



Differential resource consumption in leaf litter mixtures by native and non-native amphipods

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Abstract Leaf litter processing is an essential ecosystem function in freshwater systems, since much of the carbon and nutrients moving through freshwater food webs come from the surrounding terrestrial ecosystems. Thus, it is important to understand how the species performing this function differ, especially because many native species are being replaced by non-native species in aquatic ecosystems. We used a field experiment to examine leaf consumption rates of two common shredding macroinvertebrates (the native *Gammarus fossarum* and the non-native *Gammarus roeselii*). Leaves from three species, varying in resource quality, were added both in leaf monocultures and as a three-species mixture. Biomass-adjusted daily consumption rates were similar between the two amphipod species, and each consumed nitrogen-rich alder leaves faster than oak or beech leaves. However, because adult *G. roeselii* are approximately twice the size of *G. fossarum*, this led to systematic, though nonsignificant, differences in consumption rates at the

per-capita or population level. Furthermore, we found nuanced effects of decomposer identity on leaf decomposition in mixtures. Only *G. roeselii* showed increased consumption of the preferred resource (alder) in the mixture, while *G. fossarum* consumed all leaves at the same proportional rates as in monocultures. This is an important distinction, as most measures of macroinvertebrate leaf shredding are made in the laboratory with only a single leaf resource available. Our results, based on a field experiment which could control the presence of dominant macroinvertebrates while still providing natural, biologically realistic context, suggest that even functionally similar species may subtly shift ecosystem processes.

Keywords Biodiversity · Decomposition · Headwater streams · Ecosystem function · Meta-ecosystem · Preferential feeding

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Introduction

Freshwater is among the most threatened habitats worldwide (Carpenter et al. 2011), with streams, rivers, and lakes experiencing major ongoing documented declines in biodiversity (Darwall et al. 2018). There is no end in sight for the threats causing these declines, due to projected further increases in human

activity and water needs (Vörösmarty et al. 2010; WWAP 2018). Yet many global challenges in biodiversity topics are under-researched in freshwater compared to other ecosystem types (Jucker et al. 2018). Among these is the concept that ecosystem functioning is positively related to biodiversity (Hooper et al. 2005). This is for two reasons. First, terrestrial ecosystem functions are proportionally well represented in the field of biodiversity and ecosystem research, while research in freshwater systems is comparatively less common (Cardinale et al. 2012), despite the importance of freshwater as a resource. Secondly, some freshwater ecosystems rely heavily on detrital energy pathways due to low autochthonous production, and so decomposition is relatively very important (Webster and Benfield 1986), yet across all ecosystems the relationship between diversity and decomposition is less well studied than the relationship between diversity and productivity (Cardinale et al. 2011). Despite the relative lack of emphasis on decomposition as an ecosystem function, aquatic ecosystems are well represented among all studies of diversity and decomposition (Duffy et al. 2007; Srivastava et al. 2009; Handa et al. 2014), perhaps because the decomposition pathway can provide the bulk of resources to some freshwater systems (Gounand et al. 2018); yet this research has so far come to contrasting conclusions. As a result, evidence currently indicates that diversity has a net positive effect on decomposition in aquatic ecosystems (Handa et al. 2014), but that this conclusion is surrounded by considerable uncertainty (Tank et al. 2010). Thus, much more work is needed to fully understand how biodiversity loss affects decomposition in aquatic systems.

An important aspect of the biodiversity–decomposition relationship is taxa identity. While biodiversity *sensu* richness has not always been found to determine decomposition rates, the identity of specific members of the community, including the presence of dominant species, may be an important control (Dangles and Malmqvist 2004; Tolkkinen et al. 2013; Santonja et al. 2018). A variety of taxa contribute to decomposition in aquatic ecosystems, including bacterial, fungal, macroinvertebrate, and vertebrate communities. These different taxa can perform overlapping functions but also facilitate one another by performing complementary functions in processing detritus; hence, the identity of the species making up a diverse

community can be more informative than their number. For example, organisms with different body stoichiometries sometimes preferentially process resources with different nutrient contents, leading to higher total decomposition rates in stoichiometrically diverse communities (Ohta et al. 2016). As another example, larger shredding organisms can create fine particulate material which is then available for smaller organisms to further decompose (Tonin et al. 2018). As a result, these shredding organisms, often macroinvertebrates, serve a key functional role in the decomposition process (Wallace and Webster 1996).

Yet, troublingly, macroinvertebrate diversity is declining in freshwater worldwide along with freshwater diversity in general, and even more troublingly, we have few systematic assessments of the degree to which this is occurring, in part because macroinvertebrate species are assessed less frequently than other groups, such as vertebrates (Strayer 2006; Strayer and Dudgeon 2010; Carpenter et al. 2011). In addition, freshwater ecosystems are highly affected by non-native species (Ricciardi and MacIsaac 2000; Strayer 2010), including many non-native macroinvertebrates. Dominant species can control decomposition in multiple ways, both by contributing to decomposition themselves and by altering the effect of other diversity on decomposition rates (McKie et al. 2008; Creed et al. 2009; Little and Altermatt 2018b). Thus, it is essential to understand how the species re-arrangements, especially with regard to the identity of dominant macroinvertebrate species, could influence decomposition.

The rate of decomposition in freshwater systems also depends on the identity and diversity of the material being decomposed—that is, bottom-up diversity. While the effects of plant/producer diversity on decomposition are weak compared to the effects on biomass production, in streams they can be relatively strong (Cardinale et al. 2011). Identity of leaf litter can be very important, and in particular, macroinvertebrates consume higher-quality, nutrient-rich litter more quickly (Martínez et al. 2013; Bruder et al. 2014; Little and Altermatt 2018b). Numerous experiments have found that increasing leaf litter diversity speeds decomposition (as a few examples, Lecerf et al. 2011; Jabiol and Chauvet 2012; Handa et al. 2014). Others, however, have found that mixing leaf types has the effect of homogenizing leaf litter quality and leads to intermediate or low decomposition rates

(Swan 2011; Little and Altermatt 2018b; Santschi et al. 2018). Thus, as a whole, work on the effect of producer and detritivore diversity on decomposition has not led to clear and general conclusions, but rather suggests that these effects may be context dependent.

These differing results may arise from important differences in biological context, including varying degrees of ecological realism in experimental design. Many experiments are done in the laboratory in small- to medium-sized mesocosms which may not reflect realistic densities/biomass of detritivores, may feature highly simplified habitats, and may not include species from other trophic guilds. For example, most laboratory experiments lack the microbial and fungal communities that shredders coexist and interact with in streams, as well as signals of predation pressure that may affect foraging behavior and exert top-down control on detritivores. While laboratory experiments may lack generality, field work has other limitations. Besides the presence of potentially confounding environmental and biotic factors, it can often be more difficult to identify ecological mechanism. For example, deploying bags filled with different types and diversities of leaf litter into streams often reveals that decomposition rates differ among treatments, but does not provide information about which consumers are performing this function at a given scale (as discussed in Frainer et al. 2015).

Here, we performed a field experiment using enclosures to isolate the effects of dominant macroinvertebrate decomposer identity on decomposition, while still providing the ecological realism of natural stream conditions. We examined how the presence of two closely related shredding amphipods (Crustacea: Amphipoda) influences processing rates of three types of leaf litter, either individually or in a mixture. Native amphipods can dominate the biomass in Central European streams (Nery and Schmera 2015), but in some catchments are being replaced by non-native amphipod species (Altermatt et al. 2014; Altermatt et al. 2016; Little and Altermatt 2018a). Thus, for our experiment in Eastern Switzerland, we used the most common native species in the area (Altermatt et al. 2016), *Gammarus fossarum*, and a common non-native species, *Gammarus roeselii*, which is assumed to fill the same functional role. We addressed three questions:

- (1) Are there differences in leaf litter consumption rates of *G. fossarum* and *G. roeselii*, a non-native species which has replaced it in some Central European stream catchments?
- (2) In a natural stream setting, what effect does mixing leaf types have on decomposition rates?
- (3) Does this mixing effect differ between the native and the non-native amphipod species?

Methods

Field site and study organisms

The experiment was set up in the Imbersbach stream in Arbon, Switzerland, approximately 300 m from its outlet into Lake Constance. At the experimental site (47.524°N, 9.419°E), Imbersbach is a second-order stream about 1.5 m in width. We used two amphipod species for the experiment: *G. fossarum* and *G. roeselii*. *Gammarus roeselii* is not native to the region, but has been present around Lake Constance since roughly 1850 (Altermatt et al. 2014), and was actually the only amphipod species present in the Imbersbach stream (Little and Altermatt 2018a). *Gammarus fossarum* is native to Central Europe and is the most widespread and most common amphipod species across much of Switzerland (Altermatt et al. 2014). Though it was not present in Imbersbach at the time of our study, it was present in nearby catchments < 5 km away (Little and Altermatt 2018a). The two species differ in size, such that adult *G. roeselii* added to the enclosures were on average just over twice as large as *G. fossarum*. The average *G. fossarum* dry mass was 5.9 mg and the average *G. roeselii* dry mass 12.9 mg.

Experimental design

To measure leaf litter processing in a natural setting, we built enclosures from 28-cm-length PVC pipe (internal diameter 7 cm) with both openings covered with 1-mm polyester mesh, such that water and very small organisms could flow through, and placed them lengthwise in the stream (Fig. 1). Enclosures were stocked with amphipods and leaves, according to a factorial experimental design manipulating leaf litter identity and diversity and amphipod identity. The two amphipod treatments were *G. fossarum* and *G.*



Fig. 1 Experimental setup; a subset of the 76 enclosures deployed over a ~ 150 m stream reach are shown in the photograph. Enclosures were built from two pieces of PVC pipe, each with one end covered with 1-mm mesh. Leaves and amphipods (except in the no-amphipod control enclosures) were placed in one of the pieces of PVC pipe, and the two pieces were then slid together. Enclosures were secured to cement blocks (two enclosures per block) using zip ties and placed in the stream lengthwise with respect to the current, and in locations where they were completely covered by water. Enclosures were randomly assigned to cement blocks such that no block had two enclosures of the same treatment

roeselii. Four leaf litter treatments were applied: alder (*Alnus glutinosa*), oak (*Quercus robur*), beech (*Fagus sylvatica*), and the three-species mixture of these leaf litter species. These are three of the most common tree species in forests surrounding the study site. The three species differ in resource quality, with the nitrogen fixer alder containing higher nutrient content (Little and Altermatt 2018b) and beech and oak having higher lignin content (Frainer et al. 2015). Leaf litter had been collected the previous autumn around Dübendorf, Switzerland, and air-dried before being stored. Before the experiment, leaves were conditioned in a mix of stream and tap water for 2 weeks to provide them with a natural microbial community. We placed six alder leaves in each enclosure, or six oak leaves, or twelve beech leaves, or a mixture of two alder, two oak, and four beech leaves, according to treatment. More beech leaves were provided because each leaf was smaller, and we wished to roughly standardize the amount of leaf biomass available in enclosures, and the amount of leaf surface area available as habitat structure. Since species had different surface area/mass ratios, equalizing both metrics was not possible. Mean and standard deviation of leaf litter provisioned to the

enclosures were as follows: alder only, 1.34 g (± 0.24 g); beech only, 1.69 g (± 0.21 g); oak only, 1.90 g (± 0.12 g); and three-species mix, 2.32 g (± 0.26 g). Each amphipod treatment/leaf treatment combination was replicated six times, with twelve adult amphipods initially placed in each enclosure. We also placed three control enclosures in the stream for each leaf litter treatment, which contained no amphipods but only the leaves, so that we could account for decomposition by microbial, bacterial, and small macroinvertebrate communities and correctly assess the contribution of amphipods to decomposition. A total of 76 enclosures were placed in the stream on April 21, 2016, and retrieved on May 18, 2016.

Leaf consumption measurements

Leaves were photographed before and after the experiment to measure leaf area. Leaf area analysis was performed using the program ImageJ (National Institutes of Health, Bethesda, Maryland, USA). Scale was set for area calculations using a rectangular object of known size (2.54 cm in length). Images were converted to 8-bit grayscale, pixels were selected using a lower threshold of 1 and an upper threshold of 90 (pre-experiment photographs) and 55 (post-experiment photographs, due to different overhead lighting which altered the image metrics), and area was measured using the “analyze particles” function. A visual assessment was made of whether individual leaves had holes inside the leaf area, and the “include holes” parameter was set accordingly. For treatments with multiple leaf types, each type was analyzed separately. Area was then converted to biomass using previously developed allometric relationships for dried senescent leaves of each individual species, using leaves from the same trees where the litter for this experiment was collected (alder: mg dry mass = $10.89 + 0.055 * \text{mm}^2 \text{ area}$ ($R^2 = 0.85$); beech: mg dry mass = $54.843 + 0.019 * \text{mm}^2 \text{ area}$ ($R^2 = 0.53$); oak: mg dry mass = $0.076 * \text{mm}^2 \text{ area} - 16.34$ ($R^2 = 0.91$); Little and Altermatt 2018b), and mass loss over the course of the experiment was calculated. For leaves from amphipod treatments, the average mass loss in control enclosures for that leaf litter treatment was subtracted from the total mass loss to get an estimate of the decomposition performed only by amphipods.

After the experiment, amphipods from the enclosures were counted, sacrificed, dried in an oven at 60 °C for 48 h, and weighed. Survival was 89.8% for *G. fossarum* and 90.1% for *G. roeselii* across the entire experiment and did not vary with species, leaf litter treatments, or their interaction ($F_{7,56} = 0.30$, $p = 0.95$). At the enclosure level, leaf consumption rates were calculated per day over the course of the experiment in two ways. First, the daily total mass loss was adjusted for the density of amphipods. Because we did not have detailed information on when mortality occurred during the experiment, we used an average density of $(\text{amphipods}_{\text{initial}} + \text{amphipods}_{\text{final}})/2$. Secondly, the daily total mass loss was adjusted for the biomass of amphipods. To obtain enclosure-level biomass, the density of amphipods was multiplied by the average mass of the amphipods which were weighed from that enclosure.

Statistical analysis

All statistical analyses were performed in R version 3.5.0 (R Core Team, Vienna, Austria, 2018). Total leaf litter consumption by amphipods was analyzed as a response to amphipod species and leaf litter type. Density- and biomass-adjusted daily consumption rates were right-skewed and residuals from a linear model strongly deviated from normality (Shapiro–Wilk normality test, $p < 0.001$), even when square root or double square root transformed. Therefore, we used nonparametric tests. First, we assessed each metric of daily consumption rate as a response to combined treatment (for example, *G. fossarum*—oak or *G. roeselii*—beech) using a Kruskal–Wallis test. If this test was significant ($p < 0.05$), we followed it with a Dunn test using the “FSA” package version 0.8.20 (Ogle 2018) of four pairwise comparisons: between *G. fossarum* and *G. roeselii* consumption rates of each of the four leaf litter treatments. If any of these pairwise comparisons were significant ($p < 0.05$), we took this as an indication that there was a main effect of amphipod species and/or an interaction between amphipod and leaf litter treatments. If none of these four pairwise comparisons were significant, we concluded that amphipod species was not an important factor explaining consumption rates and performed a new Kruskal–Wallis test using only leaf litter treatment as an explanatory variable. If this test was significant ($p < 0.05$), we then performed

a Dunn test between all pairs of leaf litter treatments to find significant differences, and used a Holm–Bonferroni correction to adjust p values for multiple comparisons.

To examine the possible mechanisms explaining consumption rates in three-species mixtures, we analyzed percent area loss of leaves in monocultures versus mixtures, and whether any possible difference in these relative rates of loss could be due to amphipod species, leaf type, or an interaction between the two. Using percent area loss in this analysis was preferable to absolute consumption rates for two reasons. First, there was less leaf litter of each species available in the mixed-species enclosures, which may have affected total consumption rate and made direct comparisons inaccurate. Second, using percent area loss allowed us to include the control enclosures in the same analysis, even though area loss could not be adjusted for density because no amphipods were present. We used linear models of square-root-transformed percent area loss and performed model selection using AIC beginning from a full factorial model with the three-way interaction between leaf type, diversity level, and amphipod species. When significant treatment effects were indicated by the model, we tested planned linear contrasts using the “glht” function in the “multcomp” package version 1.4–8 (Hothorn et al. 2008). We tested all contrasts with two factor levels in common (for example, *G. roeselii* consumption of alder in mixed vs. monoculture enclosures), in order to determine whether the consumption rate under these two conditions was modified by a third condition. We did not test contrasts with one or fewer factor levels in common (for example, we did not test *G. roeselii* consumption of alder in monocultures against *G. fossarum* consumption of beech in monocultures).

Results

Factors determining total leaf litter consumption rates differed between per-capita and biomass-adjusted daily consumption rates. For biomass-adjusted daily consumption rates, the initial Kruskal–Wallis test indicated a significant effect of treatment ($\chi^2 = 33.22$, d.f. = 7, $p < 0.001$); however, none of the comparisons between amphipod species consumption rates of the four leaf treatments were significant in the Dunn test (all $p > 0.10$). Therefore, we performed a new

Kruskal–Wallis test using only leaf litter treatment as an explanatory variable. This test showed a significant effect of leaf litter type ($\chi^2 = 27.57$, d.f. = 3, $p < 0.001$). The Dunn test with a Holm–Bonferroni correction showed that consumption of beech was lower than consumption of alder ($p_{\text{adjusted}} < 0.001$) or the three-species mix ($p_{\text{adjusted}} < 0.001$), and consumption of oak was also lower than consumption of alder ($p_{\text{adjusted}} < 0.02$, Fig. 2a).

The Kruskal–Wallis test for per-capita leaf consumption rate was also significant ($\chi^2 = 39.16$, d.f. = 7, $p < 0.001$); however, in this case the Dunn test indicated that there were significant differences between *G. fossarum* and *G. roeselii* consumption rates in the three-species leaf treatment ($p = 0.02$) and the alder treatment ($p = 0.04$), indicating a main effect of amphipod species and/or an interaction between amphipod species and leaf litter treatment. To determine which of these scenarios better fit the data, we examined all pairwise comparisons from the Dunn test after adjusting for multiple comparisons using a

Holm–Bonferroni adjustment. This indicated significant differences between six different pairs of treatment combinations (Fig. 2b). *Gammarus roeselii* per-capita consumption of alder was twice that of *G. fossarum*, its consumption of oak was 4.3 times the rate of *G. fossarum*, and of the three-species mixture 3.75 times that of *G. fossarum*, although none of these differences were significant in the Dunn test.

The best linear model of square-root-transformed percent area loss included the three-way interaction between leaf type, leaf diversity level (monoculture vs. mixture), and amphipod species ($\Delta \text{AIC} = 8.8$ to the next model, leaving out the three-way interaction). Several interactive effects were significant (Table 1, Fig. 3). First of all, only for alder and only with *G. roeselii* was the percent leaf loss significantly higher in amphipod enclosures than control enclosures, a result consistent with the low levels of daily leaf consumption of beech and oak in the total consumption analysis (Fig. 2). This was true both when alder was in monoculture (planned contrasts: $t = 5.041$, d.f. = 96,

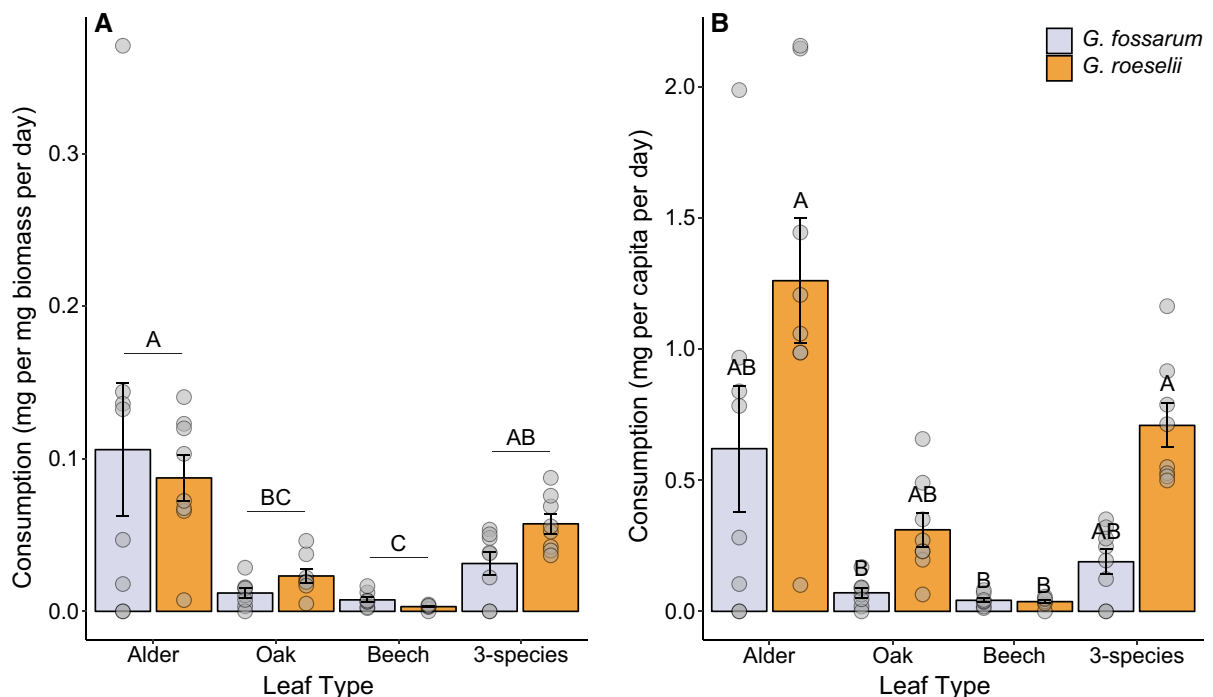


Fig. 2 Biomass-adjusted (a) and per-capita (b) daily leaf litter consumption rates by amphipods in the experiment, varying with leaf litter type and amphipod species ($n = 6$ replicates per treatment combination). Consumption rates are in terms of dry mass of leaf litter and are corrected by mass loss in control enclosures which did not contain amphipods. Error bars

represent standard error of the mean, and gray points represent raw data. Treatments with the same letter are not significantly different according to a Dunn test with a Holm–Bonferroni correction for multiple comparisons; in a, amphipod species was not important, so comparisons were only done between leaf litter treatments

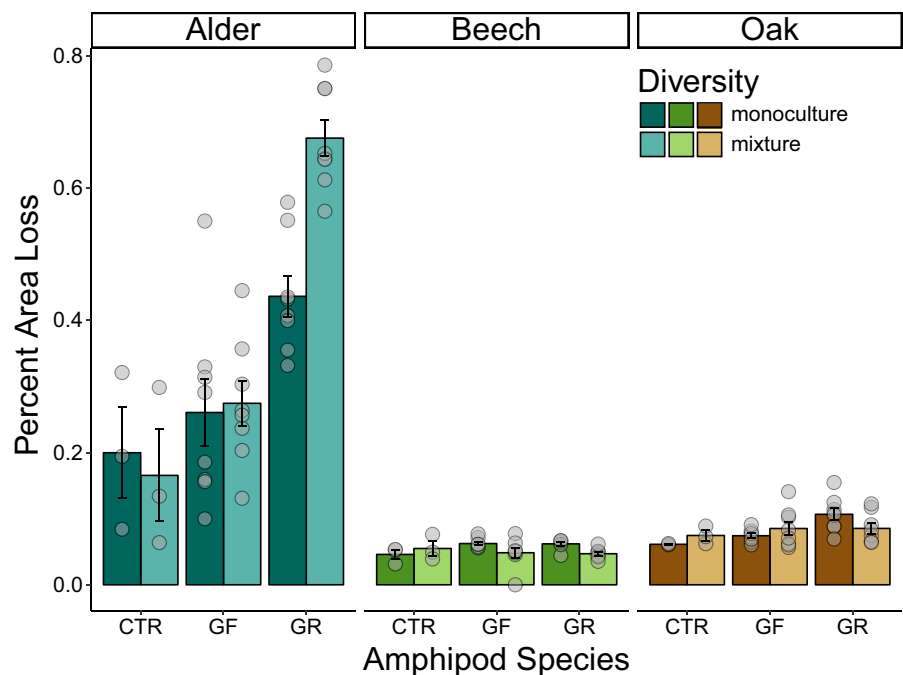
Table 1 Multiple comparisons based on planned contrasts between percent area loss of leaves in enclosures with different combinations of leaf types, amphipod species (GF = *Gammarus fossarum*, GR = *Gammarus roeselii*, and CTR = no-amphipod control), and diversity levels (mono = monoculture, mix = mixture). Asterisks denote comparisons where estimated differences in leaf consumption rates are significant at the $\alpha = 0.05$ level

Leaf–amphipod combination	Diversity comparison	Estimated difference	Std. error	<i>t</i> value	<i>p</i> value
A. Between diversity levels of same leaf type and amphipod species					
Alder–CTR	Mono–mix	0.044	0.054	0.817	1
Alder–GF	Mono–mix	– 0.022	0.033	– 0.675	1
Alder–GR	Mono–mix	– 0.163	0.033	– 4.937	< 0.01*
Beech–CTR	Mono–mix	– 0.019	0.054	– 0.355	1
Beech–GF	Mono–mix	0.044	0.033	1.340	0.9864
Beech–GR	Mono–mix	0.032	0.033	0.977	0.9996
Oak–CTR	Mono–mix	– 0.025	0.054	– 0.463	1
Oak–GF	Mono–mix	– 0.016	0.033	– 0.494	1
Oak–GR	Mono–mix	0.035	0.033	1.050	0.9991
Amphipod–diversity combination	Leaf comparison	Estimated difference	Std. error	<i>t</i> value	<i>p</i> value
B. Between leaf types with the same amphipod species and diversity level					
CTR–mix	Beech–alder	– 0.156	0.054	– 2.900	0.1426
CTR–mix	Oak–alder	– 0.116	0.054	– 2.151	0.6016
CTR–mix	Oak–beech	0.040	0.054	0.749	1
CTR–mono	Beech–alder	– 0.220	0.054	– 4.072	< 0.01*
CTR–mono	Oak–alder	– 0.185	0.054	– 3.431	0.0326
CTR–mono	Oak–beech	0.035	0.054	0.641	1
GF–mix	Beech–alder	– 0.312	0.033	– 9.440	< 0.01*
GF–mix	Oak–alder	– 0.229	0.033	– 6.921	< 0.01*
GF–mix	Oak–beech	0.083	0.033	2.519	0.3299
GF–mono	Beech–alder	– 0.245	0.033	– 7.425	< 0.01*
GF–mono	Oak–alder	– 0.223	0.033	– 6.740	< 0.01*
GF–mono	Oak–beech	0.023	0.033	0.685	1
GR–mix	Beech–alder	– 0.605	0.033	– 18.314	< 0.01*
GR–mix	Oak–alder	– 0.531	0.033	– 16.084	< 0.01*
GR–mix	Oak–beech	0.074	0.033	2.230	0.5389
GR–mono	Beech–alder	– 0.409	0.033	– 12.400	< 0.01*
GR–mono	Oak–alder	– 0.333	0.033	– 10.097	< 0.01*
GR–mono	Oak–beech	0.076	0.033	2.302	0.4837
Leaf–diversity combination	Amphipod comparison	Estimated difference	Std. error	<i>t</i> value	<i>p</i> value
C. Between amphipod species of same leaf type and same diversity level					
Alder–mix	GF–CTR	0.128	0.045	2.873	0.1509
Alder–mix	GR–CTR	0.432	0.045	9.673	< 0.01*
Alder–mix	GR–GF	0.304	0.033	9.207	< 0.01*
Alder–mono	GF–CTR	0.062	0.045	1.389	0.9804
Alder–mono	GR–CTR	0.225	0.045	5.041	< 0.01*
Alder–mono	GR–GF	0.163	0.033	4.945	< 0.01*
Beech–mix	GF–CTR	– 0.027	0.045	– 0.602	1
Beech–mix	GR–CTR	– 0.016	0.045	– 0.355	1
Beech–mix	GR–GF	0.011	0.033	0.334	1

Table 1 continued

Leaf–diversity combination	Amphipod comparison	Estimated difference	Std. error	<i>t</i> value	<i>p</i> value
Beech–mono	GF–CTR	0.036	0.045	0.816	1
Beech–mono	GR–CTR	0.036	0.045	0.795	1
Beech–mono	GR–GF	– 0.001	0.033	– 0.029	1
Oak–mix	GF–CTR	0.016	0.045	0.356	1
Oak–mix	GR–CTR	0.017	0.045	0.389	1
Oak–mix	GR–GF	0.001	0.033	0.045	1
Oak–mono	GF–CTR	0.025	0.045	0.549	1
Oak–mono	GR–CTR	0.077	0.045	1.722	0.8851
Oak–mono	GR–GF	0.052	0.033	1.589	0.937

Fig. 3 Percent area loss of alder, beech, and oak leaves in single-species (monoculture) or three-species (mixture) leaf enclosures, with no amphipods (CTR), *G. fossarum* (GF), or *G. roeselii* (GR) amphipods. Error bars represent standard error of the mean, and gray points represent raw data. Differences between treatment combinations are described in Table 1



$p < 0.01$) and when alder was in the three-species mixture (planned contrasts: $t = 9.673$, d.f. = 96, $p < 0.01$). Percent area loss of alder was not significantly different between control and *G. fossarum* enclosures, but was higher in *G. roeselii* than *G. fossarum* enclosures in both monocultures (planned contrasts: $t = 4.945$, d.f. = 96, $p < 0.01$) and when alder was in the three-species mixture (planned contrasts: $t = 9.207$, d.f. = 96, $p < 0.01$).

Consistent with the nonparametric analysis of biomass-adjusted total consumption rates, the percent

area loss was significantly higher in alder than in beech or oak, when diversity levels and amphipod species were otherwise equal (Table 1, Fig. 3). There was also a significant difference between percent leaf loss in control monocultures of beech and alder (planned contrasts: $t = -4.072$, d.f. = 96, $p < 0.01$).

Finally, the only leaf type and amphipod species combination where percent area loss was significantly different between monocultures and mixtures was *G. roeselii* in alder (planned contrasts: $t = -4.937$, d.f. = 96, $p < 0.01$), where on average 50% more leaf

area was lost in mixtures than in monocultures (Fig. 3). This suggests that *G. roeselii* potentially increase their consumption of a preferred resource when it is mixed in with less palatable food sources (Fig. 4).

Discussion

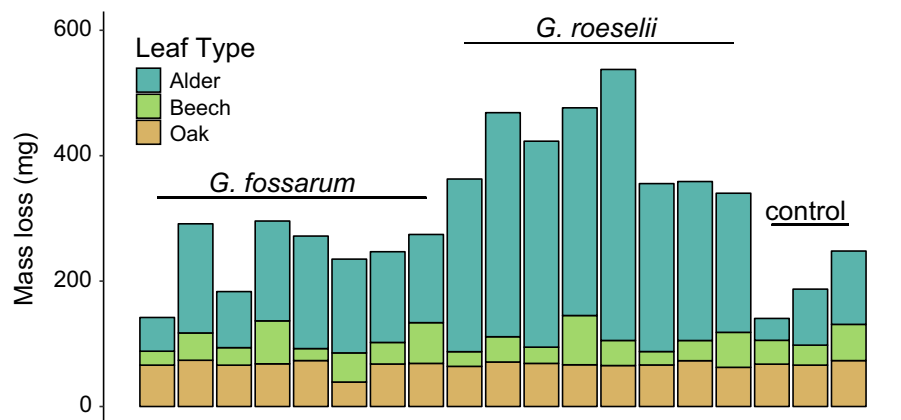
From decades of study, it is clear that freshwater decomposition depends on ecological context, that is, which other are present, including which types of leaf resources are available for consumption (Webster and Benfield 1986; Cardinale et al. 2011; Bruder et al. 2014). Field experiments represent an important strategy for gaining knowledge about such context-dependent processes, because they are intermediate between laboratory-based experiments and comparative field studies. We used an experimental approach to obtain well-controlled estimates of the contributions of individual detritivore species to decomposition, while simultaneously operating in natural, realistic stream conditions, including natural water chemistry, microbial and fungal communities, and chemical cues from species across multiple trophic levels that may influence decomposer activity. Our experiment showed that a native and a non-native species have similar biomass-specific effects on leaf litter processing, but because of differences in size between the two species, they have different effects when they are at similar densities. Furthermore, the non-native species showed a tendency to consume more of the higher-quality resource when it is in a mixture, while the native species consumed all species

in the mixture at roughly the same rate as in monocultures.

We found that after accounting for biomass, the daily leaf litter consumption rates for *G. fossarum* and *G. roeselii* were statistically indistinguishable. This aligns with another study where the biomass-adjusted consumption rates were similar (Piscart et al. 2011), but is contrary to evidence from a previous laboratory experiment, where *G. roeselii* had higher biomass-adjusted consumption rates of alder and oak compared to *G. fossarum* (Little and Altermatt 2018b). One possible explanation for the differing results in laboratory and stream experiments is that the more realistic biological context of the field experiments altered the organisms' behavior or physiological performance. The *G. roeselii* used in this experiment were about twice as large as *G. fossarum*, leading to different patterns in individual consumption rates than in biomass-adjusted rates. As a result, we did find a statistically significant interaction between amphipod species and leaf litter type in per-individual consumption rates, including a biologically meaningful difference in the species' consumption rates of alder. This is contrary to previous laboratory work which found that individual consumption rates were nearly identical between the two species (Jourdan et al. 2016).

The biomass equivalence of feeding rates of *G. fossarum* and *G. roeselii* is an interesting finding for at least two reasons. First, the equivalent biomass-adjusted consumption rates for the two species, despite their twofold difference in size, are surprising since metabolic rate and thus resource consumption scale nonlinearly with size (Brown et al. 2004), although a direct comparison between the two species which

Fig. 4 Mass loss of alder, beech, and oak leaves in the three-species mixed-leaf enclosures with *G. fossarum*, *G. roeselii*, and no amphipods present (control). Each bar represents one experimental replicate



likewise found a twofold difference in size revealed identical routine and resting metabolic rates at multiple temperatures (Becker et al. 2016). Yet previous experiments on amphipods have found that indeed, biomass-adjusted consumption rates of large amphipods (in this case *Gammarus pulex*) are lower than those of small amphipods (Reiss et al. 2011). Thus, given the body size discrepancy between our two study species, we would expect a greater difference in biomass-adjusted consumption rates and, correspondingly, a smaller difference in per-capita consumption rates. This may in fact provide some explanation for why another, much larger non-native species (*Dikerogammarus villosus*) has lower consumption rates than native species (Jourdan et al. 2016; Little and Altermatt 2018b).

Secondly, this suggests that if the two species have different typical densities in streams, this could lead to large differences in leaf litter processing, even though the species are functionally equivalent at a per-biomass level. To date, we have little information about what densities these two species are often found at. Existing data suggest that *G. fossarum* can reach slightly higher abundances where it is found in headwater streams compared to *G. roeselii*, although most survey data are based on kicknet or other effort-based metrics (Altermatt et al. 2016; Little and Altermatt 2018a) and are not standardized to areal units. In one such study, *G. fossarum* were present at 73 sites in a regional survey, with average abundance of 66 and a maximum abundance of 411 per unit sampling effort, while *G. roeselii* were present at only 43 sites, with an average abundance of 50 and a maximum abundance of per unit sampling effort (Altermatt et al. 2016). It is unclear how generalizable these data are, especially as most past studies mostly looked at the respective abundances of these species in reaches where they were present individually, and only in few cases was the occurrence of both species in the same headwater streams studied. Furthermore, other work shows that *G. roeselii* can reach higher abundances than *G. fossarum* in lake shoreline habitats (Rey et al. 2005). A more comprehensive understanding of the species' natural densities across habitats would be important for understanding the effect of species identity on leaf litter processing in stream catchments, especially as *G. roeselii* continues to expand its range (Paganelli et al. 2015) and *G. fossarum* expands into the UK (Blackman et al.

2017). In that context, it would also be interesting to know the likely effect of co-occurrence/competition between the two species. Laboratory experiments suggest that total leaf litter processing by *G. fossarum*–*G. roeselii* mixtures is roughly an average of the leaf consumption rates of these species in monocultures and that the species have no significant influence on each other's survival over short time periods (Little and Altermatt 2018b), but this would be important to verify in realistic field conditions.

We also found that both species consumed the higher-quality (nitrogen-rich, lower-lignin) alder leaves faster than the more common beech and oak leaves, which is consistent with many past results from amphipods (Foucreau et al. 2013; Little and Altermatt 2018b) and the macroinvertebrate community in general (Martínez et al. 2013; Bruder et al. 2014; Frainer et al. 2015). Here, we showed that this preference extended to leaf mixtures, but only for one of the two amphipod species. The lack of preferential feeding in mixtures by *G. fossarum* aligns with results from our own laboratory experiments (Little and Altermatt 2018b); however, overall leaf consumption of mixtures in the field was considerably higher than in the laboratory, relative to monocultures. We are not aware of any previous studies examining *G. roeselii* feeding patterns on diverse resources; thus, the finding that this species does engage in preferential feeding on the best-quality resource is novel and could have implications through the stream food web. Feeding on higher-nutrient resources could allow faster growth by *G. roeselii*, as has been shown in other detritivores (Halvorson et al. 2018), and higher nitrogen requirements are one possible explanation for their behavior. Depending on how well the leaf litter is processed—whether this nitrogen is assimilated efficiently and in what form it is excreted—the species could also have different influences on nitrogen processing and cycling in stream ecosystems, in line with the previous work on invertebrates showing that feeding rates and selective feeding can alter nutrient turnover rates (Hood et al. 2014; Liess 2014).

In our experiment, we only used one native and one non-native species, and thus, we are hesitant to generalize our conclusions to other invasion scenarios, particularly because the non-native species (*G. roeselii*) has been naturalized to the study region for over a century and is not a recent arrival. However, our results illustrate that even species which fill similar

functional roles may have important differences in how they perform these roles, and with ongoing biodiversity change, this could have consequences for ecosystem function. Thus, it will be important to measure these potential effects even for non-native species which are not considered invasive, as we try to comprehensively understand how frequently non-native species impact ecosystem function (Strayer 2012).

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Data availability Data are available from the Dryad Digital Repository at <http://dx.doi.org/10.5061/dryad.v22901d> (Little and Altermatt 2019). The R code used for analyses and to make figures is available at <http://github.com/chelseajlittle/CAGE2>.

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