Biogeosciences Discuss., author comment AC1
https://doi.org/10.5194/bg-2021-23-AC1, 2021

Reply on RC1

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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-AC1, 2021

In the study, the authors used art-of-the-state technologies to understand the transformation of pyrogenic dissolved organic matter by soil microbes. The authors reveal that the alternation of pyDOM was uniform and that a large portion of the bio-produced compounds is peptide-like. The results of this study can definitely improve the current understanding of the biogeochemical cycle of pyDOM.

We thank the referee for their review of our manuscript and are pleased that they see its value for improving the current understanding of the biogeochemical cycle of pyDOM.

1 Method. FT-MS.Positive or negative ESI? These two modes are suitable for acidic and basic compounds, respectively. If only one mode was used, only partial results could be obtained.

We apologize for omitting this critically important detail. Samples were analyzed in ***negative*** *electrospray ionization (ESI) mode. We recognize the limitations of the use of only one mode of ionization. The manuscript will be edited accordingly to specify the ionization mode as well as to include a short discussion section in the supporting information that contextualizes how the choice of ionization mode impacts the results obtained for the present study.*

2 Results 3.1 The pyDOM produced at a higher temperature is more recalcitrant than that produced at a lower temperature. The photo-irradiated pyDOM should be more labile than the fresh one. However, it was not the case in this study (as shown in Figure 1). Why? How about the results using TOC loss and CO2 respiration?

The four samples of this study behaved as expected though we avoided quantifying bio-

lability in this manuscript due to the non-quantitative character of ESI-FT-ICR-MS. The bio-lability of these samples is quantified in a companion paper using carbon loss as a proxy for lability (Bostick et al., 2021). The quantitative results do follow our expectations (Figure 1 of Bostick et al., 2021): Oak 400 Photo (48% C loss) is the most bio-labile followed by Oak 400 Fresh (45% C loss), Oak 650 Photo (44% C loss), and Oak 650 Fresh (37% C loss). As the referee notes, using the number of formulas lost as a proxy for lability here in our study, Oak 400 Photo (1242 bio-labile formulas) appears less bio-labile than its non-photo-irradiated counterpart (Oak 400 Fresh, 1646). What is also surprising is that Oak 650 Photo (1410) appears to be more bio-labile than Oak 400 Photo (1242) and we expect the opposite based on quantitative carbon loss data. Clearly, the trends observed by mass spectrometry are different.

The difference is likely due to the difference in the analytical windows of the techniques used to describe lability. From the four samples of this study, Oak 400 Photo is likely the sample with the highest number of low molecular weight (LMW) compounds. Microbes would have preferentially consumed LMW compounds before moving onto consuming larger molecules. LMW compounds are not detectable by the FT-ICR-MS either due to the analytical m/z range of 250 – 900, and/or because such molecules are generally lost during PPL extraction. We only detect acetate, formate, and methanol using proton nuclear magnetic resonance spectroscopy (Bostick et al., 2021) but there are certainly many more LMW that remain undetectable. We recognize that this has not been made clear in the manuscript and this will be corrected in the results section of the revised version of the manuscript.

Regarding CO_2 respiration, nearly all carbon (within 4%) was lost during the incubations as respired CO_2 (based on CO_2 quantification).

3 Result. As stated by the authors, the photo-degradation of pyDOM is also very interesting; why not to compare the structure change before and after photo-irradiation in the supplement.

The photochemical changes to these pyDOM samples have been a focus of two previously published manuscripts: we report the quantitative changes in Bostick et al. (2020) and the qualitative (structural and molecular) changes are described in detail in Goranov et al. (2020). As the current manuscript and its supplement are already of significant length, we prefer to not further focus on the photochemical changes of these samples. Rather, we will clarify that their photochemical trends have been previously published. This clarification will be made in the revised version of manuscript.

4 Discussion. 4.1.1 It should be very careful to draw a conclusion on the biodegradability of pyDOM with various structures. Only polar compounds can be ionized by the ESI. Therefore, the results obtained by the ESI-FT-MS are biased. Some compounds are with high aromaticity index; they are still polar if the ESI can ionize them. If possible, more ionization modes can be tested using the same samples.

We agree with the referee that the observed trends from negative-mode ESI-FT-ICR-MS are biased by the analytical window of this technique. This will be addressed in the revised version of the manuscript and we will incorporate a higher level of caution when discussing bio-labile (molecules that appear to have degraded) or bio-produced molecules. We will edit the discussion section accordingly and provide an additional section in the supporting information describing the biases of ESI pertaining to the current study. However, the fact that we observe 1000+ formulas of significant spectral magnitude being removed suggests that the sample composition had been significantly altered after the biotic treatment. This conclusion is validated by findings from the quantitative techniques (NMR, absorbance, fluorescence, etc.) employed in this and the companion study (Bostick et al., 2021). Unfortunately, due to sample and funding limitations, we were not able to employ positive-mode ESI or other types of ionization.

References

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