waters draining humid forests.

The observation that in no-CCC lakes CH₄ metabolism could be driven by phytoplankton, as opposed to CCC lakes, is based on rather weak evidence and it is rather surprising that it could be presented as a main striking contribution of the present research and placed at the beginning of the Conclusions.

The contents of the Abstract should be better aligned to the Conclusions.

The Conclusions should focus more closely on the original results achieved in this research and highlight its added value to the current scientific context/discussion, rather than continue the Discussion. It is surprising to see, in the Conclusion, citations reported to support statements.

In 272-274, the authors admit that no progress has been offered through their research about the postulated attachment between bacteria and phytoplankton. Yet this mechanism is essential to offer an explanation to the relationship between methane oxidation and Chl "a" indicated in this research. This initial statement seems to be contradicted further along. An important point of discussion in this extensive work concerns MOX dynamics in relation to Chl "a" and nutrient levels. This issue has been debated in the literature before, but the present work introduces a large number of new data. Despite this, the interpretation of this data should be phrased more explicitly. The MOX:Chl "a" relationship in CCC lakes indicates that MOX is not simply nutrient limited and that MOX could be promoted by the abundance of phytoplankton itself. At the same time, however, K:Chl-a values were positively related to PO₄³⁻ concentrations; the ambiguity therefore remains (nutrients or phytoplankton drive MOX?). The Manuscript should explain much more clearly if/how the data presented here contribute to the development of a clear line of evidence that could be considered novel (and how strong it may be).

The last paragraph of the Conclusions, in Lines 280-289, proceeds describing a hypothetical scenario, giving the impression that the results obtained in this research did not produce a clear and univocal weight of evidence that would explain the patterns revealed.

The manuscript is relatively complex and a clearer Conclusion would be beneficial to ensure that readers do not miss the main achievements that render this contribution original and innovative. How does this work contribute to the on-going debate concerning methane metabolism and its relevance to C dynamics in tropical ecosystems?

Further Minor points are highlighted below:

The Manuscript does not provide details about the actual sampling dates. This reduces the chance to use these data in future intercomparisons.

Sampling procedures are well described, however scarce description is given of the controlled laboratory measurements on pure phytoplankton cultures, and in situ oxic methane production measurements in lakes Edward, George, and Nyamusingere.

Lines 82-83 are a surprising. Why should one expect to find NO₂ in the surface waters of lakes (even if partially humic)? NO₂ is very difficult to assess even when oxygen is very low because it is rapidly oxidized during sampling and sample manipulation. It seems difficult to utilise the very low NO₂ values presented in this manuscript to try and support any argument.

Lines 88-93 describe phytoplankton groups in CCC lakes as opposed to no-CCC ones. This reads as an entirely descriptive detail and it does not contribute to explain potential methane metabolism differences between the two groups of lakes. A more explicit link to the objectives of the Manuscript should be highlighted.

In Lines 117-118, the evidence "Dissolved CH₄ concentrations in surface waters were also negatively related to the mixed layer depth" could be simply explained by higher rates of sediment methane production (a shallower mixed layer would reveal a greater influence by the hypolimnion) rather than by "the greater influence of MOX as a CH₄ sink in deeper, oxygenated mixed layers." The argument does not seem to be robustly supported by evidence and a more convincing case should be developed.

The last section of Results and Discussion proposes rather complex text discussing the contribution of oxic methane production to the diffusive CH₄ flux. This concludes in Lines 262-264. A brief summary of what is developed here should be highlighted in the Conclusion section.

In 357, stratification was defined by a $\geq 0.2^{\circ}\text{C}$ temperature gradient. This statement could benefit from a citation of an authoritative source. In general, stratification strength is related to temperature and is therefore stronger in tropical lakes; however, 0.2°C seems to be at the lowest limit of what could indicate actual separation between water masses.

(Remarks to the Author)

The manuscript, "Methane oxidation in African lakes increases with phytoplankton and outweighs aerobic methane production," is a highly comprehensive study featuring an experimental design conducted at a regional scale. This study describes a research consistent with previous works from the same research group. The study focuses on analyzing the impact of phytoplankton concentration (i.e. Chl-a) on methane oxidation in the surface waters of more than eighty lakes across Central Africa.

Compared to other studies published by this group, the objective of this research is certainly original in its scope. However, it is important to note that the core phenomenon—the link between phytoplankton and methane oxidation—has been addressed in other studies conducted by different groups, without the necessity of such a large-scale, regional experiment. The following references, which are relevant to this context, were not included in the manuscript's reference list (ChatGpt helps me in this bibliographic search):

- Oswald et al., 2015. PLoS ONE, 10(7): The abstract concludes: "Along with the detection of chlorophyll a, results suggest that aerobic methane oxidation is tightly coupled to light-dependent photosynthetic oxygen production both at the oxycline and in the anoxic bottom layer. It is likely that this interaction between oxygenic phototrophs and aerobic methanotrophs represents a widespread mechanism by which methane is oxidized in lake water, thus diminishing its release into the atmosphere."

- van Grinsven et al., 2021. Biogeosciences, 18(10), 3087–3101: The abstract includes the sentence: "Overall, this study shows that photosynthetically fueled CH₄ oxidation can be a key process in CH₄ removal in the water column of humic, turbid lakes, thereby limiting diffusive CH₄ emissions from boreal lakes."

Furthermore, several references already cited by the authors (e.g., Cerbin et al., 2022; Li et al., 2021; Milucka et al., 2015) also refer to the relationship between phytoplankton and methanotrophs.

In summary, the authors have conducted a very thorough and well-executed study, providing a wealth of novel data from many lakes in a region that has received limited previous attention. Nonetheless, their results do not diverge significantly from the mechanistic insights described in existing literature.

Given that this work is being considered for publication in a very high-impact journal, such as Nature Communications, my conclusion—regretfully—is that the manuscript does not present sufficiently novel conclusions or a paradigm shift to justify publication in a journal of this caliber.

My conclusion would likely have been different if the results had refuted or substantially challenged the established understanding from previous studies; in that case, the resulting scientific novelty would justify publication in Nature Communications.

That said, I have several substantive concerns which I will outline below:

MOX Estimates

Lines 312–315 (Incubation Details): It is unclear how many measurements were taken for each incubation. The text mentions "T1–T4": are these four distinct time points? What was the criterion for choosing incubation durations ranging from a few minutes to up to 12 hours? I interpret that all incubations were conducted in the field without the immediate capability to verify the rate of methane decrease. Is this interpretation correct? If so, what methodology allowed the determination of whether an incubation should last only a few minutes or many hours a priori?

Were the same number of incubations performed in every lake?

Lines 322–323 (MOX Calculation): The authors state that MOX was calculated by fitting the data to an exponential decay model (leaving aside the need for greater transparency regarding the statistical significance of these decays and whether the exponential function was indeed the most appropriate model). I do not understand why the MOX rate is expressed in nmol/L⁻¹/d⁻¹ when the fitted exponential model yields a decay rate with units d⁻¹.

To obtain the MOX values, do the authors multiply the decay rate by the initial methane concentration? This must be explained more clearly. The necessity for this clarification is heightened because the text (Lines 131–132) defines the parameter $K = \text{MOX}/[\text{CH}_4]$ (units of d⁻¹), implying that CH₄ represents the initial methane concentration prior to incubation. While the introduction of K is justified because the process depends on the initial reactant concentration, why introduce K when the exponential decay rate inherently accounts for this initial concentration?

The Trophic State

Line 372 (Trophic State Index - TSI): The classification of the Trophic State Index (TSI) appears to depend solely on chlorophyll concentration. If so, the results derived from this index are entirely redundant with the information already provided by the chlorophyll estimates. While one might argue that this allows for the classification of lakes into four trophic conditions, this separation seems somewhat arbitrary. Furthermore, such classification could be achieved directly using chlorophyll data (for instance a lake is oligotrophic if Chl-a < 9 ngL⁻¹). In my opinion, the introduction of the TSI is unnecessary and could be removed.

Figure 2 (Statistical Comparisons): Visually, the reported significant differences in MOX and K between trophic states are surprising. The box plots (right-hand graphs) exhibit considerable overlap, making it unexpected that the ANOVA and Tukey's post-hoc test report so many significant differences. This concern regarding visual overlap versus reported significance persists for many of the box plots shown throughout the manuscript and supplementary material.

Impact of OMP as a Methane Source

This section is interesting, but somewhat difficult to follow. I agree that methane produced through processes classified under "OMP" (Oxygenated Methane Production) cannot contribute to MOX. However, the explanation could be significantly simplified.

Figure 5: Plots "c" and "d" are essentially identical to "a" and "b" and could thus be moved to the supplementary material.

Core Utility: My greatest concern is the usefulness of incorporating OMP-Chl relationships derived from estimates obtained in laboratory incubations under presumed optimal light and nutrient conditions. These conditions are likely not representative of the in situ stress conditions that might affect methane production. I would suggest making this section more

concise and clearer, potentially by focusing only on the empirical data from the three field lakes and omitting the data derived from laboratory incubations.

MOX Drivers

Line 122 (Methane and Chlorophyll Correlation): The text states that CH₄ concentrations are positively related to chlorophyll concentration. This relationship is clear for "non-CCC" lakes but not for "CCC" lakes (according to the information at the appendix). This distinction needs to be clarified better.

Temperature and Oxygen: Given that this article describes a metabolic process highly dependent on ambient temperature and oxygen concentration, it is surprising that these two variables appear to play almost no role. How can the authors ensure that all incubations were conducted under fully oxic conditions? The study included some shallow lakes with $z < 5$ m and high DOC concentrations (>30 ppm). How can it be confirmed that the dissolved O₂ concentration was high at the start of all incubations? Upon reflection, this appears to be a critical issue that should be addressed in future submissions.

Figure S1b shows O₂ concentrations. However, to compare dissolved gas concentrations across different environments, the authors should report the percentage saturation, which accounts for both temperature and the gas's partial pressure in the atmosphere. Given that the temperatures of the different lakes surely vary, as does the partial pressure O₂ (I could not find information on the elevation of the lakes, but they are unlikely to all be at the same altitude), reporting raw concentration is insufficient.

Conclusions and Study Design

Lake Connection Focus: The "Conclusions" paragraph begins by discussing lakes "connected and unconnected" to flooded forests. However, the relevance of this aspect is not clearly presented at the beginning of the manuscript. It is absent from the Introduction and is only mentioned in the Methods and Results when referring to "CCC" and "non-CCC" lakes. If the authors consider this distinction highly relevant and wish to emphasize it in the conclusions, they must introduce it more transparently at the beginning of the manuscript.

Statistical Imbalance and Geomorphology: Furthermore, I question the emphasis on this "connection" aspect. If I am not mistaken, the "non-CCC" lakes are significantly more numerous than the "CCC" lakes (i.e., 65 vs. 16), which could introduce issues in statistical comparisons. Further, the substantial difference in the number of lakes in the two datasets is not clearly highlighted in the article, despite continuous comparisons between "CCC" vs. "non-CCC."

Geomorphological Differences: The two categories of lakes also appear to differ significantly in their geomorphology (e.g., some "non-CCC" lakes are very small in area (0.7 km²) but very deep, a characteristic not observed in "CCC" lakes). A potential way to mitigate this issue might be to remove all "non-CCC" lakes smaller than 0.7 km² from the comparative analyses.

Appendix

The Appendix is a very comprehensive and useful document. Perhaps Table S1 could also include the average methane values. Given that the text (Line 95) mentions a methane concentration as high as 95 μmol L⁻¹. One of the highest I have ever read—it would be highly interesting to know which specific lake this measurement corresponds to.

Reviewer #4

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

Dear authors,

Thanks you for addressing my questions and making modifications to the manuscript. In the attached PDF I provide additional comments.

I find your work of high significance to the community by providing methane fluxes and MOX rates from African lakes. Nevertheless I find your discussion on the MOX vs OMP not justified. The data provided for OMP comes exclusively from 13C incubations from 5 lakes out of the 79 you studied. These data covers one single mechanism. For all others, specifically demethylation of MPn and methylamines you cannot provide any data. Methylamines (Wang et al. 2021 and Bizic et al., 2018) are still ignored. N₂ fixation by iron-only nitrogenases is mentioned, but likely less significant. You develop a discussion on the abundance of phosphonates in lakes based on one study of one lake. This lake is a temperate northern hemisphere lake, such as those that you put great effort to dismiss in your rebuttal. In fact, the paper you bring shows a 20% contribution of phosphonates to total P which at peak make a relatively high P concentration. You then dismiss methylphosphonate abundance citing a marine paper. That paper (Repeta et al) in fact claims that a small fraction of these methylphosphonates can account for the entire oceanic methane flux. The sad truth is that there is no sufficient quantitative data on methylphosphonate in aquatic bodies nor on their contribution to the methane fluxes hence all quantification attempts are speculative at best.

There are other aspects that make your OMP discussion speculative. See this in the attached PDF.

I therefore suggest to highlight the attenuation effect of MOX to the total flux and leave the question of the methane source (sediment diffusion, ebullition, or OMP) to future studies targeting these exact question.

In your rebuttal you explained why the morphology vs OMP contribution curve is not to be used. My intention was not for you to use this curve as a predictor, but rather to evaluate where your own data falls on this curve. However, as your OMP data is not complete, I agree that this option should not be further explored.

You have also wondered how the "regression line" can be biased. My intention was that the evidence of correlation is biased (since you only show regression lines in case of significant correlation). If one combined two data sets one correlated and another not correlated to the parameter in question and the correlated one has significantly more data points than the other, one can still obtain a lower R (as is the case here) and a significant correlation, without the entire set being truly correlated. I believe this statistical phenomenon is called Simpson's paradox or the Yule-Simpson effect.

Overall, I am very much in support of publishing the MOX data, while leaving the OMP discussion to a point where matching data can be provided.

Reviewer #2

(Remarks to the Author)

The Manuscript significantly improved in clarity and logical coherence. The authors critically revised reviewers' comments and produced a constructive rebuttal by incorporating many of the proposed changes to the Main Text. The Initial objectives are closer to the Discussion and to the research achievements. The Conclusion now effectively highlights the most relevant contributions. A section dedicated to Uncertainties addresses most of the concerns raised by reviewers.

At this point, only Minor errors have been noted and a few "near-repetitions". Language and Manuscript structure are coherent with scientific style and format.

The relative stability of African lakes (no clear seasons, longterm persistence of dominant phytoplankton assemblages) is a clear advantage in studying processes such as OMP and MOX and this could be stated more explicitly, and it could give a reason for the lakes chosen in this research.

The last sentence in the Abstract, containing a "double-would" statement reads rather strange and it could be worth rewriting it more affirmatively by stating something like: "Available evidence suggests that OMP increase supported by eutrophication can be compensated by a stronger increase in MOX."

Error in Line 97, where "and" instead of "but" should be checked.

Lines 178-180, Lines 192-195 and then 235-238 again insist on the important contribution represented by nearby flooded soils. The argument makes sense, however the formatting reads a bit like a repetition (especially 178-180 and 192-195); perhaps rewriting this text more effectively could avoid such an impression.

Positive correlation between K and Chl"a" (Fig. 2) works very differently for the two lake types and this should be more carefully reflected in the wording within the Main Text, to avoid the impression that this could be interpreted as a major tendency for all lakes.

Error in Line 526, where NO3 is reported twice.

Error in Line 531 ", and a350"???

Error in Line 566 "The abundance ...was" instead of "were"

Please provide a full reference citation for CHEMTAX.

Reviewer #3

(Remarks to the Author)

All relevant details are provided in the attached document

Reviewer #4

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Version 2:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

Dear authors

Thank you for your replies and amendments to the paper. As mentioned in my previous comments, I think your study and results should be published as they add significant information to our understanding of the methane budget, and even more so by focusing on an understudied region on Earth on a large scale.

Nevertheless, I still cannot fully endorse your manuscript at this stage. The manuscript convincingly demonstrates that methane oxidation constitutes a major attenuation pathway for CH₄ emissions in African lakes. However, the title and several summary statements still imply that total aerobic methane production is quantitatively constrained and outweighed by MOX.

This may be the case, but in reality, the quantitative comparison is primarily based on photosynthesis-associated OMP

estimates and literature-derived assumptions regarding additional OMP pathways. Since no direct measurements of MPn concentrations, methylamine-driven OMP, or other non-photosynthetic OMP mechanisms are available for African lakes,

The cited literature demonstrates potential MPn-dependent methane production in lake incubations, primarily under amendment conditions, but does not provide direct measurements of ambient MPn concentrations or in situ MPn-derived OMP rates in African lakes. Therefore, the conclusion that MPn-based OMP cannot materially contribute to total OMP in the studied systems remains inferential rather than empirically constrained.

I recommend revising the title and overarching framing to focus on the robustly supported conclusion: that MOX substantially attenuates CH₄ fluxes from African lakes, independent of the unresolved relative contributions of specific methane production pathways.

About the correlations and regression bias that I mentioned in my previous comments.

I agree that this situation does not correspond to Simpson's paradox in its canonical statistical definition. My comment / concern was broader and relates to the interpretation of pooled regressions across heterogeneous lake groups. Specifically, the significant relationship observed in the pooled CCC + non-CCC dataset may be driven primarily by the much larger non-CCC subset, while the same relationship is absent within CCC lakes. In such cases, the pooled regression can visually and statistically suggest a general relationship across all systems despite substantial subgroup heterogeneity. My point was therefore not the strict occurrence of Simpson's paradox, but rather that aggregation of ecologically distinct groups may obscure effect modification or subgroup-specific dynamics. Given the pronounced differences between CCC and non-CCC lakes in hydrology, phytoplankton composition, CDOM, nutrient regimes, and productivity, I believe the manuscript would benefit from emphasizing subgroup-specific interpretations rather than relying primarily on pooled regressions to support generalized conclusions.

I hope these suggestions will aid in bringing the paper to a final state where it focuses on the strong available data, without extensive extrapolation from non-representative subsets.

Reviewer #2

(Remarks to the Author)

This research assembles an impressive amount of original data and combines them in a comprehensive meta-analysis. It is a milestone and a building block for future research. My former comments were fully addressed. After this last revised version, I see no more issues that could prevent acceptance.

Reviewer #4

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Version 3:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

I thank the authors for making these additional changes.
I have no further comments on the manuscripts.

Reviewer #4

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

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Manuscript#: NCOMMS-25-86285A

Title: Methane oxidation in African lakes increases with phytoplankton and outweighs aerobic methane production

Below is my revised review.

In this document, I will not go through all the authors' rebuttals to my questions and concerns in detail. When I do not comment on specific responses, it is because I accept the arguments provided by the authors in their rebuttal.

In this text, I will focus on the following three points:

1. The identification of previous studies that report results similar to those described in this manuscript
2. The use of the Thottathil model to model the methane oxidation rates (MOX_modelled)
3. A final comment regarding dissolved oxygen concentrations: % saturation or mol L⁻¹?

1) At the beginning of my first review, I mentioned that I identified two relevant references, with the assistance of ChatGPT, whose results lead to conclusions very similar to those presented in this manuscript. In their rebuttal, the authors appropriately noted that caution is needed when using AI, as it may misattribute citations. I would therefore like to clarify that AI was used only as a tool to help identify potentially relevant literature. The references themselves were verified after reading the original articles, and I have listed them again below to avoid any possible misunderstanding. These are not AI-generated fantasies, and I believe these studies are sufficiently relevant that they should be cited in the manuscript.

Methane oxidation in the waters of a humic-rich boreal lake stimulated by photosynthesis, nitrite, Fe(III) and humics

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Abstract. Small boreal lakes are known to contribute significantly to global CH₄ emissions. Lake Lovén is a small, clear, low-nutrient forested wetland with low water CH₄ concentrations up to 2 mol. However, the surface water concentrations, and thus the diffusive methane potential, was low (< 0.5 mol). We studied the biogeochemical processes involved in CH₄ removal by chemical profiling and through incubation experiments. ¹³C-CH₄ profiling of the water column revealed a methane oxidation hotspot just below the oxycline and rates of CH₄ oxidation within the anoxic water column. In incubation experiments involving the addition of light and/or oxygen, CH₄ oxidation rates in the anoxic hypolimnion were enhanced 3-fold, suggesting a major role for photochemically formed aerobic CH₄ oxidation. We observed a distinct peak in CH₄ concentrations at the chlorophyll-a maximum, caused by either in situ CH₄ production or other CH₄ inputs such as the lateral transport from the littoral zone. In the dark anoxic water column at 7 m depth, nitrite seemed to be the key electron acceptor involved in CH₄ oxidation, yet addition of Fe(III), ascorbic acid, 2,4-dichlorophenol and humic substances also stimulated aerobic CH₄ oxidation. Surprisingly, nitrite seemed to inhibit CH₄ oxidation at all other depths. Overall, this study shows that photochemically formed CH₄ oxidation can be a key process in CH₄ removal in the water column of boreal lakes.

1 Introduction

Lacustrine water bodies represent a substantial natural source of atmospheric methane (CH₄), a major contributor to global warming. They may release up to ~12 Tg CH₄ a⁻¹ (12% of total global emissions) (Sherman et al., 2011), despite covering a relatively small proportion of the land surface area (~1%). However, in situ CH₄ production and oxidation within the water column are still poorly understood. In temperate and subarctic boreal regions, small lakes generally emit more CH₄ per unit area than larger systems (Chapman et al., 2006; Kunkiewicz et al., 2009, 2010; Michener et al., 2010). Northern lakes often are considered to contribute 24.2 ± 10.7 Tg CH₄ a⁻¹ to global CH₄ emissions (Walter et al., 2007). The majority of lacustrine CH₄ is produced by anaerobic methanogenic archaea in the sediments of reservoirs.

Light-Dependent Aerobic Methane Oxidation Reduces Methane Emissions from Seasonally Stratified Lakes

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Abstract

Lakes are a natural source of methane to the atmosphere and contribute significantly to total emissions compared to the oceans. Controls on methane emissions from lake surfaces, particularly biotic processes within anoxic hypolimnia, are only partially understood. Here we investigated biological methane oxidation in the water column of the seasonally stratified Lake Rotsee. A zone of methane oxidation extending from the oxic/anoxic interface into anoxic waters was identified by chemical profiling of oxygen, methane and δ¹³C of methane. Incubation experiments with ¹³C-methane yielded highest oxidation rates within the oxycline, and comparable rates were measured in anoxic waters. Despite predominantly anoxic conditions within the zone of methane oxidation, known groups of anaerobic methanotrophic archaea were conspicuously absent. Instead, aerobic gamma-proteobacterial methanotrophs were identified as the active methane oxidizers. In addition, continuous oxidation and maximum rates always occurred under light conditions. These findings, along with the detection of chlorophyll a, suggest that aerobic methane oxidation is tightly coupled to light-dependent photosynthetic oxygen production both at the oxycline and in the anoxic bottom layer. It is likely that this interaction between oxygenic photosynthesis and aerobic methanotrophs represents a widespread mechanism by which methane is oxidized in lake water, thus diminishing its release into the atmosphere.

The sentences I highlight at the end of the abstracts of these two articles summarize their main conclusions and emphasize that aerobic methane oxidation is linked to the presence of photosynthetic organisms. This is precisely the main outcome that the present manuscript seeks to highlight, as also clearly emphasized in its title. For this reason, I conclude that this particular result of the manuscript may be not entirely novel.

I would like to emphasize that, in the manuscript under review, the evidence for the presence of methanotrophs and for their association with phytoplankton (or their allochthonous origin) is indirect rather than direct. The article does not provide data demonstrating the presence and abundance of methanotrophs attached to phytoplankton (e.g. FISH, microscopy, or other direct approaches). For this reason, the authors are deliberately cautious in the abstract and use the adverb “*presumably*” (on two occasions) to indicate that the proposed relationship is inferred indirectly—supported by evidence from the literature—but has not been directly demonstrated by the data generated in this study.

From my perspective, this is a very important point, because the manuscript does not demonstrate for the first time the “*quantitative impact of the increase of methanotrophs attached to phytoplankton on MOX rates*” (as stated by the authors in their rebuttal to my comment), simply because it does not provide any direct evidence for an actual *increase of methanotrophs*. This implies that, in Figure 7, the two green arrows linking “*phytoplankton increase*” to “*attached methanotroph increase*” and subsequently to “*MOX increase*” are also derived from the literature. The manuscript directly links “*phytoplankton increase*” (data from this study) with “*MOX increase*” (at the non-CCC sites, based on data from this study) and then indirectly infers that this pattern is due to an increase in “*attached methanotrophs*” (the intermediate compartment in Figure 7), a deduction based on the literature rather than on direct evidence from this study.

On the other hand, based on this new reading of the revised manuscript, I reach the conclusion that the relatively more novel result may be the hypothesis that *allochthonous* methanotrophs could be transported from uplands and wetlands, as inferred for the CCC lakes, also through comparison with the estimated K values in CCC rivers (I am referring mainly to Figure 4). Naturally, there are studies that propose and analyze soil–water connectivity specifically in the context of methanotrophs, and these are cited in the manuscript (and other works also exist in the literature, e.g. <https://doi.org/10.1111/mec.15223>; <https://doi.org/10.1016/j.ecolind.2005.05.002>). From my point of view, this is an interesting result. However, the authors may have chosen not to explore this topic in greater depth. Indeed, they note that a manuscript currently under review likely focuses specifically on connectivity (e.g. *Borges, A. V. et al. Methane oxidation in rivers depends on stream size and wetland connectivity. Sci. Adv., submitted (2026)*).

Returning to the studies by Oswald and van Grinsven, the authors of the manuscript state in their rebuttal that these two articles focus on “low O₂ environments” and “at the bottom of the oxycline,” whereas in their study the oxygen concentrations during the incubations could not have inhibited aerobic methane oxidation (see Fig. S21). Indeed, the studies by Oswald and van Grinsven focus on the oxycline (and not only at its lower boundary), where, according to the figures presented in those works, oxygen concentrations are sufficiently high to allow methane oxidation while methane is still present. At higher oxygen concentrations, methane (almost) disappears, and measuring MOX under such conditions is likely methodologically very challenging or simply not particularly meaningful.

In any case, in the manuscript under review, oxygen plays only a relatively minor role. Indeed in the main text, it is mentioned only sporadically, and dissolved O₂ data appear only in two supplementary figures, which serve to demonstrate that (a) anoxic conditions were not generated during the incubations (Fig. S21) and (b) the K:[Chl-a] ratio decreases at high O₂ concentrations (Fig. S13c). The latter result is, in fact,

consistent with the findings reported by Oswald and van Grinsven. For this reason, I do not fully understand why the authors emphasize this aspect to differentiate their study from those of Oswald and van Grinsven, when oxygen does not appear to be a central factor in their investigation.

On the other hand, I fully understand why the role of oxygen cannot be emphasized too strongly, as this is a methodologically delicate issue when considering microbial processes arising from associations between primary producers and aerobic methanotrophic bacteria. Such associations are very likely to generate chemical gradients—including oxygen gradients—at micrometer scales, which cannot be resolved with a field optical oxygen sensor operating at spatial scales on the order of several centimeters.

2) Thottathil model for the calculation of theoretical MOX

The authors compare their measured MOX rates with MOX values estimated using the model of Thottathil et al. (2019). This is an empirical model derived from a series of incubations performed on samples collected from six small and shallow lakes, characterized by low chlorophyll-a concentrations ($< 2.9 \mu\text{g L}^{-1}$) and water temperatures below 25°C .

What justifies the assumption that a model developed under these conditions can be used to simulate MOX in lakes (more specifically non-CCC lakes) with chlorophyll-a concentrations typically exceed $10 \mu\text{g L}^{-1}$ and water temperatures that, in many cases, exceed 25°C ? Figure 3 in the manuscript is particularly instructive in this regard: the divergence between measured MOX and MOX_modelled becomes evident above approximately $3 \mu\text{g L}^{-1}$ Chl-a, precisely where the Thottathil et al. (2019) model likely ceases to be valid and, consequently, ceases to be an appropriate reference. An empirical model should not be applied outside the range of conditions for which it was developed (see: Box, G. E. P., & Draper, N. R. (1987). *Empirical model-building and response surfaces*. John Wiley & Sons.). Therefore, in my view, the Thottathil model cannot be used to argue that chlorophyll-a needs to be included in predictive MOX models. In this sense, the exercise is uninformative.

This exercise might become more informative if the authors were to consider implementing alternative models. The model proposed by Tan et al. (2024) (ALBM; <https://agupubs.onlinelibrary.wiley.com/doi/pdf/10.1029/2024MS004275>) could represent a useful starting point. It would need to be examined carefully, as it appears to adopt the Thottathil approach for MOX. However, chlorophyll-a is included in this model. Notably, in ALBM model, chlorophyll-a is not introduced to promote the presence of aerobic methanotrophs (and thus methanotrophy); rather, it is used to stimulate aerobic methane production. It is precisely this opposite conceptual framework that makes the ALBM approach interesting. Comparing the MOX values calculated in the present manuscript with those predicted by such a model could help clarify the role of chlorophyll-a—whether as a vector facilitating the transport of aerobic methanotrophs, or as a driver enhancing aerobic methanogenesis. This is simply a suggestion, but one that may be worth further consideration.

Another interesting contribution is the recent paper that includes tropical water masses and focus on temperature: Pookkattu, A., Patel, L., Singh, R., Gowd, S. C., & Thottathil, S. D. (2025). Temperature Dependence of Methane Oxidation in Lakes: Global Trends and High-Temperature Inhibition in the Tropics. *ACS ES&T Water*, 6(1), 477-484.

3) Dissolved oxygen concentrations: % saturation or mol L^{-1} ?

This is a secondary issue and does not have major implications. However, I would like to respond to the authors' rebuttal. My original comment referred to the use of dissolved O₂ concentrations expressed in $\mu\text{mol L}^{-1}$ rather than percent saturation (%Sat) in Figure S1b when comparing CCC and non-CCC systems. The authors justify their choice of using concentrations with an argument that is entirely valid and irreproachable from a bioenergetic and microbial metabolism perspective (including aerobic methanotrophy).

That said, it is worth noting that this is not a study of microbial ecology—there are no data on methanotroph abundance or diversity—but rather a regional-scale biogeochemical study. Supplementary Figure S1 compares the biogeochemical characteristics of two groups of systems without direct reference to underlying ecological microbial/macrobial processes. From this perspective, I believe that, at least for gases, expressing concentrations as percent saturation would be more appropriate than using absolute concentrations (and, this panel could also include CH₄ percent saturation). If the bioenergetic–microbial perspective were the primary focus, then it would also be necessary to note that the dataset includes a slightly saline system (approximately 7–8 mS cm⁻¹), for which solute—and gas—activities, rather than concentrations, should technically be considered.

A last comment: Figure S1 I could also include temperature.

Reviewer #1

This study by Borges and colleagues investigated methane oxidation (MOX) patterns across 81 African lakes spanning all trophic states from oligotrophic to hypereutrophic conditions, testing drivers like trophic state and nutrient limitation. The researchers quantified MOX rates alongside CH₄ concentrations, chlorophyll-a (Chl-a), nutrients, and phytoplankton composition, comparing CCC (Cuvette Centrale Congolaise) vs. non-CCC lakes.

MOX rates increased with CH₄ and Chl-a, peaking in productive lakes due to (presumably) methanotrophs attached to phytoplankton, and in CCC lakes from wetland soil inputs. MOX dominated CH₄ removal (>70% median) over diffusive emissions, with phosphate limitation evident. Oxidic methane production (OMP), estimated from literature Chl-a relationships, contributed minimally (<12%) declining in high-CH₄ systems sustained by sedimentary sources.

The authors conclude that eutrophication boosts MOX more than it boost OMP/sedimentary production, attenuating diffusive CH₄ emissions despite rising phytoplankton biomass.

Overall the provided information that MOX, presumably attached to phytoplankton attenuates a significant portion of the methane produced in lakes is important and rather reassuring, I have made specific comments in the attached annotated PDF files of the manuscript and the supplementary material. Hence the text here addresses general issues

General assessment

The paper provides a broad scale assessment of methane concentration in a large number of African lakes and thus provides a significant contribution to our knowledge on methane emissions from lakes worldwide. The authors also provide information on controls of methane emissions from African lakes at different trophic states. This is also very important for bottom-up modelling efforts assessing the contribution of freshwater lakes to the global methane budget.

Reply(1): We thank the reviewer for their positive and constructive evaluation of our submission and for rightly pointing out an error in unit conversions in the initial submission. We noticed that 2 lakes were counted twice in Table S1 and Figure 1 of the original submission, so in fact there are 79 lakes and not 81, but the total number of MOX measurements remains unchanged. We copy-pasted into the present document the reviewer's comments embedded in the pdfs of manuscript and supplemental material to which we provide a point-by-point response here-after.

In their work, the authors also try to assess the contribution of methane produced in oxic environments (OMP) and specifically that produced as a byproduct of photosynthesis, to the total methane flux. Several aspects make this part of the paper less convincing. I generally have the feeling that the authors confuse OMP with photosynthesis associated methane production. The latter is part of OMP with varying significance that was not determined in this paper

Reply(2): The reviewer is right that there are OMP pathways independent from photosynthesis. We have now included a new section « Uncertainty on MOX and OMP estimates » where we discuss these other possible OMP pathways such as bacterial utilization of methylphosphonate (MnP), photoproduction, or N₂-fixation (also

mentioned in the Introduction). Based on available literature we made quantitative comparisons between these OMP pathways and the median of MOX measurements in our data-set. We concluded that based on these best-possible estimates, OMP supported by bacterial utilization of MnP, photoproduction, or N₂-fixation is likely to be also at least one order of magnitude lower than MOX rates in lakes.

First, the authors did not experimentally determine general OMP derived from multiple processes. The, OMP rates taken from literature for 3 out of the 81 lakes are driven from 13CO₃ uptake in the light – i.e photosynthesis and not the complete OMP (ref 19 Morana et al...Borges). Additionally, do we know if these rates measured by Morana and colleagues reflect OMP rate all year-round? Also here (like below) some number conversions seem to be wrong. Morana et al also report a 4th lake Karinda, why was this lake not included in the OMPcom?

Reply(3): The reviewer is right we did not determine experimentally OMP. The main topic/objective of the paper is in fact MOX, and most of the paper is actually devoted to discussing the drivers of MOX variability among lakes. We tried to contextualize MOX with other fluxes of CH₄ in surface waters of lakes, namely diffusive CH₄ emissions and OMP. While the comparison of MOX with OMP might be controversial, it is just one of several points of discussion of the paper, but not the central topic of the paper.

The rates of OMP reported by Morana et al. (2020) are “grab measurements” during a few sampling cruises. However, photosynthesis in tropical lakes is seasonally much less variable than in northern lakes (<https://doi.org/10.1007/BF00346388>) due to the “endless summer” in the tropics (<https://doi.org/10.1146/annurev.es.18.110187.001111>).

We initially used the OMP data in the 3 lakes where all the CH₄ fluxes at ecosystem scale were determined (Fig. 6 of Morana et al. 2020), but the reviewer is right that OMP was measured in two additional lakes (Katinda and Kyamwinda). We now use data from the five lakes where OMP was determined (George, Nyamusingere, Edward, Katinda, Kyamwinda). The resulting OMPcom are very marginally lower than those from the initial publication, and do not change our initial conclusions.

There are at least two studies by now, the latest I know of is: <https://pubs.acs.org/doi/abs/10.1021/acs.est.2c01730> that reveal a morphometric relationship between lakes and contribution of OMP to the methane flux. How do these lakes fall on that curve? Or, can this curve be used to model the contribution of OMP to the observed flux? Looking at table S1 there are lakes like lake Besako with a large area and are very shallow but also lakes like Kyamwinda or Katinda which are deeper and with a smaller surface area. The contribution of OMP in these lakes to the total CH₄ flux is expected to be different.

Reply(4): We are aware of the publications that reported OMP relationships with morphometric lake variables such as the one cited by reviewer. However, we preferred to estimate OMP from relations to Chl-a rather than applying OMP-morphometry relations for the following reasons:

- the OMP-morphometry relationships referred to by the reviewer are based on data in northern lakes and do not include data in tropical lakes. Emissions of CH₄ in tropical lakes are different from northern lakes (refer to Figure 7 in <https://doi.org/10.1126/sciadv.abi8716>) due to differences in temperature and

productivity. Primary production in tropical lakes is higher than in northern lakes (<https://doi.org/10.1007/BF00346388>), and OMP is strongly dependent on photosynthesis. It is then very plausible that the OMP morphometric relations established in northern lakes do not apply to tropical lakes.

- the OMP-morphometry relations would have provided one single value per lake that would have been uneasy to compare to MOX measurements that are variable in time and space within each lake, and for which we do not have robust upscaled estimates. For instance, in Lake Albert, MOX ranged over 4 orders of magnitude from 0.1 to 3,907 nmol L⁻¹ d⁻¹ along gradients of Chl-a and CH₄ concentration. Our approach allows to compare OMP and MOX for each individual measurement of MOX since OMP is computed from Chl-a that was determined for each MOX measurement. Since MOX itself seems to be in part dependent on Chl-a, this approach seems much more appropriate than looking at whole lake OMP estimates derived from morphometry relations.

- the OMP-morphometry relations are based OMP computed as the closing term of a CH₄ mass balance budget at the scale of the whole lake. It has been argued that the OMP values obtained in this way have been over-estimated because some terms of the mass balance were incorrectly estimated (<https://doi.org/10.1038/s41467-021-21215-2>). For instance, loss terms of the mass balance were over-estimated and input terms were under-estimated (<https://doi.org/10.1038/s41467-021-21215-2>) or some input terms were not accounted for (<https://doi.org/10.1002/lno.12652>). This seems to align with the substantial downward revision of whole lake OMP estimates in a very

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Second, while estimating the potential of phytoplankton to produce methane based on published data, it seems there is a mistake that underestimates these rates by 4 orders of magnitude (see calculations under specific comments). Even if these rates derived from cultures are an overestimate when it comes to environmental conditions, they could easily account for the mentioned “OMP” rates.

Overall the author's conclusion could be that Photosynthesis associated methane production makes up ca. 12 % of the methane flux with the general OMP likely more. In addition they show the significance of MOX in attenuating the CH₄ flux, specifically OMP and phytoplankton derived.

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Reply(5): The reviewer is right that there was an unfortunate error in conversion of the original units of the rates from the publications on phytoplankton cultures. We are very grateful to the reviewer for pointing this out. The recomputed OMP estimates are now obviously higher than in the previous submission, yet, the contribution of OMP_{phyto} to the total loss terms of dissolved CH₄ in the mixed layer (sum of diffusive emission and MOX) remains small (median = 4% and average = 17%). Our initial conclusion that MOX is much larger than OMP remains unchanged.

As stated in my specific comments the authors made what appears to be some arbitrary decision that are not so transparent to the reader. These can be numerical values like the 6.2 m MLB or 18 µg Chl-a. Why not 7 m or 20 µg.

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Reply(6): We certainly did not intend to lack transparency with readers. The threshold values are not arbitrary but correspond to the maximal values for those quantities in the CCC lakes. This then allows us to compare CCC and non-CCC lakes within ranges of Chl-a or MLD that are equivalent. If this was not the case, the comparison would have been biased by the higher MLD or Chl-a values in numerous non-CCC lakes than in CCC lakes. We realize that this was not self-evident from the figures (as we initially anticipated), so this is now explained in text.

There are also some comparisons of African lakes and rivers with European rivers, why not also European Lakes and why European and not Global? It feels like the authors chose values and data that would support their ideas.

Reply(7): We limited the comparison to our own data in rivers from a companion submitted paper, not for arbitrary reasons, because there are only a few papers on MOX in rivers and it was not possible to integrate these data in the comparison due to methodological differences and lack of appropriate auxiliary data (TSM, Chl-a, POC). One study (<https://doi.org/10.1111/gcb.13169>) is based on $\delta^{13}\text{C}\text{-CH}_4$ and this approach provides estimates that are not strictly comparable to those derived from incubations, as discussed in new section « Uncertainty on MOX and OMP estimates ». Another study (<https://doi.org/10.1016/j.watres.2024.122257>) is based on incubations but the auxiliary data (TSM, POC, Chl-a) were not acquired, so these data cannot be integrated in the comparison in Figure 4.

Regarding lakes, there are some papers in northern lakes, but data are not available in public repositories, so this would have required to scan data from the figures of papers, which is uneasy and not very precise as MOX spans several orders of magnitude. The aim of Figure 4 is not to provide an exhaustive meta-analysis of MOX in lakes or rivers but to see to which extent drivers of K we found in lakes are also relevant (or not) in rivers.

Nevertheless, we have incorporated comparisons of MOX in African lakes with Québec lakes using the model of the UQUAM group (<https://doi.org/10.1007/s10533-019-00552-x>). This comparison (Figure 3) is an interesting addition and re-enforces the idea of the importance of MOX enhancement by methanotrophs attached to phytoplankton.

Text now reads L169: “Additional evidence of the importance of phytoplankton biomass in enhancing MOX and K is provided by the comparison of measured MOX in African lakes with MOX modelled ($\text{MOX}_{\text{modelled}}$) using a parameterization developed from northern lake measurements (6), which uses dissolved concentrations of O_2 and CH_4 as drivers. Median MOX in CCC lakes was 4-fold higher than $\text{MOX}_{\text{modelled}}$, and their ratio ($\text{MOX}:\text{MOX}_{\text{modelled}}$) increased with Chl-a (Fig. 3). This pattern likely reflects the low Chl-a values ($<3 \mu\text{g L}^{-1}$) in surface waters of the northern lakes used to develop the model (6). Adding a multiplicative factor proportional to Chl-a to the original model substantially improved model performance (Supplemental Fig. S7).”

And L270: “In northern lakes, $\%\text{MOX}_{\text{removal}}$ was on average 40% (5), lower than the mean in African lakes (74%), possibly due to lower phytoplankton biomass ($\text{Chl-a} < 3.0 \mu\text{g L}^{-1}$)”

I also wonder, as the MOB are thought to be attached to phytoplankton, is the increase in MOX an effect of more MOB attached to the increased phytoplankton biomass rather than an increase in MOX activity?

Reply(8): The activity of methanotrophs in oxic conditions is mainly related to CH₄ availability and *K* is independent from CH₄ availability, since by definition it is normalized by CH₄ concentration. So, *K* should mainly be a function of the abundance of methanotrophs. Available studies comparing micro-organism abundance to MOX measurements suggest a relationship between abundance of methanotrophs and *K* (<https://doi.org/10.3389/fenvs.2022.833688>; <https://doi.org/10.3389/fmicb.2026.1789101>). We show that *K* increases with Chl-a and this is likely to be related to an increase of methanotroph abundance attached to phytoplankton, as it has been established for at least 5 decades that the majority of the abundance/activity is from attached microbes rather than free living microbes (<https://doi.org/10.1111/j.1365-2672.1977.tb00766.x>). It is difficult to imagine a situation where for a high and low Chl-a sample the methanotroph abundance would be identical but that the activity would be higher in the former and lower in the latter resulting in high and low MOX rates, respectively.

Last Could characterization of methanotrophic community help elucidate the differences in MOX? Perhaps MOX increases not only a result of an increase in MOX activity or MOB, but also because of the difference in methanotrophic communities present in each areas/lakes.

Reply(9): We fully agree that data on diversity of methanotrophic communities would be a very useful addition, but since we did not sample for these variables we can only advocate for future studies to explore this which would allow to validate some of the hypotheses proposed (L450). Note that it is not straightforward at all to relate micro-organism abundance to MOX rate measurements (<https://doi.org/10.3389/fmicb.2026.1789101>). Some studies have shown that there is a niche partitioning of methane-oxidizing bacteria (types I and II) along the oxygen–methane counter gradient of stratified lakes (<https://doi.org/10.1038/s41396-019-0515-8>). Yet, MOX can be predicted from CH₄ and O₂ concentrations along equivalent oxygen–methane counter gradients in stratified lakes (<https://doi.org/10.1007/s10533-019-00552-x>). This would suggest that “bulk” MOX measurements are not sensitive enough to distinguish the activity of different methanotrophic communities. This would also suggest that “bulk” MOX rates can be predicted from CH₄ and O₂ irrespective of differences in methanotrophic communities, as done in Québec lakes (<https://doi.org/10.1007/s10533-019-00552-x>). Nevertheless, our analysis suggests that for a complete description of MOX the effect of phytoplankton biomass needs to be included - refer to Reply (7).

Hereafter, the reviewer's comments were copy-pasted from the pdfs of the manuscript and supplementals, noted by line number of the initial submission.

L37 : I would say also methane produced in anoxic environments can contribute to the diffusive flux depending on the depth of the oxicline.

Reply(10): We fully agree and we do not see a contradiction here.

Reply: Sedimentary methanogenesis = methane produced in anoxic environments

Reply(11): Text was rephrased accordingly.

L49 : This concept has been repeatably based on a single study.

Even though other papers found evidence hinting that this may be the case, this has not been explicitly studied since. Therefore, I would rehearse this sentence to cite the paper challenging this concept:

<https://onlinelibrary.wiley.com/doi/full/10.1002/edn3.441>

"..but inhibited by NH₄... and light, though the latter concept has been recently challenged"

Reply(12): We have included this reference and changed text as suggested by the reviewer.

L 53 : I suggest separating here between Cyanobacteria, algae, plants and fungi, and to cite for each the relevant sources. The paper cited refers exclusively to Cyanobacteria.

Reply(13): The cited paper (ref 20) reports new data on Cyanobacteria as mentioned by the reviewer but also provides an in-depth literature review of CH₄ production and the compilation of rates by phytoplankton, plants, and fungi. Given that OMP by plants and fungi is outside the scope of our submission, we prefer to limit the reference to the paper cited in our original submission.

L 54 : This is only one of the proposed mechanisms. At least prokaryotes (in this case only the Cyanobacteria) can also demethylate MPN as was shown in more than one case. Other mechanisms like the methylamines demethylation (Wang et al 2020) were not demonstrated yet in phototrophs..

Reply(14): Please refer to Reply(2)

L58 : Indeed, but papers like your ref 11 are moving forward towards establishing the link between lake morphology and the contribution of OMP to the diffusive flux.

Reply(15): Please refer to Reply(4).

L69 : Please state how many lakes belong to the CCC area and how many belong to the non-CCC area and also maybe provide the differentiation between polymictic and meromictic.

Reply(16): This is now included.

L 79 : Later the lakes are separated into 3 groups CCC, non-CCC polymictic and non-CCC meromictic - why is this separation not done in Fig. S1. It makes sense to have the same grouping for comparison.

Reply(17): We initially found that some details in the patterns of dissolved CH₄ concentration between polymictic and meromictic were interesting. However, this difference of mixing regime was not relevant for the rest of the paper dealing with MOX, the core topic/objective of the submission. Following the reviewer's comments, we concluded that the difference of mixing regime was a distraction and possibly confusing since this distinction was made for CH₄ concentration but not MOX. Consequently, we have removed this aspect of the paper. The most important information on distribution of dissolved CH₄ concentrations remain unaffected: 1/ the difference between CCC and non-CCC lakes; 2/ CH₄ concentration in CCC lakes scales with surface area and depth.

L 82 : Later the lakes are separated into 3 groups CCC, non-CCC polymictic and non-CCC meromictic - why is this separation not done in Fig. S1. It makes sense to have the same grouping for comparison.

Reply(18): Please refer to Reply(17).

L87 : If they dominated one lake - were these a secondary group? Consider rephrasing for clarity

Reply(19): Text was rephrased for clarity.

L98 : The correlation with Chl-a was shown also elsewhere - worth mentioning. e.g. <https://www.nature.com/articles/s41467-019-09100-5>

Reply(20): Yes, and this paper was cited in the Introduction, but we agree it is worth including this reference again here, as suggested by the reviewer.

L 105 : This seems like an arbitrary number. See my comment for line 119.

If there is a clear reason for this value it should be clearly stated.

Reply(21): Please refer to Reply(6).

L108: This seems like an arbitrary number. See my comment for line 119. If there is a clear reason for this value it should be clearly stated.

Reply(22): Please refer to Reply(6).

L 112 ...the concentrations of dissolved CH₄ in surface water...

better formulation

Reply(23): Text was removed, the difference between meromictic and polymictic lakes of CH₄ concentrations is no longer discussed – see Reply(17) for more details.

L114 : i.e. methane accumulates in the hypolimnion, diffuses into the epilimnion and results in higher surface concentration?

Some depth profiles would be great to support this. The profiles I know, specifically coming from anoxic hypolimnia, show a decrease at the oxic anoxic interphase, followed by an OMP peak or nothing in case OMP is minimal or absent.

Reply(24): The difference between meromictic and polymictic lakes of CH₄ concentrations is no longer discussed – see Reply(17).

L115 : Please state how many lakes are in each category, especially since below the impression is given that the second group consist only of 3 lakes.

Reply(25): The difference between meromictic and polymictic lakes of CH₄ concentrations is no longer discussed – see Reply(17).

L117 : May I ask why?

Reply(26): The difference between meromictic and polymictic lakes of CH₄ concentrations is no longer discussed – see Reply(17).

L119 : This is in agreement with my comment for line 114. Does that idea still hold when comparing polymictic and meromictic lakes with greater MLD e.g. greater than the 6.2 meter chosen in Fig S2.

Reply(27): The difference between meromictic and polymictic lakes of CH₄ concentrations is no longer discussed – see Reply(17).

L 136: See my comment on Fig. 2 regarding the K - Chl-a correlation. What is stated here is correct - i.e the correlation is true for lakes outside CCC. However in the figure there is also a black correlation line which refers to "all lakes", that line is likely biased.

Reply(28): We do not understand what the reviewer means by a regression model line being "biased". The regression model is either statistically significant or not. The interpretation of a regression model can be open to discussion. However, the differences of K vs Chla between CCC and non-CCC lakes are clearly mentioned and discussed in text. We do not see a problem here.

L146: How was this value chosen?

Reply(29): Please refer to Reply(6).

L156: Could phytoplankton aggregation state play a role? For example solitary unicellular vs. microcystis like colonies? The latter allowing more MOB to attach?

Reply(30): We have added information on the occurrence of colonial and filamentous cyanobacteria (Table S2). We do not have data on colony counts, only presence/absence, so we cannot address quantitatively an enhancement of MOX by colonies. But colonies were always very abundant or dominant in high Chl-a samples. The role of aggregation of phytoplankton on the attachment of micro-organisms is complex and there is not necessarily a direct simple relationship. When normalized by Chl-a there should be more attached micro-organisms on single-cells as the surface to volume ratio is higher than in colonies. However, if colonies develop mucilage (which is not systematic) this strongly enhances the attachment of microorganisms. Mucilage production depends on a variety of processes such as nutrient limitation, light regime, and physical mixing. (e.g. <https://doi.org/10.1016/j.scitotenv.2005.09.026>). Finally, there could be cycles of sinking, deposition on the surface of sediment, and resuspension back to the water column of colonies that should "inoculate" micro-organisms from the surface of sediment on the surface of colonies. In summary, all we can say is that colonies were present in all non-CCC samples examined under the microscope (new Table S2), and it is plausible that this contributed to enhancing the abundance of attached methanotrophs.

L181 : Nyerges et al (your ref 22) for example claims that the inhibition is dose dependent. Also these authors and citing others, show that the NH₄ /NO₂ result in shifts in methanotrophic communities and not necessarily inhibition.

Hence the lack of effect of NH₄ to some extent falls in line with other studies.

One line below you state that the concentrations were below those shown to result in inhibition. Since the inhibition by NH₄, if occurs, is competitive, the inhibitory values are likely not absolute, but rather related to the concentration of CH₄. This would be higher in sediments and lower in the overlaying (oxic) waters.

Reply(31): We agree with the reviewer. Most of the information on inhibition of MOX by NH₄⁺ is related to research on soils or sediments where NH₄⁺ concentrations are typically much higher than the concentrations of NH₄⁺ encountered in the mixed layer of lakes. Nevertheless, given that NH₄⁺ was different in CCC and non-CCC lakes, we

tested the effect of NH_4^+ on K , and it was not significant. We think this information was relevant enough to be mentioned, and hence would prefer to keep this.

L190 : Why not global rivers, as best as data is available?

I understand the comparison of African lakes to African rivers, but if unrelated water bodies are brought as European Rivers then why were the lakes not compared to European or global lakes?

This question is relevant for all similar comparisons throughout the paper.

Reply(32): please refer to Reply(7).

L197 : In Fig. S10c, K from African CCC rivers is shown significantly higher as oli, meso and eutrophic lakes but not compared to hypereutrophic lakes. In the text, it is mentioned that K from African CCC rivers is higher than in oligo- to hypereutrophic lakes.

Reply(33): The reviewer is right; text was changed accordingly.

L227 : Citation of the sources? Are these species similar to those in the African lakes? As far as I know - data for photosynthesis driven phytoplankton derived OMP is scarce and there is no information on the effects of light, temp, nutrient concentration on these rates.

Reply(34): We have added the references. Given that in inland waters there are 20,000 species of diatoms and 2,000 species of cyanobacteria, it would have been unlikely to have literature data on OMP rates available for all of the species observed in the field. Yet, our high Chl-a samples were dominated by *Microcystis*, and *Navicula* was a genus observed among diatoms, for which OMP rates have been reported (Table S3). This information was added in the legend of Table S3.

We agree that OMP rates derived in cultures are unlikely to represent OMP in field conditions due to the influence of other factors, which explains why $\text{OMP}_{\text{phyto}}$ rates were higher than OMP_{com} rates, yet, $\text{OMP}_{\text{phyto}}$ was still low compared to MOX and diffusive CH_4 emissions.

Following a similar comment from another reviewer, we use the OMP_{com} estimates as nominal, and the $\text{OMP}_{\text{phyto}}$ estimates as an upper bound estimate in the new section "Uncertainty on MOX and OMP estimates"

L 229: The values shown for OMP_{com} taken from Ref 19 seem only to include $^{13}\text{CO}_3$ uptake i.e. photosynthesis. These values do not account for the entire community demethylation-based OMP mechanism

Reply(35): We agree, please refer to Reply(2).

L230 : Looking at table S2 there seems to be an error underestimating $\text{OMP}_{\text{phyto}}$ by 4 orders of magnitude. Please check.

If his is correct, and OMP_{com} is also correct, than $\text{OMP}_{\text{phyto}}$ is 10-50 times larger.

For example: for *Anabaena* PCC 7120 a rate of $0.75 \mu\text{molCH}_4 \text{gdw}^{-1} \text{h}^{-1}$ is equivalent to $1.8 \text{nmolCH}_4 \mu\text{gChl-a}^{-1} \text{d}^{-1}$ under the $\text{gDw} \rightarrow \text{CHla}$ conversion rates used in S2.

this is in great contrast to $0.0001790 \text{nmolCH}_4 \mu\text{gChl-a}^{-1} \text{d}^{-1}$ proposed in table S2 and 45 time larger than the OMP_{comp} of Lake Edward (for example).

Reply(36): The reviewer is right; please refer to Reply(5)

L238 : See comment above - if the calculations are incorrect this changes this paragraph.

Reply(37): The reviewer is right; please refer to Reply(5)

L256 : Refers to Fig. S4, but in Supplement there are 2 Fig. S3 and no S4.

Reply(38): The reviewer is right; figures were re-numbered.

L282 : Given the suggested error in OMPphyto calculation - MOX may be an even greater sink than estimated. Especially if attached to phytoplankton and directly consuming the methane.

Reply(39): Please refer to Reply(5).

It would have been great to assess the MOX rates using isotopes and keeping the samples in the light allowing the activity of the phytoplankton to which the MOB are attached to remain active (produce O₂).

Reply(40): This is indeed a good suggestion but would have been a whole different paper based on measurements we did not make. If samples are exposed to light, we would have expected MOX and photosynthesis driven OMP to co-occur, making the interpretation complex. Furthermore, while there is some (but very limited) information on the isotopic fractionation of CH₄ from OMP by phytoplankton, the isotopic fractionation is unknown for OMP related to MnP or to photoproduction, so the modelling based on CH₄ isotopes would have large uncertainties.

L287 : This should likely be S9

Reply(41): The reviewer is right, figures were re-numbered.

L387 : How were these decided? Were the bottles still oxic in the end. Methanogenesis (classical and MPN-Based OMP may have still be present), hence these represent gross MOX rates.

Reply(42): We decided the time intervals based on Secchi disk depth measured just before sampling for MOX. Secchi disk depth in the sampled lakes depends mostly on phytoplankton (low CDOM). We noticed early on during data collection that MOX was much faster in samples with high chlorophyll-a, so adapted the sampling intervals accordingly.

We compiled a large dataset of respiration measurements in surface waters based on incubations of O₂ decrease (n=188) during the same cruises where we acquired MOX measurements, although not systematically at each MOX measurement. We have then modelled respiration based on Chl-a and DOC relations (<https://doi.org/10.1093/acprof:oso/9780198527084.003.0007>), and estimated the decrease of O₂ during the MOX measurements based on the initial O₂ concentration and the duration of the MOX incubation. The results show that conditions remained oxic and O₂ concentrations well above the threshold of 5 μM, when MOX becomes limited by O₂ and declines (<https://doi.org/10.1139/m97-133>). Please refer to Figure S21 and text reads L315: "Conditions remained oxic throughout all MOX incubations, and O₂ concentrations at the end of the incubations were above the threshold value (5 μmol L⁻¹) below which MOX inhibition occurs (15) (Supplemental Fig. S21)."

The MnP based OMP is discussed in new section « Uncertainty on MOX and OMP estimates ». Reported rates of MnP-OMP (<https://doi.org/10.1128/aem.01097-23>) are 160-fold lower than the median of our MOX measurements. Furthermore, MnP-OMP only occurs in extreme PO_4^{3-} depletion at concentrations $<0.09 \mu\text{mol L}^{-1}$ (<https://doi.org/10.1002/loi2.70095>). So it is unlikely that MnP based OMP interfered with the measurements of MOX during the incubations.

L329: surface waters

Reply(43): Typo was corrected.

L362 : What is the basis for this low delta in temperature? Can citations be provided to show that 0.2°C is indeed the lower limit for thermal stratification?

Reply(44): Text now reads L544: “Thermal stratification was defined as a temperature difference $\geq 0.2^\circ\text{C}$ between surface and bottom waters. Although no universal definition of lake stratification exists, thresholds based on differences or gradients of temperature or density are commonly used (65). In this study, differences in dissolved O_2 concentration between surface and bottom waters were used as an additional indicator of thermal stratification, reflecting the segregation of biogeochemical processes between surface and bottom layers. In most cases, a difference in dissolved O_2 concentration $>50 \mu\text{mol L}^{-1}$ coincided with a temperature difference $\geq 0.2^\circ\text{C}$ between surface and bottom waters; this value was therefore adopted as the stratification threshold. The Inter-Sectoral Impact Model Intercomparison Project lake sector protocol defines stratified conditions globally based on a density difference of 0.1 kg m^{-3} between surface and bottom waters (66), corresponding to a temperature difference of approximately 0.4°C at 25°C . This threshold is higher than the one used in our study, reflecting the weaker vertical density gradients typically observed in tropical lakes compared with higher-latitude systems (67).”

Figure 2 : The K value from the CCC lakes is not likely to be correlated to chlA as it forms an undefined cloud. Accordingly the overall linear correlation (black line) is driven by the non-CCC lakes with a (much) lower R (R^2). I would therefore refrain to interpret it as a general rule across lakes.

Reply(45): The fact that K is independent from Chl-a in CCC lakes is clearly mentioned in text and the variations of K are attributed to inputs of soils and not to the attachment of methanotrophs to phytoplankton. Nevertheless, the linear regression model is statistically significant. There is no valid reason to hide this information from readers. However, nowhere in text do we mention such a “general rule across lakes”. The K -Chla relationship is only discussed for non-CCC lakes.

Figure S1 It is not possible to see the mean values. Maybe plot this value with a different (and more visible) color?

Reply(46): We have changed the transparency of the symbols, so the cross of the mean is visible.

Figure S3 This is a second Fig. S3 and there is no S4

Reply(47): Figures have been re-numbered.

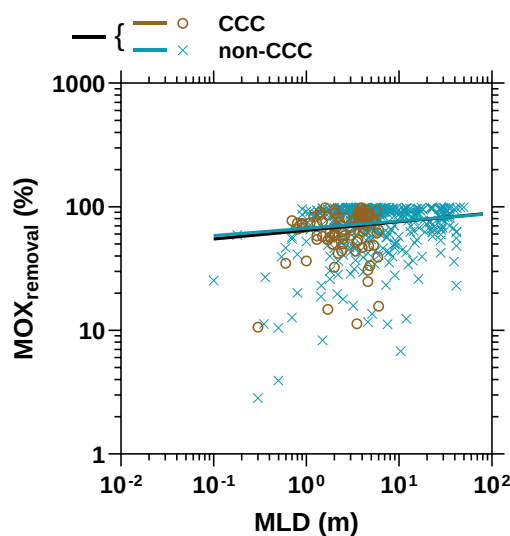
Figure S9b The combined linear regression in plots b and c are likely biased and incorrect. In plot C the combined correlation shows an opposite trend than the non-CCC because of the identical values in all CCC lakes.

Reply(48): Former plot S9c (NO_2^-) was removed following comments from reviewer 2. Regarding plot S9b, the regression model is statistically significant. However, it is not discussed as meaningful in text, on the contrary, we mention that there is no evidence of enhancement/inhibition by NH_4^+ .

d⁻¹

Reply(49): Typo corrected.

Reply(50): The figure shows $\text{MOX}_{\text{removal}}$ on a linear scale while the regression model was calculated on a log-log space. The figure below on log-log scale shows a convergence of non-CCC and CCC data.



d⁻¹

d⁻¹

Reviewer #2

The Manuscript “Methane oxidation in African lakes increases with phytoplankton and outweighs aerobic methane production” addresses a topic that has been hotly debated for >10 years, as can be assessed looking at the long and comprehensive list of papers cited, including ground-breaking publications that appeared in Nature Communications by Beaulieu, DelSontro, & Downing in 2019, Günthel et al. in 2019 and in 2021, and by Focardi et al. in 2025.

Afrotropical inland waters have been characterised as hotspots of methane production and release to the atmosphere. Drivers governing both of these processes are imperfectly understood as they involve a balance between: anoxic (termed “sedimentary”) and oxic methane production, methane oxidation, and methane diffusion into the atmosphere. The authors attempted to quantify these processes and relate them to classical limnological parameters including nutrient concentrations, Chl “a”, oxygen availability, total suspended matter. The research strategy included: 1. a large sampling campaign, with 503 samples in 81 tropical African lakes, 2. controlled measurements carried out in the laboratory on pure phytoplankton cultures, and 3. in situ oxic methane production measurements conducted in lakes Edward, George, and Nyamusingere.

Reply(1): We thank the reviewer for their positive and constructive evaluation of our submission. We noticed that 2 lakes were double counted in Table S1 and Figure 1 of the original submission, so in fact there are 79 lakes and not 81, but the total number of MOX measurements remains unchanged.

At the end of the Introduction the authors explicitly state their objectives: characterise relationships between methane oxidation and limnological parameters and quantify it in relation to methane release to the atmosphere, and to oxic methane production. The H0 being set (“MOX changes with the trophic state of lakes and is limited by nutrients”) does not sound to be particularly ambitious, as it rephrases results obtained in previous research cited in the Manuscript, for example: Nijman et al. 2021 (as well as others).

Reply(2): We strongly disagree with the reviewer’s evaluation of the ambition of our hypothesis. One paper has shown that in controlled conditions - mesocosms (not natural settings) an increase of nutrients leads to the increase in sediments of methanotroph abundance based on qPCR (<https://doi.org/10.1038/s43705-021-00026-y>). The conclusions of a study of eutrophication effects on sediments cannot provide insights on the impact of higher nutrient availability on microbial processes occurring in the water column. So far, no study has investigated variations of MOX in the mixed layer of lakes along gradients of productivity. Available information of MOX in the water column of lakes comes from a few northern lakes with low chlorophyll-a (<3 $\mu\text{g L}^{-1}$). In fact existing models of MOX in the water column of lakes (<https://doi.org/10.1007/s10533-019-00552-x>) only include CH_4 and O_2 as input variables, and we show in the amended version of the manuscript that these models strongly under-estimated MOX in African lakes (Fig. 3).

Text now reads L169: “Additional evidence of the importance of phytoplankton biomass in enhancing MOX and K is provided by the comparison of measured MOX in African lakes with MOX modelled ($\text{MOX}_{\text{modelled}}$) using a parameterization developed from northern lake measurements (6), which uses dissolved concentrations of O_2 and CH_4 as drivers. Median MOX in CCC lakes was 4-fold higher than $\text{MOX}_{\text{modelled}}$, and their ratio ($\text{MOX}:\text{MOX}_{\text{modelled}}$) increased with Chl-a (Fig. 3). This pattern likely reflects the

low Chl-a values ($<3 \mu\text{g L}^{-1}$) in surface waters of the northern lakes used to develop the model (6). Adding a multiplicative factor proportional to Chl-a to the original model substantially improved model performance (Supplemental Fig. S7)."

As a strong point, this research usefully corroborated previous findings indicating the attenuation of diffusive CH_4 emissions via enhanced methane oxidation (MOX), a process that reduced early expectations about contributions of tropical inland waters to the atmospheric methane budget. To do this, the Manuscript assembles a useful comparative database of methane concentration in African inland waters including lakes, wetlands and rivers (however a competent overview was recently offered by Cuvert et al. 2025 in Nature Water), and it discusses the relevance of methane oxidation in the overall methane budget. Drivers controlling methane oxidation were investigated through direct measurement and statistical inter-comparisons between different waterbodies.

Reply(3): We thank the reviewer for pointing out the strong points of our work. The evaluation of the mitigation of diffusive CH_4 emissions by MOX was so far done on limited data-sets in northern lakes. Our study provides for the first time such an evaluation in tropical lakes, and across nearly 80 lakes. Because the sampled African lakes were more productive than northern lakes, the sink of MOX was higher than in northern lakes (L270). We are aware of the Duvert et al paper that we co-authored but it deals with CH_4 emissions not MOX.

The Results highlight a striking difference between "lakes" comprised within the Congolese Cuvette Centrale and lakes situated elsewhere. This pattern was explained by a connection between "wetland soils" and CCC lakes that drives their limnology, as opposed to no-CCC lakes in which phytoplankton-driven processes predominate. This observation does not read as ground-breaking evidence, it does not fulfil the objectives of this research and it should be explained more explicitly that the contrast between these two groups of lakes serves as an argument that tries to identify what drivers determine methane dynamics.

Reply(4): We added the comparison on CCC and non-CCC lakes as an objective of the paper, text now reads L75: "We test the hypothesis that MOX changes with the trophic state of lakes and the connectivity with flooded forests by comparing lakes within and outside the CCC, and that MOX is limited by nutrients."

The manuscript offers a description of phytoplankton community patterns, citing main phytoplankton groups potentially associated to methane metabolism, as reported in published literature. Lines 135-140 propose mechanisms that could explain the potential association between phytoplankton and bacteria. These observations are not directly supported by the Results presented but cite adequate literature. Further on, in Lines 156-157, it is stated that this attachment does not seem to be specific to Cyanobacteria. This last observation could be given higher emphasis and be added to the Conclusions.

Reply(5):

text now reads L75: "We test the hypothesis that MOX changes with the trophic state of lakes and the connectivity with flooded forests by comparing lakes within and outside the CCC, and that MOX is limited by nutrients."

and L441: "The connectivity between wetlands and inland waters in sustaining CH_4 emissions is an increasingly recognized conceptual framework in both tropical and

higher-latitude regions. Our results provide an additional perspective on this framework. Lakes connected to flooded forests within the CCC exhibited higher *K* values than lakes outside the CCC, which are largely disconnected from extensive wetlands (within equivalent ranges of depth and Chl-a). We hypothesize that this enhancement of *K* in CCC lakes may be related to inputs of methanotrophs from the soils of flooded forests. The inoculation of methanotrophs from soils could also explain the higher *K* values in African rivers compared to African lakes at equivalent TSM and POC values. These hypotheses should be tested through future studies quantifying the abundance and diversity of methanotrophic microorganisms.”

And L427: “The photosynthesis-associated OMP pathway could become particularly important if phytoplankton communities shift toward cyanobacteria, which exhibit higher OMP rates than diatoms. However, our results show that *K* increased with Chl-a regardless of whether cyanobacteria or other phytoplankton groups dominated, indicating that MOX should partially mitigate OMP regardless of shifts in phytoplankton community composition.”

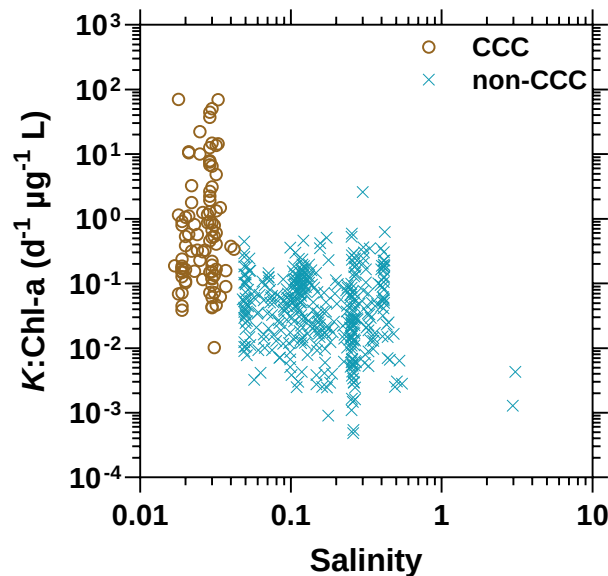
Apart for Cyanobacteria, no reference is being made to other bacterial groups (in particular Archaea) that have been related to methane metabolism in African lakes (see publications by Seymour et al. 2017 in *Nature Microbiology*, Fazi et al. 2020 in *Communications Biology*, Nijman et al. 2021 in *ISME Communications*, Li et al. 2021 in *Environmental Microbiology*, Gontijo et al. 2024 in *Environmental Microbiome*, Focardi et al. 2025 in *Nature Communications*).

Reply(6): We fully agree that data on methanotrophic bacterial communities or archaeal methanogenic communities would be a useful addition, but since we did not sample for these variables we cannot speculate on their distribution. Some studies have shown that there is a niche partitioning between different communities of methane-oxidizing bacteria along the oxygen–methane counter gradient of stratified lakes (<https://doi.org/10.1038/s41396-019-0515-8>). Yet, MOX can be predicted from CH₄ and O₂ concentrations along equivalent oxygen–methane counter gradients in stratified lakes (<https://doi.org/10.1007/s10533-019-00552-x>). This would suggest that methanotrophy rates are mainly driven by the availability of substrates (CH₄ and O₂), irrespective of the type of micro-organisms, although, these micro-organisms occupy spatially distinct niches.

The manuscript does not include salinity as a relevant factor involved in the establishment of high microorganism biomass and high methane concentration, as shown by Fazi et al. 2020 in *Communications Biology*, who reported high methane release rates in a hypersaline lake, higher than the range reported in the studied lakes (see Line 95).

Reply(7): There is no evidence that salinity affects aerobic MOX, so salinity was not tested in the first version of the manuscript that focuses on the drivers of MOX and not of dissolved CH₄ concentration. Following the reviewer’s comment, we have included an analysis of CH₄ concentration as a function of salinity. Text now reads L127: “Dissolved CH₄ concentrations in surface waters of non-CCC lakes were positively related to salinity (Supplemental Fig. S4), consistent with high CH₄ concentrations reported in an African hypersaline lake (30). This contrasts with the negative relationship between CH₄ emissions and salinity reported for northern lakes (31).”.

Yet, there was no significant relation between *K*:Chl-a and salinity, as shown below.



Trophic state (nutrient) dependency of bacterial growth is well supported in the literature, so are the nutrient dependencies of methane production and methane oxidation. The significant relationships illustrated in Figure 2 between methane oxidation and methane concentration, and between K(MOX:CH₄) and Chl "a" do not come as a surprise but corroborate expectations.

Reply(8): We strongly disagree with the reviewer that our work simply corroborates expectations.

Previous work has established the following chain of causality:

Nutrients => enhanced methanogenesis => more CH₄ => enhanced MOX (more CH₄ as substrate)

We show a different and additional chain of causality:

Nutrients => more phytoplankton => more methanotrophs attached to phytoplankton => enhanced MOX (higher abundance of attached methanotroph).

This new chain of causality is independent from an enhancement of methanogenesis, and provides novel insights into understanding and modelling of MOX in surface waters of lakes.

Existing models of MOX in the water column of lakes (<https://doi.org/10.1007/s10533-019-00552-x>) only include CH₄ and O₂ as input variables, and we show in the amended version of the manuscript that these models strongly under-estimated MOX in African lakes, because they did not account for the effect of methanotrophs attached to phytoplankton (new Figure 3). Indeed, the UQUAM model was established in oligotrophic northern lakes (Chl-a < 3 μg L⁻¹).

Refer to Reply(2) for more details on additions of text to revised manuscript.

Oxygen availability is often referred to in this manuscript as it represented a conservative value, while it is well-known that it changes widely according to the hour of the day, especially in standing or slow flowing waters rich in organic matter such as the CCC lakes. For this reason, it is difficult to interpret dissolved oxygen values

(reported as O₂) as characteristic of the limnology of different lakes. An O₂ max could be more indicative, or a daily range O₂ min-O₂ max.

Reply(9): Micro-organisms respond to the O₂ concentration in their immediate environment and not to the maximal daily O₂ or the diurnal O₂ min-max. As such, it seemed more relevant to relate the MOX rates to the actual O₂ concentration at the start of the incubation. We have now added a section looking into the relationship between K and O₂ and text now reads L230: “Surface dissolved O₂ concentrations ranged between 20 and 746 $\mu\text{mol L}^{-1}$ (mean = 259 $\mu\text{mol L}^{-1}$ median = 250 $\mu\text{mol L}^{-1}$). K:Chl-a was negatively related to O₂ concentrations in both CCC and non-CCC lakes (Supplemental Fig. S13). This negative relationship may reflect inhibition of MOX at high O₂ concentrations and the existence of an optimal O₂ range for MOX between 5 and 30 $\mu\text{mol L}^{-1}$, with inhibition of MOX for O₂ <5 $\mu\text{mol L}^{-1}$ (6,15). However, at equivalent water temperature ($\geq 25^{\circ}\text{C}$) and O₂ concentrations ($\leq 261 \mu\text{mol L}^{-1}$) (the minimum and maximum values observed in CCC lakes), K:Chl-a values remained significantly higher in CCC lakes than in non-CCC lakes, presumably due to inputs of methanotrophs from flooded forest soils.”

Reference to “electron acceptors” in Lines 44-46 is rather surprising, given that O₂ will always come first in the chain regardless of the availability of other acceptors. It is not evident that MOX could be limited by the scarcity of alternative electron acceptors in tropical lakes (convincing evidence of this is not presented). Low O₂ abundance can be caused by high organic matter loads, but the same would then presumably provide available SO₄²⁻, NO₃⁻, Mn and Fe oxides.

Reply(10): We agree with the reviewer that our initial sentence was too short to describe how the relative importance of aerobic and anaerobic MOX relates to the availability of electron acceptors. For the purpose of the paper, the dominance of aerobic MOX over anaerobic MOX in lakes is the only relevant information, and the sentence was simplified. Readers can refer to the cited papers for explanation of the causes of the dominance of aerobic MOX.

The first paragraph of the Conclusions that should emphasise the main achievements obtained during this research, highlights as main result the distinction between CCC and no-CCC lakes, attributed to the connection of CCC lakes to “wetland soils”. However, distinction between CCC and no-CCC was not the main objective presented in the Introduction.

Reply(11): We now present the influence of wetlands as a hypothesis to test. Text now reads L75: “We test the hypothesis that MOX changes with the trophic state of lakes and the connectivity with flooded forests by comparing lakes within and outside the CCC, and that MOX is limited by nutrients.”.

The Conclusions section was re-written following the reviewer’s comments.

Secondly, “wetland soils” is a rather vague term. Several of the “lakes” sampled during this research would be classified as wetlands, being shallower than 3 m (see Line 71); “wetland soils” may indicate anything between “wetland aquatic sediments” and more or less wet pastures, or waters draining humid forests.

Reply(12): Wetlands are functionally different from lakes, and none of the sampled lakes during our study can be classified as wetlands. The lakes located in the CCC were bordered by flooded forests. We referred to these flooded forests as “wetlands”. We now use the term “flooded soil” instead of “wetland soil”.

The Conclusions section was re-written following the reviewer's comments.

The observation that in no-CCC lakes CH₄ metabolism could be driven by phytoplankton, as opposed to CCC lakes, is based on rather weak evidence and it is rather surprising that it could be presented as a main striking contribution of the present research and placed at the beginning of the Conclusions.

Reply(13): We feel there is a misinterpretation here - we did not wish to state that “non-CCC lakes CH₄ metabolism could be driven by phytoplankton”. We stated that MOX was enhanced by methanotrophs fixed to phytoplankton based on the compelling relation between K and Chl-a (Fig. 2d).

The contents of the Abstract should be better aligned to the Conclusions.

The Conclusions should focus more closely on the original results achieved in this research and highlight its added value to the current scientific context/discussion, rather than continue the Discussion. It is surprising to see, in the Conclusion, citations reported to support statements.

Reply(14): The Conclusions section was re-written following the reviewer's comments.

In 272-274, the authors admit that no progress has been offered through their research about the postulated attachment between bacteria and phytoplankton. Yet this mechanism is essential to offer an explanation to the relationship between methane oxidation and Chl “a” indicated in this research. This initial statement seems to be contradicted further along.

Reply(15): We do not see a contradiction. We show that K increases with Chl-a, and the most likely explanation is the increase of micro-organisms attached to phytoplanktonic cells. It has been established that for more than 5 decades that the activity of micro-organisms attached to particles is higher than free-living micro-organisms (<https://doi.org/10.1111/j.1365-2672.1977.tb00766.x>).

Nevertheless, methanotrophs attached to phytoplanktonic cells compete with phytoplankton for PO₄³⁻ so they could be limited by PO₄³⁻ availability. This is apparent in the relationship between K normalized by Chl-a versus PO₄³⁻.

An important point of discussion in this extensive work concerns MOX dynamics in relation to Chl “a” and nutrient levels. This issue has been debated in the literature before, but the present work introduces a large number of new data. Despite this, the interpretation of this data should be phrased more explicitly. The MOX:Chl “a” relationship in CCC lakes indicates that MOX is not simply nutrient limited and that MOX could be promoted by the abundance of phytoplankton itself. At the same time, however, K:Chl-a values were positively related to PO₄³⁻ concentrations; the ambiguity therefore remains (nutrients or phytoplankton drive MOX?). The Manuscript should explain much more clearly if/how the data presented here contribute to the development of a clear line of evidence that could be considered novel (and how strong it may be).

Reply(16): We have already addressed in Replies (2) and (8) that our work does not simply confirm existing knowledge but effectively provides a new insight on how nutrients lead to an enhancement of MOX true the increase of the abundance of methanotrophs attached to phytoplankton instead of simply increasing the concentration of CH₄, the substrate for MOX, as shown in existing literature looking

into the response of sediments to eutrophication. There was no significant relationship between K and PO_4^{3-} , as K variations were overwhelmingly driven by Chl-a variations. What we did show is that K normalized by Chl-a ($K:\text{Chl-a}$) indicates a PO_4^{3-} limitation. This means that methanotrophs compete with phytoplankton for PO_4^{3-} , so they could be limited by PO_4^{3-} availability.

So, there is no contradiction but rather complementary and compatible drivers of K .

The last paragraph of the Conclusions, in Lines 280-289, proceeds describing a hypothetical scenario, giving the impression that the results obtained in this research did not produce a clear and univocal weight of evidence that would explain the patterns revealed.

Reply(17): The Conclusions section was re-written following the reviewer's comments.

The manuscript is relatively complex and a clearer Conclusion would be beneficial to ensure that readers do not miss the main achievements that render this contribution original and innovative. How does this work contribute to the on-going debate concerning methane metabolism and its relevance to C dynamics in tropical ecosystems?

Reply(18): The main conclusion of our work is that MOX increases with methanotrophs attached to phytoplankton is applicable in any eutrophic system irrespective of climate zones, and not necessarily only relevant for tropical ecosystems. The Conclusions section was re-written following the reviewer's comments.

Further Minor points are highlighted below:

The Manuscript does not provide details about the actual sampling dates. This reduces the chance to use these data in future intercomparisons.

Reply(19): The sampling dates are now included in Table S1. We did not initially include this information because the full dataset will be made public upon publication, including date and coordinates, facilitating the opportunity to use our data in future intercomparisons.

Our group has a track record of consistent open data sharing, as upon publication we systematically make public data in Tabulated form in data-repositories or as supplemental material. We are regularly complimented by our peers for this and our data are frequently re-used in meta-analysis of GHG dynamics such as:

<https://doi.org/10.1073/pnas.210632211>

<https://doi.org/10.1038/s41586-023-06344-6>

<https://doi.org/10.1890/15-1027>

<https://doi.org/10.1038/s44221-025-00532-6>

<https://doi.org/10.1038/s44221-025-00522-8>

<https://doi.org/10.1016/j.scitotenv.2024.176611>

<https://doi.org/10.1016/j.watres.2025.124320>

Sampling procedures are well described, however scarce description is given of the controlled laboratory measurements on pure phytoplankton cultures, and in situ oxic methane production measurements in lakes Edward, George, and Nyamusingere.

Reply(20): The OMP rates from cultures and field measurements were taken from literature. We have changed the text to make this even more explicit.

Lines 82-83 are a surprising. Why should one expect to find NO₂ in the surface waters of lakes (even if partially humic)? NO₂ is very difficult to assess even when oxygen is very low because it is rapidly oxidized during sampling and sample manipulation. It seems difficult to utilise the very low NO₂ values presented in this manuscript to try and support any argument.

Reply(21): Following the reviewer's comment, we have removed NO₂⁻ data and analysis from the paper.

Lines 88-93 describe phytoplankton groups in CCC lakes as opposed to no-CCC ones. This reads as an entirely descriptive detail and it does not contribute to explain potential methane metabolism differences between the two groups of lakes. A more explicit link to the objectives of the Manuscript should be highlighted.

Reply(22): Since OMP is extremely variable among different phytoplankton groups (Table S3), it is necessary to briefly present the phytoplankton composition.

In Lines 117-118, the evidence "Dissolved CH₄ concentrations in surface waters were also negatively related to the mixed layer depth" could be simply explained by higher rates of sediment methane production (a shallower mixed layer would reveal a greater influence by the hypolimnion) rather than by "the greater influence of MOX as a CH₄ sink in deeper, oxygenated mixed layers." The argument does not seem to be robustly supported by evidence and a more convincing case should be developed.

Reply(23): The reviewer is right that dissolved CH₄ concentrations in surface waters result from a complex combination of processes. We agree that higher hypolimnetic CH₄ concentrations should result in higher surface water CH₄ concentrations. However, there is evidence that seasonal variations of CH₄ in surface waters of lakes closely track the seasonal variations of MLD (<https://doi.org/10.1007/s00027-016-0491-2>), corroborating our explanation.

These two explanations are compatible and co-occur, and text was changed accordingly.

The last section of Results and Discussion proposes rather complex text discussing the contribution of oxic methane production to the diffusive CH₄ flux. This concludes in Lines 262-264. A brief summary of what is developed here should be highlighted in the Conclusion section.

Reply(24): The Conclusions section was re-written following the reviewer's comments.

In 357, stratification was defined by a $\geq 0.2^{\circ}\text{C}$ temperature gradient. This statement could benefit from a citation of an authoritative source. In general, stratification strength is related to temperature and is therefore stronger in tropical lakes; however, 0.2°C seems to be at the lowest limit of what could indicate actual separation between water masses.

Reply(25): We agree with the reviewer that conditions are warmer in the tropics but, in fact, thermal stratification is weaker in tropical lakes (<https://doi.org/10.1002/9781118470596.ch18>), because conditions are warmer all year round (“endless summer”). Consequently, bottom waters do not cool during “winter” (like in northern lakes), and so temperatures in bottom waters are much closer to temperatures in surface waters than in northern lakes. Text now reads L544: “Thermal stratification was defined as a temperature difference ≥ 0.2 °C between surface and bottom waters. Although no universal definition of lake stratification exists, thresholds based on differences or gradients of temperature or density are commonly used (65). In this study, differences in dissolved O₂ concentration between surface and bottom waters were used as an additional indicator of thermal stratification, reflecting the segregation of biogeochemical processes between surface and bottom layers. In most cases, a difference in dissolved O₂ concentration $> 50 \mu\text{mol L}^{-1}$ coincided with a temperature difference $\geq 0.2^\circ\text{C}$ between surface and bottom waters; this value was therefore adopted as the stratification threshold. The Inter-Sectoral Impact Model Intercomparison Project lake sector protocol defines stratified conditions globally based on a density difference of 0.1 kg m^{-3} between surface and bottom waters (66), corresponding to a temperature difference of approximately 0.4 °C at 25 °C. This threshold is higher than the one used in our study, reflecting the weaker vertical density gradients typically observed in tropical lakes compared with higher-latitude systems (67).”

Reviewer #3

The manuscript, "Methane oxidation in African lakes increases with phytoplankton and outweighs aerobic methane production," is a highly comprehensive study featuring an experimental design conducted at a regional scale. This study describes a research consistent with previous works from the same research group. The study focuses on analyzing the impact of phytoplankton concentration (i.e Chl-a) on methane oxidation in the surface waters of more than eighty lakes across Central Africa.

Reply(1): We thank the reviewer for their positive and constructive evaluation of our submission. We noticed that 2 lakes were counted twice in Table S1 and Figure 1 of the original submission, so in fact there are 79 lakes and not 81, but the total number of MOX measurements remains unchanged.

Compared to other studies published by this group, the objective of this research is certainly original in its scope.

Reply(2): Our group published several papers on

1) methanotrophy:

Morana et al (2015) <https://doi.org/10.5194/bg-12-2077-2015>

Roland et al. (2016) <https://doi.org/10.1016/j.chemosphere.2016.10.138>

Roland et al. (2018) <https://doi.org/10.1016/j.jglr.2018.04.003>

Roland et al. (2021) <https://doi.org/10.1038/s41598-021-81210-x>

Roland et al. (2021) <https://doi.org/10.1093/femsec/fiab124>

2) aerobic methane production

Morana et al. (2020) <https://doi.org/10.5194/bg-17-5209-2020>

3) and planktonic ecology

Morana et al. (2023) <https://doi.org/10.1007/s10021-022-00783-4>

Stoyneva-Gaertner et al. (2020) <https://doi.org/10.1016/j.jglr.2020.01.003>

Descy et al. (2017) <https://doi.org/10.1111/fwb.12851>

Morana et al. (2016) <https://doi.org/10.1002/lno.10304>

Llirós et al. (2015) <https://doi.org/10.1038/srep13803>

İnceoğlu et al. (2015) et al. <https://doi.org/10.1007/s00248-015-0612-9>

So, on the contrary, the topic of the submission is within the established expertise of our group.

However, it is important to note that the core phenomenon—the link between phytoplankton and methane oxidation—has been addressed in other studies conducted by different groups, without the necessity of such a large-scale, regional experiment.

The following references, which are relevant to this context, were not included in the manuscript's reference list (ChatGtp helps me in this bibliographic search):

- Oswald et al., 2015. PLoS ONE, 10(7): The abstract concludes: “Along with the detection of chlorophyll a, results suggest that aerobic methane oxidation is tightly coupled to light-dependent photosynthetic oxygen production both at the oxycline and in the anoxic bottom layer. It is likely that this interaction between oxygenic phototrophs and aerobic methanotrophs represents a widespread mechanism by which methane is oxidized in lake water, thus diminishing its release into the atmosphere.”
- van Grinsven et al., 2021. Biogeosciences, 18(10), 3087–3101: The abstract includes the sentence: “Overall, this study shows that photosynthetically fueled CH₄ oxidation can be a key process in CH₄ removal in the water column of humic, turbid lakes, thereby limiting diffusive CH₄ emissions from boreal lakes.” Furthermore, several references already cited by the authors (e.g., Cerbin et al., 2022; Li et al., 2021; Milucka et al., 2015) also refer to the relationship between phytoplankton and methanotrophs. In summary, the authors have conducted a very thorough and well-executed study, providing a wealth of novel data from many lakes in a region that has received limited previous attention. Nonetheless, their results do not diverge significantly from the mechanistic insights described in existing literature.

Given that this work is being considered for publication in a very high-impact journal, such as Nature Communications, my conclusion—regretfully—is that the manuscript does not present sufficiently novel conclusions or a paradigm shift to justify publication in a journal of this caliber.

My conclusion would likely have been different if the results had refuted or substantially challenged the established understanding from previous studies; in that case, the resulting scientific novelty would justify publication in Nature Communications.

Reply: Thank you for disclaiming that ChatGPT was used for these interpretations – we must obviously keep in mind that ChatGPT is known to regularly state incorrect facts, mis-interpret scientific literature, and misattribute citations (<https://doi.org/10.2196/47049>). In line with this note of caution, we strongly disagree with the interpretation of the cited papers above.

Both Oswald et al. (2015) and van Grinsven et al. (2021) suggest that in low O₂ environments at the bottom of the oxycline or even in anoxic hypolimnion, photosynthesis can provide O₂ for aerobic methanogens. However, this does not imply that MOX would increase, because the abundance of methanogens attached to phytoplankton as we have shown conclusively and quantified how this translates into changes of MOX rates.

However, the reviewer is right that there is abundant evidence that micro-organisms are attached to phytoplankton, this has in fact been a paradigm in microbial ecology for 5 decades (<https://doi.org/10.1111/j.1365-2672.1977.tb00766.x>). The fact that photosynthesis supplies O₂ for methanogens as shown by Oswald et al. (2015) and van Grinsven et al. (2021) is only relevant in very low O₂ environments at the bottom of the oxycline. This phenomenon becomes irrelevant in the well-oxygenated mixed layer where we obtained our measurements – no O₂ limitation should occur here. On the contrary, we show that inhibition by high O₂ occurs in the MLD (Fig. S13).

We show for the first time the quantitative impact of the increase of methanotrophs attached to phytoplankton on MOX rates. Indeed, existing models of MOX in the water column of lakes (<https://doi.org/10.1007/s10533-019-00552-x>) only include CH₄ and O₂ as input variables, and we show that these models strongly under-estimated MOX in African lakes (Figure 3), because they did not account for the effect of

methanotrophs attached to phytoplankton. The UQUAM model was established in oligotrophic northern lakes (with very low phytoplankton abundance: Chl-a < 3 $\mu\text{g L}^{-1}$). Our study thus provides an important step compared to the current state-of-the-art in better predicting how MOX and hence CH₄ emissions will change in future with eutrophication.

Text now reads L169: “Additional evidence of the importance of phytoplankton biomass in enhancing MOX and K is provided by the comparison of measured MOX in African lakes with MOX modelled (MOX_{modelled}) using a parameterization developed from northern lake measurements (6), which uses dissolved concentrations of O₂ and CH₄ as drivers. Median MOX in CCC lakes was 4-fold higher than MOX_{modelled}, and their ratio (MOX:MOX_{modelled}) increased with Chl-a (Fig. 3). This pattern likely reflects the low Chl-a values (<3 $\mu\text{g L}^{-1}$) in surface waters of the northern lakes used to develop the model (6). Adding a multiplicative factor proportional to Chl-a to the original model substantially improved model performance (Supplemental Fig. S7).”

And L270: “In northern lakes, %MOX_{removal} was on average 40% (5), lower than the mean in African lakes (74%), possibly due to lower phytoplankton biomass (Chl-a<3.0 $\mu\text{g L}^{-1}$)”

That said, I have several substantive concerns which I will outline below:

MOX Estimates

Lines 312–315 (Incubation Details): It is unclear how many measurements were taken for each incubation. The text mentions “T1–T4”: are these four distinct time points?

Reply: We mentioned that 5 bottles were sampled and 4 were incubated after fixing the initial one (T0). We now mention explicitly that there were five distinct time points (L540).

What was the criterion for choosing incubation durations ranging from a few minutes to up to 12 hours? I interpret that all incubations were conducted in the field without the immediate capability to verify the rate of methane decrease. Is this interpretation correct? If so, what methodology allowed the determination of whether an incubation should last only a few minutes or many hours a priori?

Reply: We decided the time intervals based on Secchi disk, that in the sampled lakes depends mostly on phytoplankton (low CDOM), as we noticed early on during data collection that MOX was much faster in samples with high phytoplankton. This information was added to the revised text, which now reads L474: “The remaining four bottles (T₁–T₄) were kept in the dark in a cool-box containing lake water and sequentially poisoned at regular time intervals ranging from 30 min to 12 h depending on the lake and chosen based on an approximate estimation of phytoplankton biomass based on Secchi depth measurements.

Were the same number of incubations performed in every lake?

Reply: No. A few lakes were sampled only once, others repeatedly, given that some lakes are more difficult to access, in an area that is in general very challenging to sample. The number of MOX measurements is given per lake in Table S1 (information already available in the initial submission).

Lines 322–323 (MOX Calculation): The authors state that MOX was calculated by fitting the data to an exponential decay model (leaving aside the need for greater

transparency regarding the statistical significance of these decays and whether the exponential function was indeed the most appropriate model). I do not understand why the MOX rate is expressed in $\text{nmol/L}^{-1}/\text{d}^{-1}$ when the fitted exponential model yields a decay rate with units d^{-1} .

To obtain the MOX values, do the authors multiply the decay rate by the initial methane concentration? This must be explained more clearly. The necessity for this clarification is heightened because the text (Lines 131–132) defines the parameter $K = \text{MOX}/[\text{CH}_4]$ (units of d^{-1}), implying that CH_4 represents the initial methane concentration prior to incubation. While the introduction of K is justified because the process depends on the initial reactant concentration, why introduce K when the exponential decay rate inherently accounts for this initial concentration?

Reply: The calculation of MOX is given in text L538 that reads: “The CH_4 concentration at each time step ($\text{CH}_{4(t)}$ nmol L^{-1}) was fitted as a function of time (t in d) through the 5 data points (T0-T4) (7):

$$\text{CH}_{4(t)} = \text{CH}_{4(T0)} e^{-Kt}$$

where $\text{CH}_{4(T0)}$ is the initial CH_4 at T0, K (d^{-1}) is the fitted coefficient corresponding to the MOX rate constant, allowing to compute MOX ($\text{nmol L}^{-1} \text{d}^{-1}$) as the product of K and $\text{CH}_{4(T0)}$.

The Trophic State

Line 372 (Trophic State Index - TSI): The classification of the Trophic State Index (TSI) appears to depend solely on chlorophyll concentration. If so, the results derived from this index are entirely redundant with the information already provided by the chlorophyll estimates. While one might argue that this allows for the classification of lakes into four trophic conditions, this separation seems somewhat arbitrary. Furthermore, such classification could be achieved directly using chlorophyll data (for instance a lake is oligotrophic if $\text{Chl-a} < 9 \text{ ngL}^{-1}$). In my opinion, the introduction of the TSI is unnecessary and could be removed.

Reply: The trophic states were attributed based on TSI values - oligotrophic ($\text{TSI} < 40$), mesotrophic ($40 \leq \text{TSI} < 50$), eutrophic ($50 \leq \text{TSI} < 70$), and hypereutrophic ($\text{TSI} \geq 70$). TSI can be calculated from several variables such as Secchi disk depth, total phosphorous, and Chl-a. The reviewer is right that, in our particular case, we could have classified lakes per trophic state based on Chl-a directly. However, Carlson (1977, <https://doi.org/10.4319/lo.1977.22.2.0361>) defined trophic states based on TSI values, and we prefer to keep this convention, as this is a widely used classification system.

Figure 2 (Statistical Comparisons): Visually, the reported significant differences in MOX and K between trophic states are surprising. The box plots (right-hand graphs) exhibit considerable overlap, making it unexpected that the ANOVA and Tukey's post-hoc test report so many significant differences. This concern regarding visual overlap versus reported significance persists for many of the box plots shown throughout the manuscript and supplementary material.

Reply: Our statistical tests are significant, we conclude that visual impression of overlap was misleading because most plots are given in log scale. For the statistical tests what's important is the comparison of mean and variance, and the reader can notice that the means (crosses) are visually separate, specially given that log scales are used.

Impact of OMP as a Methane Source

This section is interesting, but somewhat difficult to follow. I agree that methane produced through processes classified under "OMP" (Oxygenated Methane Production) cannot contribute to MOX. However, the explanation could be significantly simplified.

Reply: This is a controversial aspect of the paper, we preferred to keep a detailed description of results, as well as detailed discussion. Note however that text has been re-arranged, we are confident that the present version is easier to follow.

Figure 5: Plots "c" and "d" are essentially identical to "a" and "b" and could thus be moved to the supplementary material.

Reply: The top panels show OMP:MOX and the bottom panels show OMP:FCH₄. The plots show that OMP does not contribute significantly in sustaining respectively MOX and diffusive emissions of CH₄. This is a central message of our work, and we hence prefer to keep this in the main text.

Core Utility: My greatest concern is the usefulness of incorporating OMP-Chl relationships derived from estimates obtained in laboratory incubations under presumed optimal light and nutrient conditions. These conditions are likely not representative of the in situ stress conditions that might affect methane production. I would suggest making this section more concise and clearer, potentially by focusing only on the empirical data from the three field lakes and omitting the data derived from laboratory incubations.

Reply: We followed the reviewer's suggestion, and the OMP values derived from the lake in-situ experiments are used as nominal estimates, while the OMP rates derived from cultures are used as an upper-bound estimate in a new section « Uncertainty on MOX and OMP estimates ».

MOX Drivers

Line 122 (Methane and Chlorophyll Correlation): The text states that CH₄ concentrations are positively related to chlorophyll concentration. This relationship is clear for "non-CCC" lakes but not for "CCC" lakes (according to the information at the appendix). This distinction needs to be clarified better.

Reply: The reviewer is absolutely right, and this is actually one of the important findings of the paper. This is in fact clearly stated in text, and *K* in CCC lakes is attributed to inputs of methanotrophs from flooded soils rather than methanotrophs attached to phytoplankton as in non-CCC.

Temperature and Oxygen: Given that this article describes a metabolic process highly dependent on ambient temperature and oxygen concentration, it is surprising that these two variables appear to play almost no role. How can the authors ensure that all incubations were conducted under fully oxic conditions? The study included some shallow lakes with $z < 5$ m and high DOC concentrations (>30 ppm). How can it be confirmed that the dissolved O₂ concentration was high at the start of all incubations? Upon reflection, this appears to be a critical issue that should be addressed in future submissions.

Reply: The reviewer is right, and in the present version of the manuscript we have included variations of *K* as a function of temperature and O₂ (new Fig. S13).

Text now reads L219: “Surface water temperature ranged from 20.5 to 33.1°C (mean = 27.0°C median= 26.9°C), although most observations fell within a narrow range (interquartile range 25.5-28.5°C). These temperatures correspond to optimal growth range of type II methanotrophs (23-34°C) (49), whereas Type I methanotrophs have lower temperature optima (8-15°C) (50). In non-CCC lakes, *K*:Chl-a was negatively related to water temperature (Supplemental Fig. S13), contrary to expectations from metabolic theory. This pattern may reflect increased competition for nutrients between methanotrophs and phytoplankton in surface waters under stronger thermal stratification. The absence of a significant statistical relationship between *K*:Chl-a and water temperature in CCC lakes and across the full dataset is consistent with a study in northern lakes showing a stronger dependence of MOX on CH₄ and O₂ availability than on temperature (6).”

And L230: “Surface dissolved O₂ concentrations ranged between 20 and 746 μmol L⁻¹ (mean = 259 μmol L⁻¹ median = 250 μmol L⁻¹). *K*:Chl-a was negatively related to O₂ concentrations in both CCC and non-CCC lakes (Supplemental Fig. S13). This negative relationship may reflect inhibition of MOX at high O₂ concentrations and the existence of an optimal O₂ range for MOX between 5 and 30 μmol L⁻¹, with inhibition of MOX for O₂ <5 μmol L⁻¹ (6,15). However, at equivalent water temperature (≥25°C) and O₂ concentrations (≤261 μmol L⁻¹) (the minimum and maximum values observed in CCC lakes), *K*:Chl-a values remained significantly higher in CCC lakes than in non-CCC lakes, presumably due to inputs of methanotrophs from flooded forest soils.”

We compiled a large dataset of respiration measurements in surface waters based on incubations of O₂ decrease (n=188) during the same cruises where we acquired MOX measurements, although not systematically at each MOX measurement. We have then modelled respiration based on Chl-a and DOC relations (<https://doi.org/10.1093/acprof:oso/9780198527084.003.0007>), and estimated the decrease of O₂ during the MOX measurements based on the initial O₂ concentration and the duration of the MOX incubation. The results show that conditions remained oxic and O₂ concentrations well above the threshold of 5 μM, when MOX becomes limited by O₂ and declines.

Refer to Figure S21 and text reads L315: “Conditions remained oxic throughout all MOX incubations, and O₂ concentrations at the end of the incubations were above the threshold value (5 μmol L⁻¹) below which MOX inhibition occurs (15) (Supplemental Fig. S21).”

Figure S1b shows O₂ concentrations. However, to compare dissolved gas concentrations across different environments, the authors should report the percentage saturation, which accounts for both temperature and the gas's partial pressure in the atmosphere. Given that the temperatures of the different lakes surely vary, as does the partial pressure O₂ (I could not find information on the elevation of the lakes, but they are unlikely to all be at the same altitude), reporting raw concentration is insufficient.

Reply: While it is better to use saturation instead of concentration in certain contexts, we disagree that this would be a better way to present the data for our objectives. Micro-organisms respond to the (absolute) quantity of O₂ molecules in their environment. Thus, concentration (number of moles of O₂ per volume) is the relevant variable that will influence activity of micro-organisms. O₂ concentration at 1°C (~440 μM) will be two-fold higher than at 35°C (~220 μM), at 100% saturation in both cases.

Even though the saturation value is the same at those two temperatures (100%), we would expect micro-organisms to respond to a change of O₂ concentration by a factor of 2. So we kept O₂ expressed as a concentration.

We have added elevation to Table S1 as requested by the reviewer.

Conclusions and Study Design

Lake Connection Focus: The "Conclusions" paragraph begins by discussing lakes "connected and unconnected" to flooded forests. However, the relevance of this aspect is not clearly presented at the beginning of the manuscript. It is absent from the Introduction and is only mentioned in the Methods and Results when referring to "CCC" and "non-CCC" lakes. If the authors consider this distinction highly relevant and wish to emphasize it in the conclusions, they must introduce it more transparently at the beginning of the manuscript.

Reply: This was added as a hypothesis.

Text now reads reads L75: "We test the hypothesis that MOX changes with the trophic state of lakes and the connectivity with flooded forests by comparing lakes within and outside the CCC, and that MOX is limited by nutrients."

The Conclusions section was re-written following the reviewer's comments.

Statistical Imbalance and Geomorphology: Furthermore, I question the emphasis on this "connection" aspect. If I am not mistaken, the "non-CCC" lakes are significantly more numerous than the "CCC" lakes (i.e., 65 vs. 16), which could introduce issues in statistical comparisons. Further, the substantial difference in the number of lakes in the two datasets is not clearly highlighted in the article, despite continuous comparisons between "CCC" vs. "non-CCC."

Reply: By design, statistical tests can handle populations with different sample numbers for the comparison of means. We have added the number of lakes sampled + number of MOX measurements in text that now reads L87: "Lakes were categorized

.

The number of MOX measurements is given per lake in Table S1.

Geomorphological Differences: The two categories of lakes also appear to differ significantly in their geomorphology (e.g., some "non-CCC" lakes are very small in area (0.7 km²) but very deep, a characteristic not observed in "CCC" lakes). A potential way to mitigate this issue might be to remove all "non-CCC" lakes smaller than 0.7 km² from the comparative analyses.

Reply: We agree that morphological attributes are important, and we show that area and depth influence dissolved CH₄ concentration and Chl-a (Fig. S2,S3). MOX is analyzed as a function of CH₄ dissolved CH₄ concentration and Chl-a so inherently incorporates the influence of morphological attributes. For some of the statistical comparisons between CCC and non-CCC lakes, the data were sorted by depth (<6m) to minimize the impact of morphological attributes (in this case depth). As such, we kept the analysis of the full data-set rather than excluding data as suggested by the reviewer.

Appendix

The Appendix is a very comprehensive and useful document. Perhaps Table S1 could also include the average methane values. Given that the text (Line 95) mentions a methane concentration as high as $95\mu\text{mol L}^{-1}$. One of the highest I have ever read—it would be highly interesting to know which specific lake this measurement corresponds to.

Reply: We agree that this CH_4 concentration is high but in line with African lakes having CH_4 concentration higher than northern lakes (refer to Figure 7 of <https://doi.org/10.1126/sciadv.abi8716>). Following the reviewer's suggestion, we have added the mean CH_4 dissolved concentrations to Table S1. The manuscript focuses on MOX and not CH_4 , although we provide a brief description of drivers of CH_4 (Text L 110-134). For a more in depth discussion of CH_4 variability in African lakes, including the occurrence of high CH_4 dissolved concentrations, please refer to papers from our (<https://doi.org/10.1126/sciadv.abi8716>) and other (<https://doi.org/10.1038/s42003-021-02365-x>) groups.

Reviewer #4 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reply: We thank the reviewer for their contribution to the positive and constructive evaluation of our submission.

Reviewer #1 (Remarks to the Author):

Dear authors,

Thanks you for addressing my questions and making modifications to the manuscript. In the attached PDF I provide additional comments.

Reply: We warmly thank the reviewer for taking the time to evaluate our work extensively a second time. We have added references regarding OMP linked to the demethylation of methylamines. We have carefully checked our text to tone down some of the previous statements that might have been perceived as overemphatic. We now use the abbreviations OMP_{in-situ} instead of OMP_{com} and OMP_{cult} instead of OMP_{phyto}.

We fully agree with the reviewer that are still a lot of unknowns regarding the processes associated with OMP.

However, the relevant question for our work is if there is enough information to quantify if OMP is higher or lower than MOX ? We believe this is the case because there is a vast body of literature on OMP in lakes (as compiled by <https://doi.org/10.1016/j.watres.2025.124446>). For OMP driven by photosynthesis we used two independent estimates: one based on our own measurements in African lakes and another based on laboratory phytoplankton cultures published by the one of most prominent reasearch groups working on OMP. For OMP based on MPn and methylamines we used information from 7 papers (refs 23-29). We agree that there are still a lot of uncertainty related to these biogeochemical pathways, however, the relevant information for the purpose of our work is that the unamended (control) treatments or the treatments amended with low MPn or methylamines showed undetectable or low OMP rates, meaning that OMP mediated by MPn/methylamine demethylation cannot support the measured rates of MOX in the mixed layer. The same reasoning applies to methane photoproduction and from N₂-fixation.

We believe this conclusion is robustly supported by available literature.

I find your work of high significance to the community by providing methane fluxes and MOX rates from African lakes. Nevertheless I find your discussion on the MOX vs OMP not justified. The data provided for OMP comes exclusively from 13C incubations from 5 lakes out of the 79 you studied.

Reply: The reviewer is right. The incubations amended with ¹³C-HCO₃⁻ provide the contribution of OMP associated to photosynthesis. However, OMP associated to photosynthesis is considered as a very important if not the most important contributor to OMP (<https://doi.org/10.1093/plankt/fbab069>). Future changes of OMP in lakes are expected to be linked to changes in primary production due to eutrophication. It is more difficult to imagine large future changes in OMP linked to MPn/methylamines cycling leading to major change in CH₄ emissions. In fact, OMP linked to MPn/methylamines is expected to decreased (downregulation of utilization of those substrates) in response to eutrophication.

Incubations to measure OMP are extremely time consuming both in the field and in terms of laboratory analysis, it would have been impossible to perform such incubations in parallel to each of 503 MOX measurements in the 79 lakes we studied. Nevertheless, the OMP data reported by Morana et al. (2020) covers a wide range of productivity (Ch-a ranged 5 to 150 µg/l), and provides in our opinion a robust basis to make an estimate for the purpose of our study.

These data covers one single mechanism.

Reply: The reviewer is right that the OMP rates based $^{13}\text{C-HCO}_3^-$ reported by Morana et al. (2020) relates to photosynthesis. This comment was made by the reviewer during the previous round of review, and we amended the manuscript by mentioning the other possible processes and we provide a rough quantitative estimate. These rough quantitative estimates show that OMP associated to MPn, photo-oxidation or N_2 -fixation are of the same order of magnitude as (or lower) than OMP associated to photosynthesis. It difficult to imagine the opposite situation (other OMP processes being an order of magnitude higher than OMP linked to photosynthesis and being higher than MOX).

For all others, specifically demethylation of MPn and methylamines you cannot provide any data. Methylamines (Wang et al. 2021 and Bizic et al., 2018) are still ignored.

Reply: We now mention demethylation of methylamines.

N_2 fixation by iron-only nitrogenases is mentioned, but likely less significant. You develop a discussion on the abundance of phosphonates in lakes based on one study of one lake. This lake is a temperate northern hemisphere lake, such as those that you put great effort to dismiss in your rebuttal. In fact, the paper you bring shows a 20% contribution of phosphonates to total P which at peak make a relatively high P concentration. You then dismiss methylphosphonate abundance citing a marine paper. That paper (Repeta et al) in fact claims that a small fraction of these methylphosphonates can account for the entire oceanic methane flux. The sad truth is that there is no sufficient quantitative data on methylphosphonate in aquatic bodies nor on their contribution to the methane fluxes hence all quantification attempts are speculative at best.

Reply: We fully agree with reviewer that there is still a lot of unknowns regarding cycling of MPn. But we do not think this argument is sufficient to totally exclude a discussion on the relative importance of OMP based on MPn, even if this discussion is imperfect/incomplete. We used the available data in the ocean to estimate MPn from DOP. We agree this is not optimal but should allow to have a first estimate, as we are not aware of equivalent estimates in freshwaters where only total phosphonate concentration has been determined.

It was not our intention to give the impression of dismissing studies. However, it seems to us useful and necessary to take into account the differences (but also convergences) between biogeochemical cycling between temperate and tropical lakes.

There are other aspects that make your OMP discussion speculative. See this in the attached PDF.

I therefore suggest to highlight the attenuation effect of MOX to the total flux and leave the question of the methane source (sediment diffusion, ebullition, or OMP) to future studies targeting these exact question.

Reply: We agree with the reviewer that we still have an incomplete understanding and quantification of the numerous processes sustaining OMP in lakes. Nevertheless, there has been sustained research on OMP for the last 15 years, and a fair amount of available information has already been generated (as compiled by <https://doi.org/10.1016/j.watres.2025.124446>). Progress of scientific knowledge is incremental: theories are built at a given moment based on available information, and

challenged by ulterior studies, leading to the adjustment of the initial theory. We believe that debate should be encouraged because it is a powerful motor for the progress of knowledge.

We believe that it is not necessary or even possible to wait to measure every single aspect of multiple processes driving OMP to establish a hypothesis and then test it. The important question is whether there is enough information to reasonably test a specific hypothesis. For our work, do we have enough information to test if OMP is potentially higher or lower than measured MOX ? We believe that this is case, although admittedly the estimates have some uncertainty.

Several papers based on contemporary information on OMP made extrapolations and generalizations at global scale concluding that OMP was the major driver of CH₄ emissions from lakes (<https://doi.org/10.1038/s41467-019-13320-0>; <https://doi.org/10.1021/acs.est.2c01730>; <https://doi.org/10.1038/s41467-023-37861-7>). If confirmed, this implies a paradigm shift with important consequences on how we should model CH₄ emissions to predict future feedback on CH₄ emissions. Given the important implications, such findings need to be carefully scrutinized. One way to achieve this is to check if estimates of OMP rates in the mixed layer can sustain the CH₄ sinks – MOX and diffusive emissions to the atmosphere.

Our dataset provides a good opportunity to do so because we obtained a large dataset of MOX (n=503) in nearly 80 lakes.

We believe that this comparison is a very useful contribution to the on-going debate on the role of OMP in CH₄ cycling in lakes, even if not all the details of the complex processes driving OMP are known.

In your rebuttal you explained why the morphology vs OMP contribution curve is not to be used. My intention was not for you to use this curve as a predictor, but rather to evaluate where your own data falls on this curve. However, as your OMP data is not complete, I agree that this option should not be further explored.

Reply: We acknowledge that the reviewer thinks that we should not further explore the use of OMP-morphology relationships.

You have also wondered how the “regression line” can be biased. My intention was that the evidence of correlation is biased (since you only show regression lines in case of significant correlation). If one combined two data sets one correlated and another not correlated to the parameter in question and the correlated one has significantly more data points than the other, one can still obtain a lower R (as is the case here) and a significance correlation, without the entire set being truly correlated.

I believe this statistical phenomenon is called Simpson’s paradox or the Yule-Simpson effect.

Reply: Simpson's paradox (or Yule-Simpson effect) describes a situation where a significant trend is present in several individual groups but the trend is no longer significant or reverses in direction when the groups are combined.

In our case, it would have corresponded to a situation where there was a positive significant relationship between MOX and another variable in each group (CCC and non-CCC) but once the data merged the relationship between MOX and that variable became negative or not significant.

Simpson’s paradox does not apply to the case the reviewer pointed out: a relationship between two variables is not significant within a group (CCC) but once merged with another group (non-CCC), a significant relationship emerges. We are not aware of a statistical

principle that precludes testing the merged CCC and non-CCC data. Or else, it would require to systematically test individual datasets before merging them, which we do not think is common practise.

Overall, I am very much in support of publishing the MOX data, while leaving the OMP discussion to a point where matching data can be provided.

Reply: We thank the reviewer for pointing out the merit of our work. We believe that the comparison between OMP and MOX is a useful contribution to the on-going debate on the role of OMP in CH₄ cycling in lakes, even if not all of the details of the complex processes driving OMP are known, as mentioned by the reviewer. Indeed, our results shown that MOX will increase with phytoplankton biomass because of eutrophication, which calls for a comparison with OMP that is also supposed to increase with eutrophication (<https://doi.org/10.1093/plankt/fbab069>). Nevertheless, we have carefully checked our text to tone down some of the previous statements that might have been perceived as overemphatic. We have added references strengthening the discussion on OMP and included demethylation of methylamines.

Hereafter, the reviewer's comments were copy-pasted from the pdfs of the manuscript, noted by line number of the initial submission.

L31: This sentence is textually disconnected from the abstract. It should be rephrased to something in line with: We therefore proposes/suggest/conclude that an increase in OMP, as could be expected due to eutrophication of lakes, would be compensated for by an increase in MOX.

Reply: Last sentence of the abstract was changed accordingly, and now reads L27: "Available evidence suggests that OMP increase supported by eutrophication can be compensated by a stronger increase in MOX."

L 36: This suggests that diffusive fluxes from lakes are well constrained. This is not the case.

Reply: The reviewer is right; text was changed accordingly and now reads L30: "Diffusive fluxes account for about half of these emissions, with the remainder attributed to ebullition, although the latter is more uncertain."

L 37: To be precise with formulations - the term anoxic refers to environments not processes. A better formulation would be methanogenesis in anoxic sediments.

Oxic methane production - while falling under the same rules, became an accepted short term to describe methane production in oxic environments.

Consider rephrasing to maintain the differences in meaning between oxic/anoxic aerobic/anaerobic oxygenic/anoxigenic. This is correctly observed in the first line of the following paragraph.

Reply: The reviewer is right; we replaced "anoxic methanogenesis" by "anaerobic methanogenesis" in L33.

L46: Consider also citing this paper by Gao et al:
<https://pubs.rsc.org/en/content/articlehtml/2022/va/d2va00091a>

Reply: This reference was added.

L80: The authors claim in the rebuttal letter that the paper focuses on MOX. The OMP data is partial, 4/79 lakes, comes from literature, and quantifies a single OMP mechanisms at a different time point.

Therefore, this sentence should be removed from here or strongly rephrased.

One can generalize this and say that the overall potential of MOX to attenuate the methane flux will be evaluated.

In the discussion you can discuss how much of this flux may come from OMP based on the available 4 data points.

Reply: This sentence was removed and text now reads L74: “We compare MOX to other CH₄ fluxes in the mixed layer such as diffusive emissions to the atmosphere (FCH₄) and OMP estimated from literature data.”

L278: This is not a proper statement of the facts:

1) A correlation between CH₄ flux and Chl a was repeatedly shown, and several Chl-a related OMP mechanisms are known. But to date a quantitative correlation between OMP and Chl-a was not shown or published that would allow the estimation of OMP from Chl-a data.

Reply: The paper by Bižić et al. (2020) (<https://doi.org/10.1126/sciadv.aax5343>) - one of the most prominent publications on the topic - reports OMP data per grams of dry-weight. We converted the OMP rates per grams of Chl-a, as transparently explained in the manuscript. We could have done the computation the other way round and converted the measured Chl-a concentrations into grams of dry weight and applied the OMP ratios of Bižić et al. (2020). The result would have been identical numerically.

2) I could not find in the methods how OMP_{com} was derived.

Reply: Text now reads L602: “OMP_{in.situ} was calculated by applying the mean OMP:Chl-a ratio (0.0393 nmolCH₄ μgChl-a⁻¹ d⁻¹, Table S3), derived from incubations with ¹³C-HCO₃⁻ in five African lakes (14), to measured Chl-a concentrations, irrespective of phytoplankton composition.”

3) Stating that the incubation with tracer additions represent the entire microbial pelagic community is incorrect. The tracer was ¹³C labeled bicarbonate. This is relevant exclusively for C-fixers and for C-fixation related methane production. So it does not represent the entire microbial community.

Reply: We agree and we rephrased the sentence that now reads L290: “OMP in our lake dataset was calculated from Chl-a using an average OMP:Chl-a ratio reported in five African lakes (14, Table S3) where OMP was evaluated from incubations of lake water amended with ¹³C-HCO₃⁻ (the resulting estimates are referred to as OMP_{in.situ}).”

L282: OMP_{com} was not measured.

Measured was photosynthesis-associated methane production from which OMP_{com} was calculated.

Reply: Text was changed accordingly.

L295: OMP scales with Chl because you used CHI to calculate it, not because you showed a relationship.

Reply: We have changed text that reads L309: “Because OMP was calculated from Chl-a, OMP_{in.situ}:MOX, and OMP_{in.situ}:FCH₄ ratios were lower in lakes inside the CCC than those outside the CCC (Supplemental Fig. S18), reflecting lower Chl-a (Supplemental Fig. S1).”

L312: That is not correct as a general rule.

Calvo-Diaz et al., 2011

Baltar et al 2012

Ionescu et al 2015

These papers and more show an increased heterotrophic activity due to incubation effects.

L315: No, this paper does not question the bottle effect! It questions the role of volume in it. Meaning, whether a bottle of 1000 mL is better than 20 mL. Their conclusion was that there is no difference. I.e. the bottle effect is independent of incubation volume.

Reply: The text has been updated and now reads L326: “The biomass of heterotrophic bacteria can remain constant, but in some cases may increase during incubations in enclosed containers (63). The incubation environment may also favor certain micro-organisms while suppressing others (63). Although studies specifically testing this effect on methanotrophs are lacking, MOX may be negatively affected by depletion of O₂ during incubation.”

L322: This assumed value reflects classical methanogenesis sources. How would the model look like if the values for photosynthesis associated methane production look like?

Reply: Using of the median value reported for phytoplankton (-38 %) (<https://doi.org/10.5194/bg-16-4129-2019>) would result in negative MOX estimates in about 1/3 of our dataset.

L334: As far as I could see, this paper reports dry weight of biomass rather than Chl-a.

Reply: The reviewer is right that the paper reports dry weight as explicit from Table S3 that reports data in the original units and the converted units per Chl-a. The details of the unit conversion are given in the Table caption. It seems that adding this information would make the text unnecessary stuffy, and not strictly necessary as this is totally transparent to readers from the content and caption of Table S3.

L339: In your rebuttal, reply42, you write CDOM is low and most light attenuation is from phytoplankton.

Reply: Yes, we did mention in the rebuttal letter that CDOM was low in the context of Secchi disk depth mostly varying with phytoplankton biomass, in contrast with northern lakes where Secchi disk depth primarily reflects light attenuation by CDOM. This does not preclude that

CDOM is present in the sampled lakes and that CDOM nevertheless attenuates light. The a_{350} values are reported in Figure S1.

L345: This is a much better term to use throughout the entire paper as it describes better the literature based data used - though as mentioned also that covers one of several mechanisms.

Reply: Following the reviewer's comments, we now use the term $OMP_{in-situ}$ (instead of OMP_{com}) and OMP_{cult} (instead of OMP_{phyto}). These abbreviations reflect the methods to derive OMP rather than the organisms responsible for OMP: $OMP_{in-situ}$ refers to incubations of in-situ water and OMP_{cult} refers to laboratory cultures.

L354: MPn or better spell out methylphosphonates.

The mechanism shown in Wang et al., 2021 and Bizic et al. 2108 demethylation of methylamines, should be mentioned. This mechanism was shown so far exclusively from freshwater and therefore, may be of relevance here.

Reply: We have changed the abbreviation to MPn. We have added reference to the Wang et al., 2021 and Bizic et al. 2108 papers on the demethylation of methylamines.

L356: This paper studies a single lake.

The authors in their rebuttal letter emphasize how northern hemisphere lakes do not represent African lakes. The lake discussed in this paper is Lake Mendota in Madison Wisconsin. In Fig. 1 Phosphonates reach ca. 20% of the total P and reactive P. at peak this comes to over 50 micrograms/L - this is a lot of P.

Ref 57 cited afterwards (Repeta et al) is marine so the correlation is not obvious. There are papers from FW showing that many FW bacteria have the genes necessary to synthesize MPn. Last the Repeta paper is commonly cited for the significance of MPn stating that only a small fraction of the marine available MPn is sufficient to explain the marine CH_4 flux.

Hence, these two citations, are incorrectly used here.

Reply: The only existing paper that reports in-situ MPn concentrations is the one by Repeta et al. in oligotrophic marine waters that should give a reasonably correct order of magnitude of MPn ratio to DOP in oligotrophic lake water. In this context the reference is correctly used. There are only a few papers that have measured phosphonates in lakes, and we cite ref 65 in Lake Mendota. The relevant information is not the absolute concentration of phosphonates, but the relative concentration of phosphonates with regards to total dissolved organic phosphorus. So the reference is correctly used in the context of the paper.

Sentence was amended and now reads L373: "Phosphonates have been detected in lakes, although quantitative estimates remain scarce and suggest low relative concentrations compared to total dissolved organic phosphorus (65), and only a fraction corresponds to MPn (66)."

Bottom line, is we have no info on MPN in African lakes (or others) and it can be low but it can also be high.

Reply: There are 5 papers (refs 23-27) that have reported OMP related to MPn utilization in temperate oligotrophic lakes. The measured rates should correspond to the highest OMP related to MPn demethylation rates. Indeed, the increase of PO_4^{3-} inhibits the utilization of MPn, as expected in mesotrophic and eutrophic lakes (ref 68).

These papers reported OMP with the addition of MPn, but for which the OMP was undetectable in the natural unamended control or in experiments with an addition of low concentrations of MPn.

In our rebuttal letter we emphasized that CH₄ emissions in African lakes are higher than northern lakes because of higher primary production at tropical latitudes. This does not exclude that OMP related to MPn demethylation occurs in oligotrophic African lakes, most probably at similar rates than in temperate lakes. There is no reason to assume that MPn concentrations would be orders of magnitude higher in African lakes than temperate lakes. Available information suggests that the utilization of MnP only occurs in extremely oligotrophic conditions (ref 68), which allows to assume that the utilization of MnP in oligotrophic lakes in temperate lakes in summer should be equivalent to oligotrophic African lakes.

L386: And yet there is a methane flux that can be clearly attributed to OMP.
This statement is too generalized is not supported by the the data.

Reply: We have changed the text that now reads L409: “Estimates of OMP supported by microbial utilization of MPn or methylamines, photoproduction, or N₂-fixation, compiled from literature, are based on a limited number of measurements - mostly in temperate lakes - and should be interpreted cautiously due to their inherent uncertainty, which warrants further investigation. Nevertheless, these alternative OMP pathways estimates were equivalent or lower than the estimated OMP_{in.situ} (median=0.4, mean=1.5 nmol L⁻¹ d⁻¹) and OMP_{cult} (median=6.2, mean=27.1 nmol L⁻¹ d⁻¹) calculated in our lakes, and were lower than the measured MOX rates.”

L388: A one and half page conclusion is continuation of the discussion

Reply: The Conclusion section might be longer than usual because the paper contains numerous parts (K & connectivity to wetlands, K & attachment of methanotrophs to phytoplankton, comparison of MOX with rivers+oceans, comparison of MOX with FCH₄, comparison of MOX with OMP).

Reviewer #2 (Remarks to the Author):

The Manuscript significantly improved in clarity and logical coherence. The authors critically revised reviewers' comments and produced a constructive rebuttal by incorporating many of the proposed changes to the Main Text. The Initial objectives are closer to the Discussion and to the research achievements. The Conclusion now effectively highlights the most relevant contributions. A section dedicated to Uncertainties addresses most of the concerns raised by reviewers.

At this point, only Minor errors have been noted and a few "near-repetitions". Language and Manuscript structure are coherent with scientific style and format.

Reply: We warmly thank the reviewer for taking the time to evaluate our work extensively a second time. We implemented all the changes requested by the reviewer.

The relative stability of African lakes (no clear seasons, longterm persistence of dominant phytoplankton assemblages) is a clear advantage in studying processes such as OMP and MOX and this could be stated more explicitly, and it could give a reason for the lakes chosen in this research.

Reply: This is a good point; text now reads L488: "Although phytoplankton composition and primary production vary among African lakes (35), seasonal variability is relatively small compared to higher-latitude systems (73)."

The last sentence in the Abstract, containing a "double-would" statement reads rather strange and it could be worth rewriting it more affirmatively by stating something like: "Available evidence suggests that OMP increase supported by eutrophication can be compensated by a stronger increase in MOX.".

Reply: Text was changed accordingly - L27.

Error in Line 97, where "and" instead of "but" should be checked.

Reply: Text was changed accordingly.

Lines 178-180, Lines 192-195 and then 235-238 again insist on the important contribution represented by nearby flooded soils. The argument makes sense, however the formatting reads a bit like a repetition (especially 178-180 and 192-195); perhaps rewriting this text more effectively could avoid such an impression.

Reply: Text was changed accordingly to avoid impression of repetition.

Positive correlation between K and Chl"a" (Fig. 2) works very differently for the two lake types and this should be more carefully reflected in the wording within the Main Text, to avoid the impression that this could be interpreted as a major tendency for all lakes.

Reply: Text was changed accordingly.

Error in Line 526, where NO3 is reported twice.

Reply: Typo was corrected.

Error in Line 531 ", and a350"???

Reply: Typo was corrected.

Error in Line 566 "The abundance ...was" instead of "were"

Reply: Typo was corrected.

Please provide a full reference citation for CHEMTAX.

Reply: Reference was added.

Reviewer #3 (Remarks to the Author):

Manuscript#: NCOMMS-25-86285A

Title: Methane oxidation in African lakes increases with phytoplankton and outweighs aerobic methane production

Below is my revised review.

In this document, I will not go through all the authors' rebuttals to my questions and concerns in detail. When I do not comment on specific responses, it is because I accept the arguments provided by the authors in their rebuttal.

In this text, I will focus on the following three points:

1. The identification of previous studies that report results similar to those described in this manuscript
2. The use of the Thottathil model to model the methane oxidation rates (MOX_modelled)
3. A final comment regarding dissolved oxygen concentrations: % saturation or mol L⁻¹?

Reply: We warmly thank the reviewer for taking the time to evaluate our work extensively a second time. We have included in the discussion the findings of the papers of Oswald et al. (2015), van Grinsven et al. (2021), and Pookkattu et al. (2025). We added plots of temperature, and saturation levels (%) of O₂ and CH₄. We think that with these modifications, all the reviewer's remaining concerns have been adequately addressed.

1) At the beginning of my first review, I mentioned that I identified two relevant references, with the assistance of ChatGPT, whose results lead to conclusions very similar to those presented in this manuscript. In their rebuttal, the authors appropriately noted that caution is needed when using AI, as it may misattribute citations. I would therefore like to clarify that AI was used only as a tool to help identify potentially relevant literature. The references themselves were verified after reading the original articles, and I have listed them again below to avoid any possible misunderstanding. These are not AI-generated fantasies, and I believe these studies are sufficiently relevant that they should be cited in the manuscript. The sentences I highlight at the end of the abstracts of these two articles summarize their main conclusions and emphasize that aerobic methane oxidation is linked to the presence of photosynthetic organisms. This is precisely the main outcome that the present manuscript seeks to highlight, as also clearly emphasized in its title. For this reason, I conclude that this particular result of the manuscript may be not entirely novel. I would like to emphasize that, in the manuscript under review, the evidence for the presence of methanotrophs and for their association with phytoplankton (or their allochthonous origin) is indirect rather than direct. The article does not provide data demonstrating the presence and abundance of methanotrophs attached to phytoplankton (e.g. FISH, microscopy, or other direct approaches). For this reason, the authors are deliberately cautious in the abstract and use the adverb "*presumably*" (on two occasions) to indicate that the proposed relationship is inferred indirectly—supported by evidence from the literature—but has not been directly demonstrated by the data generated in this study.

From my perspective, this is a very important point, because the manuscript does not demonstrate for the first time the "*quantitative impact of the increase of methanotrophs attached to phytoplankton on MOX rates*" (as stated by the authors in their rebuttal to my comment), simply because it does not provide any direct evidence for an actual *increase of methanotrophs*. This implies that, in Figure 7, the two green arrows linking "*phytoplankton increase*" to "*attached methanotroph increase*" and subsequently to "*MOX increase*" are also derived from the literature. The manuscript directly links "*phytoplankton increase*" (data

from this study) with “*MOX increase*” (at the non-CCC sites, based on data from this study) and then indirectly infers that this pattern is due to an increase in “*attached methanotrophs*” (the intermediate compartment in Figure 7), a deduction based on the literature rather than on direct evidence from this study. On the other hand, based on this new reading of the revised manuscript, I reach the conclusion that the relatively more novel result may be the hypothesis that *allochthonous* methanotrophs could be transported from uplands and wetlands, as inferred for the CCC lakes, also through comparison with the estimated *K* values in CCC rivers (I am referring mainly to Figure 4). Naturally, there are studies that propose and analyze soil–water connectivity specifically in the context of methanotrophs, and these are cited in the manuscript (and other works also exist in the literature, e.g.

<https://doi.org/10.1111/mec.15223>; <https://doi.org/10.1016/j.ecolind.2005.05.002>). From my point of view, this is an interesting result. However, the authors may have chosen not to explore this topic in greater depth. Indeed, they note that a manuscript currently under review likely focuses specifically on connectivity (e.g. *Borges, A. V. et al. Methane oxidation in rivers depends on stream size and wetland connectivity. Sci. Adv., submitted (2026)*).

Returning to the studies by Oswald and van Grinsven, the authors of the manuscript state in their rebuttal that these two articles focus on “low O₂ environments” and “at the bottom of the oxycline,” whereas in their study the oxygen concentrations during the incubations could not have inhibited aerobic methane oxidation (see Fig. S21). Indeed, the studies by Oswald and van Grinsven focus on the oxycline (and not only at its lower boundary), where, according to the figures presented in those works, oxygen concentrations are sufficiently high to allow methane oxidation while methane is still present. At higher oxygen concentrations, methane (almost) disappears, and measuring MOX under such conditions is likely methodologically very challenging or simply not particularly meaningful.

In any case, in the manuscript under review, oxygen plays only a relatively minor role.

Indeed in the main text, it is mentioned only sporadically, and dissolved O₂ data appear only in two supplementary figures, which serve to demonstrate that (a) anoxic conditions were not generated during the incubations (Fig. S21) and (b) the K:[Chl-*a*] ratio decreases at high O₂ concentrations (Fig. S13c). The latter result is, in fact, consistent with the findings reported by Oswald and van Grinsven. For this reason, I do not fully understand why the authors emphasize this aspect to differentiate their study from those of Oswald and van Grinsven, when oxygen does not appear to be a central factor in their investigation.

On the other hand, I fully understand why the role of oxygen cannot be emphasized too strongly, as this is a methodologically delicate issue when considering microbial processes arising from associations between primary producers and aerobic methanotrophic bacteria. Such associations are very likely to generate chemical gradients—including oxygen gradients—at micrometer scales, which cannot be resolved with a field optical oxygen sensor operating at spatial scales on the order of several centimeters.

Reply: All new submissions build on existing knowledge and might to some extent align with previous findings, so the qualification of novelty is somewhat subjective. Nevertheless, we think that our findings are sufficiently different from what has been published before to be considered as novel, although it is always possible to find common ground with other publications.

The Oswald and van Grinsven studies hypothesize that phytoplankton provides O₂ through photosynthesis that can be used by methanotrophs. They do not mention methanotrophs attached to phytoplankton cells. We show that MOX increases with Chl-*a* that we attribute to methanotrophs attached to phytoplankton cells and not O₂ produced by phytoplankton (in fact the contrary occurs, see hereafter). So our results are different from the studies of Oswald and van Grinsven.

Our results show that K:Chl-a decreases with increasing O₂. So this is the exact opposite of the results of Oswald and van Grinsven that infer that MOX should increase with O₂ from photosynthesis. This can be reconciled by accounting for the ranges of O₂. At very low O₂ (<5 µM), methanotroph activity is inhibited by O₂ availability. In this situation, the production of O₂ by phytoplankton will be beneficial, as shown by the studies of Oswald and van Grinsven conducted at the base of the oxycline where O₂ is low. For O₂ > 30 µM, there is an inhibition of MOX by O₂ itself (ref 16). This corresponds to the majority of our measurements. So the enhancement of MOX as a function of Chl-a cannot be explained by phytoplankton providing O₂ (since O₂ is in fact inhibiting MOX) and the most likely explanation is an enhancement of methanotroph biomass attached to phytoplankton. The reviewer is right that we did not actually measure the methanotrophs on the surface of phytoplankton. This would have been a whole different study. However, it is established in literature that methanotrophs are attached to phytoplankton (refs 41,42). We do not see why this should not be the case in our sampled lakes. The sampled lakes with the highest chlorophyll-a concentrations were dominated by colonial forms of *Microcystis* that have been shown to have a diverse attached microbiome including methanotrophs (ref 42). It seems to us reasonable to assume this was also the case for our sampled lakes. The attachment of methanotrophs to phytoplankton cells provides the most logical explanation for the enhancement of MOX with Chl-a (since high levels of O₂ inhibits MOX). The fact that methanotrophs are attached to phytoplankton has indeed been shown before in literature, however, the impact on the MOX rates has not been quantified before. Our study does provide such a quantitative assessment. It can also be argued that studies that show the occurrence of methanotrophs attached to phytoplankton cells with FISH or microscopy approaches only record the presence of these organisms but do not determine if these micro-organisms are active and how they impact quantitatively the removal of CH₄ (MOX). So, it seems to us that our study provides valuable and novel complementary quantitative information to existing studies that have shown the presence of attached methanotrophs to phytoplankton cells.

Following the reviewer's comments, we now cite the work of Oswald et al. (2015) and van Grinsven et al. (2021) with regards to the phytoplankton-methanotroph interactions through O₂ supply. Text reads L164: "The association between autotrophs and methanotrophs is presumably mutually beneficial: methanotrophs supply CO₂ for photosynthesis (43), while autotrophs produce O₂ that supports aerobic MOX even under low O₂ conditions (45,46)." We also discuss the work of Oswald and van Grinsven in the context of the K:Chl-a vs O₂ relationship. Text reads L 240: "At O₂ < 5 µmol L⁻¹, photosynthetic O₂ production may enhance MOX, as observed at the base of the oxycline in temperate lakes (45,46). However, this mechanism does not explain the enhancement of K with Chl-a we observed in non-CCC lakes (Fig. 2). The negative relationship between K:Chl-a and O₂ (Supplemental Fig. S13) suggests inhibition of MOX at high O₂ concentrations (6,16), which should be more pronounced in productive lakes characterized by elevated O₂ concentrations (Supplemental Fig. S14)."

We think that this additional discussion and the references therein provide a comprehensive account of the role of O₂ in regulating MOX based on our dataset, and that the reviewer's concerns have been adequately addressed.

2) Thottathil model for the calculation of theoretical MOX

The authors compare their measured MOX rates with MOX values estimated using the model of Thottathil et al. (2019). This is an empirical model derived from a series of incubations

performed on samples collected from six small and shallow lakes, characterized by low chlorophyll-a concentrations ($< 2.9 \mu\text{g L}^{-1}$) and water temperatures below 25°C .

What justifies the assumption that a model developed under these conditions can be used to simulate MOX in lakes (more specifically non-CCC lakes) with chlorophyll-a concentrations typically exceed $10 \mu\text{g L}^{-1}$ and water temperatures that, in many cases, exceed 25°C ? Figure 3 in the manuscript is particularly instructive in this regard: the divergence between measured MOX and MOX_{modelled} becomes evident above approximately $3 \mu\text{g L}^{-1}$ Chl-a, precisely where the Thottathil et al. (2019) model likely ceases to be valid and, consequently, ceases to be an appropriate reference. An empirical model should not be applied outside the range of conditions for which it was developed (see: Box, G. E. P., & Draper, N. R. (1987). *Empirical model-building and response surfaces*. John Wiley & Sons.). Therefore, in my view, the Thottathil model cannot be used to argue that chlorophyll-a needs to be included in predictive MOX models. In this sense, the exercise is uninformative.

This exercise might become more informative if the authors were to consider implementing alternative models. The model proposed by Tan et al. (2024) (ALBM; <https://agupubs.onlinelibrary.wiley.com/doi/pdf/10.1029/2024MS004275>) could represent a useful starting point. It would need to be examined carefully, as it appears to adopt the Thottathil approach for MOX. However, chlorophyll-a is included in this model. Notably, in ALBM model, chlorophyll-a is not introduced to promote the presence of aerobic methanotrophs (and thus methanotrophy); rather, it is used to stimulate aerobic methane production. It is precisely this opposite conceptual framework that makes the ALBM approach interesting. Comparing the MOX values calculated in the present manuscript with those predicted by such a model could help clarify the role of chlorophyll-a—whether as a vector facilitating the transport of aerobic methanotrophs, or as a driver enhancing aerobic methanogenesis. This is simply a suggestion, but one that may be worth further consideration. Another interesting contribution is the recent paper that includes tropical water masses and focus on temperature: Pookkattu, A., Patel, L., Singh, R., Gowd, S. C., & Thottathil, S. D. (2025). Temperature Dependence of Methane Oxidation in Lakes: Global Trends and High-Temperature Inhibition in the Tropics. *ACS ES&T Water*, 6(1), 477-484.

Reply: It is good practise to compare the output of an existing model with independent datasets ([https://doi.org/10.1016/0304-3800\(95\)00152-2](https://doi.org/10.1016/0304-3800(95)00152-2)). If there is convergence, it validates the model and if there is divergence, it paves the way to improve the model. We agree with the reviewer that a model should not be applied outside the range of values of the drivers (predictors) to which the model was fitted. In the Thottathil et al. (2019) model of MOX, Chl-a was not used as a predictor. So, it seems to us that it cannot be argued that this model should not be applied for $\text{Chl-a} > 3 \mu\text{g L}^{-1}$ since Chl-a is not a predictor. We fully agree with the reviewer that the model of Thottathil et al. (2019) and our data diverge at $\text{Chl-a} > 3 \mu\text{g L}^{-1}$. This is exactly the point we are trying to make. We conclude that to achieve a model that can predict MOX we need to include CH_4 and O_2 as in the model of Thottathil et al. (2019) but we need to add Chl-a as an additional predictor. This seems to be an important conclusion, if we want to properly model MOX. This should not be viewed as downplaying or demeaning the Thottathil et al. (2019) work; on the contrary, we think that the Thottathil et al. (2019) paper is extremely well executed and a very important contribution to the field. But this does not mean that this paper is definitive and that the proposed model of MOX cannot be improved. The comparison of the output of an existing model with independent datasets seems to us to be the normal and desirable process for the advancement of scientific knowledge.

ALBM uses the Thottathil et al. (2019) formulation to model MOX using the same predictors (CH_4 , O_2 , temperature) and does not use Chl-a as a predictor of MOX as stated by the

reviewer. It seems to us more correct to refer to the original paper rather than the ALBM paper.

ALBM uses Chl-a to model OMP (and not MOX as stated by the reviewer) using a OMP:Chl-a ratio based on cultures (refs **21,64**). We use the same approach to compute OMP_{cult} , and again it seems to us more correct to refer to the original papers rather than the ALBM paper.

We thank the reviewer for pointing us the Pookkattu et al. (2025) paper that was published after we submitted our initial draft. This work contained relevant information to the topic of our work. We included this reference in the Introduction and we have included it in the discussion of the K:Chla vs Temperature discussion, as well as the comparison with the Thottahil et al. (2019) model.

Text reads L177: “The discrepancy between MOX and $MOX_{modelled}$ may also be attributable to higher water temperatures in African lakes compared to northern lakes ($<25^{\circ}C$, **6**).

However, the divergence between MOX and $MOX_{modelled}$ was more pronounced in non-CCC lakes, which were cooler than CCC lakes (Fig. S1). Moreover, the temperature dependence of MOX (Q_{10}) in the model used ($Q_{10} = 2.4$, **6**) is comparable to that established over a broader temperature range ($5-31^{\circ}C$) ($Q_{10} = 3.1$, **11**).”

and L228: “In non-CCC lakes, K:Chl-a was negatively related to water temperature (Supplemental Fig. S13), contrary to expectations from metabolic theory, and might reflect inhibition of K at high temperature (**11**).”

We think that with these clarification and additions to text the reviewer’s concerns have been adequately addressed.

3) Dissolved oxygen concentrations: % saturation or mol L⁻¹?

This is a secondary issue and does not have major implications. However, I would like to respond to the authors’ rebuttal. My original comment referred to the use of dissolved O₂ concentrations expressed in $\mu\text{mol L}^{-1}$ rather than percent saturation (%Sat) in Figure S1b when comparing CCC and non-CCC systems. The authors justify their choice of using concentrations with an argument that is entirely valid and irreproachable from a bioenergetic and microbial metabolism perspective (including aerobic methanotrophy). That said, it is worth noting that this is not a study of microbial ecology—there are no data on methanotroph abundance or diversity—but rather a regional-scale biogeochemical study. Supplementary Figure S1 compares the biogeochemical characteristics of two groups of systems without direct reference to underlying ecological microbial/macrobial processes. From this perspective, I believe that, at least for gases, expressing concentrations as percent saturation would be more appropriate than using absolute concentrations (and, this panel could also include CH₄ percent saturation). If the bioenergetic–microbial perspective were the primary focus, then it would also be necessary to note that the dataset includes a slightly saline system (approximately $7-8 \text{ mS cm}^{-1}$), for which solute—and gas—activities, rather than concentrations, should technically be considered. A last comment: Figure S1 I could also include temperature.

Reply: We acknowledge that the reviewer considers this issue as secondary. We agree with the reviewer that gas saturation levels are relevant to present data in some contexts. We agree with the reviewer that we did not make a microbiological study, nevertheless, it seemed to us more relevant to plot the MOX rate constant as a function of O₂ concentration rather than O₂ saturation level. This was also the case of the Thottahil et al. (2019) model.

We agree with the reviewer that O₂ saturation level and CH₄ saturation are useful variables to frame the study's settings, so these variables have been added to Supplemental Figure S1 as well as water temperature as requested by the reviewer.

We think that with these additions to Figure S1 the reviewer's concerns have been adequately addressed.

Reviewer #4 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reply: We thank the reviewer for their contribution to the positive and constructive evaluation of our submission.

Reviewer #1:

Dear authors

Thank you for your replies and amendments to the paper. As mentioned in my previous comments, I think your study and results should be published as they add significant information to our understanding of the methane budget, and even more so by focusing on an understudied region on Earth on a large scale.

Reply: We warmly thank the reviewer for taking the time to evaluate our work a third time, and for the positive recommendation.

Nevertheless, I still cannot fully endorse your manuscript at this stage. The manuscript convincingly demonstrates that methane oxidation constitutes a major attenuation pathway for CH₄ emissions in African lakes. However, the title and several summary statements still imply that total aerobic methane production is quantitatively constrained and outweighed by MOX.

Reply: We changed the title to mention “photosynthesis-driven” OMP instead of OMP alone. This change clarifies that we do not compare MOX with total OMP (including MPn-methylamine cycling) but only OMP that is related to photosynthesis. We now specify “photosynthesis-driven OMP” elsewhere in text, in Abstract (L15, L25, L28), throughout the main text (L292, L301, L351), and in the Conclusions (L462, L464, L469, L475).

This may be the case, but in reality, the quantitative comparison is primarily based on photosynthesis-associated OMP estimates and literature-derived assumptions regarding additional OMP pathways. Since no direct measurements of MPn concentrations, methylamine-driven OMP, or other non-photosynthetic OMP mechanisms are available for African lakes,

The cited literature demonstrates potential MPn-dependent methane production in lake incubations, primarily under amendment conditions, but does not provide direct measurements of ambient MPn concentrations or in situ MPn-derived OMP rates in African lakes. Therefore, the conclusion that MPn-based OMP cannot materially contribute to total OMP in the studied systems remains inferential rather than empirically constrained.

Reply: We agree with the reviewer. We now mention that no direct measurements of MPn concentrations are available (African lakes included) and that MPn-driven OMP has not been measured in African lakes.

Text now reads L378: “Only a fraction of phosphonates corresponds to MPn in oligotrophic marine waters (66), but the actual concentrations of MPn in lakes, including African lakes, remain unknown.”

And

L413: “Estimates of OMP supported by microbial utilization of MPn or methylamines, photoproduction, or N₂-fixation, compiled from literature, are based on a limited number of

measurements - mostly in temperate lakes and none in African lakes - and should be interpreted cautiously due to their inherent uncertainty, which warrants further investigation.”

I recommend revising the title and overarching framing to focus on the robustly supported conclusion: that MOX substantially attenuates CH₄ fluxes from African lakes, independent of the unresolved relative contributions of specific methane production pathways.

Reply: We have changed the title mentioning “photosynthesis-driven OMP” instead of OMP alone. We think that there is enough information in literature to make an estimate of “photosynthesis-driven OMP” and its uncertainty (since we use two independent approaches to model OMP). We think that the largest uncertainty on methane production pathways lies in the MPn-Methylamines pathway, but the usage of term “photosynthesis-driven” in title and rest of the text should avoid any confusion.

About the correlations and regression bias that I mentioned in my previous comments.

I agree that this situation does not correspond to Simpson’s paradox in its canonical statistical definition. My comment / concern was broader and relates to the interpretation of pooled regressions across heterogeneous lake groups. Specifically, the significant relationship observed in the pooled CCC + non-CCC dataset may be driven primarily by the much larger non-CCC subset, while the same relationship is absent within CCC lakes. In such cases, the pooled regression can visually and statistically suggest a general relationship across all systems despite substantial subgroup heterogeneity. My point was therefore not the strict occurrence of Simpson’s paradox, but rather that aggregation of ecologically distinct groups may obscure effect modification or subgroup-specific dynamics. Given the pronounced differences between CCC and non-CCC lakes in hydrology, phytoplankton composition, CDOM, nutrient regimes, and productivity, I believe the manuscript would benefit from emphasizing subgroup-specific interpretations rather than relying primarily on pooled regressions to support generalized conclusions.

Reply: We thank the reviewer for further clarification. We agree with the reviewer that the Yule-Simpson effect (or any other rule) does not preclude us to statistically test the merged CCC and non-CCC lakes.

We are nevertheless aware that interpreting the relationships with all data as general relationship across all systems would have many caveats, but since we systematically discussed the patterns with each group (CCC and non-CCC) separately we do not feel that any of our conclusions are solely based on the overall relationships across all systems. However, we do not find a valid reason not to statistically test and report on the merged CCC and non-CCC lakes.

We have nevertheless emphasized the differences among the CCC and non-CCC groups in the revised version more strongly.

Text reads L160: “In the CCC lakes, *K* was not significantly related to Chl-*a* (Fig. 2)”

and

L 440: “However, K was not related to Chl-a in CCC lakes, and at comparable Chl-a concentrations, K was higher in CCC lakes than in non-CCC lakes. This suggests that additional processes contribute to K and that the phytoplankton-driven enhancement of K is context dependent, potentially reflecting differences in connectivity with wetlands.”

I hope these suggestions will aid in bringing the paper to a final state where it focuses on the strong available data, without extensive extrapolation from non-representative subsets.

Reply: We warmly thank the reviewer for taking the time to provide additional guidance.

Reviewer #2:

This research assembles an impressive amount of original data and combines them in a comprehensive meta-analysis. It is a milestone and a building block for future research. My former comments were fully addressed. After this last revised version, I see no more issues that could prevent acceptance.

Reply: We warmly thank the reviewer for taking the time to evaluate our work a third time, and for the positive recommendation.

Reviewer #4:

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reply: We thank the reviewer for their contribution to the positive and constructive evaluation of our submission.

Reviewer #1 (Remarks to the Author):

I thank the authors for making these additional changes.

I have no further comments on the manuscripts.

Reply: We warmly thank the reviewer for taking the time to evaluate our work a fourth time. No further modifications were made to the submission, except for editorial requests.

Reviewer #4 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reply: We warmly thank the reviewer for taking the time to evaluate our work a fourth time. No further modifications were made to the submission, except for editorial requests.