

R1:

All suggestions for improvement were well considered and addressed.

[Reply:](#) We thank the reviewer for his/her positive feedback.

Only a minor issue - on line 225 of the revised manuscript, absorption is not clearly indicated: "log-transformed a spectra". I would recommend including the lambda subscript to make it abundantly clear to the reader that you are referring to absorption.

[Reply:](#) Done.

R2:

Comments on RV1.

The authors have addressed some of the review comments well, but the manuscript remains several arguments to be justified.

I share with Referee #1 about the concern that whether the degradation of "terrestrial DOM components" can be discerned by PARAFAC, given the fact that DOM produced during microbial process can also contribute to those components labeled as terrestrial materials in PARAFAC. The authors addressed this question by adding the sentence "Addressing this point would require the characterization of DOM at the molecular level (e.g., Kim et al., 2006)". Thus, I don't think it is suitable to emphasize "terrestrial DOM" in the title of this manuscript. I insist that the concepts of "terrestrial", "not autochthonous origin", and "Long-term reactive carbon, LTRC", should not be interchangeable.

[Reply:](#) We made significant changes in the revised manuscript to take into consideration the fact that our study does not allow indeed to discuss about changes in terrestrial DOM upon bacterial degradation. Please see our reply below for details.

Meanwhile, the results presented in this work consistently show that biological activities, both primary production and bacterial respiration, are significantly elevated in Argo-urban streams than Forestgrassland streams. I think it is better to highlight this confident information rather than presenting an arguable statement. I would recommend the title to be something like "Increased biodegradable carbon pool in the human-disturbed streams in Alpine fluvial networks".

[Reply:](#) In agreement with previous comments from R1 and R2, we followed the suggestion of reviewer 2 to change the focus of the study. More specifically, we choose to focus on the confident result that human activities have enhanced both primary production and bacterial respiration in streams and to highlight the implications of these observations, namely that human activities may have a limited impact regarding the role of inland water in the context of the global C cycle.

Major changes are:

[Title:](#) Enhanced bioavailability of dissolved organic matter (DOM) in human-disturbed streams in Alpine fluvial networks

[Discussion:](#)

- [Lines 347-355:](#) We removed the first paragraph of the discussion in which we argued that we did not observe any influence of human activities on the degradation of terrestrial DOM. In the revised manuscript, we resumed the goal of the study and highlighted the main observation of our results as follow: "The bacterial degradation of DOM along fluvial networks contributes to CO<sub>2</sub> emissions toward the atmosphere (Lapierre et al., 2013). Human activities are expected to alter the role of inland waters in the global carbon cycle by disturbing DOM sources and composition (Xenopoulos et al., 2021). Keeping in mind that our study focused mainly on small size catchments during the wet period, our results highlighted that the enhanced production and

accumulation of autochthonous DOM in human-disturbed streams was quickly cycled back to the atmosphere by heterotrophic bacteria. From a greenhouse gas emission perspective, the respiration of this highly reactive DOM pool may have a limited impact on C budgets in human-disturbed catchments.”

- The section 4.2 entitled “Biodegradability of terrestrial DOM is not related to land use” has been removed. The two paragraphs of this section dealing with the link between STRC and LTRC and with PARAFAC are now in the section 4.1.
- We also removed the sentence “we found no evidence that human land uses impact the loss of terrestrial DOM upon bacterial degradation” in the last paragraph of the previous 4.2 section.
- Lines 419-430: In order to avoid repetition and to highlight the main result of the study, we changed the first paragraph of the revised section 4.2 as follow: “The positive influence of enhanced primary production on the absolute amount of biodegradable DOM in human-disturbed streams agrees with previous studies (Hosen et al., 2014; Parr et al., 2015), but our results suggest that the impact regarding the role of inland waters in the context of the C cycle may be limited. Higher BR in agro-urban streams was indeed mostly related to the accumulation and mineralization of molecules generated by aquatic primary producers (Figure 9A), although the photodegradation of terrestrial DOM could also fuel BR through the transformation of complex and aromatic molecules into compounds of lower molecular weight (Bertilsson and Tranvik, 1998) as suggested by the positive relationship between BR and C1 (Figure 9B). Therefore, our results point to a limited effect of STRC on greenhouse gas emission as most of the C released toward the atmosphere upon bacterial respiration corresponded to atmospheric CO<sub>2</sub> previously fixed by aquatic producers and converted into biomass.”

#### Conclusion:

- Lines 448-450: We changed the sentence “Greater autochthonous production of DOM in agro-urban streams led to higher amounts of bioavailable DOM, stimulating ecosystem respiration while no influence on the loss of terrestrial DOM was observed.” by “Enhanced primary production in human-disturbed catchments led to the accumulation of highly reactive molecules of low molecular weight which in turn stimulated bacterial respiration”.
- Lines 456-467: The last paragraph of the conclusion has been modified to highlight the need of further studies taking into account spatial and temporal variations in DOM sources: “Considering that an enrichment in protein-like DOM due to greater autochthonous production is a recurrent observation in agricultural and urban catchments (Stanley et al., 2012; Xenopoulos et al., 2021), our results are likely not limited to the Lake Geneva Basin. However, seasonal and longitudinal variations in DOM sources and composition should be considered along with the fact that the net effects of agriculture and urbanization on freshwater DOM vary widely depending on the environmental context (Stanley et al., 2012). While our results are in line with previous works (Hosen et al., 2014; Parr et al., 2015), they contrast with studies reporting no influence of human land uses on the bacterial consumption of DOM (Kadjeski et al., 2020; Lu et al., 2013) or higher DOM degradability in agricultural streams (Shang et al., 2018). Therefore, additional works on the links between human activities and DOM reactivity and fate are needed in order to fully assess the future of inland waters in the context of the global C cycle.”

As I mentioned in my original review comments, this study did not include spring and summer seasons when the temperature is higher in the field. The authors have added wet/dry season information, but they should rationalize temperature effect as well. Certainly, the season information should be reflected in the abstract.

**Reply:** We changed “wet period” by “winter high flow period” in the abstract.

The authors should also explain why the field temperature for the collected samples in this study was 6~10 oC (Line 126) but the lab incubation experiments were conducted at 20 oC (Line 148), and how this would affect the results.

**Reply:** We choose to use a standardized protocol – including the fixed temperature of 20°C commonly used to standardize metabolic activities – in order to be able to compare the results obtained at different periods. Biological activities are strongly affected by temperature, so fixing incubation temperatures at field conditions may have masked patterns in degradation related to differences in the nature of DOM. This standardized protocol allowed us to investigate how DOM degradation dynamics varied among streams, once the effect of temperature was removed (del Giorgio and Davis, 2003). We added in the revised manuscript some sentences to justify the fixed temperature and to highlight that our measurements performed in laboratory conditions should not being considered as representative of field conditions:

In Material and methods, lines 148-158: “A fixed temperature was chosen in order to be able to compare DOM degradation dynamics across our sampling sites at different periods. Because biological activity is strongly impacted by temperature, using water temperature on the field for incubations may have masked patterns in degradation related to differences in the source and composition of DOM (del Giorgio and Davis, 2003). Using a fixed temperature allowed us to investigate how DOM bacterial degradation varied among streams, once the effect of temperature was removed. BDOC, BR, and the bacterial consumption of low molecular weight compounds were incubated in similar conditions (see below), ensuring comparability between water quality, bacterial metabolism, and DOM degradation dynamics. However, consumption and respiration rates should not be considered as representative of field conditions, as incubation temperature – and thus bacterial activity – was higher compared to field conditions.”

In the conclusion, lines 453-455: “However, further studies should perform incubation and respiration measurements at in situ conditions to improve our understanding of different bioreactive DOC pools to better constrain C budgets.”

Other comments:

Line 71: lead to increased CO<sub>2</sub>

**Reply:** We changed by “can lead to an increase in CO<sub>2</sub>”.

Line 383: a shift in the molecular weight to what?

**Reply:** We changed by “The loss of protein-like components paralleled by an increase in the average molecular weight during incubations also evidences the efficient degradation of this DOM from algal origin”.

Line 279-280, SRP 29.3 ug/L is not consistent with Figure 2B. It appears to be about 35 ug/L in Figure 2B.

**Reply:** Indeed, there was a mistake in values for SRP, this has been corrected.

Table 2, Figure S1: C<sub>2</sub> Max Em is 489 or 498?

**Reply:** 498, the figure S1 has been modified.

Figure 1: Denote sites with different color or label, to indicate Argo-urban and Forest-grassland type, respectively, corresponding to Table 1.

**Reply:** Done.

Figure: Suggest to include a figure to show the plots of STRC and LTRC with incubation time, and to show how the decay constant was derived. This can be included in the

supplemental materials.

[Reply: Done.](#)

Table: It would be more informative if the authors can provide a table to list the original parameters for figures 2, 3, and 5, by presenting detailed numbers rather than just showing average and range in the figures. This can be included in the supplemental materials.

[Reply: These data are now included in the manuscript in the supplemental materials.](#)

#### References:

del Giorgio, P. A. and Davis, J.: Patterns in DOM lability and consumption across aquatic ecosystems, in Aquatic ecosystems: Interactivity of dissolved organic matter, pp. 399–424., 2003.