

1 **Priming of leaf litter decomposition by algae seems of minor importance in**
2 **natural streams during autumn**

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10

11 **Abstract**

12 Allochthonous detritus from terrestrial origin is one of the main energy
13 sources in forested headwater streams, but its poor nutritional quality makes it
14 difficult to use by heterotrophs. It has been suggested that algae growing on this
15 detritus can enhance its nutritional quality and promote decomposition. So far,
16 most evidence of this "priming" effect is derived from laboratory or mesocosm
17 experiments, and it is unclear what its importance is under natural conditions. We
18 measured accrual of algae, phosphorus uptake capacity, and decomposition of
19 poplar leaves in autumn in open- and closed-canopy reaches in 3 forest and 3
20 agricultural streams. Chlorophyll a abundance did not change significantly neither
21 with stream type nor with canopy cover, although some between open and closed
22 reaches, although in some agricultural streams it was higher in open than in closed
23 canopy reaches. Canopy cover did not affect either phosphate uptake capacity or
24 microbial decomposition. On the other hand, although there was no effect of
25 canopy cover on invertebrate fragmentation rate, a significant interaction between

26 canopy cover and stream suggests priming occurs at least in some streams. Overall,
27 the results point to a weak effect of algae on litter decomposition in natural
28 streams during autumn.

29

30 **Keywords:** Canopy cover, nutrient uptake, chlorophyll, breakdown, fragmentation
31 rate.

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33 **Running title:** Stream decomposition priming

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38 **Introduction**

39 Organic detritus from terrestrial origin, and leaf litter in particular, is one of
40 the main energy sources for food webs in forested headwater streams (Fisher &
41 Likens, 1973; Gessner *et al.*, 2010), where riparian shading limits primary
42 production (Hill, Ryon & Schilling, 1995). This detritus tends to be dominated by
43 recalcitrant compounds such as lignin and cellulose, is stoichiometrically
44 imbalanced for the needs of consumers (Anderson, 1976; Hanson *et al.*, 1985;
45 Lemoine, Giery & Burkepile, 2014; Evans-white & Halvorson, 2017), and thus, has
46 low nutritional value (Brett *et al.*, 2017). Once in the water, leaf litter is colonised
47 and "conditioned" by microbes, especially bacteria and aquatic hyphomycetes
48 (Krauss *et al.*, 2011), whose lower C:N ratio enhances its overall palatability and
49 nutritional value for invertebrate shredders (Hieber & Gessner, 2002; Findlay,
50 2010), which thus choose the most nutritious leaf patches (Fuller, Evans-White &
51 Entrekin, 2015).

52 Yet, the lack of components such as essential fatty acids can still limit the
53 nutritional value of leaves even after microbial conditioning (Torres-Ruiz & Wehr,
54 2010; Guo *et al.*, 2017). Recently, Lovatt *et al.* (2014) reported that leaf-litter
55 leachate and light interact to promote algal biofilm accrual, which can in turn
56 enhance the nutritional quality of leaf litter and thus promote the consumption by
57 detritivore invertebrates (Guo *et al.*, 2016). This is an example of the *priming* effect,
58 i.e., the enhancement of decomposition of recalcitrant organic matter by addition
59 of labile carbon, which has been extensively documented in terrestrial ecosystems
60 (Kuzyakov, 2010), much less frequently in aquatic ecosystems (Guenet *et al.*, 2010;
61 Kuehn *et al.*, 2014). In the case of freshwater ecosystems, priming can involve the
62 transfer of algal-produced C to heterotrophs (Kuehn *et al.*, 2014), or the direct

63 consumption of algae by detritivore invertebrates (Ledger & Winterbourn, 2000;
64 Franken *et al.*, 2005; Guo *et al.*, 2016), which would thus grow faster and have a
65 larger effect on litter breakdown. It has even been suggested that consumers
66 would preferentially use low-quality terrestrial carbon sources (e.g. leaf litter) for
67 respiration, whereas they would preferentially use algae for secondary production
68 (Karlsson, 2007).

69 Therefore, the priming effect could have important consequences for food
70 webs, as well as for the management of stream ecosystems. Yet, most of the
71 evidence on the priming effect is derived from experiments either in the laboratory
72 or in artificial channels. For instance, Danger *et al.* (2013) in a factorial mesocosm
73 experiment with light and nutrients, showed that under low nutrient
74 concentrations diatom growth enhanced litter decomposition. The importance of
75 the priming effect under field conditions is currently unclear. In theory, priming
76 should be most important in periods of stable baseflow and high light-availability,
77 which promote the accrual of algal biomass (Biggs, 1995). However, it is possible
78 that priming could be less important in high-flow periods, in strongly shaded
79 reaches or in periods of large accumulations of detritus, as heterotrophic microbes
80 have been shown to outcompete algae for limiting nutrients (Mindl *et al.*, 2005;
81 Danger, Benest & Lacroix, 2007). In particular, in many temperate streams the bulk
82 of leaf fall occurs in autumn (Abelho, 2001), a period in which the differences in
83 light availability between open and closed reaches decrease as a consequence of
84 leaf fall and shorter daylight period, which might reduce the real effect of priming.
85 Also, it has been suggested that the priming effect is more important in nutrient-
86 poor conditions, where algae would release more labile C exudates (Guenet *et al.*,
87 2010), but there is so far little empirical evidence of this.

88 The objective of the present study was to test the importance of priming in
89 natural streams during the peak of litterfall. More specifically, we assessed the
90 effects of autumnal riparian cover on biofilm accrual and activity, and on litter
91 decomposition in streams of contrasting nutrient status. Our hypotheses were: i)
92 that biofilm accrual will be higher at open than at closed reaches, ii) that higher
93 biofilm accrual will boost litter breakdown at nutrient-poor but not at nutrient-
94 rich streams, and iii) that the effect will be higher for invertebrate fragmentation
95 than for microbial decomposition.

96

97 **Methods**

98 *Study sites*

99 The experiment was performed in six streams, 3 forested (F1 to F3) and 3
100 agricultural (A1 to A3), near Vancouver, British Columbia, Canada (Table 1). The
101 forest streams are located in the Malcolm Knapp Research Forest (Maple Ridge,
102 BC), a 52 km² area owned by the University of British Columbia. It is almost
103 entirely covered by forest, dominated by Douglas-fir (*Pseudotsuga menziesii*),
104 western hemlock (*Tsuga heterophylla*) and western red cedar (*Thuja plicata*), and
105 with red alder (*Alnus rubra*) and black cottonwood (*Populus trichocarpa*) abundant
106 in the riparian areas. The lithology is granitic, soils acidic and streams in the area
107 have extremely low conductivity and nutrient concentrations. The agricultural
108 streams are located ca. 30 km further south, in the Fraser River Delta (Surrey and
109 Aldergrove, BC), a region with intense agricultural activities, including berry farms
110 and dairies, and scattered urban areas. The same tree species occur, but the forest
111 cover is much lower than in the forest streams, grasses and bushes becoming more

112 important. Lithology is dominated by glacial till, streams are circumneutral and
113 richer in nutrients (Shupe, 2017).

114 The climate in the Vancouver region is moderate oceanic, with an annual
115 mean temperature near 11 °C and a precipitation ranging from 1500 mm per year
116 in the coast to over 3000 mm in the mountains. Summers are drier, and autumn
117 tends to be very rainy.

118 In each stream we selected two nearby (less than 200 m apart) riffle reaches,
119 one with open canopy (O), and the other with closed canopy (C).

120

121 *Biofilm*

122 To measure biofilm accrual and activity we used *biofilm carriers*. These are
123 standard materials where biofilm can attach, designed to promote self-purification
124 in aquaria by encouraging growth of bacteria inside the water pump. We used Bio-
125 filter balls (Marineland, United Pet Group, Spectrum Brand Inc., Blacksburg, VA),
126 which consist of hollow plastic spheres, 2 cm in diameter, with a surface made of
127 thin plates, the space between plates allowing water flow as to promote biofilm
128 growth. Two such balls were tied with fishing line to each of the rebars used to
129 deploy litterbags (see below), at mid depth. They were recovered together with the
130 litterbags, enclosed by pairs in 50 mL Falcon tubes with filtered stream water, and
131 carried to the laboratory in an ice chest to perform a phosphate-uptake bioassay.

132 Once in the laboratory, the stream water was replaced by an acclimation
133 solution (1 L of Perrier water in 4 L of distilled water), designed to ensure a
134 sufficient supply of micronutrients, and the Falcons were incubated for 30 min in a
135 LabRoller rotator at minimum speed inside a Cenviron environmental chamber at
136 8 °C temperature and 50 µmol of PAR (Apogee quantum sensor SQ100). After this

137 acclimation period, the water in the Falcons was replaced by a 5 μM solution of
138 PO_4^{3-} , prepared by dissolving $\text{H}_2\text{NaO}_4\text{P}\cdot\text{H}_2\text{O}$ in the acclimation solution, and were
139 incubated for 1 h. An additional set of five pairs of uncolonized biofilm carriers was
140 used as blanks. After this period, water was filtered from the Falcons (Whatman
141 GF/F) and frozen to later analyse the remaining P concentration (see below), and
142 the biofilm carriers were frozen in the Falcons. The uptake rate of phosphorus (U_p ,
143 in mg P h^{-1}) was calculated as:

144

$$145 \quad U_p = V \frac{C_b - C_c}{T}$$

146

147 where V is the volume of incubation solution (L), C_b is the final concentration of P
148 (mg P L^{-1}) in the bank treatment (uncolonized biofilm carriers), C_c is the final P
149 concentration in the colonized treatment and T is the incubation time (h).

150 Biofilm carriers were later thawed, kept by pairs in 20 mL of acetone
151 overnight at 4 °C, and their content of chlorophyll was measured fluorometrically
152 (TD-700, Turner Designs), using uncolonized biofilm carriers in acetone as a blank.

153

154 *Leaf breakdown*

155 We studied the breakdown of black cottonwood leaves, a common riparian
156 tree in the area with relatively poor-quality leaves (Crutsinger *et al.*, 2014), which,
157 thus, could be more prone to priming. Recently fallen leaves were collected below
158 five adjacent trees in Burnaby (BC, Canada), taken to the laboratory and air-dried.
159 Batches of 3.0 (± 0.1) g of dry leaves were enclosed in coarse (9 mm) or fine (250
160 μm) mesh bags. One fine bag was enclosed inside each coarse bag, thus ensuring

161 that they were subject to the same environmental conditions. Bags were taken to
162 the field and tied with cable binders simultaneously to the biofilm carriers, to five
163 metal rebars per reach (1 double bag per rebar), anchored in riffle sections. Bags
164 were retrieved after 2 months of incubation, enclosed in zip-lock bags and carried
165 to the laboratory in an ice chest. There, they were opened, the remaining leaf
166 material was cleaned with tap water on a 250- μ m sieve, and the ash-free dry mass
167 (AFDM) was measured gravimetrically after drying (60 °C, 96 h) and ashing
168 (500 °C, 4 h). The initial ash-free dry mass was calculated from 10 additional
169 batches of dry leaves, which were dried and ashed as explained. Breakdown rates
170 were calculated according to the negative exponential model (Petersen & Cummins,
171 1974) with time in degree-days. Following Lecerf (2017) we calculated the mean
172 litter fragmentation rate (λ_F) as

$$173 \quad \lambda_F = k_c - \frac{k_f - k_c}{\ln(k_f) - \ln(k_c)}$$

174 where λ_F is the mean fragmentation rate and k_c and k_f the breakdown rates
175 in coarse and fine mesh bags, respectively.

176

177 *Environmental variables*

178 On 5 occasions during the decomposition experiment, we measured wetted
179 cross-section with a ruler and a measuring tape, and water velocity (current meter
180 Swoffer 2100) to calculate stream discharge. Additionally, we measured water
181 temperature, conductivity and pH (field probe YSI Pro1030) and dissolved oxygen
182 (field probe YSI ProODO), and collected water samples, which were filtered
183 (Whatman GF/F), carried to the laboratory in an ice chest, and frozen immediately
184 for analysis of nutrients. The concentrations of nitrate, nitrite, ammonium and

185 phosphate in water were determined by an OI-Analytical “Alpkem Flow System IV”
186 automated chemistry analyser at the Department of Analytical Chemistry, British
187 Columbia Ministry of Environment and Climate Change Strategy, Victoria, British
188 Columbia.

189 Additionally, water temperature was recorded continuously at each reach by
190 Onset TidBit v2 temperature loggers tied to one of the rebars. Riparian cover was
191 estimated from zenithal photos taken with a 28 mm (35 mm equivalent) lens
192 (camera Fujifilm X20), which were later processed to maximize contrast and then
193 analysed with ImageJ, a free software downloadable from
194 <https://imagej.nih.gov/ij/> to measure total image brightness, which was converted
195 into cover.

196

197 *Statistical analyses*

198 We used linear mixed effect models to contrast stream water characteristics
199 (IBM SPSS V.24) with stream type (agricultural vs forested) and canopy cover
200 (open vs closed) as fixed factors, and stream identity as random effect with five
201 levels. Nutrient variables (PO₄-P and TIN) were log transformed to meet normality
202 assumptions. Significance for each term was assessed by model comparisons using
203 likelihood ratio test (Crawley, 2007).

204

205 **Results**

206 The experiment started in late September 2017, after an unusual dry summer
207 that caused extremely low baseflows in the streams in the area. The weather
208 suddenly changed after the 16th of October, when large storms affected the area for
209 5 days (<http://climate.weather.gc.ca/>). Floods scoured all bags and most of the

210 biofilm carriers deployed in Blaney Creek (stream F1), which led us to remove this
211 stream from the study. After these floods, weather remained fairly dry from 26th
212 October to 7th November, and then it rained almost every day until the end of the
213 experiment, although no large floods were registered.

214 Riparian cover at the beginning of the experiment ranged from 0 to 45% at
215 the open reaches, whereas it was higher than 76% at the closed reaches (Table 2).

216 It decreased along the experiment in all reaches, as deciduous trees lost their
217 leaves, but the closed reaches still maintained riparian cover higher than 55%.

218 Average discharge over the study period ranged from 129 L s⁻¹ in stream A1 to 586
219 L s⁻¹ in stream A2. The average temperature was higher in agricultural than in
220 forest streams (9.2-9.9 vs 7.0-8.5 °C, respectively), as were pH (7.0-7.4 vs 6.4-6.5)
221 and conductivity (163-252 vs 19 µScm⁻¹). On the contrary, oxygen concentration
222 tended to be higher at forest (11.0-11.9 mgL⁻¹) than at agricultural (9.3-10.1 mgL⁻¹)
223 streams (Table 2). Average TIN concentrations were below 0.30 mg N L⁻¹ in

224 forest streams, whereas they ranged from 0.47 to 1.81 in agricultural streams. SRP
225 concentrations were below 6 µg P L⁻¹ in forest streams, whereas they ranged from
226 12.8 to 35.0 in agricultural streams. Linear mixed models detected statistically

227 significant difference between agricultural and forest streams for oxygen
228 concentration ($F_{1,4} = 64.6$; $p=0.001$), pH ($F_{1,4} = 58.5$; $p=0.002$), conductivity ($F_{1,4} = 239.2$; $p<0.001$), TIN ($F_{1,4} = 16.1$; $p=0.016$) and phosphate ($F_{1,4} = 27.8$; $p=0.006$),
229 whereas differences were marginal for temperature ($F_{1,4} = 5.1$; $p=0.088$). None of
230 these variables showed statistically significant differences between open and
231 closed reaches.

233 Chlorophyll *a* per pair of biofilm carriers ranged from 0.83±0.43 µg
234 (mean±SD) in F2 to 10.53±7.99 in A1 (Fig. 1). Differences were not significant

235 between stream types (LMM, $F_{1,3} = 2.4$; $p=0.216$) nor between open and closed
236 reaches ($F_{1,3} = 3.5$; $p=0.154$), and the interaction canopy*type was neither
237 significant ($F_{1,3} = 2.12$; $p=0.239$). Nevertheless, there were statistically significant
238 differences of the stream factor ($F_{3,3} = 13.5$; $p=0.030$) as well as for the
239 canopy*stream interaction ($F_{3,37} = 3.2$; $p=0.035$), showing that the canopy effect
240 was significant at some streams. In these cases, open reaches had more chlorophyll
241 than closed ones.

242 Phosphate uptake in the bioassay performed with biofilm carriers ranged
243 from $0.96 \pm 0.28 \mu\text{g P h}^{-1}$ in reach F2C to $1.52 \pm 0.37 \mu\text{g P h}^{-1}$ in reach F3C (Fig. 2). The uptake
244 per unit of chlorophyll ranged from $32.2 \pm 12.4 \mu\text{g P mg Chl}^{-1} \text{h}^{-1}$ to $1582 \pm 382 \mu\text{g P mg Chl}^{-1} \text{h}^{-1}$ (Fig.
245 2). It was lowest in streams A1 and A2, the two ones with highest basal
246 concentrations of SRP in water. None of the factors and interactions tested by LMM
247 resulted statistically significant for phosphate uptake ($p > 0.05$ in all cases), but
248 there were significant differences in uptake per unit of chlorophyll for the factor
249 stream ($F_{3,3} = 10.3$; $p=0.044$) and the interaction canopy*stream ($F_{3,37} = 4.0$;
250 $p=0.014$).

251 After 2 months of incubation, the leaf AFDM remaining in fine mesh bags
252 ranged from $18.02 \pm 6.26 \%$ in reach A03 to $49.44 \pm 2.51 \%$ in FC2 (Fig. S1). No clear
253 differences could be detected either between agricultural and forest streams, nor
254 between closed and open reaches. The leaf AFDM remaining in coarse mesh bags
255 ranged from $1.80 \pm 1.96 \%$ in A03 to $47.17 \pm 2.32 \%$ in FO3. It was higher in forest than in
256 agricultural streams, but no obvious differences were detected between open and
257 closed reaches. The temperature-corrected breakdown rates ranged from
258 $0.0015 \pm 0.0001 \text{ dd}^{-1}$ to $0.0032 \pm 0.0006 \text{ dd}^{-1}$ in fine mesh bags and from 0.0018 ± 0.0003
259 to $0.0082 \pm 0.0021 \text{ dd}^{-1}$ in coarse mesh bags. In fine mesh bags there were no

260 obvious differences between agricultural and forest streams, but in coarse mesh
261 bags agricultural streams tended to have higher breakdown rates (Fig. 3). No clear
262 differences were seen between open and closed reaches. LMM showed the factors
263 stream type and canopy cover and their interaction to have no statistically
264 significant effect on fine-mesh decomposition rate, but stream had ($F_{3,3} = 33.8$;
265 $p=0.008$). For coarse mesh bags the only statistically significant difference was
266 attributed to the canopy*stream interaction ($F_{3,36} = 3.45$; $p=0.027$), although the
267 direction of the difference was not consistent among streams.

268 Finally, the fragmentation rate, i.e., the contribution of shredders and
269 physical abrasion to total breakdown, ranged from 0.0011 to 0.0259 d^{-1} , and
270 tended to be higher in agricultural than in forest streams (Fig. 4), although
271 differences between stream types were not statistically significant. LMM only
272 found a statistically significant effect for the interaction between canopy cover and
273 stream ($F_{3,36} = 4.83$; $p<0.006$), thus showing that fragmentation rate was higher in
274 open than in closed reaches of some streams.

275

276 **Discussion**

277 Our results point to a weak priming effect by algae on autumn litter
278 decomposition in the study streams. In agricultural streams there was a trend for
279 open reaches to have more chlorophyll than closed ones, but the differences were
280 not consistent and did not translate into faster litter decomposition. Although
281 stream*canopy interactions showed differences between open and closed reaches
282 in some streams, the direction of the difference was not consistent among streams.
283 Thus, our overall results suggest that other environmental factors override the
284 potential effect of algal growth on leaf litter dynamics.

285 Our experimental design, with open and closed reaches very close to each
286 other, aimed at reducing the potentially confounding effects of other
287 environmental variables apart from canopy cover. Among our forest streams open
288 reaches are rare and very short, whereas among our agricultural streams usually
289 there is an alternation of short open and closed reaches. Therefore, none of the
290 environmental variables measured in addition to canopy cover showed systematic
291 differences among reach types, as water quality variables usually respond to
292 riparian cover at longer scales, on the order of hundreds to thousands of metres
293 (Allan, 2004; Johnson, 2004). On the other hand, the differences in canopy cover
294 between open and closed reaches decreased but did not disappear over the study
295 period, and therefore, the changes found among reach types can be attributed to
296 canopy cover.

297 Under these conditions, algal biomass showed no response to canopy cover
298 in the forest streams, thus suggesting algal growth there to be limited by factors
299 other than light, probably nutrients. The concentration of phosphorus in our forest
300 streams was close, when not below, detection limits. Additionally, high N:P atomic
301 ratio ranged from 52 (A1C) to 164 (F3C), suggesting P to be the limiting nutrient
302 in all our sites (Elser *et al.*, 2000; Sterner *et al.*, 2008). As the winter approaches,
303 other factors such as low water temperature, decreased day length, low elevation
304 of the sun in the horizon, frequent cloudy days and high flow tend to limit algal
305 growth (Izagirre & Elosegi, 2005), which probably explains the lack of differences
306 in our forest, nutrient-limited streams. Long baseflow periods promote biofilm
307 accrual (Ponsatí *et al.*, 2015), whereas floods scour algae (Francoeur & Biggs,
308 2006), and thus, in mountain streams frequent floods can override the effects of
309 canopy cover on algal biomass (Boulêtreau *et al.*, 2008). It is likely that, even if

310 there were no differences in chlorophyll content, the algal assemblages differed
311 between open and closed reaches, as algal taxa differ in their competitive abilities
312 depending on light and nutrient availability (Franken *et al.*, 2005; Litchman &
313 Klausmeier, 2008; Lange, Townsend & Mattheai, 2016). However, even if this was
314 the case, overall it had no measurable effect on our decomposition rates.

315 Contrary to our expectations, phosphate uptake capacity, a proxy for biofilm
316 activity, did not differ between forest and agricultural streams, or between open
317 and closed reaches. In general, the metabolic activity of stream biofilm depends on
318 its biomass (Haggerty *et al.*, 2014), whereas the uptake per unit biomass tends to
319 be greatest at oligotrophic, nutrient-poor reaches (Mulholland, 1996).

320 Nevertheless, internal phosphorus recycling at the biofilm level gains importance
321 in nutrient-rich reaches (Mulholland *et al.*, 1995), which usually reduces uptake
322 rate (Proia, Romaní & Sabater, 2017) and results in our bioassay yielding highest
323 uptake rate in moderately enriched streams (Arturo Elosegi, unpublished data).

324 The small differences we found in P uptake rate per unit of chlorophyll suggest a
325 part of the algal biomass to be not very active, probably as a consequence of the
326 senescence of algal mats by the end of autumn, which reduces the biological
327 activity even if the biomass is large (Izagirre *et al.*, 2008). Interestingly, the weak
328 trends found between open and closed reaches point towards a higher nutrient
329 uptake per unit biomass in the latter, which again, was contrary to our
330 expectations, and could be related to site-specific characteristics such as flow
331 velocity.

332 Microbial decomposition, as measured by temperature-corrected breakdown
333 rate in fine-mesh bags, did not differ between agricultural and forest streams, or
334 between open and closed reaches, although the random factor stream was

335 statistically significant. This is a surprising result, as moderate nutrient
336 concentrations as those found in our agricultural streams have been shown to
337 promote litter breakdown (Gulis, Ferreira & Graca, 2006), although effects are
338 much more clear for total decomposition (Chauvet *et al.*, 2016). For coarse mesh
339 bags, although there seemed to be a trend for litter to decompose faster at
340 agricultural streams, the differences in temperature-corrected breakdown rates
341 were again not statistically significant, contrasting with clear patterns shown
342 elsewhere (Woodward *et al.*, 2012). Nevertheless, the statistically significant
343 interaction between canopy cover and stream suggested a weak priming effect in
344 some of the streams. We can only speculate about the reasons for this difference
345 occurring only in some streams, but it is likely that local factors affected differently
346 the two reaches studied in one stream, thus overriding the weak effects of priming.
347 Indeed, litter breakdown is sensitive to small differences in flow velocity (Dewson,
348 James & Death, 2007), sediment deposition (Piggott *et al.*, 2012) or biological
349 communities (Handa *et al.*, 2014), factors that can change at the micro-habitat
350 scale (Elosegi, Flores & Díez, 2011). In our case, shredders were present in all sites
351 (Arturo Elosegi, personal observation), including agricultural streams. Intense
352 human activities can result in local extinction of large shredders such as
353 crustacean gammarids, and thus impact litter breakdown (Hladyz *et al.*, 2011), but
354 our streams seem not to have crossed this impairment threshold.

355 The trend towards higher fragmentation rates suggests that invertebrates
356 were even more important in our agricultural than in our forest streams, which is
357 usually not the case (Hladyz *et al.*, 2011). Again, the interaction between stream
358 and canopy cover was statistically significant, suggesting that the priming effect
359 can be important at least in some streams.

360 Overall, our results suggest that algal priming of litter decomposition, a key
361 ecosystem process (Hagen *et al.*, 2012), is at best weak in natural streams during
362 autumn, when the environmental conditions become less favourable for algal
363 growth. The relevance of priming in streams remains a controversial effect.
364 Although the nutritional quality of biofilms tends to be greater when algae are
365 present (Huggins, Frenette & Arts, 2004) and much of the nitrogen assimilated by
366 stream consumers is of algal origin (Brett *et al.*, 2017), the effects on litter
367 decomposition are often unclear. Most of the research has been performed in the
368 laboratory or in artificial streams, and even there results are far from unequivocal.
369 For instance, a recent laboratory experiment (Guo *et al.*, 2016) found enhanced
370 nutrients but not light to promote shredder biomass and breakdown rate,
371 contrasting with other experiments (Franken *et al.*, 2005), who found no effect on
372 shredder-mediated decomposition, although light promoted the growth of *Asellus*
373 and *Gammarus* crustaceans. (Guo *et al.*, 2016) attributed the lack of effect to the
374 small difference between their two light levels (21 and 114 $\mu\text{mol m}^{-2} \text{s}^{-1}$). We did
375 not measure differences in light levels between our open and closed reaches, but it
376 is likely that they were rather small and decreased during the experiment, as a
377 consequence of leaf fall, shortening of the day and increased cloudiness. As for field
378 experiments, although some authors (Kuehn *et al.*, 2014) found exposure to light
379 to stimulate decomposition in the field, their "shadow" treatment was artificial, as
380 they covered the channel with cloth. Similarly, Vonk *et al.* (2016) found that,
381 whereas in microcosms, invertebrates showed a preference for artificial substrate
382 with poly-unsaturated fatty acids added, this effect could not be detected in the
383 field, probably because it was overruled by unknown sources of variation. Priming
384 could be more important in summer, when the differences in light availability

385 between open and closed reaches are largest and the long baseflow periods make
386 biological effects more marked.

387

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2 **Table 1.** Main characteristics of the studied streams. Land cover data obtained from the Land Use 2010 map by the Canada Government
3 (<https://open.canada.ca/data/en/>).

Stream	Name	Coordinates	Stream order	Basin (Km ²)	Forest (%)	Agriculture (%)	Urban (%)	Water (%)
F1	Blaney Creek	49°16'14" N 122°35'20" W	2	8.74	94	0	0	6
F2	Spring Creek	49°16'15" N 122°34'30" W	2	2.55	100	0	0	0
F3	Mayfly Creek	49°15'06" N 122°32'51" W	2	1.14	100	0	0	0
A1	Pepin Creek	49°00'14" N 122°28'18" W	2	15.38	33	29	35	0

A2	Bertrand Creek	49°01'58" N	3	30.87	27	34	35	0
				122°32'04" W				
A3	Little Campbell	49°01'60" N	3	28.74	56	21	21	2
	River			122°41'06" W				

5 **Table 2.** Environmental characteristics of the study reaches. F stands for forest, A for agricultural, O for Open, C for
 6 Closed. Canopy cover values are point values measured at the beginning and at the end of the experiment. The rest
 7 of the values are mean \pm SD of 5 measurements during the experiment. TIN (total inorganic nitrogen) is the sum of
 8 nitrate, nitrite and ammonium. SRP, soluble reactive phosphorus. Discharge was only measured in one site per
 9 stream, where it was most convenient, not necessarily in any of the sampling reaches.

Reach	Canopy cover (%)	Discharge (L s^{-1})	Temp ($^{\circ}\text{C}$)	pH	Cond ($\mu\text{S cm}^{-1}$)	Oxygen (mg L^{-1})	TIN (mg N L^{-1})	SRP ($\mu\text{g P L}^{-1}$)
F2O	7.2-3.2	143 \pm 142	8.4 \pm 2.2	6.4 \pm 0.3	19.1 \pm 10.0	11.8 \pm 0.6	0.22 \pm 0.10	5.8 \pm 5.2
F2C	86.3-79.1		8.5 \pm 2.3	6.5 \pm 0.4	19.2 \pm 10.1	11.7 \pm 0.7	0.22 \pm 0.13	4.0 \pm 2.5
F3O	0.7-3.1	172 \pm 253	7.1 \pm 2.1	6.6 \pm 0.4	18.7 \pm 9.1	11.0 \pm 0.7	0.20 \pm 0.04	3.0 \pm 1.9
F3C	76.5-71.4		7.0 \pm 2.0	6.5 \pm 0.2	18.9 \pm 8.9	11.8 \pm 0.8	0.20 \pm 0.07	2.7 \pm 1.1
A1O	0.0-0.2	129 \pm 92	9.2 \pm 1.7	7.3 \pm 0.2	252 \pm 97	9.3 \pm 0.2	0.82 \pm 0.36	31.8 \pm 4.0
A1C	83.9-62.2		9.2 \pm 1.7	7.4 \pm 0.1	224 \pm 63	9.3 \pm 0.3	0.82 \pm 0.41	35.0 \pm 5.3

A20	45.0-24.6	586 \pm 956	9.9 \pm 2.6	7.3 \pm 0.2	174 \pm 46	10.1 \pm 0.8	1.81 \pm 0.60	56.8 \pm 49.0
A2C	85.8-73.4		9.8 \pm 2.3	7.3 \pm 0.2	173 \pm 44	10.1 \pm 0.8	1.69 \pm 0.30	53.4 \pm 46.7
A30	0.0-0.0	640 \pm 565	9.5 \pm 2.4	7.0 \pm 0.2	163 \pm 58	10.0 \pm 0.4	0.62 \pm 0.22	15.6 \pm 7.8
A3C	79.7-55.9		9.6 \pm 2.2	7.2 \pm 0.3	163 \pm 58	10.1 \pm 0.4	0.47 \pm 0.28	12.8 \pm 8.4

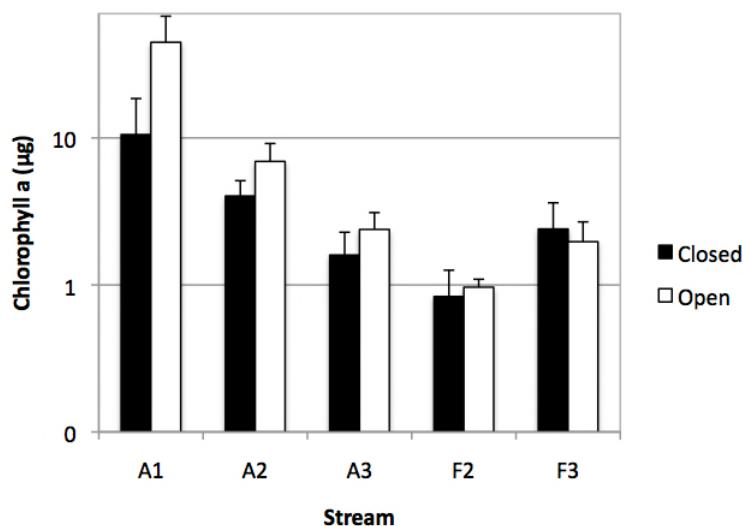
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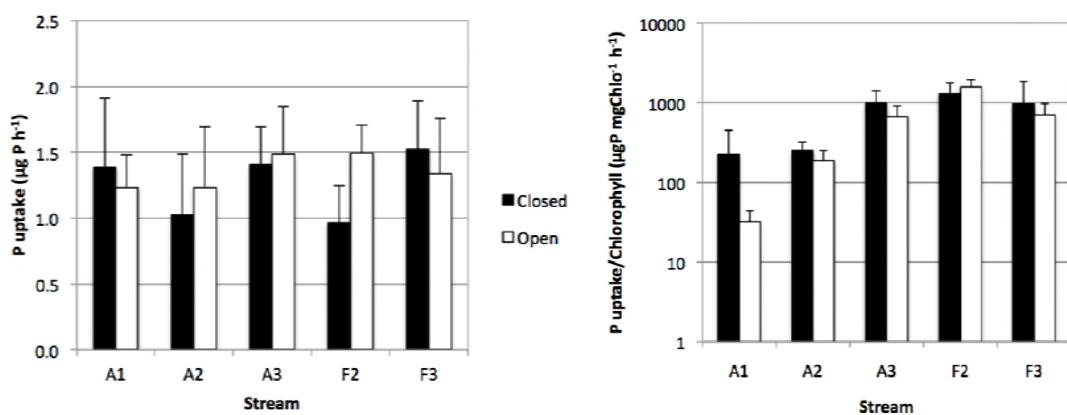
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16 **Figure 1.** Chlorophyll *a* per pair of biofilm carriers in open and closed reaches of agricultural (A)
17 and forest (F) streams. Note logarithmic scale.

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22 **Figure 2.** Left. Phosphate uptake by biofilm carriers in open and closed reaches of agricultural (A)
23 and forest (F) streams. Right. Efficiency in phosphate uptake of chlorophyll in biofilm carriers.

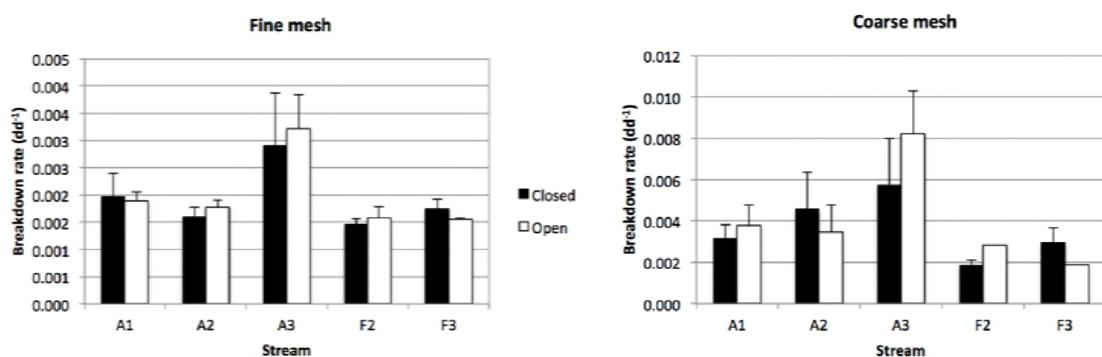
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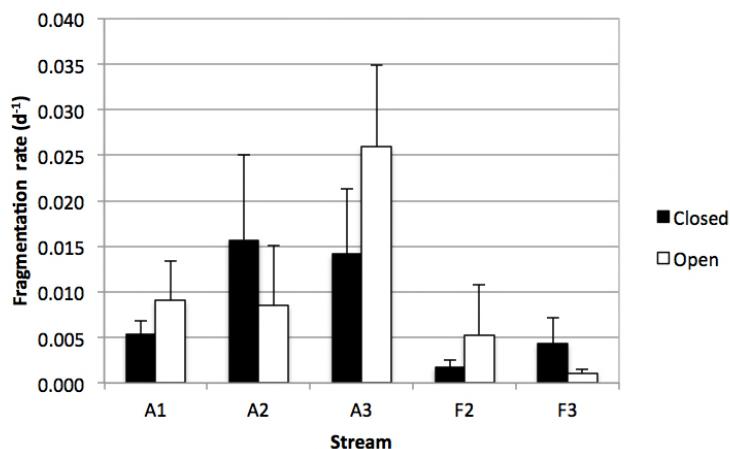
30 **Figure 3.** Temperature-corrected breakdown rates in fine (left) and coarse (right) mesh bags in
31 open and closed reaches of agricultural (A) and forest (F) streams. Note the different scale of Y axes.

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37 **Figure 4.** Fragmentation rates in coarse mesh bags in open and closed reaches of agricultural (A)
38 and forest (F) streams.

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