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# ***Interactive comment on “Partial coupling and differential regulation of biologically and photo-chemically labile dissolved organic carbon across boreal aquatic networks” by J.-F. Lapierre and P. A. del Giorgio***

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Response to anonymous Reviewer #2.

Reviewing a manuscript is a time-consuming process and we would like to thank the Reviewer for taking the time to comment on our study. We feel, however, that the review appears to have missed the main point of the study and that the comments provide very little guidance to help us understand and address the criticisms presented. This review contains rather vague statements suggesting that portions of our study may be

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seriously flawed, uncritical or biased, but without elaborating on why this would be the case or offering any alternative. The problem with such statements is not that they are critical to our work, but rather that there is no effective way to either reply or address the issues raised. Below we have attempted to interpret what the Reviewer means and to reply accordingly, but it is unfortunate that the nature of these comments has not allowed us to readily identify significant elements that we could incorporate to improve our work.

Reviewer's comments:

Reviewer:

The paper aims at evaluating the basin-scale drivers of biodegradable and photochemically reactive DOM pools. However, the paper promises much more than it delivers. The dataset comprises a large number of environmental samples covering a vast geographical area and thus gradient in DOC quantity and quality. However, the statistical analysis of this robust dataset is fairly basic and by any means not exhaustive.

Authors:

The objective of our study was to 1) assess the relationship between the concentrations of biologically and photo-chemically degradable DOC across boreal aquatic networks, 2) link the patterns in the concentrations of degradable DOC to intrinsic DOC properties, and 3) identify the environmental and landscape drivers of each. While biologically and photo-chemically degradable DOC have been extensively studied in many types of aquatic ecosystems, few if any studies have actually assessed if and how they co-vary across large environmental and landscape gradients, and whether they are regulated by the same environmental factors or not; this has important implications for the flow of carbon in inland waters. In order to address these objectives, we built on existing knowledge and used well established, routinely used chemical and optical measurements combined with controlled and standardized DOC degradation experiments to generate data that can be compared across the wide range of aquatic ecosystems

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sampled. The data and results that we present in this paper explicitly address these three stated objectives, and we never implied that we would do anything other than this. We therefore do not understand the Reviewer's comment that we "promised" more than what we delivered. What is it that we promised?

Concerning the complexity and suitability of our statistical analyses, one could argue that PARAFAC modeling, moving window regressions and model comparison are state of the art analyses techniques in empirical studies of DOM dynamics. That being said, whether or not one agrees with this statement, since when the quality of a study has depended on the complexity of the analyses used? This is a question-driven study, not an analysis-driven or a descriptive one. As stated above, the analyses aim at answering, in the cleanest way possible, the scientific question that we have clearly expressed in the introduction, not at describing all the possible relationships that may exist between the reported variables. Finally, whereas the Reviewer is adamant about the statistical weakness of our study, absolutely no suggestions on alternative or more effective approaches are provided that could help us improve our work.

Reviewer:

The paper contains several speculations about the causal links between different variables that are not supported by the statistical methods used.

Authors:

Ours is an empirical study of links between DOC lability and environmental factors that is based on regression analyses, which does not necessarily provide evidence for causality. All the patterns and links that we show here are statistically sound, and our interpretation of these patterns and links in terms of potential causal links is entirely based on an extensive literature that exists on the overall topic of our study. Since the Reviewer does not specify which "speculations" are not supported by our analysis or the published evidence, we cannot further reply or elaborate on this comment.

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Reviewer:

The authors tend to oversimplify and over interpret complex relationships between the variables.

Authors:

We would like to be able to address this criticism, but unless more specific details are provided on exactly what is it that we are oversimplifying and overstating, unfortunately we cannot. We can only state, having worked quite extensively on these topics, that we are well aware of the complexities involved, and that we are were extremely careful in developing and interpreting the ideas and results presented in the manuscript so that they are coherent with our analyses and with the current state of the knowledge.

Reviewer:

The focus of the paper is confusing. The authors claim that the main focus is on large scale patterns in bd and pd-DOC not on the estimation of both fractions. However, the former aspect of the study is not evaluated critically enough to support such a claim.

Authors:

We are not sure to understand this comment. We do not "claim" anything. We actually wrote a manuscript that focuses on the large-scale patterns in Bd- and Pd-DOC. How our analyses may or may not "support such a claim" remains obscure even after reading this and the next couple of comments.

Reviewer:

Analysis of environmental conditions is restricted to classification of source waters (river, lake, wetland) and links between DOM biodegradability and photochemical degradation inferred from simple (and not particularly strong) correlations with other chemical determinands (TN, TP).

Authors:

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The real strength of this study does not lie in any of the technique used, but rather in the large number (over 500 sites) and diversity of systems that have been sampled in a highly comparable way. We are not aware of a comparable sampling design in the aquatic literature where such a combination of variables is reported.

In previous comments the Reviewer highlighted the complexity of the processes and links that we are exploring here, and yet in this comment the Reviewer would seem to suggest that only relationships with high  $r^2$  are worth reporting or are of any value. We think that there is a essential contradiction between these positions and we fundamentally disagree with this philosophy. We set out to assess the relationship between the concentrations of biologically and photo-chemically degradable DOC across boreal aquatic networks; that this overall relationship has a relatively low  $r^2$  cannot be interpreted as a weakness of our study. It is in fact a major result, as important as if it had had a very high  $r^2$ , because both cases would be telling us something different about the nature of this relationship and of its underlying drivers.

Concerning the links between Pd- and Bd-DOC and their respective drivers, we acknowledge that  $r^2$  ranging from 0.35 to 0.70 may appear low to researchers used to work in smaller and more homogenous sets of systems, but again the value of these relationships cannot be judged on the  $r^2$  alone. Coefficients of determination typically decrease as sample size (and the underlying complexity among the systems studied) increases. The systems studied here range over several orders of magnitude in basically every possible environmental variable. The fact that single variables or a combination of 2-3 variables may explain a large portion of the variability in the concentrations of biologically and photo-chemically degradable DOC is actually surprising and remarkable in itself, suggesting that these factors are integrating fundamental underlying processes occurring across system type and spatial scales.

Reviewer:

The evaluation is based on assumptions and speculations e.g. Page 6689, lines 25-29

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and Page 6690, lines 1-2.

Authors:

We are not sure to understand what "evaluation" refers to, and the terms "assumptions" and "speculations" may have been used loosely here. The lines in question refer to a 3-step reasoning that may explain our pattern, based on 6 references (not assumptions). By elimination (based on our own results), we indeed "suggest" (p6689 L27) that one of the three scenarios is more likely than the others.

Reviewer:

TP is hardly an indicator of biological activity without information on the percentage contribution of soluble reactive phosphorus.

Authors:

Most of the studied sites are P-limited and thus SRP hardly accumulates in measurable concentrations in any of the systems sampled. In this context, what does SRP tell on the level of biological activity going on? This is in fact the case for most freshwaters, and this is why TP (and not SRP), together with chlorophyll (which we also have measured), are the most widespread indicators of system trophic status in freshwaters, extensively used in limnology for decades now. This may not be the case in oceanography, but this does not disqualify TP as an index of system trophic status, which is how we use it in our study. Finally, it is unclear to us to what specific statement this comment refers to, and how it affect its validity.

Reviewer:

One of the major flaws of the experimental setup is lack of measurements of low excitation wavelengths <270 nm; these spectral regions contain a large proportion of FDOM that is both photochemically reactive and biodegradable.

Authors:

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We are aware that there is some fluorescence lying at wavelengths  $< 270$  nm. It is well known, however, that most fluorescence components identified by the PARAFAC modeling process have two peaks in excitation, and at least one of the peaks will always be measured at wavelengths  $> 270$  nm. The fluorescence intensities reported here are based on the maximal fluorescence intensity ( $F_{\max}$ ) of a PARAFAC component within the scanned region (see Stedmon and Bro, 2008 for more detailed explanations on the calculations of  $F_{\max}$ ), not on the total fluorescence measured (i.e. area under the curve). The PARAFAC model identifies components that have exactly the same shape in every sample (only concentration changes), such that capturing or not the peak at the lowest possible wavelength would not alter the overall patterns.

Reviewer:

The paper is poorly organised with the parts of discussion appearing in all other sections (Introduction, Results). Some parts of the discussion are not relevant to the main topic of the paper e.g. page 6687, lines 14-24, as the paper does not focus on the determination of the age or freshness of DOC in water samples. Similar, conclusions (Page 6695, lines 9-11).

Authors:

The main topic of the paper is precisely the factors that may explain the apparently contradictory patterns in DOC degradability across contrasting aquatic environments that are reported in the literature, and we propose that the freshness (not the age, see p6688, L5-6) of the DOC pool may adequately place contrasting environments on a common gradient in terms of DOC biological degradability (see p.6688, L7-9, and the previous and following reasoning behind this statement). This concept has been developed in the introduction (implicitly) and discussion of the manuscript, thus it is hard to understand why the Reviewer missed that point as being the main topic of the manuscript.

That being said, we acknowledge that the concept of freshness could be more explicitly

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developed in the introduction, and that we could better explain how we used CDOM as a proxy of "freshness". This will be included in the revised manuscript.

Reviewer:

The authors confirm what is already know from previous studies, for example studies of Andy Baker and co-workers who correlated BOD with protein-like fluorescence.

Authors:

There is nothing in the work mentioned by the Reviewer that compromises the novelty of the findings reported here. We explicitly acknowledge that a positive relationship between protein-like DOM and biological lability have been observed before (p.6690, L4-7), and never claimed that this was a novel finding of our study. Perhaps what is novel, and that the Reviewers fails to acknowledge, is that those previous patterns that were found in a rather small set of rivers and lakes may extend to entire freshwater networks.

Reviewer:

Simple and not very strong correlations between variables do not confirm the causal links between DOM character and its behaviour in environment.

Authors:

See above comments on the strength of the relationships and on the causality underlying our relationships. That being said, as a general rule, even if we had developed much more complex multiple regression models and had obtained much higher  $r^2$ , these would not have confirmed causal links. Complexity and high  $r^2$  are not the window to causality.

Reviewer:

The results of this study do not support conclusions drawn by the authors on the large-scale patterns of DOC biodegradability and photochemical reactivity.

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Authors:

This is a rather critical (in more than one way) statement that we would very much like to address, but as formulated unfortunately we cannot. The Reviewer provides no rationale for this (and other) sweeping statements that our analyses and results are flawed and do not support our conclusions.

Reviewer:

The authors should critically evaluate their results, their significance and appropriateness of their experimental setup and rewrite the discussion section, largely by removing all the speculation and assumptions not supported by the data, to reflect the study itself.

Authors:

See above comments on speculations, assumptions, on the actual topic of the study and on the analyses being question-driven rather than the questions being analysis-driven.

Reviewer:

Specific comments:

Extensive parts of the introduction e.g. page 6676 lines 7-30 and page 6677 lines 15-20 are a discussion of the results and therefore should be shortened/moved to the discussion section.

Authors:

It is very hard to understand why the Reviewer would think that there is discussion of the results anywhere in p. 6676; we are simply developing our reasoning based on current state of the knowledge. We encourage the Reviewer to re-read this section.

We will remove the sentence at p.6677 L15-20

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Reviewer:

Experimental setup – why fluorescence measurements were constrained to 275-450 nm excitation wavelengths? If the aim of the study was to characterise biodegradable DOM, the authors should have considered analysing lower excitation wavelengths \_225-230 nm. Large proportions of protein-like, biodegradable DOM lie in this region. Likewise photochemically reactive DOM of humic-like origin lies in this region.

Authors:

Fluorescence was measured below those wavelengths but the rather high signal to noise ratio affected the performance of the PARAFAC model, hence we removed them. See above comment regarding the (lack of) need to include those wavelengths considering the questions being explored in this manuscript.

Reviewer:

Thus the result that component C3 was the strongest predictor of Pd-DOC can simply result from not incorporating lower excitation wavelengths in this study. This serious limitation of the study should be discussed and the results can be significantly biased.

Authors:

This comment suggests that the Reviewer does not fully understand the calculations behind the fluorescence intensities reported here, and perhaps the PARAFAC modeling process as a whole. We acknowledge that the calculations were not explained in details in the current manuscript, but we consider that this is a well-established method in the fluorescence literature. Stedmon and Bro, 2008 (cited in the manuscript) explain very well the calculations and the model.

Reviewer:

Page 6678 – TN concentrations are not reported, line 6.

Authors:

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It was actually TP that was missing. We thank the Reviewer for the careful observation and we will include the concentrations in the revised manuscript.

Reviewer:

Page 6678, line 8 – should read Strahler order.

Authors:

Agreed, we will update this term in the revised version of the manuscript.

Reviewer:

Page 6678, line 23 – please rewrite the sentence ‘TN was analysed as nitrate’ to correctly describe how TN was calculated.

Authors:

We can provide more details in the revised manuscript.

Reviewer:

Results – authors should separate results from their discussion e.g. pages 6681-2, lines 23- 3 and entire section 3.7.

Authors:

p.6681-2. We consider that this information is important in order to correctly interpret the following results.

Section 3.7: Basic interpretation of the results in this section allows to link the different results together, which will then be useful in the Discussion in order to discuss the real ecological/biogeochemical question that these results support.

Reviewer:

Results, page 6682, lines 10-16 and Figures 2 and 3: I am not convinced that the relationship between concentrations of Bd-DOC and Pd-DOC is meaningful. The ab-

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solute concentrations of both fractions simply increase with DOC concentration and this relationship should not be over interpreted to infer DOM functionality.

Authors:

It may appear intuitive that both fractions increase with total DOC, but the interesting question is why is that the case? This can only be explained by those distinct DOC pools sharing substantial sources or sinks at the landscape level, and the results we show strongly suggest that indeed they share land as a source at that scale.

Reviewer:

Page 6688, lines 9-14 – this discussion is redundant and irrelevant, as the authors analyse summer samples only.

Authors:

The concept of DOC freshness is certainly applicable over time as well as over space; we will include a statement in the discussion to highlight this notion.

Reviewer:

Page 6691, lines 6-10 – light climate? This a sweeping statement.

Authors:

We really did not think that this statement needed a tighter formulation considering the ideas being developed, but we can precise in the revised manuscript.

Reviewer:

Page 6691, lines 10-15 – this is speculation.

Authors:

If a DOM pool is lost at very high rates, there has to be high supply rates in natural environments in order to maintain measurable concentrations in all the sampled sites.

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This is not speculation, this is critical reasoning.

Reviewer:

Page 6694, lines 12-16 - this is speculation.

Authors:

Of course it is, but it is based on reasonable hypotheses which are themselves based on solid, published studies. The lines in question provide a plausible explanation for the patterns that we report here based on the current state of knowledge in the literature.

Reviewer:

Page 6695, lines 3-8 – this is speculation.

Authors:

Of course it is. We are providing reasonable hypotheses that may explain our patterns, based on current state of the knowledge and on the coherence of the different results presented in this study. Biolabile C6 is typically found in low abundance and is relatively invariable in aquatic environments, presumably because it is highly degradable and cannot accumulate (see references in the manuscript). The only way to accumulate is to go out of steady-state and have production or importation exceed removal; our collective results strongly suggest that this is the case when terrestrial influence is very high (as denoted by CDOM).

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Interactive comment on Biogeosciences Discuss., 11, 6673, 2014.

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