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Detrital Nutrient Content and Leaf Species Differenitally Affect Growth and Nutritional Regulation of Detritivores

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Resource nutrient content and identity are common bottom–up controls on organismal growth and nutritional regulation. One framework to study these factors, ecological stoichiometry theory, predicts that elevated resource nitrogen (N) and phosphorus (P) contents enhance organism growth by alleviating constraints on N and P acquisition. However, the regulatory mechanisms underlying this response – including whether responses depend on resource identity – remain poorly understood. In this study, we tested roles of detrital N and P contents and identity (leaf species) in constraining growth of aquatic invertebrate detritivores. We synthesized results from seven detritivore species fed wide nutrient gradients of oak and maple detritus in the laboratory. Across detritivore taxa, we used a meta-analytic approach quantifying effects of detrital leaf species and N and P contents on growth, consumption, and N- and P-specific assimilation and growth efficiencies. Detritivore growth rates increased on higher-N and P detritus and on oak compared to maple detritus. Notably, the mechanisms of improved growth differed between the responses to detrital nutrients versus leaf species, with the former driven by greater consumption rates despite lower assimilation efficiencies on higher-nutrient detritus, and the latter driven by improved N and P assimilation and N growth efficiencies on oak detritus. These findings suggest animal nutrient acquisition changes flexibly in response to resource changes, altering the fate of detrital N and P throughout regulation. We affirm resource identity and nutrients as important bottom–up controls, but suggest these factors act through separate pathways to affect organism growth and thereby change detrital ecosystems under anthropogenic forest compositional change and nutrient enrichment.

Keywords: shredders, macroinvertebrates, streams, assimilation, growth, consumption, excretion, egestion, homeostasis

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Introduction

Organismal nutrient budgets are key to understanding how resources affect organismal growth and regulation (Vanni 2002, Sperfeld et al. 2016). Among animals, regulation is constrained by conservation of mass, in which inputs via consumption must equal outputs via egestion, excretion, growth and others (Fig. 1). For decades, ecologists have quantified single-currency organismal energy budgets (Benke and Wallace 1980) but only recently have frameworks emerged to budget for multiple currencies like growth-limiting nutrients (Sterner and Elser 2002, Simpson and Raubenheimer 2012). One notable framework, ecological stoichiometry, focuses on mass constraints of elements like nitrogen (N), and phosphorus (P) on organismal growth (Sterner and Elser 2002). As central tools of ecological stoichiometry, organismal N and P budgets explain growth limitation by N or P availability but remain rare because they are difficult to construct, and recent studies have raised challenges for budget implementation among consumers, including problems of selective feeding, heterogeneous turnover of nutrients in consumers and resources, and dynamic nutrient demands during ontogeny (Hood et al. 2014, Dodds et al. 2014, Hertz et al. 2016). Though challenging to construct, nutrient budgets are powerful because they provide mechanistic bases for growth and fitness, and simultaneously bridge levels of organization by placing organisms within ecosystem-level processes like nutrient cycling (Bouchard and Bjorndal 2000, Mischler et al. 2016, Sperfeld et al. 2016).

Within the constraints of their nutrient acquisition budgets, animals can employ multiple regulatory strategies to acquire limiting resource nutrients while maintaining homeostasis (Fig. 1, Frost et al. 2005, Sperfeld et al. 2017).

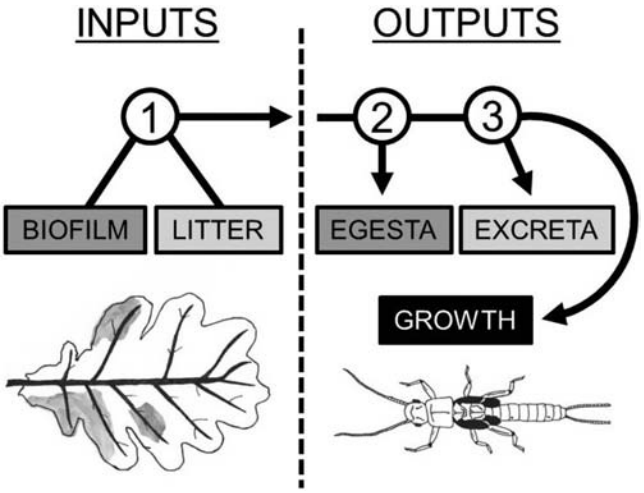

Figure 1. Conceptual summary of detritivorous animal nutrient budgets. In response to resource shifts, budgets can be altered at multiple levels of regulation including (1) the rate or amount of consumption inputs, (2) assimilation efficiency (AE_X) through altered egestion outputs, and (3) gross growth efficiency (GGE_X) through altered excretion outputs.

Animals can achieve consumption targets through selective foraging and regulated rates of food intake (Lee et al. 2008, Meunier et al. 2016). After ingestion, animals can increase efficiency of assimilating limiting nutrients (Clissold et al. 2010) and may alter excretion and metabolism (respiration) to regulate internal homeostasis and growth (DeMott et al. 1998, Anderson et al. 2005). Generally, increased resource N and P contents stimulate growth by reducing consumer–resource imbalances, but the explanatory roles at each regulatory pathway (consumption, assimilation, excretion, or others) remain elusive (Sterner and Elser 2002, Frost et al. 2005, Evans-White and Halvorson 2017). Elemental budgets, ideally from controlled studies, are needed to understand the mechanisms of homeostasis and growth that explain community responses to resource stoichiometry gradients (Jochum et al. 2017), especially in response to globally increased N and P availability that drive resource nutrient enrichment (Peñuelas et al. 2013, Yan et al. 2016). Such mechanistic responses are also important to predict how animals' ecosystem-level functions (nutrient excretion, egestion, and storage) will respond to nutrient enrichment (Atkinson et al. 2017).

Given the importance of detrital pathways in ecosystem energy flow (Moore et al. 2004), empirical studies in ecological stoichiometry increasingly broaden beyond model herbivores to include detritivorous animals (Martinson et al. 2008, Evans-White and Halvorson 2017). Key drivers of organic matter breakdown and nutrient cycling in terrestrial and aquatic ecosystems (Moore et al. 2004), detritivores face significant consumer–resource elemental imbalances and are constrained by the recalcitrance (slow decomposition and low digestibility; Webster and Benfield 1986) of detrital carbon (C), resulting in low growth rates (Cross et al. 2003, Frost et al. 2006). Nutrient enrichment increases detrital microbial biomass and nutrient contents, improving detritivore consumption rates, assimilation efficiencies, and growth rates (Danger et al. 2013, Jochum et al. 2017, Halvorson et al. 2017b). Recent work further suggests detritivore responses to detrital P content are stronger than those to N content, suggesting animal growth and regulation may be more sensitive to resource P than N enrichment (Demi et al. 2018).

Detritivore responses to nutrients may depend on leaf species because detrital recalcitrance and stoichiometry vary widely across plant species, constraining animal growth (Loureiro et al. 2006, Frainer et al. 2016). Because it contains greater proportions of low-quality C compounds such as lignin and cellulose that constrain microbial conditioning and animal digestion, recalcitrant detritus is often presumed to slow detritivore consumption rates and reduce assimilation efficiencies, leading to slower detritivore growth compared to faster-decomposing, labile detritus (Kaushik and Hynes 1971, Mehring and Maret 2011, Frainer et al. 2016). However, this paradigm is still debated because it is based primarily on microbial decomposition, and further tests quantifying consumption, assimilation, and growth are needed from a wider diversity of consumer taxa (Compson et al.

2015, Siders 2016). Roles of leaf species gain importance amid global forest compositional change, including plant species shifts, losses, and invasions that often accompany anthropogenic stressors (McCary et al. 2016, Larsen et al. 2016). Moreover, low-nutrient, recalcitrant detritus generally responds more strongly to dissolved nutrient enrichment (Scott et al. 2013, Manning et al. 2016). This suggests shifts in leaf species at the base of the food web may interact with nutrient availability, altering detrital-based ecosystems from the bottom–up (Hladyz et al. 2009, García-Palacios et al. 2016).

In this study, we synthesize results from feeding experiments across seven leaf-eating aquatic invertebrate taxa to examine how N and P gradients of two contrasting detrital leaf species – *Acer saccharum* (sugar maple) and *Quercus stellate* (post oak) – affect organismal growth, consumption, and N and P acquisition. Oak leaves generally contain greater proportions of lignin, cellulose, and tannins, resulting in slower decomposition compared to maple leaves (Aber et al. 1990, Kominoski et al. 2007). Given this, the use of maple versus oak leaves provides contrasting C quality of two co-existing plant species and addresses how replacement of oak by maple species could affect aquatic organisms in North American forests (McEwan et al. 2011). Our study focuses on shifts across the diverse assemblage of detritivore species – several of which are sensitive to landscape-level nutrient pollution, possibly due to nutritional pathways that are confounded with stressors like riparian deforestation and low dissolved oxygen (Wang et al. 2007, Evans-White et al. 2009). We predicted that increased detrital N and P content would increase animal growth due to elevated consumption rates compounded with moderate increases in assimilation and growth efficiencies (Frost et al. 2005, Jochum et al. 2017). Acknowledging that some studies indicate the opposite (Hutchens et al. 1997, Compson et al. 2015), we expected that animals would grow faster on labile, relatively more digestible maple compared to oak detritus (Mehring and Maret 2011) due to greater consumption rates and assimilation and growth efficiencies. Finally, we predicted that detritivore responses to nutrients would be greater on oak compared to maple detrituss, because nutrient enrichment would alleviate the negative effects of low C quality of the former leaf species.

Material and methods

Feeding experiments

We conditioned detritus in the laboratory following methods of Halvorson et al. (2015). Sugar maple *Acer saccharum* and post oak *Quercus stellata* were cut into 13.5 mm diameter disks for feeding to *Pycnopsyche* and *Tipula.* We later switched to whole leaf diets for all other taxa because we were unable to rear Plecoptera on leaf disks; whole leaves could increase the degree the selective feeding, but this should not confound our study because we do not make explicit comparisons across species offered different forms of leaf detritus. Leaves were leached in tap water for three days prior to incubation under one of four dissolved phosphorus P amendments as Na_2HPO_4: <5, 50, 100 or 500 µg P l^{-1} (ambient, low, medium and high P). All incubation chambers were under ambient laboratory conditions (~22°C) and contained 20 liter vigorously aerated dechlorinated tap water amended with 1000 µg l^{-1} N-NO_3 as KNO_3; water was flushed and re-amended with nutrients every 2–3 days. We inoculated incubation chambers using detrital slurry from two nearby streams (Mullins and Scull Creeks) in Fayetteville, Arkansas.

After conditioning for a minimum of seven weeks, leaf detritus was fed ad libitum to field-collected invertebrates in the laboratory. Feeding experiments were conducted separately with *Lirceus* spp. (Isopoda: Asellidae), *Strophopteryx* spp. (Plecoptera: Taeniopterygidae), *Allocapnia* spp. (Plecoptera: Capniidae), *Amphinemura* spp. (Plecoptera: Nemouridae), *Lepidostoma* spp. (Trichoptera: Lepidostomatidae), *Pycnopsyche lepida* (Trichoptera: Limnephilidae) and *Tipula abdominalis* (Diptera: Tipulidae), an assemblage of diverse phylogeny, body size and body nutrient contents (Table 1). We refer to the taxa by their genera. Results from *Pycnopsyche* and *Tipula* are published as single-species studies (Halvorson et al. 2015, Fuller et al. 2015) but have been included for the present analysis across taxa. All animals were collected from Ozark Highlands, Boston Mountains or Arkansas Valley streams in Arkansas in the winter–spring of 2012–2014; within each species, individuals were collected from the same reach, given mixed detritus, and returned to the laboratory to commence experiments within two days. At the beginning of each experiment, we collected a subsample of individuals and allowed 24 h gut clearance to determine

Table 1. Aquatic invertebrate rearing temperatures and mean ± SD initial dry mass, molar body N:C, and molar body P:C.

Species	Temperature (°C)	Initial dry mass (mg)	Body N:C	Body P:C
Allocapnia spp.	10	0.178 (0.068)	0.231 (0.017)	0.0085 (0.0021)
Amphinemura spp.	10	0.343 (0.020)	0.194 (0.009)	0.0092 (0.0007)
Lepidostoma spp.	10	2.384 (0.920)	0.120 (0.009)	0.0072 (0.0008)
Lirceus spp.	10	0.978 (0.287)	0.176 (0.014)	0.0117 (0.0006)
Pycnopsyche lepida	10	1.469 (0.383) [a]	0.177 (0.009)	0.0123 (0.0012)
Strophopteryx spp.	5	0.228 (0.126)	0.234 (0.018)	0.0074 (0.0020)
Tipula abdominalis	15	3.90 (3.16) [a]	0.199 (0.024)	0.0058 (0.0021)

[a]Initial dry mass was determined by regression and reported values are mean ± SD across individuals used in the experiment.

initial masses; we used head capsule width–mass regression (*Pycnopsyche*), blotted to dry-mass regression (*Tipula*), or mean individual dry mass across a random subset of individuals within a set range of body length (all others). Remaining individuals were disbursed among growth chambers and randomly fed one of the two leaf species conditioned under one of the four P amendments in a fully-crossed design. Growth chambers consisted of specimen cups containing 100 ml stream water (changed every five days), with mesh inserts (0.5 or 1 mm) to separate animals from their feces. Subsamples of detritus were collected in each experiment, oven dried, and homogenized to determine C, N and P contents. The feeding experiments took place over 14–33 days and differed slightly in other rearing conditions including temperature (Table 1, Supplementary material Appendix 1 Table A1).

At the end of each experiment, individuals were allowed a 24 h gut clearance period and frozen. Individuals were subsequently thawed and oven dried to determine dry mass (DM). Instantaneous growth rates over the experiment duration were calculated from Eq. 1

$$Growth\ rate\ (d^{-1}) = \frac{log(DM_{final}) - log(DM_{initial})}{time} \quad (1)$$

where time is in days. Initial and final individuals were measured for body C, N and P contents. Among large-bodied taxa, we homogenized each individual into fine powder using a spatula and subsampled resultant powder for elemental contents. Among small-bodied taxa, we could not homogenize individuals from each growth chamber because of insufficient mass; we randomly selected 1–2 individuals for C/N or P analysis from each growth chamber. Detrital and animal tissue samples were analyzed for C/N contents using an elemental analyzer and P contents by combustion, digestion in hot hydrochloric acid, and analysis for soluble reactive phosphorus (APHA 2005).

N and P budgets: per capita consumption, egestion, excretion and growth rates

We measured consumption in each growth chamber once weekly between feeding events. We used a detritus blotted to dry-mass regression, based on the difference between initial dry mass from blotted weight before consumption and measured dry weights after consumption, to calculate total consumption. We divided total consumption by trial duration and number of individuals in each growth chamber to calculate per capita rates (mg ind.$^{-1}$ day^{-1}). We then calculated average consumption rates across weekly trials for each growth chamber, and multiplied this rate by detrital %N and %P to determine per capita rates of N and P consumption. Negative consumption rates were considered immeasurable and converted to zero in 6.3% of growth chambers (mostly low-survival growth chambers, and among *Allocapnia* where high emergence rates reduced numbers of individuals present). We measured consumption by all taxa except *Amphinemura*, due to constraints on authors' time during the growth period.

Egestion rates were similarly measured once weekly between feeding events. At the start of each trial, growth chambers were given fresh, filtered stream water and animals were allowed to feed for 2–3 days. At the end of each trial, accrued egesta were filtered onto pre-weighed, combusted glass fiber filters (1 µm pore size), oven dried at 50°C, and weighed to determine total egesta mass. We cut filters in half, weighed each half, and assigned each for analysis of either N contents using an elemental analyser or P contents using combustion, hydrochloric acid digestion and soluble reactive phosphorus analysis (ascorbic acid method; APHA 2005). Total N and P egestion were calculated by dividing total measured N or P content of each half-piece by its mass proportion of total egesta mass. We then divided total N and P egested by trial duration and the number of surviving individuals to determine per capita rates of N and P consumption (µmol ind.$^{-1}$ day^{-1}). We averaged N and P egestion rates across trials for each growth chamber.

Excretion of N and P was measured among large-bodied taxa (*Pycnopsyche*; *Tipula*, only P) at the end of the growth period. We removed individuals from their growth chambers and placed each individual in 30 ml filtered stream water. After 3 h, we placed animals back in their growth chambers to undergo 24 h gut clearance. We filtered excreta (1 µm pore size) and kept filtrate on ice prior to analysis for soluble reactive phosphorus and N-NH_4 using the ascorbic acid and phenate methods, respectively (APHA 2005). Excretion rates were calculated using the difference between experimental and control chambers. Among other taxa, excretion rates were either immeasurable (*Lepidostoma*) or were not measured because animals were too small. Among these taxa, we used body mass and temperature to estimate per capita N and P excretion based on a global analysis of aquatic animal excretion (Vanni and McIntyre 2016).

We calculated N- and P-specific growth for each growth chamber using Eq. 2

$$Growth_X = [DM \times Q_X]_{final} - [DM \times Q_X]_{initial} \quad (2)$$

where DM is dry mass and Q_X is the proportion of element X in body tissues among initial or final individuals. We divided element-specific growth rates by growth duration and, where there were multiple individuals per chamber, we calculated average per capita N- and P-specific growth rates for each growth chamber.

For each growth chamber, we used the above per capita rates to calculate rates of N and P output ($Output_X$) using Eq. 3

$$Output_X = Egestion_X + Excretion_X + Growth_X \quad (3)$$

We calculated N and P assimilation and gross growth efficiencies (AE_X and GGE_X, respectively) from Eq. 4 and 5

$$AE_X = \frac{Output_X - Egestion_X}{Output_X} \quad (4)$$

$$GGE_X = \frac{Growth_X}{Output_X} \quad (5)$$

We used the denominator $Output_X$ instead of $Consumption_X$ in these calculations because $Output_X$ often exceeded $Consumption_X$, causing error (i.e. negative AE_X among growing animals) when $Consumption_X$ was used, a discrepancy that we attribute to selective feeding on N- and P-rich biofilms.

N and P budget visualization

For each detritivore species, we subsequently calculated the average magnitude of change in N and P-specific per capita consumption rates from ambient to each increasing detrital nutrient level, pooled for both leaf species (nutrient response), or from maple to oak detritus, pooled across all four nutrient levels (leaf species response). For each of N and P, we then multiplied the relative magnitude of consumption change by the mean proportions of $Output_X$ allocated to $Egestion_X$, $Excretion_X$ and $Growth_X$ at the given resource category (nutrient level or leaf species). In this way, the presented budgets visualize the collective resource-driven changes in N and P acquisition, averaged across six detritivore species (*Amphinemura* excluded due to lack of consumption data), using relative magnitudes of change as a standard across-species comparison for each budget term because per capita rates varied widely and were not readily interpretable across detritivore species, e.g. due to differences in body size.

Data analysis

Our analysis considered each detritivore species as a separate experiment, and as such, quantified standard effect sizes to test our hypotheses. To quantify responses to the resource N and P gradients, we used Pearson's *r* correlating detrital N:C or P:C to each response variable (growth and consumption rates – both N:C and P:C gradients; AE_P and GGE_P – P:C gradient; AE_N and GGE_N – N:C gradient) separately for each detrital leaf species within each detritivore species. We transformed Pearson's *r* to Fisher's *Z*-scores and used weighted one-way ANOVA to statistically compare nutrient effect sizes between the two leaf species. Subsequently, we used weighted one-sample *t*-tests to compare mean effect size on each leaf species to a null hypothesis of zero. To quantify responses to leaf species, we calculated Hedge's *d* as the difference of oak minus maple responses, and used weighted *t*-tests to compare mean leaf species effect size for each response variable (growth, consumption, AE_P, GGE_P, AE_N and GGE_N) to a null hypothesis of zero, indicating no significant effect of leaf species. Effect sizes and their variances may be found in the Supplementary material Appendix 2 Table A4–A7. Statistical analyses were weighted by the inverse of variance for each effect size. We also analyzed differences in detrital N:C and P:C contents across leaf species and P amendments using two-way ANOVA. All statistical analyses and were conducted using R ver. 3.3.1 (<www.r-project.org>).

Data deposition

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.c9g148r> (Halvorson et al. 2018).

Results

In response to dissolved P amendment, detrital N:C contents increased among the two higher P amendment treatments ($F_{3,40} = 14.0$, $p < 0.001$; Supplementary material Appendix 1 Fig. A1), but N:C contents did not differ between leaf species ($F_{1,40} = 1.7$, $p = 0.198$; Supplementary material Appendix 1 Table A2). Detrital P:C contents increased with each level of P amendment ($F_{3,40} = 159.1$, $p < 0.001$) and were greater among maple compared to oak detritus ($F_{1,40} = 11.0$, $p = 0.002$; Supplementary material Appendix 1 Table A2, Fig. A1). These responses resulted in wide gradients of oak and maple detrital N:C and P:C fed to detritivores.

Across the detritivore responses to detrital N:C and P:C, no effect sizes differed between leaf species ($p > 0.05$; Supplementary material Appendix 1 Table A3), but several responses were significantly different from zero depending on leaf species (Table 2, Fig. 2–3). Across the N gradient,

Table 2. Weighted one-sample two-tailed *t*-test results for effects of oak and maple detrital P:C and N:C gradients on growth, consumption (Cons), AE_P, GGE_P, AE_N and GGE_N. Nutrient effect sizes were calculated as Pearson's *r* and transformed to Fisher's *Z*-scores prior to analysis. Mean effect sizes were compared to a null hypothesis of zero for each leaf species separately, indicating no response to detrital N:C or P:C contents. Effect sizes did not differ between leaf species (Supplementary material Appendix 1 Table A3). Values in bold indicates mean effect size significantly different from zero ($p < 0.05$).

Response	Gradient	Leaf	*t*-value	p-value	Response	Gradient	Leaf	*t*-value	p-value[a]
Growth	P:C	oak	2.58	**0.042**	Growth	N:C	oak	2.85	**0.029**
		maple	1.49	0.187			maple	1.24	0.261
Cons	P:C	oak	3.21	**0.024**	Cons	N:C	oak	2.90	**0.034**
		maple	3.34	**0.021**			maple	2.74	**0.041**
AE_P	P:C	oak	–2.39	0.054	AE_N	N:C	oak	–0.51	0.629
		maple	–2.23	0.067			maple	–2.64	**0.039**
GGE_P	P:C	oak	1.88	0.109	GGE_N	N:C	oak	0.66	0.535
		maple	–0.28	0.790			maple	–0.03	0.978

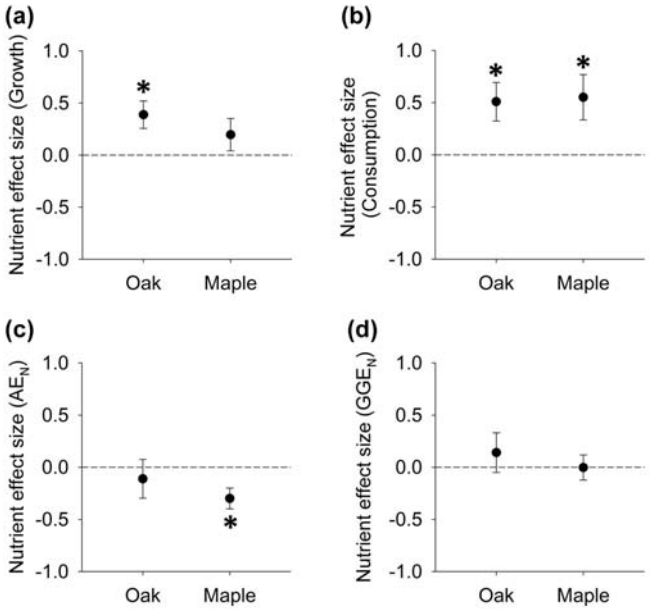

Figure 2. Weighted mean ± SE nutrient effect sizes (*Z*-scores) of oak and maple detrital N:C gradients on detritivore growth rates (a), consumption rates (b), AE_N (c), and GGE_N (d). Horizontal dotted lines indicate an effect size of zero (no response to N:C) and asterisks indicate effect sizes significantly different from zero ($p < 0.05$). Nutrient effect sizes did not differ between leaf species ($p > 0.05$; Supplementary material Appendix 1 Table A3).

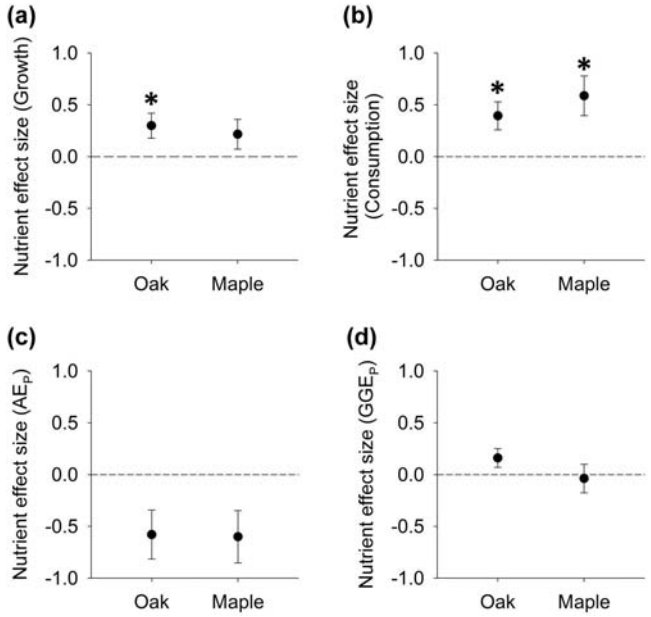

Figure 3. Weighted mean ± SE nutrient effect sizes (*Z*-scores) of oak and maple detrital P:C gradients on detritivore growth rates (a), consumption rates (b), AE_P (c), and GGE_P (d). Horizontal lines indicate an effect size of zero (no response to P:C) and asterisks indicate effect sizes significantly different from zero ($p < 0.05$). Nutrient effect sizes did not differ between leaf species ($p > 0.05$; Supplementary material Appendix 1 Table A3).

growth rates responded positively to N:C only on oak detritus ($t_{1,6} = 2.9$, $p < 0.05$; Fig. 2a). Consumption rates responded significantly positive to detrital N:C on both oak and maple detritus ($t_{1,5} = 2.9$, $p < 0.05$ and $t_{1,5} = 2.7$, $p < 0.05$ respectively; Fig. 2b). Although AE_N declined in response to N:C, only maple leaves showed a significant negative response ($t_{1,6} = 3.8$, $p < 0.01$; Fig. 2c). The response of GGE_N was not significantly different from zero on either leaf species (Fig. 2d).

Growth rates also responded positively to detrital P:C and only oak detritus produced a significant positive growth response ($t_{1,6} = 2.6$, $p < 0.05$; Fig. 3a). Consumption rates similarly responded positively to detrital P:C, and the response was significant on both oak and maple detritus ($t_{1,5} = 3.2$, $p < 0.05$ and $t_{1,5} = 3.3$, $p < 0.05$ respectively; Fig. 3b). Despite increased consumption rates, AE_P responded negatively to P:C on both leaf species, with responses similar on both leaf species and marginally significantly negative ($p = 0.054$ and $p = 0.067$, respectively). GGE_P did not respond strongly to detrital P:C on either leaf species (Fig. 3d).

Responses to leaf species, calculated using Hedge's *d*, were consistently positive and thus indicated positive responses to oak detritus compared to maple detritus (Table 3, Fig. 4). Growth rates were significantly greater on oak compared to maple detritus ($t_{1,6} = 2.5$, $p < 0.05$; Fig. 4a). Although N:C contents and consumption rates did not differ between leaf species, both AE_N and GGE_N were significantly greater on oak detritus ($t_{1,6} = 3.8$, $p < 0.01$ and $t_{1,6} = 2.66$, $p < 0.05$ respectively; Fig. 4b). AE_P was also greater on oak detritus ($t_{1,6} = 4.2$, $p < 0.01$), but GGE_P did not differ between leaf species (Fig. 4c).

The visualizations of N and P budgets demonstrate shifts in N and P consumption, egestion, excretion, and growth in response to changes in detrital nutrient content (Fig. 5a, c) and leaf species (Fig. 5b, d). Notably, budget sizes increased on higher-nutrient detritus, reflecting increased N and P consumption, while proportional egestion also increased, reflective of declining AE_N and AE_P (Fig. 2c, 3c). On oak relative to maple detritus, N and P budget sizes increased slightly while proportions allocated to egestion decreased and

Table 3. Weighted one-sample two-tailed *t*-test results for detritivore growth, consumption (Cons), AE_N, GGE_N, AE_P and GGE_P on oak versus maple detritus. Leaf species effect sizes were calculated as Hedge's *d* based on the difference of responses on oak relative to maple detritus; *t*-values assess whether effect sizes were different from a null hypothesis of zero, indicating no difference between leaf species. Values in bold indicates effect size significantly different from zero ($p < 0.05$).

Response	*t*-value	p-value[a]
Growth	2.47	**0.048**
Cons	1.45	0.208
AE_N	3.83	**0.009**
GGE_N	2.66	**0.037**
AE_P	4.21	**0.006**
GGE_P	1.72	0.136

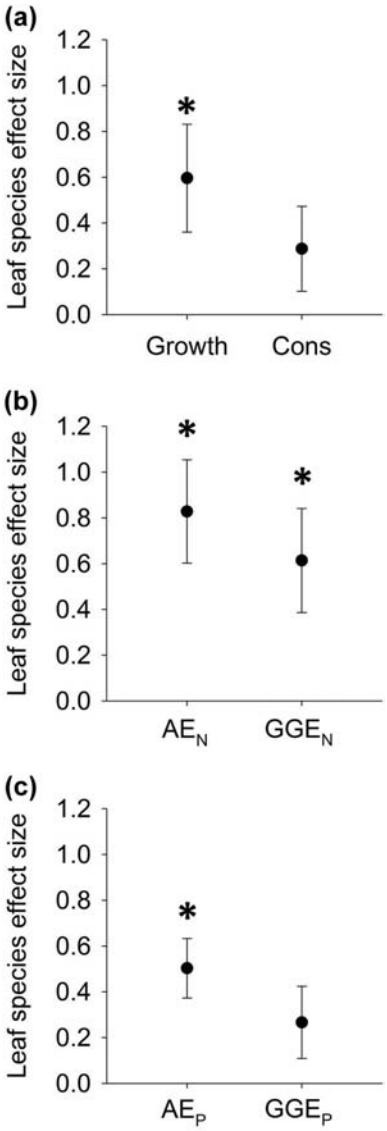

Figure 4. Weighted mean ± SE leaf species effect sizes (Hedge's *d*) on detritivore growth and consumption rates (a), AE_N and GGE_N (b), and AE_P and GGE_P (c). Positive effect sizes indicate a greater response on oak compared to maple detritus. Asterisks indicate effect sizes significantly differ from zero ($p < 0.05$).

those allocated to growth increased, reflecting increased AE_X and GGE_X (Fig. 4b–c).

Discussion

Our study synthesizes results of extended feeding experiments from diverse detritivore taxa, showing shifts in leaf-eating detritivore growth and underlying nutritional regulation in response to detrital nutrient gradients and leaf species. The data support several of our predictions from ecological stoichiometry theory, including increased consumption rates and increased growth rates with resource N and P enrichment

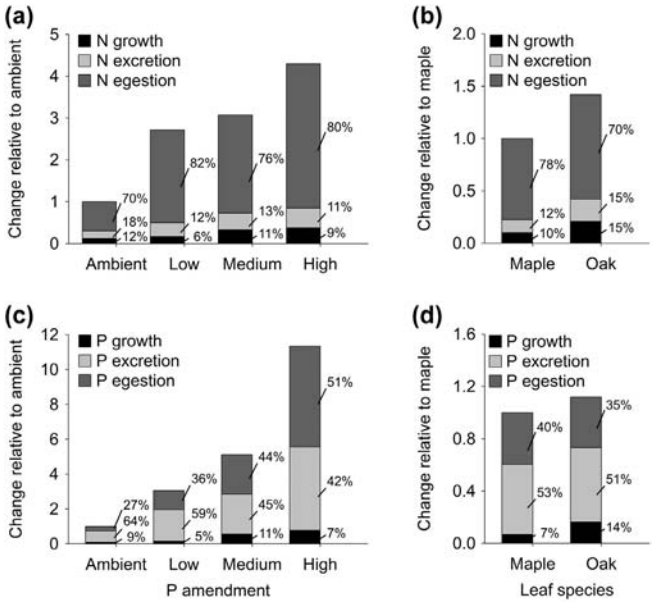

Figure 5. Shifts in N and P budgets of detritivorous invertebrates fed nutrient gradients of maple and oak detritus. Shifts were averaged across six detritivore species (*Amphinemura* excluded), calculated for each detritivore species as the change in average element-specific growth, excretion, and egestion on (a,c) higher-nutrient detritus relative to Ambient nutrient detritus or (b,d) oak relative to maple detritus. Percentages to the right of bars describe the average proportion of each budget attributable to growth, excretion, or egestion. See Supplementary material Appendix 2 Table A8–A9 for budget data.

(Sterner and Elser 2002, Frost et al. 2005). Unlike our prediction based on C quality, we found that oak conferred better detritivore growth compared to maple detritus, not through altered feeding but through improved assimilation and growth efficiencies. Our study addresses an important knowledge gap by connecting better-documented growth responses to poorly-known responses of consumption, assimilation, and growth efficiencies, providing a comparison of different regulatory levels toward growth responses to resource gradients (Fig. 1; Sperfeld et al. 2017). Moreover, our results are widely applicable because of their synthesis across diverse taxa, when most studies on bottom–up controls in detrital systems focus on single-species responses. Grounded in organismal N and P budgets, these results provide a useful bridge between the nutritional basis of detritivore growth and the functional roles of detritivores in ecosystems in response to nutrient availability and forest composition (Hladyz et al. 2009, Larsen et al. 2016, Jochum et al. 2017).

Nutrient enrichment alleviates animal growth limitation by reducing consumer–resource nutritional imbalances, but the underlying mechanisms include several possible regulatory shifts (Fig. 1; Frost et al. 2005, Evans-White and Halvorson 2017, Jochum et al. 2017). We highlight greater consumption as a main driver of improved growth with nutrient enrichment, because detritivores up-regulated total consumption rates with increased detrital nutrients (Fig. 2b, 3b), but weakly

changed or even reduced the efficiency of other pathways of nutrient acquisition with increasing nutrient availability. Compounded with increased detrital nutrient contents (33% greater detrital N:C and 270% greater detrital P:C from ambient to high P levels), the consumption response would surpass the reductions in AE_N and AE_P, increasing net N and P acquisition toward growth. These changes are evident from a visualization of N and P budgets, showing marked increases in budget size – especially of P – with increasing detrital nutrients (Fig. 5). While greater proportional egestion reflects declining assimilation efficiencies with increasing detrital nutrients, the magnitude of N and P flowing into growth increases monotonically on higher-nutrient detritus, primarily because of increased budget size due to up-regulated consumption (Fig. 5a, c).

The budgets show concomitant increases in feeding rates but lower AE_N and AE_P, indicative of shorter gut residence times on high-nutrient detritus (Golladay et al. 1983). This points to faster rates of converting detrital N and P into biomass – and notably egesta as a waste product – with nutrient enrichment (Cross et al. 2007, Halvorson et al. 2017a). These findings are in contrast to others' showing compensatory feeding on lower-nutrient detritus among detritivores (Flores et al. 2013, Jochum et al. 2017). The consistently negative nutrient effects on AE_N and AE_P versus weak nutrient effects on GGE_N and GGE_P (Fig. 2, 3) suggest detritivores most sharply increase egestion with increased nutrients and subsequently employ compensatory regulation between assimilation and growth – see no change or even reductions in post-assimilatory excretion as detrital N and P increase (Fig. 5a, c; Evans-White and Halvorson 2017) – dampening the response of GGE_N and GGE_P to nutrients. The increased egestion may partly reflect increased fragmentation of high-N and P detritus during sloppy feeding, which we did not measure but could cause underestimated AE_X. However, we note this response would be a realistic component of feeding and assimilation in response to nutrient enrichment in situ. Given the phylogenetic and stoichiometric breadth of detritivore species included in our analysis (Table 1), our results explain general observations that N and P enrichment in aquatic systems can increase secondary production (Cross et al. 2007), leaf breakdown (Ferreira et al. 2015, Manning et al. 2016), and export of fine particulate organic matter (Benstead et al. 2009).

Many studies have demonstrated detrital leaf species to affect detritivore consumption and growth, forming a direct control of forest composition on detrital ecosystems (Hutchens et al. 1997, Kominoski et al. 2011, McCary et al. 2016). Labile, fast-decomposing detritus like maple leaves are classically considered higher-quality to detritivores compared to recalcitrant, slow-decomposing leaves like oak (Kaushik and Hynes 1971, Mehring and Maret 2011, Frainer et al. 2016), although there are exceptions that recalcitrant leaf species, given sufficient conditioning, can be more amenable to shredder growth (Hutchens et al. 1997, Compson et al. 2015, Siders 2016). In our study, oak detritus conferred better growth through the mechanism of improved nutrient assimilation, because oak detritus did not strongly affect consumption rates, but enhanced AE_N, AE_P and GGE_N compared to maple detritus (Fig. 3). Our budget comparison illustrates these trends, because leaf species on average had minimal effects on N and P budget size, contrasted with marked effects on proportions of N and P allocated to growth and egestion (Fig. 5b, d). For example, mean proportions of N and P allocated to growth (GGE_N and GGE_P) increased from 7 to 14% and 10 to 15%, respectively, from maple to oak detritus. The contrasting effects of nutrients enhancing N and P budget size, versus leaf species shifting budget proportional allocation, points to the contrasting way these two factors affected growth and nutritional regulation (Fig. 5).

Increased assimilation and growth efficiency on oak detritus is surprising in light of its lower C quality, but C quality after conditioning likely did not differ as strongly between leaf species. Moreover, this finding is consistent with separate findings from isotope tracers that high-lignin and tannin *Populus* leaves conferred greater N assimilation by aquatic detritivores (Compson et al. 2015), perhaps because a greater proportion of detrital N is channeled to detritivores instead of mineralization by microbial decomposers (Siders 2016). The lower P:C contents of oak may have driven elevated AE_P as a compensatory response on this leaf species, but similar maple and oak N:C contents suggest that leaf species can affect detritivore AE_N and GGE_N, independent of leaf species' N contents. The lower AE_X on maple detritus may partly relate to easier sloppy feeding and fragmentation during feeding; however, such fragmentation was not apparent in the consumption responses, and greater growth on oak suggests this alone is not the cause of lower AE_X on maple. The leaf species effects were likely influenced by the extended duration of conditioning (>7 weeks), which could have ameliorated lower-quality C in oak while reducing the amount of digestible leaf C remaining in maple detritus, 'flipping' the comparative C quality of these species for assimilation and growth and permitting greater nutritional access of C-bound N and P in oak detritus (Suberkropp et al. 1976, Hutchens et al. 1997). We extended incubation times to ensure a wide nutrient gradient of both leaf species, but we might expect better comparative growth on maple detritus under shorter conditioning times. Though based on only two leaf species, our findings suggest resource quality for detritivore growth is not a fixed trait among leaf species, and future work should distinguish between resource quality for microbial decomposers versus detritivores (Siders 2016). At ecosystem levels, forest compositional changes could affect detrital ecosystems by altering energy and nutrient transfer into the detrital food web as well as detritivore-mediated turnover of detrital nutrients (Díaz et al. 2004, McEwan et al. 2011).

Leaf species can strongly affect detritivore growth rates, but there are still few studies comparing detritivore responses across nutrient gradients of differing leaf species. Some studies suggest detritivores' nutrient response may strengthen on recalcitrant detritus, because elevated N and P contents erase

the negative characteristics of low C quality (Greenwood et al. 2007, Fuller et al. 2015, Halvorson et al. 2015). However, decomposition studies predict nutrient enrichment may reduce the importance of leaf species by homogenizing species differences (Rosemond et al. 2010, Manning et al. 2016). Because we found no significant differences of nutrient effect sizes between maple and oak detritus, with the strongest regulatory response to nutrients (consumption) the most similar between leaf species, we suggest detritivore responses to detrital N and P contents may be generally similar on different leaf species. However, we note that oak detritus offered a marginally greater growth response to nutrients, possibly because AE_N did not decline as strongly on high-N oak compared to high-N maple detritus (Fig. 2c). Additionally, our feeding studies did not manipulate detrital quantity, which could be an important part of leaf species-specific responses to nutrient enrichment, if nutrients increase labile species' decomposition and reduce quantities of detritus available for consumption (Manning et al. 2016, Halvorson et al. 2017b). Overall, our findings across seven different taxa suggest nutrient enrichment and leaf species can independently affect detritivore growth, because these factors primarily affected consumptive versus assimilatory stages of nutrient acquisition, respectively (Fig. 5).

Conclusions

Derived from controlled experiments, our results support nutritional bases for detritivore and ecosystem-scale responses to nutrient enrichment in the field (Cross et al. 2007, Benstead et al. 2009, Manning et al. 2016). Interestingly, detritivore taxa sensitive to nutrient pollution – Plecoptera and Trichoptera that comprised the majority of species in our study – respond positively to increased detrital nutrients in the short-term, and longer-term studies show such effects result in altered energy flow to higher consumers, through disproportionate energy flow to large primary consumers (Davis et al. 2010). Additional factors associated with nutrient pollution (e.g. lower detrital quantity, altered competition, or abiotic stressors) may instead drive species declines with nutrient pollution (Wang et al. 2007, Evans-White et al. 2009). While we synthesize our findings across detritivore species, we do not compare the responses of individual species in our study, and we note this as a promising direction for future analysis to explain responses of individual taxa to resource changes. Our study shows a nutritional basis for faster detritivore growth on oak compared to maple detritus through improved AE_N, AE_P and GGE_N – a distinct mechanism of improved growth compared to the nutrient mechanism, which was based primarily on consumption rates. The absence of interactive effects between leaf species and nutrient enrichment highlights that detrital leaf species and nutrient contents can affect detritivore growth through separate regulatory pathways (Fig. 1), and are therefore likely to affect ecosystem function independently. Finally, the budgets show qualitatively similar responses of detritivore N and P acquisition to resource shifts, suggesting regulatory responses of these two elements are closely coupled during growth. However, the N and P budgets differ in proportional allocation to excretion versus egestion, with 70–82% of N lost to egestion contrasted with 27–51% of P lost to egestion (Fig. 5). This suggests detritivore effects on N cycling may operate primarily through particle pathways, whereas effects on P cycling occur more often through dissolved pathways, and particle pathways become comparatively more important with nutrient enrichment (Halvorson et al. 2017a, Atkinson et al. 2017). Our findings contribute greater understanding of detritivore nutrition, especially the basis of detrital-based community- and ecosystem-level responses to forest compositional shifts and increased nutrient availability associated with anthropogenic change (Cross et al. 2007, Larsen et al. 2016, Yan et al. 2016).

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Supplementary material (available online as Appendix oik-05201 at <www.oikosjournal.org/appendix/oik-05201>). Appendix 1–2.