We are grateful to the reviewer, Thank you so much for this constructive and detailed review. Below we provide some preliminary response to the each reviewer's comments. (Reviewer’s comments are underlined for clarity).

Specific comments:

- I am happy to see that these authors spent the time to do the analyses and writing of a technical note paper, as I believe it can prove very valuable for a wide audience and can improve the methods and results of many future studies. The use of certain chemicals to preserve samples for gas analysis is very common, and it is therefore really nice that there is research on the effects of the conservation by several methods. I therefore want to thank the authors of this manuscript for working on this topic, which I think is highly suitable for the Biogeosciences journal.

Thank you, it is nice to see that our work was appreciated.

- However, I am currently unable to assess the results of this paper, because I lack key information on the reliability of the results. I think this paper has a good potential, but the aspects that are missing are key for the interpretation of all results, so they need to be clarified before I could properly assess the quality of the whole paper.

We are sorry to read this and apologize for taking your time, we will try to improve the manuscript and clarify all unclear points raised below.

- I find it difficult that I cannot see if the actions of adding the substances to the samples itself changed something in the gas concentrations. There are differences in the gas concentrations at t0. This can be attributed to rapid effects of the interaction between the added chemicals and the sample, but it can also be due to sample contamination or degassing during handling. T0 is taken within 24 hours, but it remains unclear if this is after 1 hour for certain samples, and after 23 hours for others. There is also no miliQ or demineralized water control. To have these issues addressed, in a reply to this comment but also in the manuscript, would give me more certainty about the results.

Unfortunately, the experimental design was imperfect, and would have been nice to have some benchmark steps such as pH measurements of the samples right after preservative addition. However, we can easily rule out sample contamination and degassing during handling, we have a really high reproducibility between the 6 replicates, and the relative difference between the unfixed and fixed samples are not consistent with degassing, this will be described in the results section.

Regarding the time for T=0 samples, our wording was too vague, we apologize for that. All samples were treated at the same time within 1h or 2h (this includes transfer from the 5L bottles to the 120mL glass bottle and fixation), we will dig up the exact timing, kept dark and cold overnight and then analyzed on the GC the next day.

If this is required, we could run a simple additional field test with lake water, adding the preservatives, and measuring the pH of the sub-samples 24h after fixation (and include a demineralized water control). Although this would not be the same sample batch (the water might have a slightly different composition), we would follow the same sampling and handling procedures.

- I would think it benefits the paper if results and discussion are separated into different sections, but I leave it up to the editor to decide on this, as I know there is also personal preference involved on that topic.
I believe, we agree on this one and also think the manuscript would be clearer with results first.

- Another key issue that I would like to hear more on is why the t0 concentrations of CO2 of the different treatments is that different. You write some about it, but as I see this as a major factor for many of your interpretations, I would like to have it addressed more in depth and more clearly. Why are there no error bars on the Cu and Hg boxes of CO2?

The large difference on CO2 concentrations at T=0 is due to rapid interaction with the preservatives. Maybe this additional field test would also help document this rapid acidification of the samples with HgCl2 and CuCl2. The description of such a test would be included in the supplementary material.

- Another important overarching issue is that it seems O2 significantly decreased in the samples with CuCl2. Is this indeed the case? This is of major importance, as it would suggest incomplete inhibition of microbial processes, and would affect all results for the CuCl2 treated samples.

Yes, there is a significant decrease in O2 over time which is likely due to incomplete inhibition of microbial processes. We didn’t focus on this earlier because the acidification of the samples was already a sufficient reason for disapproving its use as preservative. We will also describe this effect in a clearer manner and warn about potential incomplete inhibition with CuCl2.

- Specific comments on certain parts of the manuscript are included below.

**Introduction**

- The first paragraph is a bit odd. I think it is most important that the reader knows why it is needed to preserve the samples, not what they are used for in the end. I would put more emphasis on the processes in the bottles that will change the concentration.

Agreed, and thank you, we can easily streamline this paragraph.

- I think it is important to also mention that ZnCl2 and CuCl2 are often used as toxic inhibitors. And give some details on why it seems certain researchers pick which inhibitor (or put that in the discussion). Problems with the disposal of HgCl2 would be good to mention as well, as well as the costs associated with it.

Thank you, we will do so and mention the problem about HgCl2 disposal.

- 48 + 49-50. Based on what did you decide to pick these papers as references?

We performed a wide literature search for studies specifically mentioning HgCl2 as preservative for dissolved gas samples in their methods.

- 75 – 81. The tone of this paragraph is also a bit odd. You state quite suddenly that CO2 concentrations will be overestimated by HgCl2, while that is not really clear from the previous paragraphs. And the last line is way too firm for an introduction. The introduction should state the current state of the art, not opinions.

We will adapt the end of the previous paragraph and adjust the tone here. Thank you for this suggestion.
• 82 – 92. I would structure this paragraph differently. First, in neutral words (so no effective, overestimation, etc) explain what you investigated. Then state in a few sentence the key findings of your research.

Agreed, and

Methods

• 96. Header ‘study area’ does not cover the content of the paragraph.

Agreed, the study sites will be introduced first and then “Sampling procedure” will be used as the section title.

• 98. What is carefully collected? Explain in more detail.

The sample procedure will be better described here.

• 100. Avoid = limit. And not gas loss, but gas exchange with the atmosphere.

Agreed.

• 107. That cannot be uniform for all Norwegian lakes, right? Do you mean that is the case for this specific lake, or do you mean this lake was like the lakes tested in that de Wit paper?

This lake is like the set of 1000 lakes, carefully selected to be representative for Norwegian lakes, presented in de Wit et al. (2023).

• 112. T=0 could be at any moment during the first 24 hours? Or was it the same for all samples? Were they randomized for the moment of analysis, or were some treatments done first and others later?

T=0 samples (as the other time points) were all treated at the same time, and also randomized, yes. This will be clearly described.

• 112. Were the same bottles measured at t0, t1 and t2, or were the bottles sacrificed?

The bottles were sacrificed to avoid introducing a headspace and sample dilution etc. This is why we had 72 bottles (6 replicates x 3 time-points x 4 treatments). This will be clarified.

• 113. ‘as the water samples were collected’. Which water samples? Is it not the same as the 5L bottles? Unclear.

Yes, but the 5L bottle was filled in the field, and up return in the lab, the 72 120mL bottles were filled.

• 123. Unclear what ‘it’ means.

“it” refers to “estimated toxicity”. This will be rephrased.

• 129. Why were they stored cold? In general, poisoned sampled are not kept cool, as far as I know.

This is how we have worked so far to be on the safe side (see e.g., Clayer et al. 2021), and this also prevents the samples to be subjected to changes in room temperature. Keeping water samples cold
and dark is the best way to optimize sample preservation. There is no guarantee that poisoned samples will be 100% inert, keep them cold helps to limit any microbial activity.

- 133. Remove ‘unfortunately’.
Will do.

- 127. Was the same amount of liquid added to all samples? Was the miliQ flushed to remove the target gasses? What was the volume of the sample bottles?
Yes, the same volume of 240µL was added to all bottles. And no miliQ water was not flushed, but given its small volume, its concentration is insignificant for the water samples. This will be clarified.

- 141. Sealed with what?
The samples were sealed with gas tight butyl rubber stoppers as for the first experiment. We used the sample bottles and caps for both experiments. This will be clarified.

**Results and discussion**

- Fig. 1. Please split the graphs of O2 and CO2 and CH4 and N2O, it is now not clear that boxes 5-8 are a different gas. 100% saturation at which temperature? That of the lake water, at the 4 degrees of the storage, or the temperature during measurement?
Everything is reported back to *in situ* temperature. This will be clarified, sorry for this oversight. In fact, saturation is only relevant for *in situ* conditions since after sampling, the water samples were never in contact with atmosphere. Thank you for the nice suggestions, we will split the graphs here and clarify the caption.

- Table 3. This table is a bit hard to read, while it does contain very interesting information. Can you add some lines or italic or bold text or something, to make it easier for the reader to focus? What is the ice free season number made up of? Please explain in caption. Also write in the caption what diff is (I know it seems obvious but still good to write down).
The style of the journal is to avoid additional line, but we can remove the “%” symbol on row 3 and 6, remove the decimal on the row 1 and 2, and arrange the alignments (probably increase the spacing a little) to improve visibility. The ice-free season is the average over the whole experiment duration from April to November. This will be clarified in the caption.

Note also that the “Preservatives” labels were inversed, lower values (1st and 4th rows) are from “unfixed” samples analyzed for DIC while the highest values were those from samples fixed with HgCl2 (2nd and 5th rows).

- Fig. 3. Is the lower panel a useful addition? Or can I already get the same info from the upper panel?

- Fig. 4. Isn’t this the exact same info as in table 3? No need to show it twice. I think the graph is much nicer, it brings across your point very clearly.

Table 3 and Fig. 4 both display the monthly mean, yes. In addition, Fig. 4 displays the observations and interpolated daily data. Table 4 also shows the relative difference between fixed and unfixed
samples in % as well as the mean for the whole duration of the experiment. Since this is a technical paper, we believe it is useful to keep both for clarity and depending on the reader’s affinity for table or figure. However, we agree that we should specifically state that these two objects display the same data. This will be clarified in the captions.

- **357-362.** Please address why the t0 concentration in the inhibited samples was so different from the control sample, and why the range in concentrations was much larger. Is it because of sample contamination with air during the addition of the inhibitors? If this is the case, that is not necessarily a bad thing, if you then also explain that that is one of the risks of inhibitor additons to samples.

The CH4 concentration at T=0 in the unfixed sample is below atmospheric saturation, as shown by the horizontal line in Fig. 1. In contrast, we expect the CH4 concentration to be oversaturated in “real conditions”, as supported by Valiente et al. 2022, Cayer et al. 2021. The CH4 concentration at t=0 in the fixed samples are consistently between 0.2 to 0.33 µM, independently of the preservative which suggests that CH4 in the unfixed samples has been consumed (which is realistic considering oxic methanotrophy reaction rates, see L. 369-371) while the concentration in the fixed samples is close to real conditions.

The reason why the range of CH4 concentrations in the fixed samples is larger is likely due to minor gas losses during handling. In fact, at the lab temperature (which is higher than in situ lake temperature) and after some storage time, the water samples will have a higher total gas oversaturation than at the start of the experiment. Under these conditions, CH4, being the least soluble of the gases, will be lost first. Such CH4 losses are much smaller for the “control unfixed” samples which show much lower CH4 concentrations. This will be clarified in the manuscript.

- **382.** Do you suggest that the microbial processes are not inhibited, or that there are abiotic proceses at play? If it’s the microbial processes, then how is it possible that these microbes are not inhibited, but the ones using O2 are?

Yes, we suggest that microbial processes are not completely inhibited. We will make this point clearer. Note that certain metabolic pathways can be selectively inhibited and N2O is likely much more sensitive (being at 10’s nM levels) to subtle microbial activity than O2 (being at 100’s µM levels).

- **386.** Please mention whether there were statistically significant changes (between timepoints or between treatments) for N2.

There were no significant changes between timepoints or between treatments for N2. This will be added.

- **408.** In the CuCl2 treated samples, you have both O2 consumption and CO2 production. Why do you think these are not linked?

Yes, good point. CO2 production in the CuCl2 treated samples is likely partially link to O2 consumption through microbial respiration. However, the CO2 production being much larger than O2 consumption, an additional source of CO2 is needed.

- I have not provided detailed comments on the later sections, as I think it important to know more about the CO2 results first, like I stated in my starting comments.
We hope, we have provided some clarifications and will continue to do so at a later stage if the manuscript is going through the next stage. Thank you for your assessment.

References:

