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

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Abstract

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

Running title: Joint stressor effects on litter breakdown.

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

Introduction

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

Freshwater ecosystems contribute disproportionately to global species richness, and their species extinction rates are among the greatest on the planet (Ricciardi & Rasmussen 1999; Dudgeon et al. 2006; Ormerod et al. 2010). Climate change can affect freshwater organisms and ecosystems mainly through changes in water temperature and hydrology (Woodward et al. 2010). However, information on ecological effects of climate change in freshwater ecosystems is relatively scarce compared to other ecosystems, particularly with respect to the interaction between climate change and other stressors (Durance & Ormerod 2007; Piggott et al. 2012). There is evidence of synergistic effects of increased water 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 cuticule 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 *Diamphipnosis samali* Illies (Plecoptera: Diamphipnoidae) in Chile and the caddisfly

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

Experimental manipulations

Detritivores were acclimated for 48 h in containers with stream water kept at stream temperature at the time of collection (8 and 13 ºC in Chile and Spain, respectively); they were then wet weighed and introduced in the experimental containers (see below). Caddisflies were gently pushed out of their cases before weighing, using a pair of soft entomological forceps, and then put back in their cases. This procedure did not cause any mortality. Leaf discs (12 mm diameter) were cut using a cork borer, air dried to constant weight and weighed.

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

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

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 7ºC 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 ºC, and the other was
147 warmed up to 15ºC 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 13ºC (the stream
157 temperature at the time of collection and the mean stream temperature at the month of
158 collection) and 18ºC (the mean stream temperature at the hottest month); a 5ºC 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 13ºC, and each channel
161 was assigned to either low (13ºC) or high (18ºC) temperature treatments; the latter were
162 provided with an aquarium heater in the downstream container, set to maintain water
163 temperature at 18ºC. 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

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

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

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

189 (individuals that were uncased and dried at 60 ºC 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 $KHCO_3$ solution (2%
204 w/v) and purified on a column Sep-pack NH_2 (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

Data analysis

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

Differences in stonefly survival among leaf treatments and temperatures were examined separately with Pearson's chi-square tests on the untransformed proportion of detritivores alive at weeks 1, 2, 3, 4 and 5. When results were significant, we further compared each pair of treatments separately. Differences in detritivore growth rate (mg day^{-1}), detritivore absolute lipid contents (mg), arcsin square root-transformed detritivore relative lipid contents, and litter breakdown rate (mg day^{-1}), were examined among leaf treatments and between temperatures with two-way ANOVAs followed by post-hoc Tukey tests. The leaf treatments compared were as follows: detritivore growth and lipid contents were compared among all treatments (N vs. E vs. N+E vs. N+EL); litter breakdown was examined separately for native leaves (N vs. N+E vs. N+EL), *Eucalyptus* leaves (E vs. N+E), and native vs. *Eucalyptus* leaves in single-species treatments (N vs. E). ANOVAs were used to compare (1) breakdown rate in controls without detritivores in Spain: two-way ANOVA with temperature and leaf treatments (*Alnus* vs. *Eucalyptus*) as factors; and (2) 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 8ºC than 15ºC (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 × temperature interaction ($F_{2,42} = 10.57$, $p = 0.0002$; Table S3, Fig. 3).
254 Breakdown was faster at 8ºC than 12ºC ($F_{1,42} = 5.88$, $p = 0.020$) 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 8ºC, while the
259 opposite was true at 15ºC ($F_{1,28} = 23.98$, $p < 0.0001$; Fig. 3, Table S3).

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

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

Discussion

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

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

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

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

We found support for our hypothesis 2, which predicted that litter breakdown is slowed down when *Eucalyptus* leaves or leachates are present. This may occur for various reasons. Firstly, if native leaves are replaced by *Eucalyptus* leaves, total litter breakdown rates are most likely reduced because the latter break down more slowly in the presence of detritivore consumers. In single-species leaf treatments in Spain, *Alnus* leaves decomposed 1.8 times faster than *Eucalyptus* leaves, and a previous study showed even higher differences (3-fold) between these two species in the presence of the detritivorous cranefly *Tipula lateralis* (Canhoto & Graça 1995), although another study showed similar breakdown rates for both species in the presence of the omnivorous shrimp *Atyaephyra desmarestii* (Duarte et al. 2011). Secondly, *Eucalyptus* leachates could retard the breakdown of other species. In Chile, *Nothofagus* leaves lost mass 1.6 times faster when they were alone than when *Eucalyptus* leaves were present, and 1.5 faster than when *Eucalyptus* leachates only were present. Interestingly, at the same time, breakdown of *Eucalyptus* was 1.3 times higher in the presence of *Nothofagus* than in the single-species leaf treatment and, when leaves of both species were present, *Eucalyptus* lost mass 1.7 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 8ºC, perhaps in relation to the greater survival
344 of detritivores at this temperature. At 15ºC, breakdown of *Nothofagus* leaves was slower
345 and similar regardless of the presence or absence or *Eucalyptus* leaves or leachates.
346 Moreover, in single-species treatments, *Nothofagus* leaves broke down faster than those of
347 *Eucalyptus* at 8ºC, while at 15ºC (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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References

Abelho M, Graça MAS (1996) Effects of *Eucalyptus* afforestation on leaf litter dynamics and macroinvertebrate community structure of streams in Central Portugal. Hydrobiologia 324:195-204.

Boyero L, Barmuta LA, Ratnarajah L, Schmidt K, Pearson RG (2012) Effects of exotic riparian vegetation on leaf breakdown by shredders: a tropical–temperate comparison. Freshwater Science 31:296-303.

Boyero L, Pearson RG, Gessner MO, Barmuta LA, Ferreira V, Graça MAS, Dudgeon D, Boulton AJ, Callisto M, Chauvet E, Helson JE, Bruder A, Albariño RJ, Yule CM, Arunachalam M, Davies JN, Figueroa R, Flecker AS, Ramírez A, Death RG, Iwata T, Mathooko JM, Mathuriau C, Gonçalves JF, Moretti M, Jinggut T, Lamothe S, M'erimba C, Ratnarajah L, Schindler MH, Castela J, Buria LM, Cornejo A, Villanueva VD, West DC (2011) A global experiment suggests climate warming will not accelerate litter decomposition in streams but may reduce carbon sequestration. Ecology Letters 14:289-294.

Brown JH, Gillooly JF, Allen AP, Savage VM, West GB (2004) Toward a metabolic theory of ecology. Ecology 85:1771-1789.

Cairns J, Heath AG, Parker BC (1975) The effects of temperature upon the toxicity of chemicals to aquatic organisms. Hydrobiologia 47:135-171.

403 Canhoto C, Graça MAS (1995) Food value of introduced eucalypt leaves for a
404 Mediterranean stream detritivore: *Tipula lateralis*. Freshwater Biology 34:209-214.
405 Canhoto C, Graça MAS (1999) Leaf barriers to fungal colonization and shredders (*Tipula*
406 *lateralis*) consumption of decomposing *Eucalyptus globulus*. Microbial Ecology
407 37:163-172.
408 Canhoto C, Laranjeira C (2007) Leachates of *Eucalyptus globulus* in intermittent streams
409 affect water parameters and invertebrates. International Review of Hydrobiology
410 92:173-182.
411 Christie WW 2003. Lipid Analysis: Isolation, Separation, Identification and Structural
412 Analysis of Lipids. The Oily Press, Bridgwater, England.
413 Dewson Z, James A, Death R (2007) Stream ecosystem functioning under reduced flow
414 conditions. Ecological Applications 17:1797-1808.
415 Díaz-Villanueva V, Albariño R, Canhoto C (2011) Detritivores feeding on poor quality
416 food are more sensitive to increased temperatures. Hydrobiologia 678:155-165.
417 Duarte S, Fidalgo ML, Pascoal C, Cássio F (2011) The role of the freshwater shrimp
418 *Atyaephyra desmarestii* in leaf litter breakdown in streams. Hydrobiologia 680:149-
419 157.
420 Dudgeon D, Arthington AH, Gessner MO, Kawabata Z, Knowler DJ, Leveque C, Naiman
421 RJ, Prieur-Richard AH, Soto D, Stiassny ML, Sullivan CA (2006) Freshwater
422 biodiversity: importance, threats, status and conservation challenges. Philosophical
423 Transactions of the Royal Society B: Biological Sciences 81:163-182.
424 Durance I, Ormerod SJ (2007) Climate change effects on upland stream macroinvertebrates
425 over a 25-year period. Global Change Biology 13:942-957.

426 Feio MJ, Vieira-Laneiro R, Ferreira V, Graça MAS (2005) The role of the environment in
427 the distribution and composition of Trichoptera assemblages in streams. Archiv für
428 Hydrobiologie 164:493-512.
429 Ferreira V, Chauvet E (2011) Synergistic effects of water temperature and dissolved
430 nutrients on litter decomposition and associated fungi. Global Change Biology
431 17:551-564.
432 Ferreira V, Elosegi A, Gulis V, Pozo J, Graça MAS (2006) *Eucalyptus* plantations affect
433 fungal communities associated with leaf-litter decomposition in Iberian streams.
434 Archiv für Hydrobiologie 166:467-490.
435 Flores L, Larrañaga A, Elosegi A (2014) Compensatory feeding of a stream detritivore
436 alleviates the effects of poor food quality when enough food is supplied. Freshwater
437 Science 33:134-141.
438 Folch J, Lees M, Stanley GHS (1957) A simple method for the isolation and purification of
439 total lipides from animal tissues. Journal of Biological Chemistry 226:497-509.
440 Folt CL, Chen CY, Moore MV, Burnaford J (1999) Synergism and antagonism among
441 multiple stressors. Limnology and Oceanography 44:864-877.
442 Graça MAS, Maltby L, Calow P (1993) Importance of fungi in the diet of *Gammarus pulex*
443 and *Asellus aquaticus*. II. Effects on growth, reproduction and physiology.
444 Oecologia 96:304-309.
445 Graça MAS, Pozo J, Canhoto C, Elosegi A (2002) Effects of *Eucalyptus* plantations on
446 detritus, decomposers, and detritivores in streams. The Scientific World Journal
447 2:1173-1185.

448 Hader DP, Helbling EW, Williamson CE, Worrest RC (2011) Effects of UV radiation on
449 aquatic ecosystems and interactions with climate change. Photochemical and
450 Photobiological Sciences 10:242-260.
451 IPCC. 2007. Climate Change 2007: Synthesis Report, Geneva, Switzerland.
452 Kashian DR, Zuellig RE, Mitchell KA, Clements WH (2007) The cost of tolerance:
453 sensitivity of stream benthic communities to UV-B and metals. Ecological
454 Applications 17:365-375.
455 Mancilla G, Valdovinos C, Azocar M, Jorquera P, Figueroa R (2009) Replacement effect of
456 riparian native vegetation on benthic macroinvertebrates community in temperate
457 climate streams, Central Chile. Hidrobiológica 19:193-203.
458 Molinero J, Pozo J (2004) Impact of a eucalyptus (*Eucalyptus globulus* Labill.) plantation
459 on the nutrient content and dynamics of coarse particulate organic matter (CPOM)
460 in a small stream. Hydrobiologia 528:143-165.
461 Ormerod SJ, Dobson M, Hildrew AG, Townsend CR (2010) Multiple stressors in
462 freshwater ecosystems. Freshwater Biology 55:1-4.
463 Parmesan C (2006) Ecological and evolutionary responses to recent climate change. Annual
464 Review of Ecology, Evolution, and Systematics 37:637-669.
465 Pearson RG, Connolly NM (2000) Nutrient enhancement, food quality and community
466 dynamics in a tropical rainforest stream. Freshwater Biology 43:31-42.
467 Perez J, Galan J, Descals E, Pozo J (2014) Effects of fungal inocula and habitat conditions
468 on alder and eucalyptus leaf litter decomposition in streams of northern Spain.
469 Microbial Ecology 67:245-255.

Piggott JJ, Lange K, Townsend CR, Matthaei CD (2012) Multiple stressors in agricultural streams: a mesocosm study of interactions among raised water temperature, sediment addition and nutrient enrichment. PLOS ONE 7:e49873.

Pozo J, Basaguren A, Elósegui A, Molinero J, Fabre E, Chauvet E (1998) Afforestation with *Eucalyptus globulus* and leaf litter decomposition in streams of northern Spain. Hydrobiologia 373/374:101-109.

Rahel FJ, Olden JD (2008) Assessing the effects of climate change on aquatic invasive species. Conservation Biology 22:521-533.

Ricciardi A, Rasmussen JB (1999) Extinction rates of North American freshwater fauna. Conservation Biology 13:1220-1222.

Stenroth P, Holmqvist N, Nyström P, Berglund O, Larsson P, Granéli W (2006) Stable isotopes as an indicator of diet in omnivorous crayfish (Pacifastacus leniusculus): the influence of tissue, sample treatment, and season. Canadian Journal of Fisheries and Aquatic Sciences 63:821-831.

Thompson RM, Beardall J, Beringer J, Grace M, Sardina P (2013) Means and extremes: building variability into community-level climate change experiments. Ecol Lett 16:799-806.

Vivanco L, Austin AT (2008) Tree species identity alters forest litter decomposition through long-term plant and soil interactions in Patagonia, Argentina. Journal of Ecology 96:727-736.

Whitworth KL, Baldwin DS, Kerr JL (2014) The effect of temperature on leaching and subsequent decomposition of dissolved carbon from inundated floodplain litter: implications for the generation of hypoxic blackwater in lowland floodplain rivers. Chemistry and Ecology 30:491-500.

494 Woodward G, Gessner MO, Giller PS, Gullis V, Hladyz S, Lecerf A, Malmqvist B, McKie
495 BB, Tiegs SD, Cariss H, Dobson M, Elosegi A, Ferreira V, Graça MAS, Fleituch T,
496 Lacoursière JO, Nistorescu M, Pozo J, Risnoveanu G, Schindler M, Vadineanu A,
497 Vought LM, Chauvet E (2012) Continental-scale effects of nutrient pollution on
498 stream ecosystem functioning. Science 336:1438-1440.
499 Woodward G, Perkins DM, Brown LE (2010) Climate change and freshwater ecosystems:
500 impacts across multiple levels of organization. Philosophical Transactions of the
501 Royal Society B: Biological Sciences 365:2093-2106.

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	36.82ºS, 73.02ºW	36.23°N, 5.61°W
Altitude (m asl)	130	150
Water temperature at time of collection (ºC)	8	13
Mean water temperature at month of collection (ºC)	8	13
Mean water temperature of the hottest month (ºC)	15	18
pH	6.2	6.1
Conductivity (µS cm^{-1})	52	180
Total dissolved solids (mg L^{-1})	79	80
Nitrates (mg L^{-1})	0.09	0.09
Dominant local riparian species	*Nothofagus obliqua, N. dombeyi, Chusquea quila, Peumus boldus, Rhaphithamnus spinosus, Fuchsia magellanica, Luma apiculata*	*Alnus glutinosa, Fraxinus angustifolia, Ficus carica, Nerium oleander, Laurus nobilis, Rhododendron ponticum, Ilex aquafolium*

506
507

Table 2. Results of Pearson's chi-square tests comparing survival of *Diamphipnosis samali* (Chile) across leaf treatments and temperatures. Chi-square statistic and p-values are shown, as well as results of comparisons of each pair of treatments (">" indicates higher survival). Leaf treatments: N = native leaves only; E = *Eucalyptus* leaves only; N+E = 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	8ºC>15ºC
Week 5	6.6	0.0101	8ºC>15ºC

516 **Figure legends**

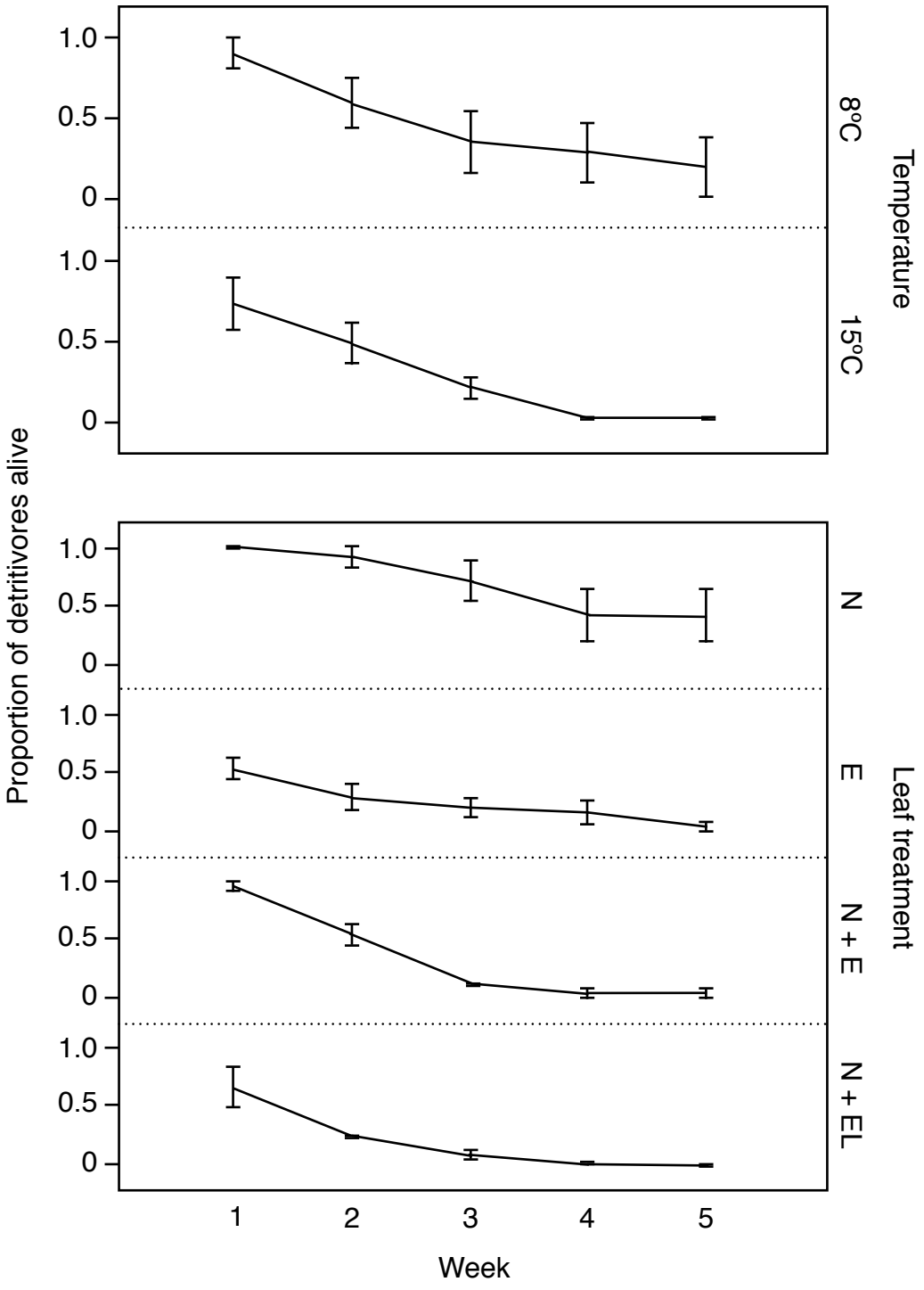
517 Fig. 1. Proportion of live individuals of *Diamphipnosis samali* (Chile) for each leaf
518 treatment (see Table 2 legend for leaf treatment definitions) and temperature during the
519 experiment (5 weeks).

520 Fig. 2. Growth rate (mg day^{-1} ± 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 (mg day^{-1} ± 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.

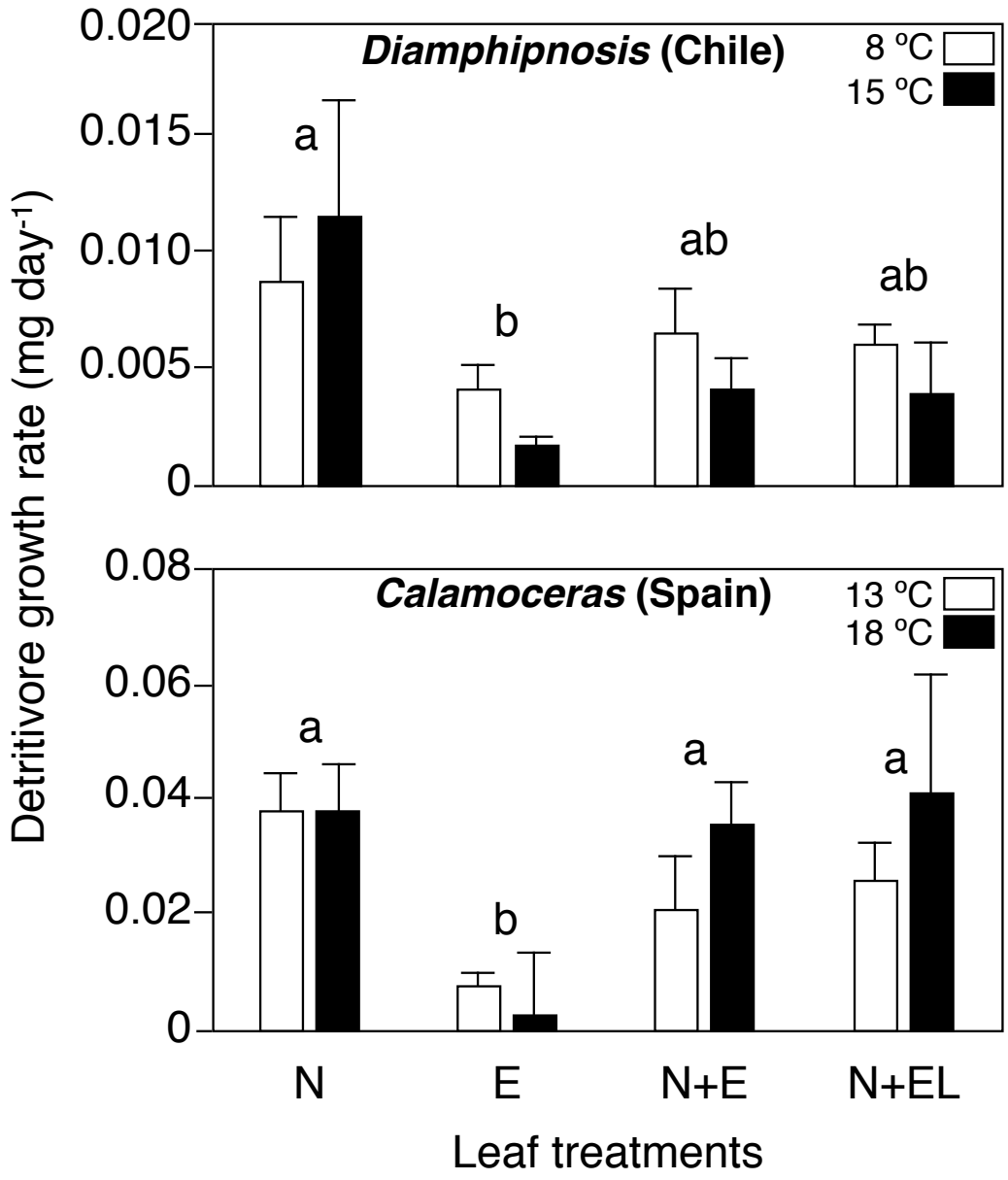
529

530 **Figure 1**



531

532 **Figure 2**



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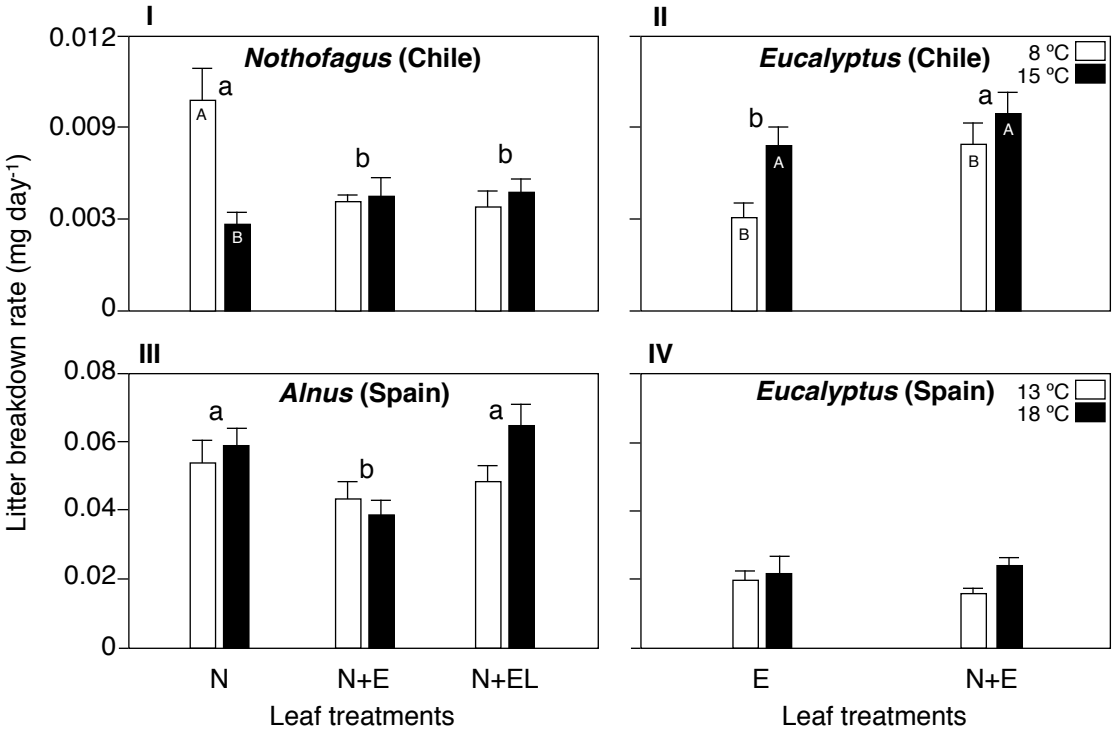
535 **Figure 3**

536



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Supplementary Material

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

Source of variation	df	SS	F	p
***Diamphipnosis* (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		
***Calamoceras* (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		

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 *Diamphipnosis 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 ºC in Chile; 13 and 18 ºC in
552 Spain).

553

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

554

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 ºC in Chile; 13 and 18 ºC 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
***Nothofagus* (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		
***Eucalyptus* (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		
***Nothofagus* vs. *Eucalyptus* (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		
***Alnus* (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		
***Eucalyptus* (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		
***Nothofagus* vs. *Eucalyptus* (Spain)**				
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 18ºC) 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 ºC in Chile; 13 and
568 18 ºC 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		
***Eucalyptus* within mesh bags (Chile)**				
Temperature	1	0.0000021	2.98	0.11
Error	14	0.0000101		
***Eucalyptus* within mesh bags (Spain)**				
Temperature	1	0.0000015	0.72	0.41
Error	16	0.0000340		

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