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How are resource consumption and growth rates of litter-consuming detritivores affected by imbalances between consumer and litter C:N:P ratios? To address this question, we offered leaf litter as food to three aquatic detritivore species, which represent a gradient of increasing body N:P ratios: a crustacean, a caddisfly and a stonefly. The detritivores were placed in microcosms and submerged in a natural stream. Four contrasting leaf species were offered, both singly and in two-species mixtures, to obtain different levels of stoichiometric imbalance between the resources and their consumers. The results suggest that detritivore growth was constrained by N rather than C or P, even though 1) the N:P ratios of the consumers' body tissue was relatively low and 2) microbial leaf conditioning during the experiment reduced the N:P imbalance between detritivores and leaf litter. This surprisingly consistent N limitation may be a consequence of cumulative N-demand arising from the production of N-rich chitin in the exoskeletons of all three consumer species, which is lost during regular moults, in addition to N-demand for silk production by the caddisfly. These N requirements are not commonly quantified in stoichiometric analyses of arthropod consumers. There was no evidence for compensatory feeding, but when offered mixed-species litter varying in C:N:P ratios, detritivores consumed more of the litter species showing the highest N:P and lowest C:N ratio, accelerating the mass loss of the preferred leaf species in the litter mixture. These results show that imbalances in consumer–resource stoichiometry can have contrasting effects on coupled processes, highlighting a challenge in developing a mechanistic understanding of the role of stoichiometry in regulating ecosystem processes such as leaf litter decomposition.

Imbalances in elemental composition between consumers and their resources can affect key processes in ecosystems, including consumer growth and consumer-mediated nutrient cycling (Sterner and Elser 2002). Ecological stoichiometry predicts that consumer growth will stay below maximum rates on diets that are nutritionally imbalanced relative to a consumer's requirements (Boersma and Elser 2006, Fink and von Elert 2006). In addition, imbalances can trigger adjustments in feeding behaviour, with effects on resource use and transformation (Frost et al. 2002). Therefore, understanding the stoichiometric relationships between consumers and their resources, and how these relationships affect resource consumption and consumer growth, is essential for understanding both current and future variation in ecosystem functioning (Elser and Urabe 1999), particularly as ecosystems worldwide experience large anthropogenic alterations in nutrient availability (Vitousek et al. 1997, Smith 2006, Woodward et al. 2012).

Nitrogen (N) and phosphorus (P) stand out among the nutrients potentially limiting consumer-mediated ecological processes related to resource consumption, due to the strong N and P enrichment in consumer body tissues relative to low background concentrations in the environment (Sterner and Elser 2002). Consumers typically require N, P and carbon (C) within a narrow range of proportions (N:P, C:N and C:P ratios), which are rarely matched in the diets of detritivores (Cross et al. 2003, Frost et al. 2006, Hladyz et al. 2009) feeding on plant litter and other organic matter strongly deficient in N and P (Hladyz et al. 2009). Furthermore, N and P concentrations of plant litter vary greatly among and within plant species (Leroy et al. 2007, Frainer et al. 2015a), whereas detritivores tend to be stoichiometrically homeostatic, meaning that the elemental composition of their bodies is relatively constant compared to that of their food (Cross et al. 2003). Across a broad phylogenetic range, N and P concentrations of invertebrate

detritivores average 10 and 1% of body dry mass, respectively, with detritivore crustaceans characterised by the lowest concentration of N (Martinson et al. 2008) and highest concentration of P (Hladyz et al. 2009). In contrast, nutrient concentrations of leaf litter typically range from 0.5 to 3% N and from 0.01 to 0.2% P of litter dry mass (Enriquez et al. 1993, Hättenschwiler et al. 2008).

Consumers are expected to show species-specific responses to nutrient imbalances with their resources, given variation in body nutrient concentrations among species (Liess and Hillebrand 2005). Consumers characterised by high N:P ratios may show a positive growth response when feeding on a high N:P diet, and the opposite response would be expected for consumers characterised by low N:P ratios (Boersma and Elser 2006, Fink and von Elert 2006). There are two strategies to cope with these imbalances. First, consumers might selectively feed on litter with N:P ratios closest to their requirements (Hood et al. 2014). This behaviour could drive variation in the decomposition of contrasting leaf species (high versus low N:P ratios) within mixed-species litter (Hladyz et al. 2009). As a consequence, synergistic or antagonistic effects of litter mixing may arise on decomposition, if consumption of the preferred resource is enhanced over the less favoured resource when both are mixed together (Vos et al. 2011, but see Frainer et al. 2015a).

The second strategy involves increased feeding rates to ensure an adequate intake of the element in shortest supply (Lincoln et al. 1986, Fink and von Elert 2006, Liess 2014). This strategy may compensate for low assimilation of the limiting nutrient (Lincoln et al. 1986, Liess 2014), but may also impose physiological costs associated with the need to increase egestion or excretion of the excess elements (Balseiro and Albariño 2006). Whereas compensatory feeding enhances consumption of the poor-quality resource, consumer growth may not benefit to the same extent, depending on the degree of negative physiological effects (Tuchman et al. 2002). Consequences of compensatory feeding at the ecosystem level include enhanced nutrient cycling by increased mineralisation and excretion of the non-limiting nutrients (Balseiro and Albariño 2006, Liess 2014).

Litter-colonising microbes also respond to stoichiometric imbalances (Güsewell and Gessner 2009, Fanin et al. 2013) and, in turn, can alter litter stoichiometry by nutrient mineralisation and immobilisation through selective mining of leaf tissue for nutrients (Hättenschwiler et al. 2005, Lummer et al. 2012, Handa et al. 2014), or nutrient capture from the surrounding environment (Cheever et al. 2013, Danger et al. 2015). Detritivores preferentially feed, and generally grow better, on litter that has been colonised by microbes (i.e. conditioned sensu Cummins 1974), due to greater accumulation of microbial biomass with high nutrient concentrations, enzymatic softening of leaf tissue, and other changes in leaf chemistry and texture (Bärlocher and Sridhar 2014). Thus, an understanding of how litter nutrient concentration affects consumer-mediated decomposition and growth also needs to consider the influences of microbially mediated nutrient dynamics.

We conducted a field experiment to determine how the growth of three stream invertebrate detritivores and the consumption of four species of leaf litter are affected by the level of imbalance between consumer and resource C:N:P ratios. As detritivores act as decomposers of organic matter, we refer to their consumption of leaf litter as consumer-mediated decomposition. We hypothesised that increasing stoichiometric imbalances between consumers and their resource 1) constrains consumer growth due to nutrient limitation but 2) enhances leaf decomposition due to compensatory feeding. Additionally, we hypothesised that 3) consumers feed preferentially on the leaf species best matching their nutrient requirements when simultaneously offered litter differing in nutrient stoichiometry; this would enhance decomposition of the preferred leaf species while slowing decomposition of the non-preferred species. Finally, we hypothesised that 4) consumers take advantage of complementary resources found in mixed-species diets, if their requirements are better matched by mixed than single leaf species, supporting increased growth relative to that achieved on either of the single-species diets.

Methods

Site description

The study was conducted between December 2009 and February 2010 in a small (~2 m wide) upland stream, Rémillassé, located in the French Pyrenees (42°56′35″N, 1°5′26″E). The stream flows through mostly native forest and is characterised by substratum dominated by cobbles and boulders. Nitrate concentration of the stream water was 0.58 mg N l^{-1} (Jabiol et al. 2013) and concentration of dissolved inorganic nitrogen (DIN) was 0.67 mg l^{-1}. Concentration of inorganic phosphate (PO_4^{3-}) was < 10 µg P l^{-1}. All nutrient analyses were made by ion chromatography (Dionex 1500i with a AS 4 A SC column). Based on previous analyses of various streams in the region, concentrations of inorganic N and P varied within a narrow range over the course of the study (Jabiol and Chauvet unpubl.). Stream water was circumneutral (pH ~7) and well oxygenated (dissolved oxygen saturation $> 90\%$), with conductivity of ~60 µS cm^{-1}. Water temperature was logged every 2 h during the study period and ranged from 0.04 to 8.40°C (mean = 3.44°C).

Experimental design and procedures

A total of 288 flow-through microcosms constructed from PVC pipes (27 cm long and 7 cm diameter) were deployed in the stream. Each contained a standard pre-weighed amount of leaf litter comprising varying leaf-species combinations and litter-consuming invertebrates. Both ends of the microcosms were closed with 330-µm nylon mesh to retain the litter and detritivores while allowing for water and microbes to circulate freely into and out of the microcosms. The microcosms were anchored to the streambed with iron bars and arranged parallel with the water flow.

Litter of four leaf species was collected shortly after abscission (Table 1): black alder *Alnus glutinosa*, English walnut *Juglans regia*, silver birch *Betula pendula* and pedunculate oak *Quercus robur*. The four species were allocated to one of two pairs according to their recalcitrance, assessed based on lignin concentrations. Pair 1 was composed of walnut and oak, which averaged 25% higher lignin concentration than pair 2 (alder and birch), and decomposes more slowly (Frainer et al. 2015a). Within each pair, leaves were chosen

Table 1. Chemical composition of the four leaf species analysed after 24 h of leaching (n = 3) and at the end of the field experiment (n = 48), of the three shredder species sampled in the same streams and at the same time of the year, but not used in the field experiment (n = 10–12) (mean ± SD). N:P, C:N and C:P are atomic ratios. DM stands for dry mass.

Species	Time	Mixture	N (mg g^{-1} DM)	P (µg g^{-1} DM)	N:P	C:N	C:P	Lignin (mg g^{-1} DM)
Alder	Initial		18.6 ± 2.6	135 ± 23	306	28	8769	226 ± 4
Birch			4.9 ± 0.5	287 ± 16	38	107	4054	254 ± 16
Walnut			10.3 ± 0.1	173 ± 28	134	51	6826	286 ± 9
Oak			9.2 ± 1.4	615 ± 133	33	58	1953	313 ± 14
Alder	Final	single	27.3 ± 1.3	707 ± 51	86	19	1641	
		mixed	32.0 ± 1.4	571 ± 23	124	16	2034	
Birch		single	8.4 ± 0.5	501 ± 21	37	62	2317	
		mixed	10.0 ± 0.2	515 ± 15	43	52	2253	
Walnut		single	15.9 ± 0.5	779 ± 30	45	33	1490	
		mixed	15.2 ± 0.6	678 ± 28	49	35	1711	
Oak		single	11.5 ± 0.4	715 ± 22	36	46	1623	
		mixed	11.5 ± 0.4	649 ± 23	39	46	1787	
Gammarus			68.0 ± 7.5	8170 ± 998	19	7	123	
Nemoura			108.5 ± 19.7	10158 ± 1263	24	6	134	
Sericostoma			85.5 ± 4.7	8760 ± 1728	22	7	166	

to maximise contrast in N:P ratios: the ratios were high in alder and walnut and low in birch and oak (Table 1). N and P were analysed (Ebina et al. 1983) initially after 24 h leaching in the laboratory and in all leaves retrieved from the field at the end of the experiment. Initial lignin concentrations of leached litter were determined with the acid-detergent fibre method developed by Van Soest (Gessner 2005). C concentrations were assumed to be 45% of litter dry mass (Singh and Mudgal 2000, Hättenschwiler et al. 2008).

Three litter-consuming detritivores were used in the experiment: the caddisfly *Sericostoma personatum* (Trichoptera, Sericostomatidae), stonefly *Nemoura flexuosa-marginata* group (Plecoptera, Nemouridae), and crustacean *Gammarus fossarum* (Amphipoda, Gammaridae). All invertebrates were collected in streams near the study site, and similar-sized individuals of each taxon were chosen for the experiment. An additional set of invertebrates was starved for 24 h to empty their guts and then frozen, freeze dried, and ground to determine C, N and P concentrations. Detritivore C and N concentrations were determined using a CHN elemental analyser and P concentration was determined spectrophotometrically at 880 nm after acid digestion (AFNOR 2004). Single specimens of *Gammarus* and *Sericostoma* constituted a replicate, whereas two individuals of *Nemoura* were pooled for a single sample (n = 10–12).

Each of the two litter pairs comprised three separate litter mixture treatments (two single-species and one mixed-species treatment) crossed with four detritivore treatments: three different single-species treatments plus a control without detritivores where only microbial colonisation occurred (Fig. 1). Leaves and detritivores were placed in microcosms and distributed in the stream in a randomised-block design; each treatment was replicated once in each of 12 distinct riffles located within a single stream reach 200 m in length. Replicates for the two litter species pairs were deployed at the same time and retrieved when the faster-decomposing species in each pair had lost about 50% of its initial dry mass. This resulted in exposure times of 37 and 63 days for the fast- and slow-decomposing litter species pair, respectively, and precluded potential confounding effects related to decomposition stage on detritivore consumption and growth. Each microcosm contained 4.00 ± 0.05 g of litter dry mass, with mixed-species treatments comprising 2.00 ± 0.05 g of each species. Upon retrieval from the field, the microcosms were returned to the laboratory and the invertebrates separated from the litter, which was then rinsed with chlorine-free water. The litter was sorted by species, oven-dried for 48 h at 60°C, and weighed to the nearest 0.01 g.

Body dry mass of the detritivores estimated on a subset of individuals prior to the experiment yielded an average (± SD) of 0.70 ± 0.18 for *Nemoura*, 3.35 ± 0.68 for *Gammarus*, and 5.20 ± 1.72 mg for *Sericostoma*. To compensate for these differences in body mass, the three taxa were placed in the microcosms at different densities: 30 *Nemoura*, 6 *Gammarus*, and 4 *Sericostoma*, resulting in the same average biomass across species treatments of ~20 mg dry mass. Four additional empty *Sericostoma* cases were placed inside each *Sericostoma* microcosm to provide building material for potential case expansion during the experiment.

Before placing the invertebrates inside the microcosms, we estimated the biomass of all individuals based on measures of body length for *Nemoura* and *Gammarus*, and anterior

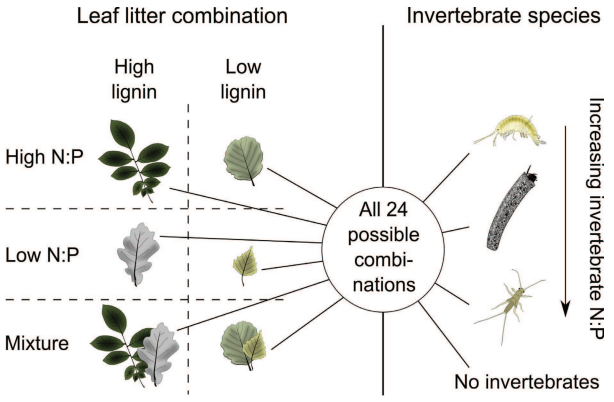

Figure 1. Experimental design in which two litter pairs, each comprising three separate litter mixture treatments (two single-species and one mixed-species treatment), are crossed with four detritivore treatments (a control without consumers plus three different single-species treatments).

case opening diameter for *Sericostoma.* Care was taken not to injure the animals during manipulations. Measurements were made on high-quality photographs using the digital image analysis software ImageJ (Abramoff et al. 2004). The conversion of body length or case-opening width to body dry mass was based on power functions derived from a sub-set of 24 (*Gammarus* and *Sericosotma*) or 60 (*Nemoura*) individuals (Supplementary material Appendix 1). Upon retrieval from the microcosms, invertebrates were oven-dried for 24 h at 60°C and weighed to the nearest 0.01 mg.

Elemental imbalance

We calculated the degree of elemental imbalance (EI) between consumers and leaf litter to assess which of the two elements (N or P) was more limiting given consumer requirements. We calculated EIs for each detritivore species, based on the quotient between consumer and resource N:P, C:N, or C:P atomic ratios, as follows:

$$EI_{X:Y}{}^{ij} = ln\left(\frac{X:Y^{i}}{X:Y^{j}}\right) \quad (1)$$

where X:Y is the atomic ratio (N:P, C:N, or C:P) of litter *i* and consumer *j*. EIs were calculated separately for the initial (i.e. prior to any microbial influence) and final nutrient concentrations of the litter. Since analyses based on final and initial litter nutrient concentrations yielded very similar results and conclusions, we present in the main text only analyses of the final concentrations.

Fungal biomass

Fungal biomass was quantified as ergosterol (Gessner and Newell 2002) in half of the replicates from each treatment (n = 6). Five leaf-discs of 11 mm diameter were freeze-dried, weighed to the nearest 0.01 mg, and extracted with KOH/methanol (8 g l^{-1} KOH) for 30 min at 80°C. The crude lipid extracts were purified by solid-phase extraction (Gessner and Schmitt 1996), and ergosterol isolated and quantified by high-performance liquid chromatography using methanol as mobile phase (1.5 ml min^{-1}, 33°C) and UV detection at 282 nm.

Data analysis

Decomposition rates (k) resulting from detritivore-mediated decomposition of litter were calculated following the model:

$$-k = \frac{\ln(M_t) - \ln(M_0)}{t} \quad (2)$$

where final litter mass (M_t) is the mass remaining in microcosms with detritivores plus the fraction of litter dry mass lost from the corresponding control microcosms (from the same riffle) to which no invertebrates had been added. M_0 corresponds to the initial litter mass and t to the time elapsed until litter retrieval from the field. Litter species from mixture treatments were separated for analyses and calculations. We also analysed detritivore-mediated decomposition rates based on alternative linear decay models [i.e. $k = (M_t - M_0)/t$], but this had little effect on the outcome of our analyses, and did not affect conclusions.

We assumed exponential detritivore growth when calculating growth rate (g) for each microcosm as:

$$g = \frac{\ln(B_t) - \ln(B_0)}{t} \quad (3)$$

where B_0 = initial body dry mass, B_t = final body dry mass, and t = elapsed time in days. Mass refers to the average detritivore dry mass per microcosm.

We constructed ANOVA mixed effect models (MEM) using the R (<www.r-project.org>) function *nlme* (Pinheiro et al. 2012) to test whether detritivore growth or detritivore-mediated decomposition were affected by elemental imbalances ($EI_{C:N}$, $EI_{C:P}$ or $EI_{N:P}$). Separate models were constructed for each detritivore species because of potentially contrasting responses to resource N:P ratios. Stream riffle (block) was included as a random effect in all models. The model testing for EI effects on detritivore-mediated decomposition rates included all 2- and 3-way interactions between EI, litter pair, and mixture type (i.e. single-species or mixed-species treatments). The model testing for EI effects on detritivore growth rate included litter pair as a fixed factor and its interaction with EI.

Calculations of EI effects on growth only used information from single-species litter treatments, because the individual contribution to consumer growth of each litter species in mixture could not be determined. To test for litter mixture effects on growth we applied separate MEMs for each treatment involving detritivore species. We included the factors litter pair and litter mixture (but not EI) as well as their interaction.

We analysed fungal biomass across all detritivore treatments, litter mixtures, and litter pairs. We also examined whether differences in litter stoichiometry (N:P, C:N and C:P) at the end of the experiment varied according to the four detritivore treatments (three treatments including invertebrates plus the control containing microbes only), litter pair, and litter mixture, and any interactions of these factors. Finally, we tested how $EI_{N:P}$ varied at the end of the experiment according to litter mixture, litter pair, detritivore species treatment, and the interactions of these factors. These analyses were based on the same MEM structure as described above. In all mixed-effect model analyses, non-significant terms were removed, and the most parsimonious model was selected based on the Akaike information criterion (AIC) (Supplementary material Appendix 2 Table A1–A2). Varying denominator degrees of freedom across our analyses reflect differences in the number of variables included: 30–33 and 70–74 for separate analyses of growth and detritivore-mediated decomposition, respectively, depending on the number of missing observations; 175 for ergosterol analyses; 251 for changes in EI between detritivore species and leaf litter, as the control chambers were not included in the analysis; and 354 for analyses of changes in litter nutrient concentration and decomposition rate, where an extra data point was available from each litter species mixture treatment.

Data deposition

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.dm94c> (Frainer et al. 2015b).

Results

Consumer elemental composition

Atomic C:N ratios of the three detritivores used in the experiment were lowest for *Nemoura*, intermediate for *Gammarus* and highest for *Sericostoma* (Table 1) ($F_{2,32} = 37.8$, $p < 0.001$). C:P ratios also differed among species ($F_{2,32} = 7.9$, $p = 0.002$), with lower ratios for *Gammarus* and *Nemoura* than for *Sericostoma* (Table 1). In contrast, N:P was lowest for *Gammarus* and higher for *Sericostoma* and *Nemoura* (Table 1) ($F_{2,32} = 5.5$, $p = 0.009$). *Nemoura* was characterised by the highest concentrations of both N and P, and *Gammarus* had the lowest N concentration, whereas P concentrations did not significantly differ between *Gammarus* and *Sericostoma* (Table 1).

Litter elemental composition and imbalance

Litter nutrient concentrations increased between the start and end of the experiment (Table 1). As a result, all litter species had lower C:N and C:P ratios at the end of the experiment than in the beginning. Increasing P concentrations in most leaf species over time were accompanied by decreases in the N:P ratios of alder and walnut, but not of oak and birch litter.

Litter mixing modified final N:P and C:N in some of the leaf species (litter pair × mixture interaction: $F > 6.7$, $p < 0.01$, for N:P and C:N), irrespective of the detritivore treatment (Fig. 2 for N:P; Table 1 for N:P and C:N). Across all detritivore treatments, including the control microcosms, alder and birch litter showed higher final N:P and lower C:N ratios when mixed than in monospecific litter packs (Fig. 2 for N:P; Table 1 for N:P and C:N), mostly due to increased N concentrations when the leaf species were mixed. Walnut and oak also had higher final N:P ratios in the litter mixture, due to small changes occurring in opposite directions in the N and P concentrations of the two species, but this mixture effect was relatively small (Table 1). The final C:P ratio of leaf litter was higher when the litter species were mixed ($F_{1,354} = 26.5$, $p < 0.001$) and on the more labile litter species pair ($F_{1,354} = 169.2$, $p < 0.001$) and it also differed across the four detritivore treatments (microbes alone, *Gammarus*, *Nemoura* and *Sericostoma*) ($F_{3,354} = 4.5$, $p = 0.004$). Leaf litter exposed to *Nemoura* had a 10% higher final C:P ratio than the litter exposed to *Gammarus* (C:P = 1972 ± 431 and 1802 ± 334, respectively; post hoc Tukey's test: $p < 0.05$), but these C:P ratios did not differ from those in treatments containing *Sericostoma* or microbes alone (all post hoc Tukey's tests: $p > 0.1$).

The observed changes in N and P concentrations also affected the EIs between the detritivores and litter, which were narrowed towards the end of the experiment (Supplementary material Appendix 3 Table A3 for EIs based on both initial and final litter nutrient concentrations). Based on final litter nutrient concentrations, the elemental imbalance of N:P ($EI_{N:P}$) was highest for alder, regardless of the detritivore species, and lowest for oak, particularly for *Sericostoma* and *Nemoura* (detritivore species × litter species interaction: $F_{6,251} = 4.89$, $p < 0.001$). $EI_{N:P}$ was also highest for alder when mixed with birch, especially for *Gammarus* (detritivore species × litter mixture interaction: $F_{3,251} = 62.4$, $p < 0.001$), whereas $EI_{N:P}$ did not vary between mixture treatments for the walnut-oak species pair.

Fungal biomass

Fungal biomass differed between litter species pairs ($F_{1,175} = 35.6$; $p < 0.001$). It was higher for the low-lignin species, alder (488 ± 155 µg ergosterol g^{-1} litter dry mass) and birch (717 ± 275 µg g^{-1}), than for the high-lignin pair, walnut (370 ± 177 µg g^{-1}) and oak (474 ± 107 µg g^{-1}). There were no differences in fungal biomass among the detritivore treatments ($F_{3,175} = 1.35$; $p = 0.26$), and no

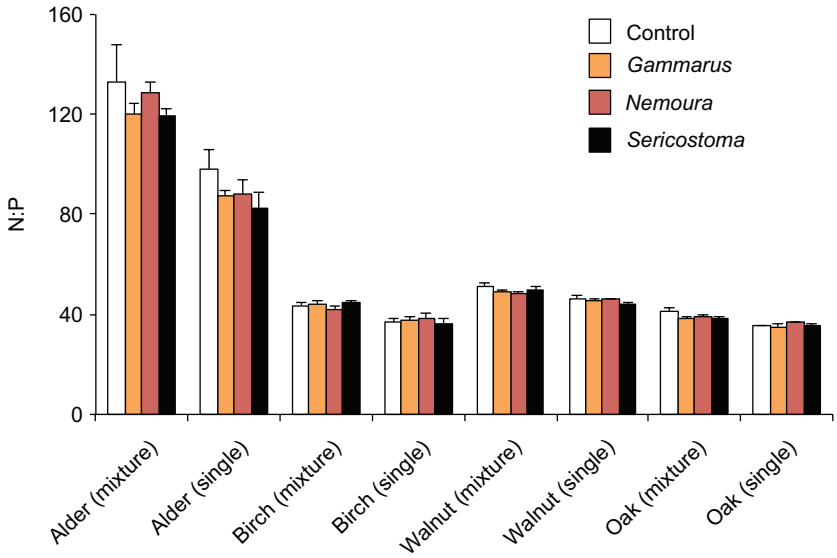

Figure 2. Nitrogen and phosphorus atomic ratios (N:P) of four leaf species in single and mixed leaf-species treatments, and across four detritivore treatments, including a control treatment where only microbes were allowed to colonise the leaf litter. Nutrient concentrations were measured at the end of the experiment.

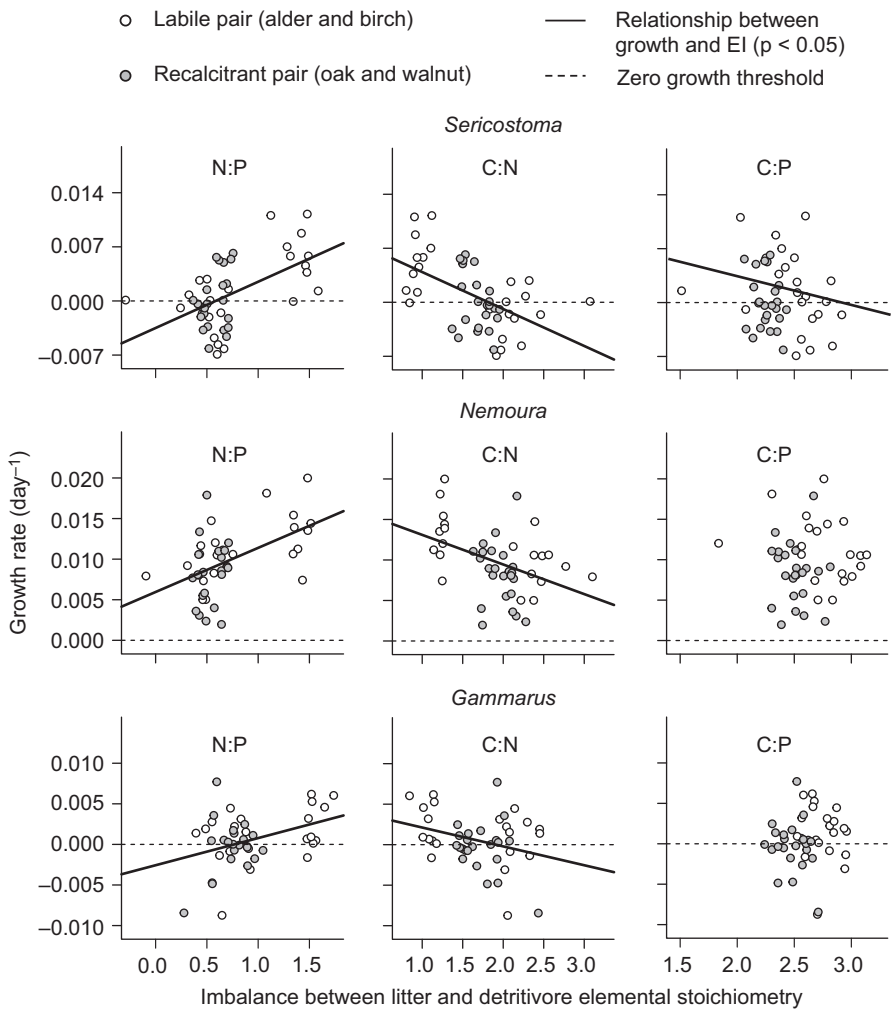

Figure 3. Growth rate of the three detritivore species used in the experiment (top row: *S. personatum*; middle row: *Nemoura* spp.; bottom row: *G. fossarum*) and the elemental imbalance (EI) between litter N:P and detritivore N:P (left column), litter C:N and detritivore C:N (middle column), and litter C:P and detritivore C:P (right column). EI is based on the final nutrient concentrations of the leaf litter. $EI_{X:Y} > 1$ indicate a higher elemental imbalance of nutrient X, $EI_{X:Y} < 1$ indicate higher elemental imbalance of nutrient Y.

evidence for differences between single- and mixed-species litter treatments ($F_{1,175} = 0.6$, $p = 0.45$) (Supplementary material Appendix 4 Fig. A1).

Elemental imbalance and detritivore growth

Growth rates of all three detritivores were positively related to $EI_{N:P}$ (all $F > 2.75$, $p < 0.01$; Fig. 3) calculated based on final litter nutrient concentrations (Supplementary material Appendix 5 Table A4), indicating stronger requirements for N than for P. *Sericostoma* responded most strongly (slope ± SE: 0.0062 ± 0.0015), followed by *Nemoura* (0.0046 ± 0.0015), and *Gammarus* showed the weakest response (0.0028 ± 0.0013). The relationship between growth and $EI_{C:N}$ was negative for all three detritivore species (all $F > 4.33$, $p < 0.045$), indicating higher demands for N than C (slope ± SE: -0.0021 ± 0.0010, -0.0034 ± 0.0011 and -0.0047 ± 0.0011 for *Gammarus*, *Nemoura* and *Sericostoma*, respectively; Fig. 3). Finally, *Sericostoma* growth rates also showed a weak negative response to $EI_{C:P}$ ($F_{1,33} = 4.39$, $p = 0.044$, slope $= -0.0058 \pm 0.0027$; Fig. 3), possibly indicating higher demands for P than C, whereas neither *Nemoura* nor *Gammarus* showed a growth response to $EI_{C:P}$ (both $F < 0.24$, $p > 0.63$; Fig. 3).

Litter mixing and detritivore growth

Sericostoma showed higher growth rates when feeding on the mixed leaf litter ($F_{1,78} = 7.18$, $p = 0.009$) (Fig. 4). Growth of *Nemoura* and *Gammarus* did not significantly differ between mixed and single-species treatments ($F_{1,79} = 0.064$, $p = 0.80$ and $F_{1,80} = 1.15$, $p = 0.29$, respectively), although tendencies for litter-mixing effects similar to those for *Sericostoma* were observed (Fig. 4).

Elemental imbalance and detritivore-mediated decomposition

Effects of EIs (based on either N:P, C:N or C:P) on detritivore-mediated litter decomposition (Supplementary material Appendix 5 Table A5, Appendix 6 Fig. A2–A4) generally varied with litter species composition, with only one significant main effect. The exception was a weak negative relationship between *Gammarus*-mediated decomposition rate and $EI_{C:P}$

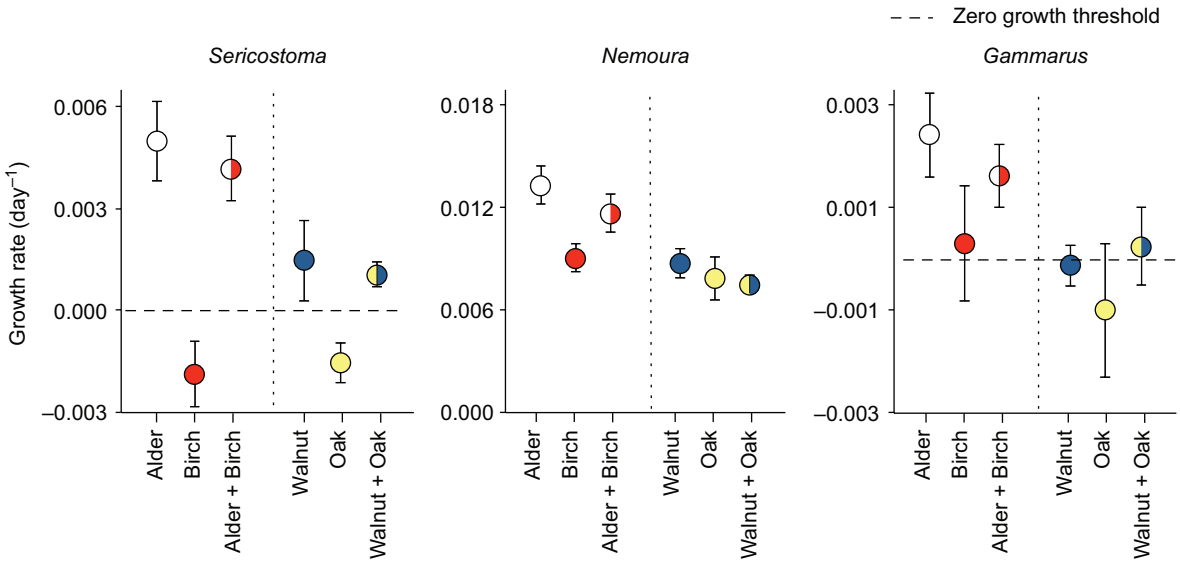

Figure 4. Growth rate of detritivores feeding on single-species and mixed-species diets of fast- and slow-decomposing leaf litter (mean ± SE). Alder and walnut are characterised by high N:P, whereas birch and oak are characterised by low N:P.

($F_{1,74} = 4.16$, $p = 0.045$, slope = –0.0029 ± 0.0012), indicating faster decomposition of litter that contained relatively more P than C (i.e. lower C:P) (Supplementary material Appendix 6 Fig. A4). *Gammarus*-mediated decomposition of the litter mixture composed of the more recalcitrant leaf species, walnut and oak, was faster than that of the mixture containing less recalcitrant species alder and birch (all litter pair × litter mixture interactions for *Gammarus*: $F > 6.22$, $p < 0.015$; Fig. 5).

Nemoura-mediated decomposition was positively affected by $EI_{N:P}$ more strongly when feeding on mixed-species litter (litter pair × litter mixture × $EI_{N:P}$: $F_{1,69} = 4.06$, $p = 0.048$; Supplementary material Appendix 6 Fig. A2). Litter species pair interacted with $EI_{C:N}$ ($F_{1,70} = 10.3$, $p = 0.002$), with a stronger negative relationship between *Nemoura*-mediated decomposition and $EI_{C:N}$ in the less refractory leaf species pair (Supplementary material Appendix 6 Fig. A3). Finally, *Nemoura*-mediated decomposition showed no relationship with $EI_{C:P}$ ($F_{1,72} = 2.40$, $p = 0.13$; Supplementary material Appendix 6 Fig. A4).

Sericostoma-mediated decomposition rate showed a stronger positive relationship with N:P (high $EI_{N:P}$) when larvae were fed on mixed-species litter than on single leaf species (litter mixture × $EI_{N:P}$ interaction: $F_{1,70} = 6.51$, $p = 0.013$; Fig. 5; Supplementary material Appendix 6 Fig. A2). Moreover, *Sericostoma*-mediated decomposition of the refractory species pair, walnut and oak, showed a stronger positive relationship with N:P than those of the less refractory pair, alder and birch (litter pair × $EI_{N:P}$ interaction: $F_{1,70} = 4.70$, $p = 0.049$; Supplementary material

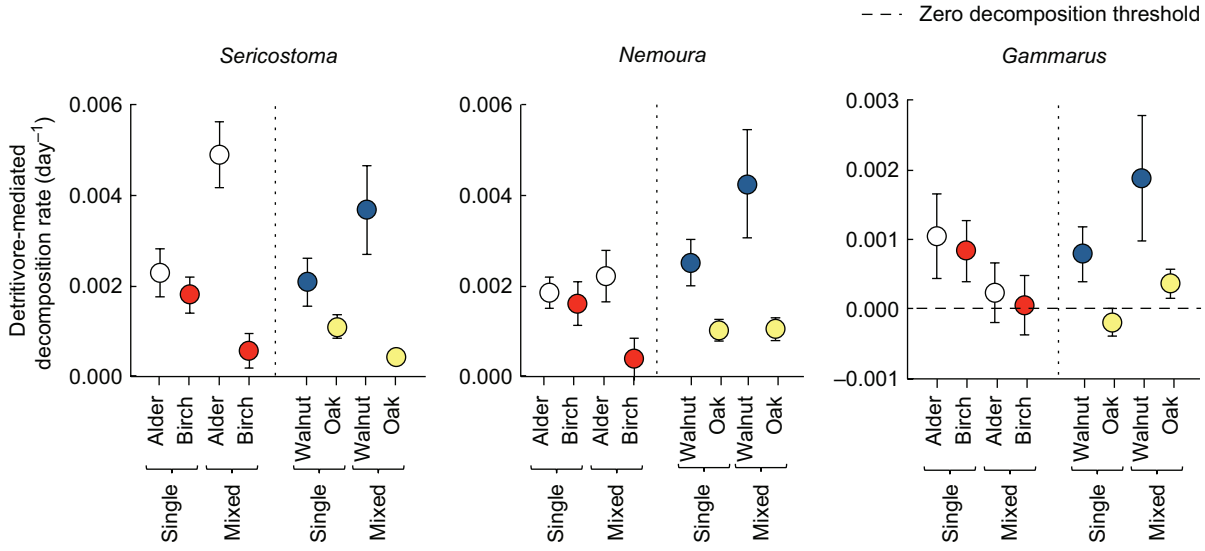

Figure 5. Detritivore-mediated decomposition rates (k) of single-species and mixed-species leaf diets composed of fast- and slow-decomposing litter species (mean ± SE). Alder and walnut are characterised by high N:P, whereas birch and oak are characterised by low N:P.

Appendix 6 Fig. A2). *Sericostoma*-mediated decomposition rates also showed a stronger negative relationship with $EI_{C:N}$ in mixed-species than in single-species litter (litter mixture $\times$ $EI_{C:N}$ interaction: $F_{1,71} = 13.0$, $p < 0.001$; Supplementary material Appendix 6 Fig. A3). Finally, *Sericostoma*-mediated decomposition rate was positively related to C:P in the mixture of the less refractory leaf-species pair, but not in the refractory pair, as reflected by a three-way interaction between litter mixture, litter pair, and $EI_{C:P}$ ($F_{1,68} = 9.68$, $p = 0.003$; Supplementary material Appendix 6 Fig. A4).

Discussion

As hypothesised, the degree of stoichiometric imbalance between consumers and resources had contrasting effects on the two processes analysed in the present study (i.e. consumer growth and consumer-mediated litter decomposition). However, the effects were more consistent for detritivore growth than decomposition. Thus, whereas detritivore growth was constrained overall by the degree of stoichiometric consumer–resource imbalance, changes in detritivore-mediated decomposition depended more strongly on litter mixture effects. This indicates that detritivores responded to variability in resource nutrient ratios through preferential rather than compensatory feeding.

Unexpectedly, growth of all three detritivores appeared to be N-limited, despite the relatively low N:P ratios of the species' body tissue. This outcome may reflect the cumulative N-demands associated with the production of exoskeleton (Huryn and Wallace 2000, Sterner and Elser 2002), which includes chitin as a major component, since repeated moulting occurred during the experiment for all three of our study species. In addition, the production of silk for use in case construction may have further exacerbated N limitation for the caddisfly (Craig et al. 1999, Huryn and Wallace 2000). We also observed a general narrowing in the stoichiometric imbalance between detritivores and leaf litter over the study period, most likely due to microbial nutrient immobilisation (Kaiser et al. 2014). Overall, these results highlight the potential key role of stoichiometric imbalances between consumers and resources in regulating ecosystem processes. However, the results also point to challenges in elucidating stoichiometric relationships for complex processes such as leaf litter decomposition that involve multiple species with distinct functional traits.

Litter stoichiometry

The spread in N:P between litter species in our study was expected to be larger than the gap invertebrates could potentially adjust for. However, changes in litter chemistry during decomposition altered resource nutrient ratios, with higher relative increases in P than N concentrations in all but one of the litter species investigated. The exception was oak, which already had the lowest N:P ratio before field incubation. For example, the increase in P concentration in alder, the most P-deficient species in our study, narrowed the C:P imbalance between alder and all three detritivore taxa from ~66 fold to ~12 fold, and also narrowed the N:P imbalance between alder and the three detritivores from ~14 fold to ~4 fold. These changes have important implications when characterising stoichiometric relationships between detritivores and leaf litter in streams, given that they greatly reduce the strong elemental imbalance common in detritus food webs (Cross et al. 2003, Lauridsen et al. 2014).

Several possible mechanisms might explain the changes in resource nutrient ratios observed across our four litter species over the study period. These include the well-known influence of microbial nutrient immobilisation, part of the processes described collectively as microbial conditioning (Bärlocher and Sridhar 2014). Alternatively, the changes might reflect mechanisms predominantly mediated by the detritivores, including selective feeding on leaf components rich in particular nutrients, or excretion of nutrients that are in sufficient supply followed by microbial uptake of those nutrients and immobilisation in litter. Overall, however, our results indicate that the changes in litter nutrient ratios primarily reflect the direct influence of microbial activity, rather than detritivores. The clearest indication is that the decreases in N:P, C:N and C:P ratios observed by the end of the experiment differed little between microcosms with and without detritivores, a result that was consistent for all three detritivore and all four litter species. There was evidence for divergence in the final C:P ratio of leaf litter offered to *Nemoura* and *Gammarus*, suggestive of a higher P intake or excretion, respectively, by *Nemoura* and *Gammarus*. However, this effect was weak, and there was also no difference in C:P ratios between microcosms containing only microbes or detrivores as well. These results indicate that changes in leaf litter stoichiometry during decomposition in streams are primarily controlled bottom–up, through microbial activity, rather than top–down through selective feeding of detritivores, at least over the short time scale of our experiment.

Litter mixing and recalcitrance both affected litter stoichiometry as well, since litter mixing had a stronger effect on final N:P ratios in the fast- than slow-decomposing litter species pair. Specifically, the N:P ratio of alder increased when alder was mixed with birch, reflecting increases in N and simultaneous decreases in P concentration. This increase in alder N concentration indicates N transfer to an N rich litter species, which may reflect elevated N immobilisation by a highly active microbial community on easily exploitable alder litter. The mechanism accounting for this seemingly counterintuitive direction of N flow may be similar to that underlying an observation by Handa et al. (2014), which suggests that nutrients in litter mixtures flow to the litter species where microbial decomposers create the greatest demand rather than from a species with a high to a species with a low N concentration. The observed similarity of fungal biomass within but not between litter pairs is also indicative of litter recalcitrance effects on fungi (Gessner and Chauvet 1994), whereas litter mixing or detritivore species had no detectable effect on fungal biomass.

Stoichiometry effects on detritivore growth

The two insect species in our study had lower N:P ratios than expected based on published data (Cross et al. 2003, Hladyz et al. 2009, Lauridsen et al. 2012). Although a low N:P was expected to drive P limitation of detritivore growth (Frost et al. 2006, Danger et al. 2013), evidence for an effect

of P limitation on growth was found only for *Sericostoma*, as seen in the negative relationship between growth rate and final $EI_{C:P}$. This weak evidence is surprising given the high P requirement in ribosomal RNA, which is positively related to growth, as posited by the growth rate hypothesis (Sterner and Elser 2002).

Rather than P limitation of growth, all our detritivores showed growth limitation in response to limited N availability, relative to C and P, irrespective of litter lignin concentration. Negative growth was seen for *Gammarus* and *Sericostoma* when their N:P or C:N stoichiometric imbalance with the leaf litter was larger than ca two or six times, respectively (i.e, $EI_{N:P}$ ~0.6 and $EI_{C:N}$ ~1.8, both on a natural log scale). The observed negative relationships might in part reflect inaccuracies in our method to estimate initial detritivore biomass (based on allometric body size–mass relationships; Supplementary material Appendix 1), but these results nevertheless highlight the higher demands of N relative to C and P for both species. The response to N availability, which was seen even when N concentration in detritivore tissue was relatively low, might reflect ontogenetic changes in nutrient demands, related to the development of the immature stages of our insect species in particular (Kerpel et al. 2006). In temperate regions, higher C:N ratios of stream detritivores occur during the early growth period in the autumn, and lower C:N ratios towards spring, which is the emergence period for most insect species (Lauridsen et al. 2012). It appears that resource–consumer imbalances in elemental ratios are likely to increase in importance during these ontogenetic changes, given that even the relatively modest C:N imbalance observed in our study resulted in reduced growth. Thus, although consumers are typically assumed to be relatively homeostatic, particularly in comparison with primary producers (Sterner and Elser 2002, Cross et al. 2003), ontogenetic changes may alter their stoichiometric characteristics (Back et al. 2008, Alves et al. 2009), changing the nutrient requirements of the detritivores as they mature.

Another potential driver of N limitation for detritivore growth could be chitin, the main component of the exoskeleton of arthropods. Chitin is relatively rich in N (C:N = 5:1) and devoid of P (Sterner and Elser 2002), and can correspond to about 25% of insect body mass (Lease and Wolf 2010). The immediate consumption of the exoskeleton after moulting (i.e. shedding of the exoskeleton) may alleviate N loss, yet the few estimates available indicate that about 40% of N present in the exoskeleton is not resorbed by the moulted invertebrate (Mira 2000). Therefore, frequent moulting during growth could greatly increase invertebrate N requirements. This might further explain why the three invertebrates included in our experiment, representing two insect orders and one crustacean, all responded positively to N availability, despite the relatively low N:P ratios of their bodies.

The caddisfly showed the strongest positive relationship between growth and N availability ($EI_{C:N}$ or $EI_{N:P}$). Caddisflies, in common with aquatic diptera and terrestrial invertebrates including spiders and Lepidoptera, require extra N to maintain silk production for the construction and expansion of cases and retreats, and/or structures for capturing resources (webs and nets) (Otto and Svensson 1980, Craig et al. 1999). Physiological investment in these structures can be substantial, and involve tradeoffs with adult fecundity and body size (McKie 2004). For example, caddisfly larvae of the family Limnephilidae have been reported to lose ~35% of their protein content when forced to rebuild their cases, delaying adult emergence by several days (Mondy et al. 2011). Notably, typical amino acids in caddisfly silk have atomic C:N ratios as low as about 2 (Craig et al. 1999). Neither the shedding of exoskeletons by insects and crustaceans, nor the silk produced by caddisflies are normally considered when body-mass elemental ratios are assessed, potentially leading to an underestimation of the cumulative N requirements of these organisms. Although the growth rate hypothesis posits that maturation and growth lead primarily to strong P-requirements on consumers, our results suggest that the limitation on growth imposed by the cumulative demands for N associated with repeated moulting and, in the case of silk-spinning taxa, silk production, could be comparable to P-effects on growth.

Stoichiometric effects on litter decomposition

Influences of stoichiometry on our other focal process, litter decomposition, were driven primarily by consumer preferences rather than compensatory feeding. In contrast to previous studies (Lincoln et al. 1986, Fink and von Elert 2006, Liess 2014), the litter offered in our experiment as food had rather high concentrations of recalcitrant lignin, which may have limited the capacity of detritivores to accelerate feeding in an attempt to overcome deficiencies in litter N and/or P. However, when two litter species with contrasting stoichiometry were offered simultaneously, selective feeding favouring the closest stoichiometric match increased the difference in decomposition between the two litter species. For *Sericostoma*, this resulted in doubled detritivore-mediated decomposition rate of the high N:P species in the mixture relative to single-species treatments, whereas detritivore-mediated decomposition of the low N:P litter was reduced to half of its rate when offered as single-species.

Although alder is commonly regarded a high-quality resource for detritivores, and is typically preferred over other litter species (Friberg and Jacobsen 1994, Carvalho and Graça 2006), detritivore preferences for alder over birch in our study depended on the degree of stoichiometric imbalance between consumer and resource. The greatest increase in alder decomposition when mixed with birch was caused by *Sericostoma*, the otherwise most N-limited of our detritivores. Similar effects were associated with consumption by *Nemoura*, whereas rates of alder decomposition mediated by *Gammarus*, which has high body concentrations of P rather than N (Hladyz et al. 2009), did not differ between single-or mixed-species litter. This mixture effect on decomposition suggests that imbalances in resource and consumer nutrient composition are a potential mechanism causing positive litter diversity effects on ecosystem functioning (Gessner et al. 2010, Vos et al. 2013).

Conclusions

Ecological stoichiometry is a key element in the development of a mechanistic understanding of how species affect ecosystem functioning. However, our results point to several challenges

in developing such generalisations for complex processes such as litter decomposition, to which many functionally distinct organisms contribute, and both the stoichiometric characteristics of the resource and the requirements of consumers may change over time. Specifically, our results suggest a potential underestimation of the N requirements of common invertebrate species, due to the importance of N usage for case-building and moulting in affecting consumer growth rates. Furthermore, our study shows that marked stoichiometric imbalances characterising consumers and their resources can have strong effects on detritivore growth, whereas effects on litter decomposition are less prominent. This finding is particularly important given globally increased availability of N, including in stream ecosystems (Woodward et al. 2012), originating from agricultural fertilisation, urban waste, and large-scale atmospheric deposition (Galloway et al. 2008, Taylor and Townsend 2010).

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Supplementary material (available online as Appendix oik-02687 at <www.oikosjournal.org/appendix/oik-02687>). Appendix 1–6.