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.

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 It is true that our campaigns were only performed during the fall and winter seasons. We focused on these periods because it corresponds to the wet period for all our sampling sites (examples given in Figure 1), except for the Rhône River for which the hydrological regime has been modified by the exploitation of the river for hydroelectrical production. Therefore, the contribution of terrestrial DOM can be expected to be greater during fall and winter compared to spring and summer periods. As hydrological pathways – and thus terrestrial DOM sources – change across the hydrological cycle, we cannot extrapolate our results to the dry season. Note that unfortunately we will not be able to provide discharge data, as detailed hydrological data are not available and limited to 2015 or 2018 depending on sampling sites.

We suggest modifying the manuscript as follow:

Abstract (line 13-15): "In this study, we investigated […] of the Lake Geneva Basin **during the wet season**"

Introduction (lines 92-93): "Water samples were collected during **the wet season** in ten independent catchments…".

Section 2.1 (l19): "Samples were collected on two occasions, at the end of autumn between the 13th and 4th 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 exploitation of the river for hydroelectrical production (Loizeau and Dominik, 2000).**"

Discussion (lines 421-422): "In our study, […] at the basin scale **during the wet season**."

Conclusion (lines 431-433): "we found that **during the wet season** human land uses had a limited effect […] by heterotrophic bacterial communities. **Further work are however required to investigate if our observations made during the wet season also apply during spring and summer, whereby changes in hydrological flowpaths during low flow may mobilize different terrestrial DOM sources (Buffam et al., 2001; Lambert et al., 2013)**"

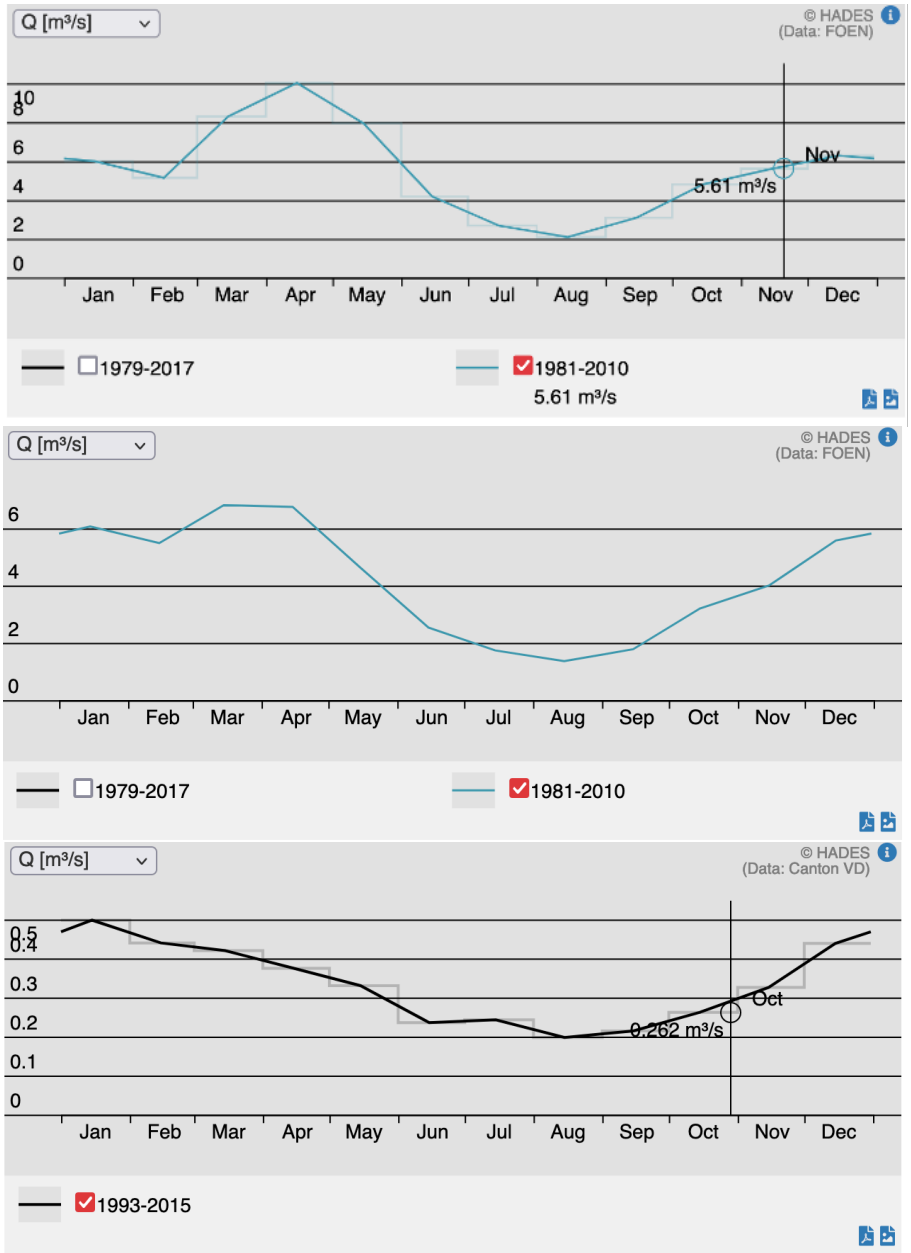

Figure 1 – Discharge variation during the hydrological cycle for three of the sampling sites. Lines represent mean discharge calculated over 1981-2010 or 1993-2015 periods. Note that our field campaigns were performed in November 2018 and March 2019, corresponding to the wet period.

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, we suggest modifying the manuscript (line 370):

"On the contrary, the similar sizes of LTRC pools between agro-urban and forest-grassland streams (Figure 5D) suggest that the bacterial degradation of terrestrial DOM was not influenced by human land uses despite higher content in inorganic nutrients, higher bacterial activity, and freshly produced autochthonous DOM in agro-urban streams. LTRC was indeed positively correlated with initial F_{Max} values of C1-C4 components but not with the protein-like components (data not shown), implying that this C originated from terrestrial inputs. However, terrestrial (C2-C4) and photoproduced (C1) components showed no significant trends during incubations (Figure 6) 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 findings that photoproduced molecules are resistant to further bacterial degradation (Tranvik et al., 2001), the lack of variation of terrestrial components may result from a net balance between bacterial consumption and production of molecules contributing to the humic-like signature (Amaral et al., 2016; Guillemette and del Giorgio, 2012). It is therefore possible that alteration in the composition of terrestrial DOM upon bacterial activity may have not 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.

We suggest modifying the manuscript:

Results (line 311): "The S_R values decreased in all experiments, indicating an increase in the average molecular weight of DOM during incubations as low molecular weight compounds were preferentially degraded (Supplementary Figure S2)"

Discussion (line 366): "Moreover, the decrease in S_R values during incubations (Supplementary Figure S2) showed that the average molecular weight of DOM increased as low molecular weight compounds were preferentially consumed".

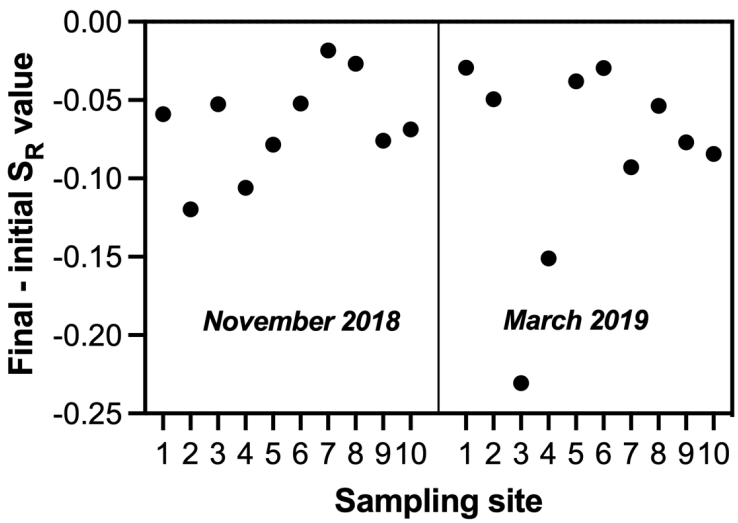

Figure 2 – Evolution of the slope ratio during incubations. Negative values mean that the average MW of DOM decreased during experiments, as LMW compounds were consumed. Numbers refer to bioassays.

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 Indeed in this study we didn't perform experiment with light conditions, however we would like to point that, based on our data, we didn't exclude a role of photo-degradation on the degradation of terrestrial DOM and its contribution to bacterial respiration (lines 389-392). That being said, we suggest modifying the manuscript as follow:

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**".

Moreover, following this comment and one of reviewer#1, we also suggest removing lines 404-412 of the manuscript. Indeed, we

following a comment of reviewer#1, we also suggest removing lines 404-412 of the manuscript. Indeed, considering the experimental setup as well as the fact that we performed campaigns only during the wet period, the DOC losses measured in our experiments cannot be extrapolated at the scale of the basin. 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 pointed 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 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 We sincerely hope that our answer will satisfy the reviewer.

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