

General Comments

Lambert et al.'s "Role of nitrogen and iron biogeochemical cycles on the production and export of dissolved organic matter in agricultural headwater catchments" provides an interesting contribution around possible mechanisms influencing dissolved organic carbon release into streams from agricultural soils. The manuscript provides a useful framework to explain why passive (water flow / discharge) transport of DOC from soils to streams does not work fully as a way to predict DOC concentrations in stream. The study proposes and investigates the role of Fe and NO₃ in soils as active regulators of DOC release throughout the hydrological cycle. The study provides a useful contribution to our understanding of environmental regulators of stream DOC concentrations. I also think the results of the study highlight very clearly the heterogeneity of the system, which raises important and novel questions about the cumulative effects of soil-water interactions, material transport, and stream conditions.

I enjoyed reading the manuscript and after reflection have a few clarify questions and comments that I hope if addressed would strengthen the manuscript.

REPLY: We thank C. Williams for its positive evaluation of our work.

1. With respect to the results and statistical analyses, I did not understand why data were averaged and then used in the PCA at level of each lysimeter. I think the cluster and PCA approach works well here to reduced noise and find patterns, but based on the temporal patterns and dynamic connections between variables, it would appear to me that using each event by lysimeter would better match the papers intent

REPLY: The aim of the PCA-clustering approach was to discriminate and group lysimeters based on the occurrence or absence of iron biodissolution in soil waters in order to investigate the temporal pattern of each cluster that would help to identify patterns compared to individual time series. For this reason, data we aggregated for each lysimeters. Otherwise, a given lysimeter would switch clusters and the temporal figure per cluster would make no sense. Although we agree that the data aggregation per lysimeter erases the temporal dimension, this is necessary for the clustering and the temporal aspect is described in the next step of the analysis. Please note that including all the dates lead to similar result compared to the "temporally-normalized PCA" used in the manuscript, although, obviously, more 'noisy' (Figure 1).

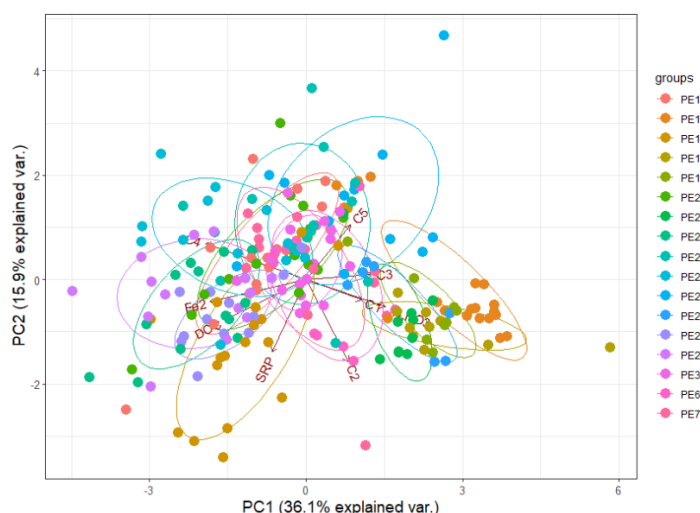


Figure 1 - PCA using all dates

The revised manuscript gives more details to justify the approach (section 2.5):

"A principal component analysis (PCA) coupled to a clustering analysis was used to discriminate and group lysimeters based on the occurrence or absence of iron biodissolution in soil waters in order to investigate the temporal pattern of each cluster that would help to identify patterns compared to individual time series. For this reason, data (DOC, NO₃⁻, SRP and Fe(II) concentrations and the relative contribution of PARAFAC components) were aggregated for each lysimeters and normalized."

1. Should flow path and spatial network be accounted for? Perhaps proximity of lysimeters to each other is important to explaining pattern?

REPLY: Lysimeters were aligned along three lines parallel to the stream channel. These lines, about 10-20 m from each other, were located at different distance from the stream with the aim to capture the heterogeneity of water flow paths and nitrates concentrations coming from the upland domain. Despite our sampling design, the distance between each lysimeter is not a variable that we could integrate in the PCA. We would need to set distance to an independent point (the nearest field? the river?) but we don't think this is would lead to an interesting pattern as no spatial pattern was visible: two neighbouring lysimeters could be more different than lysimeters on the opposite side of the transect.

Following this comment, more details were added in the Material and methods (section 2.2):

"Lysimeters were aligned along three lines parallel to the stream channel. These lines, about 10-20 m from each other, were located at different distance from the stream with the aim to capture the heterogeneity of water flow paths and nitrates concentrations coming from the upland domain. Lysimeters were all located in the hydromorphic soils unit (Figure 1)."

And in Results (section 3.3):

"Despite the fact that lysimeters were installed along three lines that were more or less closed to the stream channel, no spatial pattern was identified. Thus, two neighbouring lysimeters could be more different than lysimeters on the opposite side of the transect."

2. I agree with the use of PARAFAC components as the sole DOM identifier and not using indices or peaks. The peak shape for each component looks normal, but I was surprised that the PARAFAC model did not include a protein-like peak. This seems odd and I can't think of a study using PARAFAC that lacks some version of a protein-like peak even if contained within a multi-peak component. I am not certain what to make of this. Usually humic-like peaks dominant soils but protein-like peaks are still present and on an absolute basis can contain more protein-like materials than what would be found in a stream. Perhaps, the original uncorrected EEMs, blanks, and corrections could be revisited and verify that the model correctly represents their features. Perhaps including a few corrected-observed, modeled, and residual eem plots would be useful to highlight that protein-like peaks were not present. This way the reader can be reassured the model fit well the data. Assuming the absence of a protein-like component, I think this result needs to be discussed and clarified. Protein-like and less complex DOM forms are expected to be present in agricultural catchments and their absence would be interesting to explore more deeply in the discussion.

REPLY: We agree with C. Williams that protein-like components are commonly reported in PARAFAC models built in surface and soil waters. However, we can exclude any bias from measurement and/or from our modelling approach. First, a blank was systematically measured before samples to verify instrument calibration (e.g. Raman peak position) and noise. Second, several steps were rigorously controlled before attempting the final validation of the model including 1) the comparison of measured *versus* corrected EEM (corrections including blank subtraction, inner filter effect...as described in the Material and Methods section), 2) the randomness of residual EEMs, and 3) the correct aspect of PARAFAC components. No peaks could be visualized nor identified at low Ex/Em wavelengths all along the modelling process, and no solution proposed by PARAFAC included any protein-like component (tests were realized with a number of components ranging from 4 to 7).

That being said, we know from previous studies that soil DOM can include protein-like components. As suggested, we will discuss (in the section 4.2) the absence of these components in our model, which is related to our sampling design. Please see below a proposal of modification:

"The PARAFAC results could suggest that DOM mobilized from soil to streams is only composed by aromatic molecules of high molecular weight. Although complex organic molecules indeed dominate stream DOM export (Fellman et al., 2009), it should be noted however that protein-like components are commonly found in stream waters (Inamdar et al., 2012), including in our study site (Humbert et al., 2020). The lack of such components in our model results from our sampling approach and not from their absence in catchment soils.

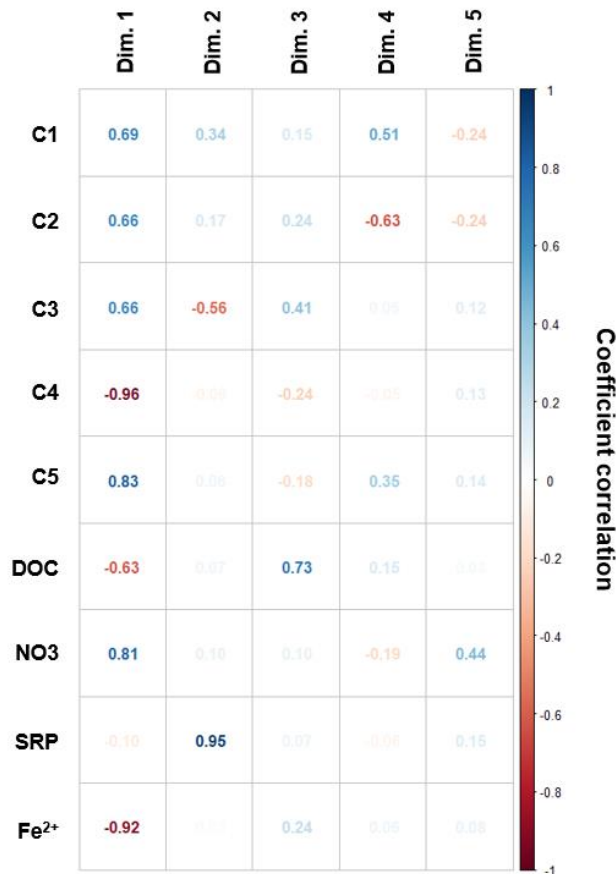
Indeed, the production of protein-like components in catchment soils is restricted to the summer hot and dry period during which a pool made of low-aromatic and microbially-derived compounds built up in riparian soils (Lambert et al., 2013). However, this DOM pool is quickly flushed and exhausted during the rewetting phase in October-November, and soil DOM during the winter period is mainly composed by highly-aromatic molecules originating from soil organic material (Lambert et al., 2014). Agricultural practices such as fertilizer applications can represent another source of protein-like DOM in the catchment (Humbert et al., 2020), but these inputs remain episodic with a low impact on DOM at the catchment scale (Humbert et al., 2015; Lambert et al., 2014). For instance, a recent one-year of monitoring of soil waters at different locations in the catchment has shown that protein-like components represent only $3.44 \pm 2.8\%$ of the total fluorescence signal in soils (Humbert et al., 2020), this contribution being particularly low in riparian areas during the winter. Therefore, the absence of protein-like components in our PARAFAC model is the consequence of our sampling design that focused on DOM production mechanisms in riparian soils (distant from agricultural inputs) during the winter period (period of production of highly aromatic compounds in soils)."

3. I think the connection between DOM quality and Fe-NO₃ interactions could be explored more fully. The PCA results are mostly explored from a Fe, DOC, and NO₃ point of view, but DOM seems to also be divided along the two clusters. I think more could be done to amplify the DOM split along PC1 within the two clusters. The discussion pulls in DOM quality as a possible mechanism and I think some of these links could be brought out more in the results. Perhaps variable influence scores for the PCA showed Fe, DOC, and NO₃ were most important and this is why DOM was only partly included as a clustering agent. If so, I think it would be useful to the reader to acknowledge these influences.

REPLY: We agree that the discussion in its current form is relatively poor regarding DOM dynamics. Following this comment, we investigated a little bit more our results to explore deeper the interactions between DOM quality and Fe-NO₃ interactions with a focus on PARAFAC component C4 that presented the most interesting and interpretable pattern. This lead to the following changes.

Results – section 3.4: “PARAFAC components had similar or even higher scores than DOC, Fe(II), and NO₃ concentrations on the two first dimensions of the PCA (Supplementary Fig. S4), illustrating the importance of DOM composition as an important factor contributing to explain the spatial variability across lysimeters. The distribution of PARAFAC components along the first dimension reflects the relationships between their relative contribution and Fe(II), concentrations. More specifically, %C4 was strongly and positively correlated with Fe(II) ($R^2 = 0.38$, Pearson $r = 0.62$) compared to other components that exhibited weakest and negative relationships with Fe(II) (R^2 from 0.09 to 0.19, Pearson r from -0.30 to -0.43).”

Discussion – section 4.1 : “The PARAFAC components identified in the model pointed to a dominance of highly aromatic and conjugated molecules, typical of DOM derived from soil organic matter and found in poorly drained soils in riparian or wetland areas (Sanderman et al., 2009; Lambert et al., 2013; Yamashita et al., 2010). The greatest proportion of C4 in the first cluster however indicates that the reduction of Fe oxyhydroxides leads to greater proportion of microbially-derived compounds within the DOM pool. In agreement with previous studies showing that the Fe(III) reduction could enhance the decomposition of organic matter in soils (Chen et al., 2020; Kappler et al., 2021), the close link between Fe(II) and C4 likely reflects an indirect effect of Fe biodissolution promoting the degradation of soil OM and the subsequent incorporation of microbially-derived compounds into the DOM pool (Dong et al., 2023). This hypothesis is well consistent with previous experimental studies performed on soils from the Kervidy-Naizin riparian area showing that the bacterial reduction of the Fe(III)-oxides to Fe(II) was concomitant with the release of large biological organic by-products upon the growth of bacterial communities (Lotfi-Kalahroodi et al., 2021).”



Supplementary Figure S4 – Matrix of coefficient correlation between variables and the dimensions of the PCA.

- The discussion did an excellent job connecting patterns and telling a story around the variables important to the release of DOC from streams into soils. I struggled a little seeing some of these patterns in the figures and I wasn't always certain how to interpret the pattern within PCA clusters. I think it would be helpful to include a little more detail in the results that explains specific patterns visualized in the figures. This then could be revisited in the more detail already present in the discussion. In addition, perhaps there might be an X vs Y type approach that could be used to amplify the patterns expressed in the timeseries plots.

REPLY: After considering this comment we recognize that our data do not fully support the statements we made in the discussion trying to link the stream DOC dynamics to what we observed in riparian soils. Although we know from previous studies that riparian soils where we installed our lysimeters are the dominant source of DOM at the catchment scale, we were limited in our ability to link stream DOC dynamics to the patterns identified in the clusters. Linking soil and stream DOC would have required a common and robust tracer, as we have done previously in this catchment using stable carbon isotopes (Lambert et al., 2013; Lambert et al., 2014). The manuscript will therefore be revised on the following points:

- Figure 7 will be modified, only stream DOC and discharge will be plotted (see below).
- The sentences relating the dynamics of stream DOC during peak flow to the specific pattern observed in the different clusters will be removed.
- The limitations of our study, namely the ability to quantify of the relative contribution of each cluster to stream DOC export, will be added in the last paragraph of the discussion.

Please find below a proposition of modification.

“The current understanding of DOM export in lowland headwater catchments is based on a two-steps conceptual model according to which the pool of DOM is built in soils during a low hydrological connectivity period and then flushed toward surface waters during the following high flow period (e.g. Tiwari et al., 2022; Ruckhaus et al., 2023; Strohmenger et al., 2020;

Raymond and Saiers, 2010). However, the high-frequency measurements of DOC in the stream do not fully support this statement. The establishment of a hydrological connection between riparian soils and the stream during the winter period showed the stream DOC to gradually decrease both at peak discharge during successive storm events and at base flow during inter-storm periods (Figure 7). This pattern, which repeats every year in this catchment (Strohmenger et al., 2020), is well consistent with the hypothesis of the mobilisation and exhaustion of an initial supply-limited pool of DOM built during the summer period (Humbert et al., 2015). However, stream DOC were found to increase slightly in March/April after the low-flow period that showed the hydrological connection between soils and the stream to decrease. It is unlikely that the mobilisation of an additional pool of DOM from upland soils may explain this small raises in stream DOC because this pool is 1) relatively small in terms of size, and 2) quickly exhausted at the beginning of the winter period (Lambert et al., 2014). Therefore, the seasonal pattern of stream DOC likely reflects the regeneration of the riparian DOM pool during the winter period as shown by our data collected in soil waters of valley bottoms.

Stable carbon isotopes have indeed demonstrated that riparian soils of the Mercy wetland – and more particularly the DOM-rich uppermost soil horizons – are the dominant source of stream DOC at the catchment scale (Lambert et al., 2014), a feature commonly shared by lowland catchments (e.g. Sanderman et al., 2009). Thus, the decline in DOC and SRP observed in soil waters, particularly in the second cluster whereby these elements became almost depleted (Fig. 6), was consistent with the general flushing behaviour of the catchment shown by stream DOC from November to February. Similarly, the large two to three fold increase in DOC concentrations in riparian soils (in cluster 1 and 2, respectively) denotes a large mobilisation of DOM between March and May despite wet and low temperature conditions, that could explain in turn the pattern observed in stream DOC at the same time.”

And in the last paragraph of the discussion:

“Taking together, our results have two important implications regarding our conceptualisation of DOM export in headwater catchments. First, it challenges the idea that the wet period acts mainly as a passive exportation period for DOC, with no or little DOC production (Strohmenger et al., 2020; Ruckhaus et al., 2023; Wen et al., 2020). Secondly, it emphasis that stream DOC dynamics at the outlet is an integrative signal, potentially masking a high heterogeneity of the system owing to complex interactions between biogeochemical cycles in soils, nutrient transfer at the soil/stream interface and hydrological functioning of catchments. While the patterns of stream DOC were consistent with what we observed in soils, our study remains however limited in its capacity to quantify the relative contribution of the cluster identified to stream DOC export. Additionally, we do not have the necessary data such as isotopes or molecular markers to elucidate the precise origin and DOM (and SRP) release in soils unrelated to iron biodissolution, and this should be the focus of future work combining experimental and field studies”

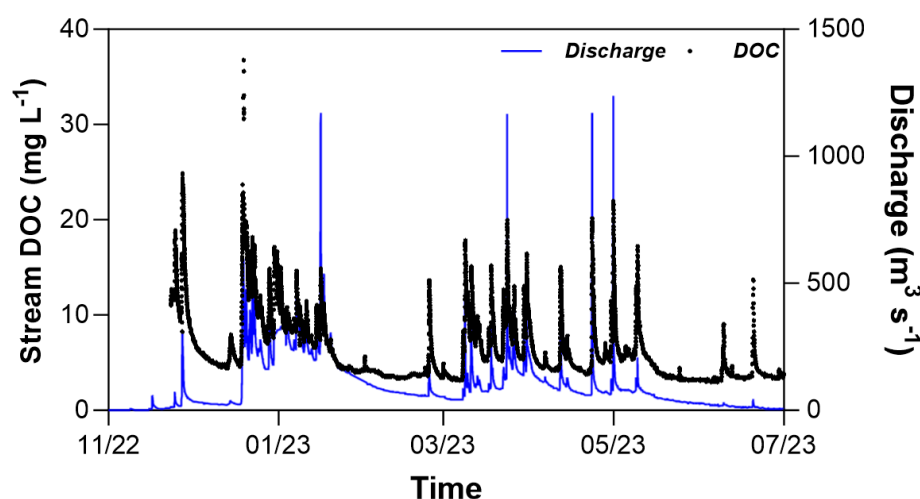


Figure 7 – Variation of DOC concentrations (black circles) in the stream based on high-frequency measurements.

Specific Comments

Introduction

The introduction framed the study and the key background ideas needed to understand the results really well.

REPLY: Thank you.

Methods

Figure 1: It seems like it would be useful to have lysimeter locations also included on the map. One of my questions was if there are spatial-correlations hidden within the clusters or across clusters that might be able to be explained by looking at location as a variable. Adding the lysimeter locations to the map, would at least help the reader see if spatial patterns seem relevant or not. The paper said some lysimeters of opposite clusters were within 1 m of each other. So their might not be a spatial pattern, but it might be useful to note this or acknowledge more fully the location of each lysimeter.

REPLY: As we mentioned in the first main comment, there was no spatial pattern. Please see our answer for more details.

PARAFAC modeling data: It is true that PARAFAC is not influenced by Fe but the optical conditions of EEMs are impacted by iron levels. It might be useful to provide the range of estimated carbon and Fe(II) levels in the diluted sample. This way they could be used to assure the reader that Fe levels are below interference levels.

REPLY: The degree of quenching due to the presence of iron varies greatly between samples depending on the iron:DOC ratio but also on DOM composition (Jia et al., 2021; Poulin et al., 2014). In our study, the Fe(II):DOC ratio was 0.30 ± 0.24 , which was in the upper range of the study by Poulin et al. (2014) but very low compared to the study by Jia et al. (2021) that investigated the effect of Fe(II) on fluorescence properties of DOM from an anaerobic aquifer containing up to 300 mg L^{-1} Fe(II), with Fe(II):DOC ratio up to 7. It should be noted however that Fe(III) also impacts DOM fluorescence (Ohno et al., 2008), limiting our ability to quantify the effect of iron on quenching.

That being said, quenching was clearly apparent in some samples ($n < 10$) that showed the fluorescence intensity to increase with dilution factor, reflecting the influence of high level of Fe that reduces DOM fluorescence (Poulin et al., 2014). The quenching was mainly affecting EEMs at low (< 270) and moderate to high (420 - 490) excitation and emission wavelengths, respectively, which is consistent with previous studies concluding that Fe mainly impacts fluorescence intensity in EEM locations associated with humic-like fluorophores, namely A and C peaks (Jia et al., 2021; Poulin et al., 2014). Thus, although we cannot rule out an effect of iron on EEMs, this would have impacted humic-like fluorophores associated with C1 and C2 components of our model. Considering that these components behaved similarly between clusters and across the season (Figure 4), we argue that Fe would have a limited impact on the conclusion of our study regarding DOM dynamics.

These details will be added in the Material and Methods section.

With respect to absorbance scans, would it be possible to clarify if scans were made on filtered or filtered and diluted soil-water samples? High levels of Fe and NO_3 have been suggested to impact absorbance scans towards the UV end of the spectrum.

REPLY: Absorbance scans were made on filtered and diluted samples. The only purpose of these measurements were to correct EEMs for inner filter effect, we did not use them for the calculation of indices for DOM composition (e.g. SUVA, slope ratio,...) due to the effect of iron and nitrates as mentioned by C. Williams. Note that the inner filter effect corrections were small, as absorbance spectra were furthermore divided by the dilution factor applied for FDOM measurements.

These details will be added in the Material and Methods section.

Line180-181: Data average then normalized by lysimeter. Does this mean temporal resolution was lost? Please explain the reasoning for this. This collapses the data to $n = 17$ with 9 variables in multivariate analysis, which could impact how clusters and relationships form. Why not run a PCA on all data and then determine the centroid and error for each lysimeter and build clusters from the full dataset? By averaging, the link between variables within the analysis seems to be broken on a case-by-case basis. It seems in order to say at each event that Fe, DOC, and NO₃ changed together, then the PCA should be able to form components with these connections available on a per event basis rather than across sampling location average for the year.

REPLY: Please see our answer to the first comment.

Figure 4 suggests correlations used but these analyses and purpose are not reported in methods. Could this approach be added to the methods and correlation coefficients be added to results?

REPLY: We agree with these suggestions. Details will be added in the Material and methods section and correlation coefficients added to the figure.

Results

When exploring the PCA clusters, I think it would be useful to include more emphasis on DOM patterns or provide the variable influence scores that demonstrate that DOM is not a key influence on the clusters

REPLY: In fact DOM has a large impact on the PCA. Please see our answer to the first comment.

I am not certain I understand Figure 7 or logic in Figure 6. The discussion suggests that the pattern highlights the role of heterogeneity in soils. Perhaps this idea could be linked here so that it's clear that the patterns don't match. Adding discharge or rainfall data to this figure might also make it easier for the reader to see the argument in the discussion around passive and active transport.

REPLY: This part of the manuscript will be revised. Please see our corresponding answer.

Discussion:

L369-372: This is a really interesting idea that very local active processes within a watershed can keep the collective view in a stream relatively constant even though the soil-water patterns are more dynamic. I wonder if there might be a way to tease this idea further out into the results so that it is clear how this idea connects to the broad level patterns in Figure 7 and the very messy lysimeter by lysimeter patterns. I wonder further how much DOC releases from a set of cluster 1 vs cluster 2 like soils is needed to maintain the DOC stream pattern once discharge (passive transport) is accounted for? Would it be possible to roughly estimate load from each cluster as a mixing model with two end members?

REPLY: C. Williams raises a very interesting point that is the possibility to quantify the relative contribution of the two clusters to stream DOC export. However, we regret that we cannot address this comment with the current dataset. In previous studies we used stable carbon isotopes ($\delta^{13}\text{C}$) to quantify the relative contribution of riparian soils to stream DOC export (Lambert et al., 2014) thanks to natural vertical and lateral gradients in $\delta^{13}\text{C}$ values. However, it is likely that DOM from cluster 1 and 2 have similar isotopic signatures, considering that variations in $\delta^{13}\text{C}$ are relatively similar in the upper most soil horizons in the riparian area. Therefore, we would have difficulties in isolating, based on an end-member approach, the two clusters. A modelling approach would be more relevant.

Technical Corrections

REPLY: We thank C. Williams for noting these technical corrections, we will amend the manuscript as suggested.

Two periods line 105

(XXX) line 137 after probe name

Line 235: “followed” should be “follow”

Line 244 % with space before number

L316: biodissolution repeated twice

Figure 1 caption, please also explain what PK3 represents.

References

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