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Dear Anonymous Reviewer 2,

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:

In their manuscript, Spray and co-authors investigated the effect of light exposure and microbial activity on the degradation of dissolved organic matter (DOM) in tropical headwater streams. They conducted in-situ experiments, with containers fulfilled with unfiltered water and covered or not by a lid to mimic dark (microbial degradation only) or ambient (photochemical + microbial degradation) conditions. Containers were anchored at each site (3 sites), and experiments were performed three times. Changes in dissolved organic carbon (DOC) and DOM composition (using absorbance and size exclusion chromatography) were investigated after 12h of incubation. In order to separate the effects of photodegradation from microbial processing on DOC losses and changes in DOM composition, "the values from the 'ambient' container, which theoretically represent the combined effects of both photochemical and microbial influences, were corrected by subtracting the values from the 'dark' container" (lines 176-178). Based on this approach, the authors observed higher DOC losses in "ambient" treatments while no clear trend was identified upon microbial degradation processes. The authors concluded that photodegradation has a larger influence on DOC degradation than microbial activity.

The relative importance of photochemical mineralization versus microbial degradation is still an unresolved question, and thus the topic addressed by this study is relevant. The experimental setup is original in that sense that more classical studies use incubation in laboratory-controlled conditions, and therefore diverge from field conditions. However, I found several issues with the approach that may affect data interpretation and conclusions. I detailed my comments below and hope that they will be useful.

General Comments:

The structure of the manuscript could be improved significantly. In its current state, the results section contains a lot of interpretation. The authors could merge the results and discussion section as a Results and Discussion section, or better differentiate the two sections.

In response to these suggestions, and similar points from reviewer 1, we propose to better delineate the results and discussions sections as follows, with interpretations being moved to the latter section:

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

My main concern is about the calculations made to separate photochemical degradation from microbial degradation outlined lines 176-183, where values from the 'ambient' container are corrected by subtracting the values from the 'dark' container. By doing so, the authors made the underlying assumptions that photochemical and microbial degradation are cumulative processes and that the microbial degradation of DOM is not affected by photochemical reactions. In other words, this approach suggests/implies that DOM microbial degradation is similar between "dark" and "ambient" treatments. However, there are clear evidence of interactions between photochemistry and biological degradation where sunlight exposure of DOM can either increase or decrease its bioavailability (Bertillon and Tranvik, 1998; Kaplan and Cory, 2016 for a short synthesis; Moran and Zepp, 1997). Given the experimental setup, the authors cannot exclude the fact that the dynamic of microbial degradation (both in terms of decay constants but also changes in DOM composition) differed across treatments. Therefore, I don't know what really mean the ambient – dark calculation from a conceptual point of view, but I am not convinced that it represents photochemical effects solely as interpreted by the authors along the manuscript (e.g. fig 2, lines 199, 206, 209-211, 225, 273, 275-277,...). In consequence, several interpretations and conclusions are not supported by the data presented. For instance, the authors concluded that there was no photo-stimulated biodegradation (lines 322-324) or that "photodegradation is a more influential process on the complete mineralization of DOC than biodegradation" (lines 418-420), yet the two processes cannot be so easily distinguish by the ambient-dark calculation. Maybe directly comparing treatments after incubations would be more appropriate to highlight the influence of light exposure on DOM degradation.

Our aim is to, as best as possible, disentangle net biotic from abiotic processes in close to 'real world' conditions, and in a remote setting where lab-based approaches are impractical. We are fully aware and accept that our approach, as any experimental and multi-proxy approach, has strengths and limitations. Our strength is the close to 'real' conditions, whereas one of our limitations may indeed lay in the not quantitative separation of photo-chemical and photo-stimulated microbiological turnover processes. We accept this fact and will work on the balanced wording of our text. The reviewer is correct to highlight that the photochemical effect we isolate may also incorporate immediate photo-stimulated microbial changes, but then other microbial control methods (mercuric chloride, autoclave, acidification and filtering) have been demonstrated to alter the DOM pool in some way and hence are equally not perfect – there is no one ideal approach. The practice of subtracting 'ambient' from 'dark' DOC loss in unfiltered river samples as a control may not be perfect and quantitative but has been applied before (Moody and Worrall, 2016) to successfully disentangle these effects. We note that in this previous study the authors' referred to this difference as "photo-induced degradation" rather than "photochemical degradation/photodegradation". We plan to pick up this revised definition and introduce the distinctions regarding DOC loss and compositional changes in our manuscript by modifying the last paragraph of the introduction:

"Approaches used to ascertain the importance of photodegradation and biodegradation of DOM in tropical headwaters isolate and incubate water, and establish how the DOC concentration and DOM composition changes over time, and by what dominant process (photochemistry versus microbial activity, or combinations thereof). These approaches tend to follow either laboratory-based methodologies, in which properties such as light and temperature can be controlled for, or are conducted in natural conditions, which are more representative but make disentanglement of multiple influences more difficult. In both approaches, photochemical and microbial influences are often separated by controlling for the latter by incubating a portion of the water sample in darkness. The further practice of treating and/or filtering the sunlight-exposed water prior to incubation can help to better control for rapidly occurring photo-induced biotic effects (e.g. Stubbins et al., 2010), but invariably alters the DOM pool, reducing representability. Incubating unfiltered, untreated water cannot control for these immediate photo-induced biotic effects, but can better characterise realistic net DOM changes (e.g. Moody and Worrall, 2016). Here the latter approach was adopted; unfiltered water samples collected from three sites in the headwaters of the Essequibo River, within the

Iwokrama Rainforest, Guyana (Fig. 1) were incubated in-situ in the river column to as close as possible achieve results representing actual in-stream processes. Splitting the initial sample and only exposing half to sunlight allows us to isolate and quantify net photo-induced processes from purely microbial ('dark') influences (Fig. 2a). Furthermore, by analysing the resulting samples using SEC, for the first time we can quantify not only changes in DOC concentrations, but also degradation-influenced compositional changes in DOM."

We also propose to stronger highlight the benefits and limitations of the approach in the newly proposed discussion (section 4.3), as follows:

"As outlined in the introduction, incubating unfiltered river water in natural conditions within the water column is beneficial in that it can quantify representative net changes in DOC concentrations and DOM compositions. It is also particularly appropriate for relatively remote locations, such as tropical headwater streams, where access to laboratories is limited. The decision to not treat water in the ambient containers to control for biota was taken to avoid altering the DOM pool, in an effort to maximise the representativeness of the results obtained and take advantage of SEC's ability to quantify a wide range of the DOM pool. The drawback of this approach, however is that the net photo-induced changes quantified likely include relatively rapid photo-stimulated microbial changes (Kaplan and Cory, 2016). In future, running a third incubation with poisoned or otherwise treated water could quantify this difference, while also revealing the effects of said treatment on the DOM pool as characterised by SEC."

Section 4.3 will also incorporate the discussions of POC-DOC, adsorption, sediment-hosted biotic processes, etc. detailed below.

Regarding our approach to quantifying 'photo-stimulated degradation,' again this was inspired by an approach and justification proposed by Moody and Worrall (2016). We recognise that this approach would be unable to quantify 'immediate' photo-stimulated biodegradation, but in the event that photoproducts were to accumulate in the water column under sunlight, then one could still expect to see some difference in the *overnight* behaviour in each container. It is still, in our opinion, therefore valid to test this concept, particularly regarding the lack of available data in tropical headwaters. Bertilisson and Tranvik (1998) found that in humic lake water, the production of photoproducts outstripped the rate at which they were consumed by bacteria during the day, leading to their accumulation in the water column (thereby leading to the possibility of the continuation of their consumption overnight). If such a trend were to have occurred at our sites, we believe that the approach taken would theoretically have been able to detect it. We propose to highlight this distinction in section 4.1 of the discussion (see new proposed structure for results and discussion) as follows:

"We explored the possibility that the accumulation of photoproducts during the day, as demonstrated by Bertilisson and Tranvik (1998) in humic lake waters, would lead to overnight photo-simulated biodegradation. Furthermore, it is possible that photoproducts may have been immediately taken up by biota in the 'ambient' container, as the water was not filtered or sterilized prior to incubation; rapid utilization may explain the scarcity of LMW acids during analysis (Fig. 3). A previous study with a similar methodological approach also found no evidence of overnight photo-stimulated bacterial degradation over a similar timescale in a temperate peatland headwater (Moody and Worrall, 2016)."

The interest of the experimental setup is that it is closed to field conditions. However, it is not possible to determine how other processes (POC-DOC exchanges, primary production) may affect the results obtained as water were unfiltered. Primary production (and DOM release) may also have been affected by light exposure. I would appreciate that the authors discuss the limitation of their study.

Our discussion highlights the point that, as stated by the reviewer, POC-DOC changes and primary productivity could have led to DOC concentration changes and some internal DOM fractionation. We

touch on this when framing the increases in DOC observed in the 'dark' incubations (line 217) and mention POC changes in the discussion (Lines 438-441). We propose to also expand this discussion to mention fractionation through adsorption, as follows:

"The approach presented here is limited in that it does not quantify POM-DOM interchange during the incubation process – filtering prior to incubation could circumvent this issue, but would reduce representability, whereas significantly larger samples would be needed to quantify POC. 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)."

We also propose to discuss that this approach only quantifies 'in-water column' changes, with sediment-hosted microbial processes being absorbed:

"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 on daily timescales. However, further exploration is needed regarding microbial degradation processes hosted within sediment, which were not considered in the present study. "

These points, alongside an overall discussion of the limitations and benefits of the in-situ, unfiltered approach (see our response to first general comment above) will be consolidated into a dedicated section of the discussion (Section 4.3; see proposed restructuring of Results and Discussion).

Finally, a direct comparison of "photodegradation rates" with previous literature should be done with caution. It is hard to compare results obtained from different studies as the protocols may be very different, especially in this study.

We fully concur with the critical view of the reviewer. In our revised discussion of photodegradation rates (also see below) compared to other tropical rivers, we propose the following:

"The mean degradation (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 is difficult however, given that river water in said study was filtered and poisoned prior to incubation."

When discussing the extension of our approach to other climate zones, we propose the following clarifications:

"The degree of DOC degradation observed in BC is higher than that observed in headwaters in other climate zones – like a boreal headwater (0.2 km^2 catchment) in Sweden (~10% decrease in DOC over 48 hrs (Köhler et al., 2002)) – but is lower than that observed in a temperate peatland headwater (0.2 km^2) in the UK (~40% decrease over 24 hrs (Moody and Worrall, 2016)) and two temperate forest streams in Japan (~21-36% over 12-13 hrs (Mostofa et al., 2007, 2005b)). 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."

Specific comments

Line 14: "from "Line 15: the authors should present the mean instead of the higher values measured and used it to compare to previous studies (keeping in mind the limitation of such comparison). Mean DOC loss are 5%, and the 9% loss is more like an outlier than a real pattern.

Line 201: "The upper limit of this degradation exceeds those observed..." Please use the mean instead of the higher value, this is not representative.

We propose the following modification 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."

We have also propose the following edit to the results section (now in Discussion section 4.1) "The mean degradation (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 is difficult however, given that river water in said study was filtered and poisoned prior to incubation."

Lines 130-137: I would not have expected significant difference across treatments during the night since photochemical reactions and biological uptake are very rapid processes.

Lines 210-211: "non-aromatic compounds were preferentially removed by sunlight" ...or consumed by microbial communities. Overall, the ambient-dark calculation is interpreted as being solely due to photochemical processes along the manuscript, but this is misleading as biological activity was also occurring. This echoes my main concern about the approach used by the authors.

Lines 322-325: as said above, I am not surprised that no difference was observed during the night because photodegradation reactions are very quick, and labile compounds potentially produced are likely consumed also quickly and thus do not accumulate in the water column. Moreover, the authors cannot conclude that there was no photo-stimulated biodegradation due to the experimental setup and the limits of their calculation.

Please see our response to this issue in the general comments above.

Lines 225-228: Sites are located in the same river networks and close to each other, DOM collected at BBR comes from BC1 and BC2 headwaters as shown by similar initial composition (table 1 and figure 3), so it is not surprising that composition and reactivity are similar despite differences in drainage areas.

It is perhaps unsurprising that initial compositions are similar given the sites' closeness, though high temporal variability in DOC concentrations and organic matter composition have been observed in this region previously, as we highlight in the introduction (Pereira et al., 2014). We still believe that it is of note to highlight the different hydrological differences of each site, however, alongside our discussion of differences in cumulative irradiation and physical properties in the lines immediately following (Lines 229-242), with the proviso that this entire section will be split to better delineate the Results and Discussions sections as discussed above.

Lines 456: fig 6?

This should refer to figure 6 and we will update it accordingly (though note that this will become Fig. 5 as we are proposing to move the DON figure to the supplementary material in response to Reviewer 1).

Figure 3: there are errors in the caption.

We propose to correct the figure caption accordingly:

““**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).**”

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