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

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Abstract

Allochthonous detritus from terrestrial origin is one of the main energy sources in forested headwater streams, but its poor nutritional quality makes it difficult to use by heterotrophs. It has been suggested that algae growing on this detritus can enhance its nutritional quality and promote decomposition. So far, most evidence of this "priming" effect is derived from laboratory or mesocosm experiments, and it is unclear what its importance is under natural conditions. We measured accrual of algae, phosphorus uptake capacity, and decomposition of poplar leaves in autumn in open- and closed-canopy reaches in 3 forest and 3 agricultural streams. Chlorophyll a abundance did not change significantly neither with stream type nor with canopy cover, although some between open and closed reaches, although in some agricultural streams it was higher in open than in closed canopy reaches. Canopy cover did not affect either phosphate uptake capacity or microbial decomposition. On the other hand, although there was no effect of 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.
32
33 **Running title:** Stream decomposition priming
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Introduction

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

Yet, the lack of components such as essential fatty acids can still limit the nutritional value of leaves even after microbial conditioning (Torres-Ruiz & Wehr, 2010; Guo *et al.*, 2017). Recently, Lovatt *et al.* (2014) reported that leaf-litter leachate and light interact to promote algal biofilm accrual, which can in turn enhance the nutritional quality of leaf litter and thus promote the consumption by detritivore invertebrates (Guo *et al.*, 2016). This is an example of the *priming* effect, i.e., the enhancement of decomposition of recalcitrant organic matter by addition of labile carbon, which has been extensively documented in terrestrial ecosystems (Kuzyakov, 2010), much less frequently in aquatic ecosystems (Guenet *et al.*, 2010; Kuehn *et al.*, 2014). In the case of freshwater ecosystems, priming can involve the 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 km^2 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 $H_2NaO_4P{\cdot}H_2O$ 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 (*Up*,
143 in mg P h^{-1}) was calculated as:
144

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

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

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

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

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

Environmental variables

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

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

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

Statistical analyses

We used linear mixed effect models to contrast stream water characteristics (IBM SPSS V.24) with stream type (agricultural vs forested) and canopy cover (open vs closed) as fixed factors, and stream identity as random effect with five levels. Nutrient variables (PO_4-P and TIN) were log transformed to meet normality assumptions. Significance for each term was assessed by model comparisons using likelihood ratio test (Crawley, 2007).

Results

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

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

Riparian cover at the beginning of the experiment ranged from 0 to 45% at the open reaches, whereas it was higher than 76% at the closed reaches (Table 2). It decreased along the experiment in all reaches, as deciduous trees lost their leaves, but the closed reaches still maintained riparian cover higher than 55%. Average discharge over the study period ranged from 129 L s^{-1} in stream A1 to 586 Ls^{-1} in stream A2. The average temperature was higher in agricultural than in 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) and conductivity (163-252 vs 19 μScm^{-1}). On the contrary, oxygen concentration tended to be higher at forest (11.0-11.9 mgL^{-1}) than at agricultural (9.3-10.1 mgL^{-1}) streams (Table 2). Average TIN concentrations were below 0.30 mg N L^{-1} in forest streams, whereas they ranged from 0.47 to 1.81 in agricultural streams. SRP concentrations were below 6 µg P L^{-1} in forest streams, whereas they ranged from 12.8 to 35.0 in agricultural streams. Linear mixed models detected statistically significant difference between agricultural and forest streams for oxygen 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), whereas differences were marginal for temperature ($F_{1,4}$ = 5.1; p=0.088). None of these variables showed statistically significant differences between open and closed reaches.

Chlorophyll *a* per pair of biofilm carriers ranged from 0.83±0.43 µg (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±0.28 µg P h^{-1} in reach F2C to 1.52±0.37 in reach F3C (Fig. 2). The uptake
244 per unit of chlorophyll ranged from 32.2±12.4 to 1582±382 µg P mg Chl^{-1} 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±6.26 in reach AO3 to 49.44±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±1.96 in AO3 to 47.17±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±0.0001 to 0.0032±0.0006 dd^{-1} in fine mesh bags and from 0.0018±0.0003
259 to 0.0082±0.0021 dd^{-1} in coarse mesh bags. In fine mesh bags there were no

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

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

Discussion

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

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

Contrary to our expectations, phosphate uptake capacity, a proxy for biofilm activity, did not differ between forest and agricultural streams, or between open and closed reaches. In general, the metabolic activity of stream biofilm depends on its biomass (Haggerty *et al.*, 2014), whereas the uptake per unit biomass tends to be greatest at oligotrophic, nutrient-poor reaches (Mulholland, 1996). Nevertheless, internal phosphorus recycling at the biofilm level gains importance in nutrient-rich reaches (Mulholland *et al.*, 1995), which usually reduces uptake rate (Proia, Romaní & Sabater, 2017) and results in our bioassay yielding highest uptake rate in moderately enriched streams (Arturo Elosegi, unpublished data). The small differences we found in P uptake rate per unit of chlorophyll suggest a part of the algal biomass to be not very active, probably as a consequence of the senescence of algal mats by the end of autumn, which reduces the biological activity even if the biomass is large (Izagirre *et al.*, 2008). Interestingly, the weak trends found between open and closed reaches point towards a higher nutrient uptake per unit biomass in the latter, which again, was contrary to our expectations, and could be related to site-specific characteristics such as flow velocity.

Microbial decomposition, as measured by temperature-corrected breakdown rate in fine-mesh bags, did not differ between agricultural and forest streams, or 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.

Overall, our results suggest that algal priming of litter decomposition, a key ecosystem process (Hagen *et al.*, 2012), is at best weak in natural streams during autumn, when the environmental conditions become less favourable for algal growth. The relevance of priming in streams remains a controversial effect. Although the nutritional quality of biofilms tends to be greater when algae are present (Huggins, Frenette & Arts, 2004) and much of the nitrogen assimilated by stream consumers is of algal origin (Brett *et al.*, 2017), the effects on litter decomposition are often unclear. Most of the research has been performed in the laboratory or in artificial streams, and even there results are far from unequivocal. For instance, a recent laboratory experiment (Guo *et al.*, 2016) found enhanced nutrients but not light to promote shredder biomass and breakdown rate, contrasting with other experiments (Franken *et al.*, 2005), who found no effect on shredder-mediated decomposition, although light promoted the growth of *Asellus* and *Gammarus* crustaceans. (Guo *et al.*, 2016) attributed the lack of effect to the small difference between their two light levels (21 and 114 µmol m^{-2} s^{-1}). We did not measure differences in light levels between our open and closed reaches, but it is likely that they were rather small and decreased during the experiment, as a consequence of leaf fall, shortening of the day and increased cloudiness. As for field experiments, although some authors (Kuehn *et al.*, 2014) found exposure to light to stimulate decomposition in the field, their "shadow" treatment was artificial, as they covered the channel with cloth. Similarly, Vonk *et al.* (2016) found that, whereas in microcosms, invertebrates showed a preference for artificial substrate with poly-unsaturated fatty acids added, this effect could not be detected in the field, probably because it was overruled by unknown sources of variation. Priming 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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397
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Table 1. Main characteristics of the studied streams. Land cover data obtained from the Land Use 2010 map by the Canada Government (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 122°32’04’’ W	3	30.87	27	34	35	0
A3	Little Campbell River	49°’01’60’’ N 122°41’06’’ W	3	28.74	56	21	21	2

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

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

A2O	45.0-24.6	586±956	9.9±2.6	7.3±0.2	174±46	10.1±0.8	1.81±0.60	56.8±49.0
A2C	85.8-73.4		9.8±2.3	7.3±0.2	173±44	10.1±0.8	1.69±0.30	53.4±46.7
A3O	0.0-0.0	640±565	9.5±2.4	7.0±0.2	163±58	10.0±0.4	0.62±0.22	15.6±7.8
A3C	79.7-55.9		9.6±2.2	7.2±0.3	163±58	10.1±0.4	0.47±0.28	12.8±8.4

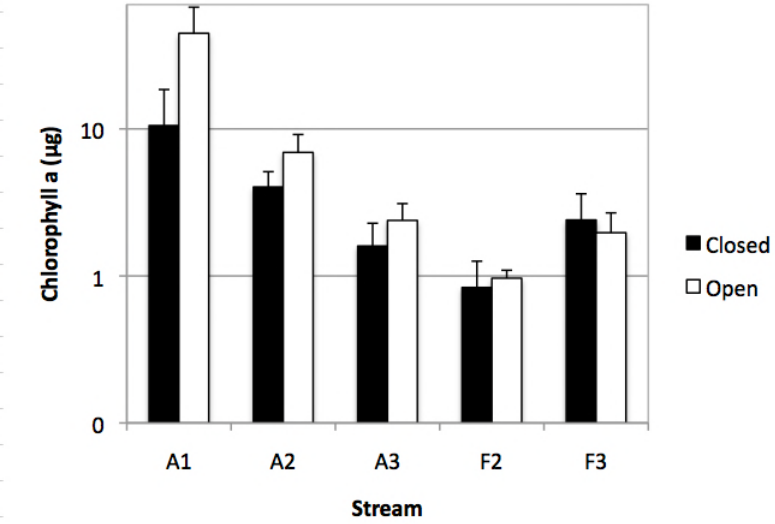

Figure 1. Chlorophyll *a* per pair of biofilm carriers in open and closed reaches of agricultural (A) and forest (F) streams. Note logarithmic scale.

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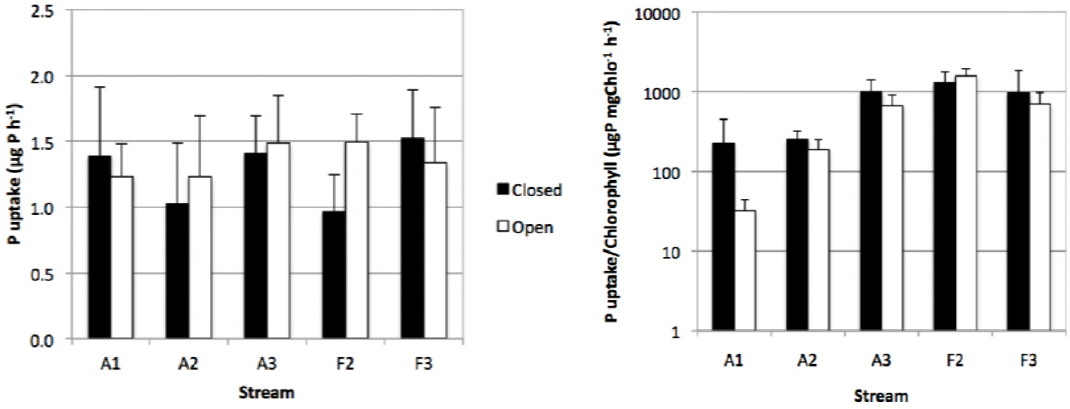

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22 **Figure 2**. Left. Phosphate uptake by biofilm carriers in open and closed reaches of agricultural (A)

23 and forest (F) streams. Right. Efficiency in phosphate uptake of chlorophyll in biofilm carriers.

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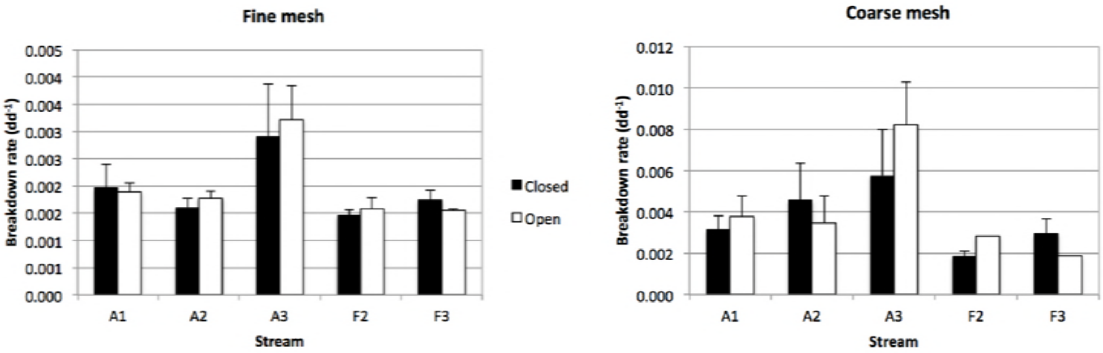

Figure 3. Temperature-corrected breakdown rates in fine (left) and coarse (right) mesh bags in open and closed reaches of agricultural (A) and forest (F) streams. Note the different scale of Y axes.

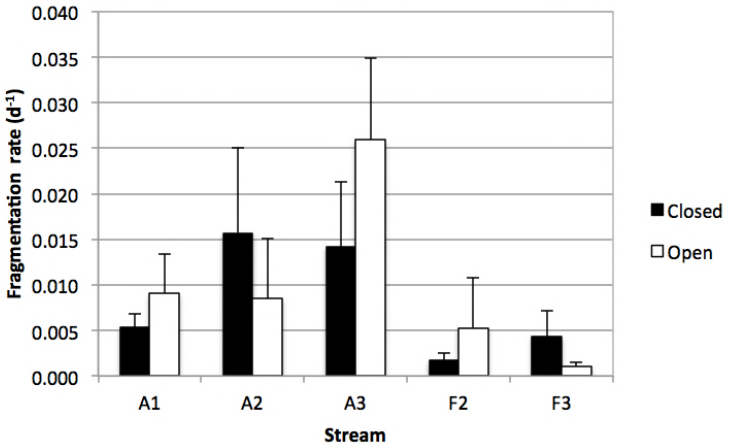

Figure 4. Fragmentation rates in coarse mesh bags in open and closed reaches of agricultural (A) and forest (F) streams.