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Comment on egusphere-2022-194

Anonymous Referee #1

Referee comment on "Potential bioavailability of representative pyrogenic organic matter compounds in comparison to natural dissolved organic matter pools" by Emily B. Graham et al., EGUsphere, https://doi.org/10.5194/egusphere-2022-194-RC1, 2022

Reviewer Summary:

In this manuscript Graham et al. explore the potential of pyrogenic DOM (pyDOM) to be bio-degraded. A purely computational approach "substrate-explicit model" and previously published data were used to estimate the energy content, metabolic efficiency, and aerobic decomposition of DOM of pyrogenic and non-pyrogenic "natural" molecules. This study provides a computational explanation of why other recent studies have discovered that pyrogenic DOM can be extensively degraded (consumed) by microbes. This work contributes to the recent paradigm shift on the knowledge on pyrogenic matter's stability and reveals that pyDOM has a comparable bio-degradability (i.e., biological lability/reactivity/consumability by microbes) to natural (e.g., fluvial) DOM. This indicates that pyrogenic molecules are not as recalcitrant as previously presumed and suggesting that part of the combustion continuum is actively involved in the global biogeochemical cycles.

Reviewer Evaluation and Recommendation: This is an excellent manuscript that contributes greatly to the literature on wildfire biogeochemistry. Very well written, flowed well, smooth read, clear visuals. The bio-degradability of pyrogenic matter is currently a hot topic though there are very few studies exploring it. The present article is a great contribution to this research trajectory and is being submitted for publication (and hopefully soon published) at a great timing for the community. Indeed, as the authors mention, this and the other studies are laying the foundations of a lot of necessary future work on pyDOM microbiology/biochemistry. Computational modeling studies, especially like this one exploring bio-degradability, are generally lacking even in the "natural" DOM

world, which makes this work very novel and interesting to readers from various communities.

Unfortunately, there is a major flaw with the design of the study preventing me from recommending it for publication. This flaw can heavily skew the conclusions of this work. However, this flaw can be addressed without too much trouble after which the study would be ready for publication. Thus, **I recommend this paper to be published after a major revision.** My other comments are minor and can be easily addressed.

Major comment:

There are multiple concerns regarding the design of the study, in particular, the choice of natural and pyrogenic data. The conclusions of this study are heavily dependent on the comparison between these two datasets (Figure 2). Thus, complete data comparability must be ensured. Issues:

- Pyrogenic data (Table S1) in this study was derived from previous studies which had utilized solvent extractions. For example, a good fraction of the pyrogenic data is extracted from the studies of Oros and Simoneit who analyzed aerosol filters and extracted pyrogenic molecules using dichloromethane. Considering that in a natural environments wildfire deposits charcoal, which charcoal is then **water-leached** by rain, there are two immediate questions arising: 1) Is aerosol data representative of charcoal on land? 2) Is solvent-extraction comparable to water-extraction? I am unsure how comparable aerosols and charcoals are, but there is likely literature on this. However, regarding solvent versus water, a recent study showed that water and solvent extracts of charcoal are extremely different (McKenna et al., 2021). By looking at Table S1, 100% of the pyrogenic data used in this study is derived from a solvent extraction. It is highly unlikely that these solvent-extracted molecules are representative of pyrogenic molecules in aquatic environments. In order to discuss bioavailability of pyDOM in aquatic systems (the goal of this study), the pyrogenic data used here must be from water extracts. Given that both charcoals are being leached by

rain, and atmospheric aerosols are deposited in waters (Wagner et al., 2021), I would say that using data of water-extracts of both charcoal and aerosols would be appropriate, but emphasis should be onto charcoal contributions as they are likely bigger.

By contrast, for "natural" DOM, authors use data fir surface waters and sediment water extracts, which data are data are appropriate for discussing aquatic biogeochemistry.

- Second issue identified is on the type of data used. For pyDOM, authors use known structures whereas for natural DOM authors use molecular formulas from FT-ICR-MS. The authors say that they focused their study on known pyrogenic compounds because "While we recognize that recent research has applied new technologies to inferring PyOM compound presence in environmental samples (e.g., FTICR-MS), there remains high uncertainty in the confidence of formula assignment and structural information with some of these techniques." However, the authors use FT-ICR-MS data for the natural DOM, and their own argument can be made for their natural DOM dataset. I agree that using known structures is much better, but at present we do not know most of them for pyDOM or natural DOM, so this may require the use of FT-MS (ICR or Orbitrap) molecular data for this study. Using known structures for pyDOM and using molecular formulas (from FT-ICR-MS) for natural DOM creates a major discrepancy in the comparison between pyrogenic and natural DOM. There are multiple reasons: 1) natural DOM samples were extracted with PPL (Garayburu-Caruso et al., 2020), which introduces a specific bias. 2) Only a specific fraction of molecules ionizes in negative ESI, which is the ionization used for the natural DOM dataset (Garayburu-Caruso et al., 2020). Negative ESI introduces another very specific bias onto natural DOM data. These biases are not accounted for in the structural pyDOM dataset. 3) There are entirely different data analysis routines for the natural and pyrogenic datasets (structure vs formula assignment). These routines can also possibly skew the analyzed data differently. For example, it is common to filter assigned molecular formulas following the constraints published by (Stubbins et al., 2010). If such step was done on the natural DOM data, should be also done on the pyDOM data.

- The environmental DOM dataset contains sediment water extracts. How are these relevant to aquatic microbial processes which are primarily occurring in surface waters away from sediments? I suggest removing the sediment DOM and only keeping the surficial DOM.

- The environmental DOM dataset is from only one study (Garayburu-Caruso et al., 2020). The authors compare pyDOM data from multiple studies to natural DOM from one study. Is the data in Garayburu-Caruso et al. (2020) representative of multiple different aquatic environments?

In summary, the comparison between pyrogenic and natural DOM seems like "apples to oranges" at present. All or some of the reasons listed above may skew the bioavailability of the two datasets to make them falsely appear with comparable bioavailability. This flaw needs to be addressed to be corrected in order to make the datasets comparable.

Suggestion for fixing this:

For natural DOM: add data from other studies and remove the sediment DOM. There is an overwhelming amount of FT-ICR-MS data published and provided in repositories. I am also sure that many research groups will be completely open to share data with you for this novel study. I do recommend mixing various surficial aquatic systems, primarily rivers, but also hopefully you can add lakes, wetlands, marine, etc. The choice of data will then allow you to determine if you can make claims strictly related to riverine environments or more like the global aquatic environment. Maybe you can compare pyDOM bioavailability to availability of DOM from different aquatic systems?

For pyDOM: I recommend using FT-ICR-MS data for ensuring comparability. Tracing truly pyrogenic molecules in natural systems is at present very challenging, so I recommend using charcoal water-extracts. There is a good number of studies that have published such data: (Chen et al., 2022; Goranov et al., 2020; Goranov et al., 2022; McKenna et al.,

2021; Smith et al., 2016; Wagner et al., 2017; Ward et al., 2014; Wozniak et al., 2020; Yan et al., 2022) – just a few of the top of my citation manager.

I think by doing this you will achieve complete comparability (ensuring data were from PPL extracts and -ESI). I can foresee one issue – if obtaining data from multiple groups, you might get molecular formulas which could be biased by the different software that groups use (ICBM, pyKrev, Formularity, etc.). What I suggest is inquiring for peak lists (m/z and intensity data) and you work up the data yourselves to avoid comparability issues from different processing routines.

Detailed Review and Specific Comments:

Abstract: Excellent. Gives a comprehensive overview of the study, information is succinctly presented. I only recommend adding one sentence somewhere around lines 29-31 that explains that this model is a computational approach using molecular formula data from mass spectrometry or molecular formulas of known DOM structures. Readers who are not familiar with this "substrate-explicit model" will likely be confused, so enhancement in clarity is needed.

Intro: Very good. Establishes the importance of wildfires and pyrogenic DOM, provides background on the modeling approach and authors identify the gaps in our knowledge of pyrogenic DOM. Authors also establish a clear objective for the study. Some minor revisions are needed:

- Terminology throughout the manuscript:

- Line 45: River corridor biogeochemistry. From the abstract and title of the paper it seems that the study is going to make claims regarding broad DOM across various aquatic systems, not just fluvial ones. I suggest replacing with "aquatic biogeochemistry" throughout the manuscript.

- pyOM is commonly used for particulate OM whereas pyDOM is used for dissolved OM. I advise using pyDOM as using pyOM for an aquatic study is confusing.

- This term needs to be more clearly defined. Authors should also consider using "bio-lability" as one may argue that all molecules surrounding microbes are available to the microbes, but only some molecules can be uptaken and consumed (i.e., labile to bio-degradation).

- BC – having BC and pyOM in the same manuscript is confusing as many readers view them as synonymous. I recommend removing BC entirely. In studies employing molecular data (from FT-ICR-MS, etc.) using a carbon term is also not appropriate as the data is reported for the whole molecules (i.e., on matter-basis) and not just for the carbon backbone (i.e., not on a carbon-basis). In simple terms, FT-ICR-MS measures DOM, not DOC. I recommend using terms that directly correspond to the structure (i.e., condensed aromatic compounds, ConAC, polycyclic aromatics, PCA, oxygenated PAHs, OPAH, or others that are used among different research groups).

- "Natural" DOM. I recognize the necessity to use this term, the problem with it is that some pyrogenic molecules exist in detectable quantities in natural DOM (Goranov et al., 2022). Or they share the same molecular formulas but are different isomers. Maybe authors should acknowledge this and clearly state in the intro that natural DOM corresponds to environmentally ubiquitous molecules obtained from fieldwork whereas the pyDOM dataset is from laboratory experiments and extractions to ensure these

molecules are truly pyrogenic. One side question – is there any overlap (i.e., common formulas) among the natural and pyrogenic DOM datasets that the authors used? Are those common formulas removed or they were kept in? Please clarify this in the methods section.

- Lines 66-68. Authors make the claim that "Yet, there has been no systematic evaluation of the bioavailability of different constituents within the heterogeneous compounds that comprise PyOM". This was actually recently done by two separate research groups (Bostick et al., 2021; Chen et al., 2022; Goranov et al., 2022) who looked at various constituents of pyrogenic DOM using various analytical approaches. Those studies also use "true" pyDOM from biochar extracts similar to Norwood et al. 2013, but of more wildfire-representative temperatures.

- Lines 69-72. I think it would be good to explain the reason for this discrepancy – most research to date assumed that pyOM/pyDOM are only comprised of condensed structures, and the latter are indeed bio-refractory (Bostick et al., 2021), but the rest of pyOM/pyDOM is likely not.

- Please expand on the substrate-explicit model text after line 82. This is the first time to my knowledge that this modeling approach is used in the wildfire biogeochemistry literature – most readers will have no background in it. Tell us about the model output: on line 99 you mention ΔG_{Cox}, λ, and CUE for the first time without providing any info on what they are. There is a lot of great text in the materials and methods that does not read like M&M text – move some of it to the intro to provide a foundation on what these parameters are.

Results and Discussion: Excellent. Results are properly discussed in the context of previously existing literature. Several comments:

- We know that behind a m/z value (or a molecular formula) there could be multiple isomers with the same elemental make-up (Leyva et al., 2019). Different isomers will have different thermodynamic properties. Do the mathematics behind the model consider this? This needs to be discussed in the last section where authors discuss the limitations.

- Does the model account for potential toxicity of molecules? Some pyrogenic molecules are toxic (Smith et al., 2016), but not sure if a mathematical proxy for toxicity can be extrapolated from previous studies and incorporated into your computation approach. This is probably something to consider and discuss in your last section too.

- **Figure 1.** Plotting λ versus ΔG_{Cox} is not very intuitive to a broader audience. Though I recognize that these plots are useful, someone who is not experienced with these parameters will be confused. I recommend improving the clarity in visualizing the model output. I recommend complementing these plots (or substituting them) with something more recognized such as van Krevelen diagrams. I suggest plotting van Krevelens for river DOM, sediment DOM, and pyDOM and color coding the markers based on lambda and/or ΔG_{Cox}. This way different compound classes can be easily identified in the H/C vs O/C space and we can see how energy content and metabolic efficiency vary per compound class (condensed molecules, phenols, lipids, etc.).

- It will be good to compare the model output *computational* results with previously published bio-degradation *experimental* studies. Bostick et al. 2021, Chen et al. 2022 and Norwood et al. 2013 provide degradation rate constants for pyDOM. Can the authors compute a similar bulk degradation rate constant using the output of their model and see how computational and experimental results compare? This will strengthen the conclusions of this manuscript.

Methods: Excellent text, but a lot of it reads like an introduction. Please move a lot of this text to other sections.

Graphical Abstract: Absolutely gorgeous design. I would only recommend enhancing font sizes (difficult to read) and not using gray color – the labels (pyOM pool, Biomass, etc.) and other subfigures (e.g., biomass particles with tails) are difficult to read/see. I suggest just converting everything to bold black and increasing the sizes (just like CO2). Also why is "Biomass" looking like bacteria? I recommend replacing Biomass with "Microbes".

Title: It is a representative title, but at present it reads a bit awkwardly. I read it as "bioavailability resembles DOM pools", which is odd. Consider rephrasing into something like "Computational modeling reveals that molecules in pyrogenic and natural dissolved organic matter pools have similar bio-lability" or something like that.

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