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1 **Title. Sampling terrigenous diffuse sources in watercourse: influence of land use and**
2 **hydrological conditions on dissolved organic matter characteristics.**

3 Amine Boukra¹, Matthieu Masson¹, Corinne Brosse¹, Mahaut Sourzac², Edith Parlanti², Cécile
4 Miège¹

5 ¹ INRAE, RiverLy, F-69625, Villeurbanne, France

6 ²Univ. Bordeaux, CNRS, Bordeaux INP, EPOC, UMR 5805, F-33600 Pessac, France

7 **Correspondence to:** matthieu.masson@inrae.fr ; +33472208706; RiverLy, INRAE, 5 rue de
8 la Doua, Villeurbanne, France, 69625

9

10 **Highlights:**

11

- 12 • This study addresses the sampling of non-point sources of DOM in watercourses
- 13 • A combination of land use markers discriminated DOM terrigenous signature in streams
- 14 • Hydrological conditions impacted the signature of watercourse DOM
- 15 • New indicators from HPSEC/UV-fluorescence improved the study of DOM
- 16 composition

17 **Abstract**

18 Diffuse and point sources of dissolved organic matter (DOM) in streams influence its
19 composition, interactions and fate in the aquatic ecosystem. These inputs can be very numerous
20 at the scale of a watershed, and their identification remains a challenge especially in the case of
21 diffuse sources related to land use. The complexity of the transfer mechanisms and the reactivity
22 of DOM throughout the soil-water column continuum raise questions about the sampling of
23 diffuse sources in watercourses. To answer this question, we compared the characteristics of
24 soil-extracted DOM influenced by a particular land use (homogenous sub-catchment of forest
25 and vineyard) and DOM collected from the watercourse adjacent to the soil samples. A 28-day
26 incubation experiment of soil extracts was designed to remove the labile fraction of DOM.
27 During the first 3 days, between 40 and 70 % of the DOC mass was lost for both types of soils.
28 A set of optical indicators (UV-Visible, EEM fluorescence and HPSEC/UV-fluorescence)
29 showed that the labile fraction was mostly composed by low (<1 kDa) and high (>10 kDa)
30 protein-like molecules. At the end of the incubation, soil-extracted DOM was mainly composed
31 of medium molecules (1-10 kDa) associated to terrigenous humic-like compounds. Its optical
32 and size molecular signature tended towards that in the adjacent watercourses and was specific
33 to land use. However, the characteristics of DOM in watercourses was also influenced by the
34 hydrological conditions, probably due to a transfer of top soil DOM during high water periods
35 and both deep soil and autochthonous DOM during low water periods. These results were
36 obtained by a set of indicators including novel ones derived from high-pressure size exclusion
37 chromatography (HPSEC/UV-fluorescence). Finally, this study demonstrated that it is possible
38 to sample the DOM representative of a land use directly in the river downstream of the
39 homogeneous sub-basin by multiplying the samples during contrasting hydrological conditions.

40 **Keywords:** Dissolved organic matter; non-point sources; land-use; optical fingerprint

41 1 Introduction

42 Dissolved organic matter (DOM) is a complex set of organic compounds operationally defined
43 as material obtained after water filtration (commonly ranging from 0.2 to 0.7 μm) (Zsolnay,
44 2003; Zark and Dittmar, 2018) with an average molecular size distribution that ranges from
45 <100 Da to >100000 Da (Nebbioso and Piccolo, 2013; Hawkes et al., 2019). DOM plays a
46 major role in environmental biogeochemistry, particularly as the main driver of terrestrial
47 carbon to the aquatic environment, as a substrate for the growth of microorganisms (Bolan et
48 al., 2011; Mladenov et al., 2022), but also in the fate and bioavailability of pollutants (Reuter
49 and Perdue, 1977; Aiken, 2014). At the scale of a watershed, DOM inputs to the rivers can
50 originate from a wide range of natural and anthropogenic sources (Nebbioso and Piccolo, 2013).
51 These sources can be either point sources (e.g. wastewater treatment plants and storm
52 overflows) or diffuse discharges (e.g. terrigenous inputs related to land use, underground inputs,
53 autochthonous production; Carstea et al., 2020; Williams et al., 2016). Point discharges of DOM
54 can be easily located and their sampling is often representative of these sources. For example,
55 DOM from wastewater treatment plant discharges, constitutes a mixture of organic molecules
56 including microbial degradation products, surfactants, pharmaceutical products, phytosanitary
57 products (herbicides, fungicides, insecticides) and disinfection by-products (Michael-Kordatou
58 et al., 2015; Verkh et al., 2018).

59 Diffuse sources of DOM are more difficult to locate and study due to the complexity of
60 geochemical interactions, degradation processes and the uncontrolled meteorological events at
61 the origin of these DOM sources. In the case of terrigenous contributions of diffuse DOM
62 sources, studies have shown that the soil type governs the amount of DOM transferred to the
63 aquatic downstream environment (Bolan et al., 2011). Organic type soils discharge more DOM
64 than mineral type soils. Land use as well as soil biomass influence the composition of DOM
65 transferred to the watercourse (Kalbitz et al., 2003). This could be explained by the fact that

66 DOM is considered to be the most active and labile part of soil organic matter (Guigue et al.,
67 2014). Low molecular weight labile compounds make up the organic matter in the surface layers
68 of the soil. Their proportion decreases in depth soils giving way to higher fraction of refractory
69 compounds of high molecular weight. In addition, the hydrology of the watershed, or more
70 specifically, the direction of water flow along the soil, determines the type of organic matter
71 transferred to rivers. Indeed, Sanderman et al., 2009 demonstrated that vertical flow would
72 favor the transfer of the more refractory denatured organic matter, whereas lateral flow would
73 transfer labile organic matter characteristic of the surface soil layers. However, establishing
74 DOM fingerprints in rivers related to land uses and occupations, driven hydrology, is a complex
75 task. The high reactivity of DOM labile fraction coupled with environmental conditions and the
76 nature of the soils make difficult the establishment of reliable DOM fingerprints. Here we
77 define a DOM fingerprint as a set of physico-chemical markers calculated from one or several
78 analytical techniques (e.g. optical characteristics, molecular size distribution), which are
79 relevant to discriminate a given source. At present, classically used techniques such as UV-
80 Visible spectroscopy and excitation emission matrix (EEM) fluorescence have brought major
81 advances in the study of chromophoric and fluorescent DOM. The application of these
82 spectroscopic techniques has allowed, for example, to differentiate between types of DOM
83 depending on the sources and the state of evolution (Li and Hur, 2017). Another analytical
84 technique that showed relevance to characterize DOM composition, is the size exclusion
85 chromatography (HPSEC). The principle of HPSEC is based on the separation of molecules
86 according to their size or hydrodynamic volume. Previous studies have shown that HPSEC can
87 be used to determine the distribution of molecular weight for DOM by calculating indicators
88 used to characterize polymers such as average molecular weight (M_w), number average
89 molecular weight (M_n) and polydispersity (p) (Huang et al., 2021). Detection by diode arrays
90 and fluorescence (HPSEC/UV-fluorescence) allowed to specifically target absorbing and

91 fluorescent compounds in organic matter, providing further qualitative information on the size
92 continuum of DOM molecules (Asmala et al., 2021; Brezinski and Gorczyca, 2019). However,
93 the combined information of molecule size and type, provided by HPSEC/UV-fluorescence,
94 could be further explored to propose relevant indicators improving DOM fingerprints. In this
95 context, the aim of this paper was to determine the representativeness of sampling a terrigenous
96 diffuse source of DOM in watercourses. More precisely, we studied the link between soil-
97 extracted DOM, characterized by a particular land use, and DOM collected in the soil-adjacent
98 watercourse. A second objective was to improve the study of DOM optical fingerprints by
99 introducing new indicators from size exclusion chromatography HPSEC/UV-fluorescence.

100 To achieve this, the characteristics of i) DOM extracted from soils defined by distinct land use
101 (forest and vineyard), and ii) DOM from adjacent watercourses collected during high and low
102 water periods were compared. . The starting hypothesis is that during the high water period,
103 there would be a greater contribution of the terrigenous source. This comparison was performed
104 by characterizing DOM properties using a combination of optical analysis techniques including
105 UV-Visible spectrophotometry, EEM fluorescence spectroscopy and HPSEC/UV-
106 fluorescence.

107

108 **2 Materials and methods**

109 **2.1 Site description**

110 All sampling areas were located in Ardière-Morcille watershed (152 km²) in eastern France.
111 The catchment area, in its downstream part, is characterized by vineyard activities representing
112 more than 32 % of total landscape (Peyrard et al., 2016). Other land uses such as forests (37 %)
113 and pastures (21 %), are also present in the upstream watershed (Figure 1).

114 The region's climate is semi-continental with a mean annual rainfall of 940 mm and episodic
115 snowfalls during winter (Gouy et al., 2021). The soils are mostly sandy and erodible and the
116 geological characteristics are an altered hercynian crystalline basement of mostly porphyric
117 biotite granite. The watershed is characterized by very steep slopes, ranging between 5 and 30-
118 35 % favoring surface runoff. In addition, numerous and fast hydrological events make the
119 riverbed, rarely dry (Rabiet et al., 2015).

120 **2.2 Sampling design and samples preparation before experimentations in laboratory**

121 Water samples were collected manually from rivers, downstream of 10 homogenous sub-
122 catchment areas characterized by 5 forest land occupations and 5 vineyard land uses (Figure 1).
123 Sub-catchment land uses were determined based on Corine Land Cover 2018 using Qgis 3.14
124 software. Sampling was carried out during two campaigns: A first sampling campaign in
125 January 2021 during a period of high water (3.65 and 0.2 m³.s⁻¹ respectively for Ardières and
126 Morcille Rivers) and a second sampling campaign in July 2021 during the period of low water
127 (0.65 and 0.03 m³.s⁻¹ respectively for Ardières and Morcille Rivers). The flow data was obtained
128 at Villié-Morgon and Beaujeau stations located downstream of the Ardières and Morcille rivers.
129 The water samples were collected in 250 mL borosilicate glass bottles previously cleaned by
130 an automatic washer at 90°C, then calcined in an oven at 500°C. The samples were filtered in
131 the field using polypropylene syringe and polyvinylidene fluoride (PVDF) filters with a

132 porosity of 0.45 μm . The filtrate was first used to rinse the 250 mL borosilicate glass bottle
133 three times before filling them completely. The samples were then stored in a cooler (at $\pm 4^\circ\text{C}$)
134 protected from light during their transport to the laboratory, then stored at 4°C in the dark until
135 analysis. Experimental blanks using ultrapure water (resistivity at 18.2 $\text{M}\Omega\cdot\text{cm}$) were prepared
136 in laboratory using the same protocol as in the field, in order to identify possible contamination
137 due to bottles, syringes or filters.

138 Soils were sampled in the forest and vineyard sub-catchment areas upstream of water samples
139 (radius of 30 to 50 m) during the high water sampling campaign. Detailed geographic
140 coordinates are available in supplementary material 1. In each site, 20 cm of top soils were
141 taken with an auger; four subsamples were randomly selected to collect approximately 1 kg of
142 soil. Each subsample was placed in a polypropylene bag. Once all 4 subsamples were collected,
143 the bulk sample was mixed in the bag. The 10 soil samples were stored at 4°C protected from
144 light until preparation in the laboratory where the soils were sieved with a 2 mm stainless steel
145 sieve, placed in aluminum trays (40x60 cm) and dried for 96 h at 40°C in an oven.

146 To summarize, for each sub-catchment area, one water sample was collected during high water
147 period as well as one soil sample (composite of 4 randomly collected subsamples). During low
148 water periods, an additional water sample was collected.

149 **2.3 Dissolved organic matter extracted from soils (soil extracts)**

150 Dissolved organic matter was extracted from soil samples by stirring 20 g (dry weight) of soil
151 in 200 mL of ultrapure water with 10 mM CaCl_2 (Supplementary Material 2). Stirring was
152 performed at 180 rotations per minute in a Lovibond thermoregulator cabinet at 20°C in dark
153 condition. After 6 h of agitation, the samples were centrifuged at 4500 g during 15 min and the
154 supernatant was filtered through 0.45 μm (PVDF filter). An initial 60 mL sample was collected
155 before the incubation experiment.

156 **2.4 Incubation experiment**

157 Two inoculums of forest and vineyard soils were prepared 48 h before the incubation
158 experiment. To obtain better microbial diversity, both forest and vineyard inoculums were
159 prepared from, respectively, a mixture of the 5 forest soils and the 5 vineyard soils (Kalbitz et
160 al., 2003; Guigue et al., 2014). For each inoculum, 5 g of the 5 corresponding soil samples were
161 added to 200 mL of ultrapure water. These preparations were then shaken at 20° for 48h in the
162 dark. A nutrient solution of $(\text{NH}_4)_2\text{SO}_4$ and KH_2PO_4 (C:N:P:S:K molar ratio of <5:1:1:1:1) was
163 also prepared 1h prior to the incubation. The addition of nutrients allows optimizing the activity
164 of the microorganisms during the following incubation.

165 Incubation of DOM extracted from soils was performed by adding 35 μL of inoculum and 1 mL
166 of nutrient solution to the samples immediately after the soil DOM extraction step (Guigue et
167 al., 2014) (Supplementary Material 2). Controls without inoculum nor nutrient solution
168 (inoculum blanks) and controls without soil extracted DOM (extraction blanks) were
169 performed. All samples and controls were incubated at 20°C in the dark, with continuous
170 shaking at 180 rotations per minute during 28 days. The incubation period of 28 days was
171 chosen based on previous work that studied the time required to degrade the labile fraction of
172 DOM from soil samples (Guigue et al., 2014). Sub-samples at 3, 7, and 14 days of incubation
173 were collected to monitor changes in DOM quantity and quality (optical properties).

174 **2.5 Analytical strategy**

175 **2.5.1 Dissolved organic Carbon**

176 Dissolved organic carbon (DOC) analyses were performed by high temperature catalytic
177 combustion according to the standard NF EN 1484 and using an Analytik Jena multi N/C®
178 3100 TOC analyzer. The calibration, rinsing, quality controls solutions, as well as samples,
179 were introduced in precombusted (at 500°C during 2 h) glass vials, where they were acidified

180 to pH=1 with hydrochloric acid (HCl 37% RPE). The detection limit was 0.2 mgC.L⁻¹ and the
181 analytical uncertainty was 12%. The accuracy of the analyses (105%) was routinely checked
182 using a certified standard of potassium hydrogen phthalate (5 mg.L⁻¹, Fluka®) different from
183 that used for the calibration (0.2–25 mgL⁻¹, Sigma-Aldrich®).

184 **2.5.2 UV-Visible spectroscopic analysis**

185 UV-Visible spectroscopy analyses were performed using a Shimadzu UV 1900
186 spectrophotometer with a 1 cm path length quartz cell (Hellma Analytics®). Absorbance
187 spectra were acquired between 200 and 800 nm with a resolution of 1 nm and a scan speed of
188 0.2 s.nm⁻¹. A baseline correction was carried out using an ultrapure water blank over the same
189 wavelength range. A series of classical descriptive indicators (SUVA₂₅₄, absorbance ratios and
190 spectral slopes) were calculated from the generated absorbance spectra (Table 1).

191 **2.5.3 EEM fluorescence spectroscopy and PARAFAC decomposition**

192 Excitation-emission matrix (EEM) fluorescence analyses were performed using an Aqualog
193 (Horiba Scientific) spectrofluorometer in a 1 cm path length quartz cuvette (Hellma
194 Analytics®) thermostated at 20°C. The Aqualog instrument was equipped with a 150 W Xenon
195 arc lamp and excitation wavelengths were scanned using a double-grating monochromator from
196 240 to 800 nm at 5 nm intervals, while the emission spectra were obtained using a high-gain
197 CCD detector at intervals of approximately 0.58-nm (1-pixel) for wavelengths from 240
198 to 800 nm. All EEM spectra were corrected for instrumental biases and normalized to the area
199 under the Raman peak of water, at 350 nm excitation, acquired daily. Fluorescence intensities
200 are therefore expressed in Raman units (R.U). In order to avoid inner filter effects, all samples
201 with maximum absorbance > 0.1 were diluted. Raman and Rayleigh scattering peaks were
202 removed by subtracting blank spectrum. The generated spectra were treated using TreatEEM-
203 V1 software developed by Dario Omanović (Ruđer Bošković Institute, Zagreb, Croatia;

204 <https://sites.google.com/site/daromasoft/home/treateem>). The fluorescence indices HIX
205 (Zsolnay), BIX, FI, YFI (Table 1) were calculated for an excitation wavelength of 250, 310,
206 370 and 280 nm respectively (Heo et al., 2016; Huguet et al., 2009; Mcknight et al., 2001;
207 Zsolnay et al., 1999)

208 The multi-way parallel factor analysis (PARAFAC) was applied to a set of 103 EEM spectra
209 (corresponding to 83 soil extracts and 20 water samples collected from the adjacent streams).
210 The PARAFAC model was run, using the drEEM toolbox under MatLab R2020a, for 4 to 8
211 components with non-negativity constraints (Murphy et al., 2013). Four components were
212 validated based on split-half analysis and compared to previously reported components using
213 the OpenFluor fluorescence database.

214 **2.5.4 Size exclusion chromatography HPSEC/UV-fluorescence**

215 High performance size exclusion chromatography (HPSEC; LC-2030C 3D Plus, Shimadzu)
216 coupled with photodiode array (Deterium D2 Lamp PDA) and fluorescence (RF-20A xs)
217 detectors was used to determine the molecular size distribution of DOM. The injection volume
218 was 10 μL with a flow rate at 0.8 $\text{mL}\cdot\text{mn}^{-1}$ of phosphate buffer mobile phase (NaCl , KH_2PO_4
219 and K_2HPO_4). Two columns (Shodex OHpak SB-803 HQ, Shodex OHpak SB-802 HQ) coupled
220 in series were used for the chromatographic separation. The first column covered an exclusion
221 range between 1 kDa and 100 kDa and the second one between 0.2 kDa and 1 kDa. Analytical
222 standards PSS (polysulfonate) were used as references of average molecular weights (0.246,
223 1.6, 4.3, 6.8, 10, 17, 32, 77 kDa). Other standards were also used to cover a wider range of
224 molecule types, such as nitrates (0.063 kDa), Bovin Serum Albumin BSA (66 kDa) and
225 Polyacrylic Acid PAA standards (1, 2.6 and 3.6 kDa). A calibration curve of molecular weight
226 (from 0.2 - to 77 kDa) according to elution volume was calculated using these analytical
227 standards (Brezinski and Gorczyca, 2019).

228 The UV-DAD detector allowed the acquisition of chromatograms on an absorbance range
229 between 200 and 450 nm with an increment of 1.2 nm every 300 ms. Regarding the fluorescence
230 detector, 3 couples of excitation-emission wavelengths (Ex-Em) were retained for all the
231 analyses The fluorescence intensities were measured for the wavelengths corresponding to the
232 humic-like components (315-443 nm), protein-type components (280-330 nm), and tryptophan-
233 type components (278-354 nm) were measured every 400 ms.

234 To optimize the use of the data generated by HPSEC/UV-fluorescence, a Gaussian
235 decomposition was performed. The aim was to decompose the size continuum obtained in each
236 HPSEC chromatogram into distinct Gaussian components (Eq. 1). For this, the toolbox
237 «peakfit.m » (O'Haver, 2020) was applied on MatLab *R2020a*.

$$238 \text{ Eq. 1 : } A(x; \mu; \sigma; \varphi) = \sum_{i=0}^{nC} \varphi_i e^{\frac{-(x-\mu_i)^2}{2\sigma^2 i}} + \varepsilon$$

239 where nC is the number of Gaussian components, ε is the residuals which represent the
240 variability not taken into account by the model, x is the peak area, μ is the peak position
241 parameter (elution volume), σ is the peak width and φ is the proportion of each fitted Gaussian
242 distribution.

243 The number of detected components was validated using the Bayesian Information Criterion (
244 Asmala et al., 2021). Areas of the identified Gaussian components were summed in order to
245 calculate the total sec absorbance (TSA) from chromatograms obtained with detection at UV
246 254 nm (Asmala et al., 2021). The total sec fluorescence TSF was calculated from
247 chromatograms obtained with fluorescence detection at three excitation-emission (Ex-Em)
248 wavelengths (nm): Ex: 315-Em: 443, Ex: 280-Em: 330 and Ex: 278-Em: 354. These
249 wavelengths are classically used to characterize DOM properties (Table 1). The calibration

250 curve allowed classifying each Gaussian component in a molecular weight fraction, based on
251 the peak position (elution volume). In this study, three molecular weight fractions were defined
252 as: low molecular weight fraction (LMW) with an average molecular weight < 1 kDa, medium
253 molecular weight fraction (MMW) with an average molecular weight ranging from 1 to 10 kDa,
254 and high molecular weight (HMW) with an average molecular weight >10 kDa. The LMW,
255 MMW and HMW fractions represent the relative percentages of respectively small, medium
256 and large molecules detected at the measured wavelengths.

257 **2.6 Mathematical and statistical analyses**

258 Anova analysis coupled with a Tukey test (packages stats and rstatix; R 4.0.2 software) were
259 used to compare both forest and vineyard soil extracts, the temporal kinetics of the incubation
260 study and also for the comparison between soil extracts and adjacent natural waters for all UV-
261 Vis, EEM fluorescence and HPSEC/UV-fluorescence indicators. Principal component analysis
262 (PCA;package factoextra, R 4.0.2 software) was also used for the comparison of soil extracts
263 vs. natural adjacent waters, and a linear discriminant analysis (LDA; packages MASS and klaR,
264 R 4.0.2 software) for the selection of the most relevant DOM markers of forest and vineyard
265 soil occupations.

266 **3 Results**

267 **3.1 Dissolved organic carbon**

268 Before incubation, dissolved organic carbon (DOC) concentrations (Figure 2.A) in the 5 forest
269 soil extracts ranged from 22.7 to 48.2 mg.L⁻¹ with a mean value of 32.7 ± 9.4 mg.L⁻¹. For the 5
270 vineyard soil extracts, DOC concentrations varied between 16.14 and 34.14 mg.L⁻¹ with a mean
271 value of 23.8 ± 6.6 mg.L⁻¹. These values are consistent with those obtained in previous studies
272 (Rinot et al., 2021). No significant difference (Tukey test, $p > 0.05$, n=50) in DOC
273 concentrations was found between forest and vineyard soil extracts before incubation.

274 The percentage of DOC (Figure 2.B) was calculated for the duration of the experiment (28
275 days). For forest soil extracts, the percentage of DOC decreased by 67 ± 8 %, with a significant
276 decrease (Tukey test, $p < 0.05$, n=50) of 42 ± 9 % within the first three days of incubation. For
277 vineyard soil extracts, the percentage of DOC decreased by 61 ± 3 %, with a significant decrease
278 of 46 ± 5%, again during the first three days of incubation. Previous studies showed DOC losses
279 range from 5 to 93% depending on soil types (forest, pasture, arable) and extraction protocols
280 (pressurized hot-water, water extraction, leaching extraction) (Guigue et al., 2014; Kalbitz et
281 al., 2003).

282 DOC concentrations of samples collected in streams adjacent to forest soils ranged from 2.2 to
283 3.0 mg.L⁻¹ during high water periods (Figure 2.A). For these same sites, DOC concentrations
284 were significantly higher (Tukey test, $p < 0.05$, n=20) ranging between 3.3 mg.L⁻¹ and 4.6 mg.L⁻¹
285 during low water periods. In the streams adjacent to vineyard soils, DOC ranged from 3.1 to
286 4.4 mg.L⁻¹ during high water. Non-significant difference was observed during low water
287 periods with DOC concentrations ranging between 3.5 and 4.6 mg.L⁻¹. In addition, DOC
288 concentrations were significantly higher in the vineyard stream samples than in the forest
289 stream samples only during high water.

290 3.2 UV-Visible

291 UV-Visible spectra (Supplementary Material 3) show a classical pattern, i.e. an exponential
292 decrease between 200 and 250nm, followed by a slight decrease between 250 and 400 nm. Then
293 the signal is close to the background for wavelength superior to 400 nm. A decrease in
294 absorption is observed between the soil extracts before and after 28 days of incubation, at
295 wavelengths from 250 to 400 nm, i.e. the range classically used to characterize the
296 chromophoric DOM. $SUVA_{254}$ (Table 1) increased significantly (Tukey test, $p < 0.05$, $n=50$)
297 over the 28-day incubation period of the experiment, from 0.98 ± 0.11 to $2.02 \pm 0.12 \text{ L.mg}^{-1}\text{cm}^{-1}$
298 ¹ for forest soil extracts, and from 1.44 ± 0.45 to $3.02 \pm 0.51 \text{ L.mg}^{-1}\text{cm}^{-1}$ for vineyard soil
299 extracts (Figure 3.A). A significant difference (Tukey, test, $p < 0.05$, $n=50$) was observed
300 between forest and vineyard soils only after the 3rd day of incubation. During the first 3 days,
301 spectral slope $S_{275-295}$ and E2.E3 index (Table 1) values decreased significantly (Tukey test, p
302 < 0.05 , $n=50$) and then stabilized during the rest of the incubation period (Figure 3.B, C).
303 Overall, values of $S_{275-295}$ for forest and vineyard extracts respectively decreased from 0.023
304 ± 0.002 to 0.017 ± 0.001 and from 0.022 ± 0.003 to 0.017 ± 0.003 ; and the E2.E3 index values
305 decreased from 7.60 ± 0.47 to 6.33 ± 0.59 and from 6.86 ± 0.92 to 5.91 ± 0.90 . The values of
306 the SR, E2.E4 and E3.E4 (Table 1) ratios did not vary significantly between the soil extraction
307 and the last day of the experiment (Figure 3.D, E, F).

308 Regarding samples collected in adjacent streams, no significant difference was observed
309 according to land use. However, the indicator SR showed significant differences between high
310 water and low water sampling periods (Tukey test, $p < 0.05$, $n=20$). Indeed, values of SR varied
311 between 0.751 ± 0.0497 to 0.808 ± 0.012 and 0.775 ± 0.027 to 0.833 ± 0.011 for both forest
312 and vineyard adjacent water samples. Previous studies on soil extracts and stream water samples
313 (Sanderman et al., 2009; Liu et al., 2021; Chen et al., 2021) have reported UV-Visible index
314 values in the same ranges as those presented in this work.

315 3.3 EEM fluorescence spectroscopy

316 The humification index (HIX; Table 1) showed a significant increasing trend during the first
317 days of incubation (Tukey test, $p < 0.05$, $n=50$). These values stabilized for vineyard soil
318 extracts while they decreased for forest soil extracts at the end of incubation (Figure 4.A).
319 Indeed, the values increased from 3.1 ± 1.0 to 3.7 ± 1.5 for the forest soil extracts and from 2.9
320 ± 0.4 to 6.6 ± 0.7 for the vineyard soil extracts. Therefore, a significant difference (Tukey test,
321 $p < 0.05$, $n=50$) is also observed between the two types of soils at the end of the experiment.
322 No particular trend was observed for the indicator of biological activity (BIX; Table 1) and the
323 Freshness index (FI; Table 1) during the incubation period (Figure 4.B, C). BIX values ranged
324 from 0.71 ± 0.08 to 0.79 ± 0.08 for forest soil extracts; and from 0.63 ± 0.05 to 0.67 ± 0.02 for
325 vineyard soil extracts; FI values from 1.40 ± 0.06 to 1.42 ± 0.04 for forest soil extracts; and
326 from 1.31 ± 0.08 to 1.36 ± 0.04 for vineyard soil extracts. The YFI indicator (Table 1), showed
327 a decrease according to incubation time (Figure 4.D). This decrease, however, is very limited
328 for both soil extracts during the incubation experiment (YFI values from 0.89 ± 0.27 to $0.71 \pm$
329 0.06 and from 0.74 ± 0.04 to 0.61 ± 0.04 respectively for forest and vineyard soil extracts).

330 For water samples, only HIX showed a significant difference between water collected during
331 high and low water (Tukey test, $p < 0.05$, $n=20$). HIX values varied between 8.44 ± 0.88 and
332 7.28 ± 1.11 for the streams adjacent to the forest, sampled during high and low water periods.
333 For vineyard streams, it varied between 9.05 ± 0.34 and 7.39 ± 0.53 during the two sampling
334 periods. The other indices BIX, FI and YFI showed no significant differences between forest
335 and vineyard waters, nor between sampling periods. Values of fluorescence indices in this work
336 are consistent with previous studies obtained on forest soils, peat soils, and subsoil extracts
337 (Hansen et al., 2016; Olshansky et al., 2018; Zhang et al., 2022)

338 **3.4 PARAFAC modeling**

339 The contour plots of the 4 components determined by PARAFAC analysis are shown in
340 supplementary material 4. Components C1 to C4 were identified from the literature and
341 successfully matched in the OpenFluor database with similarity scores above 0.97. Component
342 C1 showed two excitation maxima, a first one at ≤ 240 nm and a second one with lower intensity
343 at 320 nm with an emission maxima at 420 nm. The component C1 has been described as
344 terrestrial DOM degraded from lignin (Peleato et al., 2018) typical of soils and terrestrial
345 sources in aquatic environments (Garcia et al., 2018; Yamashita et al., 2021). This component
346 has further been attributed to bioavailable organic matter, microbially derived and/or sensitive
347 to photodegradation (Cawley et al., 2012; Eder et al., 2022). Component C2 showed an
348 excitation maxima at ≤ 240 and a lower intensity one at 390 nm with a maximum emission
349 intensity at 510 nm. Previous studies have reported the component C2 as an ubiquitous
350 terrestrial material with high molecular weight (MW) and aromatic content (Cawley et al.,
351 2012; Wünsch et al., 2018; Yamashita et al., 2021). It has also been reported as fluorophores
352 derived from higher plants and associated with photochemical (Coulson et al., 2022; Eder et
353 al., 2022) or microbial (Cory and McKnight, 2005) degradation. Component C3 showed an
354 excitation maximum at 365 nm and an emission at 443 nm. It has been reported to be high MW,
355 aromatic and hydrophobic molecules, corresponding to lignin-like compounds (Dainard et al.,
356 2015; Derrien et al., 2018; Rinot et al., 2021). Other studies reported component C3 as organic
357 matter of plant origin that has undergone little transformation in the soil after production (Catalá
358 et al., 2021; Retelletti Brogi et al., 2019). Finally, the component C4 showed an excitation
359 maximum at 280 nm and an emission at 330 nm. Numerous studies have reported this
360 component to be attributed to amino acids, either free or bound to proteins, or to phenolic-type
361 compounds (Coble, 1996; Kida et al., 2019; Parlanti et al., 2000; Wauthy et al., 2018).

362 Of the four components identified, only component C4 showed a decreasing trend during the
363 incubation experiment (Figure 5). Indeed, for both soil extracts, the maximum fluorescence
364 intensity of the C4 component decreased significantly (Tukey test, $p < 0.05$, $n=50$), from $2.6 \pm$
365 $0.3 \pm$ to 0.8 ± 0.2 for the forest soil extracts and from 1.9 ± 0.7 to 0.5 ± 0.1 for the vineyard soil
366 extracts. On the other hand, all components showed significant differences between streams
367 adjacent to the forest sampled during high and low water periods. In these samples, component
368 C1 varied between 0.62 ± 0.1 and 0.97 ± 0.19 R.U, C2 between 0.34 ± 0.06 and 0.58 ± 0.11
369 R.U, component C3 between 0.22 ± 0.03 and 0.34 ± 0.06 R.U and component C4 varied
370 between 0.10 ± 0.02 and 0.16 ± 0.01 R.U.

371 **3.5 Size exclusion chromatography and Gaussian decomposition**

372 The HPSEC/UV-fluorescence chromatograms provided additional information on the size
373 distribution of DOM molecules contained in the samples. The Gaussian decomposition of the
374 chromatograms revealed 6 to 8 fractions, depending on wavelength detection, distributed
375 throughout the chromatogram. The percentage contribution of the LMW fraction (Section
376 2.5.4), for UV detection at 254 nm (Figure 6.A) and fluorescence at 280-330 and 278-354 nm
377 (Figure 6.C, D), was significantly higher, before incubation, in forest soil extracts compared to
378 vineyard soil extracts (Tukey test, $p < 0.05$, $n=50$). At the end of the experiment, the percentage
379 of LMW was different only for UV detection at 254 nm (Tukey test, $p < 0.05$, $n=50$). For the
380 adjacent water streams, significant differences were observed on the 280-330 and 278-354 nm
381 fluorescence ranges according to the low vs. high water hydrological regime and this for both
382 soil occupations (Figure 6.C,D). The MMW fraction, which corresponds to all Gaussian
383 components classified between 1 kDa and 10 kDa, showed the least variation. Nevertheless, the
384 percentage of MMW at UV 254 nm were significantly different between the two types of soil
385 extracts at the end of the incubation period (Tukey test, $p < 0.05$, $n=50$). The HMW fraction ($>$
386 10 kDa) showed very low percentages ($< 10\%$) compared to the LMW and MMW fractions

387 for UV detection at 254 nm and fluorescence at 315-443 nm (figure 6B). However, the HMW
388 fraction was higher for 280-330 and 278-354 nm fluorescence detection with a significant
389 difference (Tukey test, $p < 0.05$, $n=50$) between forest and vineyard soil extracts before
390 incubation. For adjacent streams, the HMW fraction was also present at very low percentages
391 and no difference was observed between soil occupations nor between low and high water
392 regimes.

393 4 Discussion

394 4.1 Incubation experiment: from labile to refractory DOM in soil extracts

395 In the natural environment, the labile fraction of DOM is mainly degraded in the soil before
396 being transported to the adjacent stream. Thus, the incubation experiment was designed to
397 remove a possible labile fraction of DOM in the soil extracts before comparison with DOM
398 from adjacent streams. During the 28-day incubation, most of the DOC mass was lost in the
399 first 3 days for both soils (Figure 2A). The percentage of DOC loss stabilized between 40 and
400 70 % after 3 days incubation. These results are consistent with those obtained in previous
401 studies investigating the biodegradation of water-extracted DOM from different soil types such
402 as forest, pasture and agricultural soils (Guigue et al., 2014; Kalbitz et al., 2003). DOC loss
403 is mostly explained by the degradation of labile DOM that is easily assimilated by
404 microorganisms. It is well known that certain organic molecules, such as amino acids or
405 carboxylic acids, are considered as labile while other molecules, such as lignins, are considered
406 more recalcitrant (Eilers et al., 2010). The variability of the percentage of DOC loss observed
407 in this study (between 40 and 70 %) could be explained by differences in the microorganisms
408 communities contained in the soil samples. Indeed, it has been shown that a greater diversity of
409 microorganisms in soils favors the DOC degradation (Raczka et al., 2021). Different
410 proportions of labile and recalcitrant DOM pools in the soils could also explain the variability
411 of DOC loss. Thus, soils containing higher levels of labile DOM will be degraded to a greater
412 proportion than soils containing high levels of refractory DOM.

413 The degradation of labile molecules induced an increase in the average aromaticity content,
414 average size and molecular weight of molecules, as highlighted by the indicators SUVA₂₅₄,
415 E2.E3 and SR. Previous studies have also shown that degraded organic matter has relatively
416 higher aromaticity content and molecular weight (Guarch-Ribot and Butturini, 2016; Hansen et
417 al., 2016). The E3.E4 indicator being inversely correlated to the humification of DOM (Claret

418 et al., 2003) can be interpreted with the HIX indicator. The HIX and E3/E4 indicators show that
419 the degree of humification of soil extracts was low at the beginning of incubation, and then
420 increased to stabilize after 3 days of incubation for vineyard soils. For forest soils, a gradual
421 increase was observed throughout the incubation period until 28 days where a significant
422 decrease occurred. The unexpected decrease observed for forest soil extracts at the end of
423 incubation could be explained by the very high values of γ band (280-330 nm); linked with
424 protein-like compounds. The HIX index corresponds to the ratio of areas 435-480 nm and 300-
425 345 nm of the emission spectrum for an excitation at 255 nm, thus a high γ band intensity can
426 make the HIX values vary drastically. The other explanation could come from the humic
427 fraction of forest soils. Indeed, it is a complex fraction consisting of functional groups such as
428 phenols, quinones, methoxyls and carbonyls (Maccarthy, 2001), that are recalcitrant with a slow
429 kinetic of degradation due to its complex chemical nature (Marschner et al., 2008).
430 Humification is a process of DOM condensation resulting from several interactions such as
431 plant alteration with partially degraded lignins or the transformation of polyphenols, amino
432 acids and some sugars into quinones under the enzymatic action of microorganisms (Stevenson,
433 1995). This slow process can sometimes take years.

434 The BIX index showed a significant variability in forest soil extracts contrary to vineyard soils.
435 This could be explained by a more contrasted biological activity in forest soil extracts than in
436 vineyard soils. Based on the hypothesis that biological activity is directly related to the diversity
437 of microbial communities, vineyard soil extracts may have less microbial diversity due to
438 intensive soil exploitation allowing an homogenization of the communities present in the soil
439 (Jangid et al., 2008). Other studies mention the concept of "priming effect", which reflects a
440 greater mineralization of organic matter in soils enriched with fresh litter (Derrien et al., 2019).
441 This could be the case for forest soils which are subject to a contribution of fresh organic matter
442 through vegetation cover. In addition, the significant decrease of the YFI indicator shows a

443 lower contribution of protein-like compounds at the end of the experiment. EEM fluorescence
444 and PARAFAC decomposition bring details on the nature of the degraded molecules along the
445 incubation. The component C4 (275-350) allows to affirm that some degraded labile molecules
446 are tryptophan-like with recent biological production (Fellman et al., 2008). No significant
447 difference was observed in the evolution of the other components C1, C2 and C3 which are
448 characteristic of the following typical compounds: terrestrial humic-like (plant derived), humic-
449 like matter, commonly found in agricultural catchments and terrestrial high aromatic and
450 hydrophobic macromolecules (Derrien et al., 2018; Jørgensen et al., 2011; Rinot et al., 2021).
451 It has been reported in previous studies that the degraded molecules are generally low molecular
452 weight compounds (Fujii et al., 2010; Ondrasek et al., 2019). However, the size exclusion
453 chromatography showed a decrease in the contribution of low molecular weight molecules
454 LMW < 1k Da, and also high molecular weight macromolecules HMW > 10 kDa at the same
455 time. Based on these results, protein-like content of DOM is likely to be composed of both
456 LMW and HMW fractions. The fraction of medium molecular weight (MMW) compounds,
457 linked to C1, C2 and C3 PRAFAC components (lignin compounds and terrestrial derived
458 DOM), did not vary significantly and seemed to be recalcitrant and not easily degradable.

459 **4.2 Evolution of DOM characteristics from soils to adjacent streams**

460 The evolution of soil extracted DOM during the incubation experiment in comparison with the
461 adjacent watercourse DOM is shown in the the principal component analysis (PCA), plotted
462 with the 26 optical indicators calculated from UV-Visible, EEM fluorescence and HPSEC/UV-
463 fluorescence (Figure 7). The PCA shows that the optical fingerprints of forest and vineyard soil
464 extracts are more distinct at the end of incubation experiment (82 days) than before. . This could
465 be explained by the nature and the proportion of labile vs refractory fractions of DOM before
466 and after incubation. This suggests that the labile fraction of DOM is not so different between
467 the two soil types. For all indicators, the evolution of DOM optical fingerprints during the

468 incubation tends towards that of adjacent watercourse (Figure 7). The adjacent stream samples
469 show relatively high contribution of medium weighted molecules MMW and a lower
470 contribution of both low and high molecules of the protein and humic types (LMW and HMW).
471 This could be explained by the fact that watercourse DOM has already undergone
472 mineralization processes, mainly through microorganisms in top soil layers, before it reaches
473 the adjacent watercourses. Other abiotic ways such as photodegradation and adsorption can
474 influence DOM composition, which can explain the observed differences between samples at
475 the end of incubation and the adjacent watercourse samples. The PCA also showed that water
476 samples taken downstream of the forest soils seem more heterogeneous than downstream of the
477 vineyard soils, whatever the hydrological regime. This heterogeneity could be explained by in-
478 situ complex abiotic processes as well as by the difference in vegetation cover in the Ardières-
479 Morcille watershed. Indeed, the forest sources considered in this study are mostly influenced
480 by coniferous forests, but broad-leaved forests and natural grasslands can represent up to 35%
481 of the total sub-catchment areas (calculation based on land use maps, CLC 2018). A previous
482 study highlighted different DOM quality between deciduous and coniferous forests (Thieme et
483 al., 2019). On the contrary, the viticultural sub-catchments are very homogeneous in terms of
484 vegetation cover as they are subject to strict local regulations. The hydrologic regime also seems
485 to have an influence on DOM quality especially for vineyard adjacent watercourses. This could
486 be explained by the contribution of an autochthonous source being more heightened during low
487 water periods. Autochthonous DOM is essentially produced locally by the microbial activities
488 (Münster, 1993) and it was demonstrated previously that there is an impact of agriculture on
489 increasing carbon and nutrient inputs to downstream watercourses (Williams et al., 2010).
490 These nutrient inputs provide energy support for microbial activity which can affect DOM
491 quantity and quality.

492 **4.3 How do the fingerprints match between DOM in soil extracts after incubation and that**
493 **in downstream watercourses during high and low water periods?**

494 As discussed in the previous section, hydrological regime influences the quantity and quality
495 of the DOM in the watercourses. It is therefore more appropriate to look for markers of forest
496 and vineyard soils downstream of the waterways draining these soils considering both high and
497 low water sampling periods. To do this, a linear discriminant analysis (LDA) was applied in
498 two steps. In a first step, the construction of the LDA 1 was conducted with 3 groups: i) a first
499 group, called Forest DOM HW (high water), containing samples of degraded forest soils and
500 adjacent forest streams collected at high water. ii) A second group, called Vineyard DOM HW,
501 which contained samples of degraded vineyard soils with water from adjacent vineyard streams
502 collected at high water, and iii) a third group called low water samples, which contained all the
503 samples from adjacent watercourses taken during low water periods. The purpose of the first
504 LDA (Figure 8.A) was to identify if there was similarities between DOM markers from soil
505 extracts and DOM from the adjacent watercourse during high water period. In a second step, a
506 LDA 2 was carried out by reversing the groups of water samples, thus considering i) a first
507 group Forest DOM LW (low water), which contained the samples of forest soil extracts and
508 forest adjacent watercourses taken during low water. ii) a second group of Vineyard DOM LW
509 with samples from degraded vineyard soils and low water vineyard watercourses; and iii) a
510 third group of high water samples containing all the samples of the adjacent watercourses taken
511 during high water. The second LDA (Figure 8.B) was conducted to determine any similarities
512 between soil extracts and adjacent watercourses during low water periods.

513 Among the 26 optical indicators (UV-Visible, EEM fluorescence and HPSEC/UV-
514 fluorescence) considered in this study, 6 indicators were able to correctly classify the samples
515 into Forest DOM HW, Vineyard DOM HW and Low water samples groups (Figure 8). They
516 correspond to one UV-Visible indicator (slope ratio SR), two EEM fluorescence indicators

517 (HIX and PARAFAC component C1) and three HPSEC/UV-fluorescence indicators (LMW-
518 TSA 254, MMW TSF 278-354 and MMW TSF 280-330). The results from LDA 2 also allowed
519 the selection of 6 indicators that classified the samples into Forest DOM LW, Vineyard DOM
520 LW and High water samples groups (Figure 8.B). There were one UV-Visible indicator
521 ($SUVA_{254}$), three EEM fluorescence indicators (HIX, FI and YFI) and two HPSEC/UV-
522 fluorescence indicators (MMW TSF 278-354 and MMW TSF 280-330). Through this
523 supervised cross-sectional analysis, we have highlighted a series of indicators of terrigenous
524 contribution in both high water and low water periods. These results reveal that: (i) there is an
525 influence of land use on the quality of DOM in adjacent streams using selected optical
526 indicators, and (ii) this influence is present as well in periods of high water as in periods of low
527 water. During high water, DOM sampled downstream of forest soils comprise of relatively
528 lower molecular weight compounds with low degrees of humification and higher contribution
529 of protein-type molecules smaller than 1000 Da. On the contrary, the vineyard DOM carries a
530 higher molecular weight, a higher degree of humification and a lower contribution of small
531 protein-type molecules. During the low water period, DOM sampled downstream of the forest
532 soils has higher aromaticity and molecular weight than the vineyard DOM which contains
533 relatively low molecules. Indeed, vineyard DOM could be impacted by higher microbial
534 activity (Chavarria et al., 2021; Williams et al., 2010). The differences observed between DOM
535 sampled downstream forest and vineyard regardless the hydrological conditions can be
536 explained by several mechanisms. First, the presence of a biodegraded form of DOM in
537 vineyard soils probably due to the intensive exploitation of the soils and the low rooting
538 networks. Indeed, previous studies reported an higher organic matter oxidation in agricultural
539 soils which are more prone to erosion than forest soils and to an accelerated phenomenon of
540 open-air oxidation in the upper soil layers (Graeber et al., 2012; Shang et al., 2018). Secondly,
541 given the very steep slopes that favore surface runoff in the Ardères-Morcille watershed, the

542 differences observed between high and low water could be explained by a contribution of
543 surface soil organic matter during high water and a contribution of deeper and more degraded
544 soil organic matter during low water period.

545 It is important to note that DOM fingerprints presented in this study are not generalizable to
546 other watersheds. Considerable differences can be observed from one watershed to another
547 depending on topography, flow direction and soil characteristics. However, the fingerprints of
548 DOM sampled in a stream downstream of a homogeneous watershed while accounting for
549 hydrologic conditions provides a good representation of land use type. This sampling method
550 can be applied to other land use types with different agricultural and livestock types and
551 pressures. Moreover, given the complexity of DOM composition and reactivity, the
552 combination of optical indicators was essential. The result of the LDA, when calculated with
553 UV-Visible, EEM fluorescence and HPSEC/UV-fluorescence indicators separately, could not
554 provide a good differentiation between forest and vineyard DOM (Supplementary Material 5).
555 At last, indicators from HPSEC/UV-fluorescence proposed in this study, have shown potential
556 as good markers of diffuse sources of DOM in streams as discussed more in depth in the next
557 part 4.4 .

558 **4.4 Relevance of HPSEC/UV-fluorescence indicators to study DOM**

559 The HPSEC/UV-fluorescence indicators presented in this study provided additional
560 information regarding classical optical proxies (Table 1). For example, the SUVA indicator
561 highlight the aromatic content of DOM and the LMW TSA 254 indicator reflects the
562 contribution of molecules lower than 1 k Da (Supplementary Material 6). A negative correlation
563 (Pearson test, $p < 0.05$, $n=70$) between the two indicators demonstrates that the low molecular
564 weight compounds detected at 254 nm are poorly aromatic and that the aromatic content of
565 DOM is mostly found in compounds larger than 1 k Da. The correlation matrix (Supplementary

566 Material 6) also shows that the indicator HMW TSF 280-330 is positively correlated with the
567 component PARAFAC C4 associated with protein-like compounds. This suggests that the C4
568 component is composed of relatively large molecules (> 10 k Da). In the context of this study,
569 this showed that DOM labile pool was also composed of high molecular weight compounds in
570 contrast to what has been reported in previous studies (Curtis-Jackson et al., 2009; Derrien et
571 al., 2019). Other indicators of HPSEC/UV-fluorescence (e.g. HMW TSF 278-354) point out
572 the limitations of using classic indicators like slope ratio SR indicator (Table 1). Indeed, in
573 some soil extract, the SR indicator showed the presence of DOM mostly consisting of low
574 molecular weight compounds, while the HMW TSF 278-354 indicator showed a relatively high
575 contribution of amino-acid type compounds larger than 10 k Da. This result suggests that the
576 SR indicator could be limited to the description of certain types of molecules that exclude acid-
577 amine like compounds. Finally, the indicators from HPSEC/UV-fluorescence could be use a
578 larger scale like in monitoring studies of agricultural, industrial and urban discharges. The
579 information provided by these indicators would allow better identification of anthropogenic
580 compounds (e.g. pesticides, pharmaceuticals) that are very complicated to detect using classical
581 optical proxies as most of these compounds have almost similar optical properties (Hassoun et
582 al., 2017). Also, the establishment of databases of HPSEC/UV-fluorescence indicators specific
583 to anthropogenic compounds of interest, could facilitate their detection at lower cost than with
584 current techniques using chromatography coupled with mass spectrometry.

585 **5 Conclusion**

586 The objective of this study was to provide insight into the sampling of terrigenous diffuse
587 sources of DOM directly into streams using relevant land use markers. These markers were
588 identified by comparing DOM from soil extracts with adjacent watercourse DOM, sampled at
589 the outlet of homogenous sub-catchments during low and high water.

590 The UV-Visible, EEM fluorescence and HPSEC/UV-fluorescence techniques showed an
591 evolution of DOM extracted from forest and vineyard soils during a 28-day incubation
592 experiment. Indicators calculated from these techniques showed that the evolution of DOM
593 mainly occurred in the three first days of incubation. This resulted in the degradation of high
594 (>10 k Da) and low (<1 k Da) MW protein-like molecules (labile fraction of DOM), leading to
595 an increase of aromaticity and humification mostly observed in medium molecular weight
596 compounds (from 1 to 10 k Da). The labile fraction of DOM did not differ according to the land
597 use.

598 Adjacent stream samples had distinct optical fingerprints influenced mostly by the hydrological
599 regime, as DOM sampled during high water periods showed a large contribution of LMW and
600 HMW than DOM sampled during low water periods. At the end of the incubation study, forest
601 and vineyard soil extract tend to fingerprints of the adjacent watercourse samples. Linear
602 discriminant analysis (LDA) revealed the relevance of 9 indicators to differentiate forest DOM
603 and vineyard DOM fingerprints during high and low water sampling. These results demonstrate
604 that a representative sampling of a diffuse source of DOM in rivers should take into account
605 both the homogeneity of the upstream sub-catchments in terms of land use but also the
606 hydrological conditions. The cross exploitation of the data from these three techniques seems
607 appropriate to establish DOM fingerprints. However, the fingerprints of forest and vineyard
608 diffuse sources studied in this work are not exhaustive contrary to the methodological approach,

609 which can be applicable to other types of diffuse sources. Indeed, application of this approach
610 on a larger scale would provide specific fingerprints for terrigenous diffuse sources in streams.

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618 **Amine Boukra:** Conceptualization, Methodology, Software, Investigation, Formal analysis,
619 Data curation, Writing original draft, Visualization. **Mathieu Masson:** Conceptualization,
620 Writing-review and editing, Supervision, Project administration, Funding acquisition. **Corinne**
621 **Brosse:** Resources. **Mahaut Sourzac :** Resources. **Edith Parlanti:** Software, Writing-review
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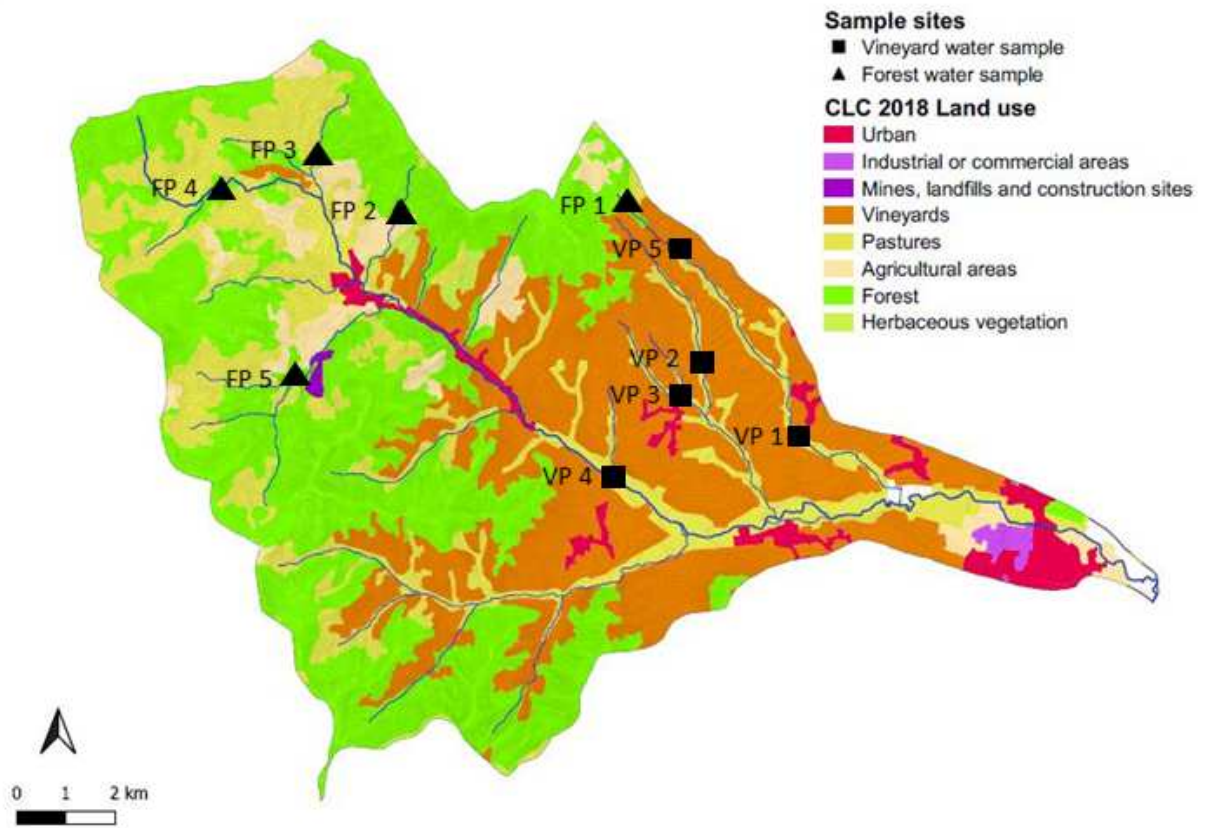
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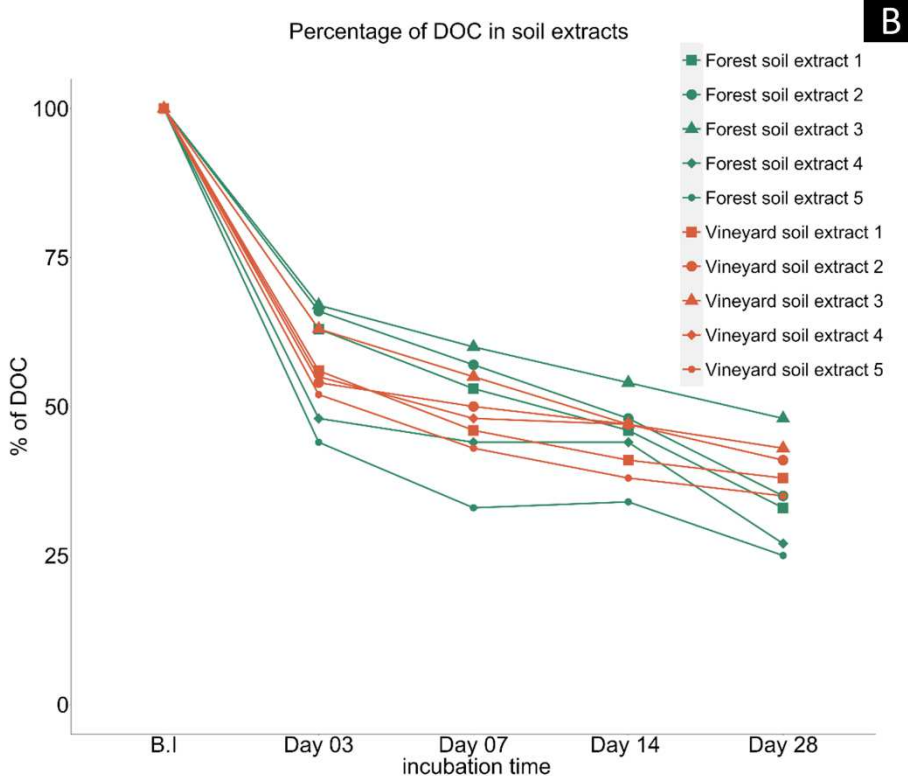
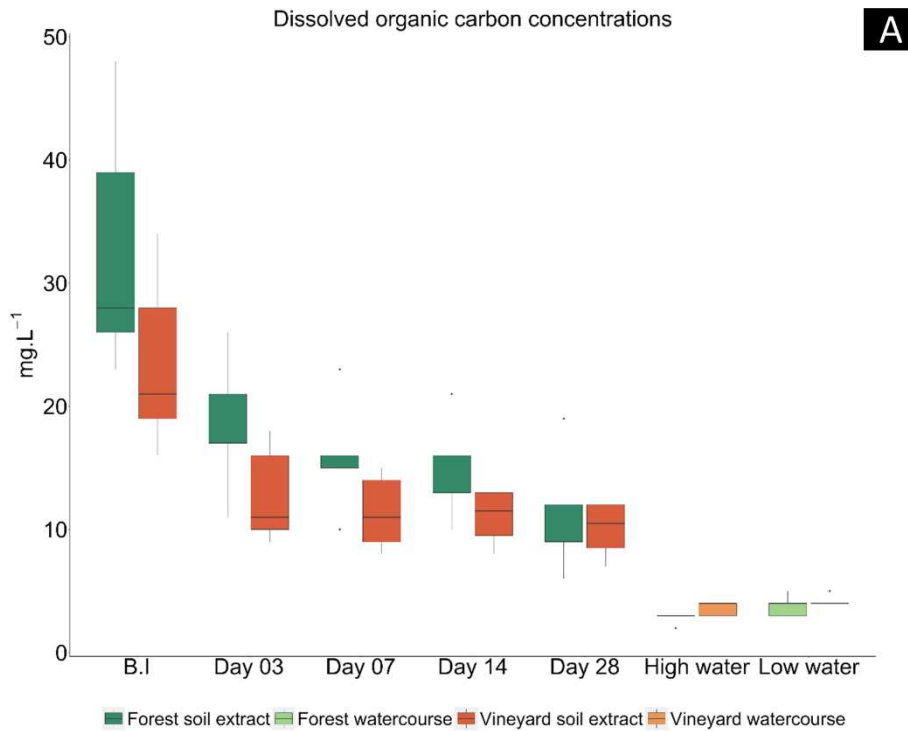


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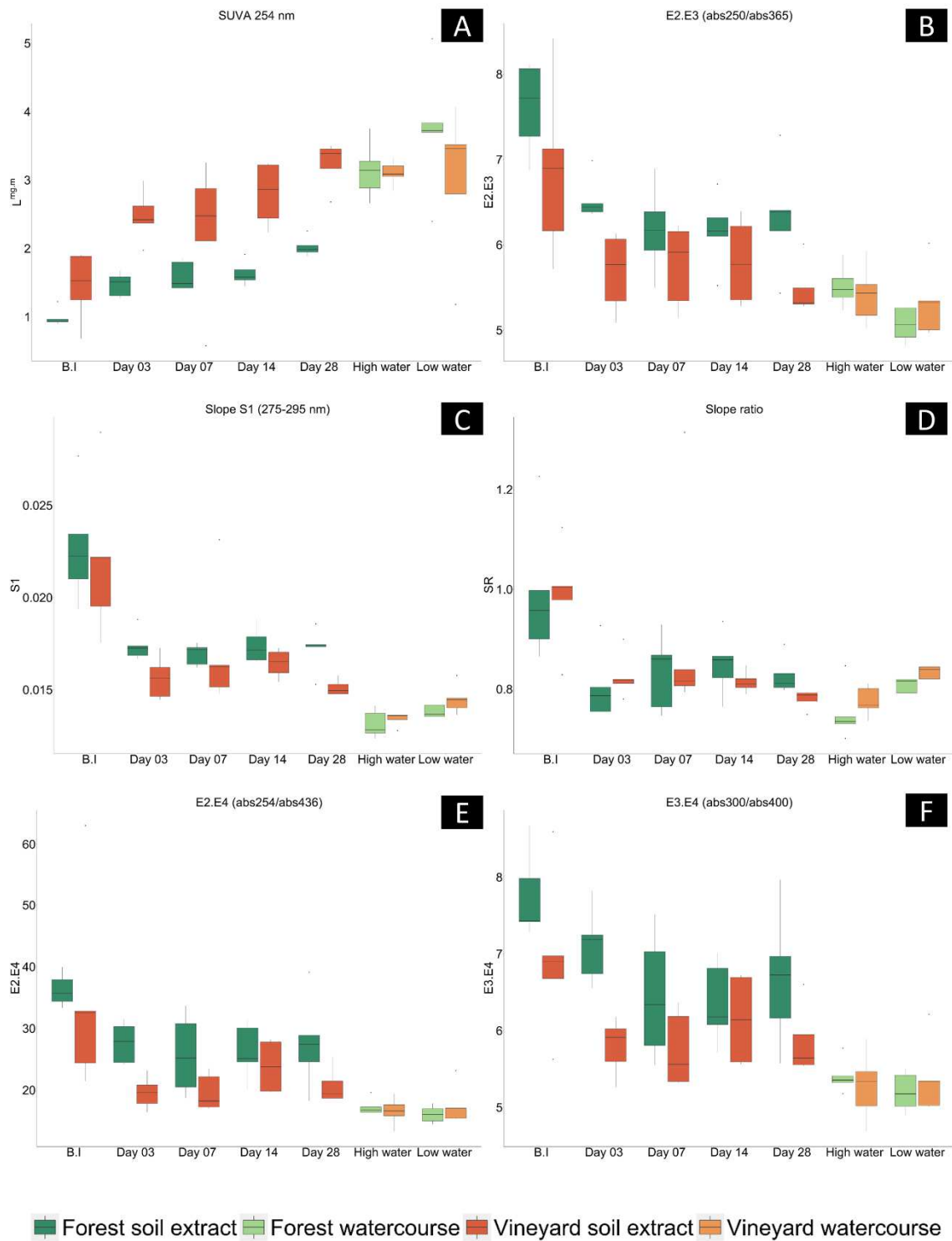
903 **Figure 1.** Map of Ardieres-Morcille catchment showing the location of the water-sampling sites
904 (FP: forest sampling points, VP: vineyard sampling points) and the land-use boundaries (Corine
905 Land Cover CLC 2018). The soil samples were collected upstream the water sampling points.

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908 **Figure 2.** A: Dissolved organic carbon (DOC) concentrations measured i) in soil extracts (forest
 909 in dark green and vineyard in dark orange) before incubation (B.I) and after 3, 7, 14 and 28
 910 days of incubation, and ii) in water samples collected from streams adjacent to the forest (in
 911 light green) and vineyard (in light orange) soils during high and low water periods. B:
 912 Percentage of DOC for soil extracts during the 28 days incubation experiment relative to the
 913 DOC concentration measured before incubation.



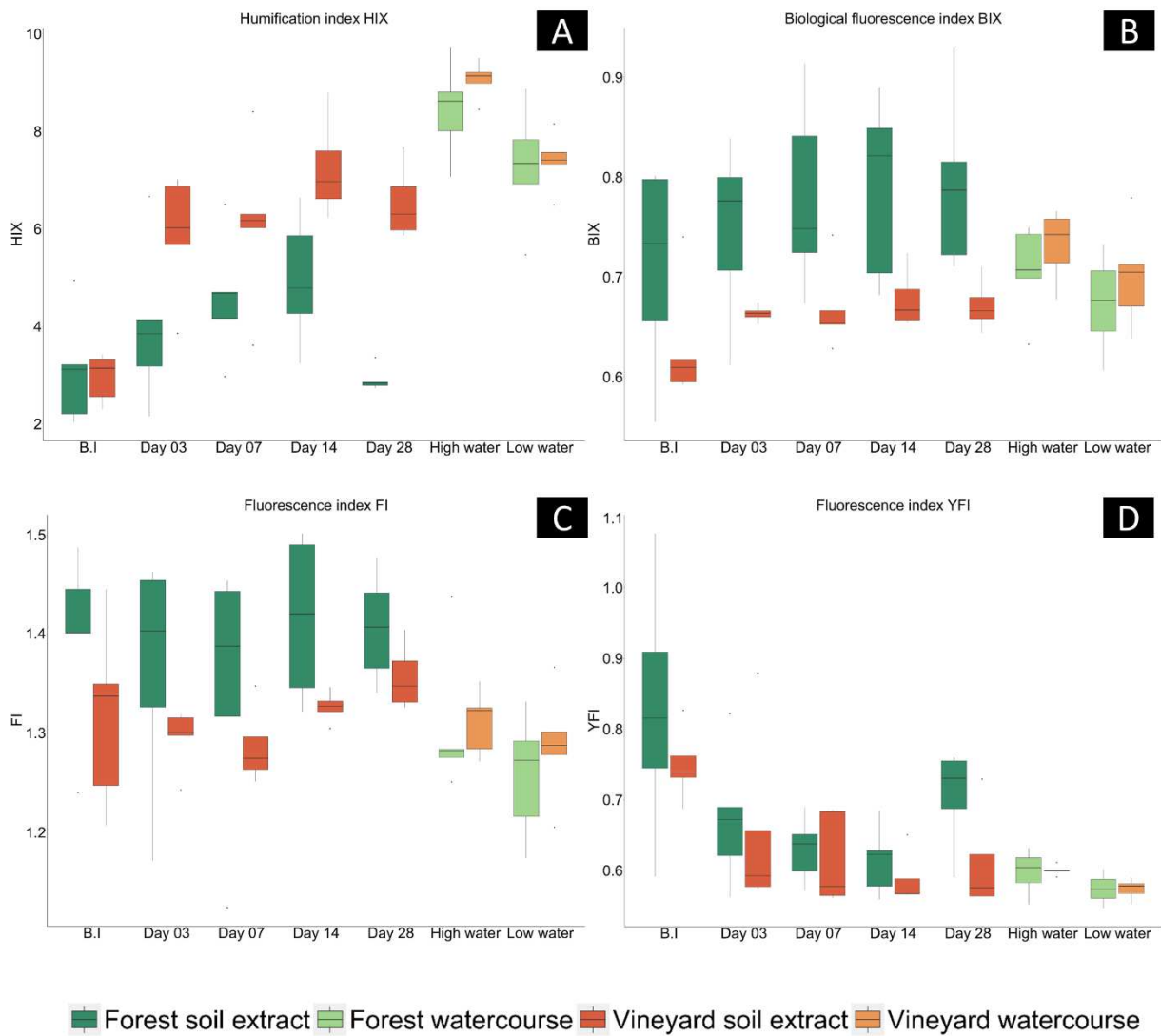
916 **Figure 3.** UV-Visible indicators measured i) in soil extracts (forest in dark green and vineyard
 917 in dark orange) before incubation (B.I) and after 3, 7, 14 and 28 days of incubation, and ii) in
 918 water samples collected from streams adjacent to the forest (in light green) and vineyard (in
 919 light orange) soils during high and low water periods. A: SUVA₂₅₄, B: E2.E3 (abs250/abs365),

920 C: Slope S1 (275_295 nm), D: Slope ratio SR (S275_295nm/S350_400nm), E: E2.E4
921 (abs254/abs436) and F: E3.E4 (abs300/abs400).

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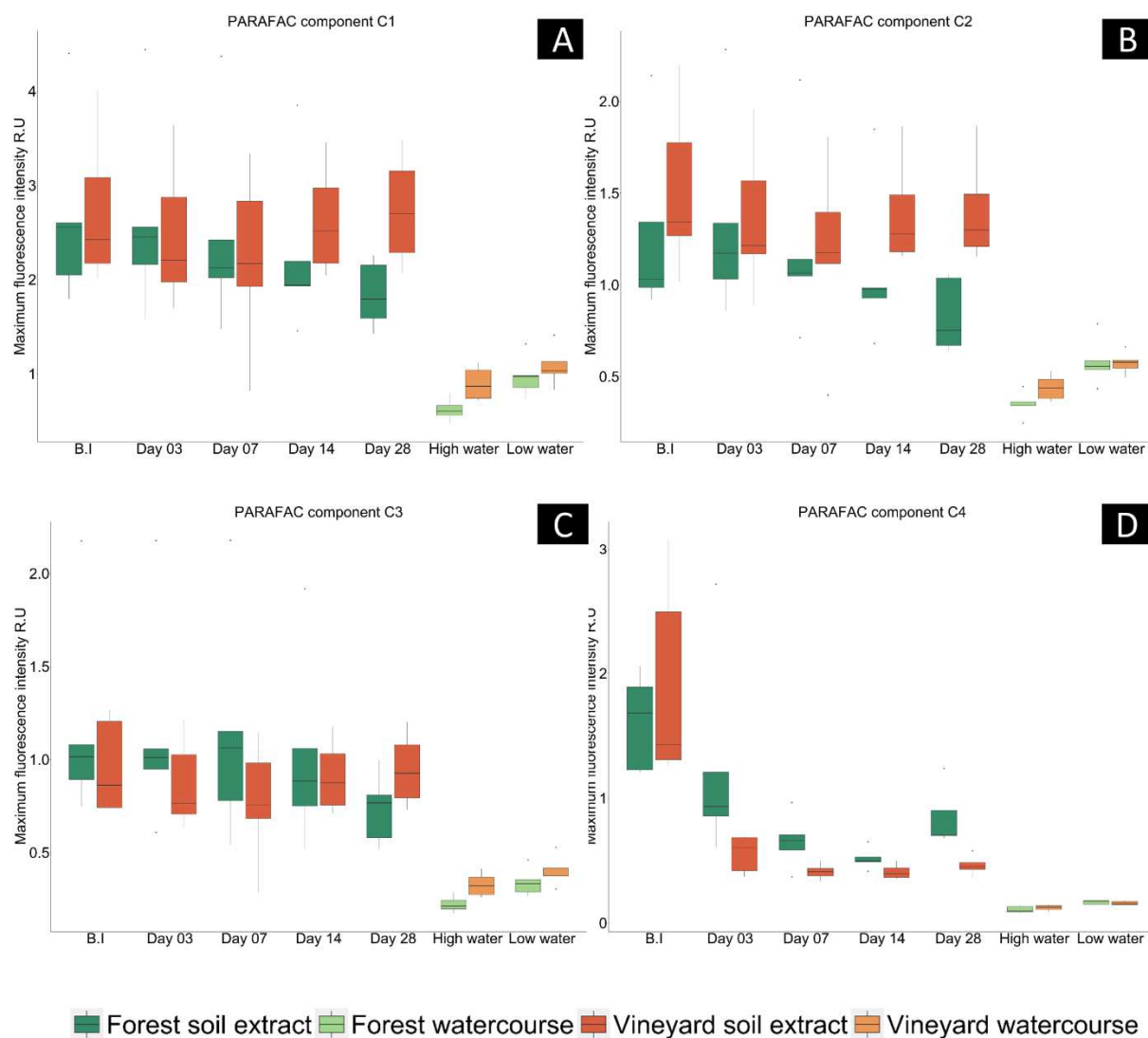


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927 **Figure 4.** EEM fluorescence indicators measured i) in soil extracts (forest in dark green and
928 vineyard in dark orange) before incubation (B.I) and after 3, 7, 14 and 28 days of incubation,
929 and ii) in water samples collected from streams adjacent to the forest (in light green) and
930 vineyard (in light orange) soils during high and low water periods. A: humification index HIX,
931 B: biological fluorescence index BIX, C: fluorescence index FI and D: fluorescence index YFI.

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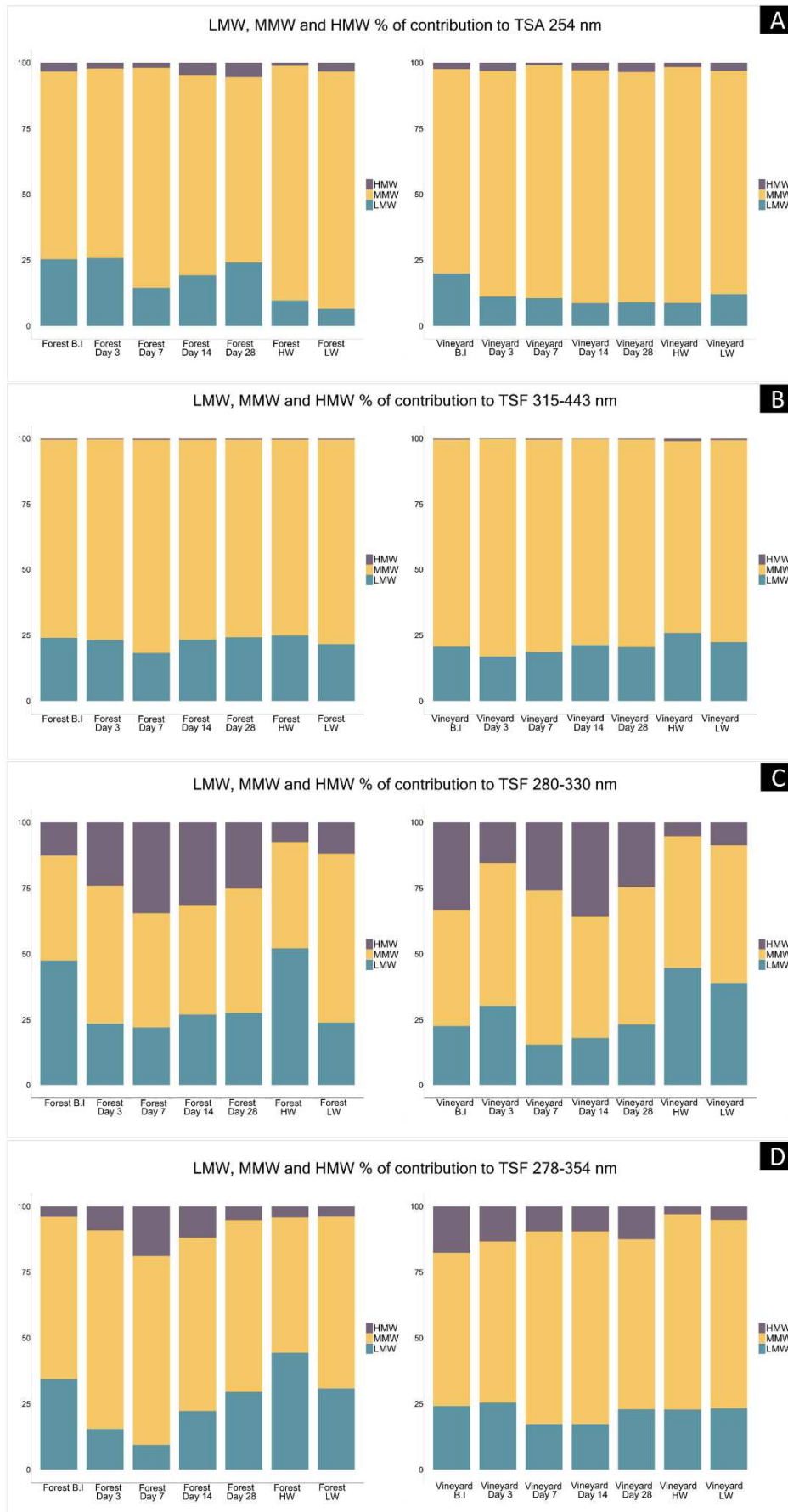


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935 **Figure 5.** PARAFAC components C1 (<240(320)-420 nm), C2 (<240(390)-510 nm), C3 (365-
 936 443 nm) and C4 (280-330 nm) measured i) in soil extracts (forest in dark green and vineyard in
 937 dark orange) before incubation (B.I) and after at 3, 7, 14 and 28 days of incubation, and ii) in
 938 water samples collected from streams adjacent to the forest (in light green) and vineyard (in
 939 light orange) soils during high and low water periods.

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943 **Figure 6.** Low Molecular Weight (LMW; blue bars), Medium Molecular Weight (MMW;
944 orange bars) and High Molecular Weight (HMW; purple bars) percentage of contribution to:
945 A: Total Sec Absorbance TSA at PDA 254 nm, B: Total Sec Fluorescence TSF at Excitation
946 Emission wavelengths 345-443 nm, C: TSF at fluorescence Excitation Emission wavelengths
947 280-330 nm, D: TSF at fluorescence Excitation Emission wavelengths 278-354 nm.. Samples
948 plotted are soil extracts before incubation (B.I) and then at 3, 7, 14 and 28 days of incubation
949 as well as for water samples collected from streams adjacent to the forest and vineyard soils
950 during high and low water periods.

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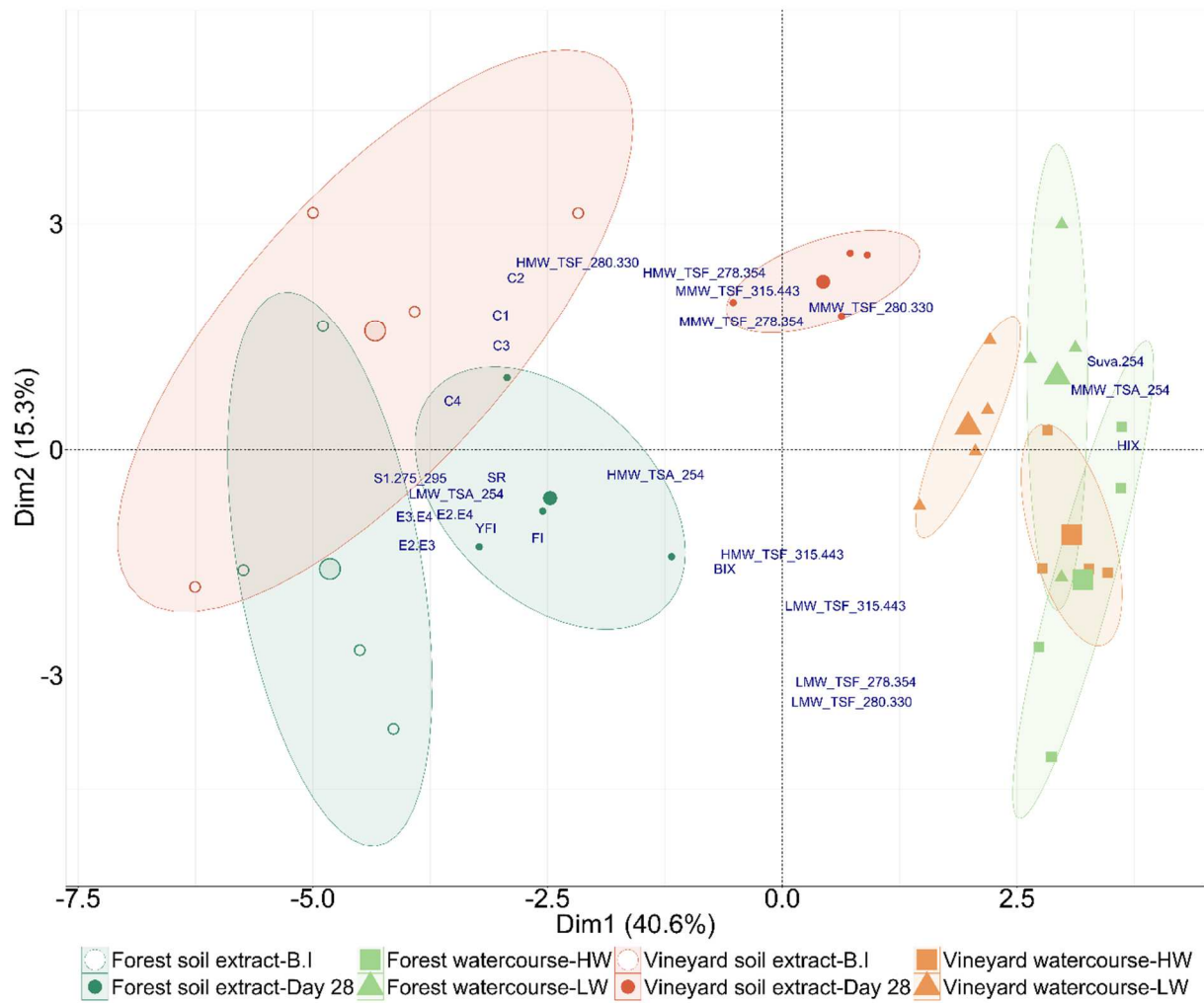
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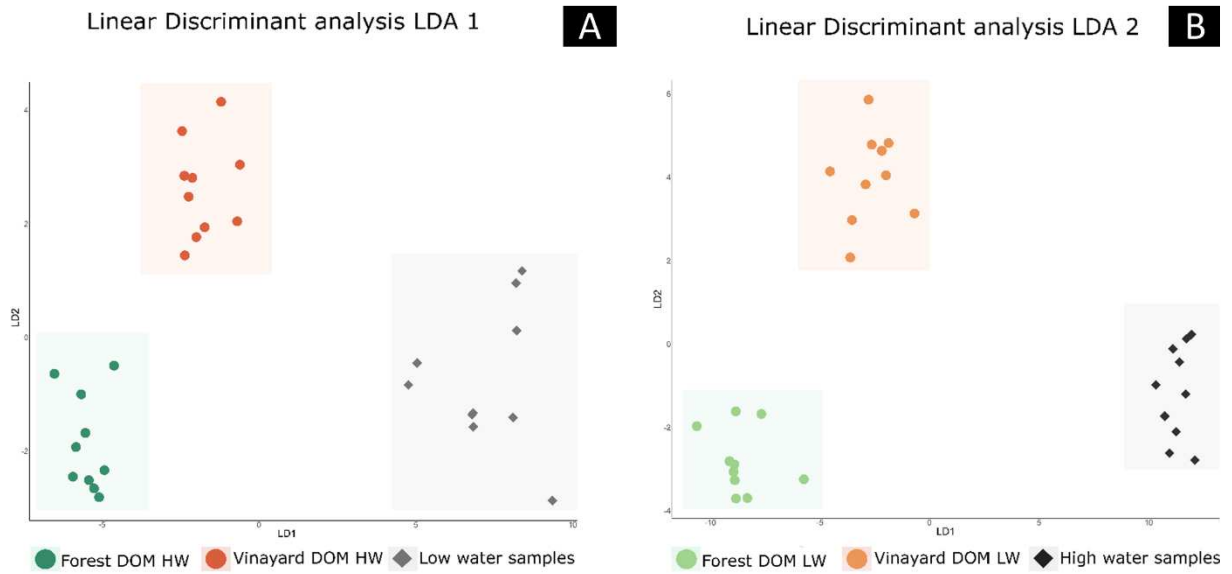
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979 **Figure 8.** A. Linear discriminant analysis (LDA1) calculated with 26 indicators from UV-
980 Visible, EEM fluorescence and HPSEC/UV-fluorescence to discriminate forest and vineyard
981 DOM taken during high water from low water DOM. B. Linear discriminant analysis (LDA2)
982 calculated with the same indicators to discriminate forest and vineyard DOM taken during low
983 water periods from High water DOM.

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987 Table 1. Classical UV-Visible and fluorescence indicators to characterize dissolved organic
 988 matter.

Indicator	Calculation	Interpretation	References
SUVA ₂₅₄	$\frac{Abs\ 254\ nm}{[DOC]}$	Correlated with the aromaticity of DOM.	(Weishaar et al., 2003)
E2.E3	$\frac{Abs\ 250\ nm}{Abs\ 365\ nm}$	Negatively correlated to organic molecules size	Helms et al., 2008
E2.E4	$\frac{Abs\ 254\ nm}{Abs\ 436\ nm}$	Autochthonous versus terrestrial DOM	Jaffé et al., 2004; Li and Hur, 2017
E3.E4	$\frac{Abs\ 300\ nm}{Abs\ 400\ nm}$	Negatively correlated to humification degree	Claret et al., 2003; Li and Hur, 2017
S ₁ (275-295 nm)	$\alpha_{\lambda} = \alpha_{\lambda_0} e^{S(\lambda_0 - \lambda)}$ α_{λ} : absorption coefficient at specific wavelength	Negatively correlated to aromatic carbon content	Stedmon et al., 2000 Helms et al., 2008
S ₂ (350-400 nm)	λ_0 : reference wavelength S: exponential slope		

SR	$\frac{S1}{S2}$	Negatively correlated to molecular weight	Helms et al., 2008
HIX	$\frac{\sum IF 435-480}{\sum IF 300-445}$ at 255 nm	Correlated to the aromatic carbon content/humification degree of DOM	Zsolnay et al., 1999
BIX	$\frac{IF 380}{IF 430}$ at 310 nm	Indicator of recent autochthonous production of DOM due to biological activity	(Huguet et al., 2009)
FI	$\frac{IF 450}{IF 500}$ at 370 nm	Terrigenous vs microbial DOM	Mcknight et al., 2001
YFI	$\frac{FEI 350-400}{FEI 400-450}$ at 280 nm FEI : average fluorescence intensities	Increases the resolution of FI indicator	Heo et al., 2016

