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Dear Anonymous Reviewer 1,

Thank you for your review of our manuscript 'Unravelling light and Microbial Activity as Drivers of Organic Matter Transformations in Tropical Headwater Rivers', authored by Spray et al., which we submitted for publication in *Biogeosciences*. Please find below our responses to each of your comments in blue text.

Yours Faithfully,

Dr James Spray and Dr Ryan Pereira,

On behalf of all co-authors (Thomas Wagner, Juliane Bischoff, Sara Trojahn, Sevda Norouzi, Walter Hill, Julian Brasche, and Leroy James)

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.

We thank the reviewer for their considered comments and suggestions; we agree that the unique strength of our approach is in that it intends to mimic DOM transformations in remote tropical headwater rivers in-situ under 'real' environmental conditions, and to analyse arising compositional DOM changes using SEC. We actually believe that the core of the criticism stems from the complex nature of the data presented, reflecting the wide range of methodologies and factors used to characterize DOM composition, the pioneering and therefore incomplete set-up of the field experiments, and the associated challenges of distilling a generic new process understanding. To remedy this, we have carefully re-analysed our presentation strategy and propose modifications to the text, figures, and manuscript structure. We outline the reasoning and proposed changes in our response to the reviewer's general and specific comments below. We would like to thank the reviewer for their criticism; it helped us to reframe and refocus the manuscript by focusing on its strengths and novelty, while also better addressing its limitations to better highlight the novelty of our study.

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.

Regarding the reviewer's first point, to our knowledge no previous study has used SEC to specifically study the photo- and/or bio-degradation of DOM. Our main point is that the observations obtained from each method are complimentary and synergistic, irrespective of methodological differences between SEC and other methods used in published studies. This concept should also apply to trends in DOM behaviour pertaining to differences in molecular weight and to differences in their optical properties. As we are all aware, each methodology has strengths and limitations and directly

comparing individual variables from different techniques and experimental set-ups remain challenging, and for specific combinations maybe not be justified. This principle also applies to our study. We argue, however, that the compositional groups identified by SEC, delineated by molecular weight (proportional to elution time) and UV absorbance, are appropriate to consider trends of photo- or bio-lability and resulting DOM properties (albeit measured by different techniques). Combined, these enable us to cautiously hypothesize whether each compositional group might be more photo- or bio-labile. We are fully aware that there are uncertainties involved in making these hypotheses as for every other analytical approach.

Regarding the reviewer's second point, our intention is not to predefine but rather to explore how the DOM component groups identified by SEC could potentially be grouped to reflect their lability or recalcitrance to either photochemistry or microbial action and build testable hypothesis around this analysis. As the reviewer correctly states, there is indeed evidence for overlap between bio-labile and photo-labile DOM pools, as highlighted by the findings of Bittar et al. (2015b), which revealed an overlap of up to 15%. In a different study, Amado (2006) concluded that photo- and biodegradation were complementary in Amazon clear waters, degrading different fractions of the DOM pool. Despite being highly relevant regarding the key question, both studies are strikingly different to the approach we have selected for our study. Bittar et al focuses on characterizing extracellular and intracellular DOM pools, while Amando analyses white waters and lake waters from the Amazon basin. We analyse in situ processes in a small black water headwater and its larger immediate receiving waters. We are therefore very cautious to expect that the results from our and other published studies can be directly compared, though all studies target at the same (complex) phenomenon, but from very different perspectives.

Our approach is intended to be a starting point (using a novel experimental set-up and analytical technique in a largely understudied (but arguably important) sub-environment, small headwaters) with which to address the compositional DOM data. This central aspect will be stronger emphasised in our revisions. We plan to go into greater detail in our results and discussion by addressing the responses of individual component groups, and recognizing their apparent reactivity to both processes (e.g. "…HS was removed by microbial activity in five of the incubations, including BC1-c and BBR-c, at a similar magnitude to that of photodegradation. It appears therefore, that even HMW molecules within this compound group are bio-available…" (Lines 305-306)). Our proposed restructuring of the results and discussion section (outlined below) will be refocused and collated within the new section 4.2 that targets the complexity/overlap of photo- and microbial influences.

To firmly address these two significant points, we suggest adding the following section to the introduction:

"The compositional groups identified through this technique are delineated based on their optical properties (i.e. their UV absorbance at 254 nm, a proxy for CDOM) and their molecular weight (Huber et al., 2011). To the authors' knowledge, SEC has not yet been applied to study compositional changes in response to photo- and biodegradation. However, as highlighted above, many studies using a variety of other approaches have characterized the relative photo- and or bio-lability of DOM. While there are limitations in our ability to directly compare to other methodological approaches, it is possible to relate the potential reactivity of SEC compositional groups to other work, based upon previous characterisations of their properties (Huber et al., 2011). For example, Spencer et al., (2009) used UV-visible spectrophotometry to demonstrate that CDOM is photodegraded at a higher rate than bulk DOC. Thus, UV-amenable component groups identified by SEC could be relatively photo-labile. Conversely, several studies with varying methodologies have shown that the biolability of DOM correlates positively to lower molecular weights and negatively to its aromaticity (Kaplan and Cory, 2016, and references therein), suggesting that LMW, optically invisible SEC components could be relatively bio-labile. Though bio- and photo-labile DOM pools may or may not overlap in different

environments (Amado et al., 2006; Bittar et al., 2015b), these hypotheses represent a starting point with which to apply SEC to the investigation of DOM transformations."

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.

We refer to our response to the previous point and propose the following alterations aligned with the suggestions by the reviewer:

1) Adding the following dedicated section to the introduction:

"In addition to DOC, the DOM pool also encompasses Dissolved Organic Nitrogen (DON), among other components. It is important to consider the role of DON as well as DOC when exploring the response of DOM to microbial and photochemical influences. In addition to influencing net DOC concentrations and DOM compositions, photodegradation can also break down DON, leading to the formation of Dissolved Inorganic Nitrogen (DIN) in the form of nitrate and ammonium, which are critical for biological productivity (Zepp, 2003). From a microbial perspective, it has also been suggested that LMW, N-containing DOM in streams is relatively biolabile (Kaplan and Cory, 2016). Furthermore, the availability of DIN has been shown to limit the biological metabolism of DOM, whereas DON has been shown to be utilized by bacteria at a higher rate than DOC, affecting the rate at which they are biologically processed (Wiegner and Seitzinger, 2001) and thereby altering the C/N ratio.

2) Trimming the existing section 3.3 as follows (with Figure 5 being moved to Supplemental information):

"As highlighted in Section 2.2, LC-OCD-OND permits the quantification of DON in the HS and biopolymer component groups, and of DIN (ammonium and nitrate). We note that the initial availability of DIN measured in our samples cannot not explain the variation in the microbial-driven changes in DOC (Fig. 2a, Table S4). The initial C/N ratios of the HS fraction were relatively high (Fig. S3), suggesting a terrigenous source for this fraction, in agreement with the HS diagram (Fig. 4; Perdue and Koprivnjak, 2007). During incubation, neither the biopolymer nor HS component groups (Fig. S3) displayed a clear trend in C/N ratios, for either photochemical or microbial influences.

Furthermore, changes in the C/N ratios from neither component group correlated with observed changes in ammonium or nitrate (Fig. S3)."

3) Adding the following paragraph to section 4.4 of the discussion (see proposed layout below):

"Though we demonstrated the photo-induced degradation of HS, this was not accompanied by the production of DIN photoproducts (ammonium or nitrate; Fig. S3). Thus, despite the prevalence of HS in tropical headwaters, their breakdown does not appear to be a significant source of DIN; N-dynamics in tropical headwaters may therefore be dominated by DON sourced from elsewhere (e.g. building blocks, LMW neutrals, or LMW acids, which LC-OCD-OND cannot quantify). Previous studies, albeit with differing techniques, have shown that N-containing compounds are more associated with LMW, aliphatic molecules (Kaplan and Cory, 2016; Kellerman et al., 2015), lending weight to the idea that N stability in the DOM pool may be controlled by these compounds; tandem SEC and FT-ICR-MS analyses could help to better reveal this. It is also possible that, (photo-induced) microbial scavenging may have outstripped the production of DIN; our study set-up captured significant degradation of DOC on daily timescales, but photochemically and/or microbial-driven N cycling between organic and inorganic pools may have occurred on timescales too rapid to be tracked by our approach (Zepp, 2003). This concept could be tested in future by filtering or otherwise altering water prior to incubation to remove bacteria, combined with hourly sampling intervals."

Regarding the methodological differences between SEC and FT-ICR-MS, optical techniques, etc., as outlined above there is still a possibility for the information that is obtained from each method to be complimentary and synergistic – particularly regarding trends in DOM behaviour pertaining to differences in molecular weight, and to differences in their optical properties.

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.

We propose to better delineate the results and discussions sections as follows, with interpretations being moved to the latter:

3. Results

3.1. Photochemical and microbial-driven changes in DOC concentrations

3.2. Photochemical and microbial driven changes in DOM composition

3.3. Organic Nitrogen Dynamics of DOM

3.4. Combined photochemical and microbial-driven changes over a day-night cycle

4. Discussion

4.1. Factors influencing photochemical and microbial-driven changes in DOC concentrations

4.2. Photo- and bio-lability of DOM component groups

4.3. Advantages and limitations of in-situ measurements

4.4. Implications and further research

Likewise, the justifications/elaborations of the methodological approach will be moved from the methods to the discussion section (Section 4.3), with the existing methods section being streamlined.

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.

In light of these points, we propose the following clarifications to the abstract:

"……on average sunlight oxidised 5% of dissolved organic carbon (DOC) over 12 hours, at rates higher than or comparable to larger tropical rivers. Larger, ultraviolet (UV)-absorbing DOM components were removed, whereas optically invisible lower molecular weight (LMW) components showed a variable response. Microbial activity had varying, less clear influences on DOC concentrations or DOM compositional groups, with no preferential consumption of LMW components; biopolymers were particularly reactive to both processes. Overall, we show sunlight has a greater potential to mineralise tropical headwater DOM than microbial processes and thus potentially influence degassing."

Introduction:

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

We propose the following revision, which groups CO_2 and methane together as greenhouse gases:

"However, only 0.9 Pg C yr^{-1} is estimated to reach the ocean; with significant volumes of this flux being mineralised and released as greenhouse gases such as carbon dioxide (CO_2) and methane (CH_4). Approximately 3.9 Pg C yr^{-1} is emitted to the atmosphere as CO_2 (Battin et al., 2008; Cole et al., 2007; Drake et al., 2018), and wetlands (in particular tropical wetlands) represent the largest non-anthropogenic source of CH_4, comprising 20-30% of total CH_4 emissions (Nisbet et al., 2014; Bousquet et al., 2011)."

Line 43: 'Tropical...emissions'—this sentence seems out of place with the previous and following sentence.

We propose the following revision, which we believe flows more naturally:

"Indeed, the annual flux of dissolved organic carbon (DOC) from tropical rivers to the ocean accounts for ~59% of global riverine DOC flux (Huang et al., 2012), and the Amazon River alone emits 470 Tg C yr^{-1} of CO_2 to the atmosphere. Up to 75% of these emissions are estimated to derive from OM of near-channel origin, which has been mobilized to, and mineralized within, the aquatic zone (Davidson et al., 2010; Richey et al., 2002)."

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.

As discussed above, we are proposing to rework the DON references as suggested. As such, we propose removing the mention of DON on line 48 and instead adding a dedicated DON section to the introduction, as outlined above.

Line 70: 'Leading to...via respiration'—it can also lead to smaller DOM compounds and by products being formed.

We propose the following alteration:

"…leading to DOM mineralization and CO_2 production via respiration, as well as to alterations in DOM composition and the formation of by products (Cory et al., 2014)."

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

We propose altering 'ignores' to 'does not capture'

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).

We propose the following alteration, referring to the issues of using FT-ICR MS regarding solid phase extraction:

"…but due to the process of solid phase extraction cannot cover the full size range of DOM molecules; it excludes biopolymers, a key DOM component."

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.

We propose removing the word 'novel'.

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

Our intention was to refer only to the fact that Voss et al. had also shown rapid variability in composition and flux, rather than implying this study had the same methodology as Pereira et al. (2014). However, we recognise that this point is already made earlier in the introduction section (Line 50), and so have removed the reference to it highlighted by the reviewer.

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.

See the proposed structural reorganization above.

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.

We believe that this issue arises from a confusion/conflation between aromaticity as indicated by $SUVA_{254}$ (i.e. DOC normalised) and colour/absorbance (as indicated by absorbance at 254 nm) – the difference in the responses of $SUVA_{254}$ and absorbance at 254 nm suggests that these two properties

are not perfectly aligned; DOC concentrations may therefore be a controlling influence in the interpretation of SUVA. Thus, it is correct to say that coloured compounds were removed as the reviewer highlights, but they appear to have been removed at a slower rate than total DOC. We have made the following edits to clarify this point:

"Interestingly, however, the specific UV absorbance at 254 nm ($SUVA_{254}$) in all but one incubation increased due to photochemical influences (~2-5%, excluding BC1-a; Table 1). It appears then that though photobleaching occurred, UV_{254} decreased at a slower rate than the DOC concentration, suggesting that CDOM compounds were not removed at a faster rate than the total DOC pool."

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.

We propose to re-word uses of "incubation" to make distinctions between replicates at each site and different incubations at different sites. We will also clarify this terminology in the methods section.

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

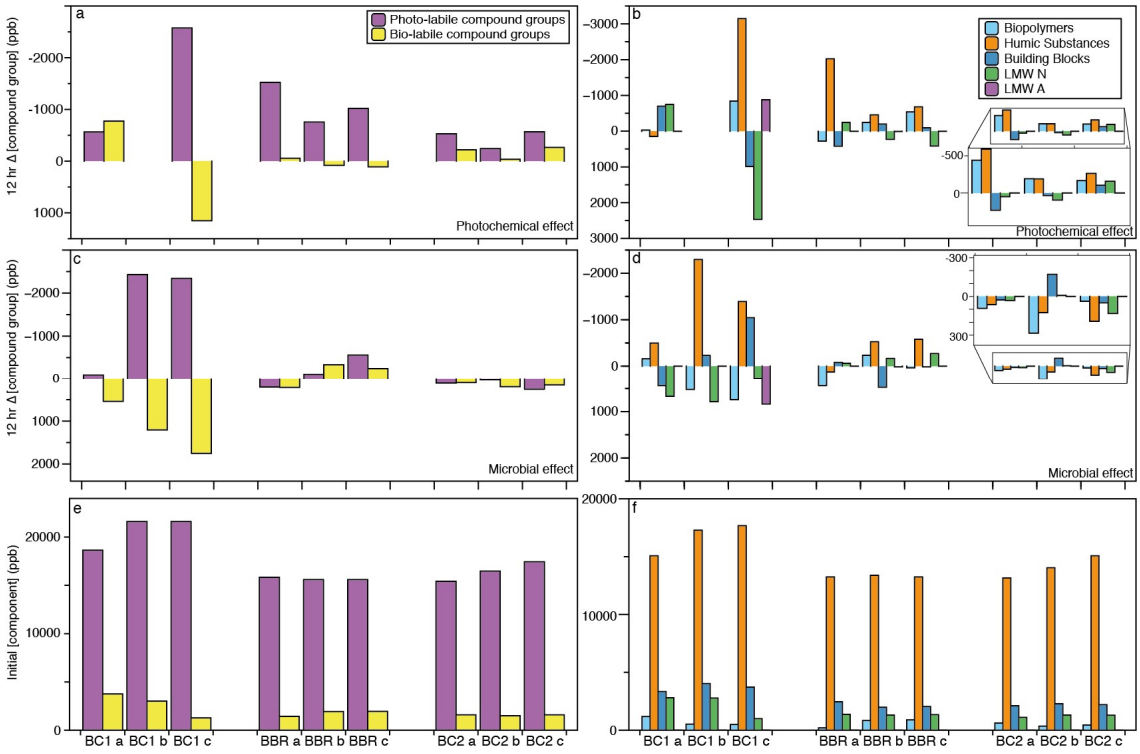
See the proposed structural reorganization above.

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 labelled 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.

We propose to alter the figure caption to correctly describe the data shown in each panel, as follows:

"**Figure 3. a) Photochemistry-induced, and c) microbial-induced changes in the concentrations of photo-labile (HS, protein-based biopolymers and building blocks) and bio-labile compounds (non-protein-based biopolymers, LMW neutrals, and LMW acids) over 12 h; e) initial concentrations of photo-labile and bio-labile compound groups. B) Photochemistry-induced, and d) microbial-induced changes in the concentration of each DOM component over 12 h; e & f) DOM compositions of initial samples for each incubation. Note inverted y axes for (a-d).**"

We propose showing the initial concentrations of each component DOM group instead of percentage changes, as shown below (e, f), with figure S2 then showing percentages. We also propose adding insets for BC2 in panels b and d, as suggested.



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

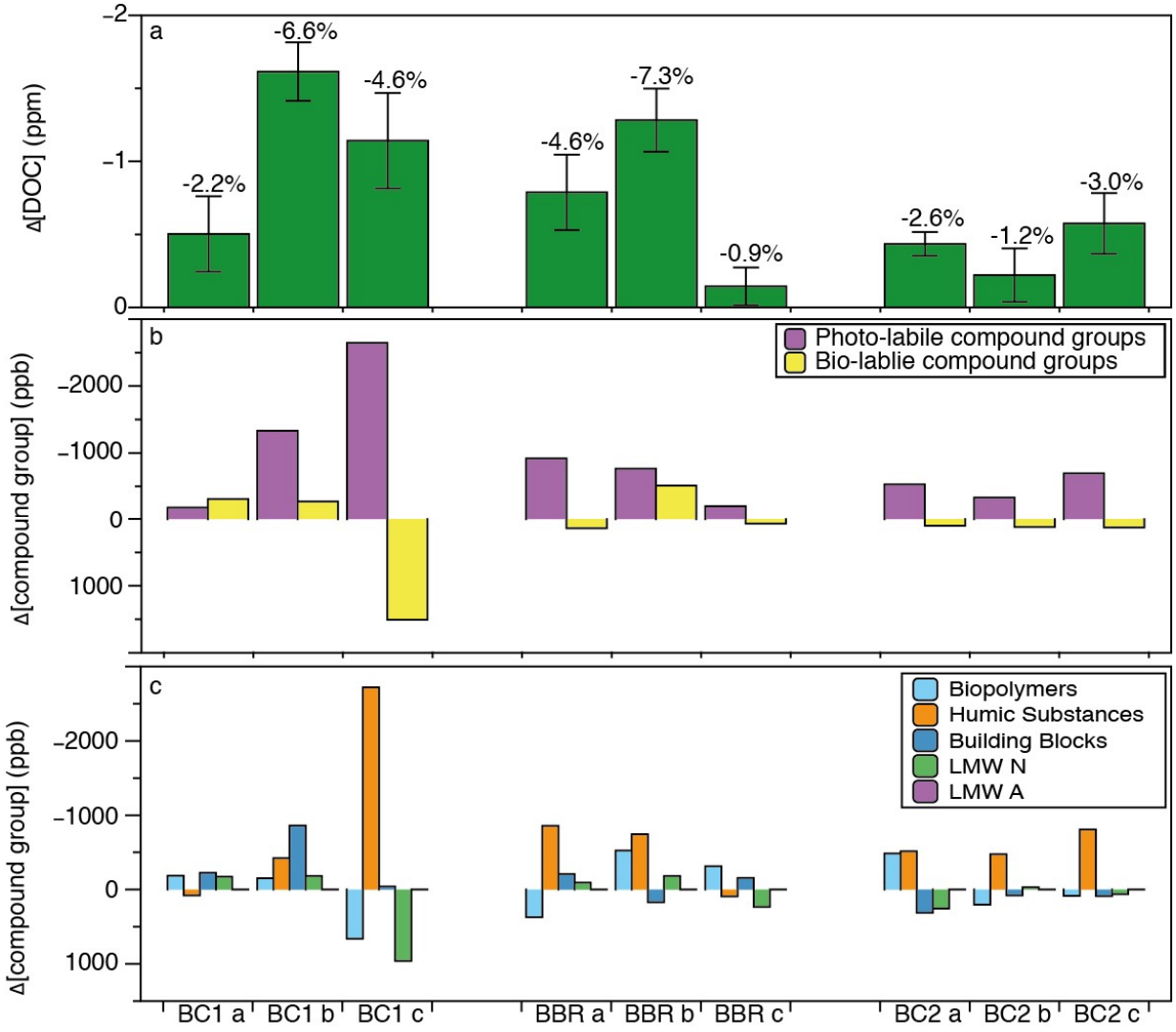
Lines 350-362: This section seems like it belongs in the discussion.

See the proposed structural reorganization above.

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).

Our rationale for placing figure 6 (now figure 5 as the DON figure has been moved to supplementary material- see above) in its original position is that it shows the combined effects (i.e. ambient response) of river water over a day-night cycle, not over 12 hours (the timescale shown in the current figure 2); we view this as secondary to the main aims of exploring photochemical vs. microbial influences on DOC concentration and compositional changes. Furthermore, this figure presents compositional changes (we have now expanded this to discuss group specific changes, see panel c), so putting it ahead of figure 2 would require restructuring to explain compositional changes. We believe that our proposed organisation of the results and discussion helps to alleviate any perceived lack of flow.



"Figure 5. Overall changes in (a) DOC concentration, (b) photo-labile and bio-labile compounds across a day-night cycle, and (c) DOM component groups (i.e. change seen in 'ambient' container over 22 hr incubation); error bars show SD. Note inverted y axes."

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).

We propose the following edit: "…That these rates are similar could suggest that the rate of transformation was relatively consistent from one location to the other."

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.

We propose rewording this as follows to clarify and show the need for further research:

"Our results do not cover longer time periods, however, they suggest that photodegradation is not necessarily limited to aromatic, HMW, and humic DOM, such that the photoreactivity of the remaining DOM pool may potentially persist if these were to be depleted over successive days of exposure, leading to similar rates of degradation downstream. There is indirect support for our assumption. The mean degradation observed in our study (5%) is comparable with those of a previous study on river water from the main stems of the Rio Negro, which reported DOC photodegradation of ~5% in unfiltered river water after ten hours of exposure to natural sunlight (Amon and Benner, 1996). It possibly exceeds that observed in the Congo River, which showed ~10% photodegradation of DOC over 48 hours of continuous exposure to a solar simulator (the intensity of which likely exceeds natural sunlight; Spencer et al., 2009). Direct comparison with the Rio Negro or our data is difficult however, given that river water in the Congo study was filtered and poisoned prior to incubation. Further investigation is needed, therefore, to determine whether photodegradation rates, and therefore potentially rates of CO_2 degassing, change along the river continuum from source to sea. This could be achieved by conducting incubations along the length of a river network – for example analysing the main stem and mouth of the Essequibo in addition to BC and BBR – to explore how DOM composition and DOC concentrations change along the river continuum (Cole et al., 2007)."

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.

We propose the following clarification:

"The findings presented here suggest that photodegradation has a higher potential for in-stream DOC removal than biodegradation, suggesting that the former may therefore be a more important driver of the degassing of CO_2 in tropical headwaters, at least on daily timescales. However, further exploration is needed regarding microbial degradation processes hosted within sediment, which were not considered in the present study. "

As discussed in the response to the reviewer's comment regarding residence times below, we believe the timeframe of the incubation is appropriate for quantifying the reactivity of processes in headwaters. As the reviewer highlights, we already discuss the need to do longer incubations to better quantify microbial processes.

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

This assumption is correct and we will revise this accordingly.

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

We will remove the first occurrence of this sentence (Lines 401-403). The first occurrence of this sentence pertains to introduce the data shown in Figure 6 (now Fig. 5), but as discussed above we are now proposing to move this figure into the new results section and re-write the text accordingly.

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.

We propose the following clarification to better characterize the typical hydrological conditions of the streams, building on our original point on Line 429 regarding the suitability of the incubation time:

"Though no dedicated studies of residence time have been conducted at either study site, the mean velocity measured near to Blackwater creek sites BC1 and BC2 across a period of one month (spanning all incubations) was ~0.29 m/s (max = 0.52 m/s; min 0.05 m/s). Given the stream length of ~6 km, we estimate a mean travel time for the catchment of ~57 h (min =~31 h; max = ~ 14 days). A study of a different tropical stream catchment ~ 1.5x bigger than Blackwater creek determined a mean transit time during the wet season of 15 days (Birkel et al., 2016), though clearly there are differences in hydrology, topography, etc. between the two catchments, making any direct connections/comparisons most difficult."

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.

We are wary of overly inflating this section of the discussion without appropriate results, but we are comfortable to propose the following expansion of our discussion to highlight the point that, as stated by the reviewer, in-incubation adsorption to suspended particles could have led to some internal DOM fractionation:

"A further consideration is that adsorption to iron hydroxides within the water column could have fractionated the DOM pool to some extent; studies have shown that aromatics and polyphenols are more likely to adsorb and become stabilized, whereas aliphatic compounds preferentially remain in solution and are more easily destabilized once adsorbed (Coward et al., 2018).The lack of appropriate data from our in situ experiments, however, does not enable us to take this discussion forward."

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.

We propose the following alteration:

"The degree of DOC degradation observed in BC is higher than that observed in headwaters in other climate zones – like …….. These comparisons are hampered however by the lack of a consistent methodology used to quantify degradation in each case. Application of the technique demonstrated here to different climate zones and different river stages could therefore help to quantify how variations in DOC concentrations, environmental conditions, and DOM composition help to modulate degradation; whereas companion lab- and field-based incubation approaches conducted at the same sites could help to inform on differences in methodological approaches. Overall, there is a need to derive consistency across approaches."

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

This should read Fig. 6 (now Fig. 5 given removal of DON figure to Supplementary info); we propose to correct this accordingly.

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.

We think that in the context of the paragraph in which this statement appears, it is obvious and clear that we are discussing the study location (there are several occurrences of 'tropical headwaters') and the timescale ('Daily, the degradation of…' (previous sentence) and '…over daily timescales…' (following sentence)). Furthermore, one of the points of our study, as we attempted to outline in our introduction, was that smaller spatial and temporal scales (i.e. headwaters, hours-days) need to be further investigated to better understand the natural system. We propose to more clearly stress this point in the conclusion.

References:

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