Plant litter diversity affects invertebrate shredder activity and the quality of fine particulate organic matter in streams

Journal:	<i>Marine and Freshwater Research</i>
Manuscript ID:	Draft
Manuscript Type:	Research paper
Date Submitted by the Author:	n/a
Complete List of Authors:	Fernandes, Isabel; University of Minho, Duarte, Sofia; University of Minho, Cássio, Fernanda; University of Minho, Department of Biology Pascoal, Cláudia; University of Minho,
Keyword:	biodiversity, ecosystem processes, freshwater, invertebrates, organic matter

1 Plant litter diversity affects invertebrate shredder activity and the quality of fine
2 particulate organic matter in streams

3

4 Isabel Fernandes, Sofia Duarte, Fernanda Cássio, Cláudia Pascoal*

5

6 Centre of Molecular and Environmental Biology (CBMA)

7 Department of Biology, University of Minho

8 Campus de Gualtar

9 4710-057 Braga

10 Portugal

11

12 * Email: cpascoal@bio.uminho.pt

13 Phone: + 351 253601543

14 Fax: + 351 253678980

15

16

17

18

19 **Abstract**

20 There is evidence that riparian plant diversity loss alters the availability and quality of
21 resources in streams, but little is known on how such effects change with time following
22 diversity loss. We used a microcosm approach with leaves of alder, oak and eucalyptus
23 previously colonized by microbes in a mixed forest stream to test how litter diversity
24 loss and time (2 and 6 months following diversity loss) affect leaf consumption by
25 invertebrate shredders, the elemental composition of shredder tissues, and the quality of
26 fine particulate organic matter (FPOM). The number and identity of leaf species
27 affected leaf consumption and FPOM production by shredders. Positive diversity effects
28 became stronger with time following diversity loss. Changes in leaf quality altered C
29 and N composition of invertebrate tissues. FPOM quality was positively correlated with
30 leaf quality. Leaf consumption by the animals decreased linearly with the increase in
31 C:N imbalance between leaf litter and invertebrate tissues. Results suggest that changes
32 in plant litter diversity affect the activity of shredders (leaf consumption and FPOM
33 production), and the quality of food resources (FPOM and shredders) to higher trophic
34 levels in streams; such effects are likely to become stronger with time following plant
35 diversity loss.

36

37 Running head: Resource diversity and quality for stream biota

38

39 Additional keywords: biodiversity loss, resource diversity and quality, leaf
40 consumption, microcosms

41

42 Introduction

43 The replacement of mixed forests by monocultures of tree species and degradation of
44 riparian corridors by agriculture, industrial activities and urban settlements have led to a
45 decrease in forest tree diversity throughout parts of the world (Graça *et al.* 2002; Foley
46 *et al.* 2005; Haines-Young 2009). In low-order forested streams, allochthonous organic
47 matter from riparian vegetation constitutes the main source of food and energy to
48 aquatic biota (Vannote *et al.* 1980). In these ecosystems, leaf-litter breakdown is a key
49 ecological process mainly driven by microorganisms and invertebrates (Graça and
50 Canhoto 2006). Among microorganisms, aquatic fungi play a major role in leaf
51 decomposition by producing an array of enzymes that soften leaf litter, making it more
52 palatable for invertebrate shredders (Suberkropp 1998). The activity of microorganisms
53 and shredders on leaf litter transforms this coarse particulate organic matter (CPOM)
54 into smaller particles. These, together with the spores released by fungi and the faeces
55 produced by invertebrates, constitute fine particulate organic matter (FPOM) (Allan and
56 Castillo 2007) which can be consumed by filter-feeders and collector-gatherers
57 (Cummins and Klug 1979). However, little is known about the nutritive value of FPOM
58 compared with CPOM in stream food webs (but see, Callisto and Graça 2013).

59 The role of individual nutrients in ecosystem processes is central in ecological
60 stoichiometry because the balance of carbon (C) in relation to the key nutrients nitrogen
61 (N) and phosphorus (P) in resources often limits the activity and growth of consumers
62 (Cross *et al.* 2005). Generally, ecological stoichiometry predicts that heterotrophs are
63 able to maintain elemental homeostasis (Sterner and Elser 2002). However, elemental
64 ratios of heterotrophs can shift when consuming food sources with very high C:nutrient
65 ratios compared with their body tissue composition (DeMott *et al.* 1998). Plant litter can
66 have diverse chemical compositions (Hladysz *et al.* 2009; Schindler and Gessner 2009),

67 resulting in large stoichiometric imbalances between stream invertebrates and detritus
68 that may alter invertebrate growth (Frost *et al.* 2006). Microbial decomposers and
69 invertebrate shredders preferentially use resources that are of high quality (low C:N),
70 and rich in labile compounds and nutrients (Canhoto and Graça 1995; Fernandes *et al.*
71 2012). Therefore, it is plausible that nutrient constraints due to changes in the diversity
72 or quality of leaf litter may result in alterations to the elemental composition of
73 invertebrates and leaf breakdown (Hladysz *et al.* 2009).

74 Effects of plant litter diversity on decomposition appear to depend more on the
75 composition of litter mixtures than on the number of litter species (Lecerf *et al.* 2007;
76 Lecerf and Richardson 2010; Fernandes *et al.* 2012; Ferreira *et al.* 2012). Indeed, the
77 decomposition of recalcitrant species in litter mixtures can be slower than that predicted
78 from individual litter species, due to preferential feeding of shredders on high-quality
79 leaves (Swan and Palmer 2006). However, invertebrate shredders colonising diverse
80 litter can also benefit from the stable substrata provided by refractory leaves, which may
81 serve as habitat or case-building resources (Sanpera-Calbet *et al.* 2009).

82 Given that leaf litter constitutes the base of detrital food webs in forested
83 streams, changes in litter diversity may result in bottom-up effects for the entire food
84 web (Kominoski *et al.* 2010). The diversity of riparian plant species has been shown to
85 be positively correlated with fungal diversity (Laitung and Chauvet 2005; Lecerf *et al.*
86 2005; Fernandes *et al.* 2013) and this, in turn, has been correlated with leaf consumption
87 by shredders (Lecerf *et al.* 2005). Litter mixture effects on leaf decomposition by
88 shredders may also result from indirect effects mediated by fungi due to a greater
89 development of certain fungal species on leaf litter (Jabiol and Chauvet 2012).

90 In a previous study, we found that the effects of leaf diversity on microbial
91 decomposers depended on leaf species number and identity, and effects become more

pronounced after longer times following diversity loss (Fernandes *et al.* 2013). This highlights the importance of incorporating longer time scales when examining the impacts of plant litter biodiversity loss on detrital food webs. In this follow up study, we tested how leaf litter diversity and time following diversity loss affect leaf consumption, the elemental composition of invertebrate shredder tissues and the quality of FPOM produced to be used by other trophic levels. Mixtures of 3 plant species common in the riparian corridors of Iberian streams (alder, oak and eucalyptus) were immersed in a stream to allow microbial colonization. Simulation of species loss was done as described by Fernandes *et al.* (2013) by placing in microcosms a set of all leaf species, retrieved from the stream, and non-colonised leaves of 3, 2 or 1 leaf species. Leaves were renewed every month throughout 6 months, and microbial inoculum was ensured by a set of colonised leaves from the previous month. After 2 and 6 months following the simulation of leaf diversity loss (as a proxy of short and long time, respectively), leaves were given to invertebrate shredders. We expected that: i) leaf consumption by invertebrate shredders would be accelerated in microcosms containing higher quality leaf litter, ii) changes in litter quality would alter the quality of FPOM produced, iii) effects of litter diversity on leaf consumption would change with time following diversity loss, iv) the elemental composition of invertebrate shredder tissues would change with the quality of leaf litter, and finally, v) C:N imbalance between animal tissues and leaf litter would affect resource consumption by the invertebrates.

112

113 **Materials and methods**

114 *Leaf conditioning*

115 Leaves of alder (*Alnus glutinosa* (L.) Gaertn.), oak (*Quercus robur* L.) and eucalyptus
116 (*Eucalyptus globulus* Labill.) were collected from trees in October 2009, immediately

117 before abscission, and dried at room temperature. Alder and oak are representative of
118 the native vegetation bordering streams in Northwest Portugal, while eucalyptus (an
119 exotic species) was introduced almost two centuries ago, and its monocultures now
120 cover vast areas of the Iberian Peninsula (Graça *et al.* 2002). Leaves were soaked in
121 deionised water and cut into disks (12 mm) with a cork borer. On 28 October 2009, sets
122 of leaf disks of equal proportion for the 3 plant species were placed in fine-mesh bags
123 (0.5-mm pore size) and immersed in the Estorãos stream, a mixed-forested stream in
124 Portugal (41°78'19.4''N, 8°63'80.00''W), to allow microbial colonization. During leaf
125 immersion, stream water had on average ($\pm$ SEM) a temperature of 14 ± 1.0 °C, a pH of
126 5.9 ± 0.06 , a conductivity of $31 \mu\text{S cm}^{-1}$ and a redox potential of 51 ± 1.5 mV (Multiline
127 F/set 3 no. 400327; WTW, Weilheim, Germany). Average ($\pm$ SEM) nutrient
128 concentrations in the stream water were: 0.30 ± 0.04 mg L⁻¹ of N-NO₃⁻, 0.003 ± 0.000
129 mg L⁻¹ of N-NO₂⁻, <0.01 mg L⁻¹ of N-NH₃ and <0.003 P-PO₄³⁻ mg L⁻¹ (HACH kit,
130 programs 351, 371, 385, and 490, respectively; HACH, Loveland, CO, USA).

131 After 2 weeks of stream immersion, mesh bags containing mixtures of alder, oak
132 and eucalyptus leaf disks were returned to the laboratory. Simulation of leaf species loss
133 was done in Erlenmeyer flasks with filtered (Macherey–Nagel MN-GF3, glass-fibre
134 membranes) and sterilised stream water (autoclaved for 20 min, at 120 °C). Sets of 30
135 autoclaved non-colonised leaf disks were enclosed in mesh bags as follows i) 3 leaf
136 species (10 disks per species) representing no loss of plant diversity (control), ii) 2 leaf
137 species (15 disks per species, 3 treatments) simulating 1 species loss, and iii) 1 leaf
138 species (30 disks, 3 treatments) simulating 2 species loss. Mesh bags with non-
139 colonized leaf disks of each treatment were placed into microcosms containing 12 leaf
140 disks (4 disks per leaf species) previously colonized in the stream (initial inoculum)
141 (Fig. 1a). After 1 month, leaf disks that comprised the initial inoculum were discarded,

and leaf disks in mesh bags were replaced by non-colonised leaf disks keeping leaf species treatment constant (3, 2 or 1 leaf species) (Fig. 1b). Microcosm inoculum was ensured by keeping 12 leaf disks from mesh bags of the previous month: 4 leaf disks of each species for 3 leaf species treatment, 6 leaf disks of each species for 2 leaf species treatments, and 12 leaf disks of each species for 1 leaf species treatments. This procedure was repeated every month during 6 months. Microcosms were kept aseptically under aeration and artificial light, at 16 °C. Every 15 days, stream water was renewed by new filtered and sterilised stream water prepared as above. As a proxy of short and long time following diversity loss, leaf disks retrieved after 2 and 6 months, respectively, were used to assess the feeding behaviour of an invertebrate shredder, as described below.

Feeding experiment

Feeding experiments were carried out with early-stage larvae of the shredder caddisfly *Allogamus* sp. (Limnephilidae), which is a common invertebrate genus in Iberian streams. Animals were collected and acclimated to the laboratory (2 weeks) and starved for 20 h prior to the experiment. Animals were placed in 250 mL Erlenmeyer flasks containing 100 mL filtered stream water and leaf disks retrieved after 2 and 6 months from microcosm treatments described in previous section. Leaf disks were freeze-dried and weighed prior to being given to invertebrates as follows: i) 18 leaf disks for 1 species treatment, ii) 9 leaf disks per species for 2 species treatments, and iii) 6 leaf disks per species for 3 species treatment. One animal was used per replicate (12 replicates per treatment). Flasks were kept under aeration with aquarium pumps, in a temperature-controlled room (16 °C), with artificial light (12h:12h photoperiod) for 8 days. Stream water was renewed every 4 days and fine particulate organic matter

167 (FPOM) was collected by centrifugation (5000 rpm, 5 min; Centrifuge 5804R,
168 Eppendorf, Madrid, Spain). At the end of the experiment, remaining leaf disks, FPOM
169 and invertebrates were freeze-dried and stored at -80 °C until analysed.

170

171 *Invertebrate initial dry mass*

172 Just before the feeding experiment, the case diameter of each animal was measured
173 under a stereoscopic microscope (Wild M8, Wild Heerbrugg, Heerbrugg, Switzerland).
174 Initial dry mass of the animals used in the feeding experiment was estimated based on
175 the relationship between the case diameter (CD; mm) and body dry mass (DM; mg) as
176 follows: $DM = 2.9 \times CD - 5.3$ ($r^2 = 0.64$, $P < 0.05$). This relationship was established based
177 on 40 animals, for which CD was measured to the nearest 0.01 mm and the animals
178 (without the case) were freeze-dried before being weighed to the nearest 0.01 mg.
179 Animals used for linear regression had a CD ranging from 2.33 to 5.05 mm and a DM
180 ranging from 1.90 to 10.21 mg.

181

182 *Leaf consumption and FPOM production*

183 Before and after the feeding experiment, leaf disks were freeze-dried for 2 days and
184 weighed to the nearest 0.01 mg. Leaf consumption was estimated as the difference
185 between initial and final leaf mass and expressed per unit of invertebrate initial dry
186 mass. FPOM was freeze-dried for 2 days before being weighed to the nearest 0.01 mg.
187 FPOM production was expressed as mass per unit of invertebrate initial dry mass.

188

189 *Nutrient content*

190 Freeze-dried samples of leaves, FPOM and invertebrates were ground before elemental
191 analysis. Four replicates of each treatment were pooled to achieve enough mass for

192 nutrient analyses. Initial elemental composition in animal tissues was quantified before
 193 the feeding experiment in 3 sets of 4 animals acclimated for 2 weeks in the laboratory.
 194 Elements in invertebrates (4 animals) and FPOM (~1-1.5 mg) were analysed using an
 195 elemental analyser (Carlo Erba EA-1108, Carlo Erba Instruments, ThermoScientific,
 196 Massachusetts, USA), using sulfanilamide as a standard. Elements in leaves (~100 mg)
 197 were analysed using an elemental analyser (LECO-CNS 2000, LECO, St. Joseph, MI,
 198 USA), using EDTA as a standard. Analyses were done at the Centro de Apoio
 199 Científico e Tecnológico á Investigación (University of Vigo, Spain). Total C and N
 200 were expressed as % dry mass, and C:N ratio was expressed on a molar basis. C:N
 201 imbalance was calculated as the arithmetic difference in elemental ratios between
 202 invertebrate tissues and leaf litter (Cross *et al.* 2003). We are aware that this calculation
 203 may overestimate C:N imbalance because invertebrates tend to conserve assimilated N
 204 more than C, but it enables comparison with other studies.

205

206 *Statistical analyses*

207 Three-way nested analyses of variance (ANOVA) were used to test if time following
 208 leaf diversity loss, leaf species diversity and identity (nested within leaf species
 209 diversity) significantly affected leaf consumption and FPOM production. Because the
 210 design was unbalanced, we applied the Type III sum of squares using the Variance
 211 Estimation and Precision (VEPAC) module in Statistica 8.0 (Statsoft, Tulsa, OK, USA).
 212 Differences between treatments were analysed by the Tukey-Kramer's post-hoc test,
 213 which is a modification of the Tukey's post-hoc test for unbalanced designs (Zar 2010).
 214 Leaf consumption data were log(x) transformed to achieve normal distribution and
 215 homogeneity of variance.

Net diversity effects of leaf litter on leaf consumption and FPOM production were estimated as the difference between observed effects in leaf species mixtures and those expected based on the sum of effects of single leaf species weighted by their initial proportion in mixtures (Duarte *et al.* 2006). The differences between observed and expected values were tested against the null hypothesis that the average difference equalled 0 (t-test).

The effects of time following leaf diversity loss and leaf species identity on FPOM quality and invertebrates tissue composition were analysed by 2-way ANOVAs followed by Tukey's post-hoc tests. Differences between final and initial invertebrate tissue composition (C, N and C:N ratio) were determined using t-tests.

Linear regressions were used to establish the relationships between: i) FPOM production and leaf consumption; ii) elemental composition (C, N and C:N ratio) in leaves and in FPOM or animal tissues; and iii) C:N imbalance and leaf consumption. The effect of time following leaf diversity loss on the relationship between C:N imbalance and leaf consumption was tested by ANCOVA (Zar 2010).

Analyses of variance were done in Statistica 8.0 for Windows (Statsoft, Inc., Tulsa, OK) and linear regressions, ANCOVA and t-tests were done in Prism 4.0 for Windows (GraphPad software Inc., San Diego, CA).

Results

Leaf consumption and FPOM production

Leaf consumption and FPOM production by invertebrate shredders was affected by leaf species number and identity, but not by the time following diversity loss (3 way-nested ANOVA, Table 1; Fig. 2). Leaf consumption and FPOM production were highest in mixtures with 3 leaf species and lowest for oak leaves alone (Tukey-Kramer's test,

241 $P < 0.05$). A positive linear relationship was found between FPOM production and leaf
242 consumption ($P < 0.001$; $r^2 = 0.954$ and $r^2 = 0.933$, for short and long time after leaf
243 diversity loss, respectively).

244 After short time of leaf diversity loss, positive net diversity effects were
245 observed in mixtures of 3 leaf species because leaf consumption and FPOM production
246 by the invertebrates were higher than expected based on data from individual leaf
247 species (t-tests, $P = 0.027$ and $P = 0.022$, respectively; Fig. 3). However, no diversity
248 effects were observed for mixtures of 2 leaf species (t-test, $P > 0.05$). A longer time
249 following diversity loss led to an increase of positive net diversity effects because
250 significant effects were found not only in mixtures of 3 leaf species for leaf
251 consumption and FPOM production (t-tests, $P < 0.001$), but also on leaf consumption in
252 mixtures of oak and eucalyptus (t-test, $P = 0.011$), and on FPOM production in mixtures
253 of alder and eucalyptus or oak and eucalyptus (t-tests, $P = 0.034$ and $P = 0.008$,
254 respectively).

256 *FPOM quality*

257 Percent C in FPOM differed with leaf species identity but not with time following leaf
258 diversity loss (2-way ANOVA, Table 2; Fig. 4a). Percent C was highest in FPOM
259 produced from eucalyptus leaves alone, and lowest in FPOM produced from oak leaves
260 alone (Tukey's tests, $P < 0.05$). A positive linear relationship was found between % C in
261 FPOM and % C in leaves, at short and long time following leaf diversity loss (Table 3;
262 Fig. 4b).

263 Percent N in FPOM varied with leaf identity but not with time following leaf
264 diversity loss (2-way ANOVA, Table 2; Fig. 4c). Percent N in FPOM was highest in
265 microcosms with alder leaves and lowest in microcosms with eucalyptus and oak leaves

266 alone or in mixture (Tukey's tests, $P < 0.05$). A positive linear relationship was found
267 between % N in FPOM and % N in leaves, at short and long time following leaf
268 diversity loss (Table 3; Fig. 4d). Percent N was always higher in FPOM than in leaves,
269 and this difference was more pronounced in leaves with low % N.

270 The C:N ratio of FPOM was only affected by leaf identity (2-way ANOVA,
271 Table 2; Fig. 4e). C:N ratio was lowest in FPOM produced from alder or mixtures of
272 alder and oak leaves, and was highest in FPOM from eucalyptus leaves alone (Tukey's
273 tests, $P < 0.05$). A positive linear relationship was found between FPOM C:N ratio and
274 leaf C:N ratio (Table 3; Fig. 4f). C:N ratio was lower in FPOM than in leaves,
275 especially for leaves with high C:N ratio.

276

277 *Elemental composition of invertebrate shredder tissues*

278 Percent C in the invertebrate tissues was affected by leaf identity and time (2-way
279 ANOVA, Table 2; Fig. 5a), with higher values at longer time following leaf diversity
280 loss (Tukey's test, $P < 0.05$). The % C was highest in invertebrates that fed on mixtures
281 of alder and eucalyptus leaves, and was lowest in invertebrates that fed on oak leaves
282 alone (Tukey's tests, $P < 0.05$). The % C in invertebrate tissues increased in treatments
283 with leaf mixtures containing eucalyptus for both times (t-test, $P < 0.05$) and in single
284 treatments with alder or eucalyptus at long time following leaf diversity loss (t-test,
285 $P = 0.017$ and $P = 0.004$, respectively). Moreover, % C in invertebrate tissues increased
286 linearly with the increase in % C in leaves (Table 3; Fig. 5b).

287 Percent N in invertebrate tissues was affected by leaf identity and time (2-way
288 ANOVA, Table 2; Fig. 5c), with lower values at long time following leaf diversity loss
289 (Tukey's test, $P < 0.05$). Invertebrates had the lowest % N when fed on mixtures of alder
290 and eucalyptus leaves (Tukey's test, $P < 0.05$). Compared with the initial tissues

composition, % N in invertebrates increased in treatments with alder or oak leaves alone (t-test, $P=0.005$ and $P=0.033$, respectively) and in mixtures with 3 leaf species at short time following leaf diversity loss (t-test, $P=0.017$). Conversely, the % N decreased in animals that fed on mixtures of alder and eucalyptus leaves at long time following leaf diversity loss (t-test, $P=0.009$). No relationship was found between the % N in invertebrate tissues and the % N in leaves (Table 3; Fig. 5d).

The C:N ratio in invertebrate tissues was also affected by leaf identity and time (2-way ANOVA, Table 2; Fig. 5e), with higher values at long time following leaf diversity loss (Tukey's test, $P<0.05$). The highest C:N ratios were found in invertebrates that were fed on mixtures of alder and eucalyptus leaves (Tukey's test, $P<0.05$). Compared to the initial invertebrate elemental composition, the C:N ratio increased in animals fed on mixtures of alder and eucalyptus leaves for both times, and on mixtures of 3 leaf species at the longer time (t-test, $P<0.05$). Conversely, invertebrates that were fed on alder leaves at short time following leaf diversity loss had lower C:N ratio than at the beginning of the experiment (t-test, $P=0.022$). No relationship was found between invertebrate C:N ratio and initial C:N ratio of leaves (Table 3; Fig. 5f).

C:N imbalance and leaf consumption

C:N imbalances between leaf litter and invertebrate tissues ranged from 6 in alder to 20 in eucalyptus leaves (Table S1, Supplementary material). Leaf consumption by the shredder decreased linearly with increasing C:N imbalance ($P<0.001$; Fig. 6), but the relationship did not change with time following leaf diversity loss (ANCOVA, $F=0.83$, $P=0.36$). Leaf consumption by the shredder decreased from approximately 45% to 14% as C:N imbalance increased from 6 to 20, corresponding to a 2% decrease in leaf consumption per unit of C:N imbalance increase.

316 Discussion

317 Our results suggest that plant litter diversity affects litter consumption and FPOM
318 production by stream invertebrate shredders, and effects become stronger as there is a
319 longer time following plant diversity loss. Positive net diversity effects were observed
320 in mixtures of 3 leaf species at short time (2 months), while at longer time (6 months)
321 positive net diversity effects also emerged in mixtures of 2 leaf species (eucalyptus with
322 oak or alder). This agrees with that reported in a recent meta-analysis which brought to
323 the forefront the increased frequency of synergistic diversity effects of plant litter on
324 carbon and nutrient dynamics in streams with time (Lecerf *et al.* 2011).

325 In our study, plant diversity effects on leaf consumption by invertebrates might
326 have resulted from changes in leaf litter identity/quality. In fact, changes in litter
327 diversity led to changes in C:N imbalances between litter and invertebrates that ranged
328 from 6 in alder leaves to 20 in eucalyptus leaves, with intermediate values for leaf
329 mixtures. The C:N imbalances found in our study were within the lower range reported
330 by others (19-21, Lauridsen *et al.* 2012; 66-75, Cross *et al.* 2003; 8-107, Hladyz *et al.*
331 2009). This was not surprising because in our study leaf litter given to invertebrates was
332 colonised by microbes, and this resulted in a decrease in leaf C:N ratio compared to
333 their initial ratios (e.g., 16 to 12 for alder, 23 to 18 for oak, 36 to 26 for eucalyptus;
334 Fernandes *et al.* 2013; this study). Similarly to that observed by Hladyz and
335 collaborators (2009), a negative linear relationship was found between C:N imbalances
336 and leaf consumption. In our study, values for C:N imbalance of leaf mixtures with the
337 highest diversity fell in the middle of the observed imbalance range, i.e. the loss of leaf
338 diversity may result in higher or lower resource quality for invertebrate shredders and
339 explain the respective increase or decrease of leaf consumption. These findings suggest

340 that changes in plant litter diversity alter consumer-resource imbalances resulting in
341 changes to stream ecosystem processes.

342 The imbalance between elemental composition of shredder tissues and their food
343 resources (detritus) are the highest among invertebrates in streams (Cross *et al.* 2003;
344 Evans-White *et al.* 2005). This suggests that shredders can feed on a wide variety of
345 detritus with different elemental compositions. In our study, invertebrate shredders
346 preferentially fed on alder leaves as found by others (Canhoto and Graça 1995, 1996),
347 probably because these leaves had high quality (low C:N ratio) minimizing nutrient
348 imbalance. Surprisingly, the consumption of leaves with the lowest quality (eucalyptus
349 with high C:N ratio) was considerably high, especially at short time following diversity
350 loss. The high invertebrate consumption of lower quality leaf litter might occur as a
351 compensatory behaviour to fulfil the need for specific nutrients (Iversen 1974; Friberg
352 and Jacobsen 1999; Albariño and Balseiro 2001), probably in an attempt to maintain
353 elemental homeostasis (Sterner and Elser 2002). However, in our study, the elemental
354 composition of invertebrate shredder tissues changed with the quality of food resources,
355 especially in terms of C, and also varied with time following leaf diversity loss. Under
356 high C:N imbalances between leaf litter and invertebrates, animals may control the
357 excess of C by regulating the uptake of C-rich compounds across the gut, excreting
358 organic C, or increasing metabolic activity and respiration along with CO₂ release
359 (Hessen and Anderson 2008). However, in our study none of these mechanisms seemed
360 to be used by the animals to cope with increasing C:N imbalances because the % C in
361 invertebrates increased with increasing % C in leaf litter. Surplus of C can sometimes be
362 used for fitness-promoting purposes, such as structural defences, protective metabolites,
363 heat gain or energy storage (Hessen and Anderson 2008), which might have occurred in
364 our study. Besides changes in % C, some leaf treatments led to changes in % N

365 suggesting that invertebrates can deviate from strict homeostasis. Actually, changes in
366 consumer tissue composition due to changes in resource quality were previously
367 reported. For instance, in P enriched streams, P content in leaf litter, epilithon and
368 consumer invertebrates increased (Small and Pringle 2010). Also, a reduction in riparian
369 vegetation canopy led to a decrease in terrestrial detrital inputs and to an increase in
370 macrophytes and filamentous algae, which have higher nutritional quality (lower C:N
371 ratio) than plant litter, resulting in a decrease of the C:N ratio in the freshwater
372 omnivorous crayfish *Cherax destructor* (Giling *et al.* 2012). Therefore, changes in
373 resources may have direct effects on invertebrate tissue composition with implications
374 for other invertebrates and fishes feeding on them.

375 Feeding activity of invertebrate shredders by fragmenting larger particles into
376 smaller ones and through the production of faeces leads to FPOM release (Allan and
377 Castillo 2007). Therefore, changes in litter quality are expected to alter the quality of
378 FPOM available for other aquatic organisms. In our study, FPOM quality was positively
379 correlated with leaf litter quality although FPOM had higher % N. In addition, FPOM
380 produced from feeding activity on leaves with lower initial % N presented
381 proportionally higher % N. Some leaf species may have N compounds that are more
382 recalcitrant and, consequently, more difficult to digest and assimilate, resulting in
383 higher % N in faeces (Balseiro and Albariño 2006) or in overall FPOM (present study).
384 Moreover, microorganisms growing on FPOM could have incorporated N from the
385 water (Suberkropp and Chauvet 1995), further increasing % N in FPOM. This suggests
386 that changes in riparian vegetation may affect the quality of FPOM available to the
387 filter-feeders and collector-gatherers, with implications to aquatic detrital food webs.

388 Overall, we found that changes in leaf diversity (both number and identity of
389 leaf species) affected leaf consumption and FPOM production by invertebrate

shredders. Leaf diversity effects increased with time because positive diversity effects were more common after long time following leaf diversity loss. Changes in leaf identity were translated into changes in C:N imbalances between invertebrate shredders and their resources, with leaf consumption decreasing with the increase in C:N imbalance. Leaf diversity altered the elemental composition of invertebrate shredder tissues and FPOM quality with possible implications to other aquatic invertebrates and fishes. Therefore, our results support that changes in plant litter diversity may affect detrital food webs directly through changes in the activity of invertebrate shredders (leaf consumption and FPOM production) and indirectly through alterations in the quality of food resources (FPOM and invertebrates shredders) to higher trophic levels in streams.

400

401

402

403 **Acknowledgements**

404 The European Regional Development Fund - Operational Competitiveness
405 Programme (FEDER-POFC-COMPETE) and the Portuguese Foundation for Science
406 and Technology (FCT) supported this study (PEst-OE/BIA/UI4050/2014, PTDC/AAC-
407 AMB/117068/2010), IF (SFRH/BD/42215/2007) and SD (SFRH/BPD/47574/2008).

408 No specific permits were required for collection of invertebrate larvae because
409 this was done at a public location and these animals are not protected or endangered.
410 Moreover, the number of specimens collected did not cause significant decline in the
411 local population.

412

413

414

415 **References**

- 416 Albariño, R. J., and Balseiro, E. G. (2001). Food quality, larval consumption, and
417 growth of *Klapopteryx kuscheli* (Plecoptera: Austroperlidae) from a south Andes
418 stream. *Journal of Freshwater Ecology* **16**, 517-526.
419 doi:10.1080/02705060.2001.9663843
- 420 Allan, J. D., and Castillo, M. M. (2007). 'Stream ecology: structure and function of
421 running waters.' 2nd edn. (Springer: Dordrecht, The Netherlands.)
- 422 Balseiro, E., and Albariño, R. (2006). C-N mismatch in the leaf litter-shredder
423 relationship of an Andean Patagonian stream detritivore. *Journal of the North*
424 *American Benthological Society* **25**, 607-615. doi:10.1899/0887-
425 3593(2006)25[607:CMITLL]2.0.CO;2
- 426 Callisto, M., and Graça, M. A. S. (2013). The quality and availability of fine particulate
427 organic matter for collector species in headwater streams. *International Review*
428 *of Hydrobiology* **98**, 132-140. doi:10.1002/iroh.201301524
- 429 Canhoto, C., and Graça, M. A. S. (1995). Food value of introduced eucalypt leaves for a
430 Mediterranean stream detritivore: *Tipula lateralis*. *Freshwater Biology* **34**, 209-
431 214. doi:10.1111/j.1365-2427.1995.tb00881.x
- 432 Canhoto, C., and Graça, M. A. S. (1996). Decomposition of *Eucalyptus globulus* leaves
433 and three native leaf species (*Alnus glutinosa*, *Castanea sativa* and *Quercus*
434 *faginea*) in a Portuguese low order stream. *Hydrobiologia* **333**, 79-85.
435 doi:10.1007/BF00017570
- 436 Cross, W. F., Benstead, J. P., Frost, P. C., and Thomas, S. A. (2005). Ecological
437 stoichiometry in freshwater benthic systems: recent progress and perspectives.
438 *Freshwater Biology* **50**, 1895-1912. doi: 10.1111/j.1365-2427.2005.01458.x

- 439 Cross, W. F., Benstead, J. P., Rosemond, A. D., and Wallace, J. B. (2003). Consumer-
440 resource stoichiometry in detritus-based streams. *Ecology Letters* **6**, 721-732.
441 doi:10.1046/j.1461-0248.2003.00481.x
- 442 Cummins, K. W., and Klug, M. J. (1979). Feeding ecology of stream invertebrates.
443 *Annual Review of Ecology and Systematics* **10**, 147-172.
444 doi:10.1146/annurev.es.10.110179.001051
- 445 DeMott, W. R., Gulati, R. D., and Siewertsen, K. (1998). Effects of phosphorus-
446 deficient diets on the carbon and phosphorus balance of *Daphnia magna*.
447 *Limnology and Oceanography* **43**, 1147-1161. doi:10.4319/lo.1998.43.6.1147
- 448 Duarte, S., Pascoal, C., Cássio, F., and Bärlocher, F. (2006). Aquatic hyphomycete
449 diversity and identity affect leaf litter decomposition in microcosms. *Oecologia*
450 **147**, 658-666. doi:10.1007/s00442-005-0300-4
- 451 Evans-White, M. A., Stelzer, R. S., and Lamberti, G. A. (2005). Taxonomic and
452 regional patterns in benthic macroinvertebrate elemental composition in streams.
453 *Freshwater Biology* **50**, 1786-1799. doi:10.1111/j.1365-2427.2005.01455.x
- 454 Fernandes, I., Duarte, S., Cássio, F., and Pascoal, C. (2013). Effects of riparian plant
455 diversity loss on aquatic microbial decomposers become more pronounced with
456 increasing time. *Microbial Ecology* **66**, 763-772. doi:10.1007/s00248-013-0278-
457 0
- 458 Fernandes, I., Pascoal, C., Guimarães, H., Pinto, R., Sousa, I., *et al.* (2012). Higher
459 temperature reduces the effects of litter quality on decomposition by aquatic
460 fungi. *Freshwater Biology*, **57**, 2306-2317. doi:10.1111/fwb.12004
- 461 Ferreira, V., Encalada, A. C., and Graça, M. A. S. (2012). Effects of litter diversity on
462 decomposition and biological colonization of submerged litter in temperate and
463 tropical streams. *Freshwater Science* **31**, 945-962. doi:10.1899/11-062.1

- 464 Foley, J. A., DeFries, R., Asner, G. P., Barford, C., Bonan, G., *et al.* (2005) Global
465 consequences of land use. *Science* **309**, 570-574. doi:10.1126/science.1111772
- 466 Friberg, N., and Jacobsen, D. (1999). Variation in growth of the detritivore-shredder
467 *Sericostoma personatum* (Trichoptera). *Freshwater Biology* **42**, 625-635.
468 doi:10.1046/j.1365-2427.1999.00501.x
- 469 Frost, P. C., Benstead, J. P., Cross, W. F., Hillebrand, H., Larson, J. H., *et al.* (2006).
470 Threshold elemental ratios of carbon and phosphorus in aquatic consumers.
471 *Ecology Letters* **9**, 774-779. doi:10.1111/j.1461-0248.2006.00919.x
- 472 Giling, D. P., Reich, P., and Thompson, R. M. (2012). Riparian vegetation removal
473 alters consumer–resource stoichiometry in an Australian lowland stream. *Marine*
474 *and Freshwater Research* **63** 1-8. doi:http://dx.doi.org/10.1071/MF11092
- 475 Graça, M. A. S., and Canhoto, C. (2006). Leaf litter processing in low order streams.
476 *Limnetica* **25**, 1-10.
- 477 Graça, M. A. S., Pozo, J., Canhoto, C., and Eloise, A. (2002). Effects of *Eucalyptus*
478 plantations on detritus, decomposers, and detritivores in streams. *The Scientific*
479 *World Journal* **2**, 1173-1185. doi:10.1100/tsw.2002.193
- 480 Haines-Young, R. (2009). Land use and biodiversity relationships. *Land Use Policy* **26**,
481 S178-S186. doi:10.1016/j.landusepol.2009.08.009
- 482 Hessen, D. O., and Anderson, T. R. (2008). Excess carbon in aquatic organisms and
483 ecosystems: physiological, ecological, and evolutionary implications. *Limnology*
484 *and Oceanography* **53**, 1685-1696. doi:10.4319/lo.2008.53.4.1685
- 485 Hladyz, S., Gessner, M. O., Giller, P. S., Pozo, J., and Woodward, G. (2009). Resource
486 quality and stoichiometric constraints on stream ecosystem functioning.
487 *Freshwater Biology* **54**, 957-970. doi:10.1111/j.1365-2427.2008.02138.x

- 488 Iversen, T. M. (1974). Ingestion and growth in *Sericostoma personatum* (Trichoptera) in
489 relation to nitrogen content of ingested leaves. *Oikos* **25**, 278-282.
490 doi:10.2307/3543945
- 491 Jabiol, J., and Chauvet, E. (2012). Fungi are involved in the effects of litter mixtures on
492 consumption by shredders. *Freshwater Biology* **57**, 1667-1677.
493 doi:10.1111/j.1365-2427.2012.02829.x
- 494 Kominoski, J. S., Hoellein, T. J., Leroy, C. J., Pringle, C. M., and Swan, C. M. (2010).
495 Beyond species richness: expanding biodiversity-ecosystem functioning theory
496 in detritus-based streams. *River Research and Applications* **26**, 67-75. doi:
497 10.1002/rra.1285
- 498 Laitung, B., and Chauvet, E. (2005). Vegetation diversity increases species richness of
499 leaf-decaying fungal communities in woodland streams. *Archiv Fur*
500 *Hydrobiologie* **164**, 217-235. doi:10.1127/0003-9136/2005/0164-0217
- 501 Lauridsen, R. B., Edwards, F. K., Bowes, M. J., Woodward, G., Hildrew, A. G., *et al.*
502 (2012). Consumer–resource elemental imbalances in a nutrient-rich stream.
503 *Freshwater Science* **31**, 408-422. doi:10.1899/11-052
- 504 Lecerf, A., Dobson, M., Dang, C. K., and Chauvet, E. (2005). Riparian plant species
505 loss alters trophic dynamics in detritus-based stream ecosystems. *Oecologia* **146**,
506 432-442. doi:10.1007/s00442-005-0212-3
- 507 Lecerf, A., Marie, G., Kominoski, J. S., LeRoy, C. J., Bernadet, C., *et al.* (2011).
508 Incubation time, functional litter diversity, and habitat characteristics predict
509 litter-mixing effects on decomposition. *Ecology* **92**, 160-169. doi:10.1890/10-
510 0315.1

- 511 Lecerf, A., and Richardson, J. S. (2010). Biodiversity-Ecosystem function research:
512 insights gained from streams. *River Research and Applications* **26**, 45-54.
513 doi:10.1002/rra.1286
- 514 Lecerf, A., Risnoveanu, G., Popescu, C., Gessner, M. O., and Chauvet, E. (2007).
515 Decomposition of diverse litter mixtures in streams. *Ecology* **88**, 219-227.
516 doi:10.1890/0012-9658(2007)88[219:DODLMI]2.0.CO;2
- 517 Sanpera-Calbet, I., Lecerf, A., and Chauvet, E. (2009). Leaf diversity influences in-
518 stream litter decomposition through effects on shredders. *Freshwater Biology*
519 **54**, 1671-1682. doi:10.1111/j.1365-2427.2009.02216.x
- 520 Schindler, M. H., and Gessner, M. O. (2009). Functional leaf traits and biodiversity
521 effects on litter decomposition in a stream. *Ecology* **90**, 1641-1649.
522 doi:10.1890/08-1597.1
- 523 Small, G., and Pringle, C. (2010). Deviation from strict homeostasis across multiple
524 trophic levels in an invertebrate consumer assemblage exposed to high chronic
525 phosphorus enrichment in a Neotropical stream. *Oecologia* **162** 581-590.
526 doi:10.1007/s00442-009-1489-4
- 527 Sterner, R. W., and Elser, J. J. (2002). 'Ecological stoichiometry: the biology of
528 elements from molecules to the biosphere.' (Princeton University Press:
529 Princeton, NJ.)
- 530 Suberkropp, K. (1998). Microorganisms and organic matter decomposition. In 'River
531 ecology and management: lessons from the Pacific coastal ecoregion'. (Eds R.
532 J. Naiman, and R. E. Bilby) pp. 120-143. (Springer: New York, USA.)
- 533 Suberkropp, K., and Chauvet, E. (1995). Regulation of leaf breakdown by fungi in
534 streams: influences of water chemistry. *Ecology* **76**, 1433-1445.
535 doi:10.2307/1938146

536 Swan, C. M., and Palmer, M. A. (2006). Preferential feeding by an aquatic consumer
537 mediates non-additive decomposition of speciose leaf litter. *Oecologia* **149**, 107-
538 114. doi:10.1007/s00442-006-0436-x

539 Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R., and Cushing, C. E.
540 (1980). The river continuum concept. *Canadian Journal of Fisheries and*
541 *Aquatic Sciences* **37**, 130-137. doi:10.1139/f80-017

542 Zar, J. H. (2010). 'Biostatistical analysis.' 5th edn. (Pearson Prentice-Hall: Upper
543 Saddle River, New Jersey.)

544

545 Table 1. Effects of leaf species number, leaf species identity (nested within species
 546 number) and time following leaf diversity loss on leaf consumption and FPOM
 547 production

Parameter	Factor	SS	df	F	P
Leaf consumption	N° of species	0.623	2	14.860	<0.001
	Identity (N° of species)	1.425	4	17.005	<0.001
	Time	0.000	1	0.000	0.992
	N° of species*Time	0.065	2	1.551	0.216
	Identity (N° of species)*Time	0.092	4	1.099	0.359
	Error	3.122	149		
FPOM production	N° of species	94.787	2	13.512	<0.001
	Identity (N° of species)	144.918	4	10.329	<0.001
	Time	6.204	1	1.769	0.186
	N° of species*Time	12.000	2	1.711	0.184
	Identity (N° of species)*Time	30.720	4	2.190	0.073
	Error	498.053	142		

548

549

550 Table 2. Effects of leaf species identity and time following leaf diversity loss on
551 elemental composition of FPOM and invertebrate tissues, as carbon (C), nitrogen (N)
552 and C:N ratio

Parameter		Factor	SS	df	F	P
FPOM	C	Identity	143.30	6	49.95	<0.001
		Time	0.54	1	1.14	0.295
		Identity *Time	4.94	6	1.72	0.153
		Error	13.39	28		
	N	Identity	15.87	6	66.35	<0.001
		Time	0.03	1	0.70	0.411
		Identity *Time	0.34	6	1.42	0.242
		Error	1.12	28		
	C:N	Identity	127.30	6	38.69	<0.001
		Time	0.77	1	1.41	0.246
		Identity *Time	4.58	6	1.39	0.252
		Error	15.36	28		
Invertebrate tissues	C	Identity	22.49	6	8.80	<0.001
		Time	9.67	1	22.69	<0.001
		Identity *Time	3.68	6	1.44	0.235
		Error	11.93	28		
	N	Identity	2.15	6	2.69	0.034
		Time	1.04	1	7.82	0.009
		Identity *Time	1.11	6	1.40	0.251
		Error	3.72	28		
	C:N	Identity	1.67	6	4.19	0.004
		Time	0.82	1	12.28	0.002
		Identity *Time	0.60	6	1.51	0.211
		Error	1.86	28		

553

Table 3. Linear regressions between elemental composition of leaf litter and FPOM or invertebrate tissues at short time (2 months) and long time (6 months) following leaf diversity loss

Time		Equation	P	r^2
Leaf litter against FPOM				
Short	%C	$Y=0.642X+18.16$	<0.01	0.84
Long		$Y=0.555X+23.29$	<0.01	0.66
Short	%N	$Y=0.642X+2.418$	<0.01	0.89
Long		$Y=0.673X+2.305$	<0.01	0.80
Short	C:N	$Y=0.376X+6.260$	<0.01	0.82
Long		$Y=0.481X+4.491$	<0.01	0.86
Leaf litter against invertebrate tissues				
Short	%C	$Y=0.195X+36.25$	<0.01	0.43
Long		$Y=0.235X+35.02$	<0.01	0.36
Short	%N	$Y=0.085X+9.157$	0.40	0.04
Long		$Y=-0.021X+9.219$	0.84	<0.01
Short	C:N	$Y=0.026X+5.276$	0.10	0.14
Long		$Y=0.001X+6.067$	0.95	<0.01

557

558 Figure legends

559

560 **Fig. 1** Scheme of leaf species treatments used in the feeding experiment: a) initial setup
561 included microcosms with mesh bags containing non-colonised leaf disks of alder (A),
562 oak (O) and eucalyptus (E) in all possible combinations (3, 2 and 1 leaf species) plus
563 disks of the 3 leaf species colonised in a stream for 2 weeks (initial inoculum); b) after 1
564 month, the old inoculum was discarded and leaf disks in the mesh bags were used as
565 inoculum for new microcosms containing new mesh bags with non-colonised leaf disks,
566 and keeping leaf species treatment constant. This procedure was repeated under aseptic
567 conditions every month for 6 months. After 2 and 6 months, leaf disks from each mesh
568 bag were used for a feeding experiment with invertebrate shredders.

569

570 **Fig. 2** Leaf consumption (a, b and c) and FPOM production (d, e and f) by the
571 invertebrate shredder after 8 days in microcosms. Invertebrates were fed on microbially-
572 colonised leaf litter harvested after 2 months (short time) and 6 months (long time)
573 following simulation of leaf diversity loss in microcosms. A, alder; O, oak; E,
574 eucalyptus. Values are mean plus standard error of the mean. Equal letters indicate no
575 significant differences between treatments (Tukey-Kramer's post-hoc tests; $P < 0.05$)

576

577 **Fig. 3** Net diversity effects of leaf litter on leaf consumption (a) and FPOM production
578 (b) by the invertebrate shredder fed on microbially-colonised leaf litter harvested after 2
579 months (short time) and 6 months (long time) following simulation of leaf diversity loss
580 in microcosms. Net diversity effects were estimated as the difference between observed
581 values in leaf mixtures and those expected based on the weighed sum of individual leaf

species. Differences were tested against zero by a t-test; * $P < 0.05$. A, alder; O, oak; E, eucalyptus. Values are mean plus standard error of the mean

584

Fig. 4 Percent C (a) and N (c), and C:N ratio (e) in the FPOM produced by the shredder during the feeding experiment. Invertebrates were fed on microbially-colonised leaf litter harvested after 2 months (short time) and 6 months (long time) following simulation of leaf diversity loss in microcosms. Linear regressions between elemental composition of FPOM and leaf litter with respect to C (b), N (d) and C:N ratio (f) at short time (black dashed lines) and long time (black solid lines) following leaf diversity loss. Regression equations are in Table 3. Grey dashed lines indicate 1:1 relationships. A, alder; O, oak; E, eucalyptus. Values are mean plus standard error of the mean (a, c, e)

594

Fig. 5 Percent C (a) and N (c), and C:N ratio (e) in the invertebrate tissues after the feeding experiment. Invertebrates were fed on microbially-colonised leaf litter harvested after 2 months (short time) and 6 months (long time) following simulation of leaf diversity loss in microcosms. Horizontal solid and dashed lines (a, c, e) indicate mean and standard error of the mean, respectively, of initial elemental composition of animal tissues before feeding. Asterisks (*) indicate significant differences in elemental composition of animal tissues before and after the feeding experiment (t-test). Linear regressions between elemental composition of animal tissues and leaf litter with respect to C (b), N (d) and C:N ratio (f) at short time (black dashed lines) and long time (black solid lines) following leaf diversity loss. Regression equations are in Table 3. Grey dashed lines indicate 1:1 relationships. A, alder; O, oak; E, eucalyptus. Values are mean plus standard error of the mean (a, c, e)

607

608 **Fig. 6** Linear regression of percentage leaf consumption against C:N imbalance between
609 invertebrate tissues (consumer) and leaf litter (resource). Invertebrates were fed on
610 microbially-colonised leaf litter from microcosms harvested after 2 months (short time,
611 dashed line) and after 6 months (long time; solid line) following simulation of leaf
612 species loss. Short time, $Y = -1.84X + 52.42$; $r^2 = 0.33$; $P < 0.0001$. Long time, $Y = -$
613 $2.20X + 59.24$; $r^2 = 0.46$; $P < 0.0001$

For Review Only

b) After 1 month

Procedure repeated every month for 6 months

Fig. 2 Leaf consumption (a, b and c) and FPOM production (d, e and f) by the invertebrate shredder after 8 days in microcosms. Invertebrates were fed on microbially-colonised leaf litter harvested after 2 months (short time) and 6 months (long time) following simulation of leaf diversity loss in microcosms. A, alder; O, oak; E, eucalyptus. Values are mean plus standard error of the mean. Equal letters indicate no significant differences between treatments (Tukey-Kramer’s post-hoc tests; $P < 0.05$)

240x124mm (300 x 300 DPI)

Fig. 3 Net diversity effects of leaf litter on leaf consumption (a) and FPOM production (b) by the invertebrate shredder fed on microbially-colonised leaf litter harvested after 2 months (short time) and 6 months (long time) following simulation of leaf diversity loss in microcosms. Net diversity effects were estimated as the difference between observed values in leaf mixtures and those expected based on the weighed sum of individual leaf species. Differences were tested against zero by a t-test; * $P < 0.05$. A, alder; O, oak; E, eucalyptus. Values are mean plus standard error of the mean
107x131mm (300 x 300 DPI)

Fig. 4 Percent C (a) and N (c), and C:N ratio (e) in the FPOM produced by the shredder during the feeding experiment. Invertebrates were fed on microbially-colonised leaf litter harvested after 2 months (short time) and 6 months (long time) following simulation of leaf diversity loss in microcosms. Linear regressions between elemental composition of FPOM and leaf litter with respect to C (b), N (d) and C:N ratio (f) at short time (black dashed lines) and long time (black solid lines) following leaf diversity loss. Regression equations are in Table 3. Grey dashed lines indicate 1:1 relationships. A, alder; O, oak; E, eucalyptus. Values are mean plus standard error of the mean (a, c, e)

Fig. 5 Percent C (a) and N (c), and C:N ratio (e) in the invertebrate tissues after the feeding experiment. Invertebrates were fed on microbially-colonised leaf litter harvested after 2 months (short time) and 6 months (long time) following simulation of leaf diversity loss in microcosms. Horizontal solid and dashed lines (a, c, e) indicate mean and standard error of the mean, respectively, of initial elemental composition of animal tissues before feeding. Asterisks (*) indicate significant differences in elemental composition of animal tissues before and after the feeding experiment (t-test). Linear regressions between elemental composition of animal tissues and leaf litter with respect to C (b), N (d) and C:N ratio (f) at short time (black dashed lines) and long time (black solid lines) following leaf diversity loss. Regression equations are in Table 3. Grey dashed lines indicate 1:1 relationships. A, alder; O, oak; E, eucalyptus. Values are mean plus standard error of the mean (a, c, e)

Fig. 6 Linear regression of percentage leaf consumption against C:N imbalance between invertebrate tissues (consumer) and leaf litter (resource). Invertebrates were fed on microbially-colonised leaf litter from microcosms harvested after 2 months (short time, dashed line) and after 6 months (long time; solid line) following simulation of leaf species loss. Short time, $Y=-1.84X+52.42$; $r^2=0.33$; $P<0.0001$. Long time, $Y=-2.20X+59.24$; $r^2=0.46$; $P<0.0001$
79x75mm (300 x 300 DPI)

Supplementary material for *Plant litter diversity affects invertebrate shredder activity and the quality of fine particulate organic matter in streams* by Fernandes *et al.*

Table S1. Elemental composition of leaf litter used to feed invertebrates and C:N imbalance between leaf litter and invertebrate tissues. Leaf litter was colonized in a stream and then transferred to microcosms to simulate leaf species loss at short time (2 months) and long time (6 months). Nitrogen (N) and carbon (C) are percent dry mass and C:N is expressed as molar ratio. A, alder; O, oak; E, eucalyptus. M $\pm$ SE.

Leaf treatment	Time	Leaf C (%)	Leaf N (%)	Leaf C:N	C:N imbalance
A	Short	52.41 $\pm$ 0.18	5.23 $\pm$ 0.18	11.73 $\pm$ 0.37	5.88 $\pm$ 0.37
	Long	53.17 $\pm$ 0.22	5.34 $\pm$ 0.17	11.65 $\pm$ 0.40	5.80 $\pm$ 0.40
O	Short	47.98 $\pm$ 0.65	3.23 $\pm$ 0.09	17.35 $\pm$ 0.61	11.50 $\pm$ 0.61
	Long	48.83 $\pm$ 0.34	3.22 $\pm$ 0.04	17.67 $\pm$ 0.25	11.83 $\pm$ 0.25
E	Short	56.83 $\pm$ 0.12	2.53 $\pm$ 0.02	26.24 $\pm$ 0.12	20.39 $\pm$ 0.12
	Long	56.17 $\pm$ 0.10	2.51 $\pm$ 0.01	26.08 $\pm$ 0.04	20.23 $\pm$ 0.04
AO	Short	50.23 $\pm$ 0.28	4.25 $\pm$ 0.05	13.78 $\pm$ 0.23	7.94 $\pm$ 0.23
	Long	51.00 $\pm$ 0.19	4.20 $\pm$ 0.13	14.19 $\pm$ 0.39	8.35 $\pm$ 0.39
AE	Short	56.44 $\pm$ 0.02	3.24 $\pm$ 0.06	20.32 $\pm$ 0.37	14.47 $\pm$ 0.37
	Long	53.02 $\pm$ 2.45	3.08 $\pm$ 0.19	20.12 $\pm$ 0.30	14.27 $\pm$ 0.30
OE	Short	54.75 $\pm$ 0.43	2.78 $\pm$ 0.03	23.00 $\pm$ 0.29	17.16 $\pm$ 0.29
	Long	53.98 $\pm$ 0.15	2.71 $\pm$ 0.05	23.24 $\pm$ 0.38	17.39 $\pm$ 0.38
AOE	Short	54.68 $\pm$ 0.28	3.54 $\pm$ 0.04	18.02 $\pm$ 0.12	12.18 $\pm$ 0.12
	Long	54.01 $\pm$ 0.21	3.25 $\pm$ 0.05	19.42 $\pm$ 0.39	13.57 $\pm$ 0.39