RC1: 'Comment on bg-2021-131', Anonymous Referee #1

The paper is organized and well-written, with a clear analysis of the data. Overall, the findings agree with the literature and advance our understanding of the impacts of land use on DOM composition and lability in watersheds.

Reply: We thank the reviewer for her/his positive evaluation of the manuscript.

My only concern is that "terrestrial" PARAFAC components are stable, which led the authors to suggest that terrestrial material is not being significantly consumed/altered (e.g., lines 407-412 of the manuscript). These components can be produced by microbes, so using these as an exclusive metric of whether terrestrial material is being consumed isn't ideal. At the least, the authors should consider literature showing alteration of terrestrial material by microbes and "maintenance" of these PARAFAC components by microbes, e.g., microbes contributing to these components so that net change is zero, while real change is happening. Due to the diversity of compounds contributing to a given optical signature, a more detailed molecular analysis would be needed to more definitively state whether the terrestrial material is being consumed or not. Thus, I would emphasize more caution when noting the degree to which terrestrial material is being altered and how that relates to land use. These are interesting and encouraging results that help illustrate these dynamics across watersheds but the full extent of terrestrial DOM change isn't fully captured with the data in hand.

Reply: Although reviewer#1 points to a relevant limitation of PARAFAC, we did not intend to suggest that terrestrial DOM is not significantly altered in our study sites. Indeed, the size of the LTRC pools was related to the rate of degradation of the terrestrial DOM (lines 376-378) and was similar in size to the STRC pools. Yet, the LTRC pools did not vary across land uses (Figure 5). That being said, we were unable to link the LTRC to changes in DOM composition as the terrestrial PARAFAC components remained stable during experiments (lines 372-375), leading us to suggest that aromatic molecules were not degraded by bacterial communities (lines 378-379). As pointed by the reviewer#1, this lack of variation does not necessary mean that terrestrial PARAFAC components were not altered, as bacterial communities are able to both consume and produce humic-like components commonly associated with terrestrial DOM. Thus, we agree that we cannot exclude some alteration of aromatic molecules during degradation processes that would have not been captured by optical measurements.

To address this comment, the manuscript has been modified as follow (**lines 404-417**):

"Although a substantial amount of terrestrial DOM was consumed by heterotrophic bacteria, the terrestrial (C2-C4) and photoproduced (C1) components showed no significant variations during incubations (Figure 7) despite the ability of bacterial communities to degrade complex aromatic molecules (Catalán et al., 2017; Fasching et al., 2014; Logue et al., 2016). While the stability of the C1 component during bioassays is consistent with the fact that photoproduced molecules may be resistant to further bacterial degradation (Tranvik et al., 2001), the lack of variation of C2-C4 components may reflect an equilibrium between the bacterial consumption and production of molecules contributing to the humic-like signatures. Experimental and field studies have shown that heterotrophic bacterial communities are able to produce molecules fluorescing in the region of EEMs commonly attributed to humic-like material from terrestrial origin (Amaral et al., 2016; Fox et al., 2017; Guillemette and del Giorgio, 2012). It is therefore possible that the alteration in the composition of terrestrial DOM upon bacterial activity may not have been captured by optical measurements. Addressing this point would require the characterization of DOM at the molecular level (e.g., Kim et al., 2006)."

Some of the specific comments refer to personification of materials, e.g. "whose" when referring to DOM or "their" when referring to carbon.

Reply: We have payed attention to this.

Specific Comments

Line 21: “whose the size increased with human disturbance”

I suggest changing this to “with relative contribution to the total DOM pool increasing with human disturbance.”

Reply: Done.

Line 29: “determine” should be “determined”

Reply: Done.

Line 35: “their travel”

I suggest changing this to “transit”

Reply: Done.

Line 49: “proportion” should be “proportions”

Reply: Done.

Line 60: “amount” should be “amounts”

Reply: Done.

Line 72: “results” should be “result”

Reply: Done.

Line 106: Remove “the” before Lake Geneva

Reply: Done.

Line 115: “forests” should be “trees”

Reply: Done.

Line 169: “weights” should be “weight” in the sub-title

Reply: Done.

Line 223: May be better to refer to the Fluorolog-3 as a spectrofluorometer.

Reply: Done.

Line 233: “prior the” should be “prior to the”

Reply: Done.

Line 234: “A eight components” should be “An eight component”

Reply: Done.

Line 285: “component” should be “components”

Reply: Done.

Line 340: “recently DOM produced” should be “DOM recently produced”

Reply: Done.

Line 344: I suggest citing Harfmann et al. 2019 JGR: Biogeosciences here to support this observation

Reply: This reference will be added.

Line 349: “investigate deeper” should be “more deeply investigate”

Reply: Done.

Line 350: “point” should be “suggest”

Reply: Done.

Line 351: “amount” should be “amounts”

Reply: Done.

Line 368: “another” should be “additional”

Reply: Done.

Line 371: “despites” should be “despite”

Reply: Done.

Line 375: The similar size of STRC and LTRC pools is intriguing – is there any evidence that the LTRC pool is related to the STRC?

Reply: A similar size of STRC and LTRC was also reported in Soares et al. 2019 (note that in their paper the LTRC corresponded to a medium-term bio-reactive DOC, defined as the amount of DOC lost between day 7 to day 23). Both the STRC and LTRC pools are significantly correlated with DOC concentrations, leading to a positive but weak relationship between STRC and LTRC pools. We think that this relationship simply reflects a higher amount of bioavailable DOM with increasing DOC concentrations due to human disturbance that affects both primary production and export of terrestrial material. Moreover, the STRC related to protein-like components while the LTRC related only to terrestrial humic-like components and there was no relationship between the decay constant k and LTRC. Therefore, we think that the positive relationship between the STRC and LTRC pools is not causal and that each pool is driven by specific drivers. This lack of relationship between STRC and LTRC has been previously reported in other freshwater ecosystems (see suggestions for modification below). The manuscript was revised as follow:

Results (lines 317-327): “Both the STRC and LTRC pools were positively correlated with DOC concentrations (Pearson $r = 0.79$, $p < 0.0001$ and Pearson $r = 0.68$, $p = 0.0013$, respectively), leading to a positive but weak relationship between the STRC and LTRC pools (Person $r = 0.49$, $p = 0.03$). STRC was correlated to all components when expressed in FMax values, but only with protein-like components when expressed as a relative contribution to the total fluorescence signal, suggesting an autochthonous origin for this reactive C. On the contrary, the LTRC related to FMax values of C1-C4 components but not with the protein-like components, implying that this reactive C originated from terrestrial inputs. The total amount of BDOC, decay constants and the size of STRC were significantly related to the C6-C8 protein-like components (Figure 6). There was however no relationship between the decay constant k and LTRC.”

Discussion (lines 389-403): “Contrary to STRC, we found no evidence that human land uses impact the loss of terrestrial DOM upon bacterial degradation. LTRC pools were indeed similar across agro-urban and forest-grassland streams despite higher content in inorganic nutrients, higher bacterial activity, and freshly produced autochthonous DOM in agro-urban streams. In line with a recent study carried out in Swedish inland waters (Soares et al., 2019), STRC and LTRC pools were comparable in size but no evidence of interaction was observed between the bioavailability of DOM on short and long timescales. The positive but weak relationship between STRC and LTRC likely reflected a greater amount of bioavailable DOM as human disturbance increased, as the latter enhanced both primary production and terrestrial export. Moreover, each pool related to specific DOM fractions. Similar observations were reported in Swedish rivers (Soares et al., 2019), in southern Québec (Guillemette and del Giorgio, 2011), or also in the Hudson River (del Giorgio and Pace, 2008). Overall, our findings are in good agreement with the idea that STRC is sustained by algal growth, whereas the consumption of DOC at longer timescales is rather related to terrestrial inputs of DOM (references above).”

Soares et al. 2019 Scientific Reports noted the role of residence time in long-term bioavailability. The discussion could be expanded a bit to consider this with the current dataset.

Reply: Unfortunately, we do not have any estimation of the water residence time (WRT) in our study sites, nor the hydrological data required to calculate it. Moreover, given the small size and limited range of variation of drainage areas in our study, it is unlikely that we could find relationship between STRC and/or LTRC and WRT as in Soares et al. 2019 where WRT ranges from < 1 day to 100 days (drainage areas from 450 to 47000 km^2). We agree with the reviewer#1 that the role of WRT in controlling the different facet of BDOM should deserve more investigation, but this requires to collect samples at a larger scale than the one of our study.

Line 374: “amount of DOC was” should be “amounts of DOC were”

Reply: This sentence has been removed in the revised manuscript.

Line 405: “closed” should be “close”

Reply: This sentence has been removed in the revised manuscript (see comment below).

Lines 407-412: I’m not sure I agree with this. It stands to reason that inland waters are well-suited to degrade terrestrial DOM, as that is a primary input to these systems and heterotrophic bacteria have arguably adapted to utilize this carbon source.

Reply: We recognized that our conclusions are exaggerated here, for several reasons. First, our sampling sites encompass mainly small streams with short WTR. Thus, our estimation of 20% of terrestrial DOM consumed is only applicable in the upper part of the basin upstream Lake Geneva and should be not extrapolated to a larger scale. Second, as mentioned by reviewer#2, we didn’t include in our experiments the effect of light and performed our campaigns during the wet season only. Therefore, given the limited spatial extent and the experimental setup, we cannot guarantee our estimation to be conservative in space and time. A proper estimation of DOC loss in the basin would have required more work/other approaches and was beyond the scope of the study. In order to account for this point as well as for a similar comment from reviewer#2, this part of the discussion has been removed in the revised manuscript.

Line 408: “timescale” should be “timescales”

Reply: This sentence has been removed in the revised manuscript (see previous comment).

Line 409: “entered in the lake Geneva” should be “in Lake Geneva”

Reply: This sentence has been removed in the revised manuscript (see previous comment).

Line 409: Remove “the” before water residence time

Reply: This sentence has been removed in the revised manuscript (see previous comment).

Line 418: Add “the” before fluvial continuum

Reply: Done.

Line 425: I would reference the findings from line 344 and citation mentioned there; this finding is largely dependent on optics which always benefits from links to other literature.

Reply: Done. Although we agree with the reviewer that PARAFAC components are often classified according to previous work, a bacterial origin for the C5 component is supported here by the fact that this compound shows an increase in fluorescence intensity during incubation.

Line 429: “amount” should be “amounts”

Reply: Done.

Line 439: “effect” should be “effects”

Reply: Done.

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RC2: 'Comment on bg-2021-131', Anonymous Referee #2

This manuscript has potential to improve our knowledge regarding the long-standing debate of whether human activities can significantly affect the biodegradation of terrestrial DOM across aquatic ecosystems. However, I have several concerns not allowing me to accept the manuscript at its current stage. I look forward to hearing from the authors' response.

Reply: We thank the reviewer for her/his good appreciation of the manuscript and hope that we will satisfy her/his comments. Line numbers refer to the revised manuscript.

First, the investigation of this work was only conducted between fall and winter seasons, with similar precipitation and water discharge condition during the two field trips. This is not reflected in the manuscript's title, as the title sounds that all conditions were examined in this work. How about spring and summer seasons? When are the wet and dry seasons, respectively? How much was represented from the samples collected in this study? Indeed, if the wet season was not included in this study at all, the contribution of terrestrial DOM could be significantly underestimated.

Reply: Our campaigns were performed during the fall and winter seasons that correspond to the wet period for all our sampling sites (examples given in Figure 1 below), except for the Rhône River for which the high flow occurs during summer. Therefore, the contribution of terrestrial DOM can be expected to be greater during fall and winter compared to spring and summer periods. Details on the period covered by the study have been provided in the following sections:

Abstract (lines 16-17): "Sites were selected along a gradient of human disturbance (agriculture and urbanization) and were visited twice during the wet season."

Introduction (lines 94-95): "Water samples were collected twice during the wet season".

Section 2.1 (lines 120-125): "Samples were collected on two occasions, at the end of autumn between the 13th and 14th of November 2018 and at the end of winter between the 5th and 7th of March 2019. Campaigns were thus carried out during the wet season, i.e., when high discharge conditions may favor greater export of terrestrial DOM (Lambert et al., 2013). The only exception was the Rhône River, that experiences higher water discharge in summer due to the glacio-nival regime of the river (Loizeau and Dominik, 2000)."

Results (lines 275-276): "Hydro-climatic conditions were similar for the two sampling campaigns that occurred during high winter base flow level"

Discussion (lines 338-339): "The spatial variability in water quality and DOM sources and composition observed in streams and rivers of the Lake Geneva basin during the wet season […]"

Discussion (lines 425):" […] they also evidenced that during the wet season and at the scale of small catchments […]"

Conclusion (lines 457-460): "Although our study focused mainly on small catchments during the wet period, our results are likely not limited to the Lake Geneva Basin considering that an enrichment in protein-like DOM due to greater autochthonous production is a recurrent observation in agricultural and urban catchments"

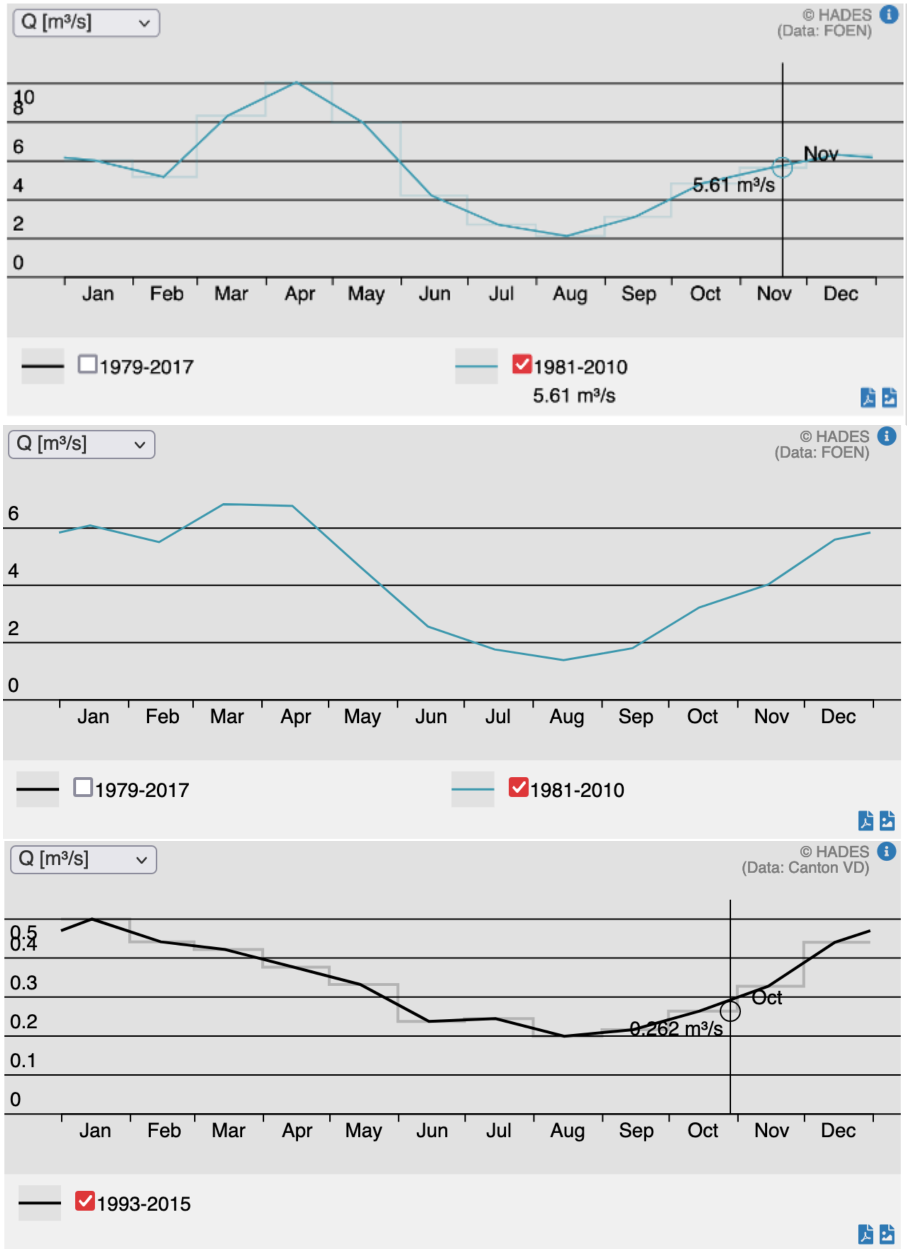

Figure 1 – Discharge variation during the hydrological cycle for three of the sampling sites. Lines represent mean discharge calculated over 1981-2010 or 1993-2015 periods. Note that our field campaigns were performed in November 2018 and March 2019, corresponding to the wet period. Note that unfortunately we are not be able to provide discharge data, as daily hydrological data are available only for visualization and furthermore limited to 2015 or 2018 depending on sampling sites.

Second, I wonder if the degradation or transformation of terrestrial DOM can be discerned by the limited analytical methods applied in this study. Some typical LMW compounds were examined in this work to show the biodegradation of autochthonous compounds. Similarly, it would be more convincing to show the constant presence of biomarkers of terrestrial DOM during the incubation, such as lignin phenols, to verify the stability of terrestrial DOM.

Reply: We agree with this comment, also pointed by reviewer#1. Unfortunately, we didn't measure specific biomarkers of terrestrial DOM as we did for LMW compounds, and it is true that PARAFAC is limited to investigate the alteration of terrestrial DOM as bacterial communities both consume and produce humic-like fluorophores. In order to take into account this comment and one of reviewer#1, the manuscript has been modified as follow (**lines 404-417**):

"Although a substantial amount of terrestrial DOM was consumed by heterotrophic bacteria, terrestrial (C2-C4) and photoproduced (C1) components showed no significant variations during incubations (Figure 7) despite the ability of bacterial communities to degrade complex aromatic molecules (Catalán et al., 2017; Fasching et al., 2014; Logue et al., 2016). While the stability of the C1 component during bioassays is consistent with the fact that photoproduced molecules may be resistant to further bacterial degradation (Tranvik et al., 2001), the lack of variation of C2-C4 components may reflect an equilibrium between the bacterial consumption and production of molecules contributing to the humic-like signatures. Experimental and field studies have shown that heterotrophic bacterial communities are able to produce molecules fluorescing in the region of EEMs commonly attributed to humic-like material from terrestrial origin (Amaral et al., 2016; Fox et al., 2017; Guillemette and del Giorgio, 2012). It is therefore possible that the alteration in the composition of terrestrial DOM upon bacterial activity may not have been captured by optical measurements. Addressing this point would require the characterization of DOM at the molecular level (e.g., Kim et al., 2006)."

The slope ratio from CDOM data was not reported for the bioassay experiments. Such information could indicate whether the molecular weight of terrestrial DOM was shifted during biodegradation.

Reply: Indeed, the slope ratio decreased during bioassays in all experiments (Figure 2), indicating a systematic decrease in the average MW of DOM as LMW compounds were preferentially consumed. These data have been added in the revised version:

Material and methods (lines 224-228):" Spectral slopes for the intervals 275–295 and 350–400 nm were determined from the linear regression of the log-transformed a spectra versus wavelength and used to determine the slope ratio (S_R). The slope ratio S_R, calculated as the ratio of $S_{275–295}$ to $S_{350–400}$, is inversely related to the molecular weight distribution of DOM (Helms et al., 2008)."

Results (lines 328-330): "The S_R values decreased in all experiments (Supplementary Figure S2), indicating an increase in the average molecular weight of DOM during incubations as low molecular weight compounds were preferentially degraded."

Discussion (lines 382-384): "The loss of protein-like components paralleled by a shift in the molecular weight to during incubations also evidences the efficient degradation of this DOM from algal origin.".

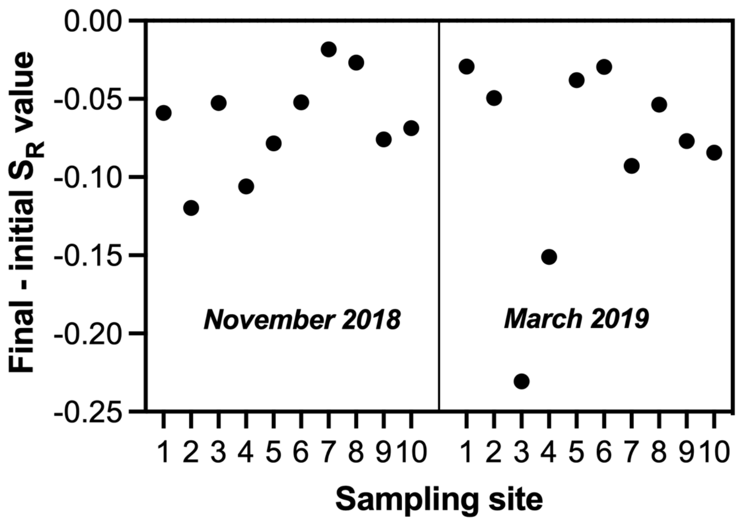

Figure 2 – Evolution of the slope ratio during incubations. Negative values mean that the average MW of DOM decreased during experiments, as LMW compounds were consumed.

Numbers refer to bioassays. This figure has been added in the revised manuscript as supplementary information.

Thirdly, the mineralization of DOM includes both photo- and bio- degradation processes. This study only conducted bioassay under dark condition. The title and abstract should reflect this fact. I would recommend to replace mineralization with biodegradation. Indeed, one scenario was overlooked in this study at all. The increased terrestrial DOM from human activities may be first photo-degraded to lower molecular weight DOM, followed by promoting the primary production and bacterial respiration. The bioassay experiment in this study could include light condition as well to examine scenarios more comprehensively.

Reply: We modified the manuscript to reflect the fact that degradation experiments were performed in dark conditions:

Title: "No evidence of a human influence on the biodegradation of terrestrial organic matter (DOM) in Alpine fluvial network"

Abstract (lines 13-15): "In this study, we investigated the impact of human land uses on the biological degradation […]"

Abstract (line 17): "[…] in parallel to DOM bioavailability in dark bioassays".

Introduction (lines 97-98): "Patterns in DOM degradation were investigated based on standardized dark degradation experiments"

In a word, this work was conducted by limited tools and limited conditions. The authors should revise the manuscript carefully to reflect these facts and to avoid exaggerating the conclusions.

Reply: The goal of this study was to investigate how human land use affect the sources and biodegradability of DOM and its potential impact on bacterial respiration (BR). This question is relevant for its implications on our understanding of the role of inland waters in the global C cycle (Xenopoulos et al., 2021), and we think that our experimental design (including dark incubations and sampling during the wet period) is adequate enough to provide some insights on this topic.

First, while we agree with the reviewer that we should have done experiments with light conditions to examine all scenarios, we don't think that it would have change the conclusions of the study. Our results clearly show that higher BR in agro-urban streams was mostly related to the accumulation and mineralization of molecules generated in-stream by aquatic primary producers. This was evidenced through the decoupling of the DOC biodegradation dynamics coupled with the identification of the most reactive fraction via fluorescence measurements. Moreover, we would like to point that an impact of photodegradation on bacterial respiration through its control on terrestrial DOM bioavailability is taken into consideration in the manuscript (lines 429-432). Indeed, BR was also correlated with C1 component, associated with photodegradation processes. However, the relationship between BR and C1 was weaker than the relationship between BR and C6-C8 components from algal origin and Figure 9). We think that the revised manuscript is in line with these observations, as we used terms such as *most* rather than *all* in our conclusions.
For instance lines 24-28: "From a greenhouse gas emission perspective, our results **suggest** that human activities **may have** a limited impact on the net C exchanges between inland waters and the atmosphere, as **most** CO2 fixed by aquatic producers in agro-urban streams is cycled back to the atmosphere after biomineralization."

Secondly, regarding the limitation of fluorescence measurements, we agree that our study do not provide information on changes of the composition of terrestrial DOM upon degradation. However, it doesn't impact the observation that the sizes of LTRC pools were similar in agro-urban and forest-grassland streams, and therefore our conclusion that human land use had no influence on the biodegradation of terrestrial DOM. Limitation of PARAFAC is now recognized in the revised manuscript but investigating changes of terrestrial DOM at the molecular level was beyond the scope of the study.

Thirdly, we made clarification in the revised manuscript to highlight that our results apply at the scale of our study sites and at a specific period (i.e,, winter). The need for further studies at different period and in other catchments is clearly emphasized in the introduction and the conclusion.

That being said, limitations of the study (dark incubations, lack of seasonality and spatial coverage) did not allow us to discuss about the amount of terrestrial DOM lost during its transit in fluvial networks nor its potential incorporation into microbial biomass (lines 400-412 of the initial version of the manuscript). Therefore, this section has been removed from the revised manuscript.

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