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Reply on EC1

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Author comment on "Microbial labilization and diversification of pyrogenic dissolved organic matter" by Aleksandar I. Goranov et al., Biogeosciences Discuss., https://doi.org/10.5194/bg-2021-23-AC2, 2021

This work investigated the molecular changes of water extracted chars (pyDOM) during microbial degradation using FT-ICR-MS and other methods. The topic is interesting and the manuscript is well written. But I have some major comments about the discussion about the role of ROS in the transformation of pyDOM.

We thank the referee for their review of our manuscript and we are pleased that they find the topic of research interesting.

The author said this is a parallel study of the same samples (Bostick et al., 2020a), and "Over the 96-day incubation, up to 48% of the carbon was respired to CO2 following first-order kinetics," However, this study only incubated 10 days. The DOC loss or mineralization is very important in the biodegradation of DOM, but I did not see any contens about this in results or discussions in this paper.

The quantitative losses have been described in detail in the companion paper by Bostick et al. (2021). After 10-days of bio-incubation, the following DOC losses were observed: Oak 400 Fresh: 16% loss, Oak 400 Photo: 25% loss, Oak 650 Fresh: 15% loss, Oak 650 Photo: 23% loss. We recognize that these results have not been clearly discussed in our manuscript and thank the referee for reminding us of their importance. These data will be incorporated in the results and discussion sections of the revised manuscript.

My biggest concern: The results and discussions about "Radical oxygenation as a potential source of molecular diversity" contained too many over-interpretations. Only the results of FT-ICR MS cannot support the obtained conclusions. (1) no data about the detection of ROS were present in this study. In addition, the control experiment by addition of ROS inhibitors during incubation was lacking. (2) the conclusions like "the bio-produced formulas could be classified as products of oxygenation reactions, likely driven by ROS species such as the hydroxyl radical (•OH)" obtained by the KMD analysis using oxygen (O) series (eg. Figure 4) are severe over-interpretation of the FT-ICR MS data. There is no evidence to support that $C_cH_hO_{o+1}$ is produced from $C_cH_hO_o$ via oxygenation by hydroxyl radical (•OH) attacks. Combined (1) and (2), no evidence support the conclusions about the pathway of radical oxygenation of pyDOM.

We agree with the referee that our results here are not definitive. During the

experimental design of our study, we did not hypothesize that radicals could play an important part in the bio-incubations we were going to perform and thus, we did not prepare any controls to include ROS quenchers to specifically test for radical reactions. As this is the first study to incubate pyDOM with microbes, our overarching study hypothesis had to be broad: ***pyDOM will be degraded/transformed by microbes*** *(H_1) vs* ***pyDOM will not be degraded/transformed by microbes*** *(H_0). Thus, our control samples were designed to allow for testing this hypothesis.*

We strongly disagree that FT-ICR-MS data cannot be used to imply radical oxygenation. Waggoner et al. (2015) performed lab-controlled Fenton reactions (producing hydroxyl radical species) on a lignin concentrate. In a sequential study, the same lignin concentrate was fractionated using HPLC and then exposed to hydroxyl radicals (Waggoner and Hatcher, 2017). Later, singlet oxygen ($^1O_2$) and superoxide ($O_2^{-\bullet}$) were also added to the lignin concentrate in lab-controlled conditions. These three studies showed that $C_cH_hO_{o+1}$ are indeed produced from $C_cH_hO_o$ molecules via radical oxygenation. As the same trends can be seen in our data, it is reasonable to speculate that the observed molecular changes to pyDOM ***potentially*** *result from radical oxygenation. Similar trends have been also observed in fungal incubations (Khatami et al., 2019). We recognize that this evaluation is not definitive and have provided an alternative in which the observed $C_cH_hO_{o+1}$ oxygen series are biologically unrelated to $C_cH_hO_o$ and the observed oxygen series are coincidental:*

- *Lines 696-700: The observed diversity can be explained by a scenario wherein the microbes secreted labile molecules whose identities differed depending on the growth medium and/or food source, yielding high variability among bio-produced formulas after the incubation of pyDOM. Additionally, it is possible that different microbial species (different bacteria, fungi, archaea, etc.) have proliferated in response to the sample-specific pyDOM composition, yielding different microbial populations growing during each different incubation, sequentially producing different bio-produced compounds (Fitch et al., 2018).*

Because we have no evidence from DOC loss or other quantitative measurements proving that there were radical oxygenation reactions in these pyDOM systems, the proposed radical oxygenation pathways were labeled as a ***potential*** *source of molecular diversity. This will be emphasized in the revised version of the manuscript and we will describe in better detail the two explanations of observed molecular diversity and $C_cH_hO_{o+1}$ oxygen series. We will also recommend that future studies perform specific experiments to test for radical reactions.*

m was converted to square, eg. line 150, line 469

We thank the referee for spotting this. We are unsure why the Greek symbols on lines 150 (μL) and 469 (β-hydrogens) were formatted into squares. These will be corrected in the revised manuscript.

Figure 1: Present bio-resistant formulas in Figure 1?

Unfortunately, when bio-resistant formulas are plotted in Figure 1, the figure becomes cluttered and the trends are very difficult to see. To overcome this, we tried using several different color schemes, marker sizes and shapes. No figure that had the bio-resistant formulas present was visually appealing. Furthermore, the bio-resistant formulas are not important for understanding the major findings in our study. Thus, if bio-resistant formulas are plotted on Figure 1 they will likely distract the readers from the main trends in the data. Therefore, we prefer to keep the bio-resistant formulas shown on Figures S3 and S7.

References

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