

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

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1 **Abstract**

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

20 Keywords: ecological stoichiometry, eutrophication, detritivore, leaf litter, shredder,
21 phosphorus

22

23

24 **Introduction**

25 Anthropogenic nutrient enrichment is occurring worldwide (Kuijper et al. 2005) and
26 is the second most common cause of freshwater ecosystem degradation in the US (EPA
27 2006). Headwater streams can have disproportionately high rates of nutrient processing
28 compared to other freshwater systems (Alexander et al. 2000, Wollheim et al. 2001), and
29 their destruction or degradation can decrease ecosystem functioning causing further
30 downstream damage to rivers, lakes, and coastal areas (Carpenter et al. 1998). Forested
31 headwater streams are increasingly imperiled due to the clearing of riparian vegetation for
32 agricultural and urban area development (Paul & Meyer 2001), which can cause amplified
33 irradiance (DeNicola & Hoagland 1996), flashier hydrology, increased sedimentation
34 (Webster & Waide 1982), and higher nutrient inputs (Likens et al. 1970) in these areas.
35 Recent studies have provided evidence that nutrient enrichment of forested headwater
36 streams causes shifts in benthic macroinvertebrate community structure due to increases in
37 detrital quality and decreases in quantity (Ramirez & Pringle 2006, Cross et al. 2006,
38 Greenwood et al. 2007, Singer & Battin 2007). However, the relationship between altered
39 diet and benthic macroinvertebrate community structure has not been examined across a
40 regional stream nutrient gradient.

41 Elevations of stream nutrient concentrations are known to differentially affect the
42 quality and quantity of detrital material. Increased nutrients in the water column such as
43 nitrogen (N) and phosphorus (P) can be immobilized by microbial decomposers (Webster et
44 al. 2009), supporting higher rates of fungal and bacterial production, microbial respiration,
45 and increased fungal biomass (Greenwood et al. 2007, Suberkropp et al. 2010). As detrital
46 resources are generally poor in N and P, increased microbial uptake can lead to increases in
47 food quality, reducing nutritional imbalances between detritus and primary consumers (Cross
48 et al. 2003, Greenwood et al. 2007, Hladyz et al. 2009). In turn, enhanced detrital quality can

49 cause decreases in detrital standing stocks due to increased feeding by microbes and
50 shredding macroinvertebrates (Rosemond et al. 2001). Therefore, unlike autotrophic systems,
51 nutrient enrichment of detritus-based heterotrophic systems can accentuate C losses by
52 stimulating C cycling rates in lower trophic levels of these systems (Rosemond et al. 2001,
53 Cebrian 2004). Thus, increases in nutrient loading can alter basal resource nutrient quality (N
54 and P) and energy (C) quantity for detritivores adapted to forested headwater streams.

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

66 Recent studies have highlighted the effects of anthropogenic P-loading on
67 macroinvertebrate consumers and their food resources. Experimental reach-level studies have
68 shown that increases in P concentrations can cause reductions in macroinvertebrate diversity,
69 by reducing the nutrient imbalance between low C:P consumers and their food resources
70 (Singer & Battin 2007, Gafner & Robinson 2007). According to the growth rate hypothesis
71 (Elser et al. 2003), low C:P consumers have a higher proportion of P-rich ribosomal
72 ribonucleic acid (RNA) that allow them to grow faster and outcompete high C:P organisms
73 under food P-saturation. These results, along with studies of experimentally enriched streams,

74 have revealed increases in abundance, biomass, and secondary production of multivoltine P-
75 rich consumers (Cross et al. 2006, Davis et al. 2010, Small & Pringle 2011). In addition,
76 regional studies have found reductions in shredder and collector-gatherer macroinvertebrate
77 community richness, diversity, and body C:P (Baldy et al. 2007, Wang et al. 2007, Evans-
78 White et al. 2009). These regional studies suggest that the community composition of aquatic
79 organisms, especially those with large elemental imbalances between their diet and body
80 composition, such as large-bodied high C:P shredding insects, can be negatively affected by
81 high stream nutrient concentrations. However, these studies do not directly examine possible
82 mechanistic changes in resource condition (i.e., increased food quality or decreased quantity)
83 that might be responsible for observed changes in shredding insect community structure. The
84 ability to predict the effects of altered detrital quality and quantity on life-history traits of
85 individual long-lived shredder species and dynamics of whole assemblages could help
86 establish more informed stream water nutrient criteria by estimating threshold responses of
87 species abundance and biomass in response to nutrient enrichment.

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

98 2006), we wanted to determine whether body C:P estimates may be used as a surrogate to
99 qualitatively predict detritivore species responses to nutrient enrichment.

100 The first objective of this study was to determine if alteration of basal resource quality
101 or quantity occurred across stream water total phosphorus (TP) and nitrate + nitrite-nitrogen
102 ($\text{NO}_3^- + \text{NO}_2^- - \text{N}$) gradients. We predicted that coarse benthic organic matter (CBOM)
103 nutritional quality would increase and that quantities would decrease with increased nutrient
104 loading. Next, we examined whether stoichiometric principles (consumer/resource nutrient
105 imbalance) could be used to predict genus-specific shifts in shredding insect abundance and
106 biomass leading to alterations in overall insect community abundance and biomass. For these
107 responses, we predicted that alteration in detrital quantity and/or quality would correlate with
108 shifts in taxon dominance altering shredder communities, providing a mechanism for
109 community composition shifts observed across larger regional scales (Wang et al. 2007,
110 Evans-White et al. 2009). We specifically predicted that abundance and biomass of low C:P
111 genera (*Tipula spp.*, *Pycnopsyche spp.*, and *Ephemerella spp.*) would increase with nutrient
112 enrichment in this study and that high C:P consumer (*Amphinumera spp.* and *Strophopteryx*
113 *spp.*) abundance and biomass would remain unchanged or that they would decrease across
114 nutrient gradients. To examine whether these changes were related to differential investment
115 of P to smaller-bodied fast growing life stages we analyzed community C:P data and
116 biomass:abundance ratios (B:A). We predicted that increased stream nutrient loading would
117 enrich food quality shifting shredder community assemblages towards taxa with higher
118 nutrient demands and that this would coincide with increased abundance of small-bodied
119 consumers. We further predicted no significant differences in population level stoichiometry
120 across streams but that shifts from high to low C:P genera would result in significant
121 reductions in shredder community B:A and C:P ratios due to the exclusion of larger-bodied
122 high C:P shredders.

123 **Methods**

124 Samples were collected between 20-Mar and 11-Apr 2009 (n= 6 streams: water
125 quality and detritus) and 2010 (n= 8 streams: water quality, detritus, and invertebrates) from
126 low-order headwater streams in the Ozark Highlands region of Northwest Arkansas, USA.
127 Samples were collected during these dates as they occurred prior to leaf out and during the
128 period of maximum invertebrate richness in this region (Evans-White et al. 2009). Land use
129 in watersheds was predominately forested (34.0%-92.0%; mean= 71.0%) and pastured areas
130 (4.0-52.0%; mean= 20.0%) (Table 1), and mean stream width and depth was 5.5m (2.0m-
131 9.3m) and 26.2cm (4.0cm-150cm), respectively. Dominant riparian vegetation was composed
132 of Red Oak (*Quercus rubra*), White Oak (*Quercus bicolor*), Sycamore (*Platanus*
133 *occidentalis*), Sugar Maple (*Acer saccharum*), and River Birch (*Betula nigra*). Study streams
134 substrata were primarily gravel and characterized by riffle-pool channel morphology
135 (Brussock et al. 1985).

136 Two composite water samples, one filtered (glass fiber filter; 0.45 μ m mesh) and one
137 unfiltered, were collected from the thalweg of a well-mixed region of each stream before
138 sampling, kept on ice, and frozen upon returning to the lab. Turbidity was determined using a
139 portable fluorometer (Turner Designs: Aquafluor 8000-010; Sunnyvale, CA) at the base of
140 each site, and dissolved oxygen (DO) was measured using a YSI probe (Model 95; Yellow
141 Springs, OH). In the laboratory, filtered water samples were analyzed for NO₃⁻+NO₂⁻-N
142 (Lachat QuikChem method 10-107-04-1-B, cadmium reduction), and ammonium (NH₄⁺)
143 (Lachat QuikChem method 10-107-06-1-C, sodium hypochlorite), and unfiltered samples
144 were analyzed for TP (Thermo Scientific; Gensys 10vis, The Netherlands) using a persulfate
145 digestion followed by an ascorbic acid reduction method (APHA, 2005).

146 Samples for CBOM quantity estimates were collected using a core and transect
147 approach to account for BOM patchiness in estimates (England & Rosemond 2004). Samples

148 were collected for CBOM nutrient analysis from three riffles and pools in each stream within
149 a 200m sampling reach. Dominant leaf species were obtained from the benthos in each
150 habitat unit, rinsed with stream water, placed into paper sacks, kept in a cooler on ice, and
151 immediately dried (< 50°C; 24-48h) upon returning to the lab.

152 Frozen CBOM samples collected with cores were thawed and picked through under a
153 dissecting scope to remove macroinvertebrates. Samples were then dried to a stable weight (<
154 50°C), weighed, ashed at 550°C, rehydrated, dried, and re-weighed to estimate ash free dry
155 mass (AFDM). Dried CBOM nutrient content samples were ground to a fine powder using a
156 Wig-L-Bug® grinder (Rinn, Crescent 3110B, Elgin, IL), and subsamples were analyzed for
157 % P, C, and N. Leaf litter P content was determined by combusting the material at 550°C and
158 incubating the material in 1N hydrochloric acid for 30 minutes at 85°C (Rosemond 1993,
159 digest efficiency standard: NIST; Peach Leaves 1547, Gaithersburg, MD; % recovery >
160 98%). Following digestion, samples were diluted to 100 mL to neutralize pH and processed
161 using an ascorbic acid reducing method (APHA 2005). Subsample C- and N-content was
162 measured using a CHN elemental analyzer (EA Analytical; NC2500, Lakewood, NJ)
163 (combustion standard: Thermo Scientific; Aspartic Acid 338 40023, The Netherlands %
164 recovery > 99%). All elemental ratios in this study are reported on a molar basis.

165 Shredding insect samples were collected from three riffles and pools in each stream
166 within a 200m reach, according to rapid bioassessment protocols developed for multi-habitat
167 macroinvertebrate sampling (Barbour et al. 1999). Insects were sampled using a 250µm mesh
168 kick net. To ensure adequate sampling of all habitats within a habitat unit, ten kicks, covering
169 an area of 0.2m², were made in each habitat, attempting to obtain samples from all sub-
170 habitats (along stream margins, thalweg, debris pools, etc.). Chironomids and two other rare
171 taxa (*Zealuctra spp.* and *Peltodytes spp.*) were excluded from the present analysis, as their
172 abundance could not be accurately estimated using this collection procedure, and our study

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

177 Shredders were thawed, rinsed, and identified to genus according to Merritt et al.
178 (2008) using a dissecting microscope. Abundances of each genus were tallied for each
179 individual riffle and pool, and lengths of each insect were measured to the nearest 0.1mm
180 using an ocular micrometer. Biomass for each genus was calculated for each habitat replicate
181 using length-mass conversions according to Benke et al. (1999). However, insects from the
182 genus *Tipula spp.* were individually dried and weighed to determine biomass. Genera
183 abundance and biomass estimates were calculated for separate riffles and pools, averaged
184 across each habitat at each site, and then used for shredder community analyses. All
185 abundance and biomass estimates are reported on a habitat-weighted basis. After
186 identification and measurement, genera from each habitat sample were pooled and analyzed
187 for C, N, P using methods reported above. Community C:P estimates were calculated from
188 the habitat-weighted means of each genus collected at each site.

189 All relationships between CBOM C:P and C:N, AFDM, and stream TP/NO₃⁻+NO₂⁻-N
190 were determined by Pearson and Spearman correlations. Differences in C:N and C:P body
191 content among individual genera were first assessed using one-way analysis of variance
192 (ANOVA) followed by Tukey-Kramer tests for unbalanced ANOVAs to confirm significant
193 differences between genera. Genus-specific responses to independent variables were first
194 analyzed using a nonparametric 2 dimensional Kolmogorov-Smirnov threshold analysis,
195 developed for detecting threshold change points for small sample sizes (Garvey 1998).
196 However, no threshold relationships were observed, so we further analyzed genera responses
197 using Pearson and Spearman correlations, after making Bonferroni corrections for multiple

198 comparisons ($p < 0.0125$). First, we performed a Shapiro-Wilk test for normality, and we
199 analyzed normally distributed data using Pearson correlations and non-normal data using
200 Spearman correlations, according to the assumptions of each test. Relationships between
201 shredder community abundance, biomass, B:A, and community C:P ratio estimates and
202 nutrient and detrital standing stock variables were determined by Pearson and Spearman
203 correlations. All statistics were calculated using SAS statistical software, version 9.2 (SAS
204 institute inc., Cary, NC.)

205 **Results**

206 Concentrations of DO ranged from 8.7-12.0 mg L⁻¹ (mean= 10.4 mg L⁻¹), and
207 turbidity was lower than values previously found to negatively affect macroinvertebrate
208 communities in this region (Evans-White et al. 2009), ranging from 0.8-2.7 nephelometric
209 turbidity units (NTU) (mean= 1.5) (Table 1). The TP gradient ranged from 8-62 µg L⁻¹
210 (mean= 27 µg L⁻¹). Levels of NH₄ were low across all sites 5-30 µg L⁻¹ (mean= 19.2 µg L⁻¹),
211 and NO₃⁻+NO₂⁻-N concentrations ranged from low to potentially toxic levels 89-4,173 µg L⁻¹
212 (mean= 1,179 µg L⁻¹) (Camargo and Alonso 2006). Stream CBOM varied from 2-20 g m⁻²
213 (mean= 8 g m⁻²).

214 No significant correlations between CBOM and nutrient/land use variables were
215 found across sites within years or when data from each year were combined ($p > 0.05$).
216 However, leaf litter C:P ratios were negatively correlated to water column TP concentrations
217 for both years considered separately 2009 [($r = 0.88$; $p < 0.01$), 2010 ($r = 0.51$; $p = 0.03$)] and
218 combined ($r = 0.56$; $p < 0.01$) (Figure 1a). Significant decreases in leaf litter C:N ratios with
219 increases in stream NO₃⁻+NO₂⁻-N were also found in 2010 ($r = 0.50$; $p = 0.03$) and when both
220 years were considered together ($r = 0.54$; $p = 0.01$) (Figure 1b). However, no significant
221 correlation between C:N and NO₃⁻+NO₂⁻-N existed in 2009 ($r = 0.07$; $p = 0.31$).

222 Shredder richness was low compared to richness found in a previous study in the
223 region (Evans-White et al. 2009) and consisted of 7 shredder genera, across four orders, with
224 body C:P ranging from 241-437 (mean= 354) and C:N from 6.2-8.2 (mean= 7.0) (Table 2).
225 Only 2 individuals representing two rare taxa (*Zealuctra spp.* and *Peltodytes spp.*) were
226 found in a single stream; therefore, we could not generate accurate stoichiometry and
227 abundance/biomass data to include them in the analysis. Average abundance of the 5 focal
228 taxa was: *Tipula spp.* (2.01 m⁻²), *Pycnopsyche spp.* (0.81 m⁻²), *Ephemerella spp.* (3.51 m⁻²),
229 *Amphinemura spp.* (5.21 m⁻²), and *Strophopteryx spp.* (0.52 m⁻²). One-way ANOVA revealed
230 a significant difference in C:P and C:N ratios for insects sampled in this study (F= 7.17; df=
231 4; P< 0.01, F=7.5; df= 4; P< 0.01, respectively). The two highest C:P shredders'
232 [*Strophopteryx spp.* and *Amphinemura spp.*] body stoichiometry differed significantly from
233 the lowest C:P insect, *Tipula spp.* Significant differences were also found between the two
234 highest C:N [*Strophopteryx spp.* and *Pycnopsyche spp.*] and lowest C:N shredders (*Tipula*
235 *spp.* and *Ephemerella spp.*) (Table 2). No insect genera responded to altered CBOM quantity;
236 however, several correlations were found between shredder genera, water quality variables,
237 and/or leaf litter stoichiometry (Table 3).

238 Four of the five genera measured responded significantly to differences in stream
239 nutrients and/or leaf litter stoichiometry. As predicted, significant positive correlations were
240 found between *Pycnopsyche spp.* abundance and TP (Figs. 2 C&D) but not between this
241 genus and any other variables. *Ephemerella spp.* stoichiometry fell in between our lowest and
242 highest C:P taxa, but abundance and biomass revealed significant positive correlations with
243 TP (Figs. 2 E&F). *Amphinemura spp.*, our second highest C:P taxon, showed no response to
244 water or leaf litter chemistry (Figs. 3 A&B). Abundance and biomass of our highest C:P
245 taxon, *Strophopteryx spp.*, showed positive correlations with leaf litter C:P and C:N (Figs. 3
246 C&D), but did not respond to water column nutrients (Table 3). Counter to our predictions,

247 the abundance and biomass of our lowest C:P genera, *Tipula spp.*, exhibited a significant
248 negative correlation with stream TP (Figs. 2 A&B), and abundance was negatively correlated
249 with stream $\text{NO}_3^- + \text{NO}_2^- - \text{N}$ (Table 2). These genus-specific responses combined to shape
250 overall shredder community structure.

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

260 **Discussion**

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

272 Detrital quality was positively correlated (i.e. decreased C:N or C:P) with increasing
273 nutrients across our study streams during the two years of study. The average leaf litter C:P
274 (2,088) and C:N (41.0) fell between those given for leaf material found across a regional
275 study conducted in Indiana, Michigan, and Wisconsin, US [(C:P~1,000); (C:N~25) Evans-
276 White et al. 2005] and those found in reference streams in North Carolina, US [(C:P= 4,858);
277 (C:N= 73) Cross et al. 2003]. Microbial community biomass or activity was not measured in
278 this study but likely increased with nutrient enrichment causing the observed trends in quality
279 (Gulis & Suberkropp 2003, Greenwood et al. 2007). Overall, evidence from this and many
280 other studies (Cross et al. 2003, Greenwood et al. 2007, Small & Pringle, 2010, Scott et al.
281 2013) indicates robust patterns of increased basal food resource quality in headwater detrital
282 systems across time and space with nutrient loading, which may potentially alter
283 macroinvertebrate assemblages.

284 We provided partial evidence for the food quality mechanism driving shifts in
285 shredder populations and communities (ie. positive correlations between in high C:P
286 *Strophoteryx spp.* and community biomass and food C:P), although water quality explained
287 more of the variation than food quality in a majority of variables. It is possible that these
288 shredders can selectively feed on more balanced C:P material (Hood et al. 2014), and this
289 would weaken the overall response found in our study. Additionally, as detrital C:P depends
290 on the initial C:P leaf litter inputs which can vary widely (Hattenschwiler et al 2008),
291 differences among inputs to different sites could also weaken consumer-resource correlation.
292 However, our study indicates negative correlations between stream nutrients and resource
293 C:N and C:P. This evidence, along with food quality explaining a large amount of
294 ecologically significant variance in shredder assemblages despite using a conservative
295 statistical analysis in a highly variable field setting, suggests that food resource enrichment
296 can have significant impacts on aquatic foodwebs. These changes could be due to either

297 variation in nutrient content of plastic consumers or from decreases in sensitive invariable
298 taxa experiencing nutrient stress.

299 Contradictory results have been found regarding the plasticity of consumer elemental
300 content in the lab and across nutrient gradients. Some studies have found significant
301 differences in taxa nutrient content when feeding across water quality or resource
302 stoichiometry gradients (Cross et al. 2003, Small & Pringle 2010, Back 2008, 2013);
303 however, others have found no deviation in elemental body composition (Evans-White et al.
304 2005, Danger et al. 2013). Our results most closely resemble Morse et al. 2012, as we found
305 no significant differences in P content of any taxa across streams; however, two taxa (*Tipula*
306 *spp.* and *Strophopteryx spp.*) in our study showed reduced body C:N with increased NO_3^-
307 $+\text{NO}_2^-$ -N (Supplementary Table 1). Due to the small sample sizes and taxonomic resolution
308 of our study, comparisons to other studies should be taken with caution. Other differences in
309 these studies are possibly due to differences in sample collection and processing (i.e.
310 separating consumers by sex and size class vs. analyzing the population as a whole) as well
311 as differences in elemental plasticity within a genus or between organisms in different
312 regions. As a majority of taxa collected from the field displayed invariable body nutrient
313 composition in our study, declines in sensitive taxa across nutrient gradients due to nutritional
314 stress could significantly alter shredder assemblages.

315 Enrichment of detrital food resources and/or increasing nutrients coincided with shifts
316 in shredding insect biomass and abundance in four out of five genera including *Tipula spp.*,
317 *Pycnopsyche spp.*, *Ephemerella spp.*, and *Strophopteryx spp.* These patterns in abundance
318 and biomass at the genus level could generally be predicted by stoichiometry theory. Previous
319 stoichiometry research suggests that organisms with low body P contain lower RNA
320 quantities and therefore may not possess the molecular capabilities necessary to elicit a
321 significant growth response to food resource enrichment (Elser et al. 2000, Cross et al. 2003,

322 Shade et al., 2003, Weider et al. 2005). In fact, metabolism of slower-growing low-P
323 organisms might be altered when consuming high-P resources, and this stress may cause
324 slower growth and altered life-history traits with resource enrichment (Boersma & Elser
325 2006). Therefore, we expected to observe either no change or decreases in high body C:P
326 shredding insect species abundance and biomass as leaf litter C:P decreased and dissolved
327 nutrients increased. Examining patterns of the two highest C:P genera, we found that
328 *Amphinemura* spp. (C:P= 385) abundance and biomass did not change across the TP and leaf
329 litter C:P gradient suggesting that this species did not respond positively to increasing food
330 quality. Further, the abundance and biomass of our highest body C:P genera, *Strophopteryx*
331 spp. (C:P= 487), actually declined with increasing detrital food quality. This decline suggests
332 either that increasing levels of P in their food have some detrimental “stoichiometric knife-
333 edge” effect on *Strophopteryx* spp. populations (Boersma & Elser 2006) or that some other
334 confounding factor is negatively affecting their populations.

335 While this study is correlative in nature and it is impossible to count out all potentially
336 confounding factors, these results reinforce the findings of negative relationships between
337 nutrient enrichment and sensitive shredding genera (Wang et al. 2007, Evans-White et al.
338 2009). If other factors such as increased turbidity or sedimentation were playing a significant
339 role shaping shredding macroinvertebrate populations in our most enriched sites, we would
340 expect most of our species to respond negatively to increasing TP as nutrient enrichment
341 correlates with these variables in this region (Evans-White et al. 2009). However,
342 *Amphinemura* spp. and *Strophopteryx* spp. did not respond to increasing TP, and
343 *Ephemerella* spp. (C:P= 321), and *Pycnopsyche* spp. (C:P= 340) responded positively,
344 confirming our prediction that lower body C:P shredders would respond positively to nutrient
345 enrichment. This evidence supports our prediction of shifting community structure to low C:P
346 organisms with nutrient enrichment; however, *Tipula* spp., the lowest C:P organism in our

347 study declined with increased nutrients in this study, possibly due to other environmental or
348 life-history factors.

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

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

387 There was an overall decrease in large-bodied shredder biomass with increasing
388 nutrient concentrations, and this shift combined with increased small-bodied consumers led to
389 an overall decrease in community B:A ratios (Fig 4C). The finding of decreasing biomass
390 differs from previous research that found overall positive relationships with P-enrichment
391 (Rosemond et al. 2002, Cross et al. 2006). In addition, we did not find a statistically
392 significant relationship between mean shredder community C:P and leaf litter C:P (Fig 4D) or
393 TP in the present study, contrary to our predictions based on data from Evans-White et al.
394 (2009), which found negative correlations between community C:P and TP. However, we
395 found abundance and biomass increases in all but one low C:P taxa along with declines of the

396 highest C:P insects with increased stream TP, suggesting that declines in community C:P
397 with enrichment might occur in this region. Results from our study are not specifically
398 comparable to previous studies however because we did not include low C:P multivoltine
399 chironomids, which can come to dominate shredder production in enriched systems.

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

413 Our study revealed an overall pattern of altered shredder communities with increasing
414 nutrient enrichment of detrital resources, possibly altering elemental flows through stream
415 foodwebs due to changes in consumer biomass. These shifts mostly favored lower C:P taxa,
416 as predicted by stoichiometric theory. The failure to accurately predict changes in *Tipula*
417 *spp.* populations highlights the need for more laboratory based measurements of differences
418 in invertebrate physiology under nutritional stress. More information gained by laboratory-
419 based TER estimates for shredding macroinvertebrates should improve predictive ability in
420 regards to stoichiometrically induced taxa shifts in aquatic environments. Information from

421 species based TERs can be used to identify and predict dietary and physiological drivers of
422 species population shifts in natural assemblages, which could lead to decreased functional
423 diversity and community resilience in affected streams. This information combined with
424 nutrient threshold analysis has proven to be a powerful tool for predicting changes in
425 macroinvertebrate community structure on regional scales and should be used to inform
426 policy decisions for water quality standards (King & Richardson 2003, Wang et al. 2007,
427 Evans-White et al. 2009) and for managing watersheds and stream networks on larger
428 regional and continental scales (King & Baker 2010, Woodward et al. 2012).

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614

Table 1. Land use, water quality variables, and organic estimates for each site sampled.¹ Dissolved Oxygen, ² Total Phosphorus, ³Ammonium, ⁴ Nitrate + Nitrite-Nitrogen, ⁵ Coarse Benthic Organic Matter.

Site	% Land use		DO ¹		Turbidity	TP ²	NH ₄ ⁺ ³	NO ₃ ⁻ +NO ₂ -N ⁴	CBOM ⁵
	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

Table 2. Shredding insect genera and mean ¹ carbon:phosphorus (C:P) and ² carbon:nitrogen (C:N) molar ratios ± standard deviation. Letters indicate species separated using Tukey-Kramer tests for significant differences ($P < 0.01$) between shredding genera C:P and C:N.

Order	Family	Genus	Sample #	C:P¹	C:N²
Diptera	Tipulidae	<i>Tipula spp.</i>	39	241±41 ^a	6.3±.052 ^{ac}
Tricoptera	Limnephilidae	<i>Pycnopsyche spp.</i>	11	321±55 ^a	7.8±1.04 ^b
Ephemeroptera	Ephemerellidae	<i>Ephemerella spp.</i>	13	340±68 ^a	6.2±0.24 ^{ac}
Plecoptera	Nemouridae	<i>Amphinemura spp.</i>	20	385±86 ^b	7±0.48 ^{abc}
Plecoptera	Taeniopterygidae	<i>Strophopteryx spp.</i>	8	437±54 ^b	8.2±1.4 ^b

Table 3. Results of correlation analysis between dependent and independent variables. Pearson and Spearman correlations are expressed as r and p -values. Significant relationships are presented in bold font after Bonferroni corrections for multiple comparisons ($p < 0.0125$). ¹Total Phosphorus, ²Nitrate + Nitrite-Nitrogen, ³Leaf litter carbon:phosphorus ratio, ⁴Leaf litter carbon:nitrogen ratio ⁵Biomass:abundance ratio, ⁶Community carbon:phosphorus ratio.

	TP¹ ($\mu\text{g/L}$)	NO₃⁻+NO₂⁻-N² ($\mu\text{g/L}$)	Leaf C:P³ Molar Ratio	Leaf C:N⁴ Molar Ratio
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 ⁵	0.85; <0.01	-0.67; 0.07	0.67; <0.01	0.86; <0.01
Community C:P ³	0.02; 0.97	-0.31; 0.46	-0.19; 0.66	-0.24; 0.57
<i>Tipula spp.</i> Abundance	-0.88; <0.01	-0.83; <0.01	0.55; 0.16	0.64; 0.09
<i>Tipula spp.</i> Biomass	-0.81; 0.01	-0.76; 0.03	0.57; 0.14	0.62; 0.10
<i>Pycnopsyche spp.</i> Abundance	0.83; 0.01	0.27; 0.52	-0.51; 0.19	-0.56; 0.15
<i>Pycnopsyche spp.</i> Biomass	0.76; 0.03	0.15; 0.73	-0.47; 0.24	-0.48; 0.23
<i>Ephemerella spp.</i> Abundance	0.96; <0.01	0.80; 0.02	-0.77; 0.03	-0.77; 0.03
<i>Ephemerella spp.</i> Biomass	0.90; <0.01	0.81; 0.02	-0.78; 0.02	-0.81; 0.02
<i>Amphinemura spp.</i> Abundance	-0.02; 0.96	-0.12; 0.78	-0.36; 0.39	-0.36; 0.38
<i>Amphinemura spp.</i> Biomass	-0.36; 0.39	-0.36; 0.39	-0.16; 0.70	-0.20; 0.64
<i>Strophopteryx spp.</i> Abundance	-0.46; 0.25	-0.33; 0.43	0.82; 0.01	0.61; 0.01
<i>Strophopteryx spp.</i> Biomass	-0.48; 0.23	-0.14; 0.75	0.83; 0.01	0.84; <0.01

Fig. 1 Correlations between total phosphorus (TP) and leaf litter carbon:phosphorus (C:P) and between $\text{NO}_3^- + \text{NO}_2^- - \text{N}$ and leaf litter carbon:nitrogen (C:N) in 2010. Results of Pearson and Spearman correlations are expressed as r and p -values.

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.

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.

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.

Figure 1

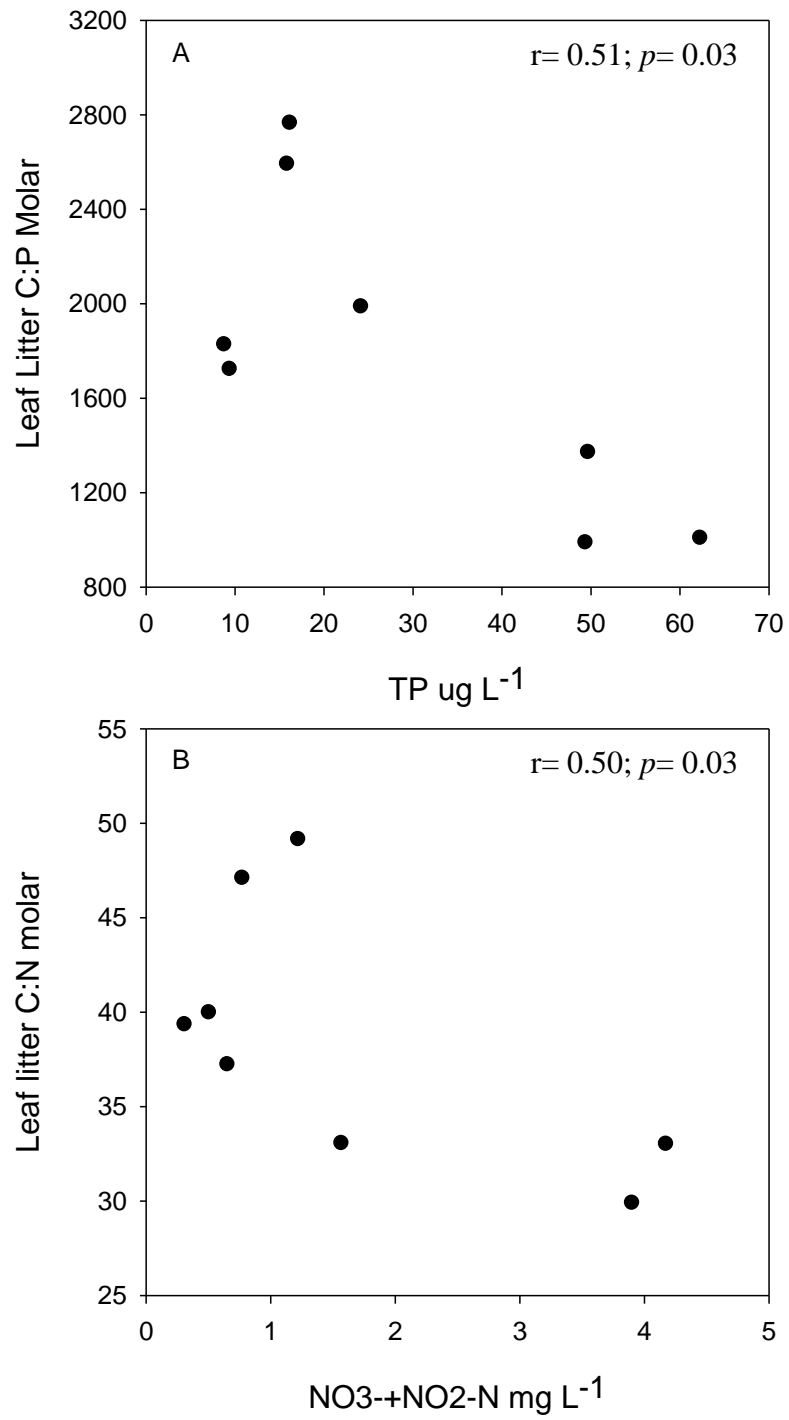


Figure 2

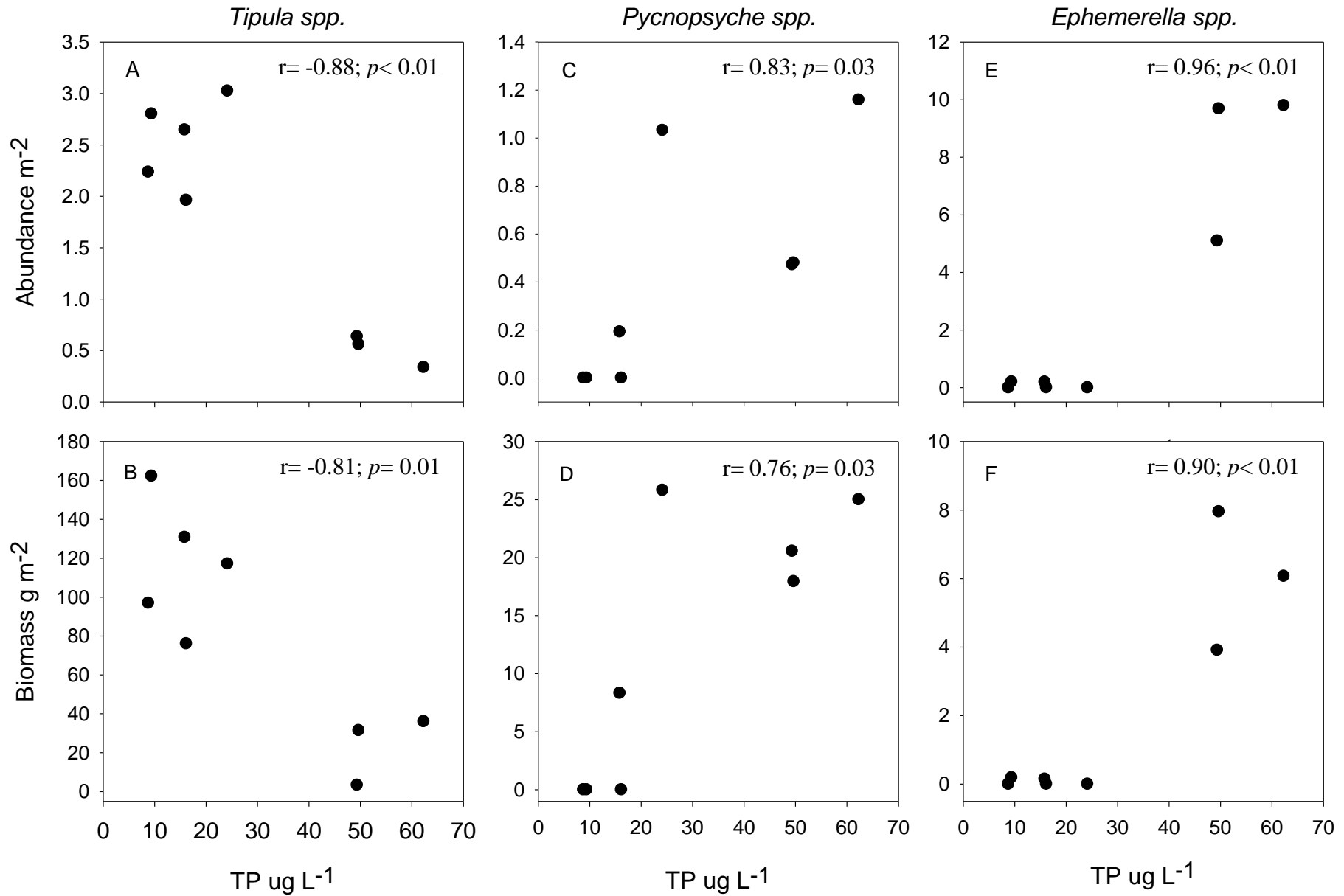


Figure 3

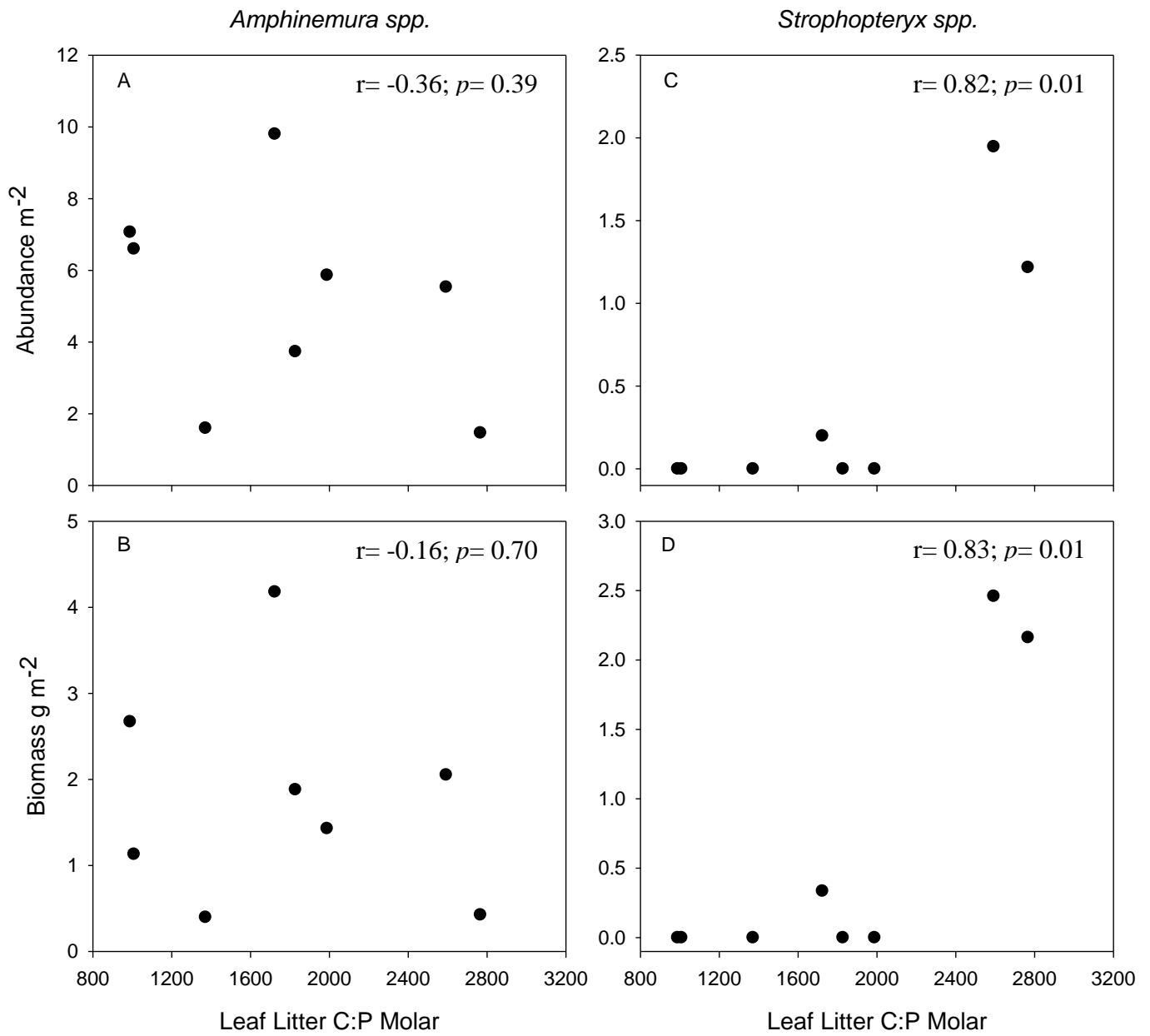


Figure 4

