

## **Response (in bold) to comments by the Referee#2**

This study investigated methane production pathways at two profundal and deltaic sites in sediment of Lake Geneva and sought to examine how the sources and compositions of organic carbon impact methanogenic potential. The authors measured stable isotopes of methane, methanogenesis rates with radiotracers, and methanogen communities and concluded that hydrogenotrophic methanogenesis was the dominant methanogenic pathway at both sites. They analyzed the concentrations and carbon isotopic compositions of long-chain fatty acids to constrain the source of organic carbon, but did not observe a clear effect of OC source on methane production. The methods were all sound, the experiments were well designed and the results were interpreted correctly. Overall, this study is important to help understand the pathways and controls of methane production in lacustrine sediments. I really appreciate this work and only have some minor comments before acceptance.

**Response: We thank the reviewer for this positive assessment.**

1. Line 177-178. Please double check labeled position of carbon in acetate. As far as I know, 2-<sup>14</sup>C-labeled acetate is <sup>14</sup>C in the methyl group rather than carboxyl group. This could be important for the interpretation of the results, as the methane carbon is derived from the methyl group during the acetoclastic methanogenesis.

**Response: We thank the reviewer for catching this. We have corrected the description of the 2-<sup>14</sup>C-acetate labeling to clarify that the <sup>14</sup>C label is in the methyl group, which is the carbon converted to methane (Line 178).**

2. Line 394-400: I am curious why DIC concentrations were much higher at the low TOC site of DS?

**Response: This is an interesting point. Possible explanations may include differences in carbonate buffering capacity, respiration pathways, or porewater exchange. However, we decided not to speculate about this aspect further.**

3. Line 560-562: The authors mentioned the production rates of H<sub>2</sub> or acetate) could impact methanogenic pathways, so I wonder if you measured hydrogen concentrations in the original sediments.

**Response: We did not measure hydrogen concentrations in this study. However, we note that while hydrogen concentration measurements can provide some insights, they do not necessarily reflect production or consumption rates. Accurate assessments of hydrogen dynamics would require dedicated incubation experiments, which were beyond the scope of this study.**

4. Line 672-675: Since a number of previous studies observed the dominance of hydrogenotrophic methanogenesis in lake sediment, this study further confirmed this conclusion with more concrete evidence, but this is not a new finding. So I would suggest to rephrase the text here.

**Response: We agree that this observation supports, rather than introduces, a novel finding. We have rephrased the text accordingly: "While the dominance of hydrogenotrophic (CO<sub>2</sub> reduction) methanogenesis in lake sediments has been reported previously, our results reinforce this conclusion with consistent geochemical and isotopic evidence across contrasting sedimentary regimes in Lake Geneva." (Line 669-672).**

5. Line 696-698: This sentence came lack of context. Please clarify.

**Response: We have revised this sentence ("Since methanogens rely on syntrophic and other heterotrophic bacteria to generate these key substrates, the activity and composition of the broader microbial community may play a significant role in shaping methane production rates", Lines 695-698), and integrated it with the following paragraph to provide better context and ensure a more logical flow of ideas.**

6. Line 742-750: It is more likely that the <sup>13</sup>δC shift of TOC reflected the source of organic matter rather than the decomposition of OM. Can you get some clue for the quality of organic matter based on the sediment age measured with tracers?

**Response:** We appreciate this insightful comment. Distinguishing whether the observed  $\delta^{13}\text{C}$  shift in TOC is primarily driven by changes in organic matter sources or by selective decomposition remains challenging. At PS, within the zone of high hydrogenotrophic activity, the low and surface-like C/N ratios suggest the persistence of relatively labile OM, rather than a major change in the OM source. However, lipid biomarker profiles, particularly the concentration of long-chain fatty acids, indicate a shift toward more autochthonous OM. We have clarified this in the revised manuscript as follows: "The  $\delta^{13}\text{C}$  shift of TOC within the most active zone at PS likely reflects either the selective loss of isotopically light OM fractions, or changes in organic matter sources, as indicated by depth-related variations in both short- and long-chain fatty acid concentrations." (Lines 746-749)

7. Line 815-816: This is somewhat not the conclusion supported by the study, I would suggest to revise here.

**Response:** We have revised this sentence to better reflect our findings and to avoid overgeneralization ("Instead, other environmental factors such as substrate availability or microbial community dynamics, may influence methane production, although the specific mechanisms remain uncertain." Line 810-812).