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# Interactions between large and small detritivores influence how biodiversity impacts litter decomposition

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## Summary

**1.** Understanding how biodiversity loss influences plant litter decomposition – i.e., the biologically mediated conversion of coarse to fine particulate organic matter – is crucial to predict changes in the functioning of many stream ecosystems, where detrital food webs are dominant. Rates of litter decomposition are influenced by detritivore diversity, but the mechanisms behind this relationship are uncertain.

**2.** As differences in detritivore body size are a major determinant of interspecific interactions, they should be key for predicting effects of detritivore diversity on decomposition. In order to explore this question we manipulated detritivore diversity and body size simultaneously in a microcosm experiment using two small (*Leuctra geniculata* and *Lepidostoma hirtum*) and two large detritivore species (*Sericostoma pyrenaicum* and *Echinogammarus berilloni*) in all possible 1-, 2- and 4-species combinations, and litter discs of *Alnus glutinosa*.

3. We expected that larger species would facilitate smaller species through the production of smaller litter fragments, resulting in faster decomposition and greater growth of smaller species in polycultures containing species of different body size. To examine this hypothesis we used a set of 'diversity-interaction' models that explored how decomposition was affected by different interspecific interactions and the role of body size, and quantified the magnitude of such effect through ratios of decomposition rates and detritivore growth between polycultures and monocultures.

**4.** We found a clear positive effect of detritivore diversity on decomposition, which was mainly explained by facilitation and niche partitioning. Facilitation of small animals by larger ones was evidenced by a 12% increase in decomposition rates in polycultures compared to monocultures and the higher growth (20%) of small species, which partly fed on fine particulate organic matter produced by larger animals. When the large species were together in polycultures decomposition was enhanced by 19%, but there were no changes in growth; niche partitioning was a plausible mechanism behind the increase in decomposition rates, as both species fed on different parts of litter discs, only one species being able to eat less palatable parts.

**5.** Our study evidences that interspecific differences in body size should be taken into account in diversity-decomposition studies. Future studies should also consider differences in species' vulnerability to extinction depending on body size and how this might affect ecosystem functioning in different scenarios of detritivore diversity and more complex food webs.

**Key-words:** body size, detritivore assemblages, ecosystem functioning, facilitation, resource partitioning, species richness, streams

## Introduction

Rapid loss of biodiversity is of major global concern, partly because of its potential consequences for ecosystem processes and the services they provide to humans (Cardinale *et* *al.* 2012). Motivated by this concern, hundreds of experimental studies have been conducted across a wide variety of organisms and systems and have confirmed that changes in species richness can alter key ecosystem process rates (Balvanera *et al.* 2006; Cardinale *et al.* 2006; Cardinale *et al.* 2011). However, evidence is not consistent for different ecosystem processes: while it is well established that plant diversity boosts primary production, the relationship between biodiversity loss and plant litter decomposition is still unclear, specially because less work has been performed on detritivore diversity than for other trophic levels and functions (Cardinale *et al.* 2011). Understanding this relationship is a crucial research goal if we are to predict the consequences of diversity loss on global carbon and nutrient cycles, as 90% of the plant biomass produced annually becomes plant litter and a considerable amount of it is ultimately decomposed (Cebrian 1999).

Decomposition is a process that involves multi-trophic biological interactions (Scherer-Lorenzen 2008) and thus can be affected by the diversity of plants, microbes and detritivores (Gessner *et al.* 2010). While there is evidence that detritivore diversity has stronger effects on decomposition than plant diversity (Srivastava *et al.* 2009), the underlying biological mechanisms behind a diversity-decomposition relationship are better known for plant diversity (e.g., Handa *et al.* 2014). This is partly because plant diversity effects are easily partitioned into complementarity and selection effects, using a statistical approach proposed almost two decades ago [i.e., the additive partitioning method (Loreau & Hector 2001)]. In contrast, assessing the contribution of different detritivore species to decomposition in an assemblage requires the use of more sophisticated and new techniques (Kirwan *et al.* 2009), which are necessary to identify the most plausible mechanisms underlying detritivore diversity effects on decomposition.

Within a detritivore assemblage, the observed net diversity effect on decomposition will depend on a balance between positive and negative interactions between species. The former may include resource partitioning (which can arise if different species exploit litter differently in space or time; Schoener 1974; Fynke & Snyder 2008; Dangles, Carpio & Woodward 2012), facilitation (if a species enhances the performance of another species or both enhance each other's performances; Bruno, Stachowicz & Bertness 2003; Wright *et al.* 2017) and a positive selection effect (if a species with large effects on decomposition dominates the assemblage; Fox 2005), while negative effects are often associated with competition (mainly when one species is a dominant competitor or shows agressive behaviour; Creed *et al.* 2009) and a negative selection effect (if a competitively dominant species does not contribute significantly to decomposition; Jiang, Pu & Nemergut 2008). Although species identity might mediate biological interactions responsible to biodiversity effects (as commonly evidenced by species assemblage main effects), species functional traits are also of great importance in predicting biodiversity effects on ecosystem functioning (Hooper *et al.* 2005). Within this context, body size is a relevant animal trait because it is linked to and can inform (1) multiple biological traits such as growth, reproduction and mortality (Peters *et al.* 1983), (2) foraging behaviour (Petchey *et al.* 2008) and (3) interspecific interactions including trophic relationships, competition and facilitation (Woodward *et al.* 2005). Thus, measuring body size is an easy way to condense a great array of biological traits into one predictor (Woodward *et al.* 2005). Remarkably, interspecific differences in body size have rarely been taken into account when exploring detritivore diversity effects on decomposition (exceptions include Reiss *et al.* 2011 and Dangles *et al.* 2012).

We explored how detritivore diversity loss affected litter decomposition – as the transformation of coarse leaf litter (> 1mm) into finer fragments (< 1mm) due to the result of both shredding and feeding activity – in stream microcosms, and investigated the potential biological mechanisms underlying such effects, with a suite of methods used novelly in this context. By manipulating detritivore species body size, and using a set of statistical models (‘diversity-interactions models’) that explicitly take into account the role of species interactions and differences in body size, we tested the hypothesis that diversity enhances decomposition when species differ in body size because litter processing by larger detritivores facilitates processing by smaller species through the production of smaller litter fragments (hypothesis 1), while diversity has weaker or no effect on decomposition when different species in the assemblage are of similar size because they are more likely to be functionally similar. Unlike the additive partitioning method, this approach does not require measuring the contribution of each species in a polyculture, but identifies the most parsimonious description of diversity effects. Further, we examined the magnitude of diversity effects on decomposition using the ratio of decomposition rates in polycultures:monocultures (an analogue of response ratios), and repeated the procedure with growth rates, as we expected that they would be enhanced in smaller detritivores when facilitation by larger detritivores occurred (hypothesis 2). Lastly, we investigated the nature of detritivore interactions by observing the feeding modes and foraging behaviours of large and small species, and behavioural differences between monocultures and polycultures that might indicate the existence of facilitation.

## Methods

### *Detritivore species*

We selected four common detritivore species in our study area (the Agüera catchment in northern Spain, 43.25ºN 3.26ºW) to represent 'small' and 'large' organisms. Small detritivores were the stonefly *Leuctra geniculata* Stephens, 1835 (Leuctridae) and the caddisfly *Lepidostoma hirtum* Fabricius, 1775 (Lepidostomatidae) (hereafter *Leuctra* and *Lepidostoma*); large detritivores were the caddisfly *Sericostoma pyrenaicum* Pictet, 1865 (Sericostomatidae) and the amphipod *Echinogammarus berilloni* Catta, 1878 (Gammaridae) (hereafter *Sericostoma* and *Echinogammarus*) (Riaño 1998; Basaguren, Riaño & Pozo 2002; Larrañaga, Basaguren & Pozo 2014). Average body dry mass ± SE was 0.7 ± 0.1 mg for *Leuctra*, 2.3 ± 0.1 mg for *Lepidostoma,* 7.5 ± 0.2 mg for *Sericostoma* and 6.1 ± 0.1 mg for *Echinogammarus.* Detritivores were collected in June 2015 from leaf litter in streams. They were transported in aerated containers within a cooler and kept in a controlled-temperature room set at 10ºC, which was lower than the average temperature of streams when detritivores were collected (approx. 13ºC) but similar to the average annual temperature of those streams and within the temperature range in June (unpubl. data), and significantly reduced evaporation during the experiment. Detritivores were starved for 48 h prior to the experiment.

### *Experimental set-up*

Our experiment included all possible 1, 2 and 4 species combinations, which resulted in 11 treatments (i.e., 4 monocultures; six 2-species polycultures, 2 with 1 and 4 with 2 body-size categories; and the single 4-species polyculture), plus a control with no detritivores (Fig. 1). All microcosms (except controls) had 8 detritivore individuals in total (i.e., 2- and 4-species polycultures had 4 and 2 individuals per species, respectively). Each treatment (including controls) was replicated 10 times, resulting in 120 microcosms.

Plastic cups (13 cm wide, 5 cm deep) were used as microcosms, each containing leaf litter, substrate, 500 mL of stream water, and aeration. Litter was provided in the form of 40 discs of black alder, *Alnus glutinosa* [L.] Gaertn. (Betulaceae). Leaves were collected just after abscission from the forest floor in the Agüera catchment in November 2014; discs were cut with a 12-mm diameter cork borer, air dried and kept in the laboratory; just before the experiment they were weighed to the nearest 0.01 mg. Substrate was provided in the form of fine sand and pebbles collected from streams, which facilitated detritivore movement and served as refuge and material for caddisfly case construction; substrate was incinerated at 550ºC for 4h and washed to remove ash before it was introduced in the microcosms. Water was taken from the stream the day before the experiment started, filtered through a 100-µm mesh, and added to each microcosm. Microcosms were aerated through pipette tips connected to an air injection system.

Litter discs were introduced in the microcosms 6 d before the addition of detritivores to allow leaching of soluble compounds and initial microbial conditioning. A previous experiment (Tonin *et al.* 2017) using a shorter conditioning period (3 d) was able to detect plant diversity effects on decomposition mediated by microorganisms, which suggests that microbial colonization was sufficient. After this period, the water was replaced and detritivores were added. Water was again replaced on days 7 and 14, using newly collected and filtered stream water. The experiment was terminated on day 21, except for *Sericostoma* monocultures, which were terminated on day 18 to avoid the underestimation of consumption because most of the labile litter material (90.57% ± 0.03 SE) had been consumed. Microcosms were monitored every 2 d to ensure that detritivores were alive (visual inspection without manipulation) and that there was litter remaining. We video-recorded 4-5 random microcosms with different species combinations daily (≈ from 8 am to 6 pm) for 1 h each day; in total, 3-4 different microcosms of each species combination were video-recorded (i.e., the same microcosm was never recorded twice over the experimental period). At the end of the experiment, all litter material [> 1mm; not including fine particulate organic matter (FPOM) or faeces] was oven dried (60ºC, 72 h), weighed to determine dry mass (DM), incinerated (550ºC, 4 h) and re-weighed to determine ash-free dry mass (AFDM). We estimated initial AFDM using 10 additional sets of 40 litter discs.

Initial detritivore body mass for each species in each microcosm was estimated from a case length (CL) – body mass (BM) relationship for *Sericostoma* ($BM = 0.170 \times CL^2 - 2.872 \times CL + 14.154$, $r^2 = 0.96$, $n = 26$) and *Lepidostoma* ($BM = 0.099 \times CL^2 - 1.091 \times CL + 3.464$, $r^2 = 0.84$, $n = 41$), and from a body length (BL) – BM relationship for *Leuctra* ($BM = -0.026 \times BL^2 - 0.515 \times BL - 1.502$, $r^2 = 0.70$, $n = 42$) and *Echinogammarus* (BM = 0.127 ×

$BL^2$ – 1.654 × BL + 9.383, $r^2 = 0.82$, $n = 28$) (Fig. S1), using additional individuals of a similar range of body mass or case length to those used in the experiment. Body length was measured from head to end of abdomen for *Leuctra* (i.e., excluding the cercus) and from head to end of abdomen with the body extended (i.e., not curved) for *Echinogammarus*. At the end of the experiment, detritivores were oven dried (60ºC, 72 h) and weighed to the nearest 0.01 mg (grouping individuals of each species from each microcosm) to determine their final body mass. Videos of detritivores were observed to investigate animal behavioural patterns that might indicate the existence of facilitation; we noted whether individuals fed on different parts of litter discs or on smaller fragments potentially produced by other species, whether individuals were more or less mobile or showed aggressive or aggregate behaviour, and whether feeding or foraging behaviour differed between monocultures and polycultures, and calculated the proportion of videos where a given species showed a particular behaviour.

### *Data analysis*

We quantified the decomposition rate mediated by detritivores (i.e., that resulting from shredding and feeding activity) as the relative daily litter mass loss = $[(LM_i - LM_f) / LM_i] / t$, where $LM_i$ and $LM_f$ were the initial and final litter AFDM in a microcosm, respectively, and t was the duration of the experiment in days. Initial AFDM was previously multiplied by the average proportion of remaining mass in control microcosms (= 0.716) to correct for leaching and microbial losses. Detritivore growth was calculated for each species as: detritivore growth = $(DM_f - DM_i) / DM_i$, where $DM_i$ and $DM_f$ were the initial and final dry mass of a species in a microcosm, respectively. When there were missing individuals, their mass was estimated as the average body mass of the remaining individuals for that species in the same microcosm.

We explored hypothesis 1 using a modelling framework that explicitly quantifies the contributions of individual species and species interactions to the diversity effect (Kirwan *et al.* 2009). This framework included the following models (Fig. 2): (1) null model (i.e., intercept only), which assumes that species perform identically and do not interact with each other; (2) species identity model, where different species have different effects on decomposition, but without interactions among species, so the performance of a polyculture can be predicted from the additive performance of each species; (3) pairwise interaction model, which augments model 2 with interactions between pairs of species, resulting in diversity effects (i.e., a difference between the performance of a polyculture and the additive expectation from the constituent monocultures); (4) species-specific model, in which interspecific interactions are due to the presence of a particular species; (5) functional-type model, which assumes that interactions between species of different functional types (i.e., large or small species) are stronger than interactions between species within a functional type; and (6) functional similarity model, where the contributions of some species to decomposition are similar (used only when model 5 showed no species interactions within a particular functional type). Model 6 was based in Kirwan's (2009) functional redundancy model, but did not assume functional redundancy (i.e., a 100% compensation of a species' function by another), but rather similar effects on decomposition.

The models were fitted using the 'gls' function and maximum likelihood method in the nlme R package in R v.3.3.1 (Pinheiro *et al.* 2016; R Core Team 2016), and they were compared through a model selection procedure based on the Akaike information criterion corrected for sample size (AICc; Zuur *et al.* 2009). Prior to running the models, Cleveland dot- and boxplots for each response variable and species combination revealed no outliers (Zuur & Ieno 2015). As boxplots showed different variances depending on detritivore species combinations for both response variables (i.e., a violation of the homogeneity assumption for parametric models), we used the *VarIdent* function of the nlme R package in the models described below to produce an appropriate variance structure (Zuur *et al.* 2009). Due to differences in the biomass of different detritivore species, we considered correcting decomposition rates by biomass. However, biomass ratios polyculture:monoculture ratios were not significantly different from 1 (Table S1), suggesting that differences between observed and expected decomposition rates in polycultures were not driven by detritivore biomass.

When significant effects of species interactions or functional types on decomposition were demonstrated, we quantified the magnitude of such effects by calculating the ratio of decomposition rate between the value of a polyculture (observed value) and the average value of the corresponding monocultures (expected value) (Appendix I). Although we used untransformed decomposition data (because assumptions of parametric models were met after the use of appropriate variance structure; see above), log-transformed data produced similar results (Table S2). We further examined whether detritivore growth differed from the additive expectation (hypothesis 2), by subtracting the relative growth of a species in a polyculture from the relative growth of the same species in a monoculture (Appendix I). We calculated ordinary non-parametric bootstrapped 95% confidence intervals (BCa method using the 'boot' function and package, and based on 1,000 bootstrap replicates; Davison & Hinkley 1997; Canty & Ripley 2016) to test whether these intervals contained the value of one (for decomposition rate) or zero (for detritivore growth) – that is, the null expectation that the response of the polyculture was not different from the mean responses of the monocultures of species present in the polyculture.

## Results

Overall survival of detritivore species was high during the experiment (mean ± SE: 74 ± 5% for *Leuctra*, 88 ± 2% for *Lepidostoma*, 94 ± 2% for *Sericostoma* and 92 ± 2% for *Echinogammarus*); when different treatments were examined separately, the only incidence of low survival for *Leuctra* was in combination with *Echinogammarus* (17.5 ± 0.1%; Table S3). Decomposition rates were lowest in the *Leuctra* monoculture (0.69 ± 0.10 mg $d^{-1}$) and highest in the *Sericostoma* monoculture (16.93 ± 0.41 mg $d^{-1}$) (Fig. S2a; Table S4). In monocultures, *Sericostoma* body mass increased by 42%, while *Lepidostoma* and *Echinogammarus* growth rates did not differ from zero, and body mass of *Leuctra* was reduced by 18% (Fig. S2b, Table S3).

The model selection procedure showed that species interacted and produced diversity effects on decomposition rates (Table S5). Two models were plausible descriptions of species interactions ($\Delta_i < 2$; Table 1): the functional-type model and the species-specific model. The functional-type model had a better fit than the species-specific model, indicating that interspecific interactions were mostly related to detritivore body size, with some influence of species identity. The bootstrap procedure showed that interactions between functional types (i.e., small and large species) produced a 12% increase in decomposition rates of the average rate of those species in monoculture (Fig. 3a). The decomposition rate of the two large species together (i.e., *Sericostoma* and *Echinogammarus*) was 19% higher than the average of their monocultures (Fig. 3a). In contrast, the interaction between the two small species did not exceed the average contribution of their monocultures (Fig. 3a), which led us to test for functional similarity within this functional type. However, the poor fit of the functional similarity model and the very different performances of *Leuctra* and *Lepidostoma* (see below) indicated that small organisms did not have similar effects on decomposition. The species-specific model and 95% confidence intervals showed that results were not driven by the presence of a single species in a polyculture, because the effect was always higher than the additive expectation (from 9% higher in interactions with *Lepidostoma* to 20% higher in interactions with *Sericostoma*; Fig. 3b).

The differences between observed and expected growth (polyculture minus monocultures) showed (i) higher growth of *Lepidostoma* and *Leuctra* when combined (Fig. 3c); (ii) similar growth of *Sericostoma* and *Echinogammarus* when combined (Fig. 3c); (iii) higher growth of small organisms, but similar growth of large organisms, when both small and large organisms were combined (Fig. 3c); and (iv) higher overall growth of *Leuctra* and *Lepidostoma* and similar overall growth of *Sericostoma* and *Echinogammarus* (Fig. 3d).

The video observations evidenced differences in feeding behaviour between monocultures and polycultures only for *Leuctra*, which was observed feeding on FPOM produced by other species in polycultures; the two caddisflies were observed shredding litter discs, but *Lepidostoma* ate only the margins, while *Sericostoma* ate the whole disc including the less palatable parts; *Echinogammarus* was a very active swimmer and was observed shredding the margins and scraping the surface of litter discs (Table S6).

## Discussion

Our study is among the first to demonstrate that body size is a key trait mediating the effects of detritivore diversity on litter decomposition in streams. Reiss *et al*. (2011) had found that within-species variation in detritivore body size had a much larger effect than diversity on decomposition rates. We show that differences in body size across species are a main determinant of interspecific interactions that mediate diversity effects on decomposition. These results are important because they can help explaining the contrasting findings of previous experiments [i.e., positive (Jonsson & Malmqvist 2000; Dangles, Jonsson & Malmqvist 2002; Boyero, Pearson & Bastian 2007; Constantini & Rossi 2010), negative or no effects (Bastian, Pearson & Boyero 2008; Creed *et al.* 2009; McKie *et al.* 2009; Reiss *et al.* 2011) of detritivore diversity on decomposition], few of which took body size into account.

We showed that diversity effects on decomposition were most evident when species of different body size were combined, which supported our first hypothesis. Leaf litter decomposed faster in polycultures containing large and small detritivores than was expected from their monocultures, indicating that interspecific interactions caused greater effects on decomposition than simple addition. Such positive effects could arise from mechanisms such as resource partitioning or facilitation, but few experimental studies have distinguished between these mechanisms (exceptions include Cardinale, Palmer & Collins 2002; Jonsson & Malmqvist 2003). The patterns we observed suggested that facilitation was an important mechanism underlying diversity-decomposition effects, as shown by the higher growth of smaller detritivores in the presence of larger species (in support of our second hypothesis). The enhanced growth and the video observations suggested that smaller detritivores could benefit from the feeding activity of larger detritivores, which would produce large amounts of smaller litter fragments and FPOM that could be used by the small species. *Leuctra* species are known to act as both litter-shredding detritivores and collectors (López-Rodríguez *et al.* 2012), and are often found in FPOM deposits in streams (Callisto & Graça 2013). The relatively small mouthparts of *Lepidostoma* compared to larger detritivores might be more efficient at handling the smaller litter fragments, although more evidence would be required to support this statement.

In contrast to the enhanced growth of small detritivores in polycultures containing species of different body size, larger detritivores showed similar growth in polycultures and monocultures, indicating that larger species did not benefit from the presence of smaller species. This could indicate that faster decomposition in polycultures was due exclusively to enhanced feeding of small species; however, this is unlikely, as the polyculture containing just the two large species also showed faster decomposition than was expected from monocultures. The absence of enhanced growth in this case, however, suggests that there was no facilitation between the large species. A plausible alternative mechanism underlying diversity effects on decomposition in this case would be resource partitioning, which is common among species belonging to distantly related taxa (Petchey & Gaston 2002), as is the case for *Sericostoma* and *Echinogammarus*, which belong to different subphyla. Gammarids are able to shred leaf litter, but can also scrape on surfaces, as observed in our videos and shown elsewhere (Mayer, Maas & Waloszek 2012); in contrast, caddisflies such as *Sericostoma* have mouthparts that are highly specialized for fragmenting leaf material, including the tougher parts, and are more obligate leaf-eaters with gut fauna adapted to breaking down cellulose (Friberg & Jacobsen 1994). Thus, *Sericostoma* was able to eat the less palatable parts of leaf discs (minor veins), as observed in our videos and elsewhere

(Tonin *et al.* 2017). In contrast, *Echinogammarus* seemed to feed only on the more palatable parts (which would better satisfy their higher energy requirements), resulting in higher consumption overall, but similar growth rates in polycultures. Also, the absence of *Echinogammarus* growth when in monocultures and their generalist feeding habits suggests leaf litter was not their preferred feeding resource and that their growth could be enhanced in a diverse food diet.

When the small species were together, decomposition was similar to that of the average monoculture, but growth of both species was enhanced. This suggests that facilitation occurred also between these two species, possibly through the mechanism described above: the feeding activity of *Lepidostoma* released high amounts of FPOM that were most likely used by *Leuctra*; it is also possible that *Lepidostoma* roughened the leaf surface, making it easier for *Leuctra* to eat it, as shown for other detritivores (Iwai, Pearson & Alford 2009). Another plausible mechanism behind detritivore diversity effects on decomposition is the reduced density of each species, which could lead to reduced intraspecific competition for specific food resources, although there is evidence of benefits of intraspecific aggregation (McKie *et al.* 2009). The negative growth of *Leuctra* in monocultures supports the generalist feeding habit of this species (i.e., probably leaf litter is not their preferred resource) and their dependence of fine particles to enhance their growth. It is unclear, however, how *Lepidostoma* could benefit from the presence of *Leuctra*; it is possible that the presence of *Leuctra* somehow enhances litter quality by increasing microbial conditioning, but this would need to be tested experimentally. Importantly, the positive diversity effect on decomposition found in polycultures containing large species, the distinct performance of small species in monocultures, and the poor fit of the functional similarity model indicated that these species were not functionally similar. It is also noteworthy that our results were not driven by the presence of one particular species with dominant effects, unlike findings elsewhere (Dangles & Malmqvist 2004). However, assemblages of different species composition may produce different results due to the existence of different interspecific interactions (McKie *et al.* 2008) or different animal traits relevant to decomposition (e.g., feeding modes, enzymatic capabilities; Frainer *et al.* 2014).

We conclude that body size is a key animal trait to take into account when exploring diversity effects on litter decomposition and related processes, as body size has the potential to mediate such effects through its influence on interspecific interactions. We show how different mechanisms of complementarity (i.e., facilitation and resource partitioning) can mediate interactions between detritivore species of different or similar size, and de-emphasize the existence of functional similarity between similar-sized species. Although microcosm experiments are much simpler than natural systems, these experiments are often crucial to understand complex ecological relationships (Fraser & Keddy 1999; Benton *et al.* 2007), and our results are supported by empirical evidence that body size is a key driver of many ecological processes (Peters 1986; Woodward *et al.* 2005). Our study suggests that, if we are to anticipate the consequences of diversity loss for decomposition in stream ecosystems, it is crucial to take into account not only the identity and biomass of detritivore assemblages but also their body-size structure. However, our results must be interpreted with caution given the limitations of microcosm experiments – i.e. the short experimental duration, lack of multi-generational responses, and artificiality compared to natural systems. The applicability of our findings to real-world ecosystems can only be addressed by running longer-term experiments at different times of year with a variety of leaf litter resources, different detritivore species, and a comparison with field conditions. Ideally, future studies should also address the potential influence of different species' vulnerability to extinction depending on body size (Petchey *et al.* 1999; Raffaelli 2004), and how this might affect ecosystem functioning on different scenarios of detritivore diversity (Boyero *et al.* 2012) and in more complex food webs (Thébault & Loreau 2003, Jabiol *et al.* 2013).

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## Author contributions

LB and JPo designed the experiment with feedback from AMT, SM and AB; AMT, SM, LB, AB, JPo and JPe conducted the field and/or laboratory work; AMT and LB analysed the data;

AMT and LB wrote the manuscript with feedback from JPo, RGP, BJC, SM, AB, JPe and JFGJr.

## Data accessibility

Data available from the Open Science Framework: https://doi.org/10.17605/OSF.IO/NTM8R (Tonin et al. 2018).

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**Table 1.** Summary of model selection for the set of diversity-interaction models used to test for diversity effects on litter decomposition rate (mg $d^{-1}$), based on the Akaike Information Criterion corrected for sample size ($AIC_c$). Models are ordered from the best to the poorest fit according to Akaike weights ($w_i$). The biological meaning of each model is described in the methods and Fig. 2. K, number of estimated parameters for each model; $\Delta_i$ (delta $AIC_c$), difference in $AIC_c$ value relative to the best model; $w_i$ , probability that a model is the best among the whole set of models. Detritivore species: Lc, *Leuctra geniculata*; Lp, *Lepidostoma hirtum*; Se, *Sericostoma pyrenaicum*; Eg, *Echinogammarus berilloni*; 2-species polyculture interactions: Lc-Lp, Lc-Se, Lc-Eg, Lp-Se, Lp-Eg, Se-Eg; diversity-interaction terms for each species: $\mathrm{Lc}_{\mathrm{INT}}$, $\mathrm{Lp}_{\mathrm{INT}}$, $\mathrm{Se}_{\mathrm{INT}}$, $\mathrm{Eg}_{\mathrm{INT}}$; diversity-interaction terms for functional types: SMALL, LARGE.

| **Model** | **K** | **$\Delta_i$** | **$w_i$** |
|---|---|---|---|
| (5) Functional type<br>Lc + Lp + Se + Eg + SMALL-LARGE + Lc-Lp + Se-Eg | 18 | 0.00 | 0.51 |
| (4) Species-specific<br>Lc + Lp + Se + Eg + $\mathrm{Lc}_{\mathrm{INT}}$ + $\mathrm{Lp}_{\mathrm{INT}}$ + $\mathrm{Se}_{\mathrm{INT}}$ + $\mathrm{Eg}_{\mathrm{INT}}$ | 19 | 0.39 | 0.42 |
| (2) Species identity<br>Lc + Lp + Se + Eg | 15 | 4.78 | 0.05 |
| (3) Pairwise interaction<br>Lc + Lp + Se + Eg + Lc-Lp + Lc-Se + Lc-Eg + Lp-Se + Lp-Eg + Se-Eg | 21 | 5.82 | 0.03 |
| (6) Functional redundancy<br>SMALL + Se + Eg + SMALL-Se + SMALL-Eg + Se-Eg | 17 | 91.89 | 0.00 |
| (1) Null<br>Intercept only | 12 | 225.28 | 0.00 |

## Figure legends

**Fig. 1.** Experimental design with four detritivore species belonging to two functional types (i.e., large and small body-sized species) in monocultures, 2-species polycultures (six species combinations of the same or different functional type) and the 4-species polyculture.

**Fig. 2.** Diversity-interaction models used to test for diversity effects on decomposition. The biological meaning of each model and model terms are described next to each box; $y$, response variable; α, intercept; β, estimated parameter of the contribution of each species; $\varepsilon_{ij}$, model residuals, which were allowed to vary with respect to each detritivore combination (see methods). Arrows linking different boxes represent an increase in model complexity. Detritivore species: Lc, *Leuctra geniculata*; Lp, *Lepidostoma hirtum*; Se, *Sericostoma pyrenaicum*; Eg, *Echinogammarus berilloni*; 2-species polyculture interactions: Lc-Lp, Lc-Se, Lc-Eg, Lp-Se, Lp-Eg, Se-Eg; diversity-interaction terms for each species: $\mathrm{Lc}_{\mathrm{INT}}$, $\mathrm{Lp}_{\mathrm{INT}}$, $\mathrm{Se}_{\mathrm{INT}}$, $\mathrm{Eg}_{\mathrm{INT}}$; diversity-interaction terms for functional types: SMALL, LARGE.

**Fig. 3.** Ratio of decomposition rates between polycultures and monocultures (a, b) and difference in detritivore growth between polycultures and monocultures (c, d) for the interaction of species of similar (Lc-Lp, Se-Eg) or different body size (small-large) or for the average interaction of each species (see Fig. 2 legend). The dashed line denotes the value of one (for decomposition) or zero (for growth), that is, the null expectation that the polyculture value is not different from the mean value of constituent monocultures. Circles are means and vertical lines denote upper and lower limits of 95% non-parametric bootstrapped confidence intervals; closed circles represent intervals that do not reject the null hypothesis (i.e., do not contain the value of one or zero) and open circles represent intervals that do reject the null hypothesis.

**Fig. 1**

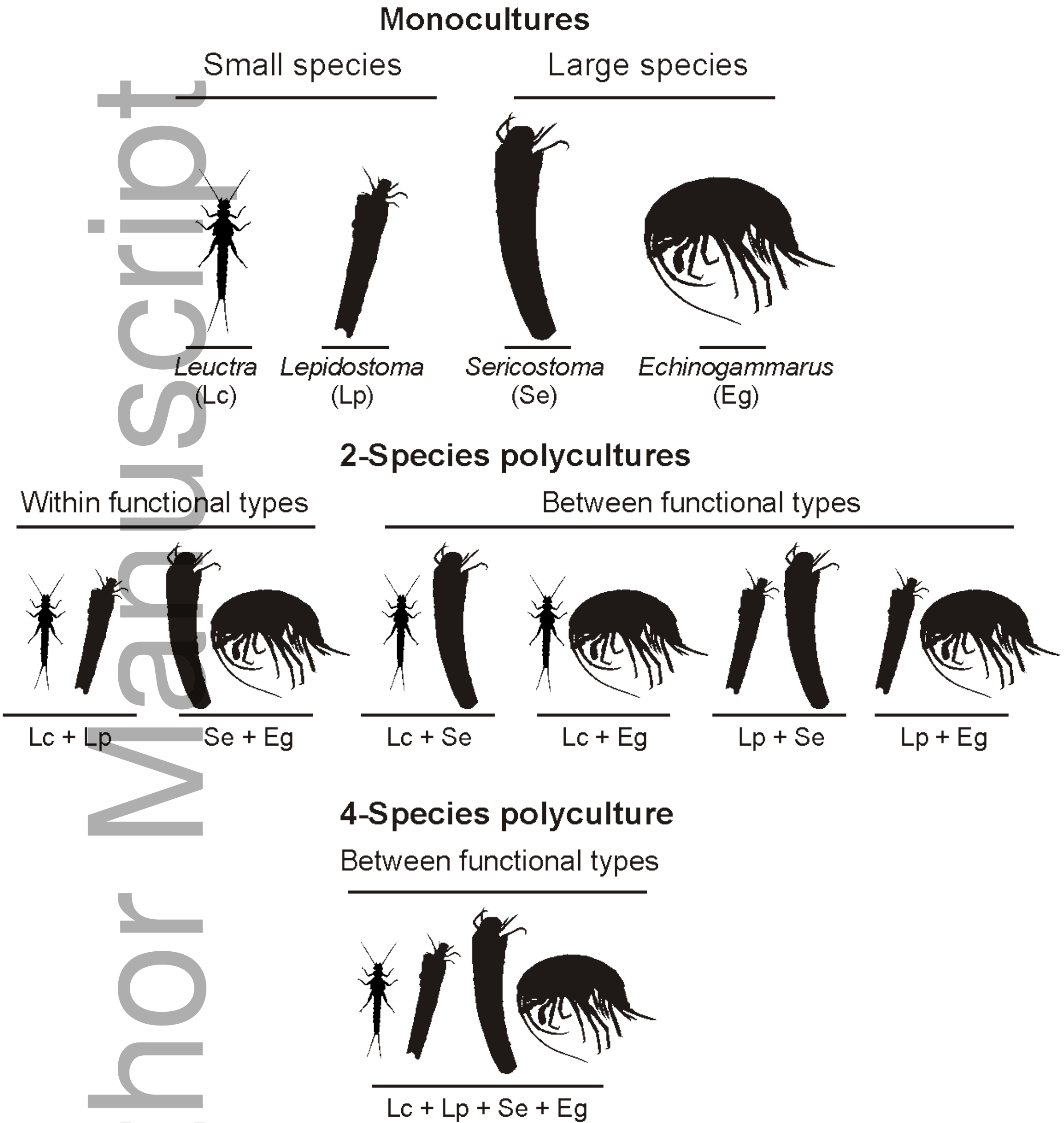

Monocultures
Small species
Large species
*Leuctra* (Lc)
*Lepidostoma* (Lp)
*Sericostoma* (Se)
*Echinogammarus* (Eg)
2-Species polycultures
Within functional types
Between functional types
Lc + Lp
Se + Eg
Lc + Se
Lc + Eg
Lp + Se
Lp + Eg
4-Species polyculture
Between functional types
Lc + Lp + Se + Eg

Fig. 2

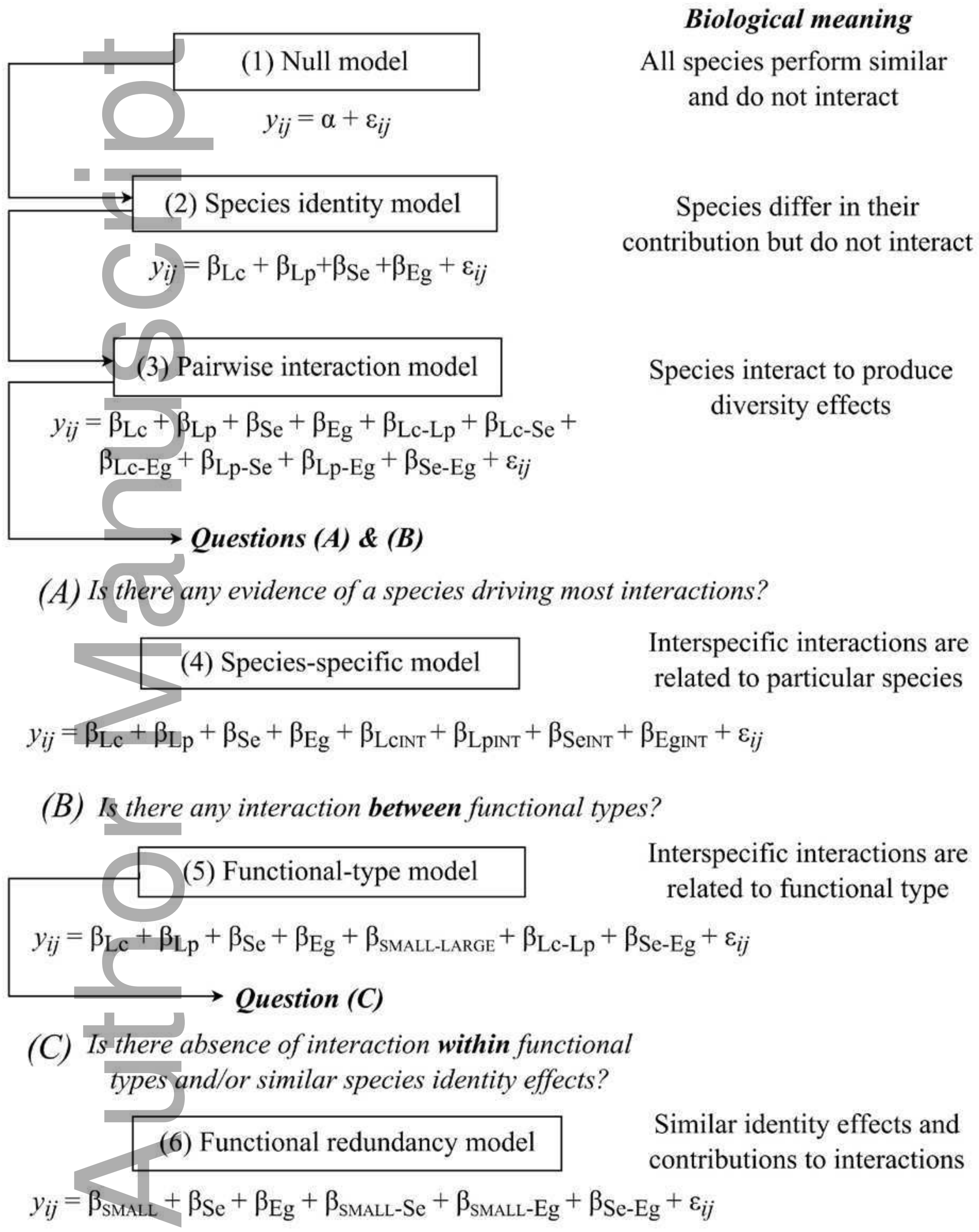
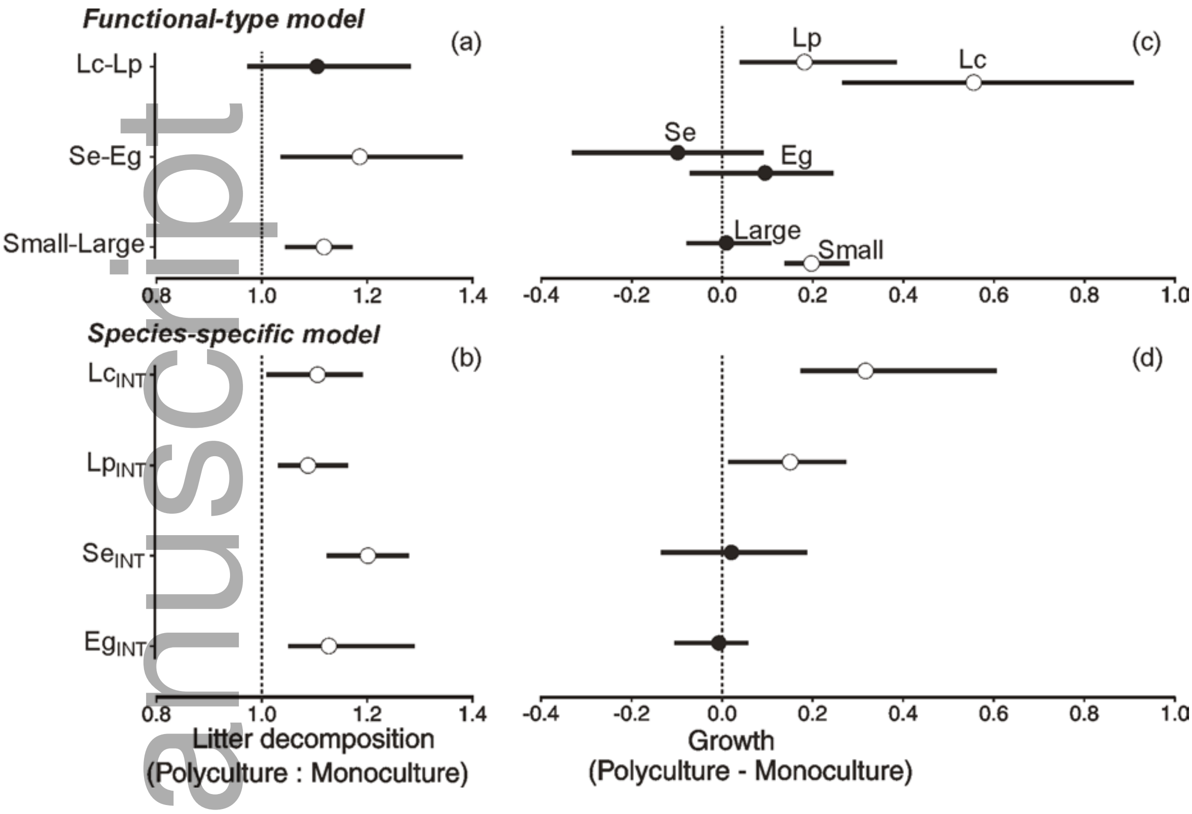

Biological meaning
(1) Null model
$y_{ij} = \alpha + \varepsilon_{ij}$
All species perform similar and do not interact
(2) Species identity model
$y_{ij} = \beta_{Lc} + \beta_{Lp} + \beta_{Se} + \beta_{Eg} + \varepsilon_{ij}$
Species differ in their contribution but do not interact
(3) Pairwise interaction model
$y_{ij} = \beta_{Lc} + \beta_{Lp} + \beta_{Se} + \beta_{Eg} + \beta_{Lc\text{-}Lp} + \beta_{Lc\text{-}Se} + \beta_{Lc\text{-}Eg} + \beta_{Lp\text{-}Se} + \beta_{Lp\text{-}Eg} + \beta_{Se\text{-}Eg} + \varepsilon_{ij}$
Species interact to produce diversity effects
Questions (A) & (B)
(A) Is there any evidence of a species driving most interactions?
(4) Species-specific model
Interspecific interactions are related to particular species
$y_{ij} = \beta_{Lc} + \beta_{Lp} + \beta_{Se} + \beta_{Eg} + \beta_{LcINT} + \beta_{LpINT} + \beta_{SeINT} + \beta_{EgINT} + \varepsilon_{ij}$
(B) Is there any interaction between functional types?
(5) Functional-type model
Interspecific interactions are related to functional type
$y_{ij} = \beta_{Lc} + \beta_{Lp} + \beta_{Se} + \beta_{Eg} + \beta_{SMALL\text{-}LARGE} + \beta_{Lc\text{-}Lp} + \beta_{Se\text{-}Eg} + \varepsilon_{ij}$
Question (C)
(C) Is there absence of interaction within functional types and/or similar species identity effects?
(6) Functional redundancy model
Similar identity effects and contributions to interactions
$y_{ij} = \beta_{SMALL} + \beta_{Se} + \beta_{Eg} + \beta_{SMALL\text{-}Se} + \beta_{SMALL\text{-}Eg} + \beta_{Se\text{-}Eg} + \varepsilon_{ij}$

**Fig. 3**


Functional-type model
(a)
Lc-Lp
Se-Eg
Small-Large
0.8
1.0
1.2
1.4
Species-specific model
(b)
$Lc_{INT}$
$Lp_{INT}$
$Se_{INT}$
$Eg_{INT}$
0.8
1.0
1.2
1.4
Litter decomposition
(Polyculture : Monoculture)
(c)
Lp
Lc
Se
Eg
Large
Small
-0.4
-0.2
0.0
0.2
0.4
0.6
0.8
1.0
(d)
-0.4
-0.2
0.0
0.2
0.4
0.6
0.8
1.0
Growth
(Polyculture - Monoculture)