Reviewer 2

General comments

In this manuscript Graham et al. investigate the bioavailability of pyrogenic organic matter (PyOM) using a substrate-explicit model, which is then compared to that of natural dissolved organic matter and water-extracted particulate organic matter. The current understanding of the impact of PyOM in freshwaters remains mainly speculative. On this note, the manuscript addresses an important topic in riverine biogeochemistry that would be of interest to the scientific community. The manuscript is also very well-written and easy to follow. I would recommend its publication after major revisions.

Thank you for the constructive and positive comments.

Specific comments

1. Based on the compounds selected as representation of PyOM, I wonder if there is any information in the literature regarding their experimental bioavailability. The same applies to DOM and POM. The authors could expand a bit more in the introduction to further clarify the contribution of the study they are presenting.

Thank you for this comment. While empirical studies are relatively rare on PyOM bioavailability, there are several that exist (Norwood et al., 2013; Bostick et al., 2021; Chen et al., 2022). We discuss this at a high level on lines 59-68, but plan to go into more detail from these studies in the introduction.

2. The rationale behind the experimental design is not completely clear and could not be adequate to test the proposed hypothesis. PyOM derived compounds mainly exhibit high Kow values that indicate their low solubility in water. In fact, some of the compounds included in Table S1 were determined after solvent extraction or CuO oxidation according to the references cited therein. However, these PyOM representative compounds were then compared to natural water-soluble organic matter (dissolved and particulate). Regardless, the authors report similar bioavailability parameters across phases, raising concerns about the model selection. This is because of the range of compounds with totally different chemical and physical properties that are being compared. I wonder why the list of PyOM derived compounds was not filtered to include just water-soluble compounds or the list of natural organic matter (dissolved and particulate) expanded to incorporate non-water-soluble compounds. These could represent an important overlooked fraction of natural organic matter, especially in the case of sediments. Also important is to include compounds that are detected beyond the 200-900 m/z analytical window or that escape the SPE procedure. I would recommend expanding the databases based on previously published literature and re-running the models. It would be interesting to see if similar results are obtained after expanding the composition of natural organic matter.

Per this comment and the reviewer 1 comments above, we will increase the database of PyOM molecules we are considering and will also note in the table of PyOM molecules how they were derived. If space allows, we will add in the methods section additional details on the selection choices made for the PyOM molecules – some of which currently exists in the SI – which will clarify how the approach addresses our research hypothesis.

3. It is interesting that the authors included sediment water-extracted organic matter. This is usually not the rule in organic matter related studies in rivers, but definitely something that should be acknowledged more often.

Thank you!

4. In the supplemental material, the authors mentioned that samples were normalised based on the concentration of dissolved organic carbon before SPE extraction. Given that the extraction efficiency of SPE cartridges is not constant, please add more information about how the organic matter extracts were normalised before FT-ICR-MS analysis or during data processing.

Thank you for this comment. We note that no new FTICR-MS data was collected for this publication. While we recognize extraction efficiencies can vary by sample, the normalization procedure used by Garayburu-Caruso et al. was intended to standardize the amount of DOM passed through each filter, which can also lead to biases. This approach has been successfully employed across a variety of published literature and is the standard operating procedure at the Environmental Molecular Sciences Laboratory. Garayburu-Caruso et al. did not report the extraction efficiencies of individual SPE cartridges.

Textor, S.R., Wickland, K.P., Podgorski, D.C., Johnston, S.E. and Spencer, R.G., 2019. Dissolved organic carbon turnover in permafrost-influenced watersheds of interior Alaska: molecular insights and the priming effect. *Frontiers in Earth Science*, *7*, p.275.

Stegen, J.C., Fansler, S.J., Tfaily, M.M., Garayburu-Caruso, V.A., Goldman, A.E., Danczak, R.E., Chu, R.K., Renteria, L., Tagestad, J. and Toyoda, J., 2022. Organic matter transformations are disconnected between surface water and the hyporheic zone. *Biogeosciences*, *19*(12), pp.3099-3110.

Danczak, R.E., Goldman, A.E., Chu, R.K., Toyoda, J.G., Garayburu-Caruso, V.A., Tolić, N., Graham, E.B., Morad, J.W., Renteria, L., Wells, J.R. and Herzog, S.P., 2021. Ecological theory applied to environmental metabolomes reveals compositional divergence despite conserved molecular properties. *Science of The Total Environment*, *788*, p.147409.

5. Please include information regarding quality controls used during FT-ICR-MS analysis.

We note that no new FTICR-MS data was collected for this publication. We will revise the text to state that we include Suwannee River Fulvic Acid (SRFA) as a quality control check in each run in the work reported by Garayburu-Caruso et al.

6. I would strongly suggest using the ranges proposed by Laszakovits & MacKay (2022) to assign compound classes via van Krevelen diagrams (DOI: 10.1021/jasms.1c00230). Please update.

There have been several iterations of Van Krevelen classes proposed in the literature since the original citation, and there is debate surrounding the optimal classification system. Since we did not generate any new FTICR-MS in this publication, we chose to use the classes assigned by Garayburu-Caruso et al. In response to this comment and reviewer 1, we will add Van Krevelen plots to Figure 1 and the supplemental material. One of our intents with these additions is to allow readers to examine alternate classification thresholds.

7. Please include the F-value of the results of the statistical analysis, when appropriate, in the main body or as supplemental material.

Thank you. We will add F-values.

8. I would recommend that the authors include a statement in the *Conclusions* addressing their previously proposed hypothesis.

Thank you. We will add a statement regarding our hypothesis in the conclusion section.

9. The authors stated the limitations of this approach well enough (e.g., lines 226-229). This is important considering the implications and future work.

Thank you!

Technical corrections

line 53: please convert to Tg or Gg or an appropriate standard unit.

We will make this change.

line 103: please use an appropriate notation (instead of p (PyOM-sediment)).

We will make this change.

line 300: Is the dataset in Garayburu-Caruso et al. (2020a) the most comprehensive assessment of DOM in rivers to date?

We will alter the wording of this sentence to weaken this language.

line 331: please include the references for the R software as well as for each package.

We will make this change.