Answer to comments of Carsten J. Schubert

High-resolution vertical biogeochemical profiles in the hyporheic zone reveal insights into microbial methane cycling

First of all, we would like to express our deep gratitude for the reviews of both reviewers.

Rev. 1 mentioned that this is a very nice manuscript. He was impressed with all the data provided by the authors and the thoroughly discussed results using a variety of techniques. He also said that the results have not been over-interpreted, as is often the case in the recent literature. In contrast, Rev. 2 was more critical and is discussed in more detail elsewhere.

Nevertheless, we have seriously considered all suggestions, addressed the highly valuable recommendations where appropriate and possible, and have provided detailed responses and explanations below. The updated manuscript with changes marked in blue color will be uploaded separately. Line numbers in this file refer to the updated manuscript.

L. 36: Please convert also to Tg
This has been converted: 756 Tg CO2 equivalents

Fig. 2: I think a continuous sampling numeration from A to E would be easier to follow.
In fact, our first intention was to sort the profiles by location along the stream. However, writing the results and discussion section we realized that the description and argumentation is better to follow when sorting the profiles by season. The season plays an important role in the interpretation of the geochemical gradients and with this order the results and discussion part could be structured more clearly. Therefore, we would prefer to stick to the order that we chose for the submitted manuscript.

L. 165: I have never heard about using a peeper for methane sampling. Could you show that in those 15 min methane did not diffuse out?
Thank you for this good comment. It is true that CH_4 diffusion out of the chambers is possible. This is why we worked as quickly as possible during sampling. We did not perform additional experiments to quantify the influence of CH_4 diffusion out of the chambers within the 15 minutes of sampling. We think that losses during this time are negligible, because measured concentrations did not show any correlation with the time they were sampled. In addition, measured CH_4 gradients seemed to be smooth and consecutive measurements in line. However, again it is still possible that measured CH_4 concentrations slightly underestimate true pore-water concentrations. Stable isotope values of CH_4 appear plausible and do not seem to be affected significantly by small losses of CH_4. We have added the information to the methods part, that small amounts of CH_4 could potentially diffuse out during sampling in the field and that we could underestimate measured concentrations (L. 160-162).

“Nevertheless, small amounts of CH_4 could diffuse out through the membrane or escape during sample injection and thus, measured CH_4 concentrations might be slightly underestimated.”

Fig. 3: This a1-a6 is a little bit confusing. The stations are called A-E why now make it a1 to 5. I would suggest to let this small letter a,b,c, out.

We included a numeration, because the Biogeosciences figure content guidelines state that "Labels of panels must be included with brackets around letters being lower case (e.g. (a), (b), etc.)". To achieve a better understanding we propose to following two solutions:

- We could leave out the numbering (a1) to (c3) completely since subpanels in the first row have headers including the profile name. This would be the preferred option by the authors, but must be clarified with the editor.
- Otherwise, we can change the order of the numbering, such that (a) to (e) are used for columns and (1) to (3) for rows. Then, (a1) to (a3) would correspond to profile A and (e1) to (e3) to profile E. This might be less confusing.

L. 454: I do not understand what you mean here, could you please explain.
We rephrased this section and hope that the argumentation is easier to follow now (L. 459-460). The section now reads as follows:

"The model is applied to find the depths of reactive production and consumption zones. Calculated reaction rates are used to compare profiles, but due to the limitations described above, absolute values should not be over-interpreted."

Fig. 5: Also here I found it weird that although profiles are labelled A-E additionally a) to c) is used. I would omit this.
Similar to Fig. 3, we used the labels to comply with the figure content guidelines. If this is accepted by the journal editor, we can remove the labels (a) to (d).

L. 516: I find this one publication showing this methane production process little convincing that this same process is happening here. Is there methanol data? Where should the methanol coming from? Can you elaborate a little more on this and maybe which other substrates could play a role.
Unfortunately, there is no methanol data. However, we have added some more detailed discussion about the potential educts for the H_2-dependent methylotrophic methanogenesis (Methanomassiliicoccales and Methanomethyliales) based on the available literature. Moreover, a relatively strong depletion in $\delta^{13}C$ in CH_4 supports the assumption that CH_4 might be formed from methanol. Changes have been done to L. 521-529:

"The Methanomasiliicoccales and the Methanomethyliales both exhibit metabolic pathways in the genome indicative of H_2-dependent methylotrophic methanogenesis (Berghuis et al., 2019; Vanwonterghem et al., 2016). In saline or sulfate-rich environments, where methylated compounds like trimethyl amine or dimethyl sulfide are available as non-competitive substrates, this pathway can be of high importance (Conrad, 2020), but it is less considered in freshwater environments. However, Methanomassiliicoccales have been linked to CH_4 production from methanol in freshwater wetlands (Narrowe et al., 2019). Methanol can be derived from pectin which is contained in terrestrial plants (Conrad, 2005) and thus, the combination of a high relative abundance of Methanomassiliicoccales combined with a high input of allochthonous plant material found in the sediment cores render this production pathway possible. The strong depletion in $\delta^{13}C$-CH_4 in the methanogenic zone supports the potential for CH_4 production from methanol. Carbon fractionation factors related to CH_4 production from methanol (ε_C = 68-77) are similar to those of hydrogenotrophic methanogenesis (ε_C = 55-58) and much higher than for acetoclastic methanogenesis (ε_C = 24-27) or CH_4 production from other methylated compounds (Whiticar, 1999)."