Litter P content drives consumer production in detritus-based streams spanning an experimental N:P gradient

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Abstract. Ecological stoichiometry theory (EST) is a key framework for predicting how variation in N:P supply ratios influences biological processes, at molecular to ecosystem scales, by altering the availability of C, N, and P relative to organismal requirements. We tested EST predictions by fertilizing five forest streams at different dissolved molar N:P ratios (2, 8, 16, 32, 128) for two years and tracking responses of macroinvertebrate consumers to the resulting steep experimental gradient in basal resource stoichiometry (leaf litter %N, %P, and N:P). Nitrogen and P content of leaf litter, the dominant basal resource, increased in all five streams following enrichment, with steepest responses in litter %P and N:P ratio. Additionally, increases in primary consumer biomass and production occurred in all five streams following N and P enrichment (averages across all streams: biomass by 1.29, production by 1.69). Patterns of both biomass and production were best predicted by leaf litter N:P and %P and were unrelated to leaf litter %N. Primary consumer production increased most in streams where decreases in leaf litter N:P were largest. Macroinvertebrate predator biomass and production were also strongly positively related to litter %P, providing robust experimental evidence for the primacy of P limitation at multiple trophic levels in these ecosystems. However, production of predatory macroinvertebrates was not related directly to primary consumer production, suggesting the importance of additional controls for macroinvertebrates at upper trophic positions. Our results reveal potential drivers of animal production in detritus-based ecosystems, including the relative importance of resource quality vs. quantity. Our study also sheds light on the more general impacts of variation in N:P supply ratio on nutrient-poor ecosystems, providing strong empirical support for predictions that nutrient enrichment increases food web productivity whenever large elemental imbalances between basal resources and consumer demand are reduced.

Key words: carbon; detritus; ecological stoichiometry; nitrogen; nutrient enrichment; phosphorus; rivers; secondary production; streams.

INTRODUCTION

A defining challenge in ecological stoichiometry is addressing how variation in nitrogen (N) and phosphorus (P) supply propagates from basal resources to higher trophic levels (Fagan and Denno 2004, Boersma et al. 2008, Malzahn et al. 2010). Ecological stoichiometry theory (EST) predicts that productivity of a given trophic level increases as resource stoichiometry becomes more balanced with consumer demand (Sterner and Elser 2002). In most ecosystems, large elemental imbalances that consequently limit production are common and may be particularly acute at the base of food webs. For example, ranges in inorganic N:P supply ratio typically exceed reported variability in tissue N:P ratios (and thus demand) of primary producers (Sardans et al. 2012). Similarly, the range of C:N:P ratios among heterotrophs is narrower than those reported for autotrophs, suggesting potential stoichiometric constraints on the productivity of herbivores, detritivores and microbial decomposers (Elser et al. 2000, Sardans et al. 2012). Only at upper trophic levels do narrower ranges of body C:N:P ratios, and a relatively higher degree of stoichiometric homeostasis (Persson et al. 2010), suggest that stoichiometric constraints on productivity become relatively attenuated.

Evidence for stoichiometric constraints on growth and production is increasingly common among a diverse array of consumer taxa and feeding habits (Bukovinszky et al. 2012, Benstead et al. 2014, Fuller et al. 2015), indicating that variation in N and P supply alters productivity of higher trophic levels via shifts in basal resource stoichiometry (i.e., C:N:P ratios). Several landscape-scale surveys have reported relationships between consumer growth rates (or biomass) and resource nutrient content (Veldbloom and Haro 2011, Ott et al. 2014, Prater et al. 2015). However, such studies cannot control for potentially confounding drivers of growth rates and/or biomass (i.e., temperature, habitat, resident community structure, population genetics), do not directly measure consumer
response to shifts in resource quality, and typically focus on primary consumer taxa (but see Ott et al. 2014). Conversely, more highly controlled feeding experiments typically lack realistic gradients in resource quality, thereby reducing the potential for selective feeding behavior as a mechanism for minimizing nutritional imbalances (Arsuffi and Suberkropp 1989, Cease et al. 2016). As such, realistic ecosystem-level manipulations of N:P supply ratios are necessary to deduce the role of N vs. P constraints on trophic interactions and to determine the extent to which the relative supply of N or P modulates patterns of productivity in natural food webs (but see Bumpers et al. 2015). That such tests are currently rare in the ecological literature highlights the need for a greater emphasis on the role of N:P supply ratios, particularly as human activities (i.e., fossil fuel combustion, application of agricultural fertilizers) continue to drive shifting patterns of N and P availability in ecosystems across the globe (Peñuelas et al. 2013).

Here, we present patterns of macroinvertebrate production from five forest streams that were continuously fertilized with N and P at different dissolved N:P ratios (ranging from 2:1 to 128:1) for two years following one year of pre-enrichment data collection. Our explicitly stoichiometric design allowed us to compare responses in annual production of a natural animal assemblage to shifts in basal resource stoichiometry across an experimental, ecosystem-scale N:P supply gradient. Detritus-based ecosystems are particularly suitable for tests of EST predictions given their characteristically large elemental imbalances in consumer–resource interactions that result from the naturally low C:nutrient ratios of vascular plant detritus (Cross et al. 2003, Martinson et al. 2008). Furthermore, elevated nutrient availability often leads to increased biomass of microbial decomposers (Gulis and Suberkropp 2003, Riggs et al. 2015), thereby increasing nutrient content, accelerating decomposition rate, and reducing turnover time of detritus (Ferreira et al. 2015, Rosemond et al. 2015). As such, the response of consumers to shifts in detrital stoichiometry is unlikely to be confounded by increases in basal resource biomass, as would be predicted following nutrient enrichment of autotroph-based food webs. Instead, accelerated rates of detrital mass loss may eventually limit consumer productivity as detrital resources become scarce, even as average resource quality increases.

Following enrichment, we predicted that the largest increases in N and P content of leaf litter, the dominant food resource, would occur in the streams with the highest dissolved N and P, respectively. The resulting steep gradient in litter N:P would allow us to test the relative importance of N vs. P limitation of animal production. Specifically, we predicted that macroinvertebrate production would exhibit a stronger response to detrital P content than to its N content, because imbalances between leaf litter and macroinvertebrate C:P have been shown to be greater than C:N imbalances in detritus-based stream food webs (Cross et al. 2003). We further predicted that the greatest response in production between pre-enrichment and nutrient-enriched conditions would occur at the dissolved N:P ratio that produced the greatest shift in detrital P content. Finally, we expected macroinvertebrate predator production to track potential prey production across streams and years, demonstrating how shifts in N and P availability can propagate to higher trophic levels via altered basal resource stoichiometry (see Bumpers et al. 2015, for related response of vertebrate predators to increasing P).

**METHODS**

**Study sites**

Five headwater streams at the Coweeta Hydrologic Laboratory (CHL) were chosen for this study (35°02′ N, 83°45′ W). CHL is a U.S. Forest Service research station in Macon County, North Carolina, USA, in the Blue Ridge physiographic province of the southern Appalachian Mountains. The 2,185-ha basin has mixed hardwood vegetation (primarily oak, maple, and poplar). A dense understory of evergreen *Rhododendron maximum* provides year-long shading of its low-order streams. The five streams chosen for the study were similarly low in soluble reactive phosphorus (SRP, mean = 2.8 μg/L, SE = 0.1) and had low, but more variable, concentrations of dissolved inorganic nitrogen (DIN, mean = 82.5 μg/L, SE = 30.8) during the year prior to the nutrient additions. The streams, which drained adjacent watersheds on the same hillside, shared similar elevation (~1,160 m above sea level), temperature, slope, and aspect (four face east, one northeast). Experimental reaches, 70 m in length, were established in each stream for sample collection and N and P fertilization (see Rosemond et al. [2015] for further details).

During July 2011, experimental additions of aqueous N (21% ammonium nitrate) and P (85% phosphoric acid) commenced and were maintained continuously for the next two years (hereafter YR1 and YR2), ending in July 2013. A year of pre-enrichment data collection (July 2010–June 2011; hereafter PRE) preceded the N and P additions. Nutrient delivery was achieved using solar-powered metering pumps (LMI Milton Roy, Ivyland, Pennsylvania, USA) and pump rate was calibrated using estimates of instantaneous discharge from a Campbell CR800 data-logger (Campbell Scientific, Logan, Utah, USA) connected to a Nanolevel pressure transducer (Keller America, Newport News, Virginia, USA). Nutrients were mixed with ambient stream water in a gravity-fed irrigation line that delivered the nutrient mixture via drip spouts located approximately every 5 m to ensure mixing throughout each reach. Each stream received N and P at different target N:P ratios (2:1, 8:1, 16:1, 32:1, 128:1; hereafter streams are referenced by their target N:P ratio), whereby N was added at increasing concentration (DIN; target range = 81–650 μg/L) and P in decreasing concentration (SRP; target range = 90–11 μg/L) across the N:P gradient, so that the highest N concentration was paired with the lowest P concentration and vice versa.
This experimental design allowed us to assess the potential for N and P limitation of macroinvertebrate production separately, as increasing concentrations of one element were not confounded by increased concentrations of the other. Our analysis assumes that the low to moderate levels of nutrient enrichment we targeted could not inhibit macroinvertebrate production (i.e., any decreases in production with increasing N concentration would be driven by the opposed P gradient, not by any inhibitory effect of high N). Target nutrient concentrations were chosen based upon regional patterns of stream water chemistry that result from shifting patterns of land use within the southern Appalachians (Scott et al. 2002). The ratio of added N:P closely tracked target ratios (linear regression, \( F_{1,8} = 978.2, P < 0.001, R^2 = 0.99 \), slope = 0.81) across all five streams during the two years of nutrient enrichment. See Bumpers et al. (2015) for a comprehensive summary of target and measured nutrient concentrations in these experiments. Though this experiment lacked a continuous control reach, our experimental design allowed us to identify patterns in macroinvertebrate abundance, biomass, and production across a range of N and P concentrations (and ratios) using a regression approach. Furthermore, our design allowed us to assess the magnitude of response to the nutrient additions across gradients of both N and P using a response-ratio analysis that incorporated stream-specific pre-enrichment data (see Data analysis).

**Benthic sampling for basal resources and macroinvertebrates**

Quantitative sampling for macroinvertebrates and leaf litter began in July 2010 and continued at monthly intervals until June 2013. Leaf litter was collected from the wetted width of eight randomly selected 0.15 m wide transects along each reach and processed for quantification of leaf litter biomass and C, N, and P content following methods outlined in Rosemond et al. (2015) and Manning et al. (2015). Briefly, leaf litter collected from each transect was weighed, then subsampled and dried for \(<24 \text{ h at } 60^\circ \text{C} \) and reweighed to determine dry mass (DM). Dried material was then ground using an 8000-D ball mill (Spex SamplePrep, Methuchen, New Jersey, USA) and a subsample combusted for \(-4.5 \text{ h at } 500^\circ \text{C} \). A subsample of dried material was also weighed and run through a Carlo Erba NA 1500 CHN Analyzer (Carlo Erba, Milan, Italy) at the University of Georgia Analytical Chemistry Laboratory (Athens, Georgia, USA) to determine C and N content. Phosphorus content was determined by the ascorbic acid method on a Shimadzu UV-1700 spectrophotometer (Tokyo, Japan; Allen 1974, APHA 1998) after digesting a subsample of the combusted litter using the plant dry ash/acid extraction method. An additional subsample of the dried leaf material was weighed, combusted (500°C), and re-weighed to determine ash-free dry mass on an areal basis (g AFDM/m²). We present annual means calculated from the 12 monthly mean values for all three years of the study. All elemental ratios are presented as molar ratios. Each month four benthic macroinvertebrate samples were collected from mixed-substrate habitat (sand, gravel, and cobble) at random locations within each stream reach using a stovepipe core sampler (490 cm²). Detailed description of the sample processing procedure can be found in Lugthart and Wallace (1992; also see Appendix S1).

**Secondary production**

Depending on the life history and abundance of each taxon, we calculated macroinvertebrate production (g AFDM m⁻² yr⁻¹) using the most appropriate of the following three methods. We used the instantaneous growth method for taxa in which discrete cohorts could be followed through time. Daily growth rates were estimated between sampling intervals by dividing the natural log of the proportional change in mean individual body mass by the duration (in days) of the sampling interval (Benke and Huryn 2006). For taxa in which individual cohorts were difficult to identify, due to asynchronous development or the presence of multiple overlapping cohorts, the size-frequency method (Hamilton 1969), corrected for cohort production interval (CPI; Benke 1979), was used to estimate production. CPIs for each taxon were taken from published studies from CHL (Hurry and Wallace 1987, Wallace et al. 1999). Annual production for the remaining taxa was estimated by multiplying mean annual biomass by annual production/biomass (P/B) values obtained from earlier production studies at CHL (Hurry and Wallace 1987) or assumed from known life-history characteristics (e.g., voltinism).

We generated 95% confidence intervals for annual production using bootstrap analysis (Benke and Huryn 2006, Cross et al. 2011). Briefly, we randomly resampled, with replacement, size-specific abundances from the four replicate samples collected on each date 1,000 times. The bootstrapped data sets were then used to generate a vector of 1,000 annual production estimates. The upper and lower 2.5% of values from the resulting vector were then discarded to produce a 95% confidence interval around the mean annual production estimate.

**Data analysis**

We restricted consideration of basal resources to leaf litter based on its dominance of organic matter inputs (Benstead et al. 2009) and established relationships with invertebrate production in CHL streams (Wallace et al. 1999, 2015). Leaf detritus and associated fungi accounted for \(\sim78\%\) of total primary consumer production in the five streams over the study period (Demi 2016). We tested for relationships between leaf litter biomass or elemental content and macroinvertebrate abundance, biomass, and production using a combination of linear and non-linear regression techniques. Regression analyses were performed using the average of the annual values from the
two enrichment years in each stream and were modeled using the natural log of target N:P ratio. We tested potential relationships between each macroinvertebrate response variable and three litter stoichiometry variables (\%N, \%P, and N:P) by comparing linear, second-order polynomial, and quadratic models (Gherardi and Sala 2015). The chosen models illustrate three potential responses to nutrient enrichment. The linear model reveals nutrient limitation across the range of litter stoichiometry values, while the polynomial model suggests attenuation of nutrient limitation at either end of the range in leaf litter nutrient concentrations or ratios. The quadratic model illustrates the potential for inhibitory effects at either end of the range in leaf litter stoichiometry values, similar to those reported by Boersma and Elser (2006) and Benstead et al. (2014). Akaike’s information criterion (AIC) corrected for small sample size (AICc), was employed using the R package AICmodavg (Mazerolle 2014) to select which of these three models best described the relationship between each response variable and potential predictor variables. We present only the models that had the lowest AICc value and that were statistically significant. Additionally, quadratic fits with positive quadratic coefficients (i.e., U-shaped relationships) were considered to lack a meaningful biological mechanism and were ignored.

We calculated log response ratios (hereafter lnRRs) by taking the natural log of the ratio between YR1, or YR2, and PRE (i.e., RR1 = ln[YR1/PRE]) values for each measure of leaf litter chemistry (\%N, \%P, and N:P), biomass, and macroinvertebrate production in order to characterize the response of each variable to N and P enrichment in each of the five streams. Using lnRRs facilitates more meaningful investigation of the influence of dissolved N:P ratios by using a standardized response, thus reducing the influence of among-stream variation. In order to remove the influence of large proportional responses by relatively rare taxa, lnRRs were only calculated for taxa that were present in >10% of samples. A mean weighted lnRR was then calculated for each stream and year (YR1 and YR2 relative to PRE), whereby each taxon was weighted based on its contribution to total production averaged across all three years in each stream. The weighted lnRRs thus reduce the influence of less productive taxa on average community lnRRs and provide a more functionally explicit measure of community response to nutrient enrichment (i.e., one based on production). We analyzed the relationship between dissolved N:P ratio and the response of leaf litter chemistry (lnRRs of \%N, \%P, and N:P) and macroinvertebrate production (weighted production lnRRs) by using a combination of linear and non-linear models, followed by the model selection process described above. Additionally, to further investigate the role of leaf litter chemistry and quantity in driving patterns of production, we employed the same combination of linear and non-linear models and model selection to characterize the relationships between lnRRs of leaf litter chemistry (\%N, \%P, and N:P), and biomass and weighted primary consumer production lnRRs. To explore responses of predator production to our nutrient treatments via the effects of enrichment on prey production, we used linear regression to analyze the relationship between primary consumer and predator production, and weighted primary consumer production lnRRs and weighted predator production lnRRs. All statistical analyses were performed using the R statistical platform (version 3.10; R Core Team 2016).

**RESULTS**

*Leaf litter stoichiometry and quantity*

Mean annual leaf litter P content across the five streams was 0.039% (\( n = 5, \text{SD} = 0.003 \)) prior to enrichment and increased to 0.054% (\( n = 5, \text{YR1 SD} = 0.005, \text{YR2 SD} = 0.005 \)) in both enrichment years 1 and 2 (Table 1). The response (expressed as lnRR) of leaf litter P content to N and P fertilization varied across the dissolved N:P gradient and was best described by the second-order polynomial model (Fig. 1a; Appendix S2: Table S1; \( F_{1,3} = 11.11, P < 0.05, R^2 = 0.79 \)). Mean annual leaf litter N content was 0.82% (\( n = 5, \text{SD} = 0.06 \)) across the five streams prior to nutrient enrichment and increased to 0.88% (\( n = 5, \text{SD} = 0.05 \)) and 0.87% (\( n = 5, \text{SD} = 0.09 \)) during enrichment YR1 and YR2, respectively (Table 1). Response ratios for leaf litter N content did not vary systematically across the experimental N:P gradient (Fig. 1b; Appendix S2: Table S1; \( P > 0.88 \)). Annual means for leaf litter N:P were driven primarily by changes in litter P content (\( F_{1,3} = 45.4, P < 0.001, R^2 = 0.77 \)). Average leaf litter N:P across the five streams was 51 (\( n = 5, \text{SD} = 1.5 \)) during the pre-enrichment year and decreased to 41

<table>
<thead>
<tr>
<th>Year</th>
<th>Target N:P</th>
<th>%N</th>
<th>%P</th>
<th>N:P</th>
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<tr>
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<td>0.82</td>
<td>52.0</td>
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<tr>
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<td>8:1</td>
<td>0.82</td>
<td>0.86</td>
<td>39.0</td>
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<tr>
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<td>16:1</td>
<td>0.81</td>
<td>0.78</td>
<td>33.9</td>
</tr>
<tr>
<td>YR1</td>
<td>32:1</td>
<td>0.81</td>
<td>0.86</td>
<td>33.6</td>
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<tr>
<td>YR2</td>
<td>128:1</td>
<td>0.81</td>
<td>0.86</td>
<td>37.8</td>
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<tr>
<td>Mean</td>
<td></td>
<td>0.81</td>
<td>0.88</td>
<td>41</td>
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*Note: Mean represents mean across all five streams.*
(n = 5, SD = 3.6) and 38 (n = 5, SD = 4.5) during YR1 and YR2, respectively (Table 1). Response of leaf litter N:P was greatest at the lowest dissolved N:P ratios and diminished with increasing dissolved N:P (Fig. 1c). This relationship was best described by the second-order polynomial model (Appendix S2: Table S1; F2,2 = 139.1, P < 0.01, R2 = 0.98). Mean leaf litter biomass was higher during the pre-enrichment year (286 g AFDM/m2 across all five streams, n = 5, SD = 85) than the two years of enrichment (~150 g AFDM/m2 in both years of enrichment, n = 5, YR1 SD = 33, YR2 SD = 12, Table 1). Analysis of response ratios revealed variation in the response of leaf litter biomass to nutrient enrichment, with the largest magnitude responses typically occurring at low target N:P ratios. This relationship was best described by the second-order polynomial model (Appendix S2: Table S1; F2,2 = 139.1, P < 0.01, R2 = 0.98).

Primary consumer abundance, biomass, and production

Primary consumer abundance ranged from ~8,800 to ~21,800 individuals/m2 prior to enrichment and from ~10,000 to ~21,700 individuals/m2 during enrichment (Fig. 2a). Primary consumer abundance was not significantly related to leaf litter biomass, %P, %N, or N:P (Appendix S2: Table S2). Primary consumer biomass consistently increased across the five streams following nutrient enrichment (Fig. 2c; PRE range ~0.5 to ~0.9 g AFDM/m2; YR1 and YR2 range ~0.7 to ~1.5 g AFDM/m2) and was significantly positively related to leaf litter %P and negatively related to leaf litter N:P, but was not related to leaf litter biomass or %N (Appendix S2: Table S2). AIC model selection identified the linear N:P model (Appendix S2: Table S2; F1,13 = 9.89, P < 0.01, R2 = 0.43) as the best model for describing patterns of primary consumer biomass.

Primary consumer production generally increased across the five streams as a result of enrichment (Fig. 2e, PRE range ~3.0 to ~4.7 g AFDM m-2 yr-1; YR1 and YR2 range ~4.1 to ~9.8 g AFDM m-2 yr-1). Primary consumer production was negatively related to leaf litter N:P (Fig. 3a) and positively related to leaf litter %P (Fig. 3b; Appendix S2: Table S2). AIC model selection identified the linear leaf litter N:P model (Appendix S2: Table S2, F1,13 = 24.96, P < 0.001, R2 = 0.66) as the best predictor of primary consumer production.
Mean weighted response ratios of annual production were positive across the five study streams (excepting YR2 in the 128:1 stream; Fig. 4a), suggesting that the average primary consumer experienced an increase in production at most levels of nutrient enrichment. Weighted response ratios revealed systematic variation in the strength of primary consumer response across the dissolved N:P gradient. This pattern was best described by the second-order polynomial model (Fig. 4b; Appendix S2: Table S3; \( F_{1,3} = 7.77, P = 0.069, R^2 = 0.72 \)). Additionally, lnRRs of primary consumer production were significantly related to changes in leaf litter N:P, but not leaf litter %N, %P, or biomass (Appendix S2: Table S4), thus showing that the largest increases in primary consumer production were associated with the largest decreases in leaf litter N:P relative to pre-enrichment conditions. The linear model (Fig. 4c; Appendix S2: Table S4; \( F_{1,8} = 10.18, P < 0.05, R^2 = 0.56 \)) best explained the relationship between lnRRs of leaf litter N:P and primary consumer production.

Predator abundance, biomass and production

Predator abundance exhibited a variable response to nutrient enrichment (Fig. 2b), though it was significantly positively related to leaf litter %N, but not leaf litter %P or N:P (Appendix S2: Table S5). The linear model (Appendix S2: Table S5; \( F_{1,13} = 7.40, P = 0.018, R^2 = 0.36 \)) was the best model for predicting predator abundance based on leaf litter %N. Though predator biomass (Fig. 2d) and production (Figs. 2f, 5a) tended to be greater during enrichment YR1 and YR2 than the pre-enrichment year, there was no systematic response of

![Fig. 2](image-url)
predator production to nutrient enrichment across the dissolved N:P gradient (Fig. 5b; Appendix S2: Table S6). However, both predator biomass and production were significantly positively related to leaf litter %P and marginally positively related to leaf litter %N, but were not related to leaf litter N:P or biomass (Appendix S2: Table S5). The polynomial leaf litter %P model best predicted patterns of predator biomass (Appendix S2: Table S5, $F_{1,13} = 7.73$, $P < 0.05$, $R^2 = 0.37$) and production (Fig. 3b; Appendix S2: Table S5, $F_{1,13} = 6.58$, $P < 0.05$, $R^2 = 0.34$). Predator production was not related to primary consumer production ($F_{1,13} = 0.34$, $P = 0.57$, $R^2 = 0.03$); neither were weighted predator and primary consumer lnRRs related to each other ($F_{1,8} = 0.03$, $P = 0.86$, $R^2 = 0.00$).

**DISCUSSION**

The majority of global primary production is not consumed as living tissue, instead being channeled either to detrital food webs or to long-term carbon storage (Cebrian 1999). Nevertheless, and despite global increases in N and P availability, the effects of altered nutrient supply on detritus-based ecosystems remain understudied, particularly at realistically large spatial scales. Here, elevated N and P concentrations increased the productivity of five detritus-based food webs via reduced detrital N:P ratios, despite reduced detrital biomass and increased rates of detrital mass loss (Rosemond et al. 2015). Detrital stoichiometry in this study responded more to P than to N availability, while good fits to polynomial models suggested the potential for an attenuated response at very low N:P ratios, possibly resulting from N limitation of fungal biomass and production (Gulis et al. 2017). Overall, our results demonstrate an important role for resource quality, and specifically of resource P content, in modulating patterns of food web productivity in ecosystems that have previously been viewed as limited primarily by resource quantity (but see Cross et al. 2006, 2007).

**Leaf litter stoichiometry**

The shifts in leaf litter chemistry reported in this study were likely driven by increases in microbial biomass as mostly fungal decomposers were released from nutrient...
limitation (Stelzer et al. 2003, Suberkropp et al. 2010). Long-term nutrient enrichment (at N:P of ~16:1) resulted in increased leaf litter %N and %P (Cross et al. 2003) and increased fungal biomass and production (Gulis and Suberkropp 2003, Suberkropp et al. 2010) in another detritus-based stream at CHL. Providing greater insight into controls on N vs. P content of litter, Manning et al. (2015) reported reduced C:N and C:P of leaf litter due to increased fungal biomass, together with additional effects of stream water P concentration on litter C:P, in the same five streams we studied. In an associated laboratory investigation of the effects of N and P ratios on fungal biomass, production, and stoichiometry, Gulis et al. (2017) report that fungal biomass and production were N-limited and did not respond to variation in P supply. The same authors reported that aquatic hyphomycetes exhibit a higher degree of stoichiometric homeostasis with respect to N than to P content, suggesting that luxury P uptake may explain the stronger relative response of leaf litter P content to nutrient enrichment in our study.

Nutrient and carbon limitation of consumer production

Our results build on those of earlier studies by providing strong evidence for P limitation of macroinvertebrate production. Our use of an experimental gradient in N:P supply ratio allowed a more robust investigation of the potential for N vs. P limitation of animal production than was possible in previous ecosystem-scale studies of nutrient enrichment. For example, following the experimental enrichment of a single CHL stream at a N:P ratio of 16:1, Cross et al. (2006) showed how elevated nutrient concentrations stimulated macroinvertebrate production per unit biomass of leaf litter, even though the authors were not able to attribute this response explicitly to release from N or P limitation. In that study, total macroinvertebrate production was considerably higher (118% and 334% during two years of enrichment) than predictions based on the long-term relationship between production and leaf litter biomass under ambient conditions (Wallace et al. 1999, Cross et al. 2006). Similarly, we observed increased primary consumer production and biomass per g of leaf litter under elevated nutrient conditions in this study, despite lower mean annual leaf litter biomass in these streams (Suberkropp et al. 2010, Rosemond et al. 2015).

We combined data from control and enriched conditions in the present study with those from Cross et al. (2006), Wallace et al. (1999), and Davis et al. (2010; J. M. Davis, unpublished data) to quantify the effects of nutrient enrichment on macroinvertebrate production for a given annual mean biomass of leaf litter (Fig. 6; linear model, AMB, $F_{1,17} = 112.2, P < 0.001, R^2 = 0.87$; ENR, $F_{1,12} = 1.73$, $P = 0.22$).

![FIG. 4. Mean weighted log response ratios (lnRRs) for primary consumer production and (a) the relationships between weighted primary consumer production lnRRs and (b) target N:P concentrations and (c) leaf litter N:P lnRRs. Regression analyses were performed using the average of the two values from each target N:P ratio and were modeled using the natural log of target N:P ratio, but are displayed as untransformed data on a logarithmic scale to preserve the actual value of dissolved N:P targets. Regression lines represent models that were statistically significant and had the lowest AICc score from each respective analysis. Gray and black symbols represent the lnRR for YR1 and YR2, respectively.](image-url)
The large range in production at lower levels of leaf litter biomass (~25 to ~80 g/m²) in nutrient-enriched streams is likely driven by differences in the relative contribution of both biotic (i.e., consumption and microbial decomposition) and abiotic (i.e., physical fragmentation and hydrologic export) processes in regulating leaf litter biomass. This analysis shows co-limitation of consumer production by resource quantity and quality and corroborates previous findings in these study systems that production of macroinvertebrates was limited by quality (Cross et al. 2006, Davis et al. 2010) and quantity (Wallace et al. 1999) of detritus in separate experimental manipulations. Indeed, such co-limitation of detritivore growth has been reported elsewhere and may be common in detritus-based ecosystems (Halvorson et al. 2017).

However, in CHL streams, mean leaf litter biomass appears to be a poor predictor of macroinvertebrate production in nutrient-enriched streams compared to non-enriched streams, where production per unit mass leaf litter is likely more tightly constrained by low detrital nutrient (N and P) content. This suggests a greater relative importance of leaf litter quality, particularly its P content, in driving patterns of consumer production in detritus-based headwater streams than has been previously recognized. This increased recognition of the role of resource quality is further supported by our response-ratio analysis, which revealed that shifts in primary consumer production were driven by shifts in leaf litter P content, despite reductions in leaf litter biomass along the same gradient.

Strong relationships between production and litter quality are consistent with predictions derived from the EST framework, in which large elemental imbalances between detrital resources and consumer demand are expected to result in nutrient limitation of consumer production.
growth and production in detritus-based ecosystems (Sterner and Elser 2002). For example, the C:P and C:N ratios of leaf litter in the present study ranged from ~2,100 to ~3,500 and from 54 to 67, respectively. Previous measurements of benthic macroinvertebrate stoichiometry in CHL streams revealed that primary consumer body C:P ranged from 80 to 877 and that consumer C:N ratios were typically around 6 (Cross et al. 2003). The range in C:N:P ratios of benthic macroinvertebrates in CHL streams is similar to that reported in other studies (Liess and Hillebrand 2006, Lauridsen et al. 2012, Ohta et al. 2007, Boersma et al. 2008). Across the three years of our study, biomass and production of macroinvertebrate predators was significantly related to leaf litter P content. Similarly, Bumpers et al. (2015) reported positive relationships between larval growth rates of two salamander species (the top aquatic predators) and dissolved P concentrations in our five study streams, suggesting propagation of a P effect to the highest trophic levels of the aquatic food web. However, biomass and production of predatory macroinvertebrates did not increase in all five streams following nutrient enrichment and was not directly related to primary consumer production in this study, even though predator response to nutrient enrichment is often attributed to shifts in prey availability (Deegan and Peterson 1992, Cross et al. 2006). As such, the specific mechanisms by which macroinvertebrate predators are released from nutrient limitation deserve further investigation (but see Bumpers et al. [2017] for salamander responses).

Previous efforts to characterize the role of nutrient availability, and basal resource stoichiometry, on the efficiency of energy transfer between trophic guilds suggest increased efficiency under elevated nutrient regimes. Rowland et al. (2015) reported increasing food chain efficiency with decreasing algal C:P in experimental mesocosms subjected to different combinations of nutrient and light availability. Similarly, Dickman et al. (2008) reported increased trophic transfer efficiency under elevated nutrient conditions in similar mesocosms. Increased transfer efficiencies in these and analogous studies was likely a response to both increased prey quality and quantity resulting from increased algal P content (Malzahn et al. 2007, Boersma et al. 2008, Schoo et al. 2012). However, the range of C:N:P stoichiometry of primary consumers and predators in CHL streams broadly overlaps, suggesting limited potential for nutrient limitation of macroinvertebrate predators (Cross et al. 2003).

One potential explanation for the contrasting response of macroinvertebrate predator efficiencies between previous studies and ours is the difference in body size composition of consumer assemblages at each trophic level. Dickman et al. (2008) and Rowland et al. (2015) focus on strongly size-structured pelagic food webs. In contrast, CHL streams contain primary consumer and predator taxa that span a broad and overlapping range of body sizes, and many primary consumers are relatively resistant to predation, at least at later stages of development (Davis et al. 2010). Increased primary consumer biomass, coupled with the lack of a consistent increase in abundance following nutrient enrichment, suggests a post-enrichment shift towards larger-bodied individuals in this study. Shifts towards larger prey taxa may mean a greater proportion of primary consumer production is unavailable to invertebrate predators (Davis et al. 2010). However, Cross et al. (2006) reported that predator production tracked prey production over the first two years of enrichment in that study, a result that differs from the present study, despite the similar duration of enrichment and broad similarities
in both prey and predator assemblages. Short-term responses by invertebrate predators may also be constrained by the relatively long aquatic life-stages of some predatory invertebrates in these streams, many of which are semi-voltine (Huryn and Wallace 1987).

In summary, our simultaneous ecosystem-scale manipulations of both N and P availability provided robust experimental evidence of P-limited secondary production in model detritus-based ecosystems. Our experimental manipulation of both N and P concentrations, and supply ratios, allowed us to relate patterns of animal production to the P content, and C:N:P ratio, of the dominant food source. This study suggests that consumer production in detritus-based food webs may be more susceptible to variation in P supply than to that of N, although the potential for N limitation of fungi (the dominant microbial decomposers) may dampen the response of metazoan consumers at low N:P ratios. As human activities continue to perturb global biogeochemical cycles, thus altering the relative availability of N and P to the biosphere and increasing the N:P ratio of depositional cycles, therefore altering the relative availability of N and macroinvertebrate taxa. Oecologia 79:30–37.


Riggs, C. E., S. E. Hobbie, E. M. Bach, K. S. Hofmockel, and C. E. Kazanski. 2015. Nitrogen addition changes grassland...

Supporting Information
Additional supporting information may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/ecy.2118/supinfo