

The manuscript by van Grinsven et al “*Methane oxidation in the waters of a humics-rich boreal lake stimulated by photosynthesis, nitrite, Fe (III) and humics*” presents a detailed study on the magnitude and control of methane (CH₄) oxidation in a small humic lake in Southern Finland. This study revisits some of the previously shown mechanisms regulating CH₄ oxidation in lakes –light, oxygen, nitrite, Fe (III), and humic substance. Although all these factors are known to stimulate CH₄ oxidation in freshwater lakes, assessing their roles and extent in a single lake water column is rarely attempted. Moreover, authors combined multiple tools – physico-chemical profiling, stable carbon isotopic ($\delta^{13}\text{C}\text{-CH}_4$) signature, near-ambient incubations with and without the addition of the above stimulants of CH₄ oxidation, and molecular assessment on the dynamics of CH₄ oxidizing bacteria (MOB). This article is well-written, and methods are adequately described. However, I feel that Introduction needs little more clarity. I suggest to re-write the introduction in such a way that objectives of the study are stated clearly and contextualized better. Moreover, I am missing a thorough discussion on the effect various factors on CH₄ oxidation; for example, light is stimulating CH₄ oxidation in this brown water lake – which is opposite to our general understanding that CH₄ oxidation is inhibited by light (Murase and Sugimoto, 2005, Dumestre et al 1999, Shelly et al 2017). I would suggest authors to build the discussion based on these previous studies. Probably, light play differently across depths within a lake and across the lakes based on the extent of “browning” (increase of DOC in aquatic systems) and if so, what could be the effect of ongoing “browning” on future CH₄ oxidation?

Specific comments

L7: I do not see any discernible CH₄ oxidation “hotspot” in the anoxic water column based on the ¹³C-CH₄ profile, although evident at ~3.0 m (Figure 2B).

L53: ...and classical MO – methane oxidation (MO)?

L69-70: Please rephrase

L71-73: I feel that the problem is not very well-defined.

L79-83: I would suggest rephrasing of this entire sentence to bring clarity.

L82: TEAa

L127: Please give little more details on the headspace equilibration; for example, how did you transfer the headspace gas into vials (?). Did you add water into the bottle to replace and take out the 20 ml headspace? Please mention how exactly you did it.

L137-142: I do not see any results on the fraction oxidized (f) or fractionation factor (α). Either you may provide the results or remove it from the Methods.

L206: Please provide the reasons for selecting these depths – or why you did not consider surface layers for oxidation measurements?

L230: Please provide the detection limit of the LI-CORE in the methods – I doubt how much we can rely on $0.01\mu\text{E m}^{-2} \text{ s}^{-1}$.

L262: How do you define epilimnion – if it is the well-mixed surface zone, it surprising to see such a large variability in $\delta^{13}\text{C-CH}_4$ (-50‰ to -35‰) within the epilimnion. Or do you think the first value (1m?) is erroneous? Please check.

L311: “Potential Methane Oxidation Rates” instead of “Methane oxidation rates incubations”?. If you are providing mass-balance based estimation of oxidation (based on $\delta^{13}\text{C-CH}_4$), it makes sense to have “Methane oxidation rates – incubations” followed by “Methane oxidation rates – isotopic mass balance”

L333: Sentence reads strange to me – “...down to a depth of 3.1 m (oxycline) in the surface..”

L341: “control methane oxidation rates were...” rephrase the sentence.

L345: “...shallow stratified lakes,...” Please be careful with the cited references – Lake studied by Blees et al 2014 (Lake Lugano) is 288 m deep, not a shallow lake. Similarly, check other references too.

L357: “pending light availability”?

L363: 0.99 ± 0.06 – space, similarly L365.

L364-365: It seems to me that O_2 is consumed within a short period of time, far before the termination of incubation around 48 hrs since the initial O_2 concentration is only $15\mu\text{M}$ (Table S2), while light incubations continue to provide O_2 through photosynthesis throughout the incubation. Please look into the time course of $^{13}\text{C-DIC}$ and see whether the pattern is linear or not. Please consider this aspect.

L371: I am not convinced – Table S2 suggest CH₄ concentration is 15 μM, which seems to be the “optimal concentration” for highest methanotrophic activity (*see*, Thottathil et al 2019), not at a level to induce O₂ inhibition.

L377-378: “..perhaps attributable to the smaller methanotrophic community” – this is in contrast to L484-485 where you states that “water column methane oxidation rates therefore seems not necessarily coupled to methanotroph cell number, but rather to cell activity rates instead”

L388-389: The local peak of CH₄ cannot be attributed to aerobic CH₄ production – for example, *see* Donis et al 2018 which showed that such metalimnetic peaks can only be a “physically driven accumulation”.

L399-401: You are suggesting two contradictory (and much debated) processes to explain the same phenomena of sub-surface CH₄ peaks. Based on the data from L. Lovojärvi and other similar systems, what is the most probable explanation?

L432: “It may be reasonably to assume...” requires rephrasing?

L454-459: I am not quite understanding why do pulses of CH₄ (that too bubble fluxes!) require to support alpha-MOB in the surface layers. In fact, recent studies have shown that Alpha-MOB are known to be regulated by oxygen concentration, particularly Alpha MOB shows high abundance at high O₂ concentration of ~200μM (*see* Reis et al 2019). Please look into those possibilities. Also, I doubt whether ebullition occurs at depth of 17.5 m (sampling location) to support the hypothesized bubble pulses. If bubbles are rising from 17.5 m depth, why does bubble dissolution in the water column support only the MOB at the surface layers?.

References

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