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 #1

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Response to GENERAL COMMENTS

1. *In this paper, the authors test the hypothesis that coloured dissolved organic carbon (DOC) would be selectively lost in boreal lakes, following previous observations from large-scale studies. Based on field and lab data, they found that at the individual lake scale, DOC loss is largely dependent on ambient DOC color (a420). They found that colour loss occurred in clear water lakes, whereas in brown water lakes DOC colour remained sustained over time.*

   These results have relevant implications for current debates about the role of lakes in carbon cycling and DOM processing, within the aquatic continuum as well as within the landscape, and therefore I strongly suggest this paper for publication. Findings of this paper are based on a complete data set that includes a large temporal period (7 years in Björntjärnarna catchment, as well as 3-4 years in 7 additional lakes) as well as a reasonable regional representativity of lakes with varying DOC and water transit time (WTT) conditions. The latter nicely showed how the "browning" level of lake water may be a main factor determining DOC reactivity within a lake. The authors argue that this factor may even overrule the effects of hydrology, even though I will partly question that below.

   Unfortunately the authors did not explore the temporal perspective of their data set, as they pooled all the different sampled time points under a regression analysis approach. Showing some time series, even if it is in the supplementary material, would add completeness to the study; and may support some speculative paragraphs of the discussion, as I comment below.

Reply: We thank Reviewer #1 the thoughtful comments that have helped us to improve the manuscript. On the more specific remark about showing time series, we agree that showing raw data for the response variables over calendar time is a good idea. In the revised ms we will add such time series to the supplementary material, at least for the Björntjärnarna catchment, and we will use this material to support the discussion regarding e.g. seasonal timing of the DOC export (see also reply to specific point #20 below).

Response to SPECIFIC COMMENTS

1. *2.4 Water transit time assessments P5, L 7: "The transit time, represented by the water that resides in a lake at a given moment, : : :" A time that is represented by a water volume sounds confusing. What about "The transit time of the water volume that resides in a lake at a given moment, : : :"*

Reply: Changed as suggested

> 2. *P5, L 9-10: It would help to add units of Voltotal, Flow rate and WTT.*

Reply: As long as the right type of physical quantities are entered (e.g., 'volume', 'time'), the input units of preference do in principle not matter – what goes in is what comes out. However, looking closer at our manuscript we noted that the physical quantity 'flow rate' was not well defined. Therefore, we now define this property as 'volume per unit time'.

> 3. *This section describes the calculation of WTT values for epilimnion, hypolimnion, and inlet sites, but not for outlet, even though this data is later used in Fig. 1.*

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.

> 4. *2.5 Response variables P6, L 26-29: Is this a specific finding of this study, or from a previous study?*

Reply: Both! Our results confirmed the expectation based on a handful of previous studies, among them Panneer Selvam et al (2016, JGR Biogeosci 121:829-840) and Lapierre et al (2013, Nature Comm 4: 2972). We will add one or two references to support this expectation/finding.

> 5. *P6, L 23: "Besides DOC and a420: : :" add mention that a420 is an indicator for DOC color plus associated reference(s).*

Reply: Changed as suggested

> 6. *2.6 Laboratory experiments This is the part of the paper I am less convinced of. These lab experiments, as standardised procedures, may be useful to compare the DOM reactivity from different sites/lakes, however I think their comparison with field data should be done with much care.*
>
> *Dark experiments: I wonder how representative it is to incubate water for 15 months compared to what happens in the lake, where both the DOM and the bacterial community are continuously mixed with newly arrived molecules and cells. During these 15 months, did you check/control for nutrient limitation?*

Reply: We agree with the reviewer that the laboratory incubations do not represent exactly what happens in the lakes *in situ*. 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.

Regarding nutrient limitation, Jansson et al (2001, Freshw Biol 46:653-666) showed that the bacterial metabolism in lakes of our study area (including Björntjärnarna) was decreasingly dependent upon inorganic nutrients with increasing DOC concentrations. This may seem counter-intuitive but agrees with the results by Soares et al (2017, Biogeosci 14, 1527-1539), showing that the DOM in these lakes includes large amounts of bioavailable DON and P while the humic DOC is relatively more difficult for microbes to degrade. Relatively high P bioavailability in streams of the region has also been shown by Jansson et al (2012, L&O 57:1161-1170). Thus the higher the DOC, the less likelihood of nutrient limitation. In the laboratory

experiments, the DOC was ca 15-20 mg C/L, which is a range representing conditions when nutrients are not expected to limit the bacterial metabolism in lakes of the study area (Jansson et al, 2001).

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. Finally, we will explain why there are good reasons to expect that there was no overriding nutrient limitation in our dark incubations.

7. *P7, L 26: "higher than in the dark control incubations": should it be without "control"? (no control was mentioned for the dark incubations).*

Reply: Changed as requested

8. *P7, L 29: At the end, I suggest briefly mentioning that the measurements before and after the incubations were used to calculate the "change" in DOM properties (as it is later used in the results), and how this was calculated. May I point here that different units are presented in Figs 3 and 4.*

Reply: We agree that these things need better explanation. Indeed what we calculate and present in e.g. Fig 3b is the before→after incubation difference in DOM properties. The Reviewer is also correct that we present data from the same laboratory incubations using a separate unit in Fig 4. In the case of Fig. 4 the relative (%) change in color from the beginning to the end of the incubation is shown (as shaded areas). In contrast Fig. 3b shows the absolute changes in DOM properties from beginning to end of the incubations. In the revision we will clarify and explain how the different variables were calculated from the laboratory incubation data.

In the revision clarifications/explanations with regard to the above will be implemented both in the methods section and in the results section where the data is presented, e.g. in Figs 3-4 and their captions. Additionally, as explained in response to point #6 above, our revised manuscript will be clearer about the fact that we only mean to compare experimental data and field data in a qualitative way. Thus in Figs. 3-4 the point is not to show absolute agreements in the rates of DOM property change between field and laboratory measurements, respectively. We rather mean to demonstrate patterns of agreements in the directions and relative magnitudes of the changes.

9. *2.7 Statistics P8 L 5-7: Is there a reference to support this? Also, in order to evaluate the significance of the linear regressions, I strongly suggest the additional use of the R2 (it is presented for the Björntjärnen lakes but not for the survey lakes regressions).*

Reply: We base this reasoning on standard tables of critical values for the significance of correlations. A higher R2 is needed to get significance at the 0.01 level compared to the 0.05 level. A similarly higher R2 is needed to maintain significance if the number of observations is cut to half. Therefore, changing the significance level from 0.05 to 0.01 is roughly equivalent to losing half of the independent observations. However, here we should emphasize that this is rough and not exact. In the revision, we will explain better why we consider the alpha scaling to roughly (i.e., not exactly but fairly close) compensate for the temporal autocorrelation. We believe that this alpha adjustment is the simplest and most straightforward way to avoid granting significance too generously.

There are more advanced and perhaps mathematically/statistically correct ways to correct for temporal autocorrelation (e.g., based on bootstrapping), but then more complicated statistical procedures would have to be added. If (and only if) the Reviewers/Editor think it is worth spending the extra manuscript space on advanced correction procedures for temporal autocorrelation, then we would follow the recommendation and add this. It would not change the results or conclusions in our manuscript in any important way.

We could also explicitly add an autocorrelation term to the mixed effects modelling, but this this could only be applied to the Björntjärnarna catchment data where we use LMER, i.e. we could only apply this to the results in Fig 1 and not to the results

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in Fig 2-3. Scaling the alpha has the advantage that we then can apply the same correction across all results, although not being the mathematically most correct choice.

We agree on showing R2 for the survey lake regressions. In the revision, we plan to put all regression details (R2s, coefficients etc.) in an appended table.

10. *3.1 Björntjärnarna chain lakes and Fig 1 Fig 1. The colours between Inlet stream and Övre Björntjärnen epi are almost indistinguishable. However, here I wonder about the inlet and outlet sites. First I wonder if it makes sense to add the inlet points to the analysis, since it is not affected by what happens in the lake. And about the outlet, I wonder to what WTT it is assigned to, since in the methods there is only a definition for the WTT of epi and hypo and inlet.*

Reply: We agree that the color fill of the inlet symbols need to be changed. This will be done in the revision.

The Reviewer is right that the inlet stream is not affected by what happens in the lakes. There might be a point with removing the inlet stream from the figure in question. For example, since we are using linear mixed effects regression models with site as random effect and WTT as fixed effect, the inlet site (which always has WTT set to 0) does not contribute to explaining any variance in the response variables (in terms of R2m). Thus, statistically, the inlet data does not play a role, in the sense that it neither contributes to nor removes explanatory value from the models. However, we think that displaying the inlet data serves a graphical purpose. It helps the reader get a better idea of the overall changes in DOM properties that happens in the catchment. Therefore we would like to keep the inlet data displayed. We do not see that there is a problem with keeping the inlet data as part of the statistics (although as mentioned it could also be removed without any impact on R2m).

As mentioned in response to point #3 above, 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. *P18 L 3-4: This may not be needed, since the y-labels are already shown in the plots, and the variables described in the methods.*

Reply: We will look into the author guidelines for Biogeosciences to see if it would be ok to remove these explanations to the variables or not. Perhaps the Reviewer is correct.

12. *3.3 Survey lakes and Figs 2 and 3 In Fig. 2, the authors argue that there is a differentiated behaviour between brownwater lakes and clearer-water lakes. Even though this is later very neatly systematized in fig 3, I suggest adding this information somehow already in Fig 2, to help relate the plot with the description in the text. One suggestion would be to draw the lines in a colour indicating the corresponding DOC concentration, or a420, as reported in Table 1. This would also allow seeing if any two lines of epilimnion and hypolimnion are paired.*

Reply: We agree; this is a very good suggestion. We will change the graphics/color scheme of this figure to differentiate between clear and brown lakes (if possible we will display the full gradient between clear and brown lakes as suggested in the comment).

13. *On the other hand the two groups with opposite slopes, not only correspond to clear vs brown water lakes, but also they have very different ranges of variation of the WTT. For example, in Fig 2A, those lakes with negative slopes are also those with shortest ranges of variation of WTT. So here it is fair to wonder to what extent the slope is a statistical artefact resulting from the data not covering a similar range of variation. In fact, if all lakes were pooled together, the relationship between a254:a365 would be positive (we do not see the points in the graph, but I am joining the regression lines), and the same can be said for the other panels (if all points were to be pooled together, they would follow the trend described by those lakes with larger WTT ranges). With this I do not mean to invalidate the results, but maybe some more information could be added in order to emphasize the validity of these correlations, like adding the R2, or plotting their corresponding data points to evidence a clear linearity. I think it is important to solidify these results, as later they lead to intriguing interpretations like DOC concentrations increasing with longer WTT.*

Reply: As mentioned in reply to specific point #9 above, we plan to add expanded regression details (R2s, coefficients etc.) in an appended table, thereby presenting more complete statistical reporting in the new revised ms version.

In addition, to fully address the Reviewer's concern, we need to develop our discussion section with regard to what it means that our different lakes do not span the same range in WTT. The reviewer is correct that new (other) patterns would appear if data from the different lakes with different WTT spans would be pooled, but we argue here that such pooled patterns would be misleading. In fact, we see strong reasons not to pool data from the different sites as they represent ecosystems of fundamentally different character and functioning. First, the fast-turnover lakes have catchments that differ systematically in their properties compared to the catchment of the slow-turnover lakes, e.g. being much larger (0.79-3.2 km2 compared with 0.03-0.25 km2 for slow-turnover lakes) and having flatter areas with more wetlands in lower reaches close to the lakes, thus representing different hydrological functioning likely leading to DOM of different quality entering the lakes (Creed et al, 2015 Aquat Sc 72:1272-1285; Laudon et al, 2011 Ecosystems 14: 880-893). Secondly, the fast-turnover lakes themselves tend to represent a fundamentally different lake ecosystem type, i.e. brown-water, compared to the slow-turnover lakes (clear-water). Thus there is no doubt that our different study lakes represent lake ecosystems of different character, receiving water from catchments of different character. In other words, these systems are in many ways fundamentally different, and it is therefore not surprising that the dynamics of DOM composition indicators such as a254/a365 are different across these lakes.

In the revised discussion, we will expand on what it means that DOM quality variables show relationships that point in one direction in lakes that span a certain range in WTT, but point in another direction for lakes spanning another range in WTT. One possible explanation is that because lakes with different WTT ranges represent catchments that are systematically different, the DOM that enters the lakes in the different cases is of different quality and reactivity from start. For example, short-turnover lakes receive water from large catchments with probable substantial wetland contributions. The colored wetland DOM may be relatively difficult to degrade (Berggren et al 2007 GBC 21:GB4002), so the lakes may stay brown even if WTT increases. Our slow-turnover lakes on the other hand receive DOM from smaller forest catchments with high hydrological connectivity. Such DOM may be more reactive in comparison (Laudon et al, 2011 Ecosystems 14: 880-893 – references therein) so these lakes easily get clear as WTT increases. Another possible explanation is that the response in DOM transformation processes to increasing WTT is in fact not linear. Initially an increase in WTT may lead to decreased a254/a365, but as WTT increases beyond a certain threshold the relationship reverses and a254/a365 starts to increase with WTT. Such non-linear dynamics would make sense in context of the ideas presented in Fig. 5, i.e. that clear-water and brow-water systems have different DOM transformation regimes, which opens up the possibility of passing thresholds that lead to regime shifts.

14. *An interesting result that can also be drawn from figs 2-3, is the fact that those lakes with intermediate colour levels are less responsive to changes in WTT (slopes not significant). This would imply that this kind of lakes are less sensitive to hydrological variability and therefore less affected by hydrological events like rainfall or drought. This could also be mentioned/discussed in the text.*

Reply: We thank the Reviewer for an excellent suggestion, which we will add to the new discussion.

15. *3.4 Experiments P 9 L 25: "similar to the changes observed over time" according to the caption of figure 3, it is not a variation over time but as a function of WTT.*

Reply: The Reviewer is correct, and we will change accordingly

16. *3.5 Overall color loss P9 L 29-30: "we multiplied the in situ rate of epilimnetic color loss in the survey lakes (same as slopes in Fig 2d) with the mean water transit time for the respective sites to find out how much total change there was in water color upon transit": I do not understand how this becomes a percentage of color loss, I suggest that this is more explicitly stated.*

Reply: Again, the reviewer is correct. In the revised version, we need to explain better how this calculation was performed. By multiplying the rate of color loss with WTT, we obtained total amounts of color losses during transit through the different lakes. We then normalized these amounts of color loss to the mean color of the different lakes.

17. *Then, this "relative color change" is compared with the percentage change in the experiments. The calculation of the latter is never explained in the text, I suggest briefly explaining that.*

Reply: We agree, and we will change the manuscript as suggested. See response to specific comment #8 above.

18. *Discussion P10 L16-21: I think it should be taken into consideration here that the brownest lakes also had much shorter ranges of variation of WTT.*

Reply: As explained in response to specific comment #18 above, we will expand the discussion with regard to what it means that the brown-water lakes had lower WTTs than the clear-water lakes.

19. *P10 L 23-31 With the data set you have, including 3-7 year time series, you would not need to speculate about that. Why not just check how a420 and DOC change over time, or seasonally, in the inlet and outlet of the Björntjärnen lakes?*

Reply: Changed as suggested, and as explained in response to the major comment #1 above. By adding the raw data in an appendix plotted over "real" (calendar) time, we will be able to support the claims and speculations made here.

20. *Conclusions P13 L 2 "brown headwater lakes": or just "brown-water lakes"?*

Reply: Changed as suggested

21. *P 13 L 4 "Thus change in WTT, e.g. due to a potentially wetter future climate, has no universal effect on lake color": This is a too hard statement, considering that your data for brown water lakes only covered a small range of WTT values.*

Reply: We will add a sentence after this statement clarifying that a possible limitation of the study was that the brown-water lakes only covered a relatively small range in WTTs. However, we do not believe that it is an over-statement that lake color is not universally affected by WTT.

22. *Response to TECHNICAL COMMENTS*

    Some suggestions, even though I am not a native English speaker:

    P2, L 26: ": : : as a result in temporal : : :": as a result of temporal

    P3, L 1: "selective" instead of "selected".

    P3, L 6: ": : : and analysed using linear mixed effects regression" probably not necessary to be mentioned at this stage. If it is mentioned, though, it should be stated what the mixed effects regression was for.

    P5, L 8: "that passes by" not necessary.

    P7, L 30: "The response of" instead of "The response in"

    P7, L 3-4: "goes up" and "goes down": may I suggest avoiding these. Maybe that could be replaced simply for "If this ratio increases with WTT: : :" and "but if a420:DOC decreases: : :".

Reply: Changes made as suggested