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Francisco Correa-Araneda, Luz Boyero, Ricardo Figueroa, Carolina Sánchez ...+3 more authors

Institutions: University of Concepción, University of Málaga, Pablo de Olavide University, University of Coimbra

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1 **Joint effects of climate warming and exotic litter (*Eucalyptus globulus***
2 **Labill.) on stream detritivore fitness and litter breakdown**

3

4 FRANCISCO CORREA-ARANEDA^{1,2}, LUZ BOYERO^{3,4,5,6*}, RICARDO FIGUEROA²,
5 CAROLINA SÁNCHEZ², ROBERTO ABDALA⁷, ANTONIO RUIZ-GARCÍA⁸ &
6 MANUEL A.S. GRAÇA^{9,10}

7

8 ¹Environmental Department, Aquaculture Research Division, Fisheries Development
9 Institute, Puerto Montt, Chile, ²Water Quality Bioindicators Laboratory, Faculty of
10 Environmental Science, University of Concepción, Concepción, Chile, ³Wetland Ecology
11 Department, Doñana Biological Station-CSIC, Sevilla, Spain, ⁴School of Marine and
12 Tropical Biology, James Cook University, Townsville, QLD, Australia, ⁵Present address:
13 Faculty of Science and Technology, University of the Basque Country (UPV/EHU),
14 Bilbao, Spain, ⁶Present address: IKERBASQUE, Basque Foundation for Science, Bilbao,
15 Spain, ⁷Ecology Department, Faculty of Science, University of Malaga, Spain, ⁸Department
16 of Physical, Chemical and Natural Systems, University Pablo de Olavide, Sevilla, Spain,
17 ⁹Department of Life Sciences, University of Coimbra, Portugal, ¹⁰IMAR / MARE,
18 Coimbra, Portugal.

19 *Corresponding author. Email address: luz.boyero@ehu.es. Phone: +34 946 018 031. Fax:
20 +34 946 013 500.

21

22 **Abstract**

23 Joint effects of climate warming and other stressors are potentially complex and difficult to
24 predict. In stream ecosystems, exotic riparian species have the potential to alter leaf-
25 shredding detritivorous invertebrate assemblages and leaf litter breakdown due to
26 differences in the quality of litter inputs. This is the case for *Eucalyptus* plantations, which
27 are widespread, occurring along riparian corridors of streams around the world. We
28 hypothesised that the presence of *Eucalyptus globulus* (Labill.) litter (1) impairs detritivore
29 fitness both directly (i.e., through leaf consumption) and indirectly (i.e., through leaf
30 leachates in the water) and (2) impairs litter breakdown, (3) with stronger effects at higher
31 temperatures. We tested these hypotheses in microcosm experiments with two detritivore
32 species from two locations: the stonefly *Diamphipnosis samali* (Illies, 1960) in Chile and
33 the caddisfly *Calamoceras marsupus* (Brauer, 1865) in Spain. *Eucalyptus* leaves affected
34 detritivore growth mainly by direct consumption, while the presence of both *Eucalyptus*
35 leaves and leachates inhibited the breakdown of native litter. When both litter types were
36 available, breakdown of *Eucalyptus* leaves was enhanced, possibly as a means of
37 compensatory feeding. Increased temperature exacerbated the negative effect of *Eucalyptus*
38 on native litter breakdown, possibly because it reduced detritivore survival. Our results add
39 to the mounting evidence that joint effects of multiple stressors can be non-additive, and
40 suggest that the sole presence of *Eucalyptus* leaves and leachates in the water may impact
41 stream communities and ecosystem functions even if native litter is available, with further
42 negative effects to be expected under a warmer climate.

43

44 **Running title:** Joint stressor effects on litter breakdown.

45 **Key words:** climate change, exotic species, leaf litter breakdown, leaf-shredding
46 detritivores, riparian vegetation, stream ecosystems, temperature.

47

48 **Introduction**

49 Climate change predictions suggest an air temperature increase of 1.1 – 6.4 °C by the end of
50 this century (IPCC 2007), and there is growing evidence that this increase will cause
51 biodiversity losses and impair ecosystem functioning in terrestrial and aquatic habitats
52 (Parmesan 2006). Climate warming may be accompanied by changes in other stressors such
53 as eutrophication (Woodward et al. 2012), increased UV radiation (Hader et al. 2011),
54 reduced flow conditions (Dewson et al. 2007), or species introductions or invasions (Rahel
55 & Olden 2008). There is consensus about the necessity to consider the joint effects of
56 multiple stressors on organisms and ecosystems, because adapting to certain stressors may
57 increase sensitivity to others (Kashian et al. 2007), and the joint action of stressors may
58 have complex, non-additive or synergistic effects on organisms and ecosystems (Folt et al.
59 1999).

60 Freshwater ecosystems contribute disproportionately to global species richness, and
61 their species extinction rates are among the greatest on the planet (Ricciardi & Rasmussen
62 1999; Dudgeon et al. 2006; Ormerod et al. 2010). Climate change can affect freshwater
63 organisms and ecosystems mainly through changes in water temperature and hydrology
64 (Woodward et al. 2010). However, information on ecological effects of climate change in
65 freshwater ecosystems is relatively scarce compared to other ecosystems, particularly with
66 respect to the interaction between climate change and other stressors (Durance & Ormerod
67 2007; Piggott et al. 2012). There is evidence of synergistic effects of increased water
68 temperature and enhanced nutrient levels on key ecological processes (e.g. Ferreira &

69 Chauvet 2011), but interactions between temperature and exotic species, and their
70 combined effects on freshwater biota and ecosystem functions, are mostly unknown.

71 Exotic riparian species are replacing native vegetation around the world, potentially
72 altering stream ecosystem functioning and benthic invertebrate assemblages through the
73 input of allochthonous leaf litter – often of different nutritional quality – to the stream
74 (Boyero et al. 2012). In different areas of the world, including several Mediterranean
75 regions (e.g., the Iberian Peninsula, Chile, California), vast areas of deciduous forests have
76 been converted into evergreen *Eucalyptus globulus* (Labill.) plantations, which are
77 profitable for the paper industry (Graça et al. 2002; Ferreira et al. 2006). This replacement
78 affects stream ecosystems through changes in litterfall timing, quantity, quality and hence
79 breakdown rates (Pozo et al. 1998).

80 *Eucalyptus* leaves have low nutrient content (Ferreira et al. 2006), a thick and waxy
81 cuticle that retards their microbial colonization (Canhoto & Graça 1999), and high content
82 of essential oils, polyphenols and tannins which impair microbial degradation (Abelho &
83 Graça 1996; Graça et al. 2002). Some leaf-shredding detritivores are unable to grow when
84 fed exclusively on *Eucalyptus* leaves, mostly because of the oils and polyphenols that these
85 leaves contain (Canhoto & Graça 1995; Graça et al. 2002). Moreover, *Eucalyptus* leaf
86 leachates in the water could potentially affect detritivore fitness, even when these
87 organisms do not directly feed on *Eucalyptus* leaves (Canhoto & Laranjeira 2007). This
88 prediction, which remains untested, is important because *Eucalyptus* plantations could
89 negatively affect stream communities and ecosystem functioning even if riparian strips of
90 native species were maintained (Abelho & Graça 1996).

91 Here we examined the joint effects of temperature and *Eucalyptus* leaves and
92 leachates on stream detritivore fitness (survival, growth and lipid content) and on litter

93 breakdown. We hypothesised that the presence of *Eucalyptus* litter (1) impairs detritivore
94 fitness both directly (i.e., through leaf consumption) and indirectly (i.e., through leaf
95 leachates in the water) and (2) impairs litter breakdown, (3) with stronger effects at higher
96 temperatures. We tested these hypotheses in microcosm experiments in two areas, central
97 Chile and southern Spain. In these countries, *E. globulus* plantations are widespread and
98 known to supply streams with leaf litter (Graça et al. 2002; Mancilla et al. 2009).

99

100 **Material and methods**

101 *Leaf and detritivore collection*

102 We collected the detritivores and leaf litter at the Estero Nonguén stream (Reserva
103 Nacional Nonguén, central Chile) in July 2012, and the Canuto de Valdeinfierno stream
104 (Los Alcornocales Natural Park, southern Spain) in November 2012 (Table 1). Both
105 streams flow through extensive areas planted with the exotic *E. globulus*, but they maintain
106 a riparian strip composed of native plant species. We collected leaves of the dominant
107 native species *Nothofagus obliqua* (Mirb.) Oerst. (Chile) and *Alnus glutinosa* (L.) Gaertn.
108 (Spain) from the riparian vegetation, and *E. globulus* leaves from nearby areas. Leaves of
109 both native species have higher quality than *E. globulus* leaves as indicated by their lower
110 C:N ratios (*A. glutinosa*: 15, *N. obliqua*: 52; *E. globulus*: 74) (Vivanco & Austin 2008;
111 Perez et al. 2014) and the presence of oils, polyphenols and tannins in *E. globulus* leaves
112 (Graça et al. 2002). Leaves were collected from the tree in Chile (as there were no abscised
113 leaves at the time of collection) and freshly abscised from the ground in Spain.

114 The detritivore species used were the dominant leaf-shredding detritivores at the
115 sampling sites and nearby sites not affected by *Eucalyptus* plantations: the stonefly
116 *Diamphipnopsis samali* Illies (Plecoptera: Diamphipnoidae) in Chile and the caddisfly

117 *Calamoceras marsupus* Brauer (Trichoptera: Calamoceratidae) in Spain (hereafter
118 *Diamphipnosis* and *Calamoceras*, respectively). Stoneflies were collected from natural leaf
119 litter packs using 250- μ m mesh Surber and hand-net samplers, and caddisflies were taken
120 manually from the stream substrate. In both cases, they were taken to the laboratory in
121 aerated containers that were kept cool within ice chests.

122

123 *Experimental manipulations*

124 Detritivores were acclimated for 48 h in containers with stream water kept at stream
125 temperature at the time of collection (8 and 13 °C in Chile and Spain, respectively); they
126 were then wet weighed and introduced in the experimental containers (see below).

127 Caddisflies were gently pushed out of their cases before weighing, using a pair of soft
128 entomological forceps, and then put back in their cases. This procedure did not cause any
129 mortality. Leaf discs (12 mm diameter) were cut using a cork borer, air dried to constant
130 weight and weighed.

131 Both experiments used the following leaf treatments: native leaves only (N);
132 *Eucalyptus* leaves only (E); both native and *Eucalyptus* leaves (N+E); and native leaves
133 plus *Eucalyptus* leaves enclosed in 55- μ m mesh bags, so only native leaves were available
134 for detritivores but the water contained *Eucalyptus* leachates (N+EL). Each replicate had 6
135 leaf discs available for detritivores, which were either of the same species (6N or 6E) or
136 both species (3N + 3E); treatment N+EL contained 6 discs of the native species plus 3 discs
137 of *Eucalyptus* enclosed in the mesh bags. Total leaf dry mass initially available for
138 detritivores was 0.26 mg \pm 0.08 SD in Chile and 0.22 mg \pm 0.02 SD in Spain.

139 In Chile, we ran the experiment in two large polystyrene containers (95 \times 50 \times 25
140 cm), which were half filled with tap water and kept at different temperatures (8 and 15 °C).

141 The low temperature treatment was chosen because it was the stream temperature at the
142 time of collection as well as the mean stream temperature at the month of collection, and
143 the high temperature treatment was chosen because it was the mean stream temperature at
144 the hottest month; a 7°C temperature increase is only slightly above the likely temperature
145 increase range predicted for this century under climate change scenario A1FI (IPCC 2007).
146 Room temperature was set so water in one container was kept at 8 °C, and the other was
147 warmed up to 15°C using aquarium heaters. In each container we introduced 32 glass jars
148 containing 110 mL of filtered (0.45 µm) stream water, the leaf discs, and one stonefly. Each
149 combination of leaf litter and temperature was thus replicated 8 times. A 55-µm mesh net
150 on the top was used to preclude emerging adults from escaping. Oxygen levels were kept
151 high within jars using aquarium pumps and 5-mm diameter tubing.

152 In Spain, we ran the experiment in eight 100 × 11 × 6 cm PVC channels filled with
153 dechlorinated tap water (80%) and stream water (20%) which had been mixed one week
154 before the beginning of the experiment. Water flowed from the channel into a downstream
155 container, from which it was returned to the upstream end of the channel through plastic
156 tubing using a pump. The two temperature treatments in this case were 13°C (the stream
157 temperature at the time of collection and the mean stream temperature at the month of
158 collection) and 18°C (the mean stream temperature at the hottest month); a 5°C temperature
159 increase falls within the upper confidence interval of the A2 scenario within IPCC (IPCC
160 2007). Room temperature was set to maintain water temperature at 13°C, and each channel
161 was assigned to either low (13°C) or high (18°C) temperature treatments; the latter were
162 provided with an aquarium heater in the downstream container, set to maintain water
163 temperature at 18°C. Each channel was also assigned to a certain leaf treatment. Channels
164 were divided into 9 sections by 0.5-mm mesh, and each section (replicate) was provided

165 with the leaf discs and one caddisfly. The tops of the channels were covered with plastic
166 sheets to prevent emerging adults from escaping. Sections within each channel were
167 considered independent because animals and leaf material did not pass through the mesh in
168 any case, temperature was constant across the channels, and flow was very low and
169 constant across the channels.

170 In Spain, we set up four additional channels that contained leaf discs within each
171 section but no caddisflies, to control for microbial decomposition. Each channel contained
172 either *Alnus* or *Eucalyptus* leaf discs (0.21 ± 0.01 SD mg per section) and was maintained
173 at either 13 or 18°C, so that we had 9 replicates (sections) of each combination of
174 treatments. We used the *Eucalyptus* leaf discs within mesh bags in the N+EL treatments as
175 additional controls for microbial decomposition, both in Chile and Spain. We did not have
176 controls for microbial decomposition of *Nothofagus* in Chile.

177 Note that, although we used summer temperatures (high temperature treatment) and
178 detritivores were collected in fall/winter, both detritivore species occur all year round (Feio
179 et al. 2005; F.J. Correa-Araneda, pers. obs.). A natural photoperiod of 10:14 hours of
180 light:dark was maintained throughout the duration of both experiments. Every 48 h we
181 monitored water temperature and oxygen levels and collected any dead animals, which
182 were kept in 70% ethanol. No insects emerged during the experiments. The experiments
183 were terminated after 35 d (Chile) or 10 d (Spain), when > 50% of all leaf material had
184 been lost in some treatments; treatments with the lowest breakdown rates had lost 3% of
185 leaf material in Chile and 7% in Spain. Leaf discs were oven dried at 60 °C for 48 h and
186 weighed. All detritivores were kept in 70% ethanol and wet weighed. Ten extra individuals
187 of each species (not used in the experiments) were used to calculate a relationship between
188 wet mass (individuals that were just collected in the field and uncased) and dry mass

189 (individuals that were uncased and dried at 60 °C for 48 h); this relationship was used to
190 estimate initial detritivore dry mass. Another 10 individuals of each species were used to
191 calculate a relationship between wet mass of individuals kept in ethanol for 48 h and dry
192 mass; this relationship was used to estimate final detritivore dry mass.

193

194 *Lipid content analysis*

195 A random subsample of three detritivore individuals per treatment combination were
196 ground using a mortar and pestle, and analysed for lipid contents following Folch et al.
197 (1957). Each sample was homogenized in chloroform:methanol (2:1) for 5 minutes. The
198 lipid fraction was separated by centrifugation, and the total lipid content was calculated
199 gravimetrically once the solvent (chloroform) was completely evaporated from the lipid
200 fraction. Lipids were dissolved in toluene and methyl esters obtained from fatty acids
201 (FAMES) by transesterification with sulfuric acid (1%) in methanol (Christie 2003). The
202 reaction was held at 50 °C for 16 hours in darkness and molecular nitrogen. The methyl
203 esters were extracted with hexane:diethyl ether (1:1 v/v), washed with KHCO₃ solution (2%
204 w/v) and purified on a column Sep-pack NH₂ (Waters, Milford, Massachusetts, USA) with
205 hexane as a diluent. FAMES were separated by gas chromatography using helium as carrier
206 gas provided with a BPX70 column (70% cyanopropyl polysilphenylene-siloxane) 60 m ×
207 0.25 mm (SGE Analytical Science). The initial column temperature was 140 °C for 10
208 minutes, then was increased to 240 °C at 2.5 °C min⁻¹ and finally maintained at 240 °C for
209 10 minutes. The FAMES detection was performed using a flame detector and the peaks
210 were identified with a standard pattern of FAMES.

211

212 *Data analysis*

213 We examined differences among treatments in several variables: 1) detritivore survival:
214 quantified as the proportion of detritivores that were alive at the end of each week (only for
215 stoneflies, as no caddisflies died during the experiment); 2) detritivore growth rate:
216 quantified as $(FDM - IDM) / IDM$, where FDM and IDM are the final and initial
217 detritivore dry mass in mg, respectively, divided by the number of days of the experiment;
218 3) detritivore lipid content: quantified as lipid absolute (mg) and relative content (i.e.,
219 proportion of body mass made of lipids); and 4) litter breakdown rate: quantified as $(FLM -$
220 $ILM) / ILM$, where FLM and ILM are the final and initial leaf dry mass in mg, respectively,
221 divided by the number of days of the experiment.

222 Differences in stonefly survival among leaf treatments and temperatures were
223 examined separately with Pearson's chi-square tests on the untransformed proportion of
224 detritivores alive at weeks 1, 2, 3, 4 and 5. When results were significant, we further
225 compared each pair of treatments separately. Differences in detritivore growth rate (mg
226 day^{-1}), detritivore absolute lipid contents (mg), arcsin square root-transformed detritivore
227 relative lipid contents, and litter breakdown rate (mg day^{-1}), were examined among leaf
228 treatments and between temperatures with two-way ANOVAs followed by post-hoc Tukey
229 tests. The leaf treatments compared were as follows: detritivore growth and lipid contents
230 were compared among all treatments (N vs. E vs. N+E vs. N+EL); litter breakdown was
231 examined separately for native leaves (N vs. N+E vs. N+EL), *Eucalyptus* leaves (E vs.
232 N+E), and native vs. *Eucalyptus* leaves in single-species treatments (N vs. E). ANOVAs
233 were used to compare (1) breakdown rate in controls without detritivores in Spain: two-way
234 ANOVA with temperature and leaf treatments (*Alnus* vs. *Eucalyptus*) as factors; and (2)
235 breakdown rate in *Eucalyptus* leaf discs within mesh bags: one-way ANOVA with

236 temperature as factor. Finally, we examined any potential effect of detritivore initial
237 biomass and survival on native and *Eucalyptus* litter breakdown rates, as well as any
238 potential relationship between detritivore survival and growth, by means of linear
239 regression; for this analysis survival was quantified as the week (from 1 to 5) each
240 detritivore was last observed alive.

241

242 **Results**

243 In Chile, survival of *Diamphipnosis* differed among leaf treatments in every observation
244 (weeks 1 to 5), being always highest in the N treatment, and among temperature treatments
245 in weeks 4 and 5, being higher at 8°C than 15°C (Table 2, Fig. 1). In Spain, survival of
246 *Calamoceras* was 100% in all cases. Detritivore growth rate differed among leaf treatments
247 both in Chile (N > E; $F_{3,56} = 3.41$, $p = 0.024$) and Spain (N, N+E, N+EL > E; $F_{3,60} = 4.08$, p
248 $= 0.010$), but not between temperatures, and the interaction between both factors was not
249 significant in any case (Table S1, Fig. 2). Detritivore lipid contents did not differ between
250 leaf or temperature treatments in any of the experiments (Table S2).

251 In Chile, breakdown rate of the native *Nothofagus* differed between temperatures,
252 but this difference occurred only within the N treatment, as shown by the significant leaf
253 treatment \times temperature interaction ($F_{2,42} = 10.57$, $p = 0.0002$; Table S3, Fig. 3).
254 Breakdown was faster at 8°C than 12°C ($F_{1,42} = 5.88$, $p = 0.020$) and, although variation
255 among leaf treatments was not significant ($F_{2,42} = 3.10$, $p = 0.056$), there seemed to be a
256 trend towards faster breakdown in the N treatment. In single-species treatments, breakdown
257 rate differed between *Nothofagus* and *Eucalyptus* leaves but this difference depended on
258 temperature: breakdown was faster in *Nothofagus* than *Eucalyptus* at 8°C, while the
259 opposite was true at 15°C ($F_{1,28} = 23.98$, $p < 0.0001$; Fig. 3, Table S3).

260 In Spain, breakdown rate of the native *Alnus* differed among leaf treatments (N,
261 N+EL > N+E; $F_{2,48} = 6.38$, $p = 0.004$) but not between temperature treatments (Table S3,
262 Fig. 3). There were no differences in *Eucalyptus* breakdown rate among leaf or temperature
263 treatments in Spain, and none of the interactions were significant (Fig. 3, Table S3). In
264 single-species treatments, breakdown was faster for *Alnus* than for *Eucalyptus* ($F_{1,32} =$
265 39.53 , $p < 0.0001$; Fig. 3); there were no differences between temperatures, and the
266 interaction was not significant (Table S3).

267 In control channels in Spain, litter breakdown was faster for *Eucalyptus* than for
268 *Alnus* leaf discs ($F_{1,32} = 10.13$, $p = 0.003$), but there were no differences between
269 temperatures and the interaction was not significant (Table S4). Breakdown rate of
270 *Eucalyptus* leaf discs within mesh bags did not vary between temperature treatments in
271 Chile or Spain (Table S4). There was a significant but weak positive relationship between
272 initial biomass of caddisflies and *Alnus* litter breakdown rate ($r^2 = 0.08$, $p = 0.033$), and
273 between stonefly survival and *Nothofagus* breakdown rate ($r^2 = 0.18$, $p = 0.003$), while
274 *Eucalyptus* breakdown rates were not affected by detritivore initial biomass or survival.
275 There was no relationship between stonefly survival and growth ($r^2 = 0.03$, $p = 0.14$).

276

277 **Discussion**

278 Our study is among the first demonstrating that multiple stressors (in this case, increased
279 temperature and the presence of exotic *Eucalyptus* leaves or leachates) can have complex
280 and non-additive joint effects on litter breakdown, a key stream ecosystem process (see also
281 Ferreira & Chauvet 2011). In contrast, detritivore fitness was only affected by single
282 stressors, with no interactive effects arising in our experiments.

283 We first predicted that detritivore fitness would be impaired by the presence of
284 *Eucalyptus* litter through direct consumption, but also through their leachates in the water
285 (hypothesis 1). In fact, both *Eucalyptus* leaves and leachates reduced survival of the
286 stonefly *Diamphipnosis* in Chile. This result is consistent with other studies suggesting that
287 *Eucalyptus* leaves are a low quality resource for leaf-shredding detritivores (reviewed by
288 Graça et al. 2002) and that high concentrations of leachates have the capability to affect
289 invertebrate survival (Canhoto & Laranjeira 2007). In contrast to *Diamphipnosis*, survival
290 of the caddisfly *Calamoceras* in our experiment was 100%, not being affected by the
291 presence of *Eucalyptus* leaves or leachates. This result should be taken with caution due to
292 the short experimental time in this case (10 days), which was chosen based on the
293 remaining leaf material but may not have been sufficient to observe deleterious effects on
294 this detritivore species, although up to 75% of individuals of *Diamphipnosis* per treatment
295 had died by day 10.

296 Growth rates of both detritivore species were affected by direct consumption of
297 *Eucalyptus* leaves, but not by the presence of leachates. Growth rates of *Diamphipnosis* and
298 *Calamoceras* larvae were 2.5 and 8 times greater, respectively, when fed native leaves only
299 than when fed *Eucalyptus* leaves only. Canhoto and Graça (1995) also reported high growth
300 rates of *Tipula lateralis* fed *Alnus* but no growth in specimens fed *Eucalyptus*, which led to
301 100% mortality. As we observed larvae feeding on *Eucalyptus* leaves during the
302 experiments, reduced growth was likely due to the lower nutritional quality of *Eucalyptus*
303 leaves compared to native leaves. *Eucalyptus* leaves have higher C:N ratios than the other
304 species, a thick cuticle, and oils that can be toxic for invertebrates (Graça et al. 2002). In
305 contrast, the presence of *Eucalyptus* leaves or leachates, provided that native leaves were
306 available, had no significant effect on the growth rate of any of the detritivores, suggesting

307 that leachates do not interfere with the nutritional value of other leaves. It is noteworthy,
308 however, that *Eucalyptus* leachates increased mortality of *Diampinopsis* but did not affect
309 its growth rate, suggesting that leachates may affect vital functions other than growth. We
310 did not find differences in detritivore lipid contents depending on whether they fed on
311 native or *Eucalyptus* leaves, or depending on the presence of leachates. Other studies have
312 shown that detritivore lipid contents do not vary significantly depending on nutrient
313 ingestion (Pearson & Connolly 2000). This suggests that lower growth rates when fed on
314 *Eucalyptus* leaves are at the expense of different body tissues, not only lipids.

315 We found support for our hypothesis 2, which predicted that litter breakdown is
316 slowed down when *Eucalyptus* leaves or leachates are present. This may occur for various
317 reasons. Firstly, if native leaves are replaced by *Eucalyptus* leaves, total litter breakdown
318 rates are most likely reduced because the latter break down more slowly in the presence of
319 detritivore consumers. In single-species leaf treatments in Spain, *Alnus* leaves decomposed
320 1.8 times faster than *Eucalyptus* leaves, and a previous study showed even higher
321 differences (3-fold) between these two species in the presence of the detritivorous crane fly
322 *Tipula lateralis* (Canhoto & Graça 1995), although another study showed similar
323 breakdown rates for both species in the presence of the omnivorous shrimp *Atyaephyra*
324 *desmarestii* (Duarte et al. 2011). Secondly, *Eucalyptus* leachates could retard the
325 breakdown of other species. In Chile, *Nothofagus* leaves lost mass 1.6 times faster when
326 they were alone than when *Eucalyptus* leaves were present, and 1.5 faster than when
327 *Eucalyptus* leachates only were present. Interestingly, at the same time, breakdown of
328 *Eucalyptus* was 1.3 times higher in the presence of *Nothofagus* than in the single-species
329 leaf treatment and, when leaves of both species were present, *Eucalyptus* lost mass 1.7
330 times faster than *Nothofagus* (note that, in controls with no detritivores in Spain,

331 *Eucalyptus* lost mass 1.1 times faster than *Alnus*). These results suggest that *Eucalyptus*
332 leachates inhibited consumption of *Nothofagus* and, at the same time, consumption of
333 *Eucalyptus* leaves may have been enhanced as a means of compensatory feeding, as shown
334 for other detritivores (Graça et al. 1993; Flores et al. 2014). It is possible that detritivores
335 used *Eucalyptus* leaves as an energy (carbon) source, but not for nutrient assimilation, as
336 carbon and nutrients can come from different food sources (Stenroth et al. 2006); stable
337 isotope studies could shed some light on these potential mechanisms. The lower breakdown
338 of *Nothofagus* could also have been mediated by the lower detritivore survival in treatments
339 with *Eucalyptus*, as survival and *Nothofagus* breakdown rate were directly related.

340 Temperature and the presence of exotic litter can interact in their effects on litter
341 breakdown, as predicted by hypothesis 3 and supported by our results in Chile. Leaves of
342 the native *Nothofagus* broke down faster when no *Eucalyptus* leaves or leachates were
343 present, but this difference only occurred at 8°C, perhaps in relation to the greater survival
344 of detritivores at this temperature. At 15°C, breakdown of *Nothofagus* leaves was slower
345 and similar regardless of the presence or absence of *Eucalyptus* leaves or leachates.
346 Moreover, in single-species treatments, *Nothofagus* leaves broke down faster than those of
347 *Eucalyptus* at 8°C, while at 15°C (where detritivore survival was lower) *Eucalyptus* broke
348 down faster, as occurred in control channels (with no detritivores) in Spain. These results
349 supported our hypothesis that increased temperature enhances the negative effects of
350 *Eucalyptus* on native litter breakdown, and such interaction could be mediated by
351 detritivores, because at higher temperatures mortality increases and thus detritivore control
352 on litter breakdown is reduced, while microbial activity and/or leaching of *Eucalyptus* litter
353 possibly increase (although we found no effect of temperature on *Eucalyptus* breakdown in
354 controls). Detritivores could become more sensitive to toxic substances at higher

355 temperatures (Cairns et al. 1975) because of their expected accelerated metabolism rate
356 (Brown et al. 2004) and because exposure to one stressor often leads to higher sensitivity to
357 other stressors (Kashian et al. 2007). For example, Díaz-Villanueva et al. (2011) showed
358 that larvae of *Sericostoma vittatum* fed poor-quality leaves (including *Eucalyptus*) were
359 more sensitive to increased temperatures than larvae fed high-quality leaves (including
360 *Alnus*), with consequences on nutrient assimilation efficiencies and mass balances.
361 Temperature may accelerate the leaching of oils and polyphenols in *Eucalyptus* leaves (see
362 also Ferreira & Chauvet 2011), as occurs with the leaching of organic carbon (Whitworth et
363 al. 2014). Other studies have demonstrated that microbial breakdown strongly depends on
364 temperature (Boyero et al. 2011), that such temperature effects can interact with those of
365 other stressors (Ferreira & Chauvet 2011), and that microorganisms are affected by the
366 toxic oils and thick cuticle of *Eucalyptus* leaves (Graça et al. 2002).

367 Our study adds to the mounting evidence that joint effects of multiple stressors can
368 be non-additive and difficult to predict (Folt et al. 1999). Although impacts of *Eucalyptus*
369 plantations on streams are known to be lessened if a native riparian corridor is maintained
370 (Abelho & Graça 1996; Graça et al. 2002; Molinero & Pozo 2004), we have shown that the
371 presence of *Eucalyptus* leaves and leachates in the water may still impact stream
372 communities and ecosystem functions in this situation, and that these effects can be
373 exacerbated by increased temperatures. We may thus expect that *Eucalyptus* plantations
374 will cause further biodiversity loss and ecosystem impairment (Graça et al. 2002) as the
375 climate warms. Future experiments should take into account temperature variability
376 associated with future climate scenarios, as extreme temperatures are more likely to have
377 ecological effects than changes in means alone (Thompson et al. 2013).

378

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383

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502

503 **Table 1.** Environmental characteristics of streams where invertebrates and leaf litter were
 504 collected and dominant local riparian tree species (all native).

505

	Estero Nonguén (Chile)	Canuto Valdeinfierno (Spain)
Geographic position	36.82°S, 73.02°W	36.23°N, 5.61°W
Altitude (m asl)	130	150
Water temperature at time of collection (°C)	8	13
Mean water temperature at month of collection (°C)	8	13
Mean water temperature of the hottest month (°C)	15	18
pH	6.2	6.1
Conductivity ($\mu\text{S cm}^{-1}$)	52	180
Total dissolved solids (mg L^{-1})	79	80
Nitrates (mg L^{-1})	0.09	0.09
Dominant local riparian species	<i>Nothofagus obliqua</i> , <i>N. dombeyi</i> , <i>Chusquea quila</i> , <i>Peumus boldus</i> , <i>Rhaphithamnus spinosus</i> , <i>Fuchsia magellanica</i> , <i>Luma apiculata</i>	<i>Alnus glutinosa</i> , <i>Fraxinus angustifolia</i> , <i>Ficus carica</i> , <i>Nerium oleander</i> , <i>Laurus nobilis</i> , <i>Rhododendron ponticum</i> , <i>Ilex aquafolium</i>

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508

509 **Table 2.** Results of Pearson's chi-square tests comparing survival of *Diamphipnosia samali*

510 (Chile) across leaf treatments and temperatures. Chi-square statistic and p-values are

511 shown, as well as results of comparisons of each pair of treatments (">" indicates higher

512 survival). Leaf treatments: N = native leaves only; E = *Eucalyptus* leaves only; N+E =513 native and *Eucalyptus* leaves; N+EL = native leaves and water with *Eucalyptus* leachates.

Source of variation	Chi-square	p	Pairwise comparisons
Among leaf treatments			
Week 1	18.1	0.0004	N>(E, N+E, N+EL); N+E>(N+EL)
Week 2	14.8	0.0020	N>(E, N+EL); N+E>N+EL
Week 3	15.6	0.0014	N>(E, N+E, N+EL)
Week 4	12.8	0.0051	N>(N+E, N+EL)
Week 5	19.9	0.0002	N>(E, N+E, N+EL)
Among temperatures			
Week 1	3.7	0.0547	–
Week 2	1.0	0.3164	–
Week 3	2.0	0.1570	–
Week 4	10.5	0.0012	8°C>15°C
Week 5	6.6	0.0101	8°C>15°C

514

515

516 **Figure legends**

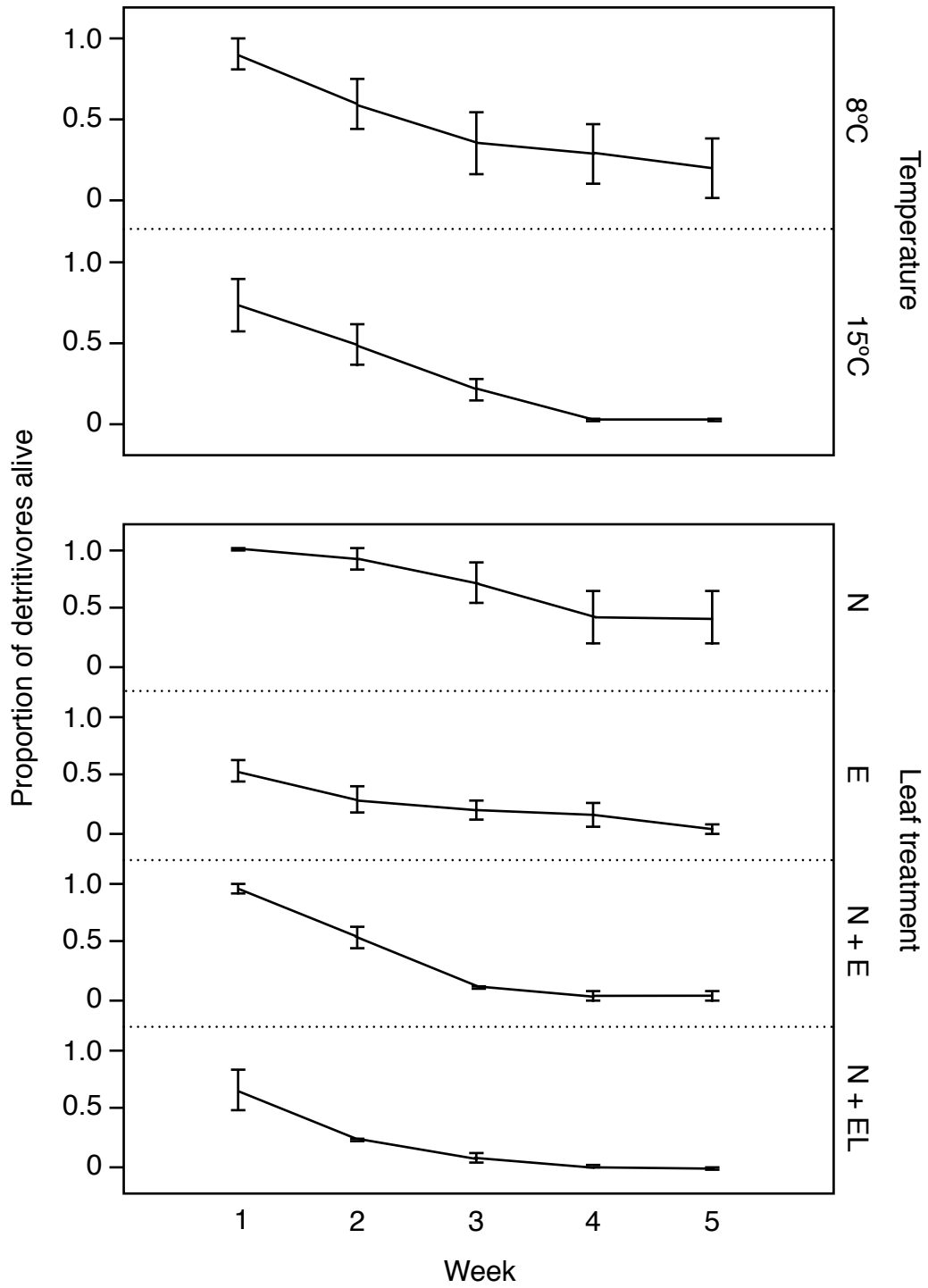
517 Fig. 1. Proportion of live individuals of *Diamphipnosis samali* (Chile) for each leaf
518 treatment (see Table 2 legend for leaf treatment definitions) and temperature during the
519 experiment (5 weeks).

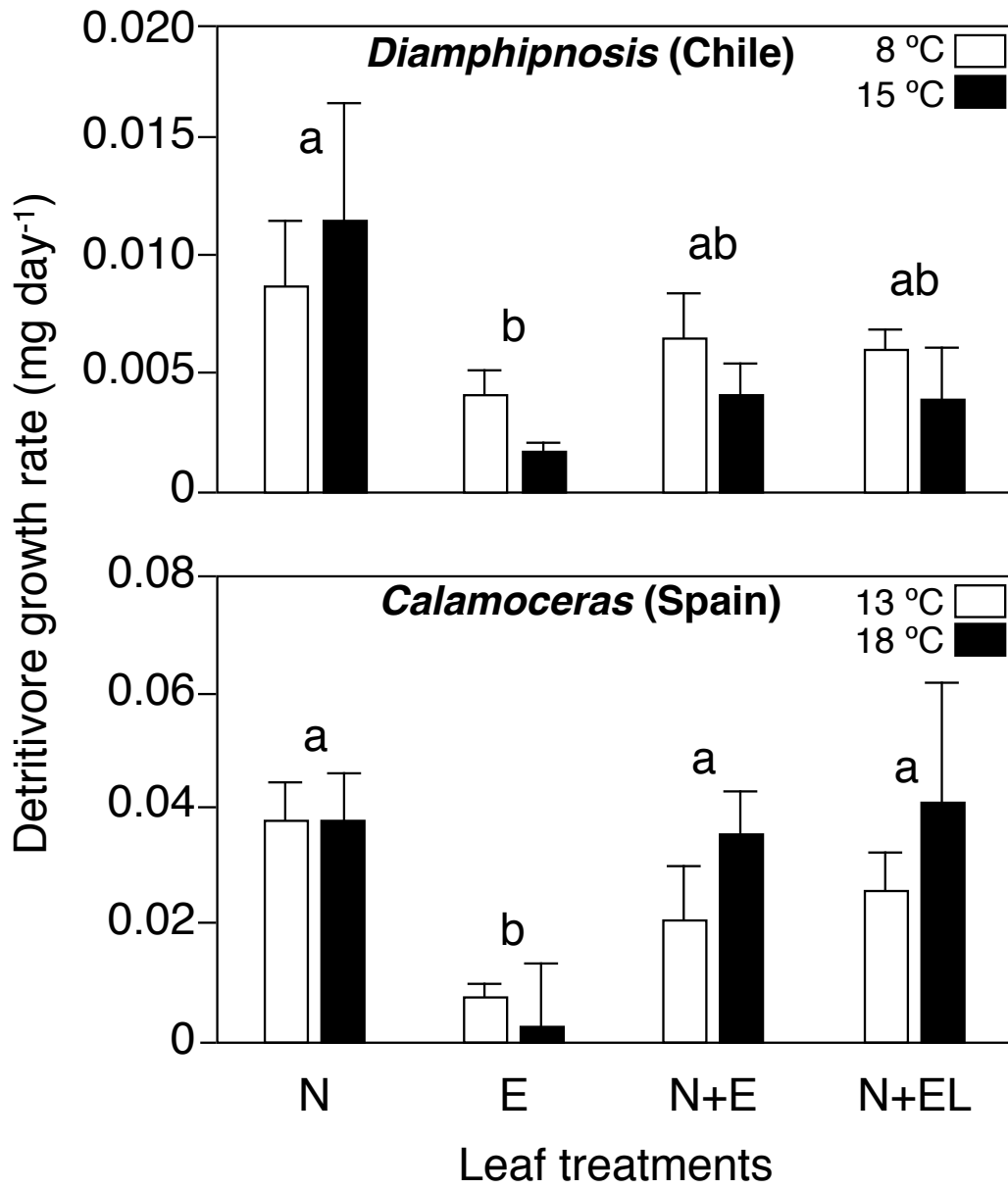
520 Fig. 2. Growth rate ($\text{mg day}^{-1} \pm \text{SE}$) of (a) *Diamphipnosis samali* (Chile) and (b)
521 *Calamoceras marsupus* (Spain) for each leaf treatment (see Table 2 legend for leaf
522 treatment definitions) and temperature. Same lowercase letters indicate no significant
523 differences across leaf treatments (there were no differences across temperature
524 treatments).

525 Fig. 3. Litter breakdown rate ($\text{mg day}^{-1} \pm \text{SE}$) of (I, III) native plant species and (II,IV)
526 *Eucalyptus* for each leaf treatment (see Table 2 legend for leaf treatment definitions) and
527 temperature in (I,II) Chile and (III,IV) Spain. Same lowercase and uppercase letters
528 indicate no significant differences across leaf and temperature treatments, respectively.

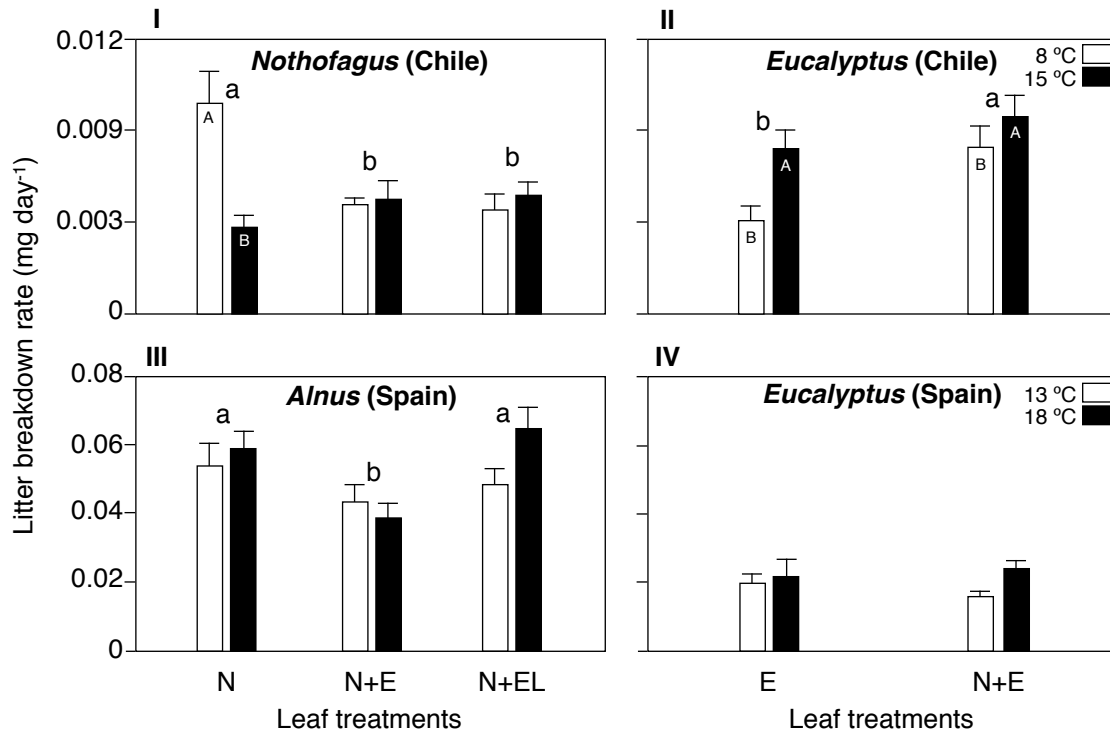
529

530 **Figure 1**





535 **Figure 3**
536



537

538

539 **Supplementary Material**

540 **Table S1.** Results of two-way ANOVA comparing growth rate (mg day⁻¹) of
 541 *Diamphipnosis samali* (Chile) and *Calamoceras marsupus* (Spain) among leaf treatments
 542 (N, E, N+E, N+EL; see Table 2 legend for definitions) and temperatures (8 and 15 °C in
 543 Chile; 13 and 18 °C in Spain). Df = degrees of freedom, SS = sums of squares, F = F-
 544 statistic and P = p-values.

545

Source of variation	df	SS	F	p
<i>Diamphipnosis</i> (Chile)				
Leaf treatment	3	0.00047	3.41	0.024
Temperature	1	0.00001	0.31	0.575
Leaf treatment × Temperature	3	0.00008	0.58	0.628
Error	56	0.00259		
<i>Calamoceras</i> (Spain)				
Leaf treatment	3	0.0021	4.08	0.010
Temperature	1	0.0001	0.66	0.420
Leaf treatment × Temperature	3	0.0002	0.49	0.692
Error	60	0.0133		

546

547

548 **Table S2.** Results of two-way ANOVA comparing invertebrate lipid absolute content (mg)
549 and arcsin square root-transformed lipid relative content (prop.) of *Diamphipnosia samali*
550 (Chile) and *Calamoceras marsupus* (Spain) among leaf treatments (N, E, N+E, N+EL; see
551 Table 2 legend for definitions) and temperatures (8 and 15 °C in Chile; 13 and 18 °C in
552 Spain).
553

Source of variation	df	SS	F	p
Lipid absolute content (mg)				
<i>Diamphipnosia</i> (Chile)				
Leaf treatment	3	20.4×10^{-6}	1.84	0.18
Temperature	1	0.5×10^{-6}	0.14	0.72
Leaf treatment \times Temperature	3	1.28×10^{-6}	0.12	0.95
Error	16	59.0×10^{-6}		
<i>Calamoceras</i> (Spain)				
Leaf treatment	3	4.3×10^{-5}	0.71	0.56
Temperature	1	0.3×10^{-5}	0.14	0.71
Leaf treatment \times Temperature	3	9.8×10^{-5}	1.62	0.22
Error	16	32.2×10^{-5}		
Lipid relative content (prop.)				
<i>Diamphipnosia</i> (Chile)				
Leaf treatment	3	9.3×10^{-3}	0.61	0.62
Temperature	1	0.3×10^{-3}	0.07	0.80
Leaf treatment \times Temperature	3	18.1×10^{-3}	1.19	0.35
Error	16	81.3×10^{-3}		
<i>Calamoceras</i> (Spain)				
Leaf treatment	3	0.029	0.42	0.74
Temperature	1	0.009	0.41	0.53
Leaf treatment \times Temperature	3	0.169	2.48	0.10
Error	16	0.363		

555 **Table S3.** Results of two-way ANOVA comparing litter breakdown rate (mg day⁻¹) among
556 leaf treatments (N, E, N+E, N+EL; see Table 2 legend for definitions) and temperatures (8
557 and 15 °C in Chile; 13 and 18 °C in Spain). Separate analyses were done for native leaves
558 (in Chile, *Nothofagus obliqua*; in Spain, *Alnus glutinosa*; treatments compared: N, N+E,
559 N+EL); *Eucalyptus* leaves (treatments compared: E, N+E); and native vs. *Eucalyptus*
560 leaves in single-species treatments (treatments compared: N, E).
561

Source of variation	df	SS	F	p
<i>Nothofagus</i> (Chile)				
Leaf treatment	2	0.000028	3.10	0.0557
Temperature	1	0.000027	5.88	0.0197
Leaf treatment × Temperature	2	0.000095	10.57	0.0002
Error	42	0.000189		
<i>Eucalyptus</i> (Chile)				
Leaf treatment	1	0.000042	8.15	0.0080
Temperature	1	0.000041	7.80	0.0093
Leaf treatment × Temperature	1	0.000006	1.14	0.2956
Error	28	0.000146		
<i>Nothofagus</i> vs. <i>Eucalyptus</i> (Chile)				
Leaf treatment	1	0.000006	0.99	0.3283
Temperature	1	0.000011	1.80	0.1910
Leaf treatment × Temperature	1	0.000147	23.98	<0.0001
Error	28	0.000172		
<i>Alnus</i> (Spain)				
Leaf treatment	2	0.00053	6.38	0.0034
Temperature	1	0.00009	2.06	0.1557
Leaf treatment × Temperature	2	0.00019	2.27	0.1100
Error	48	0.00202		
<i>Eucalyptus</i> (Spain)				
Leaf treatment	1	0.000001	0.10	0.7462

Temperature	1	0.000044	3.32	0.0786
Leaf treatment × Temperature	1	0.000014	1.05	0.3205
Error	32	0.000423		
<i>Nothofagus vs. Eucalyptus (Spain)</i>				
Leaf treatment	1	0.008128	39.53	<0.0001
Temperature	1	0.000116	0.57	0.4573
Leaf treatment × Temperature	1	0.000015	0.07	0.7918
Error	32	0.006579		

562

563

564 **Table S4.** Results of two-way ANOVA comparing litter breakdown rate (mg day⁻¹) among
 565 leaf treatments (native and *Eucalyptus*) and temperatures (13 and 18°C) in control channels
 566 in Spain; and one-way ANOVAs comparing litter breakdown rate (mg day⁻¹) of *Eucalyptus*
 567 leaf discs within mesh bags between temperature treatments (8 and 15 °C in Chile; 13 and
 568 18 °C in Spain).

569

Source of variation	df	SS	F	p
Control channels (Spain)				
Leaf treatment	1	0.0000264	10.13	0.003
Temperature	1	0.0000087	3.34	0.08
Leaf treatment × Temperature	1	0.0000002	0.09	0.77
Error	32	0.0000832		
<i>Eucalyptus</i> within mesh bags (Chile)				
Temperature	1	0.0000021	2.98	0.11
Error	14	0.0000101		
<i>Eucalyptus</i> within mesh bags (Spain)				
Temperature	1	0.0000015	0.72	0.41
Error	16	0.0000340		

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