Towards a better identification of dissolved organic matter from diffuse sources: comparative study between watercourse and soil leachate to search for specific markers

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Towards a better identification of dissolved organic matter diffuse sources: comparative study between watercourse and soil leachate to search for specific markers

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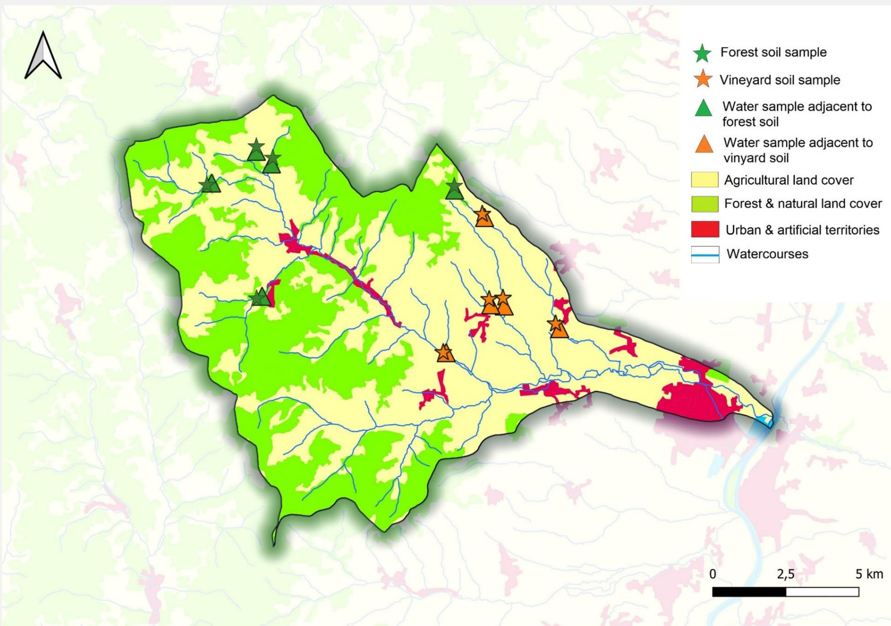
Introduction

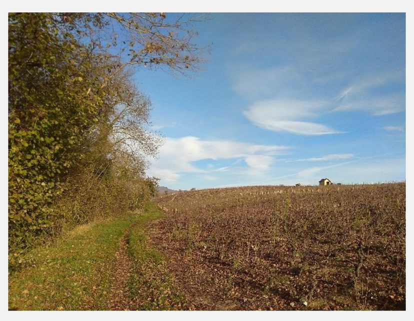
At a watershed scale, Dissolved Organic Matter (DOM) inputs to watercourses can originate from a wide range of natural and anthropogenic point or diffuse sources.
Diffuse sources related to land use and occupation influence nature and composition of organic matter and therefore its impact on the environment. These sources are difficult to identify as they are spread over time and space, making the sampling of a representative sample complicated.
The aim of the study was to better define the sampling methodology for terrestrial diffuse sources, through the identification of specific markers related to a land occupation and uses. The study was conducted in two steps. First, water extracted DOM (WEDOM) from soils and its degradation were studied to search for specific conservative markers. The second part consisted to compare WEDOM markers with the aquatic DOM collected from the adjacent watercourse. This allowed to validate diffuse sources sampling directly into the stream.

Material and methods

1- Sampling collection and preparation

The Ardières-Morcille watershed is located in the Beaujolais region (Rhône department). The watershed is characterized by vine growing activities which represent more than 70% of the total area.





Samples collected :
5 soils from forest land cover with 5 adjacent watercourses
5 soils from vinegrowing land use with **5** adjacent watercourses

2- DOM extraction and degradation

Soil samples

WEDOM extraction protocol

- CaCl2 10 mM
- Soil/water ratio 1:10 (w:v)
- Agitation 6 h

WEDOM

- Centrifugation: 4500g
- Filtration: 0.45 µm
- First sampling Day 0

Degradation study: after Day 0, soil inoculum and nutrient solution were added to WEDOM samples

Sample day 3	Sample day 7	Sample day 14	Sample day 28

3- Analytical strategy

Both aquatic and water extractible DOM were characterized by a set of analytical techniques

Global composition
- Dissolved organic carbon DOC
- Proteins

Optical properties
- UV-Visible
- 3D Fluorescence
- Size exclusion chromatography HPSEC

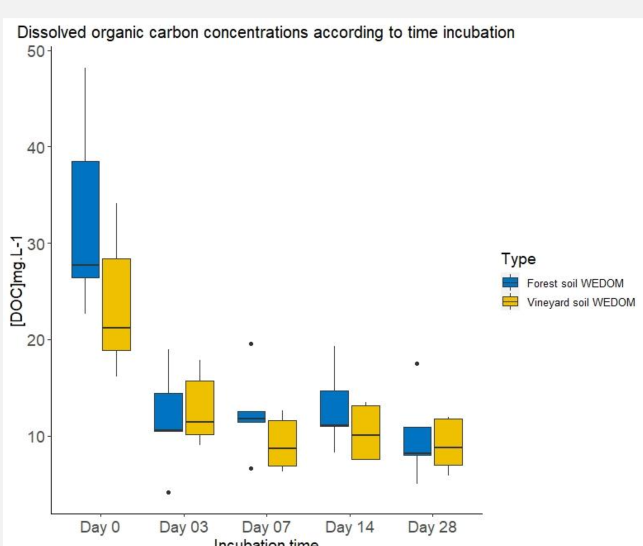
Molecular composition

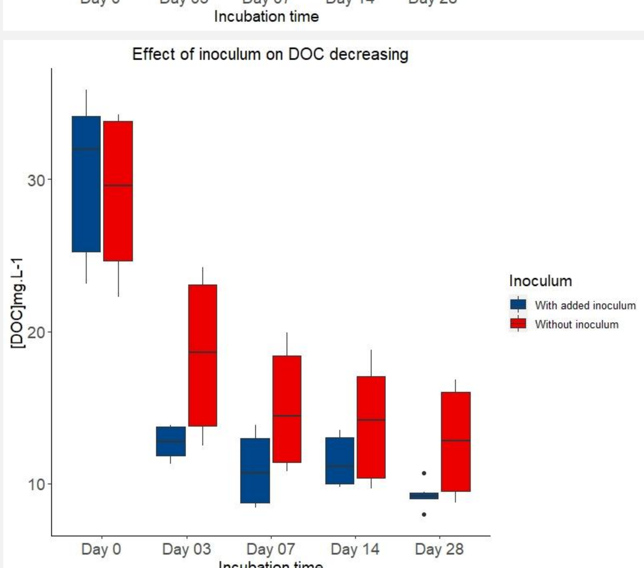
- Liquid chromatography coupled with high-resolution mass spectrometry LC-HRMS

Results and discussion

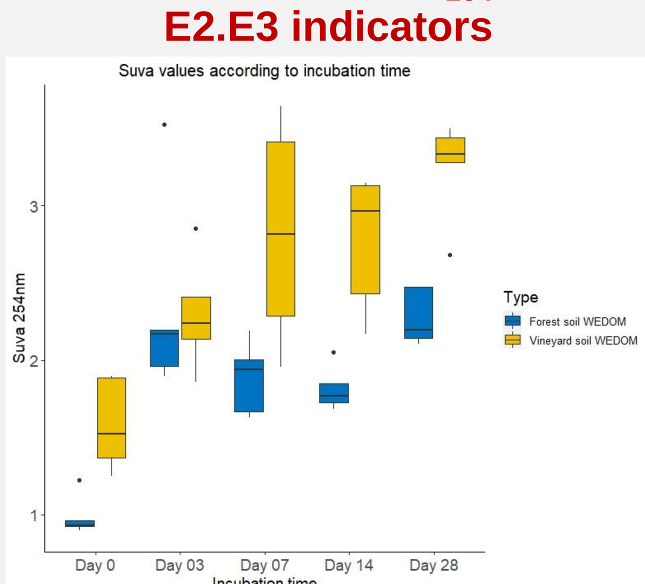
1-Soil WEDOM conservative and refractory signature

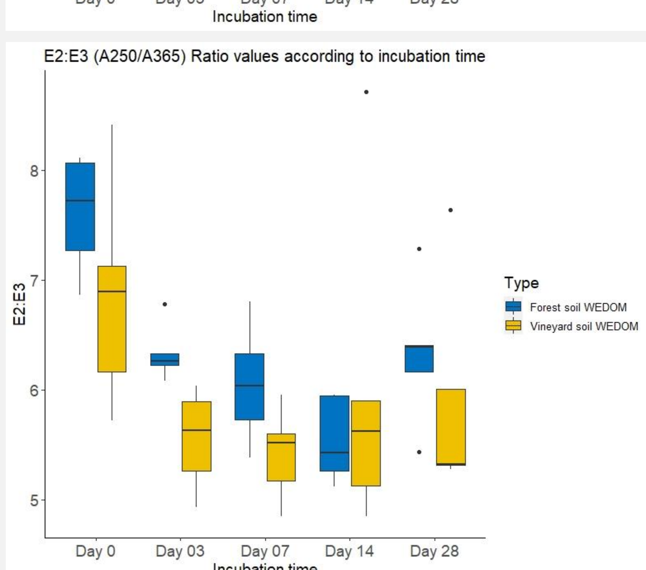
1-DOC concentrations and inoculum effect



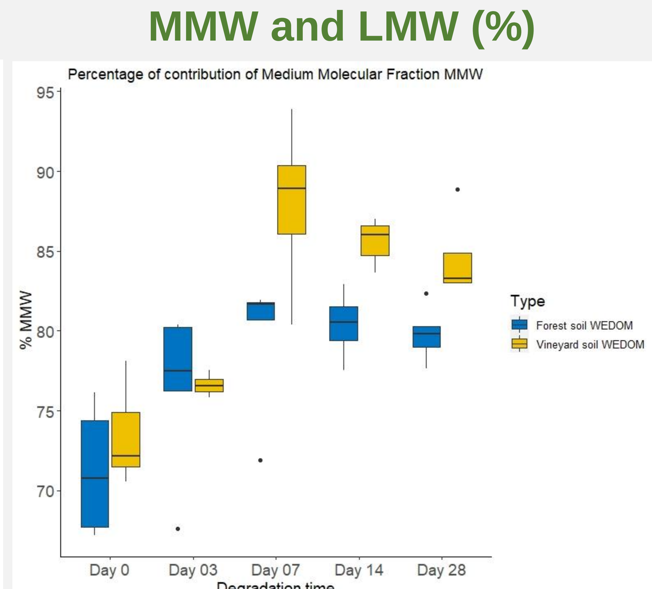


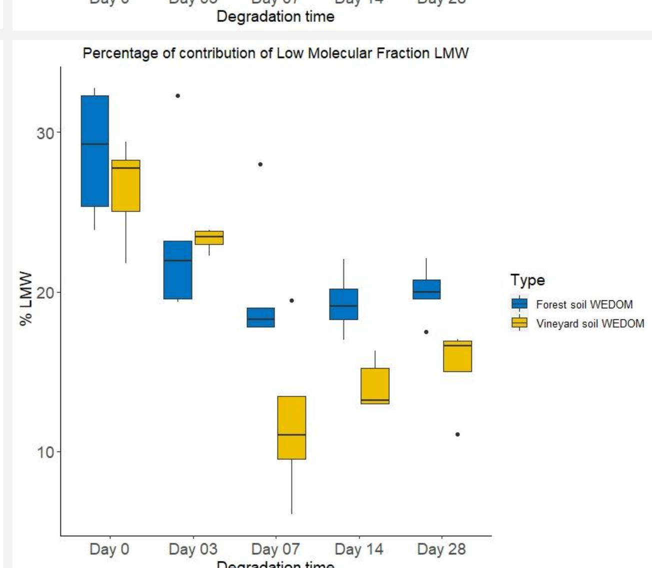
2- UV-Visible $Suva_{254}$ and E2.E3 indicators





3- HPSEC contribution of MMW and LMW (%)





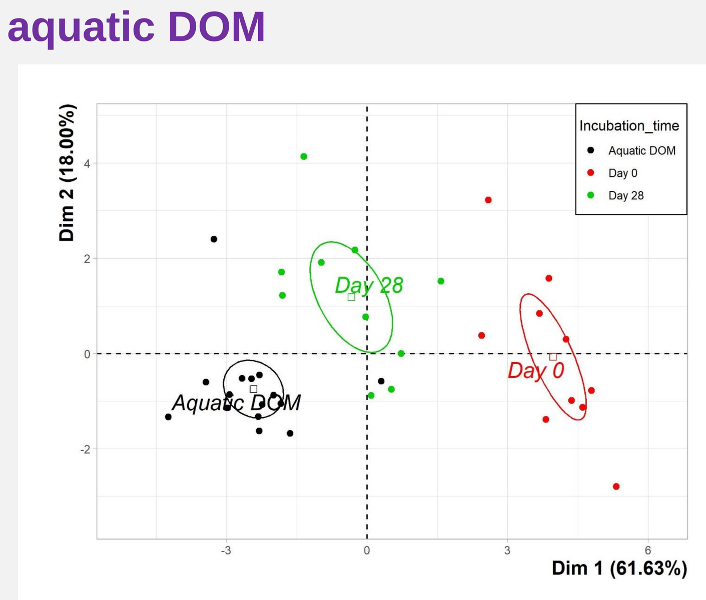
- DOC decreasing with time incubation indicates that the degradation of DOC occurs mainly within the first hours of incubation. Indeed, it was observed in similar studies, that labile fractions of DOC were rapidly degraded and the remaining fractions correspond to the refractory ones. These results explain the DOC stability observerd after 3 days (*Guigue et al., 2014 ; Mangal et al., 2020*).
- When comparing the conditions with and without the addition of inoculum, it is observed that the addition of inoculum accelerates the decreasing of carbon.

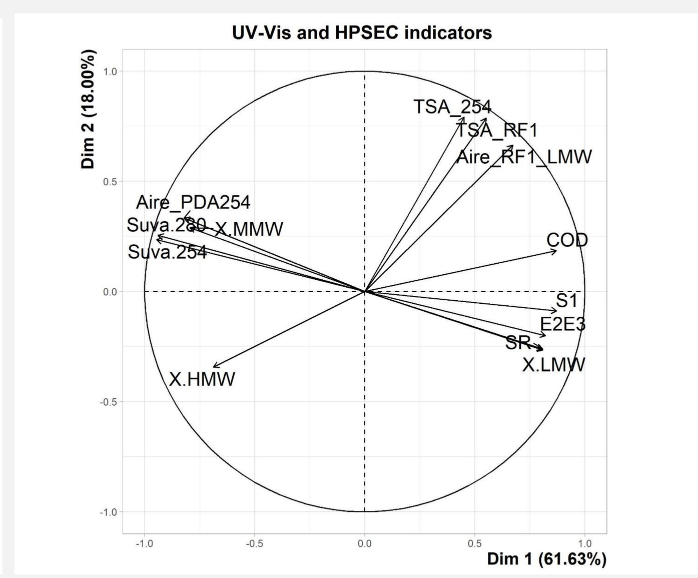
- $SUVA_{254}$ is an indicator of the aromaticity content of the MOD (Croue et al., 2003) and the absorption ratio E2:E3 (A250/A365) is inversely correlated to the size of organic molecules and macromolecules (Santos et al., 2016).
- The increase of the $SUVA_{254}$ with time incubation indicates an aromaticity increase of the WEDOM. The variability of the ratio E2:E3 shows a size increase of the molecules and macromolecules.
- These results are consistent with the decreasing of DOC. Indeed, the degradation of labile fractions exacerbates the aromatic refractory fractions of WEDOM.

- The percentage contribution of MMW (1K-10K Da) and LMW (< 1K Da) fractions are calculated from the Gaussian decomposition of HPSEC chromatograms.
- While the contribution of low molecular weight fraction LMW decreases, the medium molecular weight fraction MMW increases. Considering all these results, it seems that low molecular weight fractions are the most labile and easily degradable.

2- Soil WEDOM and aquatic DOM comparison

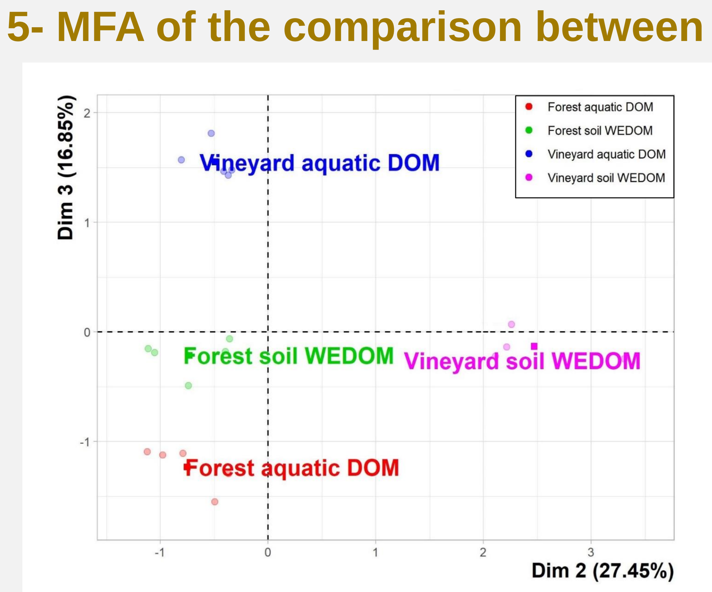
4- PCA of the comparison between soil WEDOM at day 0 and day 28 with aquatic DOM

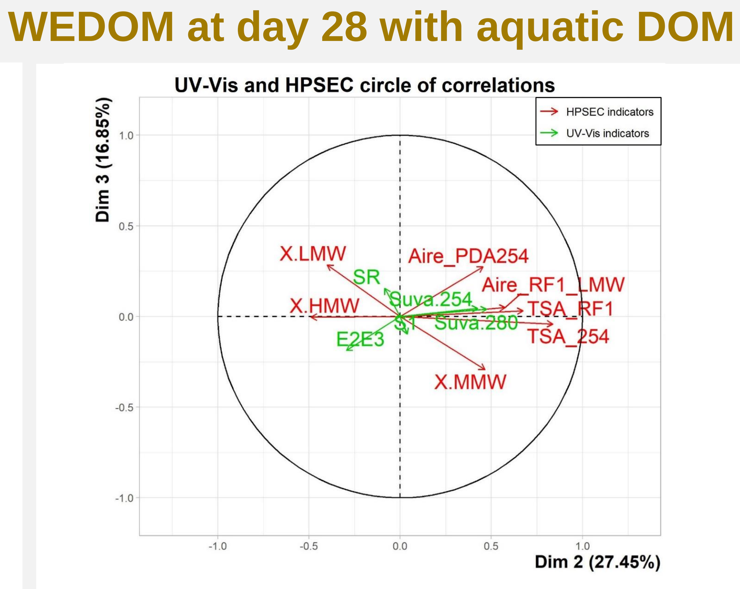




- The distribution of points, explained by 89.63 of the total inertia percentage, shows a signature of the aquatic DOM closer to the degraded soil WEDOM after 28 days of incubation.
- The graph of variables shows a coherence in the distribution of UV-Vis and HPSEC indicators. For example, the $SUVA_{254}$ is inversely correlated to the % contribution of the LMW fraction and the SR (ratio of spectral slopes, inversely correlated to the molecular weight of DOM)

5- MFA of the comparison between WEDOM at day 28 with aquatic DOM





- Multiple factor analysis (MFA) of soil WEDOM samples at day 28 and aquatic DOM, explained by 44.3% of the total percentage inertia, shows a forest soil WEDOM signature close to that of the adjacent aquatic DOM. However, the signature of the vineyard aquatic DOM appears different from the vineyard soil WEDOM.
- The difference observed for vineyard soils could be explained by the accumulation of inputs at vineyard water points regarding the downstream positioning of these points in the watershed.

Conclusion and perspectives

- This study aimed to identify DOM specific markers to soil occupations in order to better define sampling strategies for diffuse sources.
- The study of WEDOM revealed the presence of specific signatures for forest and vineyard soil occupations. The signatures evolve in the first days with a degradation of the labile and low molecular weight fractions. This degradation induces an increase of WEDOM aromaticity and molecules size, leading to a signature that tends towards the aquatic DOM in the adjacent watercourses.
- The comparison of WEDOM at day 28 and aquatic DOM shows a similarity of signature for forest soils only. The difference observed in vineyard waters could be explained by the multiplicity of inputs downstream in the watershed that would distort the quality of the sampled DOM.

❖The specific markers of diffuse sources will be reinforced with the molecular results brought by LC-HRMS.