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Relationships among nutrient enrichment, detritus quality and quantity, and large-bodied shredding insect community structure

Clay Prater · Eric J. Norman · Michelle A. Evans-White

Abstract Anthropogenic nutrient enrichment of forested headwater streams can enhance detrital quality, decrease standing stocks, and alter the community structure of detrivorous insects, reducing nutrient retention and decreasing ecosystem functioning. Our objective was to determine if stoichiometric principles could be used to predict genus-specific shifts in shredding insect abundance and biomass across a dissolved nutrient and detritus food quality/quantity gradient. Detritus, insect, and water samples were collected from 12 Ozark Highland headwater streams. Significant correlations were found between stream nutrients and detrital quality but not quantity. Abundance and biomass responses of four out of five tested genera were accurately predicted by consumer-resource stoichiometric theory. Low carbon:phosphorus (C:P) shredders responded positively to increased total phosphorus and/or food quality, and high C:P shredders exhibited neutral or negative responses to these variables. Genus-specific declines were correlated with decreased overall biomass in shredder assemblages, potentially causing disruptions in nutrient flows to higher level consumers with nutrient enrichment. This work provides further evidence that elevated nutrients may negatively impact shredding insect communities by altering the stoichiometry of detritus–detritivore interactions. A better understanding of stoichiometric mechanisms altering macroinvertebrate populations is needed to help inform water quality criteria for the management of headwater streams.

Keywords Ecological stoichiometry · Eutrophication · Detritivore · Leaf litter · Shredder · Phosphorus

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Introduction

Anthropogenic nutrient enrichment is occurring worldwide (Kuijper et al., 2005) and is the second most common cause of freshwater ecosystem degradation in the US (EPA, 2006). Headwater streams can have disproportionally high rates of nutrient processing compared to other freshwater systems (Alexander et al., 2000, Wollheim et al., 2001), and their destruction or degradation can decrease ecosystem functioning causing further downstream damage to rivers, lakes, and coastal areas (Carpenter et al., 1998). Forested headwater streams are increasingly imperiled

due to the clearing of riparian vegetation for agricultural and urban area development (Paul & Meyer, 2001), which can cause amplified irradiance (DeNicola & Hoagland, 1996), flashier hydrology, increased sedimentation (Webster & Waide, 1982), and higher nutrient inputs (Likens et al., 1970) in these areas. Recent studies have provided evidence that nutrient enrichment of forested headwater streams causes shifts in benthic macroinvertebrate community structure due to increases in detrital quality and decreases in quantity (Ramirez & Pringle, 2006, Cross et al., 2006, Greenwood et al., 2007, Singer & Battin, 2007). However, to our knowledge the relationship between altered diet and benthic macroinvertebrate community structure has not been examined across a regional stream nutrient gradient.

Elevations of stream nutrient concentrations are known to differentially affect the quality and quantity of detrital material. Increased nutrients in the water column such as nitrogen (N) and phosphorus (P) can be immobilized by microbial decomposers (Webster et al., 2009), supporting higher rates of fungal and bacterial production, microbial respiration, and increased fungal biomass (Greenwood et al., 2007, Suberkropp et al., 2010). As detrital resources are generally poor in N and P, increased microbial uptake can lead to increases in food quality, reducing nutritional imbalances between detritus and primary consumers (Cross et al., 2003, Greenwood et al., 2007, Hladyz et al., 2009). In turn, enhanced detrital quality can cause decreases in detrital standing stocks due to increased feeding by microbes and shredding macroinvertebrates (Rosemond et al., 2001). Therefore, unlike autotrophic systems, nutrient enrichment of detritus-based heterotrophic systems can accentuate C losses by stimulating C cycling rates in lower trophic levels of these systems (Rosemond et al., 2001, Cebrian, 2004). Thus, increases in nutrient loading can alter basal resource nutrient quality (N and P) and energy (C) quantity for detritivores adapted to forested headwater streams.

Aquatic shredding insects have evolved in forested headwater streams under characteristically low nutrient conditions (Boersema & Elser, 2006). Undisturbed temperate headwater streams typically have high dissolved C:P and N:P ratios, potentially making these systems P-limited (Elwood et al., 1981). Therefore, these consumers have theoretically not evolved the ability to process excess P because of weak selective pressure for luxury P-uptake (Boersema & Elser, 2006). Consequently, shredders with different life-history traits and stoichiometric needs could be differentially affected by changes in quality and quantity of their basal food resources induced by nutrient enrichment. For instance, slower growing, large-bodied, and high C:P consumers might be negatively affected and outcompeted by faster growing low C:P consumers (Danger et al., 2009), altering shredder assemblages in individual streams and across large regional scales.

Recent studies have highlighted the effects of anthropogenic P-loading on macroinvertebrate consumers and their food resources. Experimental reach-level studies have shown that increases in P concentrations can cause reductions in macroinvertebrate diversity, by reducing the nutrient imbalance between low C:P consumers and their food resources (Singer & Battin, 2007, Gafner & Robinson, 2007). According to the growth rate hypothesis (Elser et al., 2003), low C:P consumers have a higher proportion of P-rich ribosomal ribonucleic acids (rRNA) that allow them to grow faster and outcompete high C:P organisms under food P saturation. These results, along with studies of experimentally enriched streams, have revealed increases in abundance, biomass, and secondary production of multivoltine P-rich consumers (Cross et al., 2006, Davis et al., 2010, Small & Pringle, 2010). In addition, regional studies have found reductions in shredder and collector-gatherer macroinvertebrate community richness, diversity, and body C:P (Baldy et al., 2007, Wang et al., 2007, Evans-White et al., 2009). These regional studies suggest that the community composition of aquatic organisms, especially those with large elemental imbalances between their diet and body composition, such as large-bodied high C:P shredding insects, can be negatively affected by high stream nutrient concentrations. However, these studies do not directly examine possible mechanistic changes in resource condition (i.e., increased food quality or decreased quantity) that might be responsible for observed changes in shredding insect community structure. The ability to predict the effects of altered detrital quality and quantity on life-history traits of individual long-lived shredder species and dynamics of whole assemblages could help establish more informed stream water nutrient criteria by estimating threshold responses of species abundance and biomass in response to nutrient enrichment.

Estimates of threshold elemental ratios (TERs) combine information on consumer nutrient assimilation efficiencies and body nutrient composition to predict when growth limitation switches from one element to another. Furthermore, shredding macroinvertebrates generally have relatively high C:P TERs ($TER_{C:P}$) compared to other functional feeding groups (Frost et al., 2006) and thus can be adversely affected by elevated P concentrations Evans-White et al., 2009). Using stoichiometric insights, $TER_{C:P}$ can be used to make logical quantitative a priori predictions regarding shifts in natural populations and communities with nutrient enrichment. Unfortunately, very few direct measurements of element-specific assimilation or production efficiencies exist that would allow for calculation of $TER_{C:P}$ for many shredder species. However, as body C:P is positively related to $TER_{C:P}$ (Frost et al., 2006), we wanted to determine whether body C:P estimates may be used as a surrogate to qualitatively predict detritivore species responses to nutrient enrichment.

The first objective of this study was to determine if alteration of basal resource quality or quantity occurred across stream water total phosphorus (TP) and nitrate + nitrite-nitrogen ($NO_3^- + NO_2^- - N$) gradients. We predicted that coarse benthic organic matter (CBOM) nutritional quality would increase and that quantities would decrease with increased nutrient loading. Next, we examined whether stoichiometric principles (consumer/resource nutrient imbalance) could be used to predict genus-specific shifts in shredding insect abundance and biomass leading to alterations in overall insect community abundance and biomass. For these responses, we predicted that alteration in detrital quantity and/or quality would correlate with shifts in taxon dominance altering shredder communities, providing a mechanism for community composition shifts observed across larger regional scales (Wang et al., 2007, Evans-White et al., 2009). We specifically predicted that abundance and biomass of low C:P genera (*Tipula* spp., *Pycnopsyche* spp., and *Ephemerella* spp.) would increase with nutrient enrichment in this study and that high C:P consumer (*Amphinemura* spp. and *Strophopteryx* spp.) abundance and biomass would remain unchanged or that they would decrease across nutrient gradients. To examine whether these changes were related to differential investment of P to smaller-bodied fast-growing life stages, we analyzed community C:P data

and biomass:abundance ratios (B:A). We predicted that increased stream nutrient loading would enrich food quality shifting shredder community assemblages toward taxa with higher nutrient demands and that this would coincide with increased abundance of small-bodied consumers. We further predicted no significant differences in population level stoichiometry across streams but that shifts from high to low C:P genera would result in significant reductions in shredder community B:A and C:P ratios due to the exclusion of large-bodied high C:P shredders.

Methods

Samples were collected between 20-Mar and 11-Apr 2009 ($n = 6$ streams: water quality and detritus) and 2010 ($n = 8$ streams: water quality, detritus, and invertebrates) from low-order headwater streams in the Ozark Highlands region of Northwest Arkansas, USA. Samples were collected during these dates as they occurred prior to leaf out and during the period of maximum invertebrate richness in this region (Evans-White et al., 2009). Land use in watersheds was predominantly forested (34.0–92.0%; mean = 71.0%) and pastured areas (4.0–52.0%; mean = 20.0%) (Table 1), and mean stream width and depth was 5.5 m (2.0–9.3 m) and 26.2 cm (4.0–150 cm), respectively. Dominant riparian vegetation was composed of Red Oak (*Quercus rubra*), White Oak (*Quercus bicolor*), Sycamore (*Platanus occidentalis*), Sugar Maple (*Acer saccharum*), and River Birch (*Betula nigra*). Study streams substrata were primarily gravel and characterized by riffle-pool channel morphology (Brussock et al., 1985).

Two composite water samples, one filtered (glass fiber filter; 0.45 μm mesh) and one unfiltered, were collected from the thalweg of a well-mixed region of each stream before sampling, kept on ice, and frozen upon returning to the lab. Turbidity was determined using a portable fluorometer (Turner Designs: Aquaflu-or 8000-010; Sunnyvale, CA) at the base of each site, and dissolved oxygen (DO) was measured using an YSI probe (Model 95; Yellow Springs, OH). In the laboratory, filtered water samples were analyzed for nitrate + nitrite-nitrogen $NO_3^- + NO_2^- - N$ (Lachat QuikChem method 10-107-04-1-B, cadmium reduction) and ammonium (NH_4^+) (Lachat QuikChem method 10-107-06-1-C, sodium hypochlorite), and unfiltered

Table 1 Land use, water quality variables, and organic matter estimates for each site sampled

Site	% Land use		DO[a]		Turbidity	TP[b]	NH$_4^{+}$[c]	NO$_3^{-}$ + NO$_2^{-}$ − N[d]	CBOM[e]
	Forest	Pasture	(mg/L)	(%)	NTU	(µg/L)	(µg/L)	(µg/L)	g/m^2
Jones (09)	90.0	6.6	9.14	93.3	2.7	8	17	534	No Data
Sweet Water (09 & 10)	61.2	28.1	10.8	98.0	1.0	9	16	503	8
Illinois (09 & 10)	61.2	28.1	10.5	93.3	0.9	9	29	653	4
Cannon (10)	81.6	12.5	10.7	95.1	1.0	16	5	772	20
NFWOC (10)	56.1	27.3	12.0	101.2	1.6	16	30	278	2
Possum (09)	90.2	6.5	10.8	97.4	1.1	20	12	146	2
Fane (10)	92.2	4.7	11.0	90.8	0.8	24	23	1220	6
Ogden (09)	87.6	7.4	10.2	95.9	2.2	26	24	89	17
Crosses (09)	81.6	12.5	10.7	98.4	2.4	41	16	317	6
Wildcat (10)	67.2	27.9	8.7	80.3	1.9	49	21	3901	13
Flint (10)	34.9	52.7	10.4	94.4	1.2	50	22	4173	4
Chambers (10)	58.7	36.2	10.4	88.6	1.7	62	15	1567	7

[a] Dissolved oxygen

[b] Total phosphorus

[c] Ammonium

[d] Nitrate + nitrite − nitrogen

[e] Coarse benthic organic matter

samples were analyzed for TP (Thermo Scientific; Gensys 10vis, The Netherlands) using a persulfate digestion followed by an ascorbic acid reduction method (APHA, 2005).

Samples for CBOM quantity estimates were collected using a core and transect approach to account for BOM patchiness in estimates (England & Rosemond, 2004). Samples were collected for CBOM nutrient analysis from three riffles and pools in each stream within a 200 m sampling reach. Dominant leaf species were obtained from the benthos in each habitat unit, rinsed with stream water, placed into paper sacks, kept in a cooler on ice, and immediately dried (<50°C; 24–48 h) upon returning to the lab.

Frozen CBOM samples collected with cores were thawed and picked through under a dissecting scope to remove macroinvertebrates. Samples were then dried to a stable weight (<50°C), weighed, ashed at 550°C, rehydrated, dried, and re-weighed to estimate ash-free dry mass (AFDM). Dried CBOM nutrient content samples were ground to a fine powder using a Wig-L-Bug® grinder (Rinn, Crescent 3110B, Elgin, IL), and subsamples were analyzed for % P, C, and N. Leaf litter P content was determined by combusting the material at 550°C and incubating the material in 1 N hydrochloric

acid for 30 min at 85°C (Rosemond et al., 1993, digest efficiency standard: NIST; Peach Leaves 1547, Gaithersburg, MD; % recovery >98%). Following digestion, samples were diluted to 100 mL to neutralize pH and processed using an ascorbic acid reducing method (APHA, 2005). Subsample C- and N-content was measured using a CHN elemental analyzer (EA Analytical; NC2500, Lakewood, NJ) (combustion standard: Thermo Scientific; Aspartic Acid 338 40023, The Netherlands % recovery >99%). All elemental ratios in this study are reported on a molar basis.

Shredding insect samples were collected from three riffles and pools in each stream within a 200 m reach, according to rapid bioassesment protocols developed for multi-habitat macroinvertebrate sampling (Barbour et al., 1999). Insects were sampled using a 250-µm mesh kick net. To ensure adequate sampling of all habitats within a habitat unit, ten kicks, covering an area of 0.2 m^2, were made in each habitat, attempting to obtain samples from all sub-habitats (along stream margins, thalweg, debris pools, etc.). Chironomids and two other rare taxa (*Zealuctra* spp. and *Peltodytes* spp.) were excluded from the present analysis, as their abundance could not be accurately estimated using this collection procedure, and our

study focused on slower growing long-lived shredders as potential indicators for long-term nutrient stress. Insects were picked from the net in the field, placed in plastic cups containing stream water, and transported back to the lab on ice. Insects were kept in growth chambers at 10°C overnight to allow gut evacuation. Afterward, insects were frozen until identification.

Shredders were thawed, rinsed, and identified to genus according to Merritt et al. (2008) using a dissecting microscope. Abundances of each genus were tallied for each individual riffle and pool, and lengths of each insect were measured to the nearest 0.1 mm using an ocular micrometer. Biomass for each genus was calculated for each habitat replicate using length–mass conversions according to Benke et al. (1999). However, insects from the genus *Tipula* spp. were individually dried and weighed to determine biomass. Genera abundance and biomass estimates were calculated for separate riffles and pools, averaged across each habitat at each site, and then used for shredder community analyses. All abundance and biomass estimates are reported on a habitat-weighted basis. After identification and measurement, genera from each habitat sample were pooled and analyzed for C, N, P using methods reported above. Community C:P estimates were calculated from the habitat-weighted means of each genus collected at each site.

All relationships between CBOM C:P and C:N, AFDM, and stream $NO_3^- + NO_2^- - N$ were determined by Pearson and Spearman correlations. Differences in C:N and C:P body content among individual genera were first assessed using one-way analysis of variance (ANOVA) followed by Tukey–Kramer tests for unbalanced ANOVAs to confirm significant differences between genera. Genus-specific responses to independent variables were first analyzed using a nonparametric 2-dimensional Kolmogorov–Smirnov threshold analysis, developed for detecting threshold change points for small sample sizes (Garvey et al., 1998). However, no threshold relationships were observed, so we further analyzed genera responses using Pearson and Spearman correlations, after making Bonferroni corrections for multiple comparisons ($P < 0.0125$). First, we performed a Shapiro–Wilk test for normality, and we analyzed normally distributed data using Pearson correlations and non-normal data using Spearman correlations, according to the assumptions of each test. Relationships between shredder community abundance, biomass, B:A, and community C:P ratio estimates and nutrient and detrital

standing stock variables were determined by Pearson and Spearman correlations. All statistics were calculated using SAS statistical software, version 9.2 (SAS institute inc., Cary, NC.)

Results

Concentrations of DO ranged from 8.7 to 12.0 mg l^{-1} (mean = 10.4 mg l^{-1}), and turbidity was lower than values previously found to negatively affect macroinvertebrate communities in this region (Evans-White et al., 2009), ranging from 0.8 to 2.7 nephelometric turbidity units (NTU) (mean = 1.5) (Table 1). The TP gradient ranged from 8 to 62 µg l^{-1} (mean = 27 µg l^{-1}). Levels of NH_4 were low across all sites 5–30 µg l^{-1} (mean = 19.2 µg l^{-1}), and $NO_3^- + NO_2^- - N$ concentrations ranged from low to potentially toxic levels 89–4173 µg l^{-1} (mean = 1179 µg l^{-1}) (Camargo & Alonso, 2006). Stream CBOM varied from 2 to 20 g m^{-2} (mean = 8 g m^{-2}).

No significant correlations between CBOM and nutrient/land use variables were found across sites within years or when data from each year were combined ($P > 0.05$). However, leaf litter C:P ratios were negatively correlated to water column TP concentrations for both years considered separately 2009 [($r = 0.88; P < 0.01$), 2010 ($r = 0.51; P = 0.03$)] and combined ($r = 0.56; P < 0.01$) (Fig. 1A). Significant decreases in leaf litter C:N ratios with increases in stream $NO_3^- + NO_2^- - N$ were also found in 2010 ($r = 0.50; P = 0.03$) and when both years were considered together ($r = 0.54; P = 0.01$) (Fig. 1B). However, no significant correlation between C:N and $NO_3^- + NO_2^- - N$ existed in 2009 ($r = 0.07; P = 0.31$).

Shredder richness was low compared to richness found in a previous study in the region (Evans-White et al., 2009) and consisted of 7 shredder genera, across four orders, with body C:P ranging from 241 to 437 (mean = 354) and C:N from 6.2 to 8.2 (mean = 7.0) (Table 2). Only 2 individuals representing two rare taxa (*Zealuctra* spp. and *Peltodytes* spp.) were found in a single stream; therefore, we could not generate accurate stoichiometry and abundance/biomass data to include them in the analysis. Average abundance of the 5 focal taxa was as follows: *Tipula* spp. (2.01 m^{-2}), *Pycnopsyche* spp. (0.81 m^{-2}), *Ephemerella* spp. (3.51 m^{-2}), *Amphinemura* spp. (5.21 m^{-2}), and *Strophopteryx* spp. (0.52 m^{-2}). One-way ANOVA
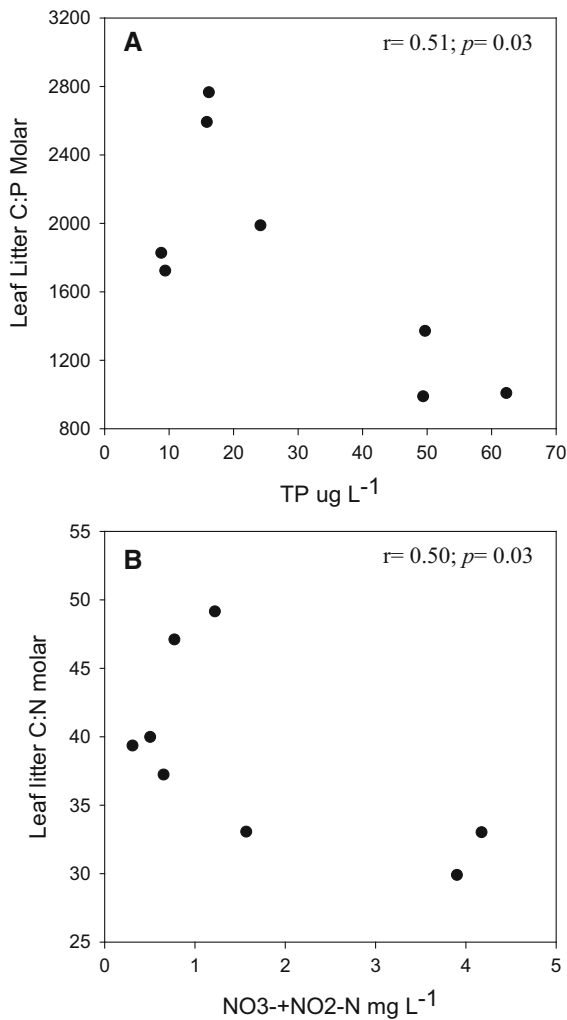
Fig. 1 Correlations between total phosphorus (TP) and leaf litter carbon:phosphorus (C:P) and between nitrate + nitrite-nitrogen $NO_3^- + NO_2^- - N$ and leaf litter carbon:nitrogen (C:N) in 2010. Results of Pearson and Spearman correlations are expressed as r and P values

revealed a significant difference in C:P and C:N ratios for insects sampled in this study ($F = 7.17$; df = 4; $P < 0.01$, $F = 7.5$; df = 4; $P < 0.01$, respectively). The two highest C:P shredders' [*Strophopteryx* spp. and *Amphinemura* spp.] body stoichiometry differed significantly from the lowest C:P insect, *Tipula* spp. Significant differences were also found between the two highest C:N [*Strophopteryx* spp. and *Pycnopsyche* spp.] and lowest C:N shredders (*Tipula* spp. and *Ephemerella* spp.) (Table 2). No insect genera responded to altered CBOM quantity; however, several correlations were found between shredder genera, water quality variables, and/or leaf litter stoichiometry (Table 3).

Four of the five genera measured responded significantly to differences in stream nutrients and/or leaf litter stoichiometry. As predicted, significant positive correlations were found between *Pycnopsyche* spp. abundance and TP (Fig. 2C, D) but not between this genus and any other variables. *Ephemerella* spp. stoichiometry fell in between our lowest and highest C:P taxa, but abundance and biomass revealed significant positive correlations with TP (Fig. 2E, F). *Amphinemura* spp., our second highest C:P taxon, showed no response to water or leaf litter chemistry (Fig. 3A, B). Abundance and biomass of our highest C:P taxon, *Strophopteryx* spp., showed positive correlations with leaf litter C:P and C:N (Fig. 3C, D) but did not respond to water-column nutrients (Table 3). Counter to our predictions, the abundance and biomass of our lowest C:P genera, *Tipula* spp., exhibited a significant negative correlation with stream TP (Fig. 2A, B), and abundance was negatively correlated with stream $NO_3^- + NO_2^- - N$ (Table 2). These genus-specific responses combined to shape overall shredder community structure.

Table 2 Shredding insect genera and mean [1]carbon:phosphorus (C:P) and [2]carbon:nitrogen (C:N) molar ratios ± standard deviation

Order	Family	Genus	Sample #	C:P[1]	C:N[2]
Diptera	Tipulidae	*Tipula* spp.	39	241 ± 41a	6.3 ± .052ac
Tricoptera	Limnephilidae	*Pycnopsyche* spp.	11	321 ± 55a	7.8 ± 1.04b
Ephemeroptera	Ephemerellidae	*Ephemerella* spp.	13	340 ± 68a	6.2 ± 0.24ac
Plecoptera	Nemouridae	*Amphinemura* spp.	20	385 ± 86b	7 ± 0.48abc
Plecoptera	Taeniopterygidae	*Strophopteryx* spp.	8	437 ± 54b	8.2 ± 1.4b

Letters indicate species separated using Tukey–Kramer tests for significant differences ($P < 0.01$) between shredding genera C:P and C:N

Table 3 Results of correlation analysis between dependent and independent variables

	TP[a]	$NO_3^- + NO_2^- - N$[b]	Leaf C:P[c]	Leaf C:N[d]
Community Abundance	0.76; 0.03	0.55; 0.16	−0.64; 0.09	−0.55; 0.16
Community Biomass	−0.78; 0.02	−0.69; 0.06	**0.82; 0.01**	0.81; 0.01
Community B:A[e]	**0.85; <0.01**	−0.67; 0.07	**0.67; <0.01**	**0.86; <0.01**
Community C:P[f]	0.02; 0.97	−0.31; 0.46	−0.19; 0.66	−0.24; 0.57
Tipula spp. Abundance	**−0.88; <0.01**	**−0.83; <0.01**	0.55; 0.16	0.64; 0.09
Tipula spp. Biomass	**−0.81; 0.01**	−0.76; 0.03	0.57; 0.14	0.62; 0.10
Pycnopsyche spp. Abundance	**0.83; 0.01**	0.27; 0.52	−0.51; 0.19	−0.56; 0.15
Pycnopsyche spp. Biomass	0.76; 0.03	0.15; 0.73	−0.47; 0.24	−0.48; 0.23
Ephemerella spp. Abundance	**0.96; <0.01**	0.80; 0.02	−0.77; 0.03	−0.77; 0.03
Ephemerella spp. Biomass	**0.90; <0.01**	0.81; 0.02	−0.78; 0.02	−0.81; 0.02
Amphinemura spp. Abundance	−0.02; 0.96	−0.12; 0.78	−0.36; 0.39	−0.36; 0.38
Amphinemura spp. Biomass	−0.36; 0.39	−0.36; 0.39	−0.16; 0.70	−0.20; 0.64
Strophopteryx spp. Abundance	−0.46; 0.25	−0.33; 0.43	**0.82; 0.01**	**0.61; 0.01**
Strophopteryx spp. Biomass	−0.48; 0.23	−0.14; 0.75	**0.83; 0.01**	**0.84; <0.01**

Pearson and Spearman correlations are expressed as *r* and *P* values. Significant relationships are presented in bold font after Bonferroni corrections for multiple comparisons ($p < 0.0125$)

[a] Total phosphorus

[b] Nitrate + Nitrite − Nitrogen

[c] Leaf litter carbon:phosphorus molar ratio

[d] Leaf litter carbon:nitrogen molar ratio

[e] Biomass:abundance ratio

[f] Community carbon:phosphorus ratio

Shredder community abundance was not significantly correlated to independent variables (Table 3; Fig. 4A). Biomass was negatively related with leaf litter C:P (Fig. 4B) but was not correlated with any other variables. We tested the prediction that average body size decreased as nutrients increased and leaf litter C:P and C:N decreased by examining relationships between these variables and B:A ratios. Ratios of shredder B:A were negatively correlated with TP (Table 3) and positively correlated to leaf litter C:P (Fig. 4C) and C:N (Table 3). However, no significant correlations were found for mean shredder community C:P (Fig. 4D), although the mean and variation in community C:P tended to decrease with increasing TP and with decreasing leaf litter C:P (Table 3).

Discussion

This study is the first to examine changes in shredding insect population and community abundance, biomass, and stoichiometry in conjunction with detritus standing stocks and resource stoichiometry across a multi-stream nutrient gradient. We provide correlative evidence collected across a nutrient gradient that shredding insect population responses to nutrient enrichment were related to differences in stream nutrients and detrital quality and independent of decreased detrital quantity. Further, shredding insect population responses to enrichment were species dependent and generally predictable by shredding insect stoichiometry. These results taken together with results from manipulative enrichment experiments (Cross et al., 2003, 2006) provide a convincing body of evidence that changes in food quality may be in part responsible for shifts in macroinvertebrate community structure with enrichment (Evans-White et al., 2009).

Detrital quality was positively correlated (i.e., decreased C:N or C:P) with increasing nutrients across our study streams during the two years of study. The average leaf litter C:P (2088) and C:N (41.0) fell between those given for leaf material found across a regional study conducted in Indiana, Michigan, and Wisconsin, US [(C:P ∼ 1,000); (C:N ∼ 25) Evans-
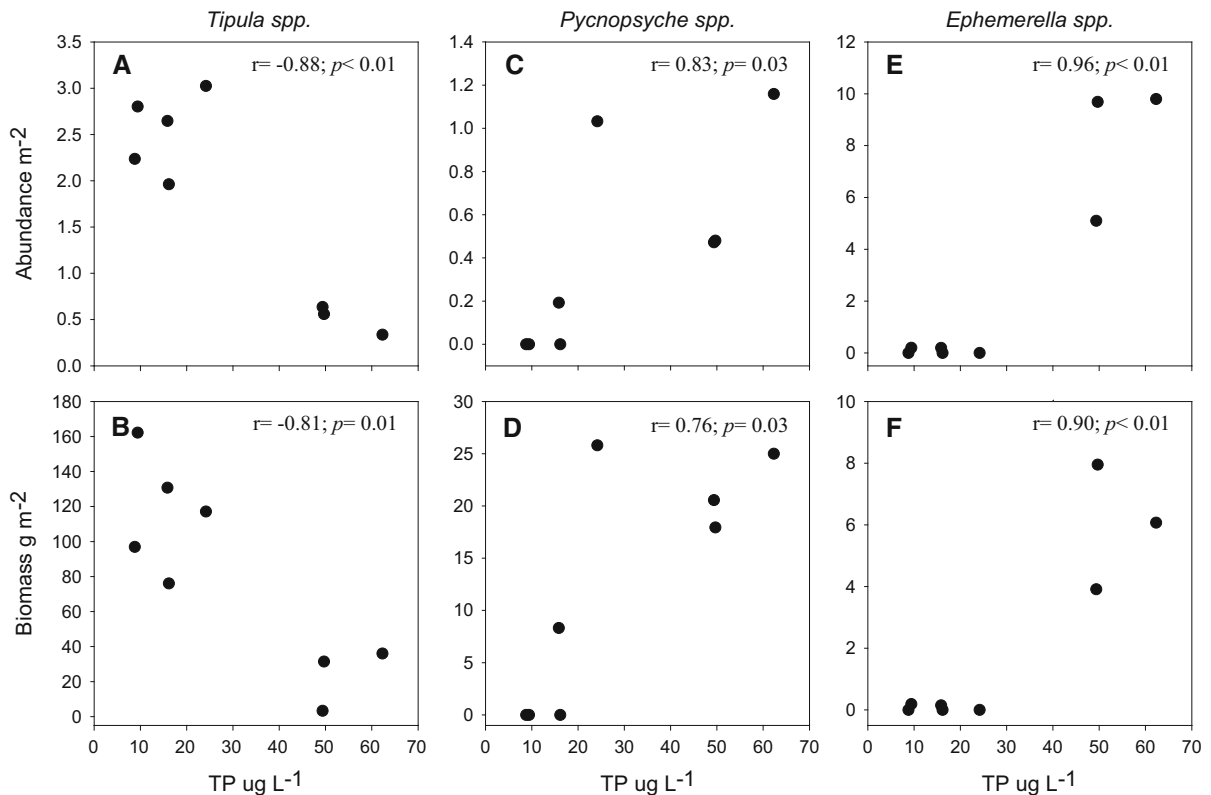
Fig. 2 Correlations between *Tipula* spp., *Pycnopsyche* spp., and *Ephemerella* spp. abundance and biomass and total phosphorus (TP). Results of Pearson and Spearman correlations are expressed as *r* and *P* values

White et al., 2005] and those found in reference streams in North Carolina, US [(C:P = 4,858); (C:N = 73) Cross et al., 2003]. Microbial community biomass or activity was not measured in this study but likely increased with nutrient enrichment causing the observed trends in quality (Gulis & Suberkropp, 2003, Greenwood et al., 2007). Overall, evidence from this and many other studies (Cross et al., 2003, Greenwood et al., 2007, Small & Pringle, 2010, Scott et al., 2013) indicates robust patterns of increased basal food resource quality in headwater detrital systems across time and space with nutrient loading, which may potentially alter macroinvertebrate assemblages.

We provided partial evidence for the food quality mechanism driving shifts in shredder populations and communities (i.e., positive correlations between in high C:P *Stophopteryx* spp. and community biomass and food C:P), although water quality explained more of the variation than food quality in a majority of variables. It is possible that these shredders can

selectively feed on more balanced C:P material (Hood et al., 2014), and this would weaken the overall response found in our study. Additionally, as detrital C:P depends on the initial C:P leaf litter inputs which can vary widely (Hattenschwiler et al., 2008), differences among inputs to different sites could also weaken consumer-resource correlation. However, our study indicates negative correlations between stream nutrients and resource C:N and C:P. This evidence, along with food quality explaining a large amount of ecologically significant variance in shredder assemblages despite using a conservative statistical analysis in a highly variable field setting, suggests that food resource enrichment can have significant impacts on aquatic foodwebs. These changes could be due to either variation in nutrient content of plastic consumers or from decreases in sensitive invariable taxa experiencing nutrient stress.

Contradictory results have been found regarding the plasticity of consumer elemental content in the lab
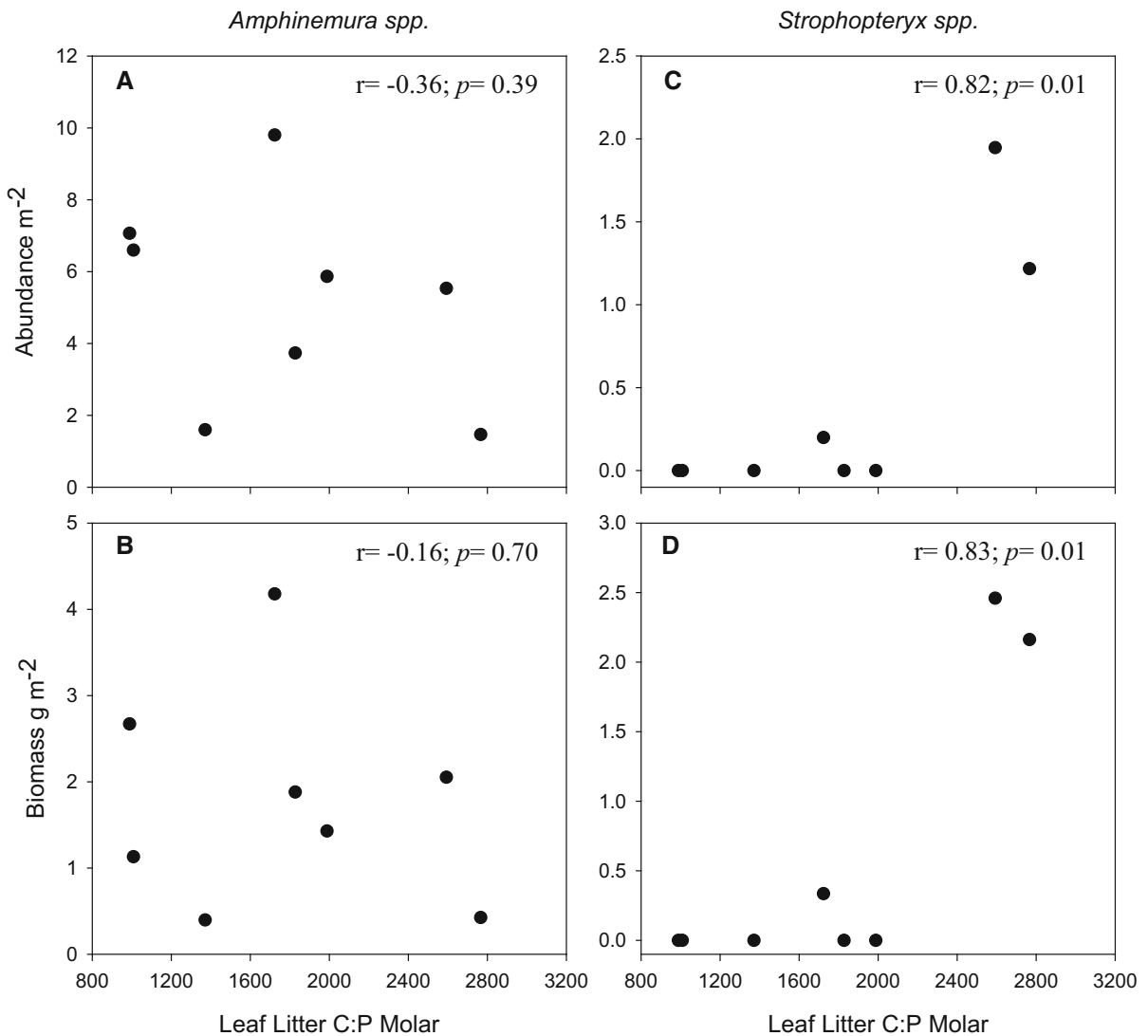
Fig. 3 Correlations between *Amphinemura* spp. and *Strophopteryx* spp. abundance and biomass and leaf litter carbon:phosphorus (C:P). Results of Pearson and Spearman correlations are expressed as *r* and *P* values

and across nutrient gradients. Some studies have found significant differences in taxa nutrient content when feeding across water quality or resource stoichiometry gradients (Cross et al., 2003, Back et al., 2008, Small & Pringle, 2010, Back & King, 2013); however, others have found no deviation in elemental body composition (Evans-White et al., 2005, Danger et al., 2013). Our results most closely resemble Morse et al., 2012, as we found no significant differences in P content of any taxa across streams; however, two taxa (*Tipula* spp. and *Strophopteryx* spp.) in our study showed reduced body C:N with increased $NO_3^- + NO_2^- - N$

(Supplementary Table 1). Due to the small sample sizes and taxonomic resolution of our study, comparisons to other studies should be taken with caution. Other differences in these studies are possibly due to differences in sample collection and processing (i.e., separating consumers by sex and size class vs. analyzing the population as a whole) as well as differences in elemental plasticity within a genus or between organisms in different regions. As a majority of taxa collected from the field displayed invariable body nutrient composition in our study, declines in sensitive taxa across nutrient gradients due to
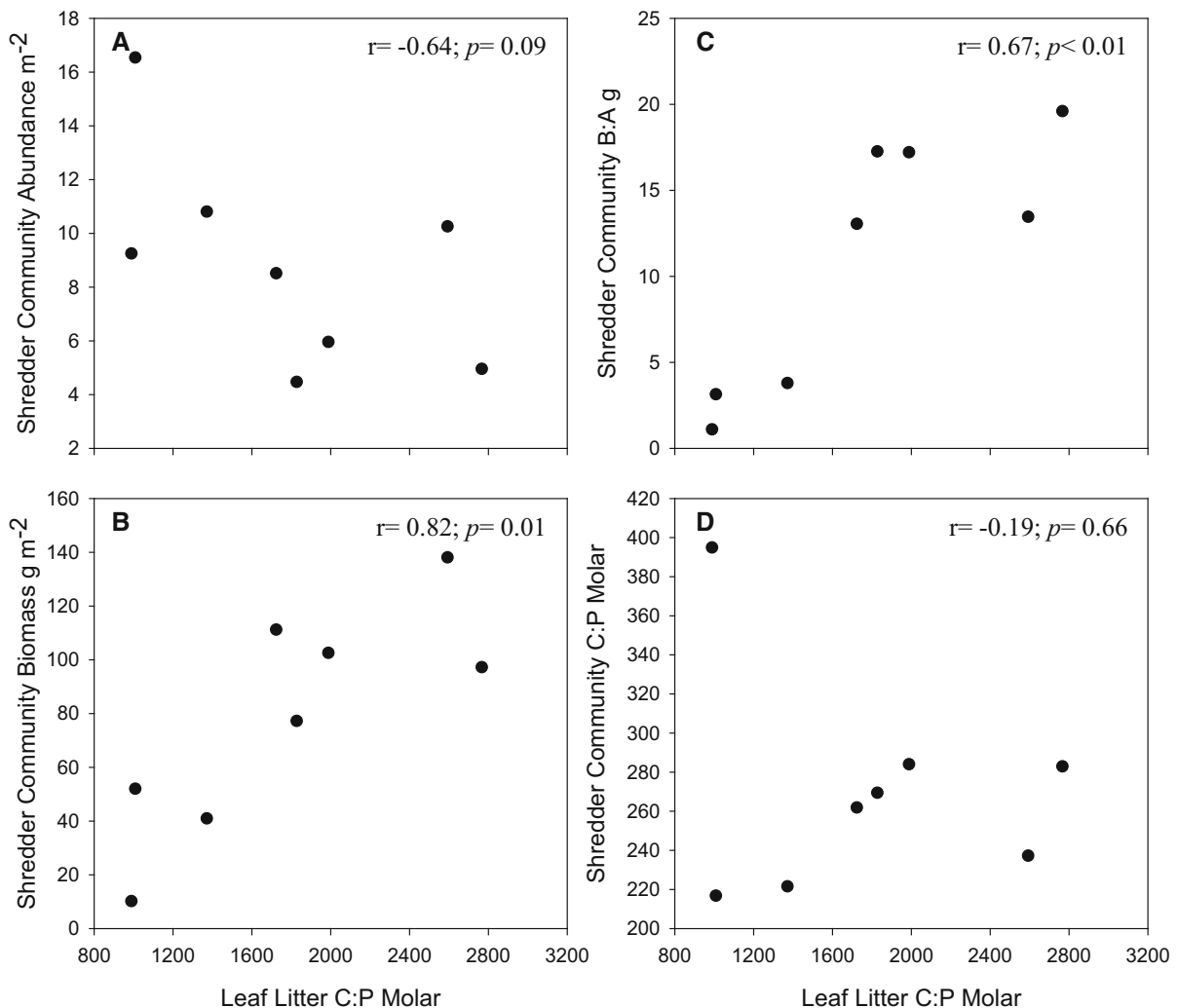
Fig. 4 Relationships between shredding insect community abundance, biomass, community biomass:abundance ratios (B:A), community carbon:phosphorus (C:P) ratios, and leaf litter C:P. Results of Pearson and Spearman correlations are expressed as *r* and *P* values

nutritional stress could significantly affect shredder assemblages.

Enrichment of detrital food resources and/or increasing nutrients coincided with shifts in shredding insect biomass and abundance in four out of five genera including *Tipula* spp., *Pycnopsyche* spp., *Ephemerella* spp., and *Strophopteryx* spp. These patterns in abundance and biomass at the genus level could generally be predicted by stoichiometry theory. Previous stoichiometry research suggests that organisms with low body P contain lower rRNA quantities and therefore may not possess the molecular capabilities necessary to elicit a significant growth

response to food resource enrichment (Elser et al., 2000, Cross et al., 2003, Shade et al., 2003, Weider et al., 2005). In fact, metabolism of slow-growing low-P organisms might be altered when consuming high-P resources, and this stress may cause slower growth and altered life-history traits with resource enrichment (Boersema & Elser, 2006). Therefore, we expected to observe either no change or decreases in high body C:P shredding insect species abundance and biomass as leaf litter C:P decreased and dissolved nutrients increased. Examining patterns of the two highest C:P genera, we found that *Amphinemura* spp. (C:P = 385) abundance and biomass did not change across the TP

and leaf litter C:P gradient. Further, the abundance and biomass of our highest body C:P genera, *Strophopteryx* spp. (C:P = 487), actually declined with increasing detrital food quality. This decline suggests either that increasing levels of P in their food have some detrimental "stoichiometric knife-edge" effect on *Strophopteryx* spp. populations (Boersema & Elser, 2006) or that some other confounding factor is negatively affecting their populations.

While this study is correlative in nature and it is impossible to count out all potentially confounding factors, these results reinforce the findings of negative relationships between nutrient enrichment and sensitive shredding genera (Wang et al., 2007, Evans-White et al., 2009). If other factors such as increased turbidity or sedimentation were playing a significant role shaping shredding macroinvertebrate populations in our most enriched sites, we would expect most of our species to respond negatively to increasing TP as nutrient enrichment correlates with these variables in this region (Evans-White et al., 2009). However, *Amphinemura* spp. and *Strophopteryx* spp. did not respond to increasing TP, and *Ephemerella* spp. (C:P = 321), and *Pycnopsyche* spp. (C:P = 340) responded positively, confirming our prediction that lower body C:P shredders would respond positively to nutrient enrichment. This evidence supports our prediction of shifting community structure to low C:P organisms with nutrient enrichment; however, *Tipula* spp., the lowest C:P organism in our study declined with increased nutrients in this study, possibly due to other environmental or life-history factors.

The resource quality hypothesis did not accurately predict patterns in abundance and biomass for *Tipula* spp. Previous work at using experimental nutrient enrichment actually found slight increases in *Tipula* spp. abundance, biomass, and production with P enrichment of detrital resources (Cross et al. 2006 Appendix A). However, as mean detrital C:P of the enriched stream of the previous study (3063) was much higher than in our region (2088), it is possible that different responses between studies could point to *Tipula* spp. TER$_{C:P}$ falling within this range of litter enrichment or that unique species could respond differently to nutrient enrichment within this genera. Other factors such as [DO], stream drying, or water quality might also contribute to differences in population level responses on regional levels. However, we measured early morning [DO] in study streams during the winter and spring, and no streams were lower than 8.7 mg l^{-1} (80.3%). While this is only one time of year, *Tipula* spp. are known to burrow into banks and enter diapause in mid-April/early May, and abundance and biomass of this genus in Arkansas, US is not significantly affected by stream drying (McCord et al., 2006). Finally, rapid bioassessment protocols suggest that *Tipula* spp. are more tolerant to degraded water quality than the other taxa sampled in this study (Barbour et al., 1999). Mean tolerance values from the different regions were 5.8, 3.4, 1.9, 2.8, and 2.6 for *Tipula* spp., *Pycnopsyche* spp., *Ephemerella* spp., *Amphinemura* spp., and *Strophopteryx* spp., respectively, with a score of zero being least tolerant and 10 being most tolerant. Therefore, if other confounding pollutants were affecting shredder populations in our study, it is not clear why *Tipula* spp. would be more sensitive than *Strophopteryx* spp. It is possible that differences in other life-history traits could be important factors controlling *Tipula* spp. populations in this study.

Cross et al. (2005) found that short-lived multivoltine chironomids responded significantly to nutrient enrichment, whereas long-lived semivoltine *Tallaperla* spp. stoneflies responded weakly under similar circumstances. Previous work conducted in the Interior Highlands, US suggests that some tipulid species are semivoltine (Pritchard, 1983, McCord et al., 2006). Semivoltine life-histories could put *Tipula* spp. at a competitive disadvantage in headwater areas due to reductions of organic matter at key periods, such as early fall, due to feeding of other quicker growing insect larvae (Cross et al., 2006). Intra-guild competition could cause C-starvation in tipulids and explain the patterns of decreases in biomass and abundance, and declines in overall organic matter standing stocks could explain the differences in *Tipula* spp. abundance and biomass shifts between this study and others. As tipulids have been recognized as one of the major shredders in the Interior Highlands in terms of biomass (McCord et al., 2006), their sensitivity to nutrient enrichment could have significant impacts on detrital trophic processes in these systems. Lab-based studies are needed to further examine species-specific tipulid physiological responses to nutrient enrichment changes as their abundance and biomass were found to drive shifts in community patterns.

There was an overall decrease in large-bodied shredder biomass with increasing nutrient concentrations, and this shift combined with increased abundances of small-bodied consumers led to an overall decrease in community B:A ratios (Fig. 4C). The finding of decreasing biomass differs from previous research that found overall positive relationships with P enrichment (Rosemond et al., 2002, Cross et al., 2006). In addition, we did not find a statistically significant relationship between mean shredder community C:P and leaf litter C:P (Fig. 4D) or TP in the present study, contrary to our predictions based on data from Evans-White et al. (2009), which found negative correlations between community C:P and TP. However, we found abundance and biomass increases in all but one low C:P taxa along with declines of the highest C:P insects with increased stream TP, suggesting that declines in community C:P with enrichment might occur in this region. Results from our study are not specifically comparable to previous studies, however, because we did not include low C:P multivoltine chironomids, which can come to dominate shredder production in enriched systems.

Chironomids have been shown to exhibit variable body stoichiometry when feeding on resources in different streams (Small & Pringle, 2010), and increases in their biomass or feeding behavior could lead to altered detrital stoichiometry or standing stocks. Therefore, shifts from high to low C:P organisms may accompany enrichment, and chironomids could be superior competitors in high-P environments causing declines in shredder community C:P and biomass (Baldy et al., 2007, Evans-White et al., 2009). Differences between studies highlight the need to consider life-history traits in addition to consumer stoichiometry when determining species responses to nutrient enrichment. Overall, this work suggests that while production might increase with nutrient enrichment (Cross et al., 2006), it comes at the expense of larger slow-growing taxa and that their absence may indicate nutrient enrichment of detrital resources. Further research into the physiology behind shredder knife-edge responses is needed to better understand the effects of dietary P saturation on slow-growing high C:P consumers.

Our study revealed an overall pattern of altered shredder communities with increasing nutrient enrichment of detrital resources, possibly altering elemental flows through stream foodwebs due to changes in consumer biomass. These shifts mostly favored lower C:P taxa, as predicted by stoichiometric theory. The failure to accurately predict changes in *Tipula* spp. populations highlights the need for more laboratory-based measurements of differences in invertebrate physiology under nutritional stress. More information gained by laboratory-based TER estimates for shredding macroinvertebrates should improve predictive ability in regards to stoichiometrically induced taxa shifts in aquatic environments. Information from species-based TERs can be used to identify and predict dietary and physiological drivers of species population shifts in natural assemblages, which could lead to decreased functional diversity and community resilience in affected streams. This information combined with nutrient threshold analysis has proven to be a powerful tool for predicting changes in macroinvertebrate community structure on regional scales and should be used to inform policy decisions for water quality standards (King & Richardson, 2003, Wang et al., 2007, Evans-White et al., 2009) and for managing watersheds and stream networks on larger regional and continental scales (King & Baker, 2010, Woodward et al., 2012).

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