Overview:

The authors present a study of DOM reactivity via photodegradation and microbial decomposition of headwaters of the tropical Essequibo River, quantified using bulk DOC and classes derived from Size Exclusion Chromatography (SEC). Incubations were performed in the river using both light and dark control chambers to separate the different effects of photochemistry and biodegradation. The authors attribute the most apparent changes in DOM composition to occur rapidly due to photodegradation with microbial processing resulting in unclear and often inconsistent trends. I appreciate the experimental approach in trying to mimic in situ riverine conditions and the careful use of replicate incubations; however, the manuscript contains serious flaws in the data presentation and interpretations that prevent it from being publishable in its current form. I have done my best to outline areas of improvement and offered suggestions in the comments below.

General Comments:

Lability:

My biggest issue with this manuscript is the definition and use of 'photo-labile' and 'bio-labile' molecular categories. From the manuscript it seems like the authors are predefining what 'photo-labile' and 'bio-labile' SEC classes are based on the literature and then tracking their increases and decreases throughout the incubation experiments. This is problematic for several reasons. First, the authors assume that certain SEC compound classes, which are considered not 'photo-labile' in certain studies (Line 263), must therefore by default be 'bio-labile'. Neither the Stubbins et al. (2010) nor Koehler et al. (2012) studies that are cited used SEC to classify DOM compositions, so how can the authors use them to define certain SEC-specific compound classes as being 'bio-labile' (line 265)? Second, from figure 3, LMW N and LMW A both decrease in some of replicates attributed to photochemical effects (even though the authors consider them to be 'bio-labile') and HS and building blocks both decrease in some of replicates attributed to the microbial effect (even though the authors consider them to be 'photo-labile'). These arbitrary classifications are forcing compound classes to be either 'photo-labile' or 'bio-labile' and don't allow for the possibility of DOM to be both or neither. In reality, photo- and bio-lability are complex concepts and DOM compounds are rarely either one or the other. I suggest reading papers such as Bittar et al. (2015a,b) for guidance on how to navigate these classifications.

It would be better to present the compound class data first and use that as a framework for identifying which classes seem to be 'bio-labile' and/or 'photo-labile' in this system rather than trying to force the compounds into these categories beforehand without any justification.

DON:

I appreciate the authors' efforts in addressing the organic N composition of DOM in the incubations (section 3.3), but the way it is presented does not make it seem like a relevant part of the manuscript. The introduction does not sufficiently prepare the reader for the importance or context of N cycling in any way, mentioning only that DON is a part of DOM (Line 49). The analysis and treatment of DON is interesting, but on their own the results do not indicate that DON is an important part of the story that the authors are trying to convey, stating that there were no clear trends in C/N ratios and speculating that the changes must be due to other processes without any proper justification or support. In fact, the Kellerman et al. (2015) reference that the authors cite (Line 390) does not even use SEC for their analysis, yet the authors still try to force their SEC-specific compound classes into incompatible frameworks to explain their data. Furthermore, there is very little effort to try to relate the DON results to the trends seen with DOM, given that the authors claim that DON is an important subset of DOM in the introduction. Outside of a single sentence about investigating future N-cycling (Lines 429-431), there are no mentions of DON in the discussion section, conclusion, or even the abstract.

If the authors wish to include DON in the manuscript, I suggest: 1) properly explaining its importance and context in tropical rivers in the introduction section; 2) extensively trim section 3.3 to a single concise paragraph; 3) move figure 5 and any other peripheral details in section 3.3 to the supplemental data; and 4) relate the DON results to the changes in DOM composition in the discussion section.

Structure and organization:

There are several instances in the manuscript where sentences unrelated to the topics of the paragraphs are spliced in and derail any sort of flow that begins to develop. There are also areas in the methods and results sections where the authors begin lengthy discussions on the interpretations and implications of their data that are better suited for the discussion section. In both cases, I have done my best to identify these areas in the specific comments below. While I understand that these stylistic conventions may have been made intentionally by the authors, they make the manuscript difficult to read and understand. Fixing the organization will make the main findings clearer for the reader to comprehend.

Specific Comments:

Abstract:

Line 16: What does 'supposedly photo-resistant' mean? Why not just say 'photo-resistant' since you already say 'photo-sensitive' right before?

Line 16: 'Microbial activity...bio-labile components'—this sentence is really vague, and I don't think it presents any useful information to the reader.

Lines 18-19: ‘Biopolymers…degassing’—These two sentences don't flow with the preceding or proceeding sentences and seem disjointed.

Introduction:

Lines 25-29: These sentences jump from CO_2 to methane back to CO_2 without any reasoning or flow.

Line 43: ‘Tropical…emissions’—This sentence seems out of place with the previous and following sentence.

Line 48-49: ‘DOM…DON’—it also includes dissolved organic S and organic P. If you're not going to mention them then just cut the DON out of it because it doesn’t seem like you discuss DON anywhere else in the introduction. If you are presenting data regarding DON, then please add a section describing its importance in tropical rivers to the introduction.

Line 70: ‘Leading to…via respiration’—it can also lead to smaller DOM compounds and byproducts being formed.

Line 80: ‘Ignoring’ implies that it detects it and doesn't account for it. UV-vis simply can't detect optically invisible DOM.

Line 82: ‘…difficult to align with quantitative DOC changes…’—Not really, several papers have demonstrated the quantitative nature of FT-ICR MS such as for organic S and organic N (e.g., Poulin et al., 2014; Kurek et al., 2020).

Line 83: I wouldn’t call the technique ‘novel’ if it has been implemented since 2011 with over 1,000 citations of the original paper.

Line 86: ‘…for example…Arctic river’—The Voss et al. (2015) paper doesn’t even use SEC, why even bring it up here?

Methods:

Lines 133-136: ‘to address…container’—Much of this material seems like it should go in the discussion. Please summarize for the methods.

Lines 183-187: ‘Previous studies…of HS’—Much of this material seems like it should go in the results. Please summarize for the methods.

Results:

Line 210: I wouldn't say non-aromatic compounds were 'preferentially removed' because overall absorbance did decrease during the incubations, meaning aromatic compounds must have been removed in enough quantity to decrease the absorbance.

Line 213 and throughout: 'four incubations…five of the incubations'—This terminology is misleading and makes it sound like you have 9 different incubation studies. You have 3 incubations each having 3 replicates. I would recommend just referring to them as replicates throughout the manuscript, but if the authors prefer to refer to the replicates as 'incubations', then this terminology needs to be clarified in the Methods section.

Lines 230-251: This section seems like it belongs in the discussion.

Figure 3: This figure is a little misleading. The reader doesn't know the starting concentrations of each DOM group unless they go digging through the supplemental and thus has no context for the concentration changes. I would make figure S2 the main text figure with figure 3 in the supplemental and make the percent changes the main focus, or find a better way to convey the context of the concentration changes to the reader. Also, the caption does not match figure 3. A and B are both photochemical effects in the figure, C and D are labeled as microbial effects. E and F look like initial compositions. Finally, I suggest making an inset for BC2 and possibly BBR, I can't tell what's happening there due to the way the y axes are scaled.

Lines 310-329: This section seems like it belongs in the discussion.

Lines 350-362: This section seems like it belongs in the discussion.

Discussion:

Lines 403:405 and figure 6: It seems like you should present this figure before figure 2 as these are the overall changes you observe and figure 2 investigates where all the changes come from (e.g., either microbial or photo-induced).

Lines 409-410: Why would the addition of new OM be less influential in larger tributaries than in headwaters? Large rivers such as the Amazon receive greater OM during periods of high precipitation/discharge which increases the overall DOC concentration and changes the DOM composition (e.g., Seidel et al., 2016).

Lines 410-412: 'Our results suggest…successive days of exposure'—The authors only incubated for less than a day making it difficult to speculate on the long-term kinetics of DOM degradation. Please elaborate and justify this claim with relevant literature.

Lines 418-420: I agree that the authors demonstrated a greater DOC loss in their incubations due to photochemistry, but I would not say that this study suggests that photodegradation is a more important driver of degassing in tropical headwaters. This study omits the sediment where biodegradation also occurs. It also only sampled within 22 hours which likely does not accurately represent the kinetics of biodegradation for this system, as the authors recognize in lines 434-437. There also may be more biolabile sources of DOM upstream that have been mineralized before the sampling sites in this study.

Line 423: 'decease'—I assume the authors meant to write 'decrease'.

Lines 427-429: This is almost an exact repetition of the first sentence in the discussion (Lines 401-403).

Lines 437-438: 'This approach…can become lentic'—I agree that this approach better represents systems with standing water, but I simply don't know if this approach represents the study site since the authors never provide or discuss water residence time and discharge at the time of sampling and the typical conditions for these streams. If water residence time is short at these sites, the incubations likely don't represent the actual conditions as the authors are effectively isolating a parcel of water that would have normally interacted with different light, temperature, sediment, groundwater, and microbial communities over the course of 22 hours.

Lines 438-441: 'The approach…to quantify POC'—I appreciate the authors' recognition of potential DOM-POM interactions, but I would like more of a discussion of the effects of adsorption to DOM in these incubations. Since these incubations are unfiltered, DOM is likely adsorbing to suspended particle and mineral surfaces over time, fractionating the remaining DOM composition. This process can occur rather quickly and drastically change the composition of the remaining DOM compounds in solution (e.g., Lv et al., 2016; Coward et al., 2019). I realize that there are likely fewer iron hydroxides and other minerals at these sites than in the cited studies, so the effects of adsorption are probably not as extreme, but I still think they are worth considering given the changes in DOM classes you observed and are unable to explain. b

Lines 446-450: This may be true, but all these cited experiments have been under different conditions with different methodology making it difficult to compare directly with your results. If the authors wish to compare their findings to these studies, please also discuss how differences in methodology and experimental conditions may have impacted the ranges reported for DOC degradation.

Line 456: 'Fig. 7b'—I do not see figure 7 anywhere in the manuscript or supplemental info.

Conclusion:

Lines 465-466: 'Photodegradation has a larger influence on DOC transformation than microbial activity, which had a more varied and inconclusive response.'—I think this is only true based on

the timescales and conditions of this study but may or may not be true of the natural system. Please clarify that these results are specific to the experimental conditions of the study.

References:

Bittar, T. B., Stubbins, A., Vieira, A. A., & Mopper, K. (2015a). Characterization and photodegradation of dissolved organic matter (DOM) from a tropical lake and its dominant primary producer, the cyanobacteria Microcystis aeruginosa. *Marine Chemistry*, *177*, 205-217.

Bittar, T. B., Vieira, A. A., Stubbins, A., & Mopper, K. (2015b). Competition between photochemical and biological degradation of dissolved organic matter from the cyanobacteria Microcystis aeruginosa. *Limnology and Oceanography*, *60*(4), 1172-1194.

Coward, E. K., Ohno, T., & Sparks, D. L. (2018). Direct evidence for temporal molecular fractionation of dissolved organic matter at the iron oxyhydroxide interface. *Environmental science & technology*, *53*(2), 642-650.

Kellerman, A. M., Kothawala, D. N., Dittmar, T., & Tranvik, L. J. (2015). Persistence of dissolved organic matter in lakes related to its molecular characteristics. *Nature Geoscience*, *8*(6), 454-457.

Koehler, B., Von Wachenfeldt, E., Kothawala, D., & Tranvik, L. J. (2012). Reactivity continuum of dissolved organic carbon decomposition in lake water. *Journal of Geophysical Research: Biogeosciences*, *117*(G1).

Kurek, M. R., Poulin, B. A., McKenna, A. M., & Spencer, R. G. (2020). Deciphering Dissolved Organic Matter: Ionization, Dopant, and Fragmentation Insights via Fourier Transform-Ion Cyclotron Resonance Mass Spectrometry. *Environmental Science & Technology*, *54*(24), 16249-16259.

Lv, J., Zhang, S., Wang, S., Luo, L., Cao, D., & Christie, P. (2016). Molecular-scale investigation with ESI-FT-ICR-MS on fractionation of dissolved organic matter induced by adsorption on iron oxyhydroxides. *Environmental science & technology*, *50*(5), 2328-2336.

Poulin, B. A., Ryan, J. N., Nagy, K. L., Stubbins, A., Dittmar, T., Orem, W., ... & Aiken, G. R. (2017). Spatial dependence of reduced sulfur in Everglades dissolved organic matter controlled by sulfate enrichment. *Environmental science & technology*, *51*(7), 3630-3639.

Seidel, M., Dittmar, T., Ward, N. D., Krusche, A. V., Richey, J. E., Yager, P. L., & Medeiros, P. M. (2016). Seasonal and spatial variability of dissolved organic matter composition in the lower Amazon River. *Biogeochemistry*, *131*(3), 281-302.

Stubbins, A., Spencer, R. G., Chen, H., Hatcher, P. G., Mopper, K., Hernes, P. J., ... & Six, J. (2010). Illuminated darkness: Molecular signatures of Congo River dissolved organic matter and its photochemical alteration as revealed by ultrahigh precision mass spectrometry. *Limnology and Oceanography*, *55*(4), 1467-1477.