Discussion of “Pathways of CH_4 formation and emission in the subsaline reed wetland of Lake Neusiedl”

Baur et al.

In the following, the reviewer’s comments are set in *italic* and the authors’ responses are marked in blue.

Authors’ response to Referee 1

Review of “Pathways of CH4 formation and emission in the subsaline reed wetland of Lake Neusiedl”

We thank the reviewer for the time and the valuable comments for improving our manuscript.

Here, Baur et al. continued the extensive research previously conducted by this scientific group in a reed wetland located at a lake in Austria, the title argue that they were focusing on understanding CH4 formation pathways. Although the title attempts to encompass this complex objective, I believe the methodology employed does not fully address such broad scenarios. Instead, I recommend emphasizing diel variations across different seasons rather than the overall CH4 formation processes in the studied ecosystem. I mention this because you are inferring CH4 pathways without performing an appropriate experimental analysis. Microbial assessments combined with isotopic measurements from emissions alone might not be sufficient to clearly identify these pathways. For robust conclusions, incubation experiments using substrates or more detailed analyses would be necessary. I am currently providing general comments on the manuscript, in the next review round, I will likely offer more detailed feedback. At this stage, however, I think the manuscript needs substantial improvement.

We thank the reviewer for the comment and agree that the title was perhaps a bit too broad and that not everything could be fully answered with this study. However, we wanted to provide insights into the various emission pathways, seasonal isotopic source signatures, methanogenic characterization (following Whiticar et al. (1986)), and the microbial communities involved in this ecosystem. We suggest changing the title to “Temporal dynamics of CH_4 emission pathways in the subsaline reed wetland of Lake Neusiedl”.

Firstly, the Introduction appears poorly organized. Although I understand you might be addressing topics similar to those from previous manuscripts, here the Introduction is brief and omits important details. Regarding isotope information, please ensure that you clearly describe and contextualize isotopic methods and their relevance in terms of diel variations, and their respective emission. Additionally, having paragraphs consisting of only one sentence is inadequate, please expand or combine such sentences with relevant information to provide greater clarity. Several process definitions are merely mentioned but insufficiently explained. For example, you need to clearly introduce the effects of sulfate and oxygen availability on sediments, as well as their potential impacts on CH4 emissions. Not all variations in CH4 emissions can be explained by oxidation alone; rather, the microbial community may also utilize alternative substrates for respiration. This aspect is currently missing and should be addressed explicitly in the Introduction.

Thank you for your recommendations. We will improve the Introduction chapter by expanding the explanations of the various processes in the sediments involved, including the isotopic methods, and by reorganizing the chapter structure. However, we must mention that the diel variability of isotope values (e.g. from gas in the atmosphere) was not part of this study, only the seasonal change of the isotopic source signature of CH_4 (in the sediments).

Secondly, I understand that the methodology was adopted from previous studies, however, you need to briefly include specific details about your own methods. For example:

(i) Please include, either as an appendix or supplementary material, information about the observed flux determined using the chambers and the Picarro analyzer, clearly indicating instances when data were too erratic. Additionally, specify the Picarro measurement mode and the frequency of data acquisition. For isotope analyses, clarify how long measurements were stabilized before recording values, as well as the measurement uncertainties (I remember it is necessary like 6 min to confirm the isotopic value).

Yes, these details are mentioned in Baur et al. (2024b), but for better traceability, we will add more information in the appendix, e.g. about the Picarro measurement mode, Picarro measurement frequency, isotope analysis, and flux equations/determination.

(ii) Table 1 currently provides limited information; please add the sample sizes corresponding to the reported uncertainties. Lastly, regarding the laboratory-analyzed samples (although not clearly described), could you clarify if the reported standard deviations for sulfate, TOC, and chlorophyll-a resulted from temporal sampling conducted over a 24-hour period?

Yes, most parameters in Table 1 show the temporal means and standard deviation over 24 h, but it also includes 3 spatial replicates (see the 3 water sample locations in Figure 1d). Only for two parameters (water level and leaf area index), which are not assumed to change over 24 h, only spatial means and standard deviation are given. We will make this clearer by including this information in the caption of Table 1 and also add the sample sizes. This table is located in the subchapter 2.2 “Measurement setup” of the Methods chapter and describes the environmental conditions at the site during each 24 h campaign. The intention of this table was also to show and allow their comparability with other days/nights in the same season. In order to keep the manuscript concise, we have refrained from including the water sample data in the results chapter. However, as the reviewer question indicates, that this might be of great interest for the reader, so we will add the lab methods (of water analysis) and detailed data, including diel variability of these parameters, e.g. in the appendix.

Also, I am curious about how you obtained ebullition data during periods of very low water levels, particularly in summer when this emission pathway appears most prominent in your results.

In the Baur et al. (2024b) study, the bubble traps were permanently installed from March to July 2021 until the water level in the reed belt was no longer above the surface. As the traps were installed at a time of higher water levels (early March), we were able to measure ebullition (collection of trapped gases) with these traps at time of very low water levels (June/July). However, for our study here, the gas collection interval of the bubble traps was every 6 h to investigate if there was a diel pattern, to quantify the ebullition pathway during 24 h and also to compare it with the other pathways. For the rest of the time, the trapped gas was collected once a week and used for the study of Baur et al. (2024b). These results show an increase in the ebullition rate in the reed belt from March to July. Contrary to your comment, we do not see the ebullition pathway as the most prominent emission pathway in summer due to two high flux rates (out of 36), but rather the plant-mediated transport due to the maximum and highest median flux rate (see Table 2 and Figure 2).

Furthermore, I am surprised by the high chlorophyll-a values observed in winter, are you certain these measurements represent chlorophyll-a and not turbidity caused by shallow water levels?

In the reed belt, the surface water has a brownish color, but it is clear and not turbid (unlike the open lake water of Lake Neusiedl). The relatively high chlorophyll-a concentrations in winter are probably more likely due to algae and low water levels (higher concentration per liter).

(iii) Ebullition measurements using bubble traps appear highly biased due to the short deployment duration. Given that ebullition is a very stochastic process, it is possible that significant emissions were missed simply because the traps were placed away from emission hotspots. I understand the difficulty involved, as this is one of the major challenges when studying ebullition. In previous studies, you deployed bubble traps for longer periods, however, the focus on diel variations in this study limited that possibility. Do you think a single measurement day per season provides sufficient data to establish a representative ebullition pattern? Looking into the data I would not discard as the main source of emission.

Since we had 9 bubble traps (always 3 traps on one board) collecting bubbles in the reed belt, we believe that the spatial heterogeneity of ebullition and ebullition hotspots are well reflected. In order to investigate the diel pattern in different seasons in this study, the gas collection time of the bubble trap had to be shorter. Combined with the other study on ebullition rates at different sites and over a longer period of time (Baur et al., 2024b), we have a better picture of ebullition at Lake Neusiedl. Of course, it is always better to have more measurement days, but since 24 h campaigns are very resource intensive, we were not able to conduct more such campaigns. In addition, the drying out of the reed belt was not foreseen, otherwise longer measurements of the bubble traps would have been possible (after July).

The soil chambers faced several issues related to ebullition (as you commented in the method section), suggesting that significant amounts of CH4 accumulated in the surface sediments,which may have been missed due to the short deployment time of the bubble traps (in this case absence of water, but still emission). Given this limitation, why did you not consider using the ebullition events detected by the Picarro analyzer for fluxes to determine other sources of CH4? With proper data management, you could potentially quantify ebullition as part of the total CH4 flux using these measurements.

Yes, in the summer campaign there were ebullitions at the beginning or during chamber closure in 8 out of 24 sediment chamber measurements. These ebullitions were likely triggered by the movement of the chamber closure, because the top sediment layer (“fluffy layer”) was very mobile, as mentioned in the Methods chapter in L160ff. Thus, we consider this release not representative. Since one aim of the study was to investigate the diel pattern of the naturally released methane emissions for each pathway and season (and not the total ecosystem emissions, which were investigated in Baur et al. (2024a)), we refrained from using the possibly artificially triggered ebullitions at the beginning or during the sediment chamber closure. As the experimental design has foreseen to measure the diffusion pathway with sediment chambers and the ebullition pathway at the same time (in summer) with the bubble traps, we did not want to overestimate this pathway by double counting. Since the bubble traps collected a lot of bubbles during the summer campaign, we believe that we are taking the accumulation of methane in the top sediment layer into account, at least to a certain extent.

Thirdly, the presented results represent measurements performed over just 1 day per season. I understand this limitation, it is because you were also collecting data on additional topics, which have been integrated into other published manuscripts. Although you made a substantial effort to collect this data, some results may still be significantly biased due to the short sampling period, particularly for ebullition as previously mentioned.

Yes, only one 24 h campaign was conducted per season. This may lead to some limitations, but we also had to consider the feasibility and the purpose of this study. We still believe that this study contributes to understand the diel variations in this ecosystem and its emission pathways, despite only one year of measurements. In addition, we have published a study with continuous eddy covariance measurements that provide information on the total methane fluxes of this ecosystem (see Baur et al. (2024a)). As mentioned in L362ff, it makes sense to extend the ebullition measurement time intervals, especially in spring. However, since higher ebullition rates are associated with higher temperatures, e.g. in summer, shorter measurement time intervals of 6 h are also useful, as the measured values show (67% of the ebullition rates greater than zero, see L247ff). Since the focus of this study is on the different emission pathways, we wanted to compare the ebullition rate with the other emission rates and their diel pattern.

But also, plant-mediated emissions are not fully isolated, as total emissions originating from sources external to the plants could have contributed.

We are not sure if we understood your comment the way you meant. Here are our responses on how the emissions from the vegetation chambers or the plant-mediated transport itself might have been affected.

We agree that for vegetation chambers, the plant-mediated transport of reeds within the chambers cannot be completely isolated from diffusion from the underlying sediment or water surface. However, the major contribution or dominance of plant-mediated transport in the emission rates in the vegetation chamber compared to the diffusion pathway (at the water-air or sediment-air interfaces) is quite clear, as can be clearly seen from the much higher emission rates from the vegetation chambers in each season compared to the sediment or floating chambers. Therefore, we believe that the effect on the total emissions in the vegetation chambers should be relatively small. Furthermore, vegetation chambers are a common method for estimating plant-mediated transport of reed plants, see also van den Berg et al. (2020) or Jeffrey et al. (2019).

We also agree that the reed stems are probably well connected to each other by their rhizomes and are most likely all form “one” plant. However, this makes it almost impossible to isolate one reed stem by any measures (e.g. clipping, etc.) without destroying the reed ecosystem in that area. We had the opportunity to conduct research in the core zone of a national park with an exceptional permit and were therefore instructed to disturb the ecosystem as little as possible. We kept the chamber closure time as short as possible to minimize artefacts. Due to the installation of the bottom frame of the chambers in advance (in the winter before the campaigns), we had to cut the sediment at least 10 cm deep, which of course also meant that the rhizome connections with the surrounding area might have been severed.

The figures require additional information, as it is currently difficult to interpret the origin of the presented data. For instance, in Figure 2, it's unclear what each data point represent, are *these individual measurements or average? If they represent average values, please clearly indicate this and the uncertainties. Additionally, if a polynomial function has been applied, specify its order. However, I question whether using a polynomial fit is appropriate in this context; please clearly justify its application as a fit rather than presenting the raw temporal data directly. Also what is the point to fit to this polynomial?*

In Figure 2, each point represents a single flux rate. We will make this clear in the caption and also indicate the order of the polynomial function. Since the data points represent the raw flux time series, we used the polynomial fit (with the 95% confidence interval) to visualize the average (and uncertainty) diel pattern of each emission pathway, including the peak (if available). An advantage of a polynomial fit is that it can also show smaller temporal variations, and in our case, it gave us the opportunity to show the temporal pattern with the raw data points and the exact measurement time without aggregating over time (e.g. one measurement round). We consider this advantageous as for example, in summer we had to measure 3 different manual chamber types with 3 spatial replicates each, it took quite some time to measure all of them one after the other (including flushing time of the cable and the measuring cell of the gas analyzer) and therefore the light or other meteorological conditions could have changed during one measurement round.

Regarding Figure 3, the source of the data is unclear, and I am particularly interested in understanding why these results differ substantially from those previously published in your earlier manuscript in Baur et al. (2024, DOI: 10.1016/j.scitotenv.2023.169112). There is no clear oxidation pattern in Figure C. So you need to expand the information about it. Does this figure include all isotopic signature data from Figures A and B in the four points? Additionally, could you clarify at what value is used during the Picarro measurements?

The data points shown in Figures 3a and b (Keeling plots) are in the x axis the reciprocal of the mean dry mole fraction of CH_4 (or CO_2) and in the y axis the mean stable carbon isotope ratio $\delta^{13}C$ of CH_4 (or CO_2), measured with the Picarro G2201-i during the closure of each chamber measurement. These points are used to calculate the seasonal isotopic source signatures (y-intercept of the regression line) using the Keeling plot approach. This approach is explained in the Methods chapter in L118ff and L125ff. However, we will clarify this with a better description in the caption of Figure 3.

The methanogenic characterization plot after Whiticar et al. (1986) in Figure 3c uses the four calculated seasonal isotopic source signature pairs, which were determined in Figures 3a and b as the y-intercept values of the regression lines and their standard errors. The winter source signature with its uncertainty (standard error) colored in black in Figure 3c is partly in the range of methane oxidation. This means that in this plot the carbon fractionation factor α_c is between 1.005 and 1.03. To facilitate understanding of the values used in Figure 3c, we will adjust the explanation in the caption.

In Baur et al. (2024b), in contrast to the manuscript submitted here, the directly measured $\delta^{13}C$-CH_4 values of the collected gas from the bubble traps are used in the methanogenic characterization plot. Ebullition is known to have no isotopic C fractionation, unlike other processes such as diffusion or methane oxidation. Therefore, the isotopic values of the ebullition gases can be used directly as an indicator of the methane source in the sediments without calculating the source signatures with a Keeling plot, as it was done here for all chamber measurements. Our study confirms the results of the methanogenic characterization plot (according to Whiticar et al. (1986)) from the previous study that acetoclastic activity is indicated as the main pathway of methanogenesis in this reed ecosystem, not only for the months of March to July (Baur et al., 2024b), but for all four seasons (see Figure 3c). In addition, this study was the first to determine the seasonal source signatures for CH_4 and CO_2 for all four seasons in a reed wetland (y-intercepts in Figures 3a and b).

We will make these points clearer in the revised manuscript.

References

Baur, P. A., Maier, A., Buchsteiner, C., Zechmeister, T., and Glatzel, S.: Consequences of intense drought on CO2 and CH4 fluxes of the reed ecosystem at Lake Neusiedl, Environ. Res., 262, 119907, https://doi.org/10.1016/j.envres.2024.119907, 2024a.

Baur, P. A., Henry Pinilla, D., and Glatzel, S.: Is ebullition or diffusion more important as methane emission pathway in a shallow subsaline lake?, Sci. Total Environ., 912, 169112, https://doi.org/10.1016/j.scitotenv.2023.169112, 2024b.

Jeffrey, L. C., Maher, D. T., Johnston, S. G., Maguire, K., Steven, A. D. L., and Tait, D. R.: Rhizosphere to the atmosphere: contrasting methane pathways, fluxes, and geochemical drivers across the terrestrial–aquatic wetland boundary, Biogeosciences, 16, 1799–1815, https://doi.org/10.5194/bg-16-1799-2019, 2019.

van den Berg, M., van den Elzen, E., Ingwersen, J., Kosten, S., Lamers, L. P. M., and Streck, T.: Contribution of plant-induced pressurized flow to CH4 emission from a Phragmites fen, Sci. Rep., 10, 12304, https://doi.org/10.1038/s41598-020-69034-7, 2020.

Whiticar, M., Faber, E., and Schoell, M.: Biogenic methane formation in marine and freshwater environments: CO2 reduction vs. acetate fermentation—Isotope evidence, Geochim. Cosmochim. Acta, 50, 693–709, https://doi.org/10.1016/0016-7037(86)90346-7, 1986.