

1 **Heterogeneity in leaf litter decomposition in a temporary Mediterranean stream**
2 **during flow fragmentation.**

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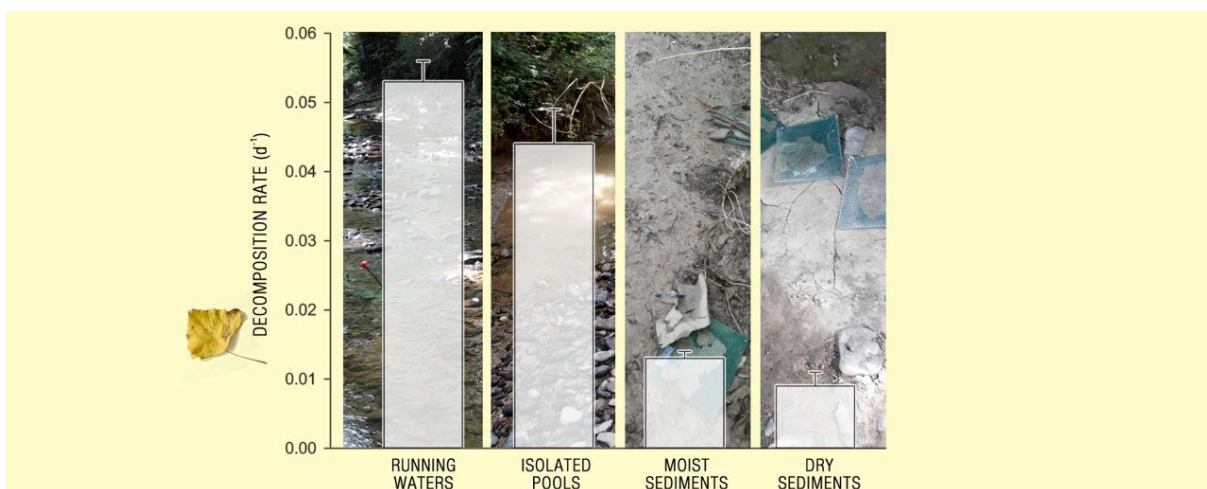
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7 **Abstract**

8 In temporary Mediterranean streams, flow fragmentation during summer droughts originates an ephemeral
9 mosaic of terrestrial and aquatic habitat types. The heterogeneity of habitat types implies a particular ecosystem
10 functioning in temporary streams that is still poorly understood. We assessed the initial phases of leaf litter
11 decomposition in selected habitat types: running waters, isolated pools and moist and dry streambed sediments.
12 We used coarse-mesh litter bags containing *Populus nigra* leaves to examine decomposition rates, microbial
13 biomass, macroinvertebrate abundance and dissolved organic carbon (DOC) release rates in each habitat type
14 over an 11-day period in late summer. We detected faster decomposition rates in aquatic (running waters and
15 isolated pools) than in terrestrial habitats (moist and dry streambed sediments). Under aquatic conditions,
16 decomposition was characterized by intense leaching and early microbial colonization, which swiftly started to
17 decompose litter. Microbial colonization in isolated pools was primarily dominated by bacteria, whereas in
18 running waters fungal biomass predominated. Under terrestrial conditions, leaves were most often affected by
19 abiotic processes that resulted in small mass losses. We found a substantial decrease in DOC release rates in both
20 aquatic habitats within the first days of the study, whereas DOC release rates remained relatively stable in the
21 moist and dry sediments. This suggests that leaves play different roles as a DOC source during and after flow
22 fragmentation. Overall, our results revealed that leaf decomposition is heterogeneous during flow fragmentation,
23 which has implications related to DOC utilization that should be considered in future regional carbon budgets.

24 **Keywords:** drought, flow fragmentation, habitat heterogeneity, black poplar, microbial biomass, DOC release.



25 **1. Introduction**

26 Temporary rivers and streams are defined as those watercourses that cease to flow at some point in space and
27 time along their course (Arthington et al., 2014). Often overlooked in global inventories (Acuña et al., 2014),
28 these ecosystems are widespread around the globe and are the dominant surface water type in Mediterranean
29 climate regions (Bonada and Resh, 2013). Temporary Mediterranean streams are characterized by high
30 hydrological variability governed by dry and wet periods throughout the year (Acuña et al., 2005). A dry period
31 typically occurs in summer, when a decrease in precipitation combined with increases in evapotranspiration and
32 water use drastically reduces water flow until the hydrological connectivity is lost (Bernal et al., 2013). This
33 contraction phase implies the fragmentation of stream ecosystems and ultimately leads to the emergence of a
34 changing and ephemeral mosaic of terrestrial and aquatic habitat types along the streambed (Larned et al., 2010).
35 These habitat types include areas with running waters, isolated pools of different sizes and dry streambed
36 sediments. The dynamics of this habitat mosaic are controlled by the frequency and duration of the dry period
37 (Stanley et al., 1997; Bunn et al., 2006), which lasts until hydrological connectivity is restored (Tockner et al.,
38 1999).

39 Flow variability is a determining factor in aquatic ecosystems (Richter et al., 2003), and in temporary streams
40 the alternation between dry and wet periods influences all ecological processes (Sabater and Tockner, 2010).
41 One of these processes is the decomposition of organic matter, a key ecosystem process that governs the entry of
42 energy and nutrients into streams (Webster and Benfield, 1986). During dry periods, water stress may impact
43 riparian vegetation by causing early leaf abscission that coincides with flow fragmentation (Sabater et al., 2001;
44 Sanpera-Calbet et al., in press). Then, leaf litter falls into the streambed and is primarily retained in dry
45 streambed sediments and isolated pools (Acuña et al., 2007) where different factors affect its processing.

46 Decomposition rate is usually slower in dry streambed sediments than under aquatic conditions (e.g.,
47 Langhans et al., 2008; Schlieff and Mutz, 2011), primarily because the absence of water limits the activity of
48 decomposers. In dry sediments, invertebrates play a minor role in decomposition because aquatic shredders are
49 inhibited by emersion (Datry et al., 2011; Riedl et al., 2013; Martinez et al., 2015) and terrestrial detritivores are
50 scarce (Maamri et al., 1997; Corti et al., 2011). Microbial biomass and activity are also reduced in dry conditions
51 (Boulton, 1991), although the importance of this factor seems to be closely linked to sediment moisture levels
52 (Amalfitano et al., 2008; Gómez-Gener et al., 2015). In this regard, some studies have reported an increase in

53 microbial decomposition when litter is partially rehydrated (Langhans and Tockner, 2006; Bruder et al., 2011;
54 Foulquier et al., 2015). Nevertheless, little is known about the effect of moisture on decomposition and some
55 authors have highlighted the importance of assessing this process at different stages of drying to better
56 understand the mechanisms involved (e.g., Larned et al., 2010).

57 In isolated pools, decomposer communities are exposed to harsh physicochemical conditions. In these
58 habitats, detritus accumulation increases nutrient concentrations and stimulates heterotrophic activity that causes
59 a gradual decrease in oxygen levels and pH (Stanley et al., 1997; von Schiller et al., 2011). Moreover, the
60 accumulation of leaf leachates in pools may be toxic for some decomposer communities (Schlief and Mutz,
61 2007; Canhoto et al., 2013). Though only a few studies have evaluated leaf litter decomposition in pools, they
62 reported lower decomposition rates in comparison to running waters and related it to conditions unfavourable to
63 the development of aquatic fungi and shredders (Baldy et al., 2002; Schlief and Mutz, 2009). However, studies
64 of leaf litter decomposition in pools have been typically carried out in microcosms because the unpredictable
65 dynamics of temporary streams make it difficult to develop a larger scale experimental framework.

66 This heterogeneity of detritus dynamics along fragmented streams may also have implications for other
67 ecological processes that rely on the products of decomposition. Leaf litter leachates supply a large proportion of
68 riverine dissolved organic carbon (DOC), an essential component of the carbon cycle that fuels stream
69 metabolism (Meyer et al., 1998). Previous studies in temporary streams have found changes of the dynamics of
70 DOC in relation to the hydrology (Vázquez et al., 2015; von Schiller et al., 2015); however, the contribution of
71 leaf litter as a DOC source and its implications for the carbon cycle in these systems remains largely unexplored
72 (but see Casas-Ruiz et al., *in press*). Moreover, the retention of organic matter during flow fragmentation may be
73 an important source of carbon and nutrients for downstream reaches when flow returns (Ylla et al., 2010). In this
74 way, the different biotic and abiotic factors involved in organic matter decomposition during flow fragmentation
75 may determine the availability of organic resources when flow connectivity is re-established, thereby influencing
76 ecosystem's metabolism and related food webs (Riedl et al., 2013; von Schiller et al., 2015).

77 Despite the abundance of temporary streams and their predicted increase due to climate change (Larned et al.,
78 2010), our knowledge of the dynamics of ecosystem processes such as litter decomposition in these systems is
79 still limited. This limits our capacity to understand ecosystem processes in a large proportion of fluvial networks

80 worldwide, hindering the accurate assessment of ecological conditions and preventing the effective management
81 of these systems (Acuña et al., 2014; Arthington et al., 2014; Leigh et al., 2015).

82 The aim of our study was to assess the initial phases of leaf litter decomposition within different habitats
83 resulting from flow fragmentation in a temporary Mediterranean stream: running waters, isolated pools and
84 moist and dry streambed sediments. We used litter bags containing *Populus nigra* L. leaves to examine
85 decomposition rates, carbon to nitrogen molar ratios, microbial biomass, macroinvertebrate abundance and DOC
86 release rates associated with leaves in each habitat type over a period of 11 days in late summer. A short
87 incubation period was set due to the importance of the first stages of decomposition in the release of DOC from
88 the litter (Yoshimura et al., 2010) and to avoid a drastic shift in habitat conditions during the experiment. We
89 hypothesized that higher decomposition rates would be observed in aquatic than in terrestrial habitats because
90 microbial decomposers and detritivores would be more abundant under aquatic conditions. Regarding aquatic
91 habitats, we expected lower decomposition rates in isolated pools than in running waters because the absence of
92 flow in pools promotes harsh conditions for shredders and aquatic fungi. Regarding terrestrial habitats, we
93 anticipated moisture level to be an important factor driving leaf litter decomposition, with higher microbial
94 biomass and, thus, higher decomposition rates in moist than in dry sediments. Finally, we predicted a more
95 pronounced decrease in the DOC release rate during the decomposition process in aquatic habitats compared to
96 terrestrial habitats because of the utilization of DOC by microbial communities associated with leaf litter and the
97 effect of leaching in aquatic habitats.

98 **2. Material and Methods**

99 *2.1. Study site*

100 This experiment was conducted on the Fluvià River, located in the north-eastern Iberian Peninsula. Its
101 watershed drains an area of 990 km² and has a mainstem that is 97 km long and flows into the Mediterranean
102 Sea. The watershed is characterized by a Mediterranean climate, with dry, warm summers and scarce
103 precipitation occurring primarily in the spring and autumn.

104 In the upper part of this watershed, we selected an experimental reach of approximately 2 km along a fourth-
105 order stream (Strahler, 1957). The reach included both a permanent and a temporary section. The reach is
106 situated between 42°8'33.61''N; 2°26'59.83''E and 42°7'35.54''; 2°26'59.83''E with the temporary section

107 located in the upstream portion of this range. The permanent and temporary sections were selected as close as
108 possible to minimize the potential interference of other environmental variables different from hydrology in our
109 response variable. The land cover in the sub-watershed associated with the study reach is comprised primarily of
110 forest (78%), with some agricultural (19%) and urban (3%) areas (Land Cover Map of Catalonia 2009, CREAM).
111 The riparian vegetation along the reach is dense and dominated by *Populus nigra*, *Fraxinus angustifolia* and
112 *Platanus hispanica*.

113 The experiment was carried out during the August-September 2013 summer drought, when the temporary
114 stream section was fragmented into isolated pools of different sizes and exposed streambed sediments with
115 different moisture levels. Along this section, we selected three replicate sites for each of the following habitat
116 types: isolated pools, moist sediments and dry sediments. In the isolated pools, the wetted area ranged from 12 to
117 20 m² and the maximum depth from 30 to 80 cm, as measured on the first sampling date. At the streambed
118 sediment sites, we identified three points that were completely dry and three points that were moist. We also
119 selected three running water sites from the permanent stream section. Water flowed at a constant rate
120 continuously during the experiment, with a discharge of 0.03 m³ s⁻¹, a maximum wetted width of 5 m and a
121 maximum depth of 20 cm.

122 During the study period (29 August -9 September 2013), the average daily air temperature was approximately
123 23°C (min. 11°C - max. 29°C) and the accumulated precipitation was 17.3 mm (Meteorological Service of
124 Catalonia, METEOCAT). This occurred in a single precipitation event just two days before the experiment
125 ended.

126 2.2. Habitat characteristics

127 A WTW multi-parametric sensor (Weilheim, Germany) was used to measure water temperature (accuracy of
128 $\pm 0.1^\circ\text{C}$), conductivity (accuracy of $\pm 1 \mu\text{S cm}^{-1}$), pH and the dissolved oxygen concentration (accuracy of ± 0.1
129 mg L^{-1}) in the permanent section and isolated pools three times during the study period. Current velocity and
130 discharge were also determined in situ. Water samples were taken in triplicate from each of these aquatic
131 habitats on the third sampling day (after 8 days of leaf incubation) for nutrient analyses. Water was filtered
132 through nylon membrane filters (0.45 μm pore size; Millipore, USA), transported to the laboratory under cool
133 conditions, and stored at -20°C in the dark until analysis. The concentrations of dissolved nitrite, nitrate,
134 ammonium, soluble reactive phosphorus (SRP), chloride, sulphate, calcium and sodium were analysed using

135 ionic chromatography (IC5000, DIONEX, USA) with an average accuracy of $\pm 2.8\%$ at 1 ppm. The dissolved
136 organic and inorganic carbon concentrations in water (DOC and DIC, respectively) were measured using a total
137 organic carbon analyser (TOC-V CSH, Shimadzu, Japan) that had an accuracy of $\pm 4.4\%$ at 5 ppm.

138 In the moist and dry sediments, soil temperature and moisture were measured in triplicate at each replicate
139 site using portable sensors (ECH2O 10HS, Decagon, USA and Hi93500, Hanna, USA) on three sampling dates
140 (4, 8 and 11 days of leaf incubation).

141 2.3. Leaf decomposition

142 Black poplar (*Populus nigra* L.) leaves were collected just after abscission in the fall of 2012. Leaves were
143 air-dried to constant weight and stored at room temperature until needed. Portions of 3.27 ± 0.01 g (mean \pm SE)
144 of these leaves were weighed, moistened with distilled water using a garden atomizer, and enclosed in coarse-
145 mesh nylon bags (15 x 20 cm, 5 mm mesh openings).

146 During the August-September 2013 summer drought, a total of 12 leaf bags were placed in each habitat type
147 (4 bags x 3 replicate habitat type). At the running water and isolated pool sites, bags were tied with nylon lines to
148 iron bars. At the streambed sediment sites, bags were put in contact with the substrate and secured using tent
149 pegs and rocks. One litter bag was retrieved from each habitat replicate 1, 4, 8 and 11 days after the experiment
150 started. The litter bags were then placed in individual plastic bags and transported in cool conditions to the
151 laboratory, where they were immediately processed. Leaf material from each bag was rinsed with distilled water
152 over a 500 μ m sieve to remove invertebrates and inorganic particles. Invertebrates remaining on the sieve were
153 preserved in 70% ethanol for later counting and identification to the lowest-feasible taxonomic level.
154 Macroinvertebrate abundance was expressed as the number of individuals (Ind.) per unit of ash-free dry mass
155 (AFDM) of leaf litter. Just after cleaning the leaves, 5 leaf discs from each bag (12 mm diameter) were cut with a
156 cork borer to determine the DOC release rate (see below). On all sampling dates except day 1, another set of 3
157 discs per bag were obtained to determine bacterial biomass (see below). These discs were preserved in vials with
158 10 ml of distilled water and 0.5 ml of 37% formalin at 4°C until analysed. On the last sampling date, one set of
159 10 discs from each bag was divided into small plastic bags and frozen at -80°C to obtain fungal biomass by
160 ergosterol determination (see below).

161 The remaining material was oven-dried (60°C, 72 h) and weighed to determine dry mass. An extra set of 5
162 leaf discs from each habitat type and for each sampling date was dried in the same way as the remaining material
163 to estimate the mass used in the microbial biomass and DOC release determinations. Then, a subsample of each
164 dried material sample was incinerated (450°C, 5 h) to remove the inorganic components and to obtain the
165 AFDM. The results were expressed as a percentage of the remaining initial AFDM. The initial AFDM was
166 determined from an extra set of 5 leaf bags that were transported into the field and returned to the laboratory on
167 the same day. These bags were processed as described above to create a conversion factor between the initial air-
168 dry mass and the initial AFDM, taking into account manipulation losses.

169 Another subsample of dried material was ground into a fine powder (~ 1 mm pore size), and the nitrogen (N)
170 and carbon (C) concentrations were analysed. Both elements were determined using a Perking Elmer series II
171 CHNS/O elemental analysis. The results were expressed in terms of C: N molar ratios. We also obtained the C:
172 N molar ratio from non-incubated leaves in the field.

173 2.4. Microbial biomass

174 2.4.1. Fungal biomass

175 To calculate the fungal biomass at each site, frozen leaf discs were lyophilized, weighed to determine the dry
176 mass and used in the ergosterol analyses (Gessner, 2005). Lipid extraction and saponification were performed
177 using KOH methanol 0.14 M (8 g L⁻¹) at 80°C for 30 min in a shaking bath. Extracted lipids were purified using
178 solid-phase extraction cartridges (Waters Sep-Pak®, Vac RC, 500 mg, tC18 cartridges, Waters Corp., Milford,
179 MA, USA), and ergosterol was eluted using isopropanol. Ergosterol was detected and quantified via high
180 pressure liquid chromatography (HPLC) by measuring absorbance at 282 nm. A Jasco HPLC system (USA)
181 equipped with a Gemini-NX 5 µm C18 250 x 4.6 mm column (Phenomenex, UK) was used. The mobile phase
182 was 100% methanol and the flow rate was set to 1.2 ml min⁻¹. Ergosterol was converted to fungal biomass using
183 a conversion factor of 5.5 mg ergosterol per gram of fungal mycelium (Gessner and Chauvet, 1993). The results
184 were expressed in mg of fungal biomass per unit of leaf litter AFDM.

185 2.4.2. Bacterial biomass

186 For bacterial biomass, one leaf disc of each fixed sample was transferred to a new glass vial filled with 10 ml
187 of pure water previously filtered through 0.2-µm pore size nitrate cellulose membranes (Whatman, Germany).

188 Samples were mixed with a vortex and then sonicated for 15 min at low power (10 W) using an ultrasonic
189 homogenizer (Sonic Ruptor 250, Omni International) to detach bacteria from the leaves. During sonication,
190 samples were kept on ice to minimize cell damage. After appropriate dilution, bacterial suspensions were stained
191 with DAPI (4, 6-diamidino-2-phenylindole hydrochloride; Sigma-Aldrich, Germany) for 5 min in the dark to a
192 final concentration of $2 \mu\text{g ml}^{-1}$. Then, the suspensions were filtered through polycarbonate membranes (0.2 μm
193 pore size; Whatman, Germany) and the filters were mounted on a slide between two drops of immersion oil.
194 Bacteria were counted using an epifluorescence microscope (Olympus BX-60, 100x objective and UV
195 excitation/long-pass filter set). From each filter, a minimum of 200 bacterial cells were counted in at least 20
196 random fields. The bacterial biomass was estimated in terms of carbon at $2.2 \times 10^{-3} \text{ g C } \mu\text{m}^{-3}$ (Bratbak and
197 Dundas, 1984) and considering a bacteria cell biovolume in *Populus nigra* of $0.147 \mu\text{m}^3$ (Gaudes et al., 2009).
198 The results were expressed as mg of bacterial biomass per unit of leaf litter AFDM.

199 2.5. DOC release rate

200 Leaf discs were incubated in 100 ml Erlenmeyer flasks with 50 ml filtered stream water (0.45 μm pore size,
201 Nylon membrane; Millipore, USA) in a shaker (60 rpm) for 48 h at the stream's water temperature. Flasks were
202 covered with perforated aluminium foil to allow air exchange. After 48 h, 30 ml of 0.45- μm filtered water was
203 taken from each microcosm, acidified and stored in pre-combusted vials at 4°C for DOC determination using a
204 total organic carbon analyser (TOC-V CSH, Shimadzu, Japan). The stream water used in the microcosms was
205 collected from the running water habitat on each sampling date, during which time three 30 ml aliquots were also
206 sampled for initial DOC determination. Obtained DOC release rates corresponded to the net release of DOC
207 (production – consumption) in the water during the incubation time, as corrected using the initial DOC
208 concentration in the stream water at the beginning of the incubation (Baldy et al., 2007). Values were expressed
209 as mg of carbon produced per gram of leaf litter AFDM per day.

210 2.6. Data analyses

211 Physicochemical characteristics were compared within aquatic (running waters and isolated pools) and
212 terrestrial (moist and dry sediments) conditions using Student's test.

213 Leaf litter decomposition rates were calculated assuming an exponential decay. Linear regressions between
214 the ln-transformed proportion of AFDM remaining and time were used to estimate the decomposition rate given
215 by the slope. To compare the slopes of these regressions between habitats, we performed an analysis of

216 covariance (two-way ANCOVA) using the ln-transformed proportion of AFDM remaining as a dependent
217 variable, time as a covariate and habitat as a categorical variable. Subsequent pair-wise comparisons were
218 performed using Tukey's Honest Significant Difference (HSD) test.

219 Bacterial biomass, the C: N molar ratio, macroinvertebrate abundances and leaf litter DOC release rates were
220 compared among treatments using a two-way analysis of variance (ANOVAs) with time and habitat as
221 categorical variables. For macroinvertebrate abundance and DOC release rates, subsequent pair-wise
222 comparisons were performed using Tukey's HSD test. For the C: N molar ratio and bacterial biomass two
223 samples lost during processing unbalanced the data; therefore, we applied the Tukey-Kramer HSD post-hoc test
224 to these data. To meet assumptions for normality and equality of variances, bacterial biomass and DOC release
225 rate data were ln-transformed and macroinvertebrate abundances were $\log_{10} + 1$ transformed. In the case of
226 fungal biomass, the data were $\log_{10} + 1$ transformed and compared between habitats using a one-way ANOVA
227 and Tukey's HSD test.

228 We used Pearson's correlation to test for potential relationships among variables and a simple linear
229 regression to build predictive models of these relationships.

230 Before the statistical analysis, the distributional properties of the data were assessed to identify outliers. The
231 Shapiro-Wilk test was applied to assess normality and the Bartlett's test to assess equality of variances. All
232 statistical analyses were conducted using R version 2.15.3 (R Core Team 2013), with a significance level set at
233 $p < 0.05$ for all tests.

234 **3. Results**

235 *3.1. Physical and chemical characteristics of habitats*

236 In the streambed sediments, temperature remained stable at an average $17.9 \pm 0.4^\circ\text{C}$ during the study period,
237 without significant differences between the moist and dry sediments (t-test, $t=0.61$, $p=0.54$). By contrast, soil
238 moisture differed between these habitats (t-test, $t=-5.07$, $p<0.001$), with an average soil water content of $19.6 \pm$
239 2.5% in moist sediments and $4.3 \pm 1.7\%$ in dry sediments. In the dry sediments, soil water content reached a
240 minimum value of 0% during the third sampling and a maximum of 13.1% on the last sampling date, which may
241 have been related to a precipitation event (17.3 mm) that occurred just two days before the end of the
242 experiment. In the moist sediments, the maximum moisture level (27.3%) was registered during the second

243 sampling date, when the soil water content across all replicated sites averaged $25.0 \pm 1.3\%$ and we found the
244 greatest difference for dry sediments ($6.4 \pm 3\%$).

245 Regarding the aquatic habitats (Table 1), water temperature differed between the running water and isolated
246 pool sites (t-test, $t=4.66$, $p=0.01$), with slightly higher values in the isolated pools ($17.8 \pm 0.3^\circ\text{C}$) than in the
247 running waters ($16.0 \pm 0.3^\circ\text{C}$). Dissolved oxygen concentrations were lower (t-test, $t=-3.0$, $p=0.04$) in isolated
248 pools ($3.4 \pm 1.3 \text{ mg l}^{-1}$) than in running waters ($7.7 \pm 0.5 \text{ mg l}^{-1}$). We found high variability in dissolved oxygen
249 content between pools, with percent saturation ranging between 8% and 58%. No current was found in isolated
250 pools, whereas running water sites had a permanent flow during the experiment. Nutrient levels differed sharply
251 between aquatic habitats. DOC (t-test, $t=25.2$, $p<0.001$), nitrite (t-test, $t=9.5$, $p<0.001$), ammonium (t-test,
252 $t=328.1$, $p<0.001$) and SRP (t-test, $t=29.9$, $p<0.001$) had significantly higher concentrations in isolated pools
253 than in running waters. On the contrary, DIC (t-test, $t=-14.4$, $p<0.001$), nitrate (t-test, $t=-144.9$, $p<0.001$),
254 chloride (t-test, $t=-14.1$, $p<0.001$), sulphate (t-test, $t=-14.3$, $p<0.001$), sodium (t-test, $t=-17.8$, $p<0.001$) and
255 calcium (t-test, $t=-6.0$, $p<0.001$) concentrations were significantly lower in isolated pools than in running waters.

256 3.2. Decomposition rates

257 Decomposition dynamics differed significantly between habitats during the study period (ANCOVA, Time x
258 Habitat, $F_{3,40}=12.71$, $p<0.0001$), with a clear distinction between aquatic and terrestrial habitats (Tukey's test,
259 $p<0.01$; Fig. 1A). After 24 h of incubation, the percentage of mass loss (as AFDM) in running waters was $13.7 \pm$
260 1.2% , and in isolated pools it was 17.9 ± 2.1 . By contrast, a mass loss of $3.1 \pm 1.5\%$ was measured in moist
261 sediments on the first sampling date, and a mass loss of only 1.8 ± 0.9 was measured in dry sediments. On the
262 following dates, mass loss in the aquatic habitats continued to be more pronounced than in sediments. Moist
263 sediments presented a constant mass loss over time, with a final remaining AFDM percentage of $84.6 \pm 2.6\%$.
264 The mass loss from the dry sediments was practically non-existent during the first 8 days ($2.4 \pm 1.2\%$ AFDM
265 loss) but increased thereafter until reaching a remaining AFDM percentage of $87.4 \pm 3.5\%$, which was probably
266 related to the precipitation event. Running waters had a slightly higher decomposition rate ($0.053 \pm 0.003 \text{ d}^{-1}$;
267 $R^2=0.95$, $p<0.001$) than isolated pools ($0.044 \pm 0.005 \text{ d}^{-1}$, $R^2=0.88$, $p<0.001$), although this difference was not
268 statistically significant. As with aquatic habitats, no significant differences were found in the rates between
269 streambed sediments, despite a higher decomposition rate in the moist ($0.013 \pm 0.001 \text{ d}^{-1}$, $R^2=0.89$, $p<0.001$)
270 than in the dry sediments ($0.009 \pm 0.002 \text{ d}^{-1}$, $R^2=0.66$, $p<0.001$).

271 Taking into account decomposition dynamics across all habitats conforming the temporary section (isolated
272 pools, moist and dry sediments), we obtained a global decomposition rate ($-0.021 \pm 0.002 \text{ d}^{-1}$, $R^2=0.95$, $p<0.001$)
273 lower than that of the permanent section (running waters; $0.053 \pm 0.003 \text{ d}^{-1}$, $R^2=0.95$, $p<0.001$).

274 3.3. Microbial biomass on leaf litter

275 3.3.1. Fungal biomass

276 After 11 days of incubation, the amount of fungal biomass colonizing the leaves was significantly different
277 between habitats (ANOVA, Habitat, $F_{3,8}=7.98$, $p=0.008$). Running waters presented the highest colonization
278 rates, with an average value of $91.6 \pm 21.9 \text{ mg}$ of fungal biomass per g^{-1} AFDM, whereas isolated pools had the
279 lowest values ($1.5 \pm 1.2 \text{ mg g}^{-1}$ AFDM). Fungal biomass was higher in moist ($15.9 \pm 2.4 \text{ mg g}^{-1}$ AFDM) than in
280 dry ($11.8 \pm 6.1 \text{ mg g}^{-1}$ AFDM) sediments. Subsequent post-hoc comparisons revealed that fungal colonization
281 was significantly different from running waters only in isolated pools (Tukey's test, $p<0.05$).

282 3.3.2. Bacterial biomass

283 Bacterial biomass on leaf litter varied significantly between habitats over time (ANOVA, Time x Habitat,
284 $F_{6,22}=8.80$, $p<0.001$; Fig. 2). Bacterial colonization was much higher in aquatic than in terrestrial habitats (Tukey
285 Kramer's test, $p<0.001$), with a maximum value of $0.71 \pm 0.22 \text{ mg g}^{-1}$ AFDM at running waters and a minimum
286 value of $0.02 \pm 0.005 \text{ mg g}^{-1}$ AFDM in dry sediments after 11 days of incubation. No significant differences in
287 bacterial biomass were found between aquatic habitats during the process; however, bacterial colonization in
288 isolated pools increased faster than in running waters, reaching a maximum value of $0.28 \pm 0.03 \text{ mg g}^{-1}$ AFDM
289 after 8 days of incubation. In running waters, we found the highest bacterial biomass after 11 days of incubation.
290 Regarding streambed sediments, post-hoc comparisons revealed significant differences in bacterial colonization
291 between moist and dry sediments (Tukey's Kramer test, $p=0.012$). These differences appeared clearly only after
292 4 days of incubation, when the bacterial biomass presented in moist sediments was 3 times higher than that in
293 dry sediments. Both habitats had maximum colonization rates on the last sampling date ($0.03 \pm 0.004 \text{ mg g}^{-1}$
294 AFDM in moist and $0.02 \pm 0.005 \text{ mg g}^{-1}$ AFDM in dry sediments).

295 After 11 days of incubation, bacterial biomass in isolated pools represented 12.9% of the total microbial
296 biomass (fungal and bacterial biomass) associated with leaves. This value was 0.8% for running waters, 0.2% for
297 moist sediments and 0.1% for dry sediments.

298 3.4. Macroinvertebrate abundance

299 Total macroinvertebrate abundance associated with leaf litter was significantly higher in aquatic than in
300 terrestrial habitats (ANOVA, Habitat, $F_{3,32}=56.99$, $p<0.001$; Tukey's test, $p<0.001$; Fig. 3). Macroinvertebrate
301 abundance in aquatic habitats increased gradually until the end of the experiment. No significant differences
302 were found in total macroinvertebrate density between isolated pools and running waters, but total abundance
303 varied considerably between pools (Fig. 3, insert), a fact likely related to the marked differences in oxygen
304 availability between sites. Approximately 90% of the macroinvertebrates in isolated pools were scrapers of the
305 genus *Physa*, whereas this proportion was lower in running waters (41%). Shredders accounted for less than 1%
306 to total macroinvertebrate abundance in both aquatic habitats.

307 3.5. C: N molar ratio

308 The elemental composition of the decomposing leaf litter varied significantly between habitats during the
309 study period (ANOVA, Time x Habitat, $F_{9,30}=2.83$, $p=0.015$; Fig. 1B). The C: N molar ratio decreased over time
310 in all habitats (ANOVA, Time, $F_{3,30}=18.63$, $p<0.001$) compared to the initial value of 110.5 ± 5.4 found on litter
311 not incubated in the field, a result related to an increase in nitrogen while carbon concentrations remained
312 constant. The lowest values were detected at running water sites (35.9 ± 2.6) after 11 days of incubation. In this
313 habitat, leaf quality differed significantly from that in dry and moist sediments (Tukey Kramer's test, $p<0.001$)
314 but not from that in isolated pools (Tukey Kramer's test, $p=0.076$). Significant differences in the C: N molar
315 ratio were also found between isolated pools and dry sediments (Tukey Kramer's test, $p=0.002$).

316 In aquatic habitats, we found a significant negative correlation between the percentage of AFDM lost and the
317 C: N molar ratio (Pearson's correlation, $r=-0.92$, $p<0.001$), which decreased in a significant linear relationship
318 (linear regression, $R^2=0.85$, $p<0.0001$; Fig. 4A) as decomposition progressed. This correlation was higher in
319 running waters (Pearson's correlation, $r=-0.97$, $p<0.001$) than in isolated pools (Pearson's correlation, $r=-0.81$,
320 $p<0.001$) and was not observed in dry or moist sediments (Pearson's correlation, $r=-0.27$, $p=0.197$; Fig. 4B).

321 3.6. DOC release rate from litter

322 DOC release rates from leaf litter differed significantly between habitats over time (ANOVA, Time x
323 Habitat, $F_{9,32}=2.70$, $p=0.018$; Fig. 1C). After 11 days of incubation, all habitats presented lower DOC release
324 rates than the initial value of 57.5 ± 8.4 mg C g⁻¹ AFDM day⁻¹ in litter not incubated in the field. However, the

325 decrease in DOC release rates followed different patterns in aquatic and terrestrial habitats (Tukey's test,
326 $p < 0.001$). A more pronounced decrease occurred in aquatic habitats, where the DOC release rate fell to $10.2 \pm$
327 $2.2 \text{ mg C g}^{-1} \text{ AFDM day}^{-1}$ in running waters and $6.2 \pm 0.9 \text{ mg C g}^{-1} \text{ AFDM day}^{-1}$ in isolated pools after only 4
328 days in the field. In terrestrial habitats, we observed a more constant decrease in the DOC release rate over time,
329 without significant differences between moist and dry sediments. After 11 days of incubation, the DOC release
330 rate was $19.7 \pm 7.6 \text{ mg C g}^{-1} \text{ AFDM day}^{-1}$ in moist sediments and $21.2 \pm 8.6 \text{ mg C g}^{-1} \text{ AFDM day}^{-1}$ in dry
331 sediments. In the case of dry sediments, we observed a drastic decrease in the DOC release rate from litter on the
332 last sampling date, which may be related to the precipitation event that occurred two days earlier.

333 Across all habitats, we found a negative correlation between DOC released rates and the proportion of
334 AFDM lost (Pearson's correlation, $r = -0.89$, $p < 0.001$). This correlation followed a linear relationship with
335 AFDM lost and explains 80% of the variation in DOC release rates (linear regression, $R^2 = 0.80$, $p < 0.001$; Fig. 5).
336 At the same time, the DOC release rates in aquatic habitats correlated positively with the C: N molar ratio
337 (Pearson's correlation, $r = 0.73$, $p < 0.001$). However, this correlation was found to be higher in running waters
338 (Pearson's correlation, $r = 0.85$, $p < 0.001$) than in isolated pools (Pearson's correlation, $r = 0.61$, $p = 0.04$). This
339 correlation was not significant in moist or dry sediments (Pearson's correlation, $r = 0.39$, $p = 0.055$).

340 4. Discussion

341 In temporary streams, the decomposition of organic matter tends to be a slower process than in perennial
342 ones (e.g., Herbst and Rice, 1982; Richardson, 1990) basically because processing efficiency is reduced during
343 drought events (Maamri et al., 1997; Pinna and Basset, 2004). However, spatial and temporal habitat
344 heterogeneity caused by flow fragmentation during these events creates disparate ecological conditions that may
345 result in a highly heterogeneous decomposition process. To assess this hypothesis, we analysed the initial stages
346 of leaf litter decomposition in different habitats resulting from flow fragmentation. After only 11 days of
347 incubation, we detected widely different decomposition rates among habitats, distinguishing faster rates for
348 leaves exposed to aquatic (isolated pools and running waters) than terrestrial (moist and dry sediments)
349 conditions. These results are consistent with previous studies (Langhans et al., 2008; Datry et al., 2011) and can
350 be primarily attributed to the fact that decomposer communities are negatively affected by emersion (Corti et al.,
351 2011; Foulquier et al., 2015; Martínez et al., 2015). However, we also found differences in factors controlling

352 leaf litter decomposition within aquatic and terrestrial conditions, suggesting a high heterogeneous
353 decomposition process that is not detectable within general (i.e., per condition) decomposition rates.

354 In aquatic habitats, contrary to our expectations, isolated pools showed similar decomposition rates to
355 running waters. Using *Populus nigra* leaves, Langhans et al. (2008) found an opposing trend, with higher rates in
356 the river channel than in pools after three months of incubation in a floodplain. Along the same lines, Schlieff and
357 Mutz (2009) simulated isolated pools using mesocosms and found slower decomposition rates of *Alnus glutinosa*
358 under reduced flow conditions. In these studies, a greater contribution of shredders to litter decomposition in
359 streams than in stagnant waters was a key factor explaining these differences. In our study, however,
360 macroinvertebrate abundance in litter bags was similar among aquatic habitats, and the relative abundance of
361 shredders was insignificant. This lack of shredders in the study reach may be related to the fact that sampling
362 was performed in late summer, when the presence of shredders is practically non-existent in Mediterranean
363 streams (Muñoz, 2003; Sabater et al., 2006). Thus, shredder feeding did not appear to play an important role in
364 leaf litter decomposition in our study. In contrast, our results were in line with Baldy et al. (2002), who found
365 similar decomposition rates for *Populus nigra* at the river channel and in a floodplain pond despite differences in
366 the microbial communities colonizing the leaves. Similarly, we found a much lower fungal biomass in pools than
367 in running waters after only 11 days of incubation. As previous studies have already described (Langhans et al.,
368 2008; Schlieff and Mutz, 2009), the reduced presence of fungi in our isolated pools may be attributed to the
369 absence of flow. A decrease in water flow limits fungal colonization because flow stimulates the sporulation
370 process (Webster and Towfik, 1972; Maamri et al., 2001) and supplies a continuous source of fungal spores to
371 detritus (Bärlocher, 1992). Furthermore, reduced flow also limits re-aeration and allows the accumulation of
372 detritus in pools, causing a reduction in oxygen and an increase in leaf leachates that can hinder fungal
373 development in these habitats (Schlieff and Mutz, 2007; Medeiros et al., 2009; Canhoto et al., 2013). Similarly,
374 our study pools exhibited high DOC concentrations, and the oxygen concentrations observed therein were lower
375 than in running waters.

376 Based on our results, bacterial biomass was similar in running water and isolated pool sites, which is in
377 accordance with the results described by Baldy et al., (2002). Physicochemical conditions in pools were likely
378 not so unfavourable to bacteria, as in the case of fungi. For example, Pascoal and Cássio (2004) reported an
379 increase in bacterial production in polluted sites, and Eiler et al. (2003) found that bacterial metabolism was
380 stimulated by large amounts of DOC in batch cultures. Along these lines, a high availability of DOC and other
381 nutrients could drive the rapid bacterial development observed in pools after just 8 days of incubation. This

382 development may also be enhanced by the large abundance of scrapers found in pools because scrapers are able
383 to stimulate microbial growth and activity through their feeding practices (Suberkropp, 1992).

384 Our results revealed a decrease in the C: N molar ratio in leaf litter during decomposition as a result of a
385 gradual increase in nitrogen that occurred while carbon concentration remained practically constant. Other
386 studies have found a similar pattern (e.g., Gulis and Suberkropp, 2003; Pascoal and Cássio, 2004; Menéndez et
387 al., 2011) and have associated it with the accumulation of microbial biomass on leaves that uptake and
388 immobilize nitrogen from the water column as carbon is mineralized (Melillo et al., 1984; Chauvet, 1987). The
389 mineralized litter carbon is replaced by microbial cells, which explains the small changes observed in carbon
390 concentration (Yoshimura et al., 2010). Along these lines, the mass loss during leaf decomposition was
391 negatively related to the C: N molar ratio in aquatic habitats, suggesting that microbial decomposers played an
392 active role in these habitats. However, a less pronounced decrease in the C: N molar ratio and a lower correlation
393 with mass loss observed in isolated pools than in running waters may indicate that microbial development and
394 activity was constrained in pools, a fact already apparent in the relatively low bacterial biomass observed at the
395 end of the incubation period and in the low presence of fungi found in this habitat. This constraint also suggests
396 that decomposition rates in isolated pools may have been lower than in running waters if a longer incubation
397 time was allowed.

398 Terrestrial habitats showed litter decomposition rates up to four times slower than in aquatic habitats, as well
399 as a much lower macroinvertebrate and bacterial biomass presence. Similar decomposition rate was recorded in
400 exposed streambed sediments, with slightly faster rates observed in the moist sediments. However, bacterial
401 biomass differed between habitats and reached higher values in leaves exposed in moist than in dry sediments.
402 These differences were larger after four days of incubation coinciding with the highest levels of soil water
403 content registered in the moist sediment sites. Previous studies have reported a positive effect of soil moisture on
404 mass loss (Cortez, 1998; Lee et al., 2014), which has mainly been attributed to the more active role microbial
405 decomposers play under wet conditions (Amalfitano et al., 2008; Manzoni et al., 2012). A decrease in moisture
406 availability results in the physiological stress of microbial communities because it constrains their osmotic
407 regulation and the diffusive transport of solutes in soil (Borken and Matzner, 2009). In contrast, we found similar
408 fungal biomass on leaf litter in moist and dry sediments after 11 days of incubation with values in agreement
409 with Langhans and Tockner (2006). These findings may be related to the fact that fungi are generally more
410 resistant to desiccation than bacteria, likely because their hyphal development facilitates the search for water and
411 nutrients (Yuste et al., 2011; Barnard et al., 2013). Nevertheless, and contrary to aquatic habitats, we did not find

412 any relationship between the C: N molar ratio and mass loss in sediments, indicating the limited role microbial
413 decomposers played in these habitats.

414 Thus, based on our results, the initial phase of leaf litter decomposition in exposed streambed sediments was
415 primarily driven by abiotic factors, whereas biotic factors were probably not important at the time scale of our
416 study. Steward et al. (2012) reviewed the ecology of dry streambeds and described an increase in the relative
417 importance of abiotic mineralization processes in these systems. Austin and Vivanco (2006) also suggested the
418 limited influence of biotic activity in leaf litter decomposition under semi-arid conditions and noted the
419 relevance of photo-degradation as an important driver of decomposition. Leaves on the sediment surface are
420 exposed to high solar radiation that promotes direct photochemical mineralization and also facilitates litter
421 biodegradability (Gallo et al., 2006; Wang et al., 2015; Almagro et al., 2015). Together with this process, the
422 negligible mass loss observed in streambed sediments also could be attributed to the leaching of water-soluble
423 compounds (Langhans et al., 2008). A high moisture level may have enhanced leaching in our study, causing the
424 slightly higher mass loss observed in moist than in dry sediments.

425 By the action of these biotic and abiotic factors involved in decomposition, a significant part of leaf mass is
426 transformed and released as DOC (Baldy et al., 1997). In our microcosms, DOC release rates obtained at each
427 sampling time represent the integration of all the processes of DOC production and loss from leaves occurring
428 inside each microcosm during the 48 h incubation period. Rates are the net balance between the DOC produced
429 by leaching and microbial degradation of leaves, and the DOC lost via microbial assimilation and physical
430 adsorption (Baldy et al., 2007; Yoshimura et al., 2010). The importance of each of these processes during
431 incubation in each microcosm was influenced by leaf preconditioning within each habitat type; and indeed, we
432 observed clear differences in the DOC release rates obtained from leaves previously incubated under aquatic or
433 terrestrial conditions. Under aquatic conditions, leaching promotes a substantial release of DOC just after
434 immersion that could account for an important decrease (10-30%) in initial mass within the first days (Petersen
435 and Cummins, 1974). As a result of this process, leaves exposed to running waters and isolated pools
436 experienced an important reduction in their DOC release rate within a few days. Then, despite the fact that
437 leaching presumably continued (Gessner et al., 1999), microbial processes likely mediated the DOC dynamics
438 (Fischer et al., 2006) and the balance between DOC production and consumption in the microcosms maintained
439 rates constant over time. By contrast, DOC release rates from leaves exposed to sediments remained high during
440 the first week, suggesting that a small amount of DOC had been previously released or assimilated from leaves
441 in the field. Contrary to the aquatic habitats, leaching under terrestrial conditions in the field was minimal over

442 our timescale (Treplin and Zimmer, 2012), as already shown with the irrelevant decrease in initial leaf mass
443 within the first days. The small differences observed between these rates and the initial DOC release rate
444 obtained from leaves not incubated in the field could be due to an additional DOC release caused by rinsing the
445 leaves with distilled water to remove invertebrates and inorganic particles after sampling. At the last sampling
446 date, however, we observed a decline in the DOC release rate, which coincided with an increase in mass loss
447 occurring in sediments after the precipitation event. This decrease was especially dramatic in dry sediments, in
448 accordance with Dieter et al. (2011), who suggested that leaf desiccation promotes greater leaching losses at first
449 water contact.

450 Summarizing all these processes, we found a negative relationship between the proportion of leaf mass lost in
451 the field and the DOC release rate obtained in the microcosms across all habitats and times. This relationship
452 evidences a decrease in the capacity to release DOC as leaves decompose, suggesting differences in the potential
453 of leaves as a DOC source during flow fragmentation and, presumably, when flow is re-established. Previous
454 studies have described an increase in DOC concentrations during the rewetting phase (e.g., von Schiller et al.,
455 2015; Vázquez et al., 2015) due to the amount of organic matter retained in the stream bed during drought
456 events. Along the same lines, we detected that the decomposition degree of this organic matter determines its
457 remaining potential as a DOC source, which is higher for leaves exposed in dry streambeds.

458 **5. Conclusions**

459 When leaves fall into a streambed during flow fragmentation, the heterogeneity of habitats governs the
460 differences in factors affecting the decomposition of this litter. Under aquatic conditions, we found that initial
461 phases of decomposition of *Populus nigra* leaves were marked by an intense leaching just after immersion and
462 early microbial colonization, which swiftly began to mineralize the litter. By contrast, under terrestrial conditions
463 leaves were mainly affected by abiotic processes that caused small mass losses. As a result, leaves in aquatic
464 habitats yielded faster decomposition rates than those in exposed streambed sediments. Despite this overall
465 decomposition rate, the environmental heterogeneity within each condition type (aquatic or terrestrial) also
466 implied differences in the decomposition factors affecting leaf litter. These results underline that in temporary
467 streams decomposition is a heterogeneous process both in space and time and is primarily determined by the
468 aquatic or terrestrial conditions that are consequence of the varying duration and frequency of droughts in the
469 basin. The reduced scale of our experiment restricts to generalize about the decomposition process in temporary
470 streams. However, our results contribute with new empirical evidences to build new hypothesis about this

471 process and clearly indicate that it is indispensable to deal with the environmental heterogeneity of temporary
472 streams to better understand their functioning. This heterogeneity also has implications for the utilization of
473 carbon in streams, suggesting a specific carbon cycle during flow fragmentation with potential effects on the
474 rewetting phase that should be considered in the carbon budgets of temporary streams, especially when
475 permanent streams are currently becoming temporary as a result of warming and water abstraction activities.

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Table captions

Table 1. Physicochemical characteristics of running waters and isolated pools during the study period (mean \pm SE; n=3).

	Units	Running waters	Isolated pools
Temperature	$^{\circ}\text{C}$	16.0 ± 0.3	17.8 ± 0.3
Conductivity	$\mu\text{S cm}^{-1}$	718 ± 12	450 ± 122
pH		7.6 ± 0.1	7.3 ± 0.1
Dissolved O_2	mg L^{-1}	7.7 ± 0.5	3.4 ± 1.3
O_2 saturation	%	84 ± 6	38 ± 15
Water velocity	m s^{-1}	0.04 ± 0.01	0
DIC	mg C L^{-1}	75.5 ± 0.2	26.2 ± 3.4
DOC	mg C L^{-1}	0.8 ± 0.0	5.1 ± 0.2
Nitrite	mg N L^{-1}	0.02 ± 0.00	0.08 ± 0.01
Nitrate	mg N L^{-1}	6.6 ± 0.0	0.9 ± 0.0
Ammonium	mg N L^{-1}	0.01 ± 0.00	0.24 ± 0.00
SRP	mg P L^{-1}	0.03 ± 0.00	0.18 ± 0.01
Chloride	mg Cl L^{-1}	18.4 ± 0.1	9.5 ± 0.6
Sulphate	mg S L^{-1}	13.1 ± 0.0	3.8 ± 0.6
Sodium	mg Na L^{-1}	12.3 ± 0.0	6.9 ± 0.3
Calcium	mg Ca L^{-1}	83.2 ± 4.8	39.7 ± 5.4

Figure captions

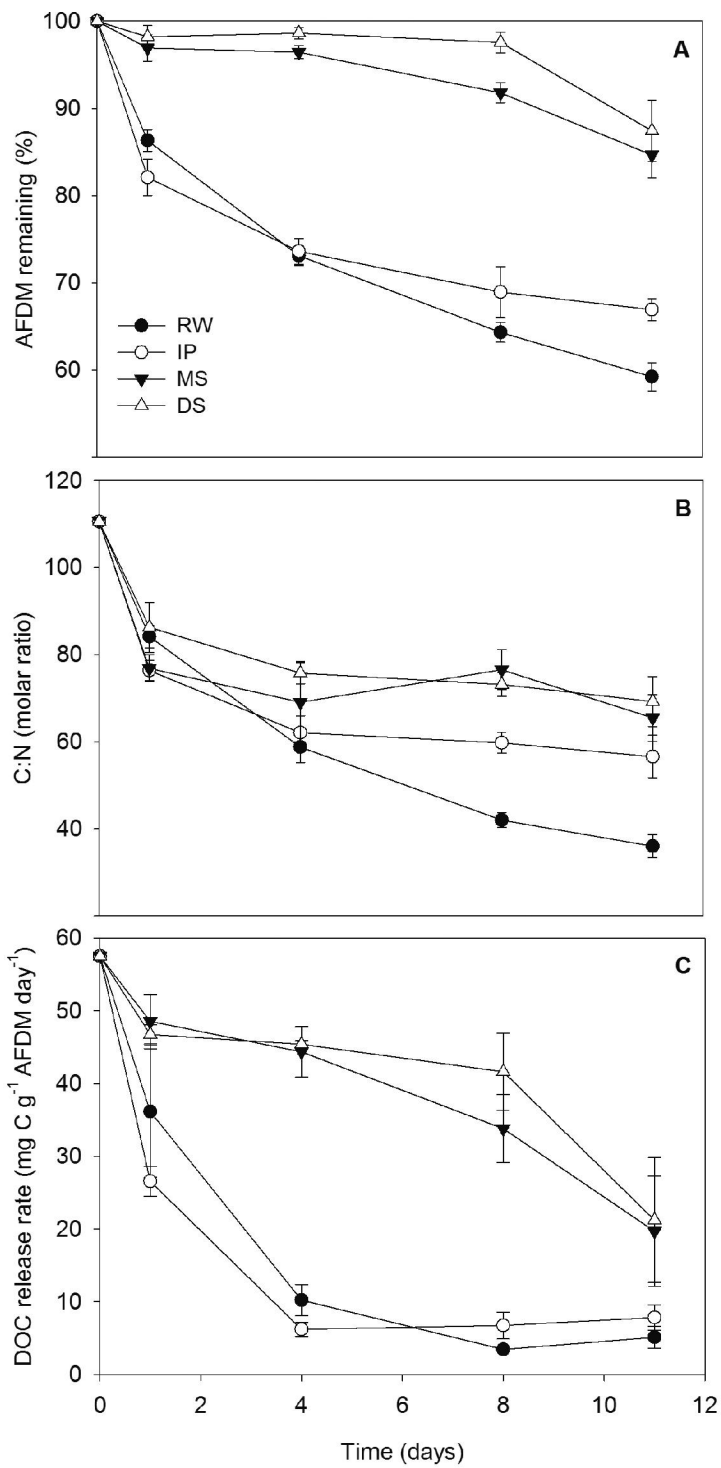


Fig 1. Percentage of ash-free dry mass (AFDM) remaining (A), C: N molar ratio in leaf litter (B) and DOC release rate (C) from leaf litter in each habitat over time (mean \pm SE, n=3): running waters (RW), isolated pools (IP), moist sediments (MS) and dry sediments (DS).

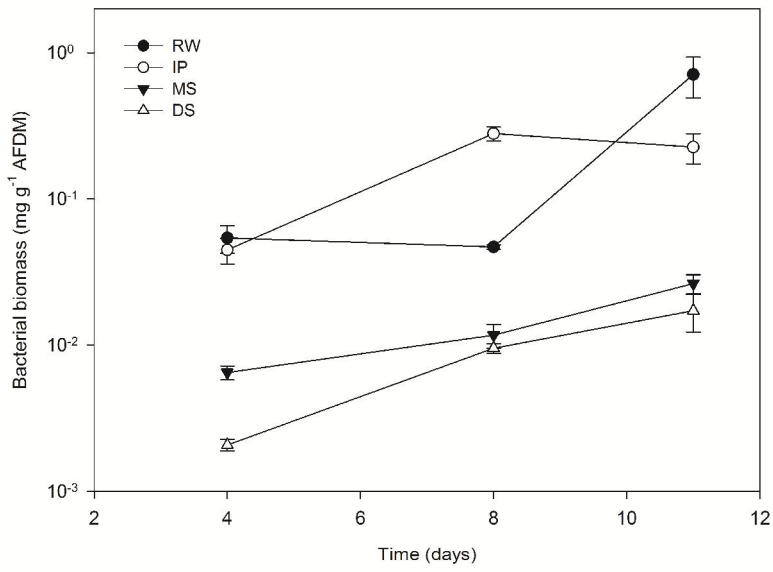


Fig 2. Bacterial biomass associated with *Populus nigra* leaves in each habitat over time (mean \pm SE, n=3): running waters (RW), isolated pools (IP), moist sediments (MS) and dry sediments (DS).

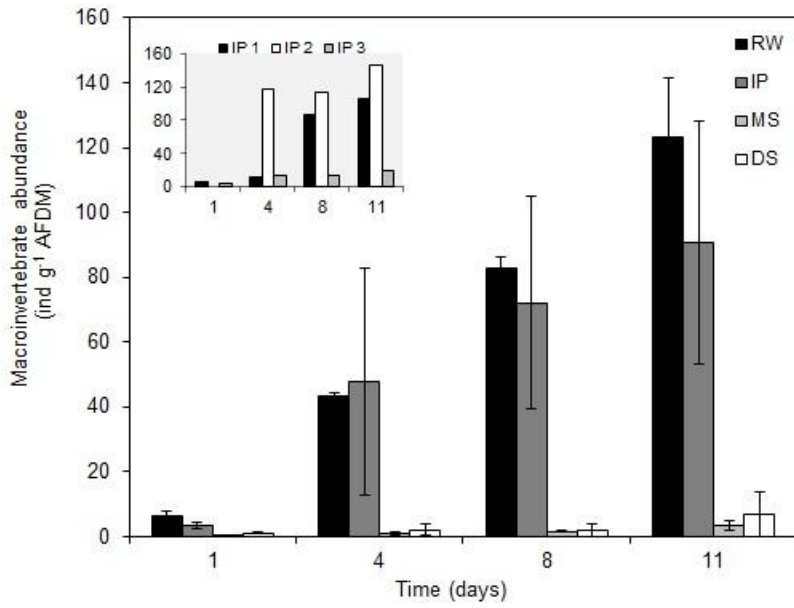


Fig 3. Total macroinvertebrate abundance in leaf bags over time for all habitat types (n=3, mean \pm SE): running waters (RW), isolated pools (IP), moist sediments (MS) and dry sediments (DS). Insert: Total macroinvertebrate abundance in each isolated pool (IP1, IP2, IP3) at each sampling time (days).

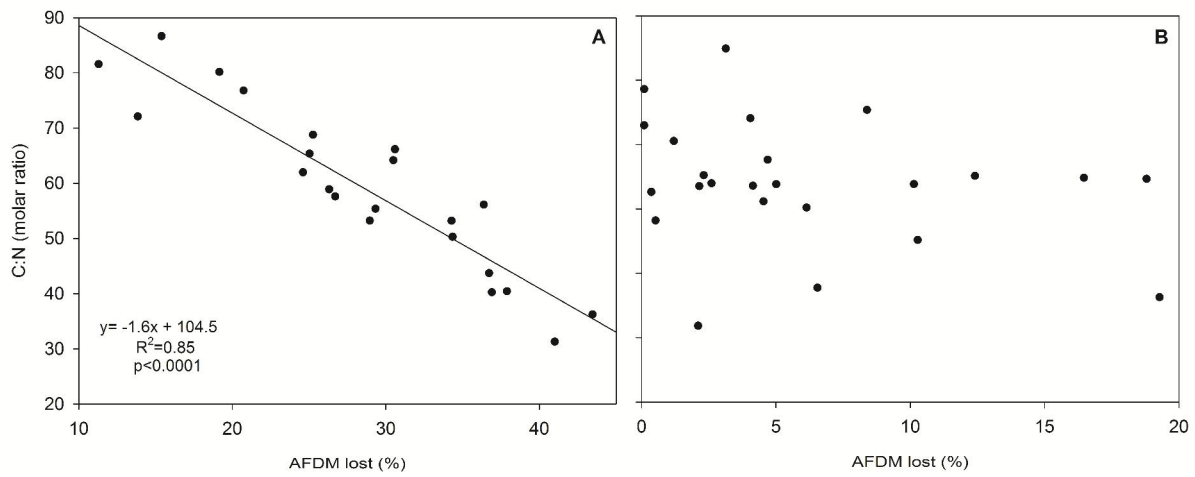


Fig 4. Relationship between the leaf C:N molar ratio and the proportion of ash-free dry mass (% AFDM) lost in aquatic (A; n=22) and terrestrial (B; n=24) habitats.

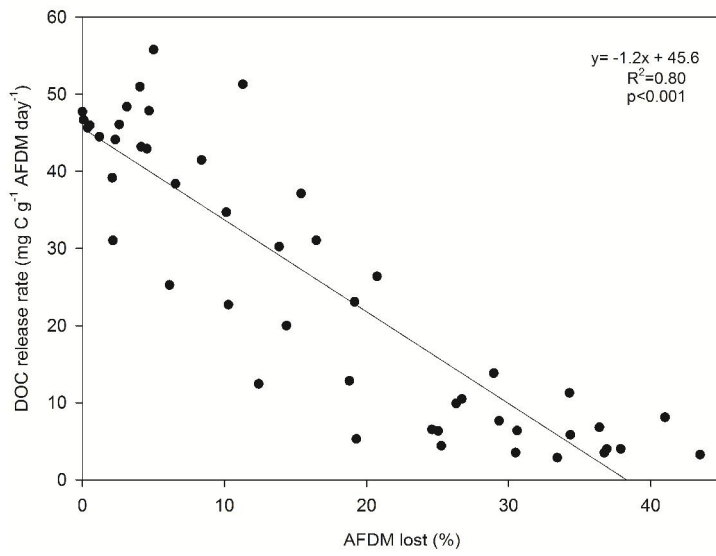


Fig 5.DOC release rate from leaf litter related to the proportion of ash-free dry mass (% AFDM) lost in all habitats: running waters, isolated pools, moist sediments and dry sediments (n = 48).