

RESEARCH ARTICLE

Experimental N and P additions alter stream macroinvertebrate community composition via taxon-level responses to shifts in detrital resource stoichiometry

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Abstract

1. Increases in nitrogen (N) and phosphorus (P) availability are changing animal communities, partly by altering stoichiometric imbalances between consumers and their food. Testing relationships between resource stoichiometry and consumer assemblage structure requires ecosystem-level manipulations that have been lacking to date.
2. We analysed patterns of macroinvertebrate community composition in five detritus-based headwater streams subject to experimental whole-stream N and P additions that spanned a steep gradient in dissolved N:P ratio (2:1, 8:1, 16:1, 32:1, 128:1) over 2 years, following a 1-year pre-treatment period.
3. We predicted that shifts in leaf litter stoichiometry would drive overall patterns of community composition via greater responses of shredders to enrichment than other taxa, as shredders dominate primary consumer biomass and experience larger consumer–resource elemental imbalances than other functional groups in stream ecosystems. Specifically, we expected litter C:P to be a significant predictor of shredder biomass given the greater relative imbalances between shredder and litter C:P than C:N. Finally, we tested whether shredder responses to enrichment were related to other taxon-level traits, including body size and stoichiometry, larval life span and growth rate.
4. Whole-community composition shifted similarly across the five streams after enrichment, largely driven by increased shredder and predator biomass. These shifts were limited to the autumn/winter seasons and related to decreased leaf litter C:P, highlighting important links between the quality of seasonal litter subsidies and community phenology.
5. Among 10 taxa that drove structural shifts, two declined while other taxa from the same functional/taxonomic groups responded positively, suggesting that specific life-history traits may determine sensitivity to enrichment.
6. Increases in total shredder biomass, and in biomass of several common shredders, were associated with lower litter C:P. Body C:P did not predict shredder response to enrichment. However, weak negative relationships between shredder response and body size, and larval life span, suggest that small-bodied and short-lived taxa may be more responsive to shifting resource stoichiometry.

7. Moderate anthropogenic increases in N and P availability affect resource stoichiometry and can alter animal communities, influencing additional food web and ecosystem properties. We provide support for ecological stoichiometry as a framework for predicting such outcomes based on changes in the elemental composition of resource pools.

KEYWORDS

community structure, detritus, ecological stoichiometry, nitrogen, phosphorus, stream

1 | INTRODUCTION

Availability of the frequently limiting elements nitrogen (N) and phosphorus (P) has long been recognized as an important driver of ecosystem structure and function (Elser et al., 2007; Halpern et al., 2008). Shifts in the relative supply of N and P may alter the identity of the biologically limiting nutrient, thereby influencing processes ranging from individual growth rates to overall ecosystem productivity. Ecological stoichiometry theory (EST; Sterner & Elser, 2002) is a key framework for predicting such outcomes based upon the balance of carbon (C), N and P availability and the relative requirements for these elements by autotrophic and heterotrophic biota. As nutrient requirements vary among species, different taxa are not expected to respond uniformly to shifts in resource C:N:P stoichiometry. Instead, altered C:N:P supply should influence community composition predictably via direct effects on the dynamics of individual populations (such as mortality and recruitment), as well as by influencing the outcome of interspecific competitive interactions (Danger, Daufresne, Lucas, Pissard, & Lacroix, 2008; Moe et al., 2005; Sterner & Elser, 2002).

Despite the potential for EST to inform predictions of the effects of altered nutrient availability on community composition, past work has largely focused on broad patterns of biodiversity (i.e., species richness; Bobbink, Hornung, & Roelofs, 1998; Hillebrand et al., 2007) and has typically lacked an explicitly stoichiometric perspective (Hillebrand, Cowles, Lewandowska, Waal, & Plum, 2014). Moreover, tests of assemblage-level EST predictions in natural communities are rare, as most previous efforts have utilized experimental mesocosms, which lack the ecological complexity of natural ecosystems (Danger et al., 2008; Moe et al., 2005; Teurlincx et al., 2017). Furthermore, these studies are largely biased towards primary producers, with some notable exceptions (McCarthy & Irvine, 2010; Prater, Norman, & Evans-White, 2015; Teurlincx et al., 2017). As such, the role of resource stoichiometry in modulating the composition of natural consumer communities at higher trophic levels remains understudied, leaving doubt about the extent to which EST predictions scale from the base of food webs to entire consumer assemblages.

Here, we present patterns of macroinvertebrate community composition from five detritus-based headwater streams subject to continuous whole-reach experimental N and P fertilization. After one year of reference monitoring at ambient nutrient concentrations, each of

the five streams received dissolved N and P at different target N:P ratios (ranging from 2:1 to 128:1 molar) for two years. Compared to systems supported primarily by in situ autotrophic production, detritus-based ecosystems provide an ideal opportunity to investigate the effects of basal resource quality on consumer dynamics. In producer-based ecosystems, increased nutrient availability often leads to increased rates of primary production and higher autotrophic biomass (Elser et al., 2007), thus confounding the attribution of changes in consumer dynamics solely to shifts in resource stoichiometry. In contrast, nutrient enrichment of detritus-based ecosystems increases activity of microbial decomposers, thereby accelerating rates of decomposition and reducing detrital resource quantity (Benstead et al., 2009; Rosemond et al., 2015). At the same time, increased microbial biomass typically produces large shifts in detrital stoichiometry in response to nutrient enrichment (Manning et al., 2015).

We predicted that N and P fertilization, and subsequent shifts in basal resource stoichiometry, would alter the taxonomic structure of macroinvertebrate communities in the study streams by differentially affecting individual consumer populations based upon principles of EST. Overall, we expected a greater response to reduced resource C:P, resulting from increased stream water P concentrations (Demi, Benstead, Rosemond, & Maerz, 2018) than to reduced C:N, due to the greater relative imbalances between body and resource C:P for most consumers in detritus-based headwater streams (Cross, Benstead, Rosemond, & Wallace, 2003).

We further predicted that changes in community structure would be driven by a greater response of leaf-shredding macroinvertebrates to nutrient enrichment than of consumers from other functional feeding groups (hereafter FFGs), as shredders are likely to experience the largest imbalances between body and resource stoichiometry among FFGs (due to high C:N:P of leaf litter compared to other resources; Cross et al., 2003; Martinson et al., 2008; Tant, Rosemond, & First, 2013). Furthermore, weaker responses among other FFGs, including detritivorous collector-gatherers, were expected given the relatively limited stoichiometric plasticity of some resources in response to nutrient enrichment (i.e., fine particulate organic matter; Tant et al., 2013), the limited contributions of other FFGs to total community biomass (i.e., scrapers and filtering collectors) and the relative scarcity and minimal contributions of other food resources (i.e., diatoms and wood) to consumer diets in detritus-based streams (Cross, Wallace, & Rosemond, 2007).

As such, we hypothesized that leaf litter C:P would be a significant driver of overall patterns of community composition, given its characteristically high C:P (Cross et al., 2003; Martinson et al., 2008), generally greater response to elevated nutrient concentrations than other common resource pools (Tant et al., 2013), and large role in driving patterns of invertebrate production in forested streams (Cross et al., 2007; Demi et al., 2018; Wallace, Eggert, Meyer, & Webster, 1999). Furthermore, we predicted that leaf litter C:P, but not C:N, would be a significant driver of shredder biomass, both at the assemblage-level and among common shredder taxa, and that among those taxa, those with low body C:P would exhibit greater responses to nutrient enrichment than those with high body C:P (due to their larger elemental imbalances under ambient conditions). Lastly, we predicted that responses to nutrient enrichment would be related to other taxon-level traits. Specifically, we predicted that shredder response would be positively related to body size and growth rates, as large and fast-growing taxa were expected to experience greater P limitation. Conversely, we predicted a negative relationship between shredder response to enrichment and both body C:P and time to maturation, as individuals with low body C:P and rapid maturation times were expected to experience greater P limitation.

2 | MATERIALS AND METHODS

2.1 | Study sites

This study was performed at the Coweeta Hydrologic Laboratory (hereafter Coweeta), a Long Term Ecological Research (LTER) Site and United States Forest Service research station located in Macon County, North Carolina, USA. A detailed description of the study sites can be found in Supporting information Appendix S1. Briefly, we established 70-m reaches in five headwater streams for continuous experimental N (21% ammonium nitrate) and P (85% phosphoric acid) additions, which commenced in July 2011 and were maintained continuously until June 2013 (hereafter YR1 = July 2011–June 2012; YR2 = July 2012–June 2013). We characterized reference conditions in each of the five streams by collecting a year of PRE-enrichment data at monthly intervals, beginning in July 2010 and continuing until June 2011 (hereafter PRE).

Each of the five streams received dissolved inorganic N (DIN) and soluble reactive P (SRP) at different target concentrations (DIN range: 96–472 µg/L; SRP range: 10–85 µg/L), in which the low SRP concentrations were paired with high DIN concentrations and vice versa. As a result, each of the five streams received N and P at different molar N:P ratios (target ratios: 2:1, 8:1, 16:1, 32:1 and 128:1). A comprehensive summary of target and measured DIN and SRP concentrations and ratios can be found in Bumpers, Maerz, Rosemond, and Benstead (2015). N and P fertilization was achieved using solar-powered metering pumps (LMI Milton Roy, Ivyland, PA, USA), which delivered the concentrated nutrient mixture to a gravity-fed irrigation line in which it was mixed with ambient stream water and dispersed via drip spouts at roughly 5-m intervals throughout each 70-m reach. Pump rate was maintained in proportion to instantaneous discharge measured by a Nanolevel pressure transducer

(Keller America, Newport News, VA, USA) connected to a Campbell CR800 data logger (Campbell Scientific, Logan, UT, USA). More details on the dosing infrastructure can be found in Manning et al. (2015) and Rosemond et al. (2015).

2.2 | Benthic macroinvertebrates and basal resources

We began collecting monthly benthic macroinvertebrate samples from each stream during July 2010 and continued through June 2013. Each month, we collected four quantitative samples from mixed-substrate habitat (i.e., sand, gravel, cobble) at random locations within each 70-m experimental reach using a stovepipe core sampler (490 cm²). Sampling and sample processing procedure largely followed that of Lugthart and Wallace (1992), as summarized in the Supplementary Methods (Appendix S1).

We constrained our analysis of basal resources to leaf litter biomass and stoichiometry as previous studies have identified leaf litter as the dominant basal resource input for benthic macroinvertebrate communities in Coweeta streams (Cross et al., 2007; Wallace et al., 1999) and because patterns in leaf litter stoichiometry in these streams are similar to those of other resource pools (i.e., wood and biofilm; A. D. Rosemond, *unpublished data*), which contribute little to total organic matter consumption by the invertebrate communities (wood = ~11%, diatoms ~1%; L. M. Demi, *unpublished data*). Bulk leaf litter samples for quantification of biomass and C, N and P content were collected and processed monthly (from randomly assigned transects) following methods detailed in Rosemond et al. (2015) and Manning et al. (2015). All elemental ratios are presented as molar ratios.

2.3 | Data analysis

All analyses, except for the SIMPER and linear regression analysis (see below), were performed using abundance and biomass data. We present only the biomass results here but provide results for the abundance analyses as Supporting information (Table S1 and Figure S1). We chose to present biomass rather than abundance because accumulation of biomass represents an important ecosystem function and is therefore a more functionally explicit metric than abundance alone. Furthermore, changes in biomass observed in this study are largely a function of altered abundance rather than the effect of nutrient enrichment on consumer body size (Demi et al., 2018). As such, analyses performed with biomass and abundance data produced similar results and interpretation. A summary of the effects of nutrient enrichment on species richness is also provided in the Supporting information.

We used analysis of variance (ANOVA) to test for differences in species richness among years (PRE, YR1 and YR2; results provided in Appendix S2). Additionally, we used ANOVA to test for differences in total biomass, as well as biomass of each FFG, among years. To test for nutrient effects, treatments YR1 and YR2 were reduced into a single level (ENR) and compared to PRE-enrichment communities

(factor = treatment). Differences between the two treatment years were tested using a nested design, in which enrichments YR1 and YR2 were nested within the treatment level ENR (factor = year). These analyses were performed using annual, autumn/winter and spring/summer biomass estimates. Data were natural log-transformed as necessary to satisfy model assumptions of normality and homogeneity of variance.

We used non-metric multidimensional scaling (NMDS, function "metaMDS" in R package "vegan"; Oksanen et al., 2015) to graphically represent patterns of macroinvertebrate community composition in response to nutrient enrichment (see Appendix S1). We related patterns of community composition to measures of leaf litter stoichiometry (C:N, C:P) and biomass, water chemistry (DIN and SRP) and discharge by overlaying appropriate vectors onto the NMDS ordinations using the "envfit" function in the R package "vegan" (Oksanen et al., 2015). The "envfit" function tests fit by generating squared correlation coefficients and *P*-values based on random permutations ($n = 999$) of the data (Oksanen et al., 2015). Only vectors for which $p < 0.05$ were added to the NMDS plots.

We tested for the effect of nutrient enrichment, season and among-stream differences in macroinvertebrate community composition using permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) with the "adonis" function in the R package "vegan" (Oksanen et al., 2015). To test for nutrient enrichment effects on macroinvertebrate community composition, treatments YR1 and YR2 were reduced into a single level (ENR) and compared to PRE communities. Differences between the two treatment years were tested using a nested design as above. Seasonal differences in community composition were tested using a nested design in which both year (YR1 and YR2) and season (autumn/winter and spring/summer) were nested within treatment (PRE and ENR). We performed a similarity percentage (hereafter SIMPER) analysis using the function "simper" in the R package "vegan" (Oksanen et al., 2015) to assess contributions of individual taxa to pairwise community dissimilarities (PRE vs. ENR) based on autumn/winter biomass data. The decision to use autumn/winter biomass was based upon the outcome of the NMDS and PERMANOVA analyses (see Section 2).

Further tests of ecological stoichiometry theory were performed using shredder biomass. We performed linear regression analysis to test for significant slopes in the relationship between autumn/winter biomass and leaf litter C:P for the entire shredder assemblage, and each of the nine individual shredder taxa that were collected during all 3 years, in each of the five study streams. Additionally, we calculated natural-log response ratios of annual biomass during the two years of enrichment for the nine individual shredder taxa by taking the natural log of the ratio between either YR1 or YR2, and PRE-enrichment biomass (e.g., $\ln[YR1/PRE]$). We then performed linear regression analyses to test for significant slopes in the relationship between biomass response ratios and the following four predictor variables estimated for each taxon: body C:P (using data from Cross et al., 2003), mean body size (g AFDM), cohort production interval (a measure of life span in days;

hereafter CPI, using data from Huryn & Wallace, 1987a) and approximate growth rate (calculated as final body size/CPI). Body C:P data were measured from individuals collected from a nearby stream at Coweeta (Cross et al., 2003) and used with the assumption that they provide an accurate representation of body C:P of taxa from the five streams in the present study during ambient (i.e. PRE-enrichment) conditions, given the close spatial proximity and similarities in nutrient chemistry among Coweeta streams. For taxa in this analysis for which body C:P was not provided in Cross et al. (2003), the average body C:P reported by Cross et al. (2003) for that taxon's order was used. Additionally, for taxa from which Cross et al. (2003) provide more than one estimate of body C:P for a given taxon (i.e., for multiple size classes), we used the mean of those estimates. All data used in linear regression analyses were natural log-transformed as needed to accommodate model assumptions.

3 | RESULTS

3.1 | Nutrient effects on patterns in community composition

Overall community composition based on annual biomass shifted following nutrient enrichment (Figure 1a), with significant differences between PRE-enrichment and nutrient-enriched communities (Table 1), but no differences in community composition between enrichments YR1 and YR2 (Table 1). Leaf litter C:P and biomass, SRP, DIN and discharge were significantly correlated with the community ordination (Table 2, Figure 1a), where communities from the two years of enrichment were associated with elevated SRP and DIN and reduced leaf litter biomass and leaf litter C:P. There were additional differences in community composition among streams that persisted during the two years of nutrient enrichment (Figure 1a, Table 1) that were best described by variation in discharge, with communities from the 8:1 and 2:1 target N:P ratio streams associated with high and low discharge, respectively.

Seasonal biomass patterns revealed differential effects of nutrient enrichment on community composition among seasons. Specifically, autumn/winter, but not spring/summer, communities changed in composition with nutrient enrichment relative to pre-enrichment conditions (Figure 1b,c, Table 1). Overall, patterns of macroinvertebrate community composition during autumn/winter were similar to those for mean annual biomass, as shifts in community composition following enrichment were driven by elevated N and P concentrations and reduced leaf litter C:P (Figure 1c, Table 2). Significant stream effects on community composition were present during both spring/summer and autumn/winter seasons, again highlighting among-stream variation in macroinvertebrate community composition (Table 1). Among the environmental variables measured, patterns of discharge best-described among-stream variation in community composition during the spring/summer (Figure 1b, Table 2), but were not significantly correlated with the community ordination during autumn/winter (Figure 1c, Table 2).

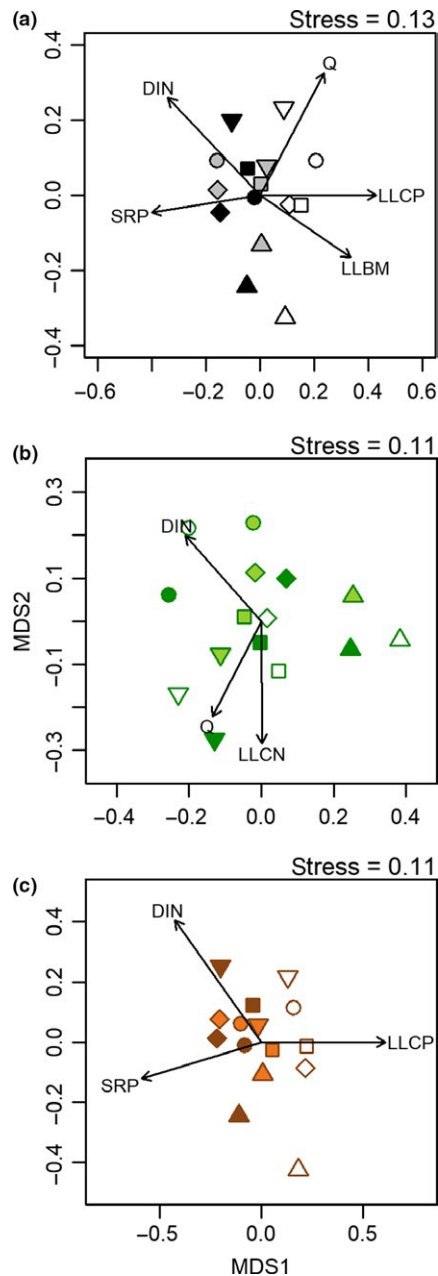


FIGURE 1 Two-dimensional community ordinations based on annual biomass (a), spring/summer biomass (b) and autumn/winter biomass (c). In all panels open symbols represent PRE-enrichment communities. In panel a, grey and black symbols represent YR1 and YR2 of enrichment, respectively. In panel b light green and dark green symbols represent spring/summer biomass during YR1 and YR2 of enrichment, respectively. In panel c light brown and dark brown symbols represent autumn/winter biomass during YR1 and YR2 of enrichment, respectively. Triangles represent the 2:1 target N:P ratio stream, inverted triangles the 8:1 stream, diamonds the 16:1 stream, circles the 32:1 stream and squares the 128:1 stream. Vectors represent environmental variables that were significantly correlated ($\alpha = 0.05$) with the community ordination (see Table 2). SRP: soluble reactive phosphorus; DIN: dissolved inorganic nitrogen; Q = discharge; LLCN, LLCP and LLBM: leaf litter C:N, C:P and biomass, respectively. Stress values are a measure of goodness of fit for the two-dimensional community ordination, where values <0.2 are considered robust representations

TABLE 1 Summary of PERMANOVA tests of differences in community composition among streams (factor = stream) and in response to nutrient enrichment

Timeframe	Factor	df	F-stat	R ²	p-value
Annual	Stream	4,10	2.26	0.47	0.001*
	Treatment	1,12	2.12	0.14	0.002*
	Year	1,12	0.73	0.05	0.38
Spring/summer	Stream	4,10	2.89	0.54	0.001*
	Treatment	1,12	0.97	0.07	0.49
	Year	1,12	0.93	0.07	0.47
Autumn/winter	Stream	4,10	1.73	0.41	0.013
	Treatment	1,12	3.38	0.21	0.003*
	Year	1,12	1.03	0.06	0.38

Notes. Treatment tests for nutrient enrichment effects (PRE vs. ENR), while year tests for differences between the two treatment years (YR1 and YR2), includes annual biomass and seasonal biomass.

Bold font indicates statistical significance at $\alpha = 0.05$.

*P-value <0.01

3.2 | Nutrient effects on total community biomass and functional feeding group composition

Total annual macroinvertebrate biomass averaged 1.0 g AFDM/m^2 (range = $0.8\text{--}1.3 \text{ g AFDM/m}^2$) across the five streams prior to nutrient enrichment and increased to an average of 1.5 g AFDM/m^2 (range = $1.1\text{--}2.3 \text{ g AFDM/m}^2$; Table 3, Figure 2) during the two years of nutrient enrichment. Biomass of both shredders and scrapers significantly increased following nutrient enrichment (Table 3, Figure 2), though scraper biomass remained a relatively small proportion of total biomass ($<5\%$) during the three years of study. Neither shredder nor scraper biomass differed between the 2 years of enrichment (Table 3). Mean predator biomass showed a similar pattern of increase between PRE and ENR years (Figure 2); however, the ENR response by predators was highly variable, ranging from -38% to 293% during the two years of enrichment, and not statistically significant (Table 3). Biomass of the remaining FFGs did not respond to nutrient enrichment (Table 3, Figure 2).

When analysed by season, total biomass increased due to enrichment during autumn/winter (mean = 2.0 g AFDM/m^2 ; range = $1.1\text{--}3.0 \text{ g AFDM/m}^2$; Table 3, Figure 2), but not spring/summer (Table 3). Increases in autumn/winter biomass were again largely driven by increased biomass of shredders (% change range = $31\%\text{--}300\%$; Table 3, Figure 2), which accounted for $\sim 40\%$ (range = $14\%\text{--}64\%$) of total autumn/winter biomass. Apparent increases in predator biomass during autumn/winter were not statistically significant (Table 3, Figure 2), again reflecting the variable response of predators to nutrient enrichment (% change range in autumn/winter = $-16\%\text{--}598\%$). Though there was no significant difference in assemblage-level predator biomass following enrichment, predators and shredders accounted for 27.7% and 26.3% of community dissimilarities between PRE and nutrient-enriched communities based upon autumn/winter biomass (Table 4). The largest individual contributors to shifts

Timeframe	Factor	NMDS1	NMDS2	r^2	p -value
Annual	DIN	-0.795	0.606	0.63	0.002*
	SRP	-0.936	-0.113	0.56	0.008*
	Q	0.590	0.807	0.56	0.004*
	LL CN	0.994	-0.112	0.16	0.36
	LL CP	1.000	0.000	0.63	0.002*
	LL BM	0.896	-0.443	0.48	0.012
Spring/summer	DIN	-0.724	0.690	0.52	0.013
	SRP	0.456	0.890	0.03	0.81
	Q	-0.520	-0.854	0.42	0.034
	LL CN	0.007	-0.100	0.50	0.016
	LL CP	-0.025	-0.100	0.10	0.50
	LL BM	-0.768	0.641	0.08	0.58
Autumn/winter	DIN	-0.724	-0.690	0.66	0.005*
	SRP	-0.980	0.200	0.69	0.003*
	Q	0.372	-0.929	0.36	0.064
	LL CN	0.948	0.318	0.31	0.11
	LL CP	1.000	0.000	0.71	0.001*
	LL BM	-0.998	-0.058	0.02	0.87

Notes. NMDS1 and NMDS2 represent coordinates of vector arrow endpoints. Plotted vectors (Figure 1) are scaled by multiplying these coordinates by the correlation coefficient (r), so that stronger relationships are represented by longer vectors, and a constant multiplier to ensure that vectors fit within the plot space.

Bold font indicates statistical significance at $\alpha = 0.05$ based upon 1,000 random permutations.

* P -value < 0.01.

TABLE 2 Results of “envfit” (R package “vegan”) tests for correlations between environmental variables (dissolved inorganic N [DIN], soluble reactive P [SRP], discharge [Q], leaf litter biomass [LL BM] and stoichiometry [LL CP and LL CN]) and the macroinvertebrate community ordination

in community composition (quantified as taxon-level biomass) were two large-bodied dragonflies (*Lanthus* and *Cordulegaster*) and three common, large shredders (*Pycnopsyche*, *Tipula* and *Tallaperla*; a caddisfly, dipteran and stonefly, respectively), which cumulatively accounted for ~28% of the differences between communities in PRE and nutrient-enriched years (Table 4). Among those five taxa, only *Cordulegaster* experienced an average decrease in biomass across the five streams following enrichment.

Scrapers, though accounting for only a small fraction of biomass, were the only other FFG to experience an average increase in biomass during autumn/winter, relative to PRE-enrichment conditions (Table 3, Figure 2). Scrapers cumulatively accounted for only 7.8% of the differences between PRE and nutrient-enriched communities (Table 4). Despite the lack of an assemblage-level biomass response to nutrient enrichment (Table 3, Figure 2), collector-gatherers were cumulatively responsible for 16.4% of the dissimilarity in community composition during enrichment (Table 4). Two collector-gatherer taxa were among the ten that contributed most to community dissimilarity following enrichment, with non-Tanypodinae midges experiencing an average increase in biomass, and *Leptotarsus*, also a dipteran, a decrease in biomass, following nutrient enrichment (Table 4). Biomass of all FFGs was unaffected by enrichment during spring/summer (Table 3, Figure 2).

3.3 | Taxon-specific responses of shredders to nutrient enrichment

Biomass of the entire shredder assemblage during/autumn winter was significantly negatively related to leaf litter C:P (Table 5, Figure 3), which explained 70% of the variation in autumn/winter biomass. Among the nine common shredder taxa analysed, there was a significant negative relationship between autumn/winter biomass and leaf litter C:P (Table 5, Figure 3) for four taxa, including three stoneflies (Plecoptera) and the caddisfly *Pycnopsyche*, and a marginal negative relationship between biomass and leaf litter C:P for an additional taxon, the caddisfly *Lepidostoma* (Table 5, Figure 3). Autumn/winter biomass of the remaining taxa was unrelated to leaf litter C:P (Table 5).

Response ratios of annual biomass for the same nine shredder taxa were not significantly related to body C:P, CPI or approximate growth rates (Table 6), but were marginally negatively related to body size (Table 6). Response ratios of autumn/winter biomass were marginally negatively related to CPI (Table 6), suggesting that shorter living taxa may have been more sensitive to altered resource stoichiometry than longer lived taxa over the 2 years of nutrient enrichment. Response ratios of autumn/winter biomass were not related to either body size or C:P, or approximate growth

TABLE 3 Summary of ANOVA tests for differences in biomass for the whole community, and each FFG, following nutrient enrichment

Timeframe	FFG	Factor	df	F-stat	p-value
Annual	CF	Treatment	1,12	0.00	0.99
		Year	1,12	0.59	0.46
	CG	Treatment	1,12	0.22	0.65
		Year	1,12	0.03	0.88
	Pr	Treatment	1,12	1.26	0.28
		Year	1,12	0.02	0.88
	Sc	Treatment	1,12	5.35	0.039
		Year	1,12	3.93	0.071
	Sh	Treatment	1,12	12.01	0.005*
		Year	1,12	0.59	0.46
	Total	Treatment	1,12	5.34	0.040
		Year	1,12	0.24	0.64
Spring/ Summer	CF	Treatment	1,12	0.08	0.78
		Year	1,12	0.01	0.92
	CG	Treatment	1,12	1.57	0.23
		Year	1,12	0.85	0.37
	Pr	Treatment	1,12	0.00	0.99
		Year	1,12	0.35	0.57
	Sc	Treatment	1,12	2.61	0.13
		Year	1,12	2.47	0.14
	Sh	Treatment	1,12	0.34	0.57
		Year	1,12	1.51	0.24
	Total	Treatment	1,12	0.00	0.97
		Year	1,12	1.41	0.26
Autumn/ winter	CF	Treatment	1,12	0.05	0.83
		Year	1,12	1.48	0.25
	CG	Treatment	1,12	3.06	0.11
		Year	1,12	1.03	0.33
	Pr	Treatment	1,12	2.23	0.16
		Year	1,12	0.01	0.93
	Sc	Treatment	1,12	9.62	0.009*
		Year	1,12	11.19	0.006*
	Sh	Treatment	1,12	10.39	0.007*
		Year	1,12	4.19	0.063
	Total	Treatment	1,12	9.47	0.010*
		Year	1,12	1.71	0.22

Notes. Treatment tests for nutrient enrichment effects (PRE vs. ENR), while year tests for differences between the two treatment years (YR1 and YR2). Functional feeding groups (FFGs): CF: collector-filterer; CG: collector-gatherer; Pr: predator; Sc: scraper; Sh: shredder. Bold font indicates statistical significance at $\alpha = 0.05$.

*P-value < 0.01.

rate (Table 6). Spring/summer biomass response ratios were unrelated to body C:P, body size, CPI or approximate growth rate (Table 6).

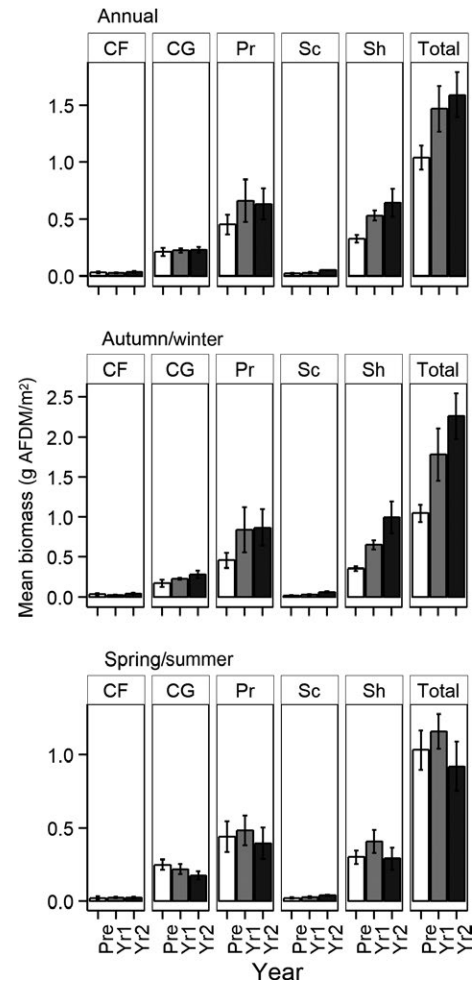


FIGURE 2 Mean biomass ($\pm$ SE) by functional feeding group using annual, autumn/winter and spring/summer biomass. PRE refers to the PRE-enrichment reference year and YR1 and YR2 the first and second year of nutrient enrichment, respectively. CF: collector filterer; CG: collector gatherer; Pr: predator; Sc: scraper; Sh: all shredders

4 | DISCUSSION

4.1 | Resource stoichiometry drives patterns of community composition

Shifts in macroinvertebrate community composition occurred following experimental nutrient enrichment and were positively correlated with reduced leaf litter C:P and reduced biomass of leaf litter. Changes in leaf litter quality and quantity in this study were a result of microbial colonization and mineralization, stimulated by N and P fertilization (Demi et al., 2018; Manning et al., 2016). Previous studies of macroinvertebrate communities in Coweeta streams have reported strong positive relationships between leaf litter biomass and macroinvertebrate biomass and production (Wallace et al., 1999; Wallace, Eggert, Meyer, & Webster, 2015). As such, we would expect reduced leaf litter biomass to produce shifts in community composition characterized by decreases in shredder and total consumer biomass. Nevertheless, nutrient-enriched communities were

TABLE 4 Dissimilarity percentages for the top ten taxa by per cent contribution and cumulative dissimilarity percentages for all taxa from each functional feeding group (FFG), based on autumn/winter biomass from PRE and ENR communities (both enrichment years combined)

	Order	FFG	Dissimilarity Contribution	Cumulative contribution	PRE	ENR	% Change
Taxon							
<i>Lanthus</i>	O	Pr	9.8	9.8	242	597	147
<i>Pycnopsyche</i>	T	Sh	5.0	14.8	23	120	422
<i>Tipula</i>	D	Sh	4.4	19.1	177	345	95
<i>Tallaperla</i>	P	Sh	4.3	23.4	34	130	282
<i>Cordulegaster</i>	O	Pr	4.0	27.5	86	54	-37
<i>Leptotarsus</i>	D	CG	3.5	31.0	34	25	-26
Non-Tanyptodinae	D	CG	2.7	33.6	46	106	130
<i>Allocaupnia</i>	P	Sh	2.5	36.1	2	19	850
<i>Fattigia</i>	T	Sh	2.2	38.4	49	84	71
<i>Hexatoma</i>	D	Pr	2.2	40.6	9	18	100
FFG							
Predators	-	Pr	-	27.7	459	854	86
Shredders	-	Sh	-	26.3	355	824	132
Collector-gatherers	-	CG	-	16.4	174	257	48
Scrapers	-	Sc	-	7.8	20	46	130
Collector-filterers	-	CF	-	7.6	34	37	9

Notes. O = Odonata; T = Trichoptera; D = Diptera; P = Plecoptera. Contribution is the per cent of community dissimilarity explained by each taxon. PRE and ENR are mean biomass (mg ash-free dry mass m⁻²) across all five streams from the PRE-enrichment year and two years of nutrient enrichment, respectively. % Change is the per cent change in biomass between PRE and ENR. Taxa with unknown functional feeding group designations account for the remaining 14.2% of community dissimilarity between PRE and ENR. Simper analysis based on Bray-Curtis dissimilarities. Cumulative contribution for FFGs considers the whole community, including taxa not listed in the table.

Taxon	Order	df	F-stat	t-value	R ²	p-value	m
All shredders	-	1,13	30.31	-5.51	0.70	0.0001*	-0.00063
<i>Allocaupnia</i>	P	1,13	11.98	-3.46	0.48	0.004*	-0.0020
<i>Fattigia</i>	T	1,13	2.06	-1.44	0.14	0.17	
<i>Lepidostoma</i>	T	1,13	4.19	-1.74	0.24	0.06	-0.0013
<i>Leuctra</i>	P	1,13	10.25	-3.20	0.44	0.007*	-0.00079
<i>Molophilus</i>	D	1,13	0.02	0.15	0.00	0.89	
<i>Psilotreta</i>	T	1,13	0.23	-0.48	0.02	0.64	
<i>Pycnopsyche</i>	T	1,13	23.34	-4.83	0.64	0.003*	-0.0015
<i>Tallaperla</i>	P	1,13	12.14	-3.48	0.48	0.004*	-0.0016
<i>Tipula</i>	D	1,13	2.37	-1.54	0.15	0.15	

Notes. Slopes (*m*) are provided only for models where *p*-value < 0.10. Bold font indicates statistical significance at $\alpha = 0.05$, and *italic* font indicates *p* < 0.10.

**p*-value < 0.01.

TABLE 5 Summary of linear regression results examining potential relationships between shredder biomass (ln[mg ash-free dry mass m⁻²]) during autumn/winter and leaf litter C:P

characterized by higher shredder, predator and total biomass than communities during the year prior to nutrient enrichment, suggesting that the observed changes in community composition were most likely driven by reduced stoichiometric constraints on consumer biomass, which is consistent with principles of EST.

Though our analysis provides compelling evidence of a role for resource C:P in modulating patterns of community composition, we

failed to see evidence of an effect of dissolved N:P ratio along our experimental gradient, as the responses of individual streams to nutrient enrichment were similar in direction and magnitude despite large differences in target dissolved N:P ratios (and concentrations) among the five study streams. Given that resource C:P was predicted to drive community response to nutrient enrichment, a greater response might be predicted in streams that occupied the lower end of

our N:P gradient (i.e. 2:1 and 8:1). These streams received the highest doses of SRP and therefore experienced greater reductions in leaf litter %P than the high target N:P streams (Demi et al., 2018).

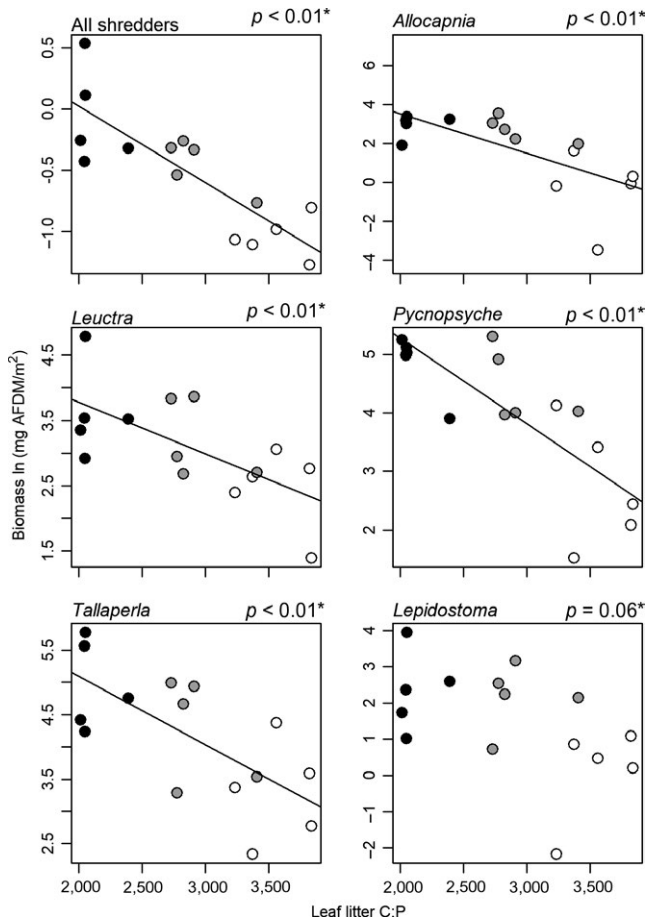


FIGURE 3 Biomass ($\ln[\text{mg AFDM}/\text{m}^2]$) during autumn/winter against leaf litter C:P for all shredders, and several common shredder taxa. Open circles represent the PRE-enrichment year, while grey and black circles represent YR1 and YR2 of nutrient enrichment, respectively

However, there was some convergence in litter C:P content among streams, despite the wide range in supply of N and P in our experimental design (Manning et al., 2016). This pattern was presumably driven by a combination of luxury P uptake and storage by fungal decomposers and nutrient effects on fungal biomass (Manning et al., 2015), as fungal uptake of P was relatively low at low P (and high N) supply, and higher at intermediate to high P (with intermediate and low N, respectively) supply, where fungal growth and biomass is more likely N-limited (Gulis et al., 2017). Thus, consumer communities were responding more to the integrated responses of microorganisms to variation in dissolved nutrients, rather than dissolved nutrient concentrations per se. Nevertheless, the consistent and similar response of community composition to enrichment among streams illustrates the primacy of P limitation for macroinvertebrate communities of detritus-based streams.

The effect of nutrient enrichment on seasonal patterns of community composition was constrained to the autumn/winter seasons. These seasonal differences may reflect the influence of phenology on community composition, as many macroinvertebrate taxa in temperate forest streams have evolved life cycles that exploit seasonal pulses of allochthonous subsidies (i.e., autumn-shed leaves; Huryn & Wallace, 1987b; Huryn & Wallace, 1988). Additionally, semi-voltine taxa may experience periods of energy limitation during spring/summer under elevated nutrient regimes, due to accelerated rates of detrital mass loss (Cross, Johnson, Wallace, & Rosemond, 2005; Rosemond et al., 2015). Similarly, long-lived predator taxa may be forced to disperse if prey availability decreases during spring/summer, which could influence their ability to respond to nutrient enrichment relative to periods when prey is more abundant. Additionally, we found no differences in community structure between the two years of nutrient fertilization, despite a clear difference between PRE-enrichment and nutrient-enriched communities. Shifts in community composition may be expected to accumulate over time as higher rates of egg production and oviposition produce continually increasing

TABLE 6 Linear regression results for models using mean response ratios ($\ln[\text{Enr}/\text{Pre}]$) of shredder biomass and body C:P, maximum individual body size ($\ln[\text{mg ash-free dry mass}]$), cohort production interval (days, CPI) and approximate growth rate ($\ln[\text{body size}/\text{CPI}]$) of the nine shredder taxa (Plecoptera: *Tallaperla*, *Leuctra*, *Allocapnia*; Trichoptera: *Pycnopsyche*, *Psilotreta*, *Fattigia*, *Lepidostomatidae*; Diptera: *Tipulidae*, *Molophilus*)

Timeframe	Predictor	df	F-stat	t-value	R ²	p-value	m
Annual	Body C:P	1,7	1.10	1.05	0.14	0.33	
	Body size	1,7	4.04	-2.09	0.37	0.074	-0.29
	CPI	1,7	2.97	-1.72	0.30	0.13	
	Growth rate	1,7	1.81	-1.26	0.21	0.25	
Spring/Summer	Body C:P	1,7	2.18	1.48	0.24	0.18	
	Body size	1,7	1.34	-1.80	0.16	0.11	
	CPI	1,7	0.56	-0.75	0.07	0.48	
	Growth rate	1,7	0.92	-1.50	0.12	0.18	
Autumn/Winter	Body C:P	1,7	0.69	0.83	0.09	0.43	
	Body size	1,7	4.34	-1.84	0.38	0.11	
	CPI	1,7	5.02	-2.24	0.42	0.060	-0.0059
	Growth rate	1,7	1.67	-0.96	0.19	0.37	

Notes. Slopes (m) are provided only for models where p-value < 0.10.
 $\alpha = 0.05$, italic font indicates $p < 0.10$.

population sizes. However, the potential for shifts in biomass and production to accumulate within the experimental reaches of our study streams may be limited by the dispersal abilities of the local species pool and their proximity to potential source populations from unfertilized upstream reaches and nearby streams. This may also explain the lack of a species richness response to nutrient enrichment in the five study streams.

4.2 | Contrasting responses to nutrient enrichment among functional groups and populations

Shifts in community composition were largely constrained to shredders and predatory macroinvertebrates, both of which increased in biomass, on average, following nutrient enrichment. The positive response of predators is likely a response to increased prey availability, although Demi et al. (2018) reported that predator production did not track primary consumer production linearly during this study. A positive response among shredders was expected given that reductions in leaf litter C:P would lower their characteristically large consumer/resource elemental imbalances (Cross et al., 2003; Tant et al., 2013). In contrast, a threshold elemental ratio analysis by Tant et al. (2013) suggests that typical collector–gatherers in Coweeta streams may be C- rather than N- or P-limited. Thus, we predicted that collector–gatherer taxa would respond positively if the C:P or C:N of food resources increased, a pattern opposite to that predicted for shredders and leaf litter. However, assemblage-level biomass of collector–gatherers did not respond to nutrient enrichment in this study, even though C:N and C:P of fine particulate organic matter (hereafter FPOM) generally increased relative to reference conditions (A.D. Rosemond, *unpublished data*). Increases in scraper biomass following enrichment were primarily driven by increased biomass of the mayfly *Maccaffertium*, which accounted for 45% of total scraper biomass averaged across all streams prior to nutrient enrichment and 61% during the two years of enrichment. Increases in scraper biomass appear to be driven by greater algal biomass in the study streams during YR1 and YR2, as nutrient enrichment resulted in increased chlorophyll *a* content of biofilms (A. D. Rosemond, *unpublished data*), as well as increased diatom consumption by *Maccaffertium* (L. M. Demi, *unpublished data*).

Shredders and predators combined to account for 54% of community dissimilarity between PRE-enrichment and enriched communities. Of the 10 taxa that contributed most to community dissimilarity, five were shredders, all of which experienced an average increase in biomass across the five streams following nutrient enrichment. The remaining five taxa were either predators (3 taxa) or collector–gatherers (2 taxa). Collector–gatherers contributed the third highest cumulative percentage to community dissimilarity among FFGs in this study despite their lack of an assemblage-level biomass response, thereby highlighting the divergent responses of individual taxa to nutrient enrichment.

Of the three predator taxa that contributed most to community dissimilarity, only *Cordulegaster* experienced an average decrease in biomass across the five streams. *Cordulegaster* has a considerably

longer aquatic life stage than the other two predators (1,140 days compared to 660 days and 365 days for *Lanthus* and *Hexatoma*, respectively; Lugthart & Wallace, 1992), which may increase its susceptibility to periods of resource scarcity, including loss of leaf pack habitat (Cross et al., 2005; Manning et al., 2016; Rosemond et al., 2015). Among collector–gatherers, the positive response of non-Tanyptodinae midges to nutrient enrichment may reflect relief from C-limitation given increases in FPOM C:nutrient ratios. However, their relatively high body P content (Cross et al., 2003) and substantial reliance on leaf litter (which accounts for >30% of annual production; L. M. Demi, *unpublished data*) suggest that their positive response may alternately be driven by reduced dietary P constraints. In contrast, biomass of the crane fly *Leptotarsus* decreased on average following nutrient enrichment, a pattern that suggests increasing N or P limitation in response to increasing FPOM C:nutrient ratios. Estimates of body P content from members of the family Tipulidae (%P = 0.35–0.80, C:P = 160–370; Cross et al., 2003) indicate the potential for P limitation of *Leptotarsus* in Coweeta streams, especially considering that FPOM biomass was unaffected by nutrient enrichment (*data not shown*; ANOVA: $F_{12,2} = 2.0$, $p = 0.18$). Nevertheless, we suggest that these patterns be interpreted with caution, as additional, non-dietary mechanisms (i.e. loss of habitat, predation, competition) cannot be ruled out as drivers of the divergent responses among some predator and collector–gatherer taxa in these streams.

We performed a detailed analysis of shredder responses to nutrient enrichment to provide a more robust test of EST on a functional group whose primary food resource has particularly high C:nutrient ratios relative to their body tissue (Cross et al., 2003). Leaf litter C:P was a strong predictor of biomass for the entire shredder assemblage, explaining 70% of the variation in biomass over the course of the study, a result that was expected given the greater imbalance between shredder and leaf litter C:P than C:N. However, among the nine common shredder taxa analysed, the relationship between autumn/winter biomass and leaf litter C:P produced negative slopes for only five. This result is consistent with those of Prater et al. (2015), which reported increased biomass of some, but not all, shredder taxa in relation to higher detrital nutrient content in streams in Arkansas, USA. One potential explanation is that the C:P ratio of bulk leaf litter may not accurately represent consumer–resource elemental imbalances for some taxa (Halvorson & Small, 2016). Many shredders are highly selective consumers of leaf litter, with the ability to differentiate among different litter species, stages of decomposition (Cummins & Klug, 1979) and individual fungal species (Arsuffi & Suberkropp, 1989). This behaviour may function as a mechanism for minimizing consumer–resource elemental imbalances.

Furthermore, rare or occasional consumption of nutrient-rich resources, such as algae or animal tissue, may supply sufficient P or N to an otherwise nutritionally poor diet (Crenier et al., 2017). In this study, the caddisfly *Psilotreta* was among the four shredders that did not respond to shifts in leaf litter C:P, and was the only common shredder taxon for which animal material was regularly found in gut contents (L. M. Demi, *unpublished data*). Finally, other factors such as habitat characteristics (i.e. discharge, substrate composition) or

additional biological interactions (i.e. predation, competition) may impose stricter constraints on biomass of some taxa than resource stoichiometry, as evidenced by the role of discharge in modulating differences in community composition among the five streams in this study.

We further tested predictions of ecological stoichiometry by analysing potential relationships between the biomass response of individual shredder taxa and their body C:P, body size, CPI and approximate growth rate. The biomass response of the nine common shredder taxa analysed was unrelated to their body C:P, which was used as a proxy for C:P demand, or to approximate growth rate. The lack of relationship between body C:P and shredder biomass response may reflect differences between the body C:P of the nine shredder taxa in our study streams and the values obtained from Cross et al. (2003), which came from a nearby stream at Coweeta. However, Evans-White, Stelzer, and Lamberti (2005) reported that the elemental stoichiometry of aquatic macroinvertebrates typically varies little, within species, across much larger spatial scales. Nevertheless, values from Cross et al. (2003) may not sufficiently account for factors such as body size, ontogeny, sex and variation in elemental content along resource stoichiometry gradients, all of which may constrain the accuracy of body stoichiometry estimates in natural systems (Halvorson & Small, 2016). Indeed, stoichiometric plasticity has been reported for some of the taxa included in this analysis (Cross et al., 2003), which may modify their response to shifts in resource stoichiometry, relative to more stoichiometrically homeostatic taxa. The marginally significant negative slopes for shredder biomass response and body size (annual) and CPI (autumn/winter) suggest that small-bodied and short-lived taxa may respond more positively to altered resource stoichiometry than larger or longer lived taxa. Small taxa, particularly those with high mass-specific growth rates, may be more likely to respond to altered resource P content than large taxa, due to their need for elevated production of P-rich ribosomal RNA to support higher relative growth rates (Elser et al., 2003). However, shredder biomass response and approximate growth rate were unrelated among the nine shredder taxa analysed in this study. Nutrient fertilization of detritus-based headwater streams may favour short-lived taxa, as increased rates of detrital mass loss (Rosemond et al., 2015) may induce periods of resource scarcity among longer lived taxa (Cross et al., 2005).

5 | CONCLUSIONS

Over the last century, human activities have significantly increased the global availability of N and P, with increases that vary widely over spatial and temporal scales (MEA, 2005; Peñuelas et al., 2013). Thus, understanding the role of resource stoichiometry in structuring consumer communities is a particularly important challenge in the face of shifting patterns of N and P availability. By utilizing a whole-system experimental N and P manipulation experiment, we identified basal resource stoichiometry as a significant driver of consumer community dynamics in detritus-based headwater streams. Interestingly, the magnitude and direction of community shifts among the five

streams was not affected by their unique N:P enrichment ratio, despite clear evidence of community wide P limitation across the five streams. Therefore, EST appears to be a powerful framework for predicting underlying mechanisms of nutrient limitation in natural systems but may have limited application in terms of predicting specific responses to altered nutrient availability across multiple systems that vary in their initial properties. Shifts in the composition of consumer communities, like those reported here, likely have consequences for a broad range of food web properties and ecosystem processes, such as predator-prey interactions (Davis, Rosemond, Eggert, Cross, & Wallace, 2010b; Davis, Rosemond, Eggert, & Wallace, 2010a; Johnson, Jin, Carreiro, & Jack, 2013), food web structure (Glibert, Fullerton, Burkholder, Cornwell, & Kana, 2011); material flows (Cross et al., 2007) and nutrient cycling (Vanni, Flecker, Hood, & Headworth, 2002). Characterizing ecological contexts in which such effects are likely to occur will enhance our understanding of how communities and ecosystems respond to further anthropogenic perturbations to global patterns of C, N and P availability.

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AUTHOR'S CONTRIBUTIONS

A.D.R., J.P.B. and J.C.M. conceived the ideas, designed methodology and acquired funding for this project. L.M.D. collected and analysed the data and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

All data are available via the Dryad Digital Repository <https://doi.org/10.5061/dryad.f8qm2d7> (Demi, Benstead, Rosemond, & Maerz, 2019).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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