

Interactive comment on “Quality transformation of dissolved organic carbon during water transit through lakes: contrasting controls by photochemical and biological processes” by Martin Berggren et al.

Response to Anonymous Referee #2

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Response to GENERAL COMMENTS

In this work, the authors aim to determine the relevance of bio-and photo-degradation processes during the water transit time in individual lakes. The authors hypothesize that each process will prevail as a function of the color of the DOC compounds, so that biodegradation will target non-colored DOC while photo-degradation, colored DOC compounds. Using a complex data set at different temporal and spatial scales and including both field and experimental data, the authors found brown-water lakes to be dominated by biodegradation processes (not photo-degradation), which leads to their persistent brown-water color.

The authors present these results as contrasting with the current paradigm of loss of colored constituents of DOC along the inland waters continuum. However, they do not provide such a continuum (i.e. accumulated water residence time along the landscape), they do not evaluate the molecular composition of DOC and, the presented here are net changes (i.e. including production and degradation of DOC) but they are not discussed as so. I consider the partitioning between photo and bio-degradation processes a key question to complete our knowledge on the pathways of C processing in inland waters. But because of this relevance, I ponder indispensable that the authors clarify those concerns above and the ones specified below (such as properly assessing the role of hydrology, improving the characterization of DOC or providing the complete results -the last specially affecting Figure 2-) before this manuscript can be considered for publication. I hope these comments are helpful and constructive.

Reply: We are thankful for the Reviewer’s constructive and much-thorough review that has helped us to improve the manuscript. We agree on the points mentioned in this general comment. Therefore, as explained in detail below (under specific comments), the revised paper will, compared with the original submission: 1) be more careful when discussing what our study suggests about loss of colored DOC along the inland water continuum; 2) discuss more explicitly what our results suggest about the molecular composition of DOC and the role of hydrology; 3) be clearer about the fact that our study addresses net changes in DOM properties; 3) provide appendices with more complete results, in terms of both statistical details and reporting/plotting of raw data.

All of the Reviewer’s comments can and will be adequately addressed in our revised manuscript. However, the DOC characterization that we have at hand is limited to information that can be extracted from UV-VIS absorbance and compound-specific analyses performed using LC-MS. We get the impression that the Reviewer would have preferred to see additional DOC composition analyses (e.g. FT-ICR-MS molecular analyses or fluorescence EEM/PARAFAC), but such data

do unfortunately not exist for this data set. Nonetheless, in the revision we will go deeper into the discussion of what our data suggest about patterns in molecular DOC composition. We will also provide justifications and explanations to why we present and analyze the absorbance data the way that we do. We strongly believe that our manuscript has sufficient data to present an original and important story about how the properties of DOC change with transit time in lakes.

Response to SPECIFIC COMMENTS

1. *Abstract P1 L17: “photo-chemistry qualitatively dominated”...what does qualitatively mean here? That the changes in DOC quality were dominated by photo-decay? That you assess that in a qualitative (i.e. non-quantitative) way? Clarify in the text. Also, photo-chemistry dominated the DOC or the CDOM transformation in headwater lakes? How is the production of non-colored DOC evaluated? Clarify in the text.*

Reply: In the revised abstract, we have changed this phrase to clarify that ‘changes in DOC quality were dominated by photo-decay’, according to the first suggestion by the Reviewer. However, it is actually also true that we draw this conclusion based on a qualitative line of reasoning, i.e. we observed that the directions of change in the DOC quality *in situ* were matching the directions of DOC quality change observed in light exposure experiments (as opposed to dark conditions where the directions of DOC quality change were the opposite). In other words, we do not make a quantitative assessment here (e.g., % dominance by photo-processing), but rather we note the qualitative agreement between *in situ* and laboratory data. The revised methods description will be changed such that this becomes clearer.

2. *P1 L19: Was there a systematic relationship between color loss and WTT in Clearwater lakes? Add this information also.*

Reply: Yes, in clear lakes the color loss was systematic. We will add this information as suggested.

3. *Introduction P2 L17: Maybe biodegradation processes do not affect colored DOC preferentially, but that they do affect it at all has a stronger impact on the inland waters C budget than the consumption of in-situ produced DOC. Add information on the DOC sources and their relevance on the C budget here.*

Reply: This comment is not completely clear, language wise, but we think the Reviewer means that we should expand the text to explain that bacteria do indeed remove colored DOC – they just don’t remove it as efficient as they remove non-colored DOC. In the revision, we will further mention additional DOC sources (we assume the Reviewer means ‘other than terrestrial’) and their relevance as suggested. We will cite one or a few references showing that boreal unproductive brown-water systems mainly have terrestrially-derived DOC, i.e. other sources play minor roles, although autochthonous production can be relatively more important in clearer and more nutrient-rich systems.

4. *P2 L22: Available references on “efficient” photo processing, showing how polyphenolic, aromatic compounds are mostly affected by photo reactivity (assessed at a molecular level) in black and boreal waters, are missing (e.g. Stubbins et al. 2010 L&O, Kellerman et al. 2014 Nat. Comm. and references therein).*

Reply: We will insert the two suggested references. It appears most appropriate to cite these references after the statement ‘UV light oxidation could theoretically explain losses of colored DOC’, in the preceding sentence.

5. *P2 L27: I agree with the authors that the assessment of the variability of WTT within systems is very relevant. However, without assessing how that variability is linked to changes in color of runoff DOC, it is hard to attribute the changes in the lake just to in situ biogeochemical processing. Clarify that here and incorporate that perspective throughout the text -see comments below-.*

Reply: We will re-write this section to clarify that the export of DOC from small headwater catchments in the region is strongly episodic. There are several classical papers from the Krycklan Catchment Study to exemplify this; for example

Laudon et al (2004 *Aquat. Sci.* 66:223-230) showed that 50-70% of the entire annual organic carbon export comes just during a short period of snowmelt in spring, and we know that much of the remaining export happens during discrete autumn rains. Given this pulsed nature of inflowing water and DOC, we do not agree with the Reviewer that it matters how the DOC or color varies temporally during other situations than high-flow. If the total DOC export is negligible during low-flow, then this carbon will not contribute significantly to the DOC that resides and gets processed in the recipient lakes, and thus it is not relevant to know the properties of such DOC entering during low-flow. It would be more critical if there is large variability in DOC concentrations and color within the high-flow episodes, but this does not appear to be the case.

We do agree with the Reviewer that we need to incorporate this perspective better, both here in the introduction and elsewhere in the manuscript. In the new revised introduction and methods parts we will explain why we expect that (in our specific study lakes) it is the transit times through the lakes that will matter for the color – not differences in color levels of the water that comes in from the catchment during different times. Moreover, we will test and confirm that this assumption is true, as explained in response to specific comment #25 below, with added results/discussion parts related to this. For example, we will present data showing how much (%) of the total DOC exports that takes place during episodes, defined as flow rates above a certain percentile. We will be able to show that: 1) most of the DOC and color enters the lakes during high-flow conditions and; 2) DOC and color variations are relatively small during these high-flow conditions. Together these two circumstances imply that colored DOC enters the lakes mainly in distinct high-flow pulses, and it is removed during in-lake processing during low-flow periods when the catchment plays a negligible role in adding new DOC and color to the lakes. See more details in our response to specific comment #25.

6. *Methods P3 L16: modify this sentence into “lakes are located in the boreal region, where nutrients” and provide a reference of that distribution.*

Reply: We will make the change as suggested and cite the distribution by Verpoorter et al (2014, *GRL* 41: 6396-6402).

7. *P3 L30-32: Although, low effects of pH on the optical properties of DOM have been reported at the most frequent inland water's pH range (i.e. 5.5-7.5), they can be important at lower pH values (< 4.5), such as the ones included in this study. Accordingly, add a paragraph in the discussion stating which lakes presented these low pH values (i.e. 3.4) and how could that affect your absorbance measurements (some useful literature: Pullin and Cabanis et al., 2003, *Geochim. Cosmochim. Act.*; Patel- Sorrentino et al., 2002 *Wat. Res.*; , Spencer et al., 2007, *Wat. Res.*).*

Reply: We will add this discussion as requested. The Reviewer is correct that there can be optical effects due to low pH values, and that we presently do not give attention to such effects. In principle, as explained in these references that the reviewer provides, an extremely low pH causes a very high degree of protonation of the molecules, which in turns means that they physically shrink into a compact mode. In their most compact/protonated state, the overall light absorption by the DOC molecules may not be at the highest, but specifically the short-wavelength UV radiation that has most energy is efficiently absorbed. This can lead to marginally higher photo-reactivity at an extremely low pH compared to moderately acidic conditions. We will discuss how this might have influenced our results in the new manuscript version.

8. *P4 L6: Consider reporting Catchment area/ Lake area ratio as a more relevant variable to discuss epilimnetic WTT than catchment area alone.*

Reply: Since our study sites are similar in size, it is mainly the catchment area that is important for the WTT. To be more precise, the variation in lake area (1-5 ha) is small compared to the 100-fold variation in catchment area (Table 1). Therefore, we do not consider that it is necessary to also report catchment to lake area ratios. We will explain this in the revised ms version.

9. *P5 L3: Why using only 3 wavelengths if the whole spectra were available? Given the aim of the study, much more robust conclusions could be reached if other widespread descriptors such as SUVA254 and slope analysis were included, and I recommend their inclusion. Those descriptors are widespread, and in particular, spectral slope analysis, is recognized to provide further insight into DOM composition than absorption coefficients alone (see*

Helms et al. 2009 L&O, Loiselle et al. 2009 L&O). Package “cdom” in R could be a useful tool to perform that exploration.

Reply: We agree that SUVA₂₅₄ is a relevant variable, and we did use this in previous manuscript versions. However, since SUVA essentially showed the same patterns as the a₄₂₀/DOC ratio, we removed it to avoid redundant data that does not add to the story. We will explain in the revised version that these two variables are strongly correlated. Similarly, while we could use a number of different spectral slope indicators, it would not be meaningful since all of them would correlate strongly with the spectral slope indicator that we already have, i.e. the a₂₅₄/a₃₆₅. However, what we can do in the revision is to explain better why certain choices were made, and what these choices mean. Part of this choice is a matter of research tradition, or even taste, but we think it is important to address how the metrics that we have chosen relate to other metrics that are common in the literature. Thus, we will add such explanations to the revised ms version.

10. P5: Calculations for outflow are nor provided but they are presented in Figure 1. Add this information here.

Reply: In the revision we will clarify that complete mixing of the epilimnion is assumed, such that outlet water is equal in its properties (including WTT – time spent in lake) to epilimnetic water.

11. P6 L17: Are all the other catchments spatially independent? Even if the inlet streams are considered negligible, what about the accumulated time in the catchment (sensu Müller et al. 2013 Aq. Sci.)?

Reply: With regard to the first question: yes, all other catchments are spatially independent. Regarding the second question: we are interested in the accumulated time in the freshwater network itself, sensu Berggren et al (2009, L&O 54:1333-1342). This is in our case the same as the accumulated time in the view of Müller et al. (2013), because the streams are headwaters even in the strictest definition, i.e. there are no upstream lakes that would add residence time. Thus the drainage dynamics is strongly pulsed, and water is flushed more or less directly from soils to the lakes. These aspects will be explained in our revision.

12. P6 L27: The relative contribution of LMWC to total DOC (%) should be used instead of the total concentration of organic acids. A higher total sum of organic acids could be just due to a higher DOC concentration. Thus, to clarify if samples have a higher relative contribution of LMWC compounds or just higher DOC, the relative contribution of LMWC to total DOC (%) should be used, and ideally both (LMWC for each sample and in % and in mgC L⁻¹) shown in the Supplementary Information. Also, is the correlation between a₂₅₄:a₃₆₅ and the organic acids positive or negative? Should be stated.

Reply: In the revised ms, we will show LMWC both as absolute amounts and as percentages of total DOC. These variables will show similar patterns. In the new manuscript, we will clarify that the correlation in question is positive, as suggested by the reviewer.

13. P7 L13: Bacteria might dominate the biomass, but still be predated by heterotrophic flagellates. How does the bacterial abundance looked during the experiments? Moreover, 450 days is a very long period, which effects would have both the predation and the death of the bacterial community and subsequent mineralization of that biomass on the results? How fair is it to consider that these results reproduce the biodegradation process occurring in the field where lakes behave like chemostats not like batch incubations? Justify in the text, and discuss later the implications and assumptions that have to be done to compare both results in the discussion.

Reply: Since there is an overlap between this comment and concerns by Reviewer #1, we like to start by pasting part of the reply to specific comment #6 by Rev 1:

“This comment helped us see that the purpose of our laboratory experiments was not sufficiently well described in the original submission. Briefly, what we wanted to achieve was experimental conditions during which either 1) photochemical reactions strongly and dominantly influenced the DOM transformation, or 2) microbial degradation strongly dominated the DOM transformation. Thus the experiments were designed such that the response to a large light dose or a long microbial

process time in the dark was measured. While we don't believe that such experiments mimic lake *in situ* conditions in an adequate way, they do provide qualitative information about how the DOM responds to the isolated effects of photochemical and biological decay. Interestingly, the patterns of DOM transformation found in dark experiments well matched the *in situ* DOM quality changes observed in dark (brown or hypolimnetic) environments, while our light experiments matched the qualitative patterns in DOM transformation in clearer and more light-exposed environments *in situ*. These findings are supporting our interpretations. [...] Therefore, based on the above, in the revised manuscript we will provide a clearer rationale for the experimental design of our study. We will also highlight that the experimental results only provide qualitative information about how the DOM responds to different types of decay – it is not possible to make quantitative comparisons.”

On the specific comment about biomass, the present discussion paper cites Daniel et al (2005) on the rough biomass contribution of 90% by bacteria in food webs (microbial communities) developed in the dark in humic water. It is a reasonable assumption that bacteria were similarly abundant in our incubations, but as we did not measure biomass this can only be speculated on. We did monitor bacterial production (not shown), and as expected it decreased systematically with increasing incubation time. This is much expected as bacterial production has been shown to decrease with increasing water residence times *in situ*, in lakes of the study area (Bergström & Jansson 2000, *Microb Ecol* 39:101-115; Berggren et al 2009, *L&O* 54:1333-1342).

In the revised discussion we will give attention to the fact that our incubations involved batch DOM degradation performed by an artificial microbial ‘bottle community’ that may be different from the *in situ* community. We will however maintain that these dark incubations fulfilled their purpose of showing how (qualitatively) DOM properties change in response long-term biological processing.

14. P7 L18: *I agree that microbial processing can happen in the entire water column, but I believe the simultaneous action of UV and biodegradation cannot be discarded. On the one side and mainly, because photo-mineralization rates are faster than biodegradation rates. On the other side, because there are several situations where the entering water will be exposed to both (i) water in the hypolimnion, would have been initially exposed to both UV and microbes when entering the lake, ii) under ice conditions, microbial activity would also be minimal due to low water temperature iii) during the ice-free period and at that latitude, daylight is almost for 24h). Thus, both processes are likely to occur also simultaneously or following the inverse sequence (photodeg --> biodeg). Justify that, considering the number of papers using the opposite approach. The authors could also perform a much deeper exploration of the changes between layers with the temporal data available and in light of the results shown in Fig.2 on that direction.*

Reply: This is a relevant point brought up by the Reviewer – there are certainly numerous interactions between microbial and photochemical processes in nature, but with our experimental approach we are not able to address these interactions. As mentioned in response to the preceding comment (#13), we plan to expand the discussion with a section that deals with limitations in the experimental approach that we chose for this study. In this new section we will also bring up the aspects mentioned in the comment above, i.e. potential interactions between light and dark processes that we currently do not recognize in the discussion paper. We will link this discussion to what results from the different layers, as hypolimnetic waters have very little light intrusion also in the clearest of the sites. Thus differences in patterns between the depth strata of the same lakes can be used to discuss the impact of the light processing *in situ*.

15. P7 L25: *Similarly for photo-decay than for bio-decay: even if a radiation equivalent to two years was applied, there was no water renewal considered. Discuss how well you expect this results to reproduce the process in the field.*

Reply: This will be discussed as suggested. Again, the Reviewer is right that we did not perfectly reproduce *in situ* conditions during our experiments. However, the pulsed nature of DOM input to the lakes makes the *in situ* processing function in a similar way as ‘batch processing’. Thus a similar response could be expected.

16. P8L8: *Where the assumptions fulfilled?*

Reply: Yes, it was fulfilled since there was generally no temporal autocorrelation for two time steps. We will re-write this section to clarify our approach as explained in response to specific comment #9 by Rev #1.

17. *P8L11: Specify which variables are set as the fixed effects and as the random effects here.*

Reply: We will specify that WTT is the fixed effect and site is the random effect.

18. *Results P8 L24: Is “the most dynamic lake” also the smaller lake (volume)? The one with bigger catchment? I missed that in the discussion later and to discuss the controls on the trends on WTT and color in the epi- and hypolimnion.*

Reply: We will remove this mentioning of ‘most dynamic’ and ‘least dynamic’ lakes as it could be misinterpreted. Moreover, we will clarify that lakes with large dynamics spans in WTT are generally those that have intermediate turnover times. These lakes can build up long residence times during extended dry periods, but when an exceptionally large discharge pulse comes, then much of the water can be renewed and the WTT may drop dramatically. In our case it is not so much the lake size that determines the WTT (all lakes are small) but rather the catchment size.

19. *P9 paragraph 3.4: There are no details provided on what is considered “change” in the incubations. Also, changes in DOC and ideally DOC decay rate should be shown in Fig. 3*

Reply: With regard to the comment about lacking explanation to how ‘change’ was calculated, the Reviewer is correct, and we will change accordingly. When it comes to the DOC decay, we need to stress that these incubations were not performed for quantification purposes, but only for seeing the changes in DOC quality upon light irradiation and biological decay respectively. Thus we do not consider that it is relevant to add decay rates to Fig. 3, which would remove the focus from what is important in this Figure, diluting the message. However, we will include more details about the incubation decay elsewhere in the manuscript, in the results text (at least ranges) and possibly in the supplemental materials.

20. *P9 L30: Provide details (e.g. units) of this calculation. Also, only the ones in Fig. 2 were included, or all the sites? Clarify. Also, looking at these figures, how does the reader know which are the “clearest” and “darkest” lakes? different symbols should be used. Moreover, that categorization should be clearly defined and the cut-off between both justified previously and based on values previously reported in the literature. Also, in Table 1, it should be an additional categorical variable stating if a lake is “clear” or “brown”.*

Reply: All these changes will be carried out as suggested. All of the sites were included, as will be explained.

21. *Discussion P10 L10: Which impact could it have that WTT does not span a whole hydrological year? Discuss here.*

Reply: This means that much of the entire lake volume is renewed during the snow melt period alone (since typically a majority of the entire annual water budget is flushed out at this time). During the low-flow period that follows in summer the lakes will typically act as a reactor that carry out batch processing of ‘spring flood’ water. We will discuss this in the revised paper.

22. *P10 L13: “the quantitative photo-bleaching in the Björntjärna catchment”, what do the authors mean? Was there a quantitative evaluation of that? What is the total DOC photo-bleached in the catchment? Also were those studies (Lindell et al. 2000; Vachon et al. 2016) using a similar approach?*

Reply: We will remove the word ‘quantitative’ as it causes confusion. We will also change the word ‘catchment’ to ‘lakes’ as this is a typographical error. With regard to the cited references, we do not claim that these used a ‘similar approach’ in relation to our study or in relation to each other. We merely point out that that these studies suggest that ‘recent inputs of humic materials from the catchment represent a relatively photo-reactive DOC source’.

23. P10 L17: *If I am correct, now comes the only available definition of “brown” lakes. Also...what other variables define a brown or clear- water lake?? Could the authors relate these categories with e.g. morphological variables? (e.g. volume, catchment/lake area, peatland presence, etc). It feels somehow poor to discuss the change in color using a categorical variable built upon that same parameter. I recommend to provide a full multi-parametrical characterization of the two groups.*

Reply: We agree with the Reviewer. In the revision, we will bring in more catchment descriptors into Table 1 (peatland presence, morphometric indices) and present/discuss the gradient from brown to clear lakes in a multi-dimensional way. In short the browner lakes are those with larger catchments and thus larger catchment areas to lake areas. However, also peatland cover might contribute to color, which we will discuss more clearly as suggested.

24. P10 L20: *Müller et al. 2013 evaluated the influence of lateral water inputs. Could later inputs explain the patterns found here? Was there some assessment of lateral fluxes in the systems (e.g. groundwater inputs) so as to discard that from happening in some of the other brown-water lakes?? Discuss in the text.*

Reply: In our analysis no distinction is made between diffuse and inlet stream fluxes. It is assumed that the entire catchment contributes with the same areal runoff to the lake, as explained in the supplementary methods. Four of the lakes have no permanent inlets, so here the groundwater inflow is up to 100%, but in the Björntjärnarna lakes there are inlet streams draining ca 90% of catchment. All cases, however, fall under the same assumptions.

We agree with the Reviewer that the discussion should bring up the possible impact of groundwater inflow more clearly. Possibly in a site like Stortjärnen (the lake in which color and DOC increased during low flow, where there is no permanent inlet but instead large amounts of peat with diffuse flow paths around the lake), we might be underestimating the amount of water and DOC that enters during baseflow. This aspect will be added to the revised discussion.

25. P10 L30: *How is it in Fig. S1b evaluated the contribution of runoff to total water and DOC? The authors do not explicitly evaluate this and they should do so. According to that figure, as runoff increased, WTT decreased. Therefore, we could expect the exported water/DOC during episodic flows to be flushed away from lakes also. As WTT turns longer after the flow, the DOC sources and thus composition, should also recover. To avoid that interpretation, the authors should explicitly evaluate the contribution of runoff to the budget, and discuss more in depth differences found in that sense between the different type of lakes (i.e. above and below one hydrological year, clear and brown) and their layers (epi vs. hypolimnion).*

Reply: We thank the Reviewer for pointing out this weakness in our manuscript. Indeed it is not clear from Fig. S1 how important hydrological episodes are for DOC input to the lakes. Because the figure is integrating a lot of data, the pattern appears smoothed out, and the readers cannot clearly see how the episodes play, especially not in fall.

We will follow the suggestion and report numbers saying how much of the total DOC budget that entered the different sites during different types of hydrological situations (at different flow percentile ranges, parts of the year etc.). We will also discuss whether or not high-flow water was flushed away from the lakes, as mentioned by the Reviewer. In short, the assumption that we make is that outflow is equal to total inflow, implying that some of the water that enters the mixed layer always will be flushed out. However, the major annually reoccurring high-flow events happen during parts of the year when the lakes are non-stratified (spring and autumn) which means that this inflowing water will mix with the entire lake volume and thus is relatively less likely to be flushed out compared to inflowing water in summer moving through the epilimnion.

26. P11 L13: *I consider the authors cannot conclude this, as there cannot be confident on the evaluation of the inputs performed, and that should be discussed at that point. Thus, “DOC accumulation can overcome degradation even in some small individual unproductive lakes” and it can be due to reduced degradation or to lateral terrestrial inputs. Add that discussion.*

Reply: The Reviewer is correct. We will add the suggested phrase and the potential different explanations that the Reviewer brings up.

27. P11 L17: *The authors should evaluate these processes always as a net result of production vs consumption. Thus, in brown-water lakes, the apparent decrease in LMWC is due to consumption above production. Opposite would hold true for Clearwater lakes. Implications of acknowledging that are apparent and results need to be discussed under that light.*

Reply: The fact that these processes are a result of net production vs consumption will be mentioned here as suggested.

28. P12 L1: *Thus, the total color loss might be the same in both type of lakes, but the relative loss in brown water much lower. So... if the brown water lakes correspond to the headwater and lower WTT lakes, terrestrial inputs being more important and frequent (lower WTT), could that color loss in brown lakes (even if just representing a small fraction of the total color) be indeed more important at the landscape level? Discuss, and as previously stated, provide a better characterization (including morphology and relation with the catchment, especially with terrestrial inputs) of the two lake types (clear vs brown).*

Reply: Based on our actual data, it is difficult to push the discussion into the direction that the Reviewer suggests here. However, we can change the discussion to highlight that it is possible that color loss in brown-water lakes is more important at the landscape level than what it appears to be in our study lakes.

29. P12 L20: *What does it mean that it eventually “takes over”? Which mechanism could then explain it? Are there no other environmental or morphological factors that can explain that? Which could be the temporal threshold and could that be related with the hydrology? Include these questions in the discussion.*

Reply: We agree that the phrase ‘takes over’ is unclear, and it should be removed. What we mean is that the threshold is passed when the directions of DOM quality change reverse as shown in Fig 3a-b. Somewhere around the a420 of 7 m⁻¹ there is a change from DOM processing characteristic of dark conditions (biology) to DOM processing characteristic of light conditions (photo-chemistry). It is very clear in Fig 3a-b that the 0 line is crossed at a certain distinct point. A long extended period of low flow could possibly induce passage of this threshold. It would then be expected that color is lost at an accelerated speed. However, a new high-flow episode with brown water entering the lake could push the system back across the same threshold again, into the brown-water state. We will develop this discussion in the revision.

30. P11 L23: *I believe it is very bold to interpret the incubation results that way. They give us an idea of the changes caused by one mechanism, but they do not exclude other mechanisms to happen. All the potential processes that could produce these changes in in-situ lake CDOM should be discussed.*

Reply: We agree with the Reviewer again. However, we did not intend to claim that “excretion of humic-like chromophoric molecules by bacteria” is the only process that can produce CDOM in lakes. Moreover, we do not propose that this specific process is significant, because this we do not now. The idea was just to put all cards on the table and mention this as a possible mechanism that might have played together with several other mechanisms. We will tone this part down further, to not give the readers the idea that we suggest bacterial color excretion to be major. Instead we will link this discussion more clearly to other possible causes of CDOM increase.

31. *Summary and conclusions The first sentence sounds contradictory. If only headwater lakes are being evaluated, then, it cannot be assessed a general freshwaters pattern. I believe the fact that headwater streams present “a sustained level of pigmentation regardless of WTT variations” is extremely interesting, and the relationship of that with hydrology and input sources deserves a much deeper exploration, and I encourage the authors to move towards that direction. Otherwise, the affirmation that “the results may not conform to the general reported pattern of selective removal of colored constituents” without providing an evaluation of the DOC sources variability, does not hold firmly.*

Reply: We will change the phrasing to make it even clearer that we do not propose a general freshwater pattern based on our study. We consider that it is relevant to contrast our findings with other studies showing continuous color loss along the freshwater continuum. However, our point is neither to refute such previous studies, nor to suggest new dynamics for the

whole land-sea continuum. Our results have important implications for the color dynamics of small headwater lakes, but this is where the scope of our study ends.

32. *Tables and figures Table 1: Provide volume or depth information. Provide the categorical variable: clear or brown.*

Reply: Changed as suggested

33. *Figure 1: use different symbol for inlet or black color, it cannot be distinguished. Also, add definition of the outlet calculation in methods. Without that information... Shouldn't "out" WTT be longer than "epi" WTT? Answer and clarify in the text.*

Reply: Changed as suggested. See also response to specific comment #10 above regarding the outlet WTT.

34. *Figure 2: I recommend fully re-working this figure and splitting it in two if needed. Above all, all data should be provided, for all lakes and layers, significant or not, so that the relationships not shown here could be evaluated by the reader. Moreover:*

- *The reader should be able to identify the lakes, to assess if the trends in the two layers are opposed or similar in each system.*
- *Also, it is impossible to assess the adequacy of the fittings without the points even if p-value is reported, and that is very important information.*
- *It is not clear which are the clear and which the brown water lakes, include that information in the legend.*
- *There seems to be two groups also as a function of WTT, how does that influence the results? e.g. in Fig 2d, where epilimnion and hypolimnion present completely opposite trends for the two age groups.*
- *Consider providing a summary table with the results of all the regressions, so the reader realizes how many fittings and which were not significant also.*

Reply: In the revised files, we will provide a table with detailed regression details (coefficients R2 values etc) for all the different relationships. We will also denote clear and brown lakes (or if possible the whole spectrum) in Fig 2. If the lakes would not be individually identifiable in the figure itself, then at least they will be so in the supplementary material.

However, adding all raw data to Fig 2 points will not be possible as the figure will become a complete mess with so many scattered points. Instead, we can show the individual relationships with raw data points in the supplementary information.

The fact that epilimnetic and hypolimnetic patterns sometimes are opposite is something that is already brought up in the results, e.g. section 3.3. However, we agree with the reviewer that this could be given more attention, especially in the discussion. For example, hypolimnia are darker, so it is not surprising that changes in DOM properties down there may be indicative of dark microbial DOM processing even in clear lakes.

35. *Figure 4. It is not clear how that % is calculated (see previous comment). Also, are these changes significantly different from zero? Add that information as well as a zeroline. Clarify also in the caption that the slopes correspond to the ones in Fig. 2d. The reader should be able to identify to which line in Fig. 2d corresponds each dot in Fig. 4, modify accordingly.*

Reply: We thank the Reviewer for pointing this out. Explanations and the zero line added as suggested.

36. *Figure 5: The presence and contents of this figure should be re-evaluated once the suggested changes have been taken into account. Also, as it reads now, it is a bit like the chicken or the egg dilemma: are brown regime lakes*

brown because they have high water color? Or do they have color because of their brown regime? In other words, what is the progress on defining color regime only based on color?

Reply: We believe that we already have an extensive discussion related to the ‘chicken/egg’ dilemma in section 4.4 of the discussion paper. However, we could highlight even clearer the key importance of the color of the inlet water for the trajectory of any given lake. Another aspect that plays is the degree to which the lake water is renewed during the spring flood. For example, if a lake annually is filled with spring flood water black as coffee, there is no room for dynamics that would allow such a lake to develop into a clear-water lake. Conversely, if only a small part of lake water is renewed annually, and if the inlet water itself is relatively clear, then it could be expected that the lake would remain clear at all times. In cases between these two extremes, we would expect to see more dynamics and shifts in color and DOM processing. In the revised section 4.4 we will discuss this deeper.

Response to TECHNICAL COMMENTS

P1 L13: “DOC quality and color”...if color and quality are considered separately, which variables are being used to describe quality besides absorbance? Isn’t color quality of DOC? I suggest modifying into “changes in DOC color”, as it most accurately describes the approach used here.

P1 L17: “Photo-chemistry” includes all the chemical effects of light, so that is not incorrect, but, as a “dominant process in DOC transformation in the epilimnia”, do the authors specifically mean “photo-decay” or “photo-degradation”?

P1 L20: Would “moreover” be more appropriate than “instead”?

P2 L2: Consider changing “and to cause” into “and cause”

P3 L1: Consider changing “selected” into “selective”

P3 L28: absorbance or absorption coefficient?

P6 L27: Fig. A2 should be Fig. S2?

P7 L29: “was” should be “were”

Reply: Changed as suggested