

## ***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***

**J.-F. Lapierre and P. A. del Giorgio**

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Reviewer:

Partial coupling and differential regulation of biologically and photo-chemically labile dissolved organic carbon across boreal aquatic networks J.-F. Lapierre<sup>1\*</sup>, P. A. del Giorgio<sup>1</sup>

While this manuscript clearly reflects a significant amount of field and lab effort, I found it extremely difficult to follow the thread of the author's points both due to its overall

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poor organization and overuse of ill-defined technical terms. Because of this, I found it challenging to provide specific comments (its hard to just say, “I didn't follow”). I hope the authors find the below review useful.

Authors:

This comment echoes comments from Reviewer#2, but is in direct opposition with those of Reviewer#5. Although it may appear to some readers that we have loosely formulated our ideas with vague terms, we have actually been very careful in the choice of words in the different parts of the manuscript (although we acknowledge the ambiguity regarding concentrations of degradable DOC vs DOC degradability). For example, we use broader words in the "Abstract", "Introduction" and end of the "Discussion" where we discuss the broader context of our study (and the contribution of our study to this broader context), whereas we have used more technical wording in the "Methods" and "Results" sections (we will comment more specific examples as they are brought up individually).

We have, on purpose, written our manuscript in the least descriptive way possible in order to really highlight the results (and their meaning) that support our main research questions, while putting very little emphasis on secondary results that may help understand the environmental context of the systems studied but did not represent novel, conceptual advances. This has been appreciated by some, but clearly not all the reviewers. This highlights that some criticisms are more a matter of opinion than actual flaws in the analysis or writing process, but also suggests that some precisions in the text are needed to make it more accessible to a wider audience.

The comprehensive nature of this Reviewer's comments as well as the overlap with comments from other reviewers allowed us to identify portions of the manuscript requiring precisions in order to clarify our main message. Below we address all of the Reviewer's points, identifying edits that have been made to the manuscript in response to those points and explaining why we disagree with some others. We would like to

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thank the Reviewer for the thorough and constructive review.

Note: This Reviewer's comments refer to lines that do not represent those present in the online pdf. We thus refer to sections and paragraphs when discussing specific parts of the manuscript in order to ease the comparison process for readers who may have different versions of the manuscript in hands. Also, the online system for submitting the comments does not allow font formatting, so in some instances we use capital fonts for emphasis.

Reviewer:

GENERAL COMMENTS (in no particular order): If the authors are clear on what the main objectives of this study were, it was not made clear to me as a reader. Is it to look at whether Bd-DOC and Pd-DOC pools different across lakes/streams/wetlands? If they are linked more or less in lakes vs. streams vs. wetlands? Is it to use Bd-DOC and Pd-DOC measurements to gain insight into DOC source? To its diagenic state? Is it to think about C processing prior to reaching oceans? Is it to understand how "landscapes" control DOM composition? To use optical properties to predict Bd-DOC and Pd-DOC concentration or percent? Even though the headers in the discussion try to break out the different topics, the introduction and results did not adequately set-up these questions. The paper requires significant rewriting to convey the authors' points in a clear, and much more concise, manner.

Authors:

The main point is much broader. The above points noted by the Reviewer were sub-objectives which collectively, allowed to better understand the role of land in delivering biologically and photo-chemically degradable DOC pools to boreal aquatic networks, and ultimately, the potential for terrestrial organic carbon to participate to aquatic ecological and biogeochemical processes. We believe that this main point have been clearly laid out in the first paragraphs of the Introduction and at the end of the Discussion and we respectfully disagree with the Reviewer (in accordance with Reviewer#5)

C4034

with the need to rewrite our manuscript in order to clearly convey our main message.

Reviewer:

Investigating links between different DOM (or DOC, whatever term you choose) pools is challenging, because it is a heterogeneous pool of compounds and we typically measure bulk properties (e.g. DOC concentration), or sub-pools (Bd-DOC, Pd-DOC, A440, Ex370/Em460, PARAFAC components), or bulk composition (SUVA, %Components, Abs ratios, %bioavailable, %photodegradable), and/or net changes in these pools or properties and try to say something about DOM origin. I think it would help the authors to make a venn diagram depicting how they imagine these different parameters relate. For example, Bd-DOM and Pd-DOM both would fit within a large circle representing the total DOM pool, and these two sub-sets of DOM would overlap to a variable extent depending on DOM source and environmental processing. Then for each parameter you measure, determine how that fits within that model. For example, the DOM that absorbs at A440 should largely overlap with the Pd-DOM pool (since both are by definition photoreactive), and probably overlap much less with the Bd-DOM pool.

Authors:

We are well aware of the complexities noted by the Reviewer, and we have performed a similar conceptual exercise before writing the paper. The key points mentioned by the Reviewer here are actually much comparable to the key points that we discuss in the manuscript: There is a partial coupling (i-e "overlap") between the concentrations of Bd- and Pd-DOC. However, our different results suggest that it is mostly because they share land as an origin across the diverse systems studied, although the pools of DOM involved actually differ. We use DOM fluorescence properties in order to associate different DOM pools to the biologically and photo-chemically degradable pools.

Reviewer:

I also imagine "fresh" - meaning un-altered not necessarily of a certain age - DOM

C4035

largely overlaps with the Bd-DOM pool (80%?) and depending on its source (algal vs. plant) can have a variable Pd-DOM overlap but still much lower as a percent compared to humified DOM. Assuming terrestrial derived DOM has already been degraded and is no longer fresh (an assumption I believe the authors make, although not explicitly), the reverse would be true.

Authors:

This and several other comments (in this and other reviews) highlight the need to better define what we mean by "Freshness". What we mean is "freshly imported", as this DOC can be old and diagenetically altered, and/or be protected from degradation in aquatic organisms or in soils, but still be both bio- and photo-degradable once released in the aquatic environment.

We revised our initial definition (second par. of section 4.1): " Origin and age, however, have different implications and different links to the notion of "freshness" (defined as the time relative to when it was imported into the aquatic environment) in different types of systems."

to better explain our view of DOC freshness:

" Origin and age, however, have different implications and different links to the notion of "freshness" (defined as the time relative to when DOM left its site of production (i.e. soil, aquatic organisms) and was imported into the aquatic environment) in different types of systems. "

Reviewer:

Overall lakes, wetlands and rivers have DOM from both terrestrial and algal sources, but the proportions differ, HOWEVER more importantly how "fresh/alterd/environmentally processed/humified" the DOM is varies. This changes the sizes and overlap of all of the circles in the venn diagram.

Authors:

C4036

We certainly agree with this statement.

Reviewer:

Another common challenge when investigating DOM pools is being aware of when you are dealing with CONCENTRATION based parameters (parameters that reflect the AMOUNT of DOC in your sample) and COMPOSITION based parameters (parameters that reflect the TYPE of material in the sample). The AMOUNT of Pd-DOC and Bd-DOC in the sample provides completely different information than the PERCENT in the samples. #1: Please come up with a clear way to distinguish between when you refer to conc and percent – please use different abbreviations!

Authors:

Unless specified otherwise, we always mean concentration. We have edited section titles and several sentences throughout the manuscript to clarify this ambiguity.

Reviewer:

I did not understand why the authors spend so much time pointing out there is a strong correlation between [Pd-DOC] and [Bd-DOC], and between these terms an other concentration based measurements. You would expect that given MORE of something (DOC) , the sub-pools would also generally increase. To me the more interesting question is how does %Pd-DOC and %Bd-DOC correlate. For the PCA analysis, I think including both concentration based and composition based terms in the analysis is confusing. Again, I would expect all of the concentration based parameters to be highly correlated. I think it would be much more informative to run the PCA analysis, and also the multiple linear regression analyses, on the COMPOSITIONALLY based parameters alone.

Authors:

We have addressed the point regarding the intuitive correlation between Bd- and Pd-DOC in our reply to Reviewer #2. Bd-DOC and Pd-DOC would not co-vary "just be-

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cause" there is more DOC overall; we explain in the intro that Bd-DOC and Pd-DOC may be expected to co-vary if they share sources or sinks at the landscape level, and we bring back this notion later in the discussion. We recognize, however, the need to better acknowledge that although a coupling between those two DOC pools may appear intuitive, there is still a fundamental interest in exploring the DEGREE of their coupling across very diverse aquatic environments.

We have revised the first and last sentences of section 4.4 in order to highlight these points: " Although it may appear intuitive that both Bd-DOC and Pd-DOC increase with overall DOC, the coupling between the concentrations of Bd-DOC and Pd-DOC implies that they share at least some basic sinks, or more likely, sources, at the landscape level. "

" The collective evidence discussed above thus rather suggests that Bd-DOC and Pd-DOC partly share land as a common origin, and the relatively weak coupling between both DOC pools suggest that the relative contribution of land-derived DOC to Bd-DOC largely differs across systems."

Finally, we agree that it would be interesting to explore the drivers of the %Bd-DOC or %Pd-DOC, but this is a different question and would not add much to the scientific questions being addressed in our study.

Reviewer:

I do not follow how the authors believe they can separate terrestrial from aquatic derived DOM pools based on the sampling approach and methods used. They seem to suggest that "CDOM" (which I assume refers to A440, please define on first use and remind the reader later too), is a measure of terrestrial DOM, however that is certainly not the case. This is a major flaw in the paper if this is being assumed.

Authors:

We really need to clarify this key point: We have never pretended to separate "terrestrial

C4038

from aquatic derived DOM pools". We have explained our reasoning for using CDOM as a proxy for terrestrial influence in Lapierre et al. 2013 (Supporting Information); we have inserted a reference in the last sentence of the revised introduction.

In short, it has been shown numerous times that land is the main source of colored DOC in freshwater environments, and because colored DOC is gradually (and preferentially) lost along continental watersheds due to cumulative processing, the amount of CDOM remaining indicates some level of connection with the surrounding landscape. We are well aware that this is not a perfect proxy (see Lapierre et al. 2013, SI), but it is adequate for the questions being addressed here, considering the environmental gradients covered.

Furthermore, we would like to point out that we used a quantitative (CDOM "concentration") rather than a qualitative (e.g. SUVA, spectral slope) proxy, precisely because we did not want to discriminate terrestrial from aquatic derived DOM, but rather evaluate how tightly a given environment was connected to its catchment. Very productive systems (e.g. some wetlands that we have sampled) could have a high proportion of autochthonous DOM yet have very high terrestrial influence; the amount of CDOM (and terrestrial influence) would still be high in this example regardless of the amount of autochthonous, uncolored DOC.

Finally, CDOM was already defined at the beginning of section 2.3: " We report CDOM as the absorption coefficient at 440 nm (in m<sup>-1</sup>, naperian units), calculated by dividing the optical absorbance at 440 nm by the path length in meters and multiplying by 2.303". We have further included reminders in the "Results" and "Discussion" sections the first time we used the abbreviation, as suggested by the Reviewer.

Reviewer:

It is not clear how the authors separate out terrestrial vs. aquatic DOM, especially in wetland environments. Freshly leached plant DOM (e.g. likely present in wetlands) can look very similar to freshly produced phytoplankton derived DOM. Where does aquatic

C4039

vegetation fall?

Authors:

As stated above, we had no intention of separating those DOM pools. Whether the DOM originates from soils, macrophytes or phytoplankton makes no difference for our analyses. What we show is that over the whole CDOM gradient, we tend to observe higher bio-labile DOM when terrestrial influence (see above) is very high.

It is possible that in some cases, there is also greater primary production within the system when terrestrial influence is very high, as noted by the Reviewer, but we discuss why it is unlikely that this would be an important driver of our patterns. For example, this could explain why there was typically more bio-labile C6 per unit CDOM in (typically productive) wetlands compared to (typically not very productive) rivers (see Fig. 7a), yet the pattern is exactly the same for unproductive rivers except that there appears to be an offset in the relationship. We deliberately kept this point out of the manuscript since, although it goes along the lines of our main results, we did not have strong support from our measurements to overly speculate on that point.

Reviewer:

Please be more careful and far more sparing in the use of technical sounding terms, and clearly define their meanings. Many of the terms included, though frequently used in the literature, mean different things to different people.

For example: \*regulation: this term is in the title, but was not used very often in the paper (except the abstract and last page). Regulation by what?

\*Landscape scale – this term is used, but never clearly explained what/how any landscape scale parameters were collected or examined. When I hear this term I think of things like soil type, vegetation type and cover, precipitation, climate, anthropogenic influence (fertilizer inputs, erosion, etc. ): : : If you just mean lakes vs. wetlands vs. rivers, I would take out this term entirely.

C4040

\*Landscape gradients – gradients of what landscape features? This term is used so broadly it isn't useful.

\*Allocthonous/autocthonous – how does this relate to terrestrial/algal? Most often the authors seem to compare autocthonous to terrestrial, so why use authocthonous at all? I found the discussion of "age" and "freshness" useful, but wasn't sure how this then relates back to the idea of initial source/origin. Regarding age, what types of environmental processing besides biodeg. and photodeg. do you think occur?

\*coupled/coupling: is this synonymous with correlated, or linked? I also like to use this term to think about pools of DOM, but there were times I wasn't certain what was meant by these terms. Especially when you use the term "partial coupling". Please use more specific (clear) language.

\*functional pools: what does this mean? instead of using different terms to mean the same thing, I suggest you try to define specific terms and use them consistently.

Authors:

We have considerably simplified the wording and standardized the expressions used throughout the manuscript to comply to this comment.

Reviewer:

This sentence from the last paragraph is an example of how the authors used complex language that may sound good, but I can't figure out what they are trying to say: "This result also suggested an additive behavior of DOC degradability along a gradient of terrestrial influence, and here we show that the role of land as a major source of both biologically and photo-chemically degradable DOC to boreal aquatic networks results in a pattern of co-variation between these two key pools of carbon at the whole landscape scale, despite fundamental contrasts in terms of their composition and regulation". I suggest you write in a more concise manner.

Authors:

C4041

We have reworded to:

" This result also suggested an additive DYNAMIC of DOC degradability along a gradient of terrestrial influence, and here we show that the role of land as a major source of both biologically and photo-chemically degradable DOC to CONTINENTAL WATER-SHEDS results in a pattern of co-variation between these two key pools of carbon ACROSS BOREAL AQUATIC NETWORKS, despite fundamental contrasts in terms of their composition and regulation."

and we believe that precisions provided in this reply and in the revised manuscript will help better appreciate the statement in question.

Reviewer:

Why suddenly use "water retention time" "DOC freshness" and "regulation" in the last page when you barely used these terms prior to this? What do you mean by these terms? Do have you have any information about residence times for these samples?

Authors:

See our reply to the Reviewer's first comment.

Reviewer:

There was recently an article in Nature about the reliance of P values indicating significance, when in cases where R2 values were in fact so low that the relationship was so weak that the significance is meaningless. I suggest the authors take a look at this paper: Nature, 152 vol 506 13Feb2014 by Regina Nuzzo.

Authors:

We are perfectly aware of such statistical considerations and have interpreted our relationships accordingly.

Reviewer:

C4042

I felt the total N and P data was included more of an aside and never satisfactorily presented. Did you look at C:N, C:P or N:P ratios for insight into DOM source and processing?

Authors:

TN and TP were indeed presented as secondary results (see our reply to the Reviewer's first comment). Elemental ratios would have been an interesting avenue to explore, but we did not think that our measurements allowed to do that in a satisfactory way, considering that we did not have SRP and that we had inorganic N only for a subset of the samples. In our opinion, the additional information would not counterbalance the loss in coherence in the data reported here.

Reviewer:

Please add a correlation matrix for all of the parameters discussed: preferably one matrix that contains only concentration based parameters, and one that contains compositionally based parameters. Unless, for some reason you want to see how composition trends with concentration – in which case please explain/justify the difference between comparing SUVA to [Pd-DOC] and %Pd-DOC.

Authors

A PCA (Fig. 5) is basically a correlation matrix, with the advantage of visually presenting the distribution of the sites along the different gradients. We presented the PCA precisely to give the reader a sense of the relationships that exist among the reported variables.

Reviewer:

By definition absorbance and fluorescence measures the photoreactive pool of DOM, thus it is well-known that these measurements should be linked to the pool of C that is photodegradable. It is interesting to examine which specific optical measurement can best predict Pd-DOC and Bd-DOC, however the authors did not take a clear statistical

C4043

approach to this question (or if you did, I didn't focus on that). Again, a correlation matrix of all parameters would be useful, followed by the multiple linear regression results shown in Table 1. As mentioned above, I found it puzzling that parameters of both concentration and composition were included in the same analysis [C6] and %C6. Is this confounded?

Authors:

It is true that optical measurements target the photo-reactive DOM pool, but several of the wavelengths involved in absorbance and fluorescence (i.e.  $\lambda < 300$  nm) are meaningless for photochemical degradation in natural (or experimental) environments. It is thus not obvious that optically active DOM is necessarily photo-reactive, and our results certainly go in that direction (see Fig. 6). Our whole interpretation of the MLR models involving Pd-DOC (Table 1) is based on this differential photochemical degradability of the different (optically active) FDOM pools. In particular, C3 was by the far the most reactive (Fig. 6), and was the best quantitative predictor of Pd-DOC (Table 1).

The use of [C6] vs % C6 is not confounded; the concentrations in C6 were a good predictor of Bd-DOC concentrations (no other fluorescence component performed as well), and when a quantitative estimate of DOM was included in MLR models for prediction of Pd-DOC, only composition-related estimates improved the model.

Reviewer:

Overall, this data set has a tremendous amount of potential, but these statistical analyses, interpretation, and discussion require significant reworking.

Authors:

We agree that this dataset has tremendous amount of potential, and its potential is certainly not limited to the current study. Here we have constrained the analyses to well-defined research questions, but there is no way that we can address all the possible relationships and complexities present in this data in a single study.

C4044

Reviewer:

SPECIFIC COMMENTS:

13 The first sentence of the abstract mentions the flow of carbon from continents to oceans – I do not believe the paper addresses this (suggest it is removed). I did not find that after reading this paper I had an understanding of the “large scale patterns in BDOC and PDOC” across continental watersheds”.

Authors:

We believe that our revisions, in the light of the reviewers comments, now more clearly reflect how our findings have implications for the flow of carbon from continents to oceans.

Reviewer: 17 Pd-DOC concentration or percent? Or both? Please specify.

Authors: both. now specified

Reviewer:

18 What do you mean by system trophy and terrestrial influence? What “landscape level” parameters did you examine?

Authors:

System trophy refer to nutrient and terrestrial influence refer to CDOM concentration. “At the landscape level” is implicit here; one does not have to have landscape level “parameters” to report results at the landscape level. In any case, as per the above replies, we have considerably revised the wording throughout the manuscript to make those points more explicit.

Reviewer:

21 Don't you use absorbance as well as fluorescence to identify DOM pools? Do you mean differences in DOM composition, not just different pools?

C4045

Authors: We do use both, but in this particular sentence we refer to the different FDOM pools.

Reviewer:

22 What nutrients? Nutrient concentration or content? N:P ratios? Very unclear. Should say "protein-like DOM fluorescence". However, fluorescence in the "Peak T and Peak B" regions are not necessarily proteins!! Tannins and other compounds also fluoresce here. Perhaps use "fluorescence in the lower UV region". See for example, Hernes et al, 2009; Beggs et al, 2011.

Authors:

We have reworded this sentence to: "Concentrations of nutrients and protein-like fluorescent DOM (FDOM) explained nearly half of the variation in Bd-DOC, whereas Pd-DOC was exclusively predicted by DOM optical properties, consistent with the photochemical degradability of specific FDOM pools that we experimentally determined."

We are certainly aware that protein-like (in optical terms) material is not necessarily proteins (hence the "-like" in protein-like), and we have been very careful in our interpretation throughout the manuscript; we even have a section explicitly discussing this point (last two par. of section 4.3).

Reviewer:

25 Please state what you mean by CDOM here (A440?) I have never seen CDOM used as a proxy for terrestrial influence! This is a major flaw if the authors are making this assumption. Many papers show a good relationship between DOC concentration and "CDOM" (A254, A440, etc.), and it is not surprising that if you have higher concentrations of DOC you have higher concentrations of degradable DOC too.

Authors:

Please see above reply considering our clarifications of our use of CDOM throughout

C4046

the manuscript.

We have previously reported our own relationships of CDOM vs DOC for most of the systems studied here (see Lapierre et al. 2013, Fig. S1), and the relationship is really not as straightforward as the Reviewer appears to believe, at least when very contrasting systems are considered together. We have a manuscript in preparation that will address this point.

The point that we discuss in Lapierre et al. 2013, however, is that the current state of knowledge suggests that DOC and CDOM are indeed coupled when terrestrial influence is high that that this coupling decreases with increasing importance of autochthonous processes (thus lower terrestrial influence); this is precisely how we used CDOM as a proxy of terrestrial influence in the current study.

Reviewer:

But does the fraction (%) of these pools differ and why? To me it is more interesting why the %s change: why in some cases is there still labile/photoreactive DOC in water.

Authors:

Again, we have reported the relationship between CDOM and %Bd-DOC and %Pd-DOC in Lapierre et al. 2013; this is not the point of the current study, and we respectfully disagree with the Reviewer that this would be a more interesting question to address.

Reviewer:

The most simple explanation to high %BDOC or %PDOC is that there have been recent inputs of fresh DOM, or the water has been cold and there is no light so it hasn't been altered, or the bacterial community is nutrient limited, or: : : (This type of discussion is missing).

Authors:

This is precisely the point of the first section (4.1) of the "Discussion".

C4047



Reviewer:

27 Putative bio-labile fluorescence component? What does this mean? Please do not use the term "browner"; use an optically measured property. Avoid using autochthonous without defining it clearly.

Authors

As the Reviewer mentions above, there are complexities revolving around the use of "protein-like" FDOM as a measure of 1) actual protein material and 2) bio-labile material, thus we wanted to mention that it is generally considered as bio-labile (implicitly hinting that it may be more complex than this simple statement); we discuss those complexities in the "Discussion" section.

We replaced "browner" by "darker". We believe that "autochthonous" is sufficiently self-explanatory and used in the literature to be used without having to define it in an abstract.

Reviewer:

31. After reading the paper, there is still no indication the authors examined any type of "landscape gradients". The only classes I saw were river vs. wetland vs. lake, which I would not classify as landscape gradients but rather simply three types of water bodies.

Authors:

We define at the beginning of the "Methods" section the large (and diverse) landscape gradients covered.

Reviewer:

31 I do not know what this last sentence is saying, and I read it many times. It is already well known that terrestrial sources of DOM are major contributors to surface waters DOM. I am not convinced that the authors can in any way determine the origin of DOM: terrestrial vs. autochthonous, per their definitions.

C4048

Authors:

As mentioned above, we never intended to determine the source of DOM per se, but the very strong coherence in the different patterns reported strongly suggests that much of the degradable DOC in the aquatic environment is actually originating from the terrestrial landscape. While this has indeed been reported in previous studies (but contradicted in others), the environmental gradients covered here allow to place apparently conflicting findings on the degradability of terrestrial DOM on common environmental gradients, in particular, on a wide gradient of terrestrial influence.

Reviewer: 38 what is the difference between sources and origins? I believe these are synonyms.

Authors: "source" and "origin" are defined a few lines below in the manuscript.

Reviewer:

50 I am not clear on how Pd-DOC pools relate to the aquatic food web. Upon photoxidation, isn't this DOC "lost" to the foodweb if it is converted completely to CO<sub>2</sub>?

Authors:

We never intended to suggest that Pd-DOC was supporting aquatic foodwebs, although some biogeochemical pathways could, in theory, make that possible. In this sentence we are discussing the overall (bio + photo) degradability of terrestrial DOC, and its overall contribution to ecological AND biogeochemical processes.

Reviewer:

56 "factors that are intrinsic to DOC" is too vague. This point needs to be elaborated – and linked to the optical properties subsequently measured and discussed.

Authors:

We respectfully disagree with the Reviewer on this semantic issue: This formulation

C4049

has been used in the study that we refer to and we believe that it conveys well our point.

Reviewer:

51-63: I agree with the authors' first point that the two pools overlap. Then they suggest the pools are "broadly" distinct?

Authors:

We mentioned in this section that bio- and photo-degradation PROCESSES CO-OCCUR in natural environments, not that bio- and photodegradable DOC POOLS "overlap"; we are not entirely sure what the Reviewer means here by "overlap". We indeed make the point that current literature suggests that Bd-DOC and Pd-DOC pools are broadly distinct.

Reviewer:

While this might be true in some cases, I would argue that it certainly does not hold up for less biologically degraded (ie "fresh") material. Certainly after biological processing of the DOM pool has been exhausted the remaining pool can still have high photolability. I don't believe anyone would suggest the sources and origins of BDOC and PDOC differ, since they both originate from photosynthesis by plants (terrestrial and/or aquatic). The authors are clearly aware of these complexities, as they reference a lot of key papers that cover these topics.

Authors:

There appears to be some confusion here, which may relate to the above point. We certainly agree with the Reviewer's point here but we do not fully understand how this may relate to (or affect) some of our statements.

Reviewer:

62. Here you define source vs. origins, but I actually don't understand your definitions.

C4050

The words are nouns but the definition for sources is a verb? What "environments" are you considering? How does the aquatic environment relate to the terrestrial environment? Terrestrial derived DOM is being added to water all the time, not just at the headwaters.

Authors:

Related to the last comment, we believe that the word "synthesized" may have created confusion here. We reworded the sentence to:

: "These broad chemical properties have thus been associated to distinct pools of DOC with distinct sources (i.e. the process that imported DOC in the environment, e.g. leaching, exudation) and origins (i.e. the environment where these processes take place, e.g. terrestrial, riverine, marine)."

We entirely agree that terrestrial DOM is being added to water all the time. This is not in contradiction with our reasoning or findings, and we actually acknowledge that point explicitly in the discussion (2nd par. section 4.1)

Reviewer:

82 I am still not clear on the authors' conceptual model of "sources and origins" and how this relates to PDOC and BDOC, bulk DOC concentration, and "functional pools" of DOC.

Authors: We believe that the revised sentence referred to above should help clarifying our ideas.

Reviewer:

Methods:

Please summarize clearly the sample numbers and how many were analyzed for DOC concentration, nutrients, BDOC, PDOC, etc. Preferably in a table. Define CDOM, TN, TP There is a fair amount of results included in the Methods (e.g. lines 107-114)

C4051

Authors:

We do not consider these ranges as result as those numbers in no way address our scientific questions; they are introduced here in order to describe the environmental gradients covered across the sampling area, and most of this range has been reported in previous studies from our group. The measurement methods are provided later in the "Methods".

Reviewer:

115: I don't understand where samples were collected from. Why the deepest measured point of lakes? Why not near the outflow or center? Near the shore of streams and wetlands from what depth? Were these just grab samples?

Authors:

The deepest measured point of the lake matched very well (most of the time) with the center of the lake; depth was always 0.5m from the surface.

Reviewer: 117 I suggest you look at C:N, C:P, and N:P ratios.

Authors: We would have liked to add this analysis to our manuscript, but please see above reply to a similar comment.

Reviewer:

120 Two weeks is a fairly long time to store samples for optical analysis, but okay.

122 Please provide references for all of these analyses.

130 Please report stdev or error associated with the duplicates.

138 Please check this. Didn't Spencer use A250:A365? (although A254 is practically the same).

Authors: done.

C4052

Reviewer: 147 blank corrected too? How many samples were ambient versus photochemically degraded?

Authors: Blank-corrected is implicit as we used the standard procedure in the FDOM-corr toolbox.

Reviewer: 163 How was %BDOC calculated?

Authors: We added "Percent degradable DOC was calculated as the fraction represented by the difference between DOC concentration at the start of the experiment and Bd-DOC, multiplied by 100." in the first par. of section 2.4.

Reviewer: 177 Add "Photoexposure incubation time". You might like to state that you assume this light exposure represents the "maximum potential" for photodegradation for each sample, if you think that is the case.

Authors: Added. We are not sure we fully agree with the Reviewer's statement, as "maximum potential" would depend on several variables, including exposure time (considering that we discuss in terms of the concentrations of degradable DOC).

Reviewer: 192: Did you consider combining the results and discussion? I usually find that makes it easier to write and read. There is a fair amount of discussion in the results.

Authors: We considered it, but the "Discussion" is already rather long so we decided it was better to separate it from the "Results". There is some degree of basic interpretation in the "Results", but we believe that it is necessary to understand well our reasoning throughout the section, and that this basic interpretation is very different from the level of discussion being developed in the "Discussion" section.

Reviewer:

196. This is an impressive range!

203: "typically lower" "no relationship at all" is not very satisfying or specific. What did

C4053

the statistics say?

Authors:

We respectfully disagree with the Reviewer here: We personally think that this kind of formulation is more satisfying than a purely technical one on the statistical aspects of the results presented.

This is what we meant by "basic interpretation" of the results, as explained in our previous reply. The statistical details are clearly presented in the Figure legend, but we deliberately went away from the statistical jargon in order to emphasize our reasoning.

Reviewer:

209 Here although the stats say the relationship is significant, the  $r^2$  values are low ( $<0.4$ ). I think the POOR relationship is surprising and warrants discussion. However, I would be more interested in discussion of the data in terms of % (i.e., proportion, fraction). You could plot % BDOC and PDOC against DOC concentration, to show whether there is a relationship between CONC and quality.

Authors:

We have extensively addressed the point regarding the (arguably) low  $r^2$  in our reply to Reviewer #2.

We agree that the strength of the coupling is intriguing, and the core of the discussion actually revolves around that point.

Furthermore, it is surprising that here the Reviewer suggests that the degree of coupling between Bd-DOC and Pd-DOC warrants additional discussion, whereas above in this review it is mentioned that:

" I did not understand why the authors spend so much time pointing out there is a STRONG correlation between [Pd-DOC] and [Bd-DOC], and between these terms an other concentration based measurements. You would expect that given MORE

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of something (DOC) , the sub-pools would also generally increase."

Finally, as mentioned above, we respectfully disagree with the Reviewer that exploring the drivers of the % degradable DOC represents a more interesting question. We invite the Reviewer to read the supplementary information of Lapierre et al. 2013 (referred to in the manuscript), where we present the relationships that exist between CDOM and percent Bd- and Pd-DOC. The relationship is non-significant for %Bd-DOC and has a  $r^2$  of 0.3 for %Pd-DOC; this is coherent with our results in the present study.

Reviewer:

220 Be careful: Peak T and Peak B like fluorescence are also associated with polyphe-nols, and should not be assumed to represent proteins only. When discussing your PARAFAC results, it would be helpful to link your components to more familiar peaks like C, A, T, B (put them into context). Also point out how your components are similar /different to commonly identified PARAFAC components.

Authors:

According to a comment by Reviewer#3, we now compare our PARAFAC components more quantitatively to previous studies using the OpenFluor dataset. Above we mentioned that the current text already acknowledges that we are aware of material other than proteins being associated to this fluorescence region.

Reviewer: 224: by "extreme" do you mean high? Do you need to include these 43 samples?

Authors:

We replaced "extreme" by "highest".

We believe that, overall, we gain more in including these samples than we lose in having to describe the potential, individual sample bias, considering that the whole dataset ranged several orders of magnitude (see our replies to Reviewer #1). Furthermore,

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a recent study by Kothawala et al. (now cited in the manuscript) demonstrated that inner-filter is adequately corrected for absorbances up to 1.5 cm<sup>-1</sup>; all our samples fall below this threshold.

Reviewer:

236 I do not see much distinction between lakes, wetlands, rivers in Fig 5. It seems obvious, and already known, that they would group along a concentration gradient. I believe seeing how they would group by "composition/reactivity" alone would be more informative.

Authors:

As stated above, the PCA was meant to provide a visual portrait of the relationships that exist between the reported variables, as a correlation matrix would do. We never mentioned that there were novel or unexpected results in Fig. 5, yet we are not aware of a comparable figure reporting this kind of measurements across such a vast range of aquatic environments.

Reviewer: 241-244: This seems to state the obvious: more DOC means more PDOC and BDOC. More interesting/informative is how does DOC conc. relate to %PDOD and %BDOC?

Authors: We have addressed this point several times already.

Reviewer: 247 remind the reader what A254:A365 is a proxy for.

Authors: done.

Reviewer: 252: this PCA analysis does not convince me PDOC and BDOC are distinct pools.

Authors: This was not the objective of the PCA.

Reviewer:

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259 I see that the authors looked at pH, Chl-a, TP, TN, but I do not see how this translates to "systematic ecosystem-specific or region-specific" interpretations. The only grouping I am aware of are their 3 aquatic environments (lake, river wetland). I strongly encourage the authors to avoid fancy sounding terms and instead stick with "the three different aquatic groupings" they use. Similarly, what is meant by "drivers"? No drivers (light exposure, temperature, residence time) have been defined let alone presented.

Authors:

The section in question also refer to the regions presented in Figure 1; we have changed the sentence in question to:

" There was thus no systematic ecosystem- or region-specific differences in the relationship between Bd-DOC its PREDICTORS, suggesting that the amount of biologically degradable DOC IN THE STUDIED SYSTEMS in the studied systems is mainly a function of DOM composition and nutrients regardless of the type of aquatic environment and the region where they lie.."

in order to better acknowledge that it is a result from our sampling effort that does not necessarily apply to all possible types of ecosystems or regions. We believe, however, that our results do support an absence of "ecosystem- or region-specific" differences in the relationship between the concentrations of biologically degradable DOC that we report here. Please note that, in accordance with a previous Reviewer's comment, we have replaced "DOC degradability " by "the amount of biologically degradable DOC" in this sentence.

Reviewer:

260 This sentence does not make any sense to me. Do you mean "DOC composition, including nutrient content"? DOC composition (its chemical makeup and structure) is determined by its source, type of aquatic environment, region it is found in. This seems

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circular.

Authors:

We did not write "including" nutrient content, but rather "and" nutrient content. One could argue that there could be ecosystem or regional specificities (e.g. hydrology, bacterial community structure, irradiance etc.) that could affect the concentrations of degradable DOC independent of nutrient and DOM composition.

Reviewer: 263 Please consistently use DOC vs. DOM. Please include a full correlation matrix. How correlated was C5 to C6? C3 to C2?

Authors:

As stated above, we believe that the PCA effectively addresses the need of including a correlation matrix.

Regarding the use of DOC vs DOM, we have been very careful throughout the manuscript; we have measured DOC loss in our experiments, so we consistently refer to the degradable "DOM" as degradable "DOC". We use DOM when referring to the bulk or specific pools, as those pools do not only contain carbon.

Reviewer: 266 One expects the photodegradable pool to be more highly correlated with optical properties, since by definition you are limited to measuring the photoreactive pool of DOM with this approach.

Authors:

Indeed. We acknowledged this point explicitly in the "Discussion" in the last par. of section 4.2.

Yet, as mentioned above, one has to consider the wavelengths involved when bridging "optically active" to "photo-chemically active".

Reviewer: 273-276 This is interesting.

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Authors: This is one of the core result of this study.

Reviewer: 277-294 Interesting, but already established in the literature. Include references to prior work.

Authors: Indeed, and we refer to previous work in the "Discussion".

Reviewer: 294-313 I am lost here: what evidence is there that CDOM at A440 represents terrestrial derived DOM? I assure you that if you extract algae it will absorb at A440, especially if you let it degrade.

Authors:

Please see above replies mentioning that we did not use CDOM as a measure of terrestrial DOC.

We are aware that different autochthonous sources may potentially contribute colored DOM to aquatic environments. We acknowledge in the "Discussion" (2nd par. section 4.3) that there may be colored DOM produced in situ, but based on current knowledge (see also the discussion in Lapierre et al. 2013, SI), it seems unlikely that this input would be significant compared to the massive loadings of terrestrial, colored DOM.

Reviewer: I am concerned that your "low-CDOM" relationship is an artifact of your analysis and detection limits. What are the %C6 values associated with this low [C6] values? Could this just be PARAFAC picking up noise?

Authors:

Visual examination of the residuals for those samples reveals that the model extracted the signal very well, even in the clearest samples.

The %C6 are typically the highest at both ends of the CDOM gradient; %C6 typically represents 30% of the total fluorescence in the lowest CDOM (and [C6]) samples. We are not sure, however, how this relates to the PARAFAC potentially picking up noise.

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