

Reply to ‘Oxic methanogenesis is only a minor source of lake-wide diffusive CH₄ emissions from lakes’

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REPLYING TO F. Peeters & H. Hofmann *Nature Communications* <https://doi.org/10.1038/s41467-021-21215-2> (2021)

The prevailing paradigm in methane research is that biological methane production is exclusive to anoxic or near-anoxic habitats such as sediments and oxygen-deficient bottom waters in lakes. Paradoxically, methane supersaturation in oxic lake waters is widely reported. To resolve this paradox while preserving the paradigm, some researchers assume this methane originates entirely from anoxic sources and is then transported to the oxic waters through physical processes^{1–3}. However, multiple recent studies have repeatedly shown, methane production can and does occur under oxic conditions on land, in the seas and in freshwaters, driven by diverse organisms within different life domains (Table 1 and references therein) and via photochemical conversion⁴. These findings raise legitimate questions about the nature of the environmental dynamics and global budget of methane. Because oxic methane production (OMP) is a recent discovery, its contribution to atmospheric emission is unknown. We conducted a whole-lake basin methane mass balance and analysed relevant literature data to estimate the contribution of OMP to surface emission versus lake morphometry.

Because the dynamics of methane concentration and isotope signal in lake waters are influenced by different and opposing processes simultaneously, one cannot meaningfully deduce the presence or absence of OMP without properly accounting for modulations by physical and biological processes. For example, underestimating surface emission or ignoring oxidation would lead to incorrect interpretation of methane concentration and isotope data and incorrect dismissal of OMP (Supplementary Note 1).

By balancing the gains and losses of epilimnetic methane in a stratified water column, we estimated the contribution of oxic versus anoxic methane to surface emission (Supplementary Fig. 1). Epilimnetic methane may originate from lateral and

vertical transport from anoxic zones, ebullition, and internal oxic production (OMP); surface emission and oxidation are the loss terms.

Surface methane emission can be measured directly using a flux chamber, or, in the absence of direct measurements, it is often modelled from surface-water methane concentrations and wind speeds. Both methods are commonly used but the results can differ considerably, and there exist many different wind-based models (for a more detailed discussion we refer readers to the literature^{5,6}). Notably in their manuscript, Peeters and Hofmann excluded our direct measurements of methane fluxes to the atmosphere and exclusively rely on modelling approaches (Supplementary Note 2). We instead combined direct measurements with models that were established for the target lake. Therefore, we consider that our direct measurement approach minimises methodological and model biases, and better represents reality.

For Lake Hallwil, we used the littoral sediment-to-water methane flux as determined by Donis et al.⁷ who implemented two littoral sediment core measurements sampled at 3 and 7 m depth and applying Fick’s law. In contrast, Peeters and Hofmann implemented only the upper sediment core into their re-analysis. They justify this choice by stating the cores’ methane isotope signature vary. As the depth of Lake Hallwil’s surface mixed layer increased over the seasonal progression⁷, both sediment cores should be considered in the mass balance especially in the light of natural variability. For Lake Stechlin, we used data from two mesocosms and the open-water to resolve littoral methane input (Supplementary Notes 3 and 4). We estimated ebullitive methane fluxes as negligible in Lake Stechlin^{8,9}. We further applied an ebullitive flux of $1.2 \pm 0.8 \text{ mmol m}^{-2} \text{ d}^{-1}$ to Lake Hallwil¹⁰, giving a total sediment

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Table 1 Literature examples of oxic methane production (OMP) in different habitats and by different domains of life.

Organism	Domain	CH ₄ production rate	Evidence	Reference
TERRESTRIAL				
Plants	Eukaryote		INC, ISO	Keppler et al. (2006) ²⁰
Plants	Eukaryote		INC	Messenger et al. (2009) ²¹
Methanogens	Archaea		INC, ISO, OMIC	Angel et al. (2011) ²²
Fungi	Eukaryote		INC, ISO	Lenhart et al. (2012) ²³
Plants	Eukaryote		INC, ISO	Althoff et al. (2014) ²⁴
Methanogens	Archaea		MB, OMIC, PHYS	Angle et al. (2017) ¹⁴
Cyanobacteria	Prokaryote		INC, ISO	Bizic et al. (2020) ¹⁵
MARINE				
Mixed assemblage			INC, OMIC	Karl et al. (2008) ¹⁷
Bacteria	Prokaryote		INC, STAT	Damm et al. (2010) ²⁵
Cyanobacteria	Prokaryote		INC	White et al. (2010) ²⁶
α-Proteobacteria	Prokaryote		INC, OMIC	Carini et al. (2014) ²⁷
Haptophytes	Eukaryote		INC, ISO	Lenhart et al. (2016) ²⁸
Bacteria	Prokaryote		INC, OMIC	Repeta et al. (2016) ²⁹
Haptophytes	Eukaryote		INC, ISO	Klitzsch et al. (2019) ³⁰
Cyanobacteria	Prokaryote		INC, ISO	Bizic et al. (2020) ¹⁵
γ-Proteobacteria	Prokaryote		INC	Ye et al. (2020) ³²
Haptophytes	Eukaryote		INC	Klitzsch et al. (2020) ³¹
FRESHWATER				
Methanogens, algae	Archaea, Eukaryote	38–58 nmol l ⁻¹ day ⁻¹ (Lake Stechlin)	INC	Grossart et al. (2011) ¹¹
Methanogens, algae	Archaea, Eukaryote	210–240 nmol l ⁻¹ day ⁻¹ (Lake Cromwell)	ISO, MB	Bogard et al. (2014) ³³
α-, γ-proteobacteria	Prokaryote		INC, OMIC	Yao et al. (2016) ¹³
Mixed assemblage		110 nmol l ⁻¹ day ⁻¹ (Lake Hallwil)	MB	Donis et al. (2017) ⁷
γ-Proteobacteria	Prokaryote	0.2–0.7 nmol l ⁻¹ day ⁻¹ (Yellowstone Lake)	INC, ISO, OMIC	Wang et al. (2017) ³⁴
Mixed assemblage			ISO, MB, PHYS	DelSontro et al. (2018) ³⁵
Proteobacteria	Prokaryote	54–257 nmol l ⁻¹ day ⁻¹ (Lake Bonney)	INC, OMIC	Li et al. (2019) ³⁶
Cyanobacteria	Prokaryote		INC, OMIC	Khatun et al. (2019) ³⁷
Mixed assemblages		72–88 nmol l ⁻¹ day ⁻¹ (Lake Stechlin) 78–138 nmol l ⁻¹ day ⁻¹ (Lake Hallwil)	MB	Günthel et al. (2019) ³⁹
Cyanobacteria	Prokaryote		INC, ISO	Bizic et al. (2020) ¹⁵
Cyanobacteria	Prokaryote		STAT	Khatun et al. (2020) ³⁸
Green algae, diatoms, cryptophytes	Eukaryote	50–210 nmol l ⁻¹ day ⁻¹ (Lake Stechlin)	INC, ISO, MB, STAT	Hartmann et al. (2020) ¹⁸
Picoeukaryotes, diatoms	Eukaryote		STAT	Leon-Palmero et al. (2020) ⁴¹
Proteobacteria	Prokaryote	24–547 nmol l ⁻¹ day ⁻¹ (5 Lakes)	INC, ISO, OMIC	Perez-Coronel and Beman (2020) ⁴²

OMP evidence type: *INC* incubation experiments, *ISO* isotope techniques, *MB* mass balance approaches, *OMIC* molecular biological methods, *PHYS* physical modelling, *STAT* statistical analyses. OMP has been observed in different limnic systems, e.g. temperate and arctic regions (DelSontro et al. 2018³⁵, Li et al. 2019)³⁶, high-elevation (Perez-Coronel and Beman, 2020)⁴², and throughout the oligo-to-eutrophic nutrient spectrum (DelSontro et al., 2018³⁵, Khatun et al., 2020³⁸, Ye et al., 2020)³².

methane input of 3 mmol m⁻² d⁻¹ when combined with the diffusive flux, which is higher than the value assumed by Peeters and Hofmann. Vertical diffusive input was calculated from empirically measured methane concentration profiles and turbulent diffusivities. We parameterised methane oxidation as 30% of internal production for Lake Stechlin; in a sensitivity analysis, we evaluated this assumption and also considered the most conservative scenario, e.g., OMP set to minimum. For Lake Hallwil, methane oxidation rates were measured by experiments.

By balancing the different input and output fluxes, we produced the first system-wide OMP estimate for Lake Stechlin, which agrees well with direct bottle incubation measurements reported earlier¹¹. To further account for (seasonal) variabilities and measurement uncertainties, we conducted Monte Carlo simulations and sensitivity analysis applying various conservative scenarios to the mass balance. It is, however, worth noting that the mass balance is sensitive to the flux parameterisation and the accuracy of its result is hinged on how

reliably one accounts for these fluxes. To better resolve OMP and allow for more general and firm statements about OMP (including different lake systems), future studies should aim to reduce uncertainties associated with the littoral methane input (e.g. methodological uncertainty in sediment core measurements¹²) and methane oxidation—two key parameters in the epilimnetic methane budget.

OMP by diverse organisms (Table 1) and pathways^{13–15} point to its wider potential relevance on a global scale. To examine how OMP may vary according to lake characteristics, we combined our results with analysis of literature data to estimate OMP contribution in relation to basin morphometry (Supplementary Note 5). The epilimnetic methane sources considered here are littoral sediment and OMP. On a whole-system level, the relative contributions of these sources are proportional to the total littoral sediment area and the epilimnion volume, respectively. Because the ratio of littoral sediment area to epilimnion volume decreases with increasing lake size, the contribution of OMP to surface emission is

expected to increase with lake size. This trend does not change even when we assume a larger littoral sediment area by decreasing the sediment slope as suggested by Peeters and Hofmann (Fig. 1). As the current OMP dataset is limited to only a few lakes (four data points based on mass balance and seven based on transport modelling), future studies should aim to increase the number and types of lakes to verify the trend on a larger scale.

Note, as Peeters' and Hofmann's re-analysis excludes internal methane modulation, their OMP estimates reflect net rates while our study presents gross rates. Accordingly, their contribution pattern of oxic versus anoxic methane source to surface emission (NOMC) cannot be directly compared to our estimates (OMC) (further discrepancy is explained by Supplementary Note 5).

Oxic methane production defies the century-old teaching of anoxic methanogenesis and the convention of considering only anoxic sources in methane research; as such, skepticism is expected. While some may dismiss OMP as irrelevant¹⁶, others take a more practical approach and investigate the phenomenon at the ecological, organismal, and molecular levels^{13,17}. However, the novelty of OMP also means researchers are still trialling different methods, each with their limitations (Table 2).

A better understanding of production, storage, consumption, and distribution processes of methane, including methane produced in oxic environments, is needed to improve the assessment of the global methane cycle. This requires better spatio-temporal data resolution and better constraints of data uncertainties by using multiple methods. For instance, OMP rates determined by bottle incubations can complement results

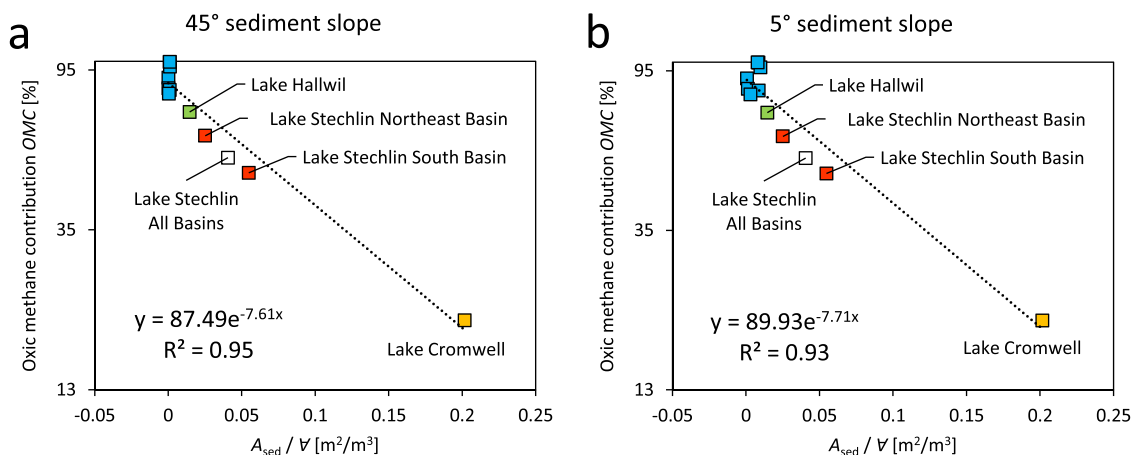


Fig. 1 Oxic methane contribution (OMC) to surface emission in relation to lake morphology. Comparison of (a) the original relationship and (b) the alternative parameterisation using a smaller sediment slope angle. A_{sed} is the littoral sediment area and V is the surface mixed layer volume. Note, OMC is defined as in our original study; the x-axis is linearly scaled, and the y-axis is scaled to $\log_{2.7}$.

Table 2 Overview on approaches to investigate oxic methane production (OMP) in lake waters.

Approach	Description	Caveats	Reference examples
Incubation of • Lake water • Enrichment cultures	Cultivating microbes in closed containers and recording CH_4 concentration over time. Additionally, the change in $^{13}C/^{12}C$ carbon isotope ratio in dissolved methane can be measured.	Bottle enclosure may alter the light and nutrient conditions versus in situ. Long-term incubations (exceeding hours) may not reflect in situ conditions due to changes to the production-consumption equilibrium (e.g., nutrient depletion, community alterations).	Grossart et al. (2011) ¹¹ , Bizic et al. (2020) ¹⁵ , Günthel et al. (2020) ⁴⁰ , Hartmann et al. (2020) ¹⁸ , Klintzsch et al. (2019, 2020) ^{30,31}
Metagenomics	Molecular analysis of relevant enzyme machinery or genes.	Qualitative evidence. Presence of relevant genes and enzymes indicates production potential, but actual production rate can be affected by inhibitors, missing precursors, unfavourable conditions, epigenetic modulation, etc.	Carini et al. (2014) ²⁷ , Yao et al. (2016) ¹³ , Perez-Coronel and Beman (2020) ⁴²
Statistical analysis	Methane concentration is measured together with other lake parameters. Statistical models are applied to test for correlative significance and predictive power.	Individual methane sources and sinks can be overlooked due to the complex lake water methane cycling. Results lack mechanistic understanding of the underlying processes.	Fernandez et al. (2016) ³ , Günthel et al. (2020) ⁴⁰ , Khatun et al. (2020) ³⁸ , Leon-Palmero et al. (2020) ⁴¹
Physical modelling	Combining physical mechanistic aspects with correlative analysis.	Underrepresentation of internal biological modulation (oxidation and OMP).	Peeters et al. (2019) ¹⁶
Mass balance of epilimnion in • Whole-lake basin or • Mesocosms/enclosures	Methane input and output fluxes for the epilimnion are experimentally determined and balanced. Discrepancy is attributed to OMP.	Accuracy of OMP production rates depends on how reliably methane fluxes have been determined. Spatio-temporal data resolution is often limited.	Bogard et al. (2014) ³³ , Donis et al. (2017) ⁷ , Günthel et al. (2019) ³⁹ , Peeters et al. (2019) ¹⁶ , Hartmann et al. (2020) ¹⁸
Methane isotope analysis • Comparing ambient signatures or • Isotope budgets	Analysing carbon (and hydrogen) stable isotope signatures of methane sources and considering isotope fractionation by biochemical and physical reactions (e.g., oxidation, OMP, phase exchange). Analogue to mass balance.	This analysis requires knowing (i) the quantity of all mass fluxes, (ii) isotope characteristics of all methane sources, (iii) isotope fractionation by biochemical and physical processes. Different precursors and biochemical production/consumption pathways can result in different isotope signatures.	Tang et al. (2014) ⁹ , DelSontro et al. (2018) ³⁵ , Günthel et al. (2020) ⁴⁰ , Hartmann et al. (2020) ¹⁸ , Tsunogai et al. (2020) ¹⁹

based on mass budgets, as we did in our study. The incorporation of methane carbon¹⁸ and hydrogen¹⁹ isotope data into mass budgets is a promising way to further tease apart the different methane sources. Omic approaches can be used to investigate the different OMP pathways and the organisms involved.

We have discussed the caveats of our mass balance analysis, such as the limited amount of OMP and littoral flux data, limited types of lakes being considered, and the influence by other compounding factors. The global significance of OMP can only be fully assessed when more relevant data become available, but this also requires researchers to look beyond the anoxic paradigm and consider OMP in future methane measurements. We hope our and others' work will continue to stimulate more research and constructive discussions on this topic.

Data availability

Data are made available in graphical or tabular form throughout the paper and Supplementary Information. Source data are provided with this paper.

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Author contributions

M.G., D.D., G.K. and D.F.M. analysed the data. M.G., D.D., G.K., D.I., M.B., D.F.M., H.-P.G. and K.W.T. discussed and wrote the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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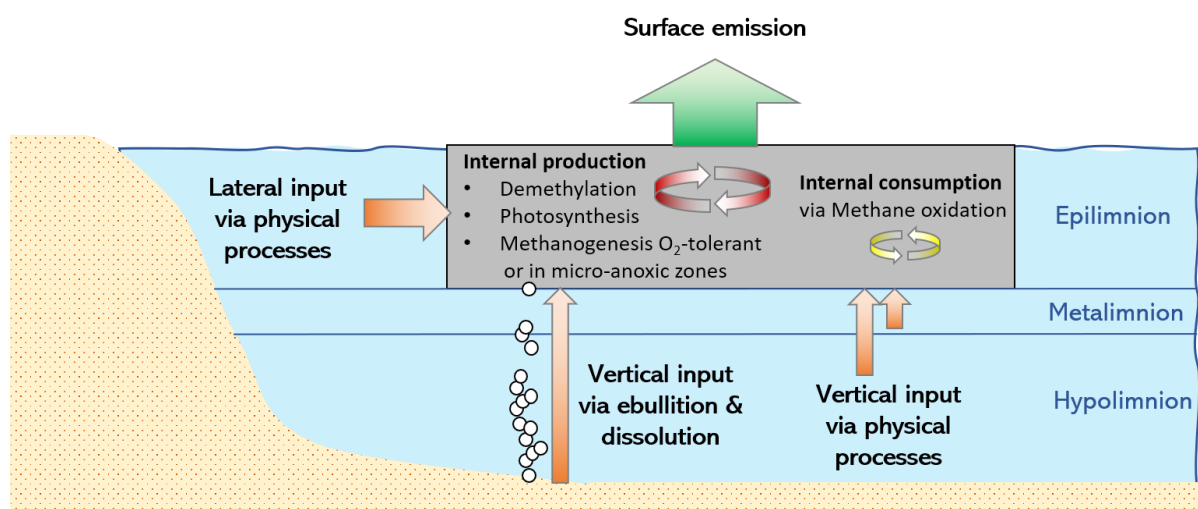
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by Günthel et al.

Supplementary Note 1:

Anoxic methanogenesis has been the paradigm in methane research for generations; questions about the novel idea of oxic methane production are expected and welcomed. Our paper acknowledges dissenting opinions in the literature including Peeters et al. 2019.

Peeters and Hofmann cited Peeters et al. as what they consider as a more valid mass balance analysis of methane dynamics in Lake Hallwil. However, Peeters et al.'s mass balance model "considers as source of CH_4 diffusive fluxes from the sediments, loss of CH_4 due to diffusive emissions from the water surface to the atmosphere, ...and lateral transport of CH_4 by turbulent mixing within the surface mixed layer"; hence, their mass balance excludes open-water biogeochemical processes such as methane oxidation, and is therefore inadequate (e.g. systematic discount of oxic methane production (OMP)). Likewise, Peeters' and Hofmann's interpretation of isotope data in Donis et al. 2017 is inadequate without proper consideration of isotopic shifts caused by physical (emission phase change) and biological processes (oxidation and OMP). Note that we did not use the isotope data for our mass balance analysis (see further discussion in Supplementary Note 5).



Supplementary Figure 1. Epilimnetic methane mass balance. The mass balance approach compares physical methane transport (lateral, vertical) and output fluxes (surface emission, oxidation) in epilimnetic waters. The difference between input and output fluxes is attributed to oxic methane production.

Supplementary Note 2:

Peeters and Hofmann alleged that Donis et al. 2017 and thence Günthel et al. 2019 overestimated surface emissions in Lake Hallwil. The emissions reported in Donis et al. were measured directly with flux chamber (see McGinnis et al. 2015) and therefore it was not necessary to calculate gas transfer constants (k_{600}), as Peeters and Hofmann claimed. These

direct emission measurements were also excluded from Peeters' and Hofmann's re-analysis. Supplementary Table 1 exemplarily displays the conversion of flux chamber readings to methane emission values which are listed by Supplementary Table 2 (mean±SD 0.7±0.4 mmol m⁻² d⁻¹).

Supplementary Table 1. Exemplary conversion of flux chamber readings to methane surface emission.

Air Pressure	Water Temperature MEAN	Slope CH ₄ (from LGR)	Chamber Temperature MEAN	Chamber volume	Bottom area of chamber	R-gas constant	Chamber Temperature	CH ₄ Flux	CH ₄ Flux
[atm]	[°C]	[ppm s ⁻¹]	[°C]	[mL]	[m ²]	[mL atm K ⁻¹ mol ⁻¹]	[K]	[mol m ⁻² d ⁻¹]	[mmol m ⁻² d ⁻¹]
1	20.5	0.0013	28	16755.72	0.126	82.0562	301.15	0.00060	0.604
1	20.5	0.0015	28	16755.72	0.126	82.0562	301.15	0.00070	0.697
1	20.5	0.0011	28	16755.72	0.126	82.0562	301.15	0.00051	0.511

Supplementary Table 2. Individual methane emission values retrieved from flux chamber measurements.

Date	Station	CH ₄ Flux [mmol m ⁻² d ⁻¹]	Date	Station	CH ₄ Flux [mmol m ⁻² d ⁻¹]
11-Jun-15	Hallwil St 8b (wp 382)	0.604	25-Jun-16	Sta 8	0.404
11-Jun-15	Hallwil St 8b (wp 382)	0.697	25-Jun-16	Sta 8	0.665
11-Jun-15	Hallwil St 8b (wp 382)	0.604	25-Jun-16	Sta 8	0.656
11-Jun-15	Hallwil St 8b (wp 382)	0.511	25-Jun-16	Sta HW1	0.778
12-Aug-15	Diffuser	0.827	25-Jun-16	Sta HW1	0.780
12-Aug-15	Diffuser	1.147	25-Jun-16	Sta HW1	1.216
12-Aug-15	Diffuser	1.434	25-Jun-16	Sta HW1	0.902
14-Apr-15	Sta 8	0.519	06-Jul-16	Sta HW 8	0.909
14-Apr-15	Sta 8	0.649	06-Jul-16	Sta HW 8	1.011
14-Apr-15	Sta HW1	0.386	06-Jul-16	Sta HW 8	0.883
14-Apr-15	Sta HW1	0.643	06-Jul-16	St HW 483 (north)	1.412
15-May-15	Sta 8	0.028	06-Jul-16	St HW 483 (north)	1.072
15-May-15	Sta 8	0.032	06-Jul-16	St HW 483 (north)	0.839
15-May-15	Sta 8	0.030	06-Jul-16	Sta HW1 (south)	1.248
15-May-15	Sta 8	0.029	06-Jul-16	Sta HW1 (south)	0.761
15-May-15	Sta HW1	0.297	06-Jul-16	Sta HW1 (south)	1.262
15-May-15	Sta HW1	0.220	06-Jul-16	Sta HW8	0.433
15-May-15	Sta HW1	0.287	06-Jul-16	Sta HW8	0.264
15-May-15	Sta HW1	0.343	06-Jul-16	Sta HW8	0.674
25-Jun-16	Sta 8	0.280			

Supplementary Note 3:

We used both mesocosm data sets when estimating the littoral sediment flux, as indicated by the mean value and standard deviation in Günthel et al. 2019. The result was in fact 50% higher than the more recent direct sediment-core measurement (Hartmann et al. 2020), meaning we may have overestimated the littoral methane contribution. While Peeters and Hofmann question the comparison to Hartmann et al.'s measurements (e.g. *“It seems unrealistic that such low sediment fluxes are representative for the average CH₄ flux from littoral sediments in the South Basin of Lake Stechlin.”*), there are independent studies indicating generally low methanogenesis activity in Lake Stechlin (Casper et al. 2003, Casper et al. 2005, Conrad et al. 2007), and that the majority happens below 20 cm of depth (Casper 1996), potentially explaining the low fluxes at the sediment-water interphase reported by Hartmann et al. (note, methane oxidation can efficiently remove the majority of methane).

Further, the mean and standard deviation of our littoral sediment flux estimate were incorporated in the Monte Carlo simulation to account for data uncertainties when computing OMP rates. When claiming that we underestimated the littoral sediment flux Peeters and Hofmann rely on the relationship between energy dissipation (ϵ) and the gas transfer constant (k_{600}). While parameterising this relation as $k_{600} \sim \epsilon^{1/4}$, Peeters and Hofmann missed to mention that the parameterisation of this relation is subject to ongoing research and that currently, there is no consensus which parameterisation produces more accurate values. Further, we did not deploy an outdated approach to deduce K_p from shear microstructure (e.g. Gregg et al. 2018) which we additionally refined by implementation of the law-of-the-wall in the unstratified epilimnion following Kirillin et al. 2012.

Peeters and Hofmann implied that we assumed sediment methane flux in Lake Stechlin was independent of temperature. Incorporating a temperature dependency of the littoral methane flux was unnecessary because we quantified the littoral methane flux in August at the same water temperature as it was in June and July (20°C) (see Supplementary Fig. 3 in Günthel et al. 2019). By applying the same value to May (<20°C), we likely had overestimated the littoral methane input. Note, we referenced the temperature dependency in the method section of our original study Günthel et al. 2019.

Instead of presenting relevant data or analysis for Lake Stechlin, Peeters and Hofmann simply mentioned the paper by Yvon-Durocher et al. Below is Fig. 2a from Yvon-Durocher et al. 2014 showing *“temperature dependence of CH₄ emissions at the ecosystem level”*:

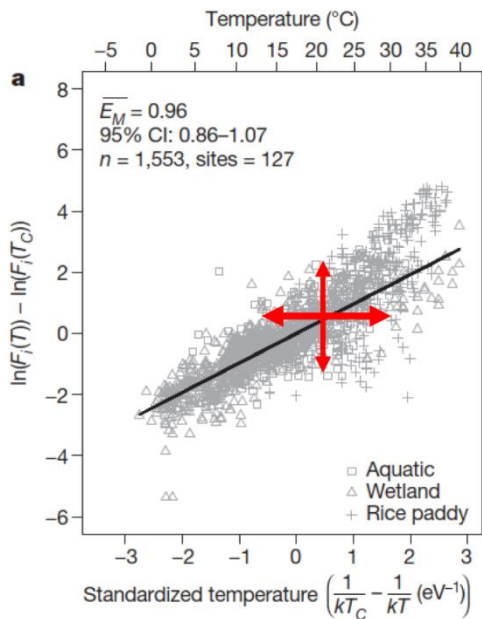


Figure 2a from Yvon-Durocher et al. 2014; red arrows are added by us to illustrate the data variability around the regression line at 20°C (approx. littoral temperature in Lake Stechlin). Note the y-variable is not the actual emission, but the difference between emission at absolute temperatures and emission at some average temperatures.

While methane emission is temperature dependent, there is a large amount of scatter around the general trend line in both x and y directions (red arrows added by us). Lake Stechlin surface mixed water temperature above the littoral was ca. 20°C (Supplementary Fig. 3 in Günthel et al. 2019). According to Yvon-Durocher et al. 2014 Fig. 2a, the corresponding y-value varies between 2.2 and -1.8 on a natural log scale, which translates to a >50-fold difference between the actual upper and lower values. Likewise, the same y-value could correspond to a temperature between 13°C and 30°C. We also refer readers to the Extended Data Figure 2 in Yvon-Durocher et al. “Correlations of average site temperatures with average CH₄ emissions and CH₄ emissions at fixed temperature for globally distributed ecosystems” which shows that temperature explains only 12% of the variance for CH₄ emissions in aquatic systems.

Supplementary Note 4:

Peeters and Hofmann speculated that the mesocosms may approach smaller CH₄ concentrations after a longer time period—We would welcome data to verify this speculation. Peeters and Hofmann also commented the central mesocosm was close to atmospheric saturation showing no indication of significant CH₄ production—This is consistent with what we discussed in the paper. Oxidic methane production is a biological process that depends on certain set of environmental conditions, including nutrients and the relevant organisms. When nutrients and the related biological activities became severely limited in the central mesocosm, oxidic methane production would become negligible as expected.

Supplementary Note 5:

We first explain why Peeters' and Hofmann's equation and their alternative oxic methane contribution to emission (OMC) estimations are incorrect, then we further clarify how we analysed DelSontro et al. 2018's data.

Peeters and Hofmann derived their version of OMC termed NOMC by comparing sediment and lake surface methane fluxes as $NOMC = (F_{surf,tot} - F_{sed,tot})/F_{surf,tot}$ (combining their equations of NOM and NOMC). By doing so, Peeters and Hofmann ignore any internal biochemical process of the aquatic methane production-consumption balance (e.g. *"This procedure neglects processes contributing to the mass balance..."*). In their calculations for additional lake estimates, Peeters and Hofmann further inadequately substitute the flux data $F_{surf,tot}/F_{litt,tot}$ with R_{CH_4} values (*"Relative [CH₄] decrease/increase due to oxidation/production"*) taken from DelSontro et al. (Supplementary Table 8) resulting in the formula $NOMC = (R_{CH_4} - 1)/R_{CH_4}$. DelSontro et al. calculated R_{CH_4} as the total change in methane concentration over the gas residence time (DelSontro et al. Table S8) relative to the background methane level along the transect (DelSontro et al. Supplementary Table 5)—this background methane was a combination of anoxic and oxic methane—as influenced by oxidation vs. production. In other words, DelSontro et al.'s calculations gave an indication of the dynamics of epilimnetic methane as influenced by the opposing processes of oxidation and production. Note that DelSontro et al. did not equate the background methane level to 'littoral methane'; to the contrary, their physical transport model predicts that littoral methane concentration decreases exponentially with distance from the shore due to dilution effect and emission loss, and in large lakes only negligible amounts of littoral methane ($\leq 1.5\%$) would reach the lake center. Actual measurements by DelSontro and colleagues showed that the concentration gradients largely deviated from this prediction (their Figs. 1, S4, S5, Table S5); accordingly, DelSontro et al. concluded that epilimnetic (oxic) methane production must be present. DelSontro et al. did not state that the observed concentrations (or background level) were due to littoral methane alone; yet, Peeters and Hofmann misrepresented the meaning of the "relative [CH₄] decrease/increase" (R_{CH_4}) in DelSontro et al. and arbitrarily set a 100% baseline value for littoral methane, then expressed any relative change as NOMC (i.e. the '1' part of their equation). Therefore, the NOMC presented by Peeters and Hofmann is misleading and unjustified. We refer readers to DelSontro et al. 2018 for details. Accordingly, Peeters' and Hofmann's NOMC cannot be compared to our OMC estimates.

We would further clarify how we analysed DelSontro et al.'s data. In addition to concentration data, DelSontro et al. inferred epilimnetic methane production vs. oxidation from

methane carbon stable isotope data by assuming that methane $\delta^{13}\text{C}$ values lower than -54 ‰ indicates epilimnetic production, whereas δ values higher than -54 ‰ indicates oxidation (their Fig. 3). This interpretation was inadequate because the precise biochemical pathways, hence the isotopic signatures of oxicly produced methane were unknown. As new information emerges (post publication of Günthel et al. 2019, e.g. Bizic et al. 2020, Günthel et al. 2020, Hartmann et al. 2020, Klintzsch et al. 2020), we now know that OMP can be linked to phototrophic fixation of inorganic carbon, which would likely yield a higher (heavier) $\delta^{13}\text{C}$ value than anoxically produced methane, thereby confounding isotopic signal of methane oxidation. To avoid uncertainties associated with the isotope data, we opted to estimate OMC based on methane concentrations along their sampling transect (data obtained via personal communications with Dr. DelSontro).

Discrepancy between observed methane concentrations and predicted methane concentrations based on their physical transport model along the transect represents contribution from oxic methane production, the rest was anoxic methane. We then extrapolated the transect data to the entire lake surface area (equivalent radius from DelSontro et al.) to obtain system-wide contributions of oxic and anoxic methane—Note this is vastly different from DelSontro et al. and Peeters and Hofmann, who only considered ‘relative $[\text{CH}_4]$ decrease/increase’ along the transect measurements.

To explore how OMC varied with lake morphometry such as surface mixed-layer volume (V) and littoral sediment area (A_{sed}), we calculated V from data in DelSontro et al. (surface area, surface mixed layer depth), and we estimated A_{sed} based on a littoral sediment slope of 45° . Even when we change the sediment slope from 45° to 5° as suggested by Peeters and Hofmann, giving higher littoral sediment areas, the overall relationship between OMC and A_{sed} over V only changes slightly (Fig. 1 in the main text).

Further, we would like to point out that Peeters and Hofmann excluded the source signature of oxicly produced methane from all of their isotope considerations.

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Lake	OMC	A_{sed}/V @ sediment slope = 45°	
	[%]	[m²/m³]	
Lake Stechlin Northeast Basin	63	0.02512	
Lake Stechlin South Basin	50	0.05483	
Lake Hallwil	73	0.01458	
Lake Cromwell	20	0.20169	
Lake Ontario	90.4	0.00009	
Lake St. Jean	84.7	0.00015	
Lake Nomingue	83.7	0.00107	
Lake Champlain	99.7	0.00105	
Lake Beauchene West-Basin	96.8	0.00122	
Lake Simard	81.8	0.00038	
Lake Camichagama	99.8	0.00098	
Lake Stechlin All Basins	55	0.04067	

A_{sed}/V @ sediment slope = 5°

[m²/m³]

0.02512

0.05483

0.01458

0.20169

0.00071

0.00125

0.00867

0.00854

0.00986

0.00312

0.00798

0.04067