

Interactive comment on “Carbon sources of benthic fauna in temperate lakes across multiple trophic states” by Annika Fiskal et al.

Anonymous Referee #1

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General: In the submitted manuscript by Annika Fiskal et al. the authors sampled sediments from lakes with different levels of eutrophication. The aim was to investigate differences in macrofauna (oligochaetes and chironomids), microbial communities in the sediment as well as on/in the macrofauna, and the contribution of methane derived carbon for macrofauna ingestion/assimilation. The authors found that methane derived carbon is a minor carbon source for macrofauna, and that macrofauna associated prokaryotes are different from sediment prokaryotes.

The conclusions drawn from the stable carbon isotope data is rather uncertain. This data was used to investigate methane derived carbon and if macrofauna ingest or assimilate this in their bodies. The authors do not know the isotope compositional values for potential food sources derived from methane (such as methane-oxidizing

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bacteria). From what I have read online methanotroph lipids can range between -45‰ to -65‰ $\delta^{13}\text{C}$ values which is very different from the macrofauna values presented in the submitted manuscript. It would therefore be good if the authors tone down the discussion and conclusions from these findings. The authors can instead focus more on the qPCR data that indicate methane cycling bacteria in the gut of the studied macrofauna. And the isotope data might then be used as supportive data to help support the findings that methane derived carbon is a minor food source.

The discussion is quite long and I think this can be shortened by almost half. The authors go into specific details about the microbiology data on ZOTU level, and paragraphs that mention previous studies with similar results can be shortened. I think the discussion can be better summarized and more focused in relevance to the aim of the study. I also think that the focus on the macrofauna associated bacteria can be shortened in the manuscript, as is the case in the Abstract where it is just mentioned briefly at the end, while a large part of the discussion is dedicated to this subject.

There is also essential information missing in the methods such as DNA extraction from the sediment, bioinformatics, and DNA sequencing. It seems that this part is instead presented in a manuscript that is in press (Han et al 2020), however there is no need to present this data as results for this manuscript. I also think it might be misleading to do so and the authors better double-check the journal guidelines for what is acceptable. Instead the authors can mention relevant findings from Han et al. (2020) in the discussion. If the results are first presented in Han et al. (2020) then it should not be presented again as new results for this manuscript. Furthermore, Han et al. (2020) is missing in the reference list so there is no way for the reviewers to read these methods or results.

I think the authors have a large and interesting dataset and it should definitely be published here or somewhere else. My opinion is that the manuscript needs to be more streamlined and focused on a single story (now it feels like two stories: one geochemical with macrofauna collection, and one microbial study).

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Additional comments: page 3 line 10: at what water depths? Maybe you can mention a range here and see more details in results.

page 3 lines 10-15: Clarify what core was used for what analysis. Right now 4 cores are mentioned but 7 analyses, and the authors end the sentence with "respectively". Were all cores used for everything? Or how was these analyses divided among the cores? How many replicates per analysis?

page 4: How was DNA extracted from sediment and chironomid larval tubes?

page 4 lines 11-14: The author state here that methane is a food source for the studied macrofauna. But considering that methane (i.e. the gas) is not a real food source for these animals, how can this model predict CH₄ contribution to their diet? The authors do not know the ¹³C isotopic compositional values of the methane derived food (i.e. methanotrophs and methanogens.)

page 5 lines 1-5: briefly write how, and with what software, the bioinformatic analyses were conducted.

page 5 lines 1-5: How and with what instrument was the DNA sequenced?

page 5 line 3: Han et al. 2020 is missing in the reference list.

page 5 line 15-16: It would be useful if that was mention earlier, i.e. which stations are oxic or hypoxic and what were the O₂ concentrations measured at each station?

page 6 lines 10-27: What were the ¹³C isotopic composition values for methanotrophs and methanogens? How can the authors know if the Macrofauna ingest or assimilate such methane derived carbon without knowing the values for these food sources?

page 8 lines 29-34: This is aims and I think it is redundant to repeat this in the discussion

page 8 line 40 - page 9 line 1: Oligochaetes is mentioned twice here, is it a mistake?

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page 9 lines 5-6: How can the authors be certain that 12% of the contributed carbon is methane derived? Any variation or differences in the ^{13}C isotope values (Fig. 4) might come from other unexplored food sources?

page 10 lines 14-16: This is the first time the radionuclide data is presented in the manuscript. This is results or should be cited if it's already published.

page 12 line 19: Are these previous findings as stated in the sentence? The supplementary data cited indicate that this is results from the current manuscript.

Figure 1: It would be useful to mention in the caption how the degree of eutrophication was defined.

Figure 1: How many cores per station? Are the error bars based on 3 or 9 data points? (i.e. 3 stations or 9 cores with 3 per station)?

Figure 3: Somewhere in the caption it needs to be mentioned that the pie charts show %.

Figure 4: Mention how many data points for each variable.

Figure 5. The authors present results from Han et al. (2020) in the figure. I think this data doesn't belong in this manuscript and can instead be discussed in relation to the results the authors present.

Figure 6 and 7: Are these figures based in all data from all lakes and sediment depths?

Tables 1 and 2: can be moved to supplementary information

Table 4: this is a bit confusing, why are two tests greater and one test less? Perhaps the authors can report the p-values in the results when this data is presented.

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