

5-2016

Dietary Effects on the Stoichiometry of Growth, Regulation, and Wastes of Ozark Stream Insect Detritivores

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Dietary Effects on the Stoichiometry of Growth, Regulation, and Wastes of Ozark Stream insect
Detritivores

A thesis submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in Biology

by

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Abstract

A widespread stressor, anthropogenic nitrogen (N) and phosphorus (P) pollution can increase resource nutrient content and alter animal community composition in freshwater ecosystems. In this dissertation, I used ecological stoichiometry theory to examine effects of diet nutrient content and leaf litter type on growth, regulation, and wastes of aquatic invertebrate detritivores. I tested effects of leaf litter diet carbon:phosphorus (C:P) on growth and stoichiometric regulation of the detritivorous caddisfly *Pycnopsyche lepida* and used results to determine a threshold elemental ratio of oak litter C:P=1620 that confers peak growth of this species. This empirical, growth-based approach provided a more accurate estimate of the threshold elemental ratio compared to current bioenergetics models. Subsequent experiments used ^{33}P and ^{14}C as microbial tracers to examine effects of diet leaf type and nutrient content, as well as taxonomic identity, on incorporation efficiency of microbial C and P by the detritivorous caddisflies *Pycnopsyche lepida*, *Lepidostoma* sp., and *Isonychia* sp. Results showed no effects of leaf type on incorporation efficiencies, however elevated litter P content reduced caddisfly incorporation efficiency of microbial P, and there were inverse relationships between caddisfly body C:P content and incorporation efficiencies of microbial C and P, suggesting stoichiometric links of detritivore growth rates and P requirements to reliance on litter microbial nutrients. Given the stoichiometry of growth and regulation can vary across diets and taxa to affect production and composition of animal wastes, I also examined effects of litter type and nutrient content on the stoichiometry of particulate wastes from the detritivores *Pycnopsyche lepida*, *Lepidostoma* sp., and *Tipula abdominalis*. Higher litter N and P content increased N and P content of particulate wastes, but the strength of effects often differed between maple and oak litter and *Tipula abdominalis* produced N- and P-deplete wastes compared to *Pycnopsyche lepida* and

Lepidostoma, indicating potential taxonomically variable effects of animals on the stoichiometry of fine particulates in streams. Finally, I conducted a long-term study of C, N, and P dynamics of decomposing egesta from the detritivorous taxa *Tipula* sp., *Lirceus* sp., and *Allocapnia* sp. fed low- or high-P litter. Egesta from *Allocapnia* and *Tipula* decomposed faster than egesta from *Lirceus*, and elevated P content of egesta increased total uptake of dissolved N by egesta during decomposition. Together, my findings provide evidence that, by increasing litter nutrient content, anthropogenic nutrient pollution alters multiple species-specific functional roles of detritivorous animals in aquatic ecosystems.

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Acknowledgments

I am greatly thankful to many friends, family, and mentors who have supported me over the last five years. I first thank Dr. Michelle Evans-White for cultivating a positive research and learning environment, for her patient and kind mentorship, and for sharing with me her passion for ecological stoichiometry theory. My committee members Drs. Steve Beaupre, Dan Magoulick, Sally Entekin, and Thad Scott each contributed uniquely to my scholarship. Thank you, Steve, for teaching me about allometry and the danger of ratios. Thank you, Dan, for introducing me to the world of statistics and experimental design. Thank you, Sally, for volunteering your wisdom and for your continual collaboration. Thank you, Thad, for being an excellent role-model and collaborator, and for so gracefully providing vital space and instruments for my research.

Many thanks go to undergraduate researchers and technicians for their assistance in the work presented here, including Andrew Sanders, Amanda Eddy, Jason Ramey, Lindsey Abel, Katharine Stewart, Delaney Hall, Grant White, Rachel Moore, Ross Shierry, and Elizabeth Moore. I especially thank fellow graduate students in the Evans-White lab – Brad Austin, Ayla Smartt, Katie Rose, Kayla Sayre, Allyn Dodd, and Brooke Howard Parker – for their camaraderie, mentorship, and contributions to my research. Special thanks to a few personal friends who brought me to and accompanied me on this journey: John Schade, Stephanie Schmidt, Eric Cole, Mark Watney, Ryan Stone, Travis Edwards, Auriel Fournier, Chris Reddin, Addison McCarver, Skippy Jennings, Marla Steele, and Eliese Ronke. Thanks to many other friends and the community at Good Shepherd Lutheran Church for keeping me grounded.

I finally thank my family, especially my parents Ron and Donna and my brothers Chris and Erik, for their constant love and support through many years and miles.

Dedication

I dedicate this dissertation to my parents, Ron and Donna. Thank you, Mom and Dad, for the strength and support you have given me throughout my life. I will always be proud and grateful to call you my parents.

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List of Published Papers

Chapter 1:

Halvorson, H.M., J.T. Scott, A.J. Sanders, and M.A. Evans-White. 2015. A stream insect detritivore violates common assumptions of threshold elemental ratio bioenergetics models. *Freshwater Science* 34: 508-518.

Chapter 2:

Halvorson HM, White G, Scott JT, Evans-White MA (2016) Dietary and taxonomic controls on incorporation of microbial carbon and phosphorus by detritivorous caddisflies. *Oecologia* 180:567-679. doi: 10.1007/s00442-015-3464-6

Chapter 3:

Halvorson H.M., Fuller C., Entrekin S.A. & Evans-White M.A. (2015) Dietary influences on production, stoichiometry and decomposition of particulate wastes from shredders. *Freshwater Biology* **60**, 466-478.

INTRODUCTION

Anthropogenic pollution of the nutrients nitrogen (N) and phosphorus (P) is a widespread stressor and leading cause of impairment of stream biotic integrity in the United States (Paulsen et al. 2008). In most freshwater ecosystems, excess nutrients originate from human sources including fossil fuel burning that drives nutrient deposition, runoff from agricultural application of fertilizers, and waste water treatment plants (Smith et al. 1999, Smith et al. 2003). Previous research regarding freshwater nutrient pollution has focused on autotroph responses to N and P enrichment, especially the process of eutrophication in which increased nutrients stimulate algal growth, eliciting diel or long-term drawdown of dissolved oxygen to negatively affect freshwater organisms (Smith et al. 1999, Biggs 2000, Dodds and Cole 2007). Nutrient addition increases both algal biomass and algal nutrient content, altering ecosystem trophic state and driving profound changes in the food web (Dodds 2007). For example, increased algal biomass and nutrient content enhances growth of herbivores such as snails and mayflies (Stelzer and Lamberti 2002, Frost and Elser 2002). Although nutrient enrichment strongly alters ecosystems via energy and nutrient pathways based on autotrophic carbon (C), nutrient enrichment can also stimulate growth of heterotrophic microbes (fungi and bacteria) and alter energy and nutrient pathways based on lesser-studied terrestrial C in freshwaters (Fig. 1; Cross et al. 2006, Rosemond et al. 2015, Carpenter et al. 2016).

In headwater stream ecosystems, allochthonous material originating from the surrounding terrestrial landscape serves as the dominant form of organic matter and supports energy and nutrient flow through the food web (Fisher and Likens 1973, Wallace et al. 1997). This dead organic matter, henceforth termed detritus, consists of leaf litter, wood, animal carcasses, and diverse other plant- and animal-derived material. The majority of detritus in streams originates from adjacent terrestrial ecosystems and is recalcitrant and deplete in nutrients, resulting in slow

rates of decomposition (Fisher and Likens 1973, Webster and Benfield 1986, Enriquez et al. 1993). For example, leaf litter in streams is typically low in N and P content because trees resorb nutrients from leaves prior to senescence and abscission of leaves (Aerts 1996). Leaf litter also contains recalcitrant forms of C including lignin, cellulose, and hemicellulose that are resistant to microbial breakdown and digestion by animals (Webster and Benfield 1986). Both C recalcitrance and nutrient content of the detrital substrate constrain the rate of colonization and subsequent decomposition by heterotrophic microbes (Taylor et al. 1989, Enriquez et al. 1993, Pastor et al. 2014). Because detrital microbes can also assimilate dissolved C and nutrients (Cheever et al. 2013, Pastor et al. 2014), dissolved nutrient availability may also constrain microbial growth on detritus. The reliance on dissolved nutrients, in particular, shapes the response of heterotrophic microbes to anthropogenic input of dissolved N and P, serving as the mechanism for enhanced microbial biomass and activity in stream ecosystems subject to elevated nutrients (Gulis and Suberkropp 2003, Suberkropp et al. 2010, Manning et al. 2015). Detritivorous animals, in turn, may respond to elevated dissolved N and P because of the significant role of microbial biomass in detritivore nutrition and growth (Cummins 1973, Findlay et al. 1986, Chung and Suberkropp 2009).

Ecological stoichiometry (ES) theory, the study of the balance of multiple elements at ecological levels of organization, provides a useful framework to address effects of dissolved N and P addition to stream ecosystems (Manning et al. 2015). Though the framework has its weaknesses, especially its simplification of organism nutritional physiology and its reliance on ratios that pose statistical and interpretive problems (Raubenheimer 1995, Raubenheimer et al. 2009), the strength of ES lies in its use of the common currency of elements, shared across scales from organisms to ecosystems, to address constraints on energy and nutrient flow among diverse

organisms and across multiple trophic levels (Sterner and Elser 2002). In streams, ES explains how addition of dissolved N and P reduces stoichiometric constraints on growth of heterotrophic microbes, increasing microbial biomass (Suberkropp et al. 2010, Tant et al. 2013) and increasing the N and P content of detritus because microbes can store excess nutrients and are nutrient-rich relative to the detrital substrate (Scott et al. 2012, Danger and Chauvet 2013, Scott et al. 2013, Pastor et al. 2014). Generally, higher dissolved N and P concentrations increase the total N and P content of detritus; however, these effects may depend on leaf litter characteristics such as recalcitrance or substrate stoichiometry, which can set limits on maximum microbial biomass or nutrient content (Fanin et al. 2013, Scott et al. 2013, Pastor et al. 2014). Elevated N and P content of detritus may affect growth and stoichiometric regulation of detritivorous animals by reducing the degree of elemental imbalance between detritivores and detritus (Cross et al. 2003). Indeed, increased detritus N and P content can increase growth and secondary production of detritivorous animals in the laboratory and in the field (Cross et al. 2006, Danger et al. 2013, Kendrick and Benstead 2013, Fuller et al. 2015). In this way, ES theory provides an explanatory framework connecting dissolved nutrient concentrations to the broader structure and function of detritus-based food webs (Evans-White et al. 2009, Manning et al. 2015).

Although studies have illustrated generally positive growth responses of detritivorous animals to nutrient enrichment, many hypotheses of ES theory regarding the stoichiometry of growth, regulation, and wastes - generated primarily from model herbivorous taxa (Sterner and Elser 2002) - remain untested among detritivorous animals. In this dissertation, I use ES theory to investigate whether the effects of nutrient enrichment on the stoichiometry of stream insect detritivore growth, regulation, and wastes depends on diet leaf litter species and is generalizable across detritivore taxa (Fig. 1). These data will advance prediction of stream ecosystem

responses to shifts in nutrient availability, tree species distribution, or detritivore community composition due to anthropogenic forces. Note this dissertation is a compilation of separate publishable papers because Chapters 1, 2, and 3 have been published in peer-reviewed journals and are each presented as re-formatted versions of the final accepted manuscripts.

An overarching concept applicable to diverse consumers, threshold elemental ratios (TERs) may inform how species respond differently to increased dietary nutrients as a consequence of nutrient pollution (Sterner and Elser 2002, Frost et al. 2006, Evans-White et al. 2009). TERs predict the elemental ratio at which consumer growth limitation switches from one element to another (Urabe and Watanabe 1993, Sterner 1997, Sterner and Elser 2002). Studies have used bioenergetics models to predict high $TER_{C:P}$ of several aquatic invertebrate detritivore species (Frost et al. 2006). However, these bioenergetics models make major assumptions about organism stoichiometric regulation and growth, because empirical growth and stoichiometric data are limited for most organisms. For example, contemporary TER bioenergetics models assume consumers exhibit high P assimilation efficiencies, negligible P excretion, and fixed consumer body C:P at peak growth (Frost et al. 2006). Chapter 1 (Halvorson et al. 2015b) uses a laboratory experiment to test effects of diet stoichiometry and leaf type on growth and stoichiometric regulation of the stream insect detritivore *Pycnopsyche lepida*, providing a novel comparison of growth-based versus bioenergetics TER calculations.

As a food resource, detritus is composed of a mixture of living, predominately heterotrophic microbial biomass and non-living detrital substrate. Termed the “peanut butter on the cracker”, microbes provide vital nutrients that contribute significantly to detritivore nutrition (Kaushik and Hynes 1971, Cummins 1973, Chung and Suberkropp 2009). While diet leaf species and background nutrient availability control detritivore growth, these effects are likely driven by

indirect effects on microbial biomass and nutrient content (Danger et al. 2013). Studies suggest microbial C may support the bulk of detritivore growth and respiratory demands, but variable incorporation of microbial C within and across taxa may be attributable, in part, to diet (Findlay et al. 1986, Van Frankenhuyzen et al. 1985, Arsuffi and Suberkropp 1989). Moreover, few studies have compared incorporation of microbial nutrients across detritivorous taxa fed similar diets. In Chapter 2 (Halvorson et al. 2016), I employ dual radiotracer experiments to measure incorporation of microbial C and P by the detritivorous caddisfly taxa *Pycnopsyche lepida*, *Isonychia* sp., and *Lepidostoma* sp. fed oak and maple litter from two distinct P concentrations, providing a test of ES theory regarding dietary and taxonomic variation in detritivore incorporation of detrital microbial nutrients.

ES theory predicts consumers modify the production and stoichiometry of wastes depending on diet elemental content to regulate stoichiometric homeostasis (Sterner and Elser 2002, Frost et al. 2005). Animal nutrient wastes such as excreta can alter ecosystem nutrient cycles, forming nutrient feedbacks between consumers and their resources, termed consumer-driven nutrient recycling (CNR; Elser and Urabe 1999). Existing CNR studies have focused disproportionately on animal dissolved wastes (excreta) because these wastes are physiologically significant in the nutrient budgets of model taxa (DeMott et al. 1998, Anderson et al. 2005), and dissolved wastes such as phosphate and ammonium are readily taken up by basal autotrophs and heterotrophs to strongly affect resource nutrient content (Evans-White and Lamberti 2005, Liess and Haglund 2007). Unlike many taxa, however, detritivores are unique for their functional role of converting coarse detritus into smaller particulate wastes via egestion and fragmentation, providing food resources for downstream collectors (Short and Maslin 1977, Cummins and Klug 1979, Cummins and Ward 1979). The significance of this particulate transformation as a form of

CNR remains largely unknown. In Chapter 3 (Halvorson et al. 2015a), I describe effects of diet leaf type and stoichiometry on production, stoichiometry, and microbial decomposition of particulate wastes from the detritivorous insects *Pycnopsyche lepida*, *Tipula abdominalis*, and *Lepidostoma* sp., testing effects of diet and species on CNR in detrital food webs via particulate pathways.

Animal egesta can contribute significantly to stream organic matter budgets (Cuffney et al. 1990, Malmqvist et al. 2001) and are a crux in the detrital processing chain linking upstream to downstream ecosystems (Heard and Richardson 1995, Navel et al. 2011, Bundschuh and McKie *in press*). However, the role of egesta in stream nutrient cycles remains understudied. Empirical data regarding long-term microbial processing of egesta are especially needed to understand the significance of egesta as a form of CNR and permit comparison to the significance of animal excreta (Liess and Haglund 2007, Halvorson et al. 2015a). Variable physical and chemical properties of egesta, such as varying fecal pellet size and nutrient content associated with diet and the source animal, may affect patterns over decomposition such as carbon and nutrient leaching, uptake, and mineralization (Joyce et al. 2007, Yoshimura et al. 2008, Yoshimura et al. 2010). In Chapter 4, I describe a 107 day decomposition experiment to examine effects of detritivore taxonomic identity and diet nutrient content on short- and long-term carbon and nutrient dynamics of microbial decomposition of egesta, providing data necessary to understand controls over the long-term fates and significance of egesta in freshwater ecosystems.

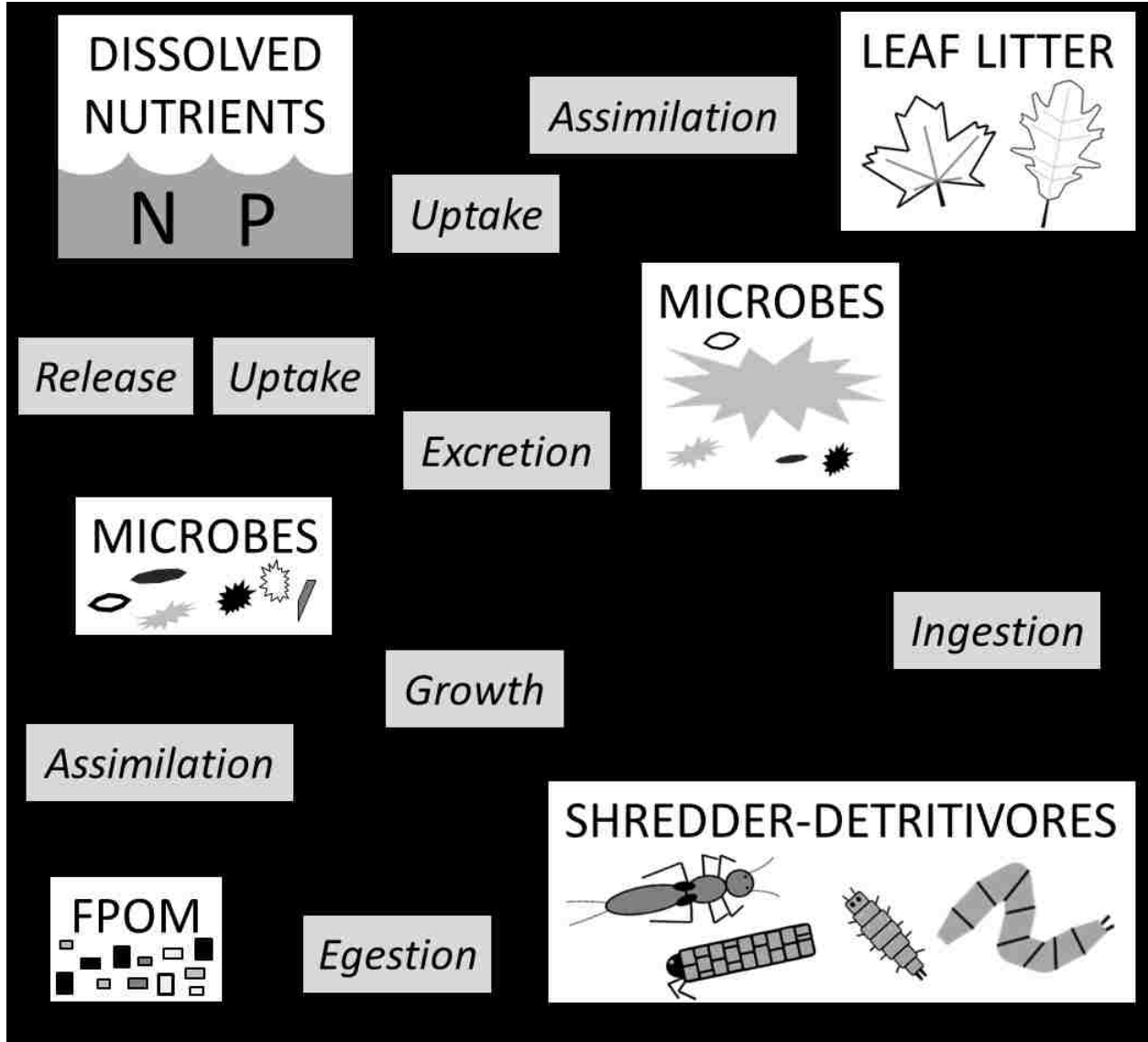


Figure 1. Conceptual diagram summarizing links between dissolved nutrients and key functional roles of invertebrate shredder-detritivore animals in stream ecosystems. Dissolved nutrient availability affects leaf litter nutrient content through microbial uptake, which can in turn alter pre- and post-ingestive regulation by detritivorous animals to affect processes of egestion, excretion, and growth. Excretion directly affects dissolved nutrient availability, whereas egestion enters the pool of fine particulate organic matter (FPOM), which is subject to microbial decomposition and associated uptake and release of dissolved nutrients.

LITERATURE CITED

- Aerts, R. 1996. Nutrient resorption from senescing leaves of perennials: Are there general patterns? *Journal of Ecology* 84:597-608.
- Anderson, T. R., D. O. Hessen, J. J. Elser, and J. Urabe. 2005. Metabolic stoichiometry and the fate of excess carbon and nutrients in consumers. *American Naturalist* 165:1-15.
- Arsuffi, T. L. and K. Suberkropp. 1989. Selective feeding by shredders on leaf-colonizing stream fungi - comparison of macroinvertebrate taxa. *Oecologia* 79:30-37.
- Biggs, B. J. F., S. N. Francoeur, A. D. Huryn, R. Young, C. J. Arbuckle, and C. R. Townsend. 2000. Trophic cascades in streams: effects of nutrient enrichment on autotrophic and consumer benthic communities under two different fish predation regimes. *Canadian Journal of Fisheries and Aquatic Sciences* 57:1380-1394.
- Bundschuh, M. and B.G. McKie. In press. An ecological and ecotoxicological perspective on fine particulate organic matter in streams. *Freshwater Biology*.
- Carpenter, S. R., J. J. Cole, M. L. Pace, and G. M. Wilkinson. 2016. Response of plankton to nutrients, planktivory and terrestrial organic matter: a model analysis of whole-lake experiments. *Ecology Letters* 19:230-239.
- Cheever, B. M., J. R. Webster, E. E. Bilger, and S. A. Thomas. 2013. The relative importance of exogenous and substrate-derived nitrogen for microbial growth during leaf decomposition. *Ecology* 94:1614-1625.
- Chung, N. and K. Suberkropp. 2009. Contribution of fungal biomass to the growth of the shredder, *Pycnopsyche gentilis* (Trichoptera: Limnephilidae). *Freshwater Biology* 54:2212-2224.
- Cross, W. F., J. B. Wallace, and A. D. Rosemond. 2007. Nutrient enrichment reduces constraints on material flows in a detritus-based food web. *Ecology* 88:2563-2575.
- Cross, W. F., J. P. Benstead, A. D. Rosemond, and J. B. Wallace. 2003. Consumer-resource stoichiometry in detritus-based streams. *Ecology Letters* 6:721-732.
- Cuffney, T. F., J. B. Wallace, and G. J. Lughart. 1990. Experimental evidence quantifying the role of benthic invertebrates in organic-matter dynamics of headwater streams. *Freshwater Biology* 23:281-299.
- Cummins, K. W. 1973. Trophic relations of aquatic insects. *Annual Review of Entomology* 18:183-206.
- Cummins, K. W. and M. J. Klug. 1979. Feeding ecology of stream invertebrates. *Annual Review of Ecology and Systematics* 10:147-172.
- Danger, M. and E. Chauvet. 2013. Elemental composition and degree of homeostasis of fungi: are aquatic hyphomycetes more like metazoans, bacteria or plants? *Fungal Ecology* 6:453-457.

- Danger, M., J. A. Funck, S. Devin, J. Heberle, and V. Felten. 2013. Phosphorus content in detritus controls life-history traits of a detritivore. *Functional Ecology* 27:807-815.
- DeMott, W. R., R. D. Gulati, and K. Siewertsen. 1998. Effects of phosphorus-deficient diets on the carbon and phosphorus balance of *Daphnia magna*. *Limnology and Oceanography* 43:1147-1161.
- Dodds, W. K. 2007. Trophic state, eutrophication and nutrient criteria in streams. *Trends in Ecology & Evolution* 22:669-676.
- Dodds, W. K. and J. J. Cole. 2007. Expanding the concept of trophic state in aquatic ecosystems: It's not just the autotrophs. *Aquatic Sciences* 69:427-439.
- Elser, J. J. and J. Urabe. 1999. The stoichiometry of consumer-driven nutrient recycling: Theory, observations, and consequences. *Ecology* 80:735-751.
- Enriquez, S., C. M. Duarte, and K. Sandjensen. 1993. Patterns in decomposition rates among photosynthetic organisms - the importance of detritus C:N:P content. *Oecologia* 94:457-471.
- Evans-White, M. A. and G. A. Lamberti. 2005. Grazer species effects on epilithon nutrient composition. *Freshwater Biology* 50:1853-1863.
- Evans-White, M. A., W. K. Dodds, D. G. Huggins, and D. S. Baker. 2009. Thresholds in macroinvertebrate biodiversity and stoichiometry across water-quality gradients in Central Plains (USA) streams. *Journal of the North American Benthological Society* 28:855-868.
- Fanin, N., N. Fromin, B. Buatois, and S. Hattenschwiler. 2013. An experimental test of the hypothesis of non-homeostatic consumer stoichiometry in a plant litter-microbe system. *Ecology Letters* 16:764-772.
- Findlay, S., J. L. Meyer, and P. J. Smith. 1986. Contribution of fungal biomass to the diet of a fresh-water Isopod (*Lirceus* sp.). *Freshwater Biology* 16:377-385.
- Fisher, S. G. and G. E. Likens. 1973. Energy flow in Bear Brook, New Hampshire - integrative approach to stream ecosystem metabolism. *Ecological Monographs* 43:421-439.
- Frost, P. C. and J. J. Elser. 2002. Growth responses of littoral mayflies to the phosphorus content of their food. *Ecology Letters* 5:232-240.
- Frost, P. C., J. P. Benstead, W. F. Cross, H. Hillebrand, J. H. Larson, M. A. Xenopoulos, and T. Yoshida. 2006. Threshold elemental ratios of carbon and phosphorus in aquatic consumers. *Ecology Letters* 9:774-779.
- Frost, P. C., M. A. Evans-White, Z. V. Finkel, T. C. Jensen, and V. Matzek. 2005. Are you what you eat? Physiological constraints on organismal stoichiometry in an elementally imbalanced world. *Oikos* 109:18-28.

- Fuller, C. L., M. A. Evans-White, and S. A. Entrekin. 2015. Growth and stoichiometry of a common aquatic detritivore respond to changes in resource stoichiometry. *Oecologia* 177:837-848.
- Gulis, V. and K. Suberkropp. 2003. Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. *Freshwater Biology* 48:123-134.
- Halvorson, H. M., C. Fuller, S. A. Entrekin, and M. A. Evans-White. 2015a. Dietary influences on production, stoichiometry and decomposition of particulate wastes from shredders. *Freshwater Biology* 60:466-478.
- Halvorson, H. M., G. White, J. T. Scott, and M. A. Evans-White. 2016. Dietary and taxonomic controls on incorporation of microbial carbon and phosphorus by detritivorous caddisflies. *Oecologia* 180:567-579.
- Halvorson, H. M., J. T. Scott, A. J. Sanders, and M. A. Evans-White. 2015b. A stream insect detritivore violates common assumptions of threshold elemental ratio bioenergetics models. *Freshwater Science* 34:508-518.
- Heard, S. B. and J. S. Richardson. 1995. Shredder-collector facilitation in stream detrital food webs - is there enough evidence? *Oikos* 72:359-366.
- Joyce, P., L. L. Warren, and R. S. Wotton. 2007. Faecal pellets in streams: their binding, breakdown and utilization. *Freshwater Biology* 52:1868-1880.
- Kaushik, N. K. and H. B. N. Hynes. 1971. Fate of dead leaves that fall into streams. *Archiv Fur Hydrobiologie* 68:465-&.
- Kendrick, M. R. and J. P. Benstead. 2013. Temperature and nutrient availability interact to mediate growth and body stoichiometry in a detritivorous stream insect. *Freshwater Biology* 58:1820-1830.
- Liess, A. and A. L. Haglund. 2007. Periphyton responds differentially to nutrients recycled in dissolved or faecal pellet form by the snail grazer *Theodoxus fluviatilis*. *Freshwater Biology* 52:1997-2008.
- Malmqvist, B., R. S. Wotton, and Y. X. Zhang. 2001. Suspension feeders transform massive amounts of seston in large northern rivers. *Oikos* 92:35-43.
- Manning, D. W. P., A. D. Rosemond, J. S. Kominoski, V. Gulis, J. P. Benstead, and J. C. Maerz. 2015. Detrital stoichiometry as a critical nexus for the effects of streamwater nutrients on leaf litter breakdown rates. *Ecology* 96:2214-2224.
- Navel, S., L. Simon, C. Lecuyer, F. Fourel, and F. Mermillod-Blondin. 2011. The shredding activity of gammarids facilitates the processing of organic matter by the subterranean amphipod *Niphargus rhenorhodanensis*. *Freshwater Biology* 56:481-490.
- Pastor, A., Z. G. Compson, P. Dijkstra, J. L. Riera, E. Marti, F. Sabater, B. A. Hungate, and J. C. Marks. 2014. Stream carbon and nitrogen supplements during leaf litter decomposition: contrasting patterns for two foundation species. *Oecologia* 176:1111-1121.

- Paulsen, S. G., A. Mayo, D. V. Peck, J. L. Stoddard, E. Tarquinio, S. M. Holdsworth, J. Van Sickle, L. L. Yuan, C. P. Hawkins, A. T. Herlihy, P. R. Kaufmann, M. T. Barbour, D. P. Larsen, and A. R. Olsen. 2008. Condition of stream ecosystems in the US: an overview of the first national assessment. *Journal of the North American Benthological Society* 27:812-821.
- Raubenheimer, D. 1995. Problems with ratio analysis in nutritional studies. *Functional Ecology* 9:21-29.
- Raubenheimer, D., S. J. Simpson, and D. Mayntz. 2009. Nutrition, ecology and nutritional ecology: toward an integrated framework. *Functional Ecology* 23:4-16.
- Rosemond, A. D., J. P. Benstead, P. M. Bumpers, V. Gulis, J. S. Kominoski, D. W. P. Manning, K. Suberkropp, and J. B. Wallace. 2015. Experimental nutrient additions accelerate terrestrial carbon loss from stream ecosystems. *Science* 347:1142-1145.
- Scott, E. E., C. Prater, E. Norman, B. C. Baker, M. Evans-White, and J. T. Scott. 2013. Leaf-litter stoichiometry is affected by streamwater phosphorus concentrations and litter type. *Freshwater Science* 32:753-761.
- Scott, J. T., J. B. Cotner, and T. M. LaPara. 2012. Variable stoichiometry and homeostatic regulation of bacterial biomass elemental composition. *Frontiers in Microbiology* 3:42.
- Short, R. A. and P. E. Maslin. 1977. Processing of leaf litter by a stream detritivore - effect on nutrient availability to collectors. *Ecology* 58:935-938.
- Smith, R. A., R. B. Alexander, and G. E. Schwarz. 2003. Natural background concentrations of nutrients in streams and rivers of the conterminous United States. *Environmental Science & Technology* 37:3039-3047.
- Smith, V. H., G. D. Tilman, and J. C. Nekola. 1999. Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environmental Pollution* 100:179-196.
- Stelzer, R. S. and G. A. Lamberti. 2002. Ecological stoichiometry in running waters: Periphyton chemical composition and snail growth. *Ecology* 83:1039-1051.
- Sterner, R. W. 1997. Modelling interactions of food quality and quantity in homeostatic consumers. *Freshwater Biology* 38:473-481.
- Sterner, R.W., and J.J. Elser. 2002. *Ecological stoichiometry: the biology of elements from molecules to the biosphere*. Princeton University Press, Princeton, New Jersey, USA.
- Suberkropp, K., V. Gulis, A. D. Rosemond, and J. P. Benstead. 2010. Ecosystem and physiological scales of microbial responses to nutrients in a detritus-based stream: Results of a 5-year continuous enrichment. *Limnology and Oceanography* 55:149-160.
- Tant, C. J., A. D. Rosemond, and M. R. First. 2013. Stream nutrient enrichment has a greater effect on coarse than on fine benthic organic matter. *Freshwater Science* 32:1111-1121.

- Taylor, B. R., D. Parkinson, and W. F. J. Parsons. 1989. Nitrogen and lignin content as predictors of litter decay rates - a microcosm test. *Ecology* 70:97-104.
- Urabe, J. and Y. Watanabe. 1992. Possibility of N-limitation or P-limitation for planktonic cladocerans - an experimental test. *Limnology and Oceanography* 37:244-251.
- Vanfrankenhuyzen, K., G. H. Geen, and C. Koivisto. 1985. Direct and indirect effects of low pH on the transformation of detrital energy by the shredding caddisfly, *Clistoronia magnifica* (Banks) (Limnephilidae). *Canadian Journal of Zoology* 63:2298-2304.
- Wallace, J. B., S. L. Eggert, J. L. Meyer, and J. R. Webster. 1997. Multiple trophic levels of a forest stream linked to terrestrial litter inputs. *Science* 277:102-104.
- Ward, G. M. and K. W. Cummins. 1979. Effects of food quality on growth of a stream detritivore, *Paratendipes Albimanus* (Meigen) (Diptera, Chironomidae). *Ecology* 60:57-64.
- Webster, J. R. and E. F. Benfield. 1986. Vascular plant breakdown in freshwater ecosystems. *Annual Review of Ecology and Systematics* 17:567-594.
- Yoshimura, C., M. Fujii, T. Omura, and K. Tockner. 2010. Instream release of dissolved organic matter from coarse and fine particulate organic matter of different origins. *Biogeochemistry* 100:151-165.
- Yoshimura, C., M. O. Gessner, K. Tockner, and H. Furumai. 2008. Chemical properties, microbial respiration, and decomposition of coarse and fine particulate organic matter. *Journal of the North American Benthological Society* 27:664-673.

CHAPTER I

A stream insect detritivore violates common assumptions of threshold elemental ratio
bioenergetics models¹

¹Halvorson, H.M., J.T. Scott, A.J. Sanders, and M.A. Evans-White. 2015. A stream insect detritivore violates common assumptions of threshold elemental ratio bioenergetics models. *Freshwater Science* 34: 508-518.

ABSTRACT

Ecologists increasingly use threshold elemental ratios (TERs) to explain and predict organism responses to altered resource carbon:phosphorus (C:P) or carbon: nitrogen (C:N). TER calculations are grounded in diet-dependent growth, but growth data are limited for most taxa. Thus, TERs are derived instead from bioenergetics models that rely on simplifying assumptions, such as fixed organism C:P and no P excretion at peak growth. I examined stoichiometric regulation of the stream insect detritivore *Pycnopsyche lepida* to assess bioenergetics model assumptions and compared bioenergetics $TER_{C:P}$ estimates to those based on growth. I fed *P. lepida* maple and oak leaf diets along a dietary C:P gradient (molar C:P range = 950–4180) and measured consumption, growth, stoichiometric homeostasis (H), and elemental assimilation and growth efficiencies over a 5-wk period in the laboratory. *Pycnopsyche lepida* responses to varying resource C:P depended on litter identity and were strongest among oak diets, on which growth peaked at diet C:P = 1620. *Pycnopsyche lepida* fed oak litter exhibited flexible body C:P during growth and in response to altered diet C:P (non-strict homeostasis; H = 4.74), low P use efficiencies, and P excretion at peak growth. These trends violated common bioenergetics model assumptions and caused deviation of estimated $TER_{C:P}$ from C:P = 1620. Bioenergetics $TER_{C:P}$ further varied among *P. lepida* of differing growth status on varying diet C:P (overall $TER_{C:P}$ range = 1030–9540). My study identifies novel effects of nutrient enrichment and litter identity on detritivore stoichiometric regulation and supports growth-based approaches for future TER calculations.

INTRODUCTION

Threshold elemental ratios (TERs) are defined as the resource ratio [(carbon:phosphorus (C:P) or carbon:nitrogen (C:N)] at which consumer growth switches from limitation by one element to the other (Sterner and Elser 2002, Frost et al. 2006). TERs remain unknown for many species, but they theoretically describe the resource C:P or C:N for optimal growth and may aid predictions of how nutrient enrichment affects communities and ecosystems. TER models predict a positive response of consumer growth to N or P enrichment as the resource ratio approaches the TER but reductions in growth as the resource ratio declines below the TER (i.e., species become C-limited, possibly because of energetic costs of excreting excess nutrients; Boersma and Elser 2006). TER theory assumes that the 2 elements under consideration (i.e., C and P) are the primary determinants of growth, and thus, TER models should be grounded in divergent growth across varying ratios of the 2 elements. Growth is implicit in the definition of the TER, but growth data across resource-ratio gradients are limited for most taxa, and instead, ecologists use models based on bioenergetics terms, such as C and P use efficiencies and body stoichiometry (hereafter referred to as bioenergetics models), to calculate TERs (Frost et al. 2006, Doi et al. 2010, El-Sabaawi et al. 2012, Tant et al. 2013).

Many bioenergetics models calculate resource consumption by dividing total growth by the gross growth efficiency (GGE) for a given resource (Benke and Wallace 1980). To render bioenergetics models stoichiometrically explicit, one must use element-specific GGE:

$$GGE_x = \frac{\text{growth}_x}{\text{consumption}_x} \quad (\text{Eq. 1})$$

where consumption_x and growth_x represent consumption and growth of element x . At optimal consumer growth and nonlimiting food availability, the ratio of GGE_P and GGE_C can be multiplied by the molar C:P of new tissue production ($\text{growth}_C/\text{growth}_P$, or growth C:P) to

estimate the molar $TER_{C:P}$ (Olsen et al. 1986):

$$TER_{C:P} = \frac{GGE_P}{GGE_C} \times \frac{growth_C}{growth_P} \quad (\text{Eq. 2})$$

Eq. 2 reduces mathematically to consumption C:P after substitution from Eq. 1. Where growth data are lacking, $TER_{C:P}$ bioenergetics models assume that consumers will achieve optimal growth when growth C:P is equal to body C:P and both GGE_P and GGE_C are maximal (max):

$$TER_{C:P} = \frac{\max GGE_P}{\max GGE_C} * \frac{Q_C}{Q_P} \quad (\text{Eq. 3})$$

where Q_C and Q_P are the fixed molar amounts of C and P in consumer dry mass, respectively (Doi et al. 2010). Some $TER_{C:P}$ bioenergetics models also assume that at optimal growth, consumer GGE_x will be interchangeable with element-specific assimilation efficiency (Olsen et al. 1986, Frost et al. 2006). Assimilation efficiency (A_x) describes the ability of an animal to absorb ingested material (Mayor et al. 2011) and can be calculated from the following:

$$A_x = \frac{\text{consumption}_x - \text{egestion}_x}{\text{consumption}_x} \quad (\text{Eq. 4})$$

where $egestion_x$ represents total egestion in element x . Eq. 3 has been used to calculate zooplankton $TER_{C:P}$ where A_P is 100% and consumer P excretion is 0 (Olsen et al. 1986).

Because the difference between A_P and GGE_P is postassimilatory P loss, such as via excretion, $GGE_P = 1$ at the $TER_{C:P}$ (Urabe and Watanabe 1992).

The current widely used $TER_{C:P}$ model (Frost et al. 2006) incorporates respiratory C losses by using GGE_C , but much like other models, assumes no P excretion by using A_P :

$$TER_{C:P} = \frac{A_P}{GGE_C} \times \frac{Q_C}{Q_P} \quad (\text{Eq. 5})$$

Frost et al. (2006) used species-specific bioenergetics data from peer-reviewed literature for all terms except A_P , which for most species was assumed to be 0.8 (80% efficiency), and calculated $TER_{C:P}$ for diverse aquatic consumers with Eq. 5 (see Table 1 for a summary of equations).

Two major challenges for the above TER bioenergetics models have not been addressed. First, many TER bioenergetics models assume that A_P is fixed and exceptionally high, that P excretion is negligible, and that consumer body C:P is fixed at peak growth, yet few data exist to support these assumptions. Studies suggest that: 1) maximal A_P for cladocerans, model organisms upon which $A_P = 0.8$ estimates appear to be based (DeMott et al. 1998, Frost et al. 2006), may fall below 0.6 on natural diets (DeMott and Tessier 2002), 2) A_P varies among cladoceran taxa (Ferrão-Filho et al. 2007), and 3) zooplankton excrete measurable quantities of P even above the estimated $TER_{C:P}$ (DeMott et al. 1998, He and Wang 2008). In addition, not all consumers are strictly homeostatic (Persson et al. 2010), and growth C:P diverges from body C:P among developing organisms (Back and King 2013). These trends violate fundamental TER bioenergetics model assumptions and could drive inaccuracy in bioenergetics $TER_{C:P}$ (Fig. 1). The second challenge is that TER bioenergetics model parameters should be constrained to optimal growth or maximum GGE_C and GGE_P diets that are unknown for most taxa, but it is unclear to what degree TER bioenergetics model estimates are sensitive to parameters drawn from organisms at differing growth status (i.e., peak vs suboptimal growth or GGE_x on diets varying in C:P).

Inaccurate bioenergetics TER estimates could have far-reaching consequences in ecology because bioenergetics models are used for diverse purposes, such as integrating ecological stoichiometry and metabolic theory (Allen and Gillooly 2009, Doi et al. 2010), assessing resource constraints on microbial C use efficiency (Sinsabaugh et al. 2013), and predicting detritivore responses to aquatic nutrient enrichment (Hladyz et al. 2009, Tant et al. 2013). Compared to taxa of other feeding modes, detritivores may be particularly responsive to nutrient enrichment because they consume high C:P and C:N resources (Cross et al. 2003). Indeed,

nutrient addition can stimulate growth and production of aquatic detritivores (Cross et al. 2006, Danger et al. 2013). However, excess dietary P can reduce growth of some species (Boersma and Elser 2006), potentially because of energetic costs of excreting excess P. As tools to predict detritivore responses to altered resource stoichiometry, TERs may explain observed detritivore community shifts, biodiversity losses, and altered ecosystem processes in enriched streams (Singer and Battin 2007, Evans-White et al. 2009, Woodward et al. 2012).

My objectives were to: 1) empirically test the assumptions of current TER bioenergetics models (Eqs 3, 5), 2) test the sensitivity of TER model estimates to parameters drawn from organisms at peak vs suboptimal growth, and 3) compare resultant TER estimates to TER based on peak growth (Eq. 2) for a nonmodel organism fed diets ranging in N and P content. I conducted this test by measuring consumption, growth, stoichiometric homeostasis, and elemental use efficiencies of an aquatic insect detritivore, *Pycnopsyche lepida*, fed diets of variable N and P content within 2 litter types of differing recalcitrance (oak and maple). *Pycnopsyche* spp. are functionally dominant shredder-detritivores in streams (Creed et al. 2009) and may respond positively to nutrient enrichment (Davis et al. 2010). I hypothesized that *P. lepida* growth would peak at intermediate litter C:P, defined as the growth-based $TER_{C:P}$. I predicted that *P. lepida* would exhibit $A_P < 0.8$, measurable P excretion ($A_P > GGE_P$), and deviation of final body C:P from initial body C:P, thus violating model assumptions to cause bioenergetics $TER_{C:P}$ to deviate from the growth-based $TER_{C:P}$. Last, I expected that error in $TER_{C:P}$ would be magnified when bioenergetics model parameters were drawn from organisms at suboptimal growth.

METHODS

Laboratory growth experiment

I incubated sugar maple (*Acer saccharum* Marshall) and post oak (*Quercus stellata* Wangenh.) leaf litter under 1 of 4 dissolved P concentrations: <5 (ambient tap water), 50, 100, or 500 $\mu\text{g/L}$ P as Na_2HPO_4 in the laboratory. All incubation chambers received 1 mg/L N- NO_3 as KNO_3 . I collected recently senesced leaves in Washington County, Arkansas (USA), air-dried them, and stored them in bags. I cut leaves into 13.5-mm-diameter disks, which I added to polypropylene mesh bags, leached in tap water for 3 d, transferred to incubation chambers every 2–3 d, and incubated for 77 d prior to feeding. Incubation chambers contained 20 L dechlorinated tap water and were flushed and re-amended with nutrients every 2–3 d. Cultures were inoculated with subsamples of leaf-litter slurry from Mullins Creek in Fayetteville, Arkansas.

I collected one hundred 3rd- and 4th-instar larvae of the detritivore caddisfly *P. lepida* Hagen (Trichoptera:Limnephilidae; Moulton and Stewart 1996) from Chamber Springs in the Ozark Highlands ecoregion on 14 November 2012 and transported them to the laboratory where I kept them in an environmental chamber (12:12 light:dark cycle, 10°C). I randomly subsampled 20 individuals and measured head-capsule width (HCW; mm) with the aid of a digital camera microscope (Leica Microsystems, Inc., Wetzlar, Germany) and dried and weighed them (as described below). I calculated a HCW–dry mass (DM; mg) regression ($\log\text{DM} = 2.685(\log\text{HCW}) - 0.019$; $R^2 = 0.621$) to estimate initial larval masses. I measured HCW of the remaining 80 larvae and randomly distributed them among 80 continuously aerated growth chambers containing 100 mL stream water. I assigned chambers to 1 of 8 diet treatments (2 litter types \times 4 P levels; $n = 10/\text{treatment}$). The bottom of each growth chamber had a 1-mm-mesh insert to separate egesta from larvae and to prevent coprophagy. I removed old litter and added fresh litter every 2 to 3 d for 33 d so that litter was always available for consumption. On each

feeding day, I subsampled leaf disks for elemental analysis. At the end of the experiment, I allowed individuals to clear their guts for 24 h before they were frozen, thawed, oven-dried (48°C), desiccated, and weighed to the nearest μg using a microbalance (Mettler Toledo, Columbus, Ohio). I calculated instantaneous growth rates as the difference between log-normalized initial and final DM divided by experiment duration (Benke and Huryn 2006).

I measured *P. lepidus* consumption and egestion weekly. Before consumption trials, I blotted 3 to 5 leaf disks/individual on paper towels, weighed them to the nearest 0.1 mg, and immediately re-wet them with stream water. A subset of disks from each treatment was oven dried, desiccated, and weighed to create a blotted weight–DM regression for maple and oak litter. All other disks were fed to *P. lepidus* for 2 to 3 d. Total consumption was calculated as the difference between post-consumption litter DM and initial DM estimated from blotted weights. Egestion trials lasted 2 to 3 d in growth chambers. At the end of each trial, I removed insects and leaf litter. I filtered particulates onto precombusted and preweighed 25-mm-diameter, 1- μm -pore glass fiber filters (GFFs; Pall Inc., Port Washington, New York). Filters were oven dried, desiccated, and weighed to the nearest 0.1 mg. I used similarly filtered particulates from 32 insect-absent chambers to measure background contributions from leaves and subtracted background contributions for each treatment. I used individual growth rates to calculate daily insect masses assuming logarithmic growth throughout the experiment. I averaged insect mass over each consumption or egestion trial, divided by trial duration, and used rates to calculate total consumption and egestion over experiment duration. To calculate whole-experiment mass-specific consumption rates, I divided total consumption by the cumulative $\text{mg} \times \text{days}$ of presence for each individual.

I analyzed initial and final *P. lepidus* larvae, leaf litter, and egesta for C, N, and P content.

I homogenized all leaf samples with a ball-bearing grinder (Wig-L-Bug; Crescent Dental Manufacturing, Elgin, Illinois) and ground larvae to a fine powder with a spatula. I cut egesta filters in half, reweighed each half, and analyzed for either P or C/N content. To measure P content, I combusted samples at 500°C for 2 h, digested them in 85°C HCl, diluted them, and measured soluble reactive P by the ascorbic acid method (APHA 2005). A CHN analyzer (Thermo Scientific, Waltham, Massachusetts) was used to measure C/N content of samples. All elemental analyses were corrected from recovery efficiencies for ground peach leaf standard (NIST SRM 1547).

Calculations and statistical analysis

For each litter diet I calculated the degree of C:P homeostasis, H , for *P. lepidus* as the inverse slope of the regression of $\log(x)$ -transformed final body and diet C:P (Persson et al. 2010):

$$\log(\text{body}_{\text{C:P}}) = \log(c) + \log(\text{diet}_{\text{C:P}}) / H \quad (\text{Eq. 6})$$

Weekly measures of bulk consumption and egestion rate were multiplied by %C, N, or P of leaf litter or egesta, respectively, to estimate total element-specific consumption and egestion over the experiment. Element-specific growth was calculated from the following:

$$\text{Growth}_x = (\text{DM}[Q_x])_{\text{final}} - (\text{DM}[Q_x])_{\text{initial}} \quad (\text{Eq. 7})$$

where DM is *P. lepidus* DM and Q_x is the proportion of element x in *P. lepidus* DM. GGE_x and A_x for each element x were calculated using Eqs 1 and 4, respectively.

To assess TER sensitivity to bioenergetics model assumptions, I compared $\text{TER}_{\text{C:P}}$ estimates for *P. lepidus* using either fixed initial body C:P, final body C:P, or growth C:P, and varying values of *P. lepidus* P use efficiency: $A_P = 0.8$, empirical measures of A_P (Eq. 5), or empirical measures of GGE_P (Eq. 3). All models used empirical GGE_C . I conducted this

comparison among individuals at both peak and suboptimal growth to assess the sensitivity of models to organism growth status. I compared these $TER_{C:P}$ estimates to those of the TER model constrained to estimation at peak growth (Eq. 2).

I analyzed leaf litter %C, N, P, and molar ratios of C:N, C:P, and N:P with 2-way analysis of variance (ANOVA) with litter species and P concentration effects as factors of interest and feeding date as a third blocked factor. *Pycnopsyche lepida* growth, consumption rates, A_x , and GGE_x were analyzed with 2-way ANOVA, which I reduced to exclude the lowest P treatment because the oak diet in this treatment did not result in measurable *P. lepida* growth and most elemental use terms were negative. For statistical analysis of A_x and GGE_x , I considered negative estimates (3–11 replicates; mostly maple diets of lowest growth) as immeasurable assimilation or growth and transformed negative values to 0. Last, I used a 1-way ANOVA to compare $TER_{C:P}$ model estimates from Eq. 2 among 3 oak diets that resulted in divergent *P. lepida* growth. Significant interaction or main effects were subsequently examined pairwise using Tukey's Honestly Significant Difference (HSD) test. Data were $\log(x)$ - or reciprocal-transformed as needed to meet assumptions of ANOVA. Statistical analyses were conducted using SYSTAT version 13.1 (SYSTAT, Inc., Chicago, Illinois).

RESULTS

Leaf litter %P responded positively to increasing P concentrations to result in 4-fold ranges and 4 distinct levels of %P, C:P, and N:P within each litter type (Table S1, Fig. S1). Litter %N similarly increased with elevated P, but the 100 and 500 $\mu\text{g/L}$ P treatments had similar litter %N and C:N (Fig. S1). Oak litter had consistently higher C:P but lower C:N than maple litter at each level of dissolved P (Fig. S1). I used measures of litter %C and %P from each feeding date separately in the estimate of total C and P consumption over the experiment because litter %C

and %P differed by feeding date (Table S1).

Dietary P enrichment and litter type interacted to affect mass-specific rates of *P. lepida* leaf-litter consumption (Table 2). Consumption increased as much as 4-fold in response to P enrichment and was greater on maple than on oak diets in the <5 µg/L P treatment (Fig. 2A). *Pycnopsyche lepida* growth rates also were affected by an interaction of litter type and nutrients. Growth was greater for oak- than maple-fed insects in the 50 and 100 µg/L P treatments (Table 2; Fig. 2B). Among oak diets, *P. lepida* growth rates peaked at 0.046/d on litter incubated at 100 µg/L P (C:P = 1620) (Fig. 2B) and declined in the 500 µg/L P treatment (C:P = 1240) despite the similarity in consumption rate between these 2 treatments. Growth was negative on the greatest-C:P oak diet and survivorship was low (30%). All other diets conferred survivorship of 80 to 100%. Based upon a positive linear relationship between $\log(x)$ -transformed mean diet C:P and final *P. lepida* body C:P (Fig. 2C), the homeostasis coefficient H for oak-fed *P. lepida* was $1/0.211 = 4.74$. No relationship was found between $\log(x)$ -transformed body C:P and diet C:P content among maple-fed insects ($H = 1/0.074 = 13.59$; $p = 0.328$; Fig. 2C).

Mean values of A_x ranged from 0.04 to 0.34, 0.06 to 0.36, and -0.29 to 0.38 for C, N, and P, respectively, across diets (Fig. 3A–C). Maple-fed insects exhibited lower A_N and A_P than oak-fed insects, but neither term varied with diet P treatment (Table 2). All GGE_x estimates were lower on maple than on oak diets but were not affected by diet P treatment (Table 2). Means ranged from -0.001 to 0.021, 0.015 to 0.093, and 0.027 to 0.30 for C, N, and P, respectively (Fig. 3D–F). Negatively growing *P. lepida* fed the lowest-nutrient oak diet exhibited negative GGE_x and A_x . All other mean GGE_x measures were below A_x measures, with the exception of P use efficiencies on 3 maple diets. Maximum values of A_P and all GGE_x were achieved on the C:P = 1620 oak diet that conferred peak growth.

Bioenergetics $TER_{C:P}$ estimates were compared among oak-fed *P. lepidus* that exhibited divergent growth to permit assessment of model sensitivity to organism growth status on varying diet C:P. Resulting $TER_{C:P}$ estimates varied with body C:P, P use efficiency parameters, and *P. lepidus* growth status such that $TER_{C:P}$ estimates diverged from C:P = 1620 by -36% ($TER_{C:P} = 1030$) to +489% ($TER_{C:P} = 9540$; Table 3). Estimates strictly from Eqs 3 and 5 ranged 7-fold across individuals of varying growth status (Fig. 4A). $TER_{C:P}$ estimates were greatest and consistently overestimated diet C:P = 1620 of peak growth when fixed $A_P = 0.8$ was assumed. $TER_{C:P}$ values were lower when bioenergetics models used empirical A_P , and often declined below 1620 when empirical GGE_P replaced A_P (Fig. 4A). The alternate model based on empirical GGE_C , GGE_P , and growth C:P (Eq. 2) predicted $TER_{C:P}$ approximately equal to diet C:P and resultant $TER_{C:P}$ estimates at suboptimal growth were different from those at peak growth ($F_{2,24} = 14,415$, $P < 0.001$; Fig. 4B).

DISCUSSION

I found that *P. lepidus* violated common assumptions of TER bioenergetics models, which led to wide error in bioenergetics $TER_{C:P}$ depending on *P. lepidus* growth status. On oak diets, *P. lepidus* growth increased steeply as diet C:P declined from 4180 to 1620, suggesting alleviation from P-limitation of growth. Below a diet C:P of 1620, *P. lepidus* growth exhibited a significant decline that probably was caused by excess dietary P and not N effects because the 2 lowest-C:P oak diets were similar in N but not P content. The C:P = 1620 oak diet resulted in peak growth among all diets. Thus, it is the closest empirical estimate to the switch between P- and C-limitation and best approximates $TER_{C:P}$ of oak-fed *P. lepidus*. However, bioenergetics $TER_{C:P}$ often did not approach 1620 because *P. lepidus* exhibited $A_P < 0.8$, measurable P excretion ($A_P > GGE_P$), and growth C:P divergent from body C:P that violated model assumptions. $TER_{C:P}$

estimates also were sensitive to parameters drawn from *P. lepidus* fed suboptimal diet C:P, indicating inaccuracy of bioenergetics models not explicitly constrained to peak growth. These findings call for reassessment of TER bioenergetics models without associated growth data, and also provide empirical evidence that nutrient enrichment may not always benefit organisms because of constraints on consumer stoichiometric regulation (Boersma and Elser 2006).

Stoichiometric regulation by P. lepidus

Previous studies indicated potential intraspecific variation in body C:P among some aquatic insects (Cross et al. 2003, Small and Pringle 2010, Kendrick and Benstead 2013). In this study, oak-fed *P. lepidus* exhibited nonstrict homeostasis ($H = 4.74$), whereas maple-fed *P. lepidus* appeared to be strictly homeostatic. Lower values of H indicate weaker homeostasis.

Pycnopsyche lepidus fed oak litter were more plastic in C:P content than many grazing zooplankton (for nonstrict species, $H = 5-14.29$) and 6 of 8 freshwater mollusks (minimum $H = 0.79$; Persson et al. 2010). *Pycnopsyche lepidus* may store excess P in hemolymph as α -glycerophosphate, as demonstrated among closely related *Manduca sexta* larvae (Lepidoptera) (Woods et al. 2002). These results suggest that H may not necessarily be a fixed property of a species but can vary with diet identity, especially where identity modulates effects of diet C:P on consumer growth. Contrasting degrees of homeostasis between litter diets may have been driven by faster growth on oak diets that enabled *P. lepidus* to incorporate a greater proportion of ingested material and, thus, to exhibit stronger diet-dependence of body C:P than maple-fed individuals that might have been focusing on maintenance. Oak-fed *P. lepidus* exhibited initial mean body C:P = 82 below final body C:P = 103, so growth C:P on oak diets (mean range = 135–141) surpassed initial body C:P 1.6-fold as expected for developing aquatic insects (Back and King 2013).

Greater A_x and GGE_x of insects fed oak diets than those fed maple diets was surprising because labile litter, such as maple, should confer better assimilation and growth (Kaushik and Hynes 1971). *Pycnopsyche* spp. larvae use leaf litter to construct cases, which are essential to growth and development (Eggert and Wallace 2003). Maple-fed *P. lepida* may have been limited in their ability to use relatively delicate maple litter for case-building compared to oak-fed individuals. To address this possibility, I compared final case DM between maple- and oak-fed insects. Case DM was higher among oak-fed insects, but the case:insect ratio did not differ between leaf diets and was within the range of field-caught 4th- and 5th-instar *P. lepida* (Table S2, Fig. S2). Nutritional factors also could explain lower growth on maple diets. Both diets were of the same conditioning age, but maple litter was further into decomposition. This condition may have rendered maple microbes more senescent or remnant maple leaf material more recalcitrant and nutrient-poor relative to oak leaf material. At Chamber Springs and throughout its wide geographic range, *P. lepida* is exposed to a diversity of litter that includes oak and maple species and probably selects a mixture of litter depending on availability and degree of conditioning (Graça et al. 2001). Later in decomposition, recalcitrant litter species, such as oak, may be important to permit growth of *P. lepida*.

I did not observe significant changes in A_x and GGE_x across diet nutrient levels as might be expected from ecological stoichiometry theory (Sternner and Elser 2002). For example, A_N and A_P did not increase on high C:N or C:P diets, perhaps because of greater digestion resistance of diet nutrients in low-nutrient litter with lower microbial biomass (Tant et al. 2013). This mechanism is similar to how greater digestive resistance of P-limited algae can cause P-limited zooplankton to display counterintuitively lower A_P than zooplankton fed P-supplemented algae (Ferrão-Filho et al. 2007). Negative A_P among some individuals may have been caused by slow

or negative growth, or individuals fed either litter diet may have selectively ingested P-rich biofilm or leaf disks from among those available. This error is a challenge in measuring consumption C:N:P that could confound TER bioenergetics terms, such as A_P , but should not confound TER estimates based on the diet that confers optimal growth.

Pycnopsyche lepida achieved maximum $A_x < 0.4$, which is below most values observed for herbivorous zooplankton (DeMott et al. 1998, DeMott and Tessier 2002, Mayor et al. 2011), and suggests that most C, N, and P ingested is lost during digestion (i.e., *P. lepida* is primarily assimilation-limited). These values may be caused by the high recalcitrance of leaf litter and may extend to additional detritivores, such as *Lepidostoma* sp. and *Pteronarcys* sp., that exhibit similarly low A_C (0.16 and 0.33, respectively; McDiffett 1970, Grafius and Anderson 1979). Observed GGE_P and GGE_N below A_P and A_N on most diets also point to substantial postassimilatory loss (excretion) of P and N, in a manner similar to respiratory loss of C. For example, given $A_P = 0.38$ and $GGE_P = 0.30$ on the C:P = 1620 oak diet, ~8% of P ingested by *P. lepida* can be lost to excretion even at peak growth. This loss increases to excretion of 21% of ingested P on the C:P = 1240 oak diet. Larger differences between A_N and GGE_N compared to A_P and GGE_P indicate proportionally more N than P is lost postassimilation.

Implications for TER bioenergetics models

TER bioenergetics models were highly sensitive to assumptions of fixed $A_P = 0.8$, negligible P excretion, and fixed consumer C:P and, therefore, did not accurately predict growth patterns for *P. lepida* across the resource C:P gradient. This finding suggests that current TER models may not be appropriately parameterized to reflect organism stoichiometric regulation. For example, strictly at peak growth (oak C:P = 1620), use of maximum $A_P = 0.8$ (Eq. 5) resulted in $TER_{C:P} = 3150$, a diet that growth trends suggest would be P-limiting. In contrast, use of

empirical GGE_P to account for P excretion and continued use of fixed body C:P (Eq. 3) resulted in $TER_{C:P} = 1030$ that would be C-limiting. At peak growth, the closest approximation to 1620 was from the model that used empirical A_P ($TER_{C:P} = 1600$; Eq. 5). This model closely approximated the diet C:P of peak growth, but it relied on demonstrably flawed assumptions of fixed body C:P and negligible P excretion. I do not endorse TER estimation using these assumptions because they do not apply to *P. lepidus* and may be prone to further error depending on organism growth status.

Bioenergetics $TER_{C:P}$ showed increased error when parameters were drawn from *P. lepidus* at suboptimal growth. The degree of variation of $TER_{C:P}$ estimates from 1620 was magnified at low growth because reduced GGE_C compounded with overestimated P use efficiencies to result in 2- to 5-fold overestimation of $TER_{C:P}$ (Table 3). When TER models used empirical GGE_P to accommodate P excretion and growth C:P to account for flexible body C:P (Eq. 2), resulting $TER_{C:P}$ estimates were strongly diet-dependent (Fig. 4B). This exploratory analysis reveals that bioenergetics TERs without simplifying assumptions will be autocorrelated with the resource ratio of consumption, highlighting the need to constrain models to peak growth. Autocorrelation will be greatest when simplifying assumptions are met. Otherwise, $TER_{C:P}$ will be skewed toward erroneous values (Table 3, Fig. 4A) depending on how significantly actual growth C:P or GGE_P diverge from assumed fixed body C:P or A_P , respectively. Thus, bioenergetics TERs may be error-prone where models are: 1) based on inaccurate assumptions, such as $A_P = 0.8$, negligible P excretion, or fixed body C:P = growth C:P at peak growth, or 2) not accompanied by growth data necessary to constrain models to peak growth.

These data also provide evidence for negative consumer responses to excess dietary P

(Boersma and Elser 2006). Consistent with my finding of increased growth as oak C:P changed from 4180 to 1620 in this study, field data indicate that *Pycnopsyche* spp. can become dominant when stream P concentrations are elevated to 80 $\mu\text{g/L}$ P and litter C:P declines to 3063 (Davis et al. 2010). However, below C:P = 1620, diets may induce C limitation to reduce growth. This induction may occur at ecologically relevant levels of P enrichment because diets fed to *P. lepida* spanned the range in mixed litter C:P in Ozark streams (Scott et al. 2013). The mechanism of reduced growth below diet C:P = 1620 is unclear, but slight decreases in consumption may play a role, perhaps because of nutrient satiation (Plath and Boersma 2001). Similar A_P and simultaneously lower GGE_P on the C:P = 1240 oak diet compared to the C:P = 1620 oak diet highlights the importance of excretion as a regulatory pathway to release excess ingested P that may exact respiratory costs to decrease GGE_C and growth (Boersma and Elser 2006). The decline in *P. lepida* growth was concurrent with increased body P storage, so detrital P enrichment may decouple the positive relationship between organism P content and growth rate (Elser et al. 2003). Future TER estimates among additional taxa could advance understanding and management of stream ecosystems subject to nutrient enrichment (Evans-White et al. 2009).

Caveats and conclusions

TERs are a powerful way to predict elemental demand of organisms and are preferable to predicting demand strictly from body stoichiometry (Sturner and Elser 2002). TER bioenergetics models appropriately account for differential use of dietary elements. Like other organisms, *P. lepida* used dietary P far more efficiently (~14-fold) than dietary C. However, certain conditions may confound bioenergetics model assumptions. For example, recalcitrant diets, such as detritus or defended algae, may reduce maximum consumer A_P and GGE_P ; organisms with complex life cycles may exhibit growth C:P divergent from body C:P; and constraining models to peak

growth may be difficult among understudied organisms. My study also indicates that organisms, such as maple-fed *P. lepida*, do not always display the nutrient-dependent growth that is implicit in TER theory. Under these circumstances, it seems inappropriate to estimate TERs, which underscores the value of growth data for assessing the most basic assumption of TER theory—that the elements in consideration determine growth. Overall, diet type may be an important determinant of organism growth that should be explored as a potential determinant of TERs.

I suggest that the most accurate estimate of $TER_{C:P}$ for oak-fed *P. lepida* is 1620 because this diet conferred maximum growth rates and efficiencies for both C and P. This $TER_{C:P}$ derivation, constrained to peak growth and grounded in Eq. 2, accommodates flexible body C:P, low P use efficiency, and P excretion by *P. lepida* even at the $TER_{C:P}$. However, the growth-based approach to TER calculations is limited in that actual $TER_{C:P}$ may have been between values of diet C:P fed to *P. lepida*. This $TER_{C:P}$ also carries some degree of error caused by variation in diet C:P across feeding dates (SE = 40). Maximum rates of *P. lepida* growth in this study (0.046/d) approached or were greater than maximum growth for *Pycnopsyche* spp. in previous studies (0.041, 0.061, and 0.030/d; Eggert and Wallace 2003, Chung and Suberkropp 2009, Kendrick and Benstead 2013, respectively), suggesting that conditions closely approximated those of optimal growth. Moreover, despite the limitations of growth-based methods, my study shows that, compared to many bioenergetics models, growth data across varying resource C:P provide a less error-prone means of estimating $TER_{C:P}$ that more accurately represents organism stoichiometry and should be more applicable to diverse taxa.

I fed *P. lepida* ad libitum, but 1620 may be the lowest C:P ratio of peak growth given that $TER_{C:P}$ may increase as food quantity becomes limiting because of background losses of C to respiration (Anderson and Hessen 2005). Accommodating background N and P excretion in TER

models probably would weaken the effect of food quantity on TERs (Anderson and Hessen 2005). Future studies should address both food quality and quantity because stream nutrient enrichment concurrently increases detrital nutrient content and decreases detrital standing stocks (Cross et al. 2003, Benstead et al. 2009), which may synergistically impose C-limitation of detritivore growth. Last, $TER_{C:P} = 1620$ is 20-fold greater than initial *P. lepida* body C:P, a stark contrast from 2.4-fold conversion factors found previously and used in $TER_{C:P}$ estimates for other detritivores (Frost et al. 2006, Hladyz et al. 2009). Future investigators of understudied taxa should calculate TERs from growth data for organisms fed along a wide diet-quality gradient, where the TER is defined as the resource ratio (C:P) conferring either 1) maximal GGE_C and GGE_P or 2) optimal growth.

ACKNOWLEDGEMENTS

Thanks to E. Scott, C. Fuller, D. Hall, J. Ramey, G. White, L. Abel, E. Grantz, C. Prater, and A. Eddy for laboratory assistance and helpful discussion. I also thank D. Bowles (National Park Service) for his help identifying *Pycnopsyche lepida*. S. Entrekin, J. M. Hood, B. Austin, A. Smartt, and 3 anonymous referees provided valuable feedback on earlier versions of the manuscript. This research was supported by the US National Science Foundation (DEB 1020722) including NSF REU programs at the University of Arkansas (DBI 1063067 and 1359188). HMM was supported by a Doctoral Academy Fellowship from the University of Arkansas.

LITERATURE CITED

- Allen, A. P., and J. F. Gillooly. 2009. Towards an integration of ecological stoichiometry and the metabolic theory of ecology to better understand nutrient cycling. *Ecology Letters* 12:369–384.
- Anderson, T. R., and D. O. Hessen. 2005. Threshold elemental ratios for carbon versus phosphorus limitation in *Daphnia*. *Freshwater Biology* 50:2063–2075.
- APHA (American Public Health Association). 2005. Standard methods for the examination of water and wastewater. 21st edition. American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, DC.
- Back, J. A., and R. S. King. 2013. Sex and size matter: ontogenetic patterns of nutrient content of aquatic insects. *Freshwater Science* 32:837–848.
- Benke, A. C., and A. D. Huryn. 2006. Secondary production of macroinvertebrates. Pages 691–710 in F. R. Hauer and G. A. Lamberti (editors). *Methods in stream ecology*. Academic Press, New York.
- Benke, A. C., and J. B. Wallace. 1980. Trophic basis of production among net-spinning caddisflies in a southern Appalachian stream. *Ecology* 61:108–118.
- Benstead, J. P., A. D. Rosemond, W. F. Cross, J. B. Wallace, S. L. Eggert, K. Suberkropp, V. Gulis, J. L. Greenwood, and C. J. Tant. 2009. Nutrient enrichment alters storage and fluxes of detritus in a headwater stream ecosystem. *Ecology* 90:2556–2566.
- Boersma, M., and J. J. Elser. 2006. Too much of a good thing: on stoichiometrically balanced diets and maximal growth. *Ecology* 87:1325–1330.
- Chung, N., and K. Suberkropp. 2009. Contribution of fungal biomass to the growth of the shredder, *Pycnopsyche gentilis* (Trichoptera: Limnephilidae). *Freshwater Biology* 54:2212–2224.
- Creed, R. P., R. P. Cherry, J. R. Pflaum, and C. J. Wood. 2009. Dominant species can produce a negative relationship between species diversity and ecosystem function. *Oikos* 118:723–732.
- Cross, W. F., J. B. Wallace, A. D. Rosemond, and S. L. Eggert. 2006. Whole-system nutrient enrichment increases secondary production in a detritus-based ecosystem. *Ecology* 87:1556–1565.
- Cross, W. F., J. P. Benstead, A. D. Rosemond, and J. B. Wallace. 2003. Consumer–resource stoichiometry in detritus-based streams. *Ecology Letters* 6:721–732.
- Danger, M., J. A. Funck, S. Devin, J. Heberle, and V. Felten. 2013. Phosphorus content in

- detritus controls life-history traits of a detritivore. *Functional Ecology* 27:807–815.
- Davis, J. M., A. D. Rosemond, S. L. Eggert, W. F. Cross, J. B. Wallace, and W. Bowden. 2010. Long-term nutrient enrichment decouples predator and prey production. *Proceedings of the National Academy of Sciences of the United States of America* 107:121–126.
- DeMott, W. R., R. D. Gulati, and K. Siewertsen. 1998. Effects of phosphorus-deficient diets on the carbon and phosphorus balance of *Daphnia magna*. *Limnology and Oceanography* 43:1147–1161.
- DeMott, W. R., and A. J. Tessier. 2002. Stoichiometric constraints vs. algal defenses: testing mechanisms of zooplankton food limitation. *Ecology* 83:3426–3433.
- Doi, H., M. Cherif, T. Iwabuchi, I. Katano, J. C. Stegen, and M. Striebel. 2010. Integrating elements and energy through the metabolic dependencies of gross growth efficiency and the threshold elemental ratio. *Oikos* 119:752–765.
- Eggert, S. L., and J. B. Wallace. 2003. Reduced detrital resource limit *Pycnopsyche gentilis* (Trichoptera:Limnephilidae) production and growth. *Journal of the North American Benthological Society* 22:388–400.
- El-Sabaawi, R. W., R. Zandonà, T. J. Kohler, M. C. Marshall, J. M. Moslemi, J. Travis, A. López-Sepulcre, R. Ferrière, C. M. Pringle, S. A. Thomas, D. N. Reznick, and A. S. Flecker. 2012. Widespread intraspecific organismal stoichiometry among populations of the Trinidadian guppy. *Functional Ecology* 26:666–676.
- Elser, J. J., K. Archarya, M. Kyle, J. Cotner, W. Makino, T. Markow, T. Watts, S. Hobbie, W. Fagan, J. Schade, J. Hood, and R. W. Sterner. 2003. Growth rate-stoichiometry couplings in diverse biota. *Ecology Letters* 6:936–943.
- Evans-White, M. A., W. K. Dodds, D. G. Huggins, and D. S. Baker. 2009. Thresholds in macroinvertebrate biodiversity and stoichiometry across water-quality gradients in Central Plains (USA) streams. *Journal of the North American Benthological Society* 28:855–868.
- Evans-White, M. A., R. S. Stelzer, and G. A. Lamberti. 2005. Taxonomic and regional patterns in benthic macroinvertebrate elemental composition in streams. *Freshwater Biology* 50:1786–1799.
- Ferrão-Filho, A. S., A. J. Tessier, and W. R. DeMott. 2007. Sensitivity of herbivorous zooplankton to phosphorus-deficient diets: testing stoichiometric theory and the growth rate hypothesis. *Limnology and Oceanography* 52:407–415.
- Frost, P. C., J. P. Benstead, W. F. Cross, H. Hillebrand, J. H. Larson, M. A. Xenopoulos, and T. Yoshida. 2006. Threshold elemental ratios of carbon and phosphorus in aquatic consumers. *Ecology Letters* 9:774–779.

- Graça, M. A. S., C. Cressa, T. M. O. Gessner, M. J. Feio, K. A. Callies, and C. Barrios. 2001. Food quality, feeding preferences, survival and growth of shredders from temperate and tropical streams. *Freshwater Biology* 46:947–957.
- Grafius, E., and N. H. Anderson. 1979. Population dynamics, bioenergetics, and role of *Lepidostoma quercina* Ross (Trichoptera: Lepidostomatidae) in an Oregon woodland stream. *Ecology* 60:433–441.
- He, X.-J., and W.-X. Wang. 2008. Stoichiometric regulation of carbon and phosphorus in P-deficient *Daphnia magna*. *Limnology and Oceanography* 53:244–254.
- Hladyz, S., M. O. Gessner, P. S. Giller, J. Pozo, and G. Woodward. 2009. Resource quality and stoichiometric constraints on stream ecosystem functioning. *Freshwater Biology* 54:957–970.
- Kaushik, N.K., and H.B.N. Hynes. 1971. The fate of the dead leaves that fall into streams. *Archiv für Hydrobiologie* 68:465-515.
- Kendrick, M. R., and J. P. Benstead. 2013. Temperature and nutrient availability interact to mediate growth and body stoichiometry of a detritivorous stream insect. *Freshwater Biology* 58:1820–1830.
- Mayor, D. J., K. Cook, B. Thornton, P. Walsham, U. F. M. Witte, A. F. Zuur, and T. R. Anderson. 2011. Absorption efficiencies and basal turnover of C, N, and fatty acids in a marine calanoid copepod. *Functional Ecology* 25:509–518.
- McDiffett, W. F. 1970. The transformation of energy by a stream detritivore, *Pteronarcys scotti* (Plecoptera). *Ecology* 51:975–988.
- Moulton, S. R., and K. W. Stewart. 1996. Caddisflies (Trichoptera) of the interior highlands of North America. American Entomological Institute, Gainesville, Florida.
- Olsen, Y., A. Jensen, H. Reinertsen, K. Yngve Børshheim, M. Heldal, and A. Langeland. 1986. Dependence of the rate of release of phosphorus by zooplankton on the P:C ratio in the food supply, as calculated by a recycling model. *Limnology and Oceanography* 31:34–44.
- Persson, J., P. Fink, A. Goto, J. M. Hood, J. Jonas, and S. Kato. 2010. To be or not to be what you eat: regulation of stoichiometric homeostasis among autotrophs and heterotrophs. *Oikos* 119:741–751.
- Plath, K., and M. Boersma. 2001. Mineral limitation of zooplankton: stoichiometric constraints and optimal foraging. *Ecology* 82:1260–1269.
- Scott, E. E., C. Prater, E. Norman, B. C. Baker, M. A. Evans-White, and J. T. Scott. 2013. Leaf-litter stoichiometry is affected by streamwater phosphorus concentrations and litter type.

Freshwater Science 32:753–761.

- Singer, G. A., and T. J. Battin. 2007. Anthropogenic subsidies alter stream consumer-resource stoichiometry, biodiversity, and food chains. *Ecological Applications* 17:376–389.
- Sinsabaugh, R. L., S. Manzoni, D. L. Moorhead, and A. Richter. 2013. Carbon use efficiency of microbial communities: stoichiometry, methodology, and modelling. *Ecology Letters* 16:930–939.
- Small, G. E., and C. M. Pringle. 2010. Deviation from strict homeostasis across multiple trophic levels in an invertebrate consumer assemblage exposed to high chronic phosphorus enrichment in a Neotropical stream. *Oecologia (Berlin)* 162:581–590.
- Sterner, R. W., and J. J. Elser. 2002. *Ecological stoichiometry: the biology of elements from molecules to the biosphere*. Princeton University Press. Princeton, New Jersey.
- Tant, C. J., A. D. Rosemond, and M. R. First. 2013. Stream nutrient enrichment has a greater effect on coarse than on fine benthic organic matter. *Freshwater Science* 32:1111–1121.
- Urabe, J., and Y. Watanabe. 1992. Possibility of N or P limitation for planktonic cladocerans: an experimental test. *Limnology and Oceanography* 37:244–251.
- Woods, H. A., M. C. Perkins, J. J. Elser, and J. F. Harrison. 2002. Absorption and storage of phosphorus by larval *Manduca sexta*. *Journal of Insect Physiology* 48:555–564.
- Woodward, G., M. O. Gessner, S. P. Giller, V. Gulis, S. Hladysz, A. Lecerf, B. Malmqvist, B. G. McKie, S. D. Tiegs, H. Cariss, M. Dobson, A. Eloegi, V. Ferreira, M. A. S. Graça, T. Fleituch, J. O. Lacousière, M. Nistorescu, J. Pozo, G. Risnoveanu, M. Schindler, A. Vadineanu, L. B.-M. Vought, and E. Chauvet. 2012. Continental-scale effects of nutrient pollution on stream ecosystem functioning. *Science* 336:1438–1440.

Table 1. Summary of stoichiometric regulation equations. In equations, x designates any element x whereas C and P designate carbon and phosphorus, respectively. See text for further explanation and sources. Max = maximum.

Term	Description	Equation	No.	Notes
A_x	Assimilation efficiency	$(\text{consumption}_x - \text{egestion}_x) / (\text{consumption}_x)$	(4)	Accounts for egestion _{x} but not excretion _{x}
GGE_x	Gross growth efficiency	$(\text{growth}_x) / (\text{consumption}_x)$	(1)	Accounts for egestion _{x} and excretion _{x}
$TER_{C:P}$	Threshold elemental ratio	$(GGE_P / GGE_C) * (\text{growth}_C / \text{growth}_P)$	(2)	Calculated at optimal growth
$TER_{C:P}$	Threshold elemental ratio	$(\max GGE_P / \max GGE_C) * (\text{body}_C / \text{body}_P)$	(3)	Assumes fixed body C:P
$TER_{C:P}$	Threshold elemental ratio	$(A_P / GGE_C) * (\text{body}_C / \text{body}_P)$	(5)	Assumes 0 excretion _P and fixed body C:P

Table 2. Two-way analysis of variance table for *Pycnopsyche lepida* response to 2 litter diets (maple, oak) incubated under contrasting concentrations of dissolved P. Terms A_C , A_N , and A_P represent assimilation efficiencies of C, N, and P, respectively, and GGE_C , GGE_N , and GGE_P represent gross growth efficiencies of C, N, and P, respectively. [P] = dissolved P concentration during leaf-litter incubation. Boldface indicates significant effects ($p < 0.05$).

Variable	Factor	F-ratio	p-value	Variable	Factor	F-ratio	p-value
Growth	[P]	6.85	0.001	Consumption ^a	[P]	28.43	<0.001
	Litter	29.43	<0.001		Litter	15.22	<0.001
	[P] × litter	13.99	<0.001		[P] × litter	12.07	<0.001
A_C ^b	[P]	0.59	0.560	GGE_C ^b	[P]	2.41	0.069
	Litter	2.46	0.124		Litter	41.75	<0.001
	[P] × litter	1.06	0.354		[P] × litter	2.84	0.102
A_N ^b	[P]	0.43	0.65	GGE_N ^b	[P]	2.05	0.141
	Litter	12.71	<0.001		Litter	16.00	<0.001
	[P] × litter	0.14	0.868		[P] × litter	0.76	0.475
A_P ^b	[P]	2.06	0.139	GGE_P ^b	[P]	1.92	0.158
	Litter	50.31	<0.001		Litter	9.26	0.004
	[P] × litter	1.33	0.274		[P] × litter	0.97	0.387

^a Reciprocal-transformed prior to analysis

^b 2-way analysis with the lowest-[P] treatment group removed

Table 3. Mean \pm SE molar C:P threshold elemental ratio (TER_{C:P}) estimates for *Pycnopsyche lepida* from bioenergetics models from varying P use efficiencies (A_P is P assimilation efficiency, GGE_P is P gross growth efficiency) and body stoichiometry (Q_C/Q_P is molar body C:P, growth_C/growth_P is C:P of growth) for oak-fed insects. TER_{C:P} was estimated for insects of varying growth status or at peak growth. All models used empirical GGE_C. Numbers in parentheses describe % deviation from C:P = 1620 of peak *P. lepida* growth.

	P use efficiency	Initial Q_C/Q_P	Final Q_C/Q_P	Growth _C /Growth _P
Across individuals of varying growth status:	$A_P = 0.8$	5770 \pm 740 (+256%) ^a	7450 \pm 1310 (+360%)	9540 \pm 2260 (+489%)
	Empirical A_P	2640 \pm 400 (+63%) ^a	3400 \pm 630 (+110%)	4670 \pm 1440 (+188%)
	Empirical GGE _P	1280 \pm 100 (-21%) ^b	1500 \pm 90 (-7%)	1770 \pm 110 (+10%) ^c
Constrained to individuals at peak growth:	$A_P = 0.8$	3150 \pm 280 (+94%) ^a	4410 \pm 560 (+172%)	6110 \pm 1770 (+277%)
	Empirical A_P	1600 \pm 280 (-1%) ^a	2250 \pm 420 (+39%)	3020 \pm 800 (+87%)
	Empirical GGE _P	1030 \pm 90 (-36%) ^b	1370 \pm 80 (-15%)	1590 \pm 10 (-2%) ^c

^a Eq. 5, Frost et al. 2006

^b Eq. 3, Doi et al. 2010

^c Eq. 2

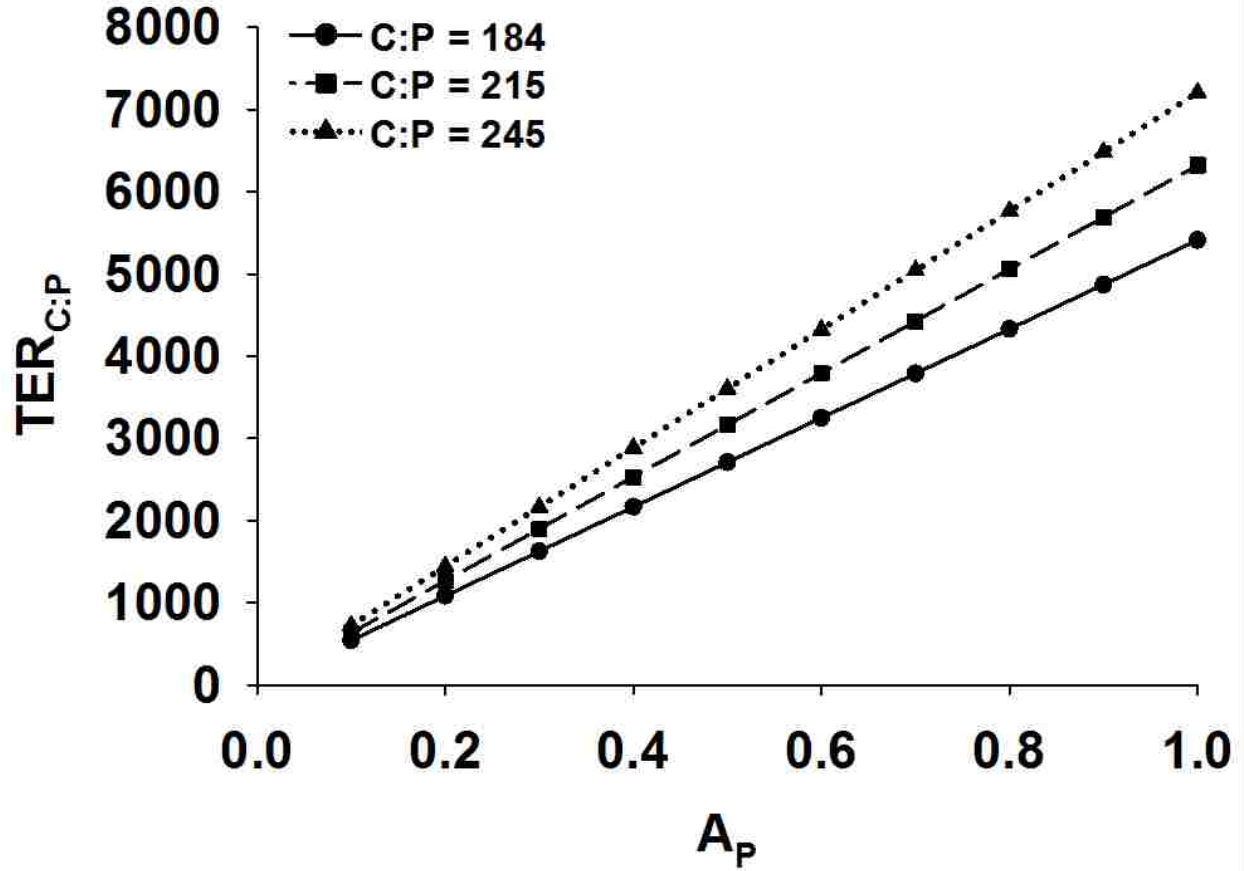


Figure 1. Estimated effect of variation in P assimilation efficiency (A_P) and body molar C:P on the molar C:P threshold elemental ratio ($TER_{C:P}$) of the detritivorous aquatic insect *Pteronarcys* sp. from TER bioenergetics models (Eq. 5). The model used initial body C:P = 184 (Evans-White et al. 2005) and mean gross growth efficiency for C (GGE_C) = 0.034 at 10°C (McDiffett 1970). Varying body C:P values of 215 and 245 were chosen to illustrate $TER_{C:P}$ sensitivity to increased body C:P as a consequence of larval development (Back and King 2013).

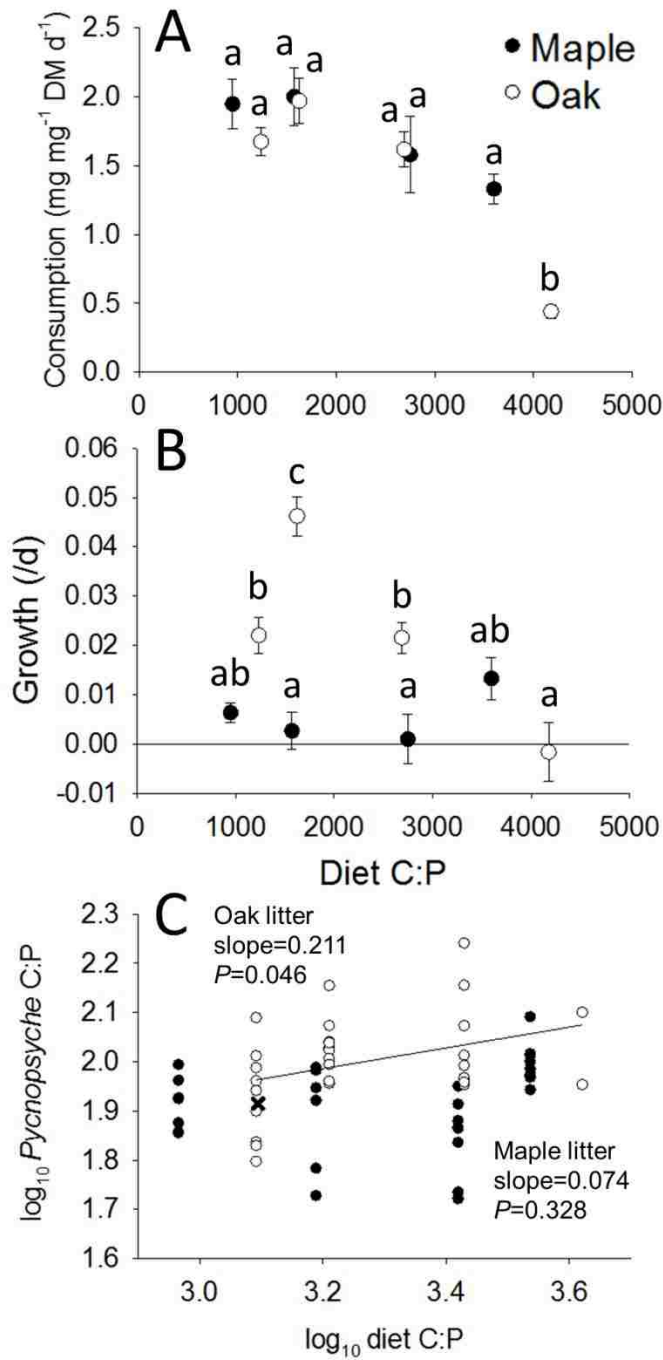


Figure 2. Mean (± 1 SE) dry mass (DM)-specific rates of consumption (A) and instantaneous growth rates (B), and log-transformed final body C:P content (C) of *Pycnopsyche lepida* fed oak and maple litter of contrasting C:P content. In panels A and B, points with the same letter are not significantly different (Tukey's Honestly Significant Difference, $p < 0.05$). In panel C, the regression line is shown only for the significant relationship (larvae fed oak diets). The black 'x' designates log-transformed mean initial larval C:P content and C:P of mixed litter from Chamber Springs.

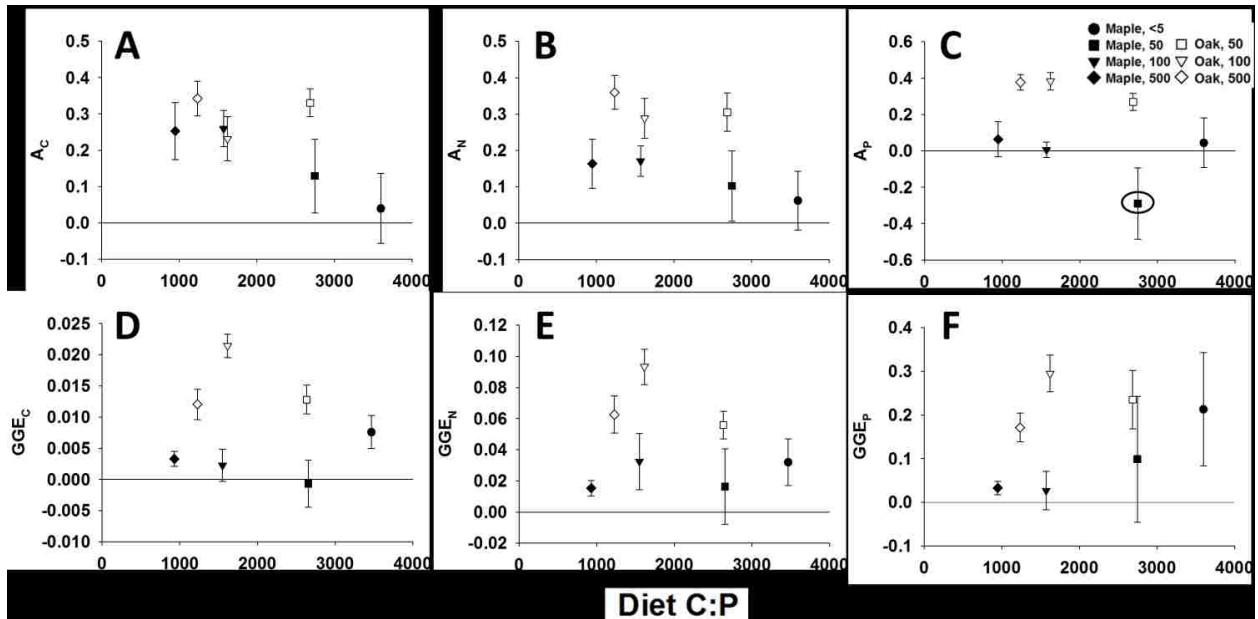


Figure 3. Mean (± 1 SE) assimilation efficiencies for C (A_C) (A), N (A_N) (B), and P (A_P) (C) and gross growth efficiencies for C (GGE_C) (D), N (GGE_N) (E), and P (GGE_P) (F) of *Pycnopsyche lepida* fed a dietary molar C:P gradient of maple and oak litter. The circled point in panel C is from maple-fed individuals with negligible growth.

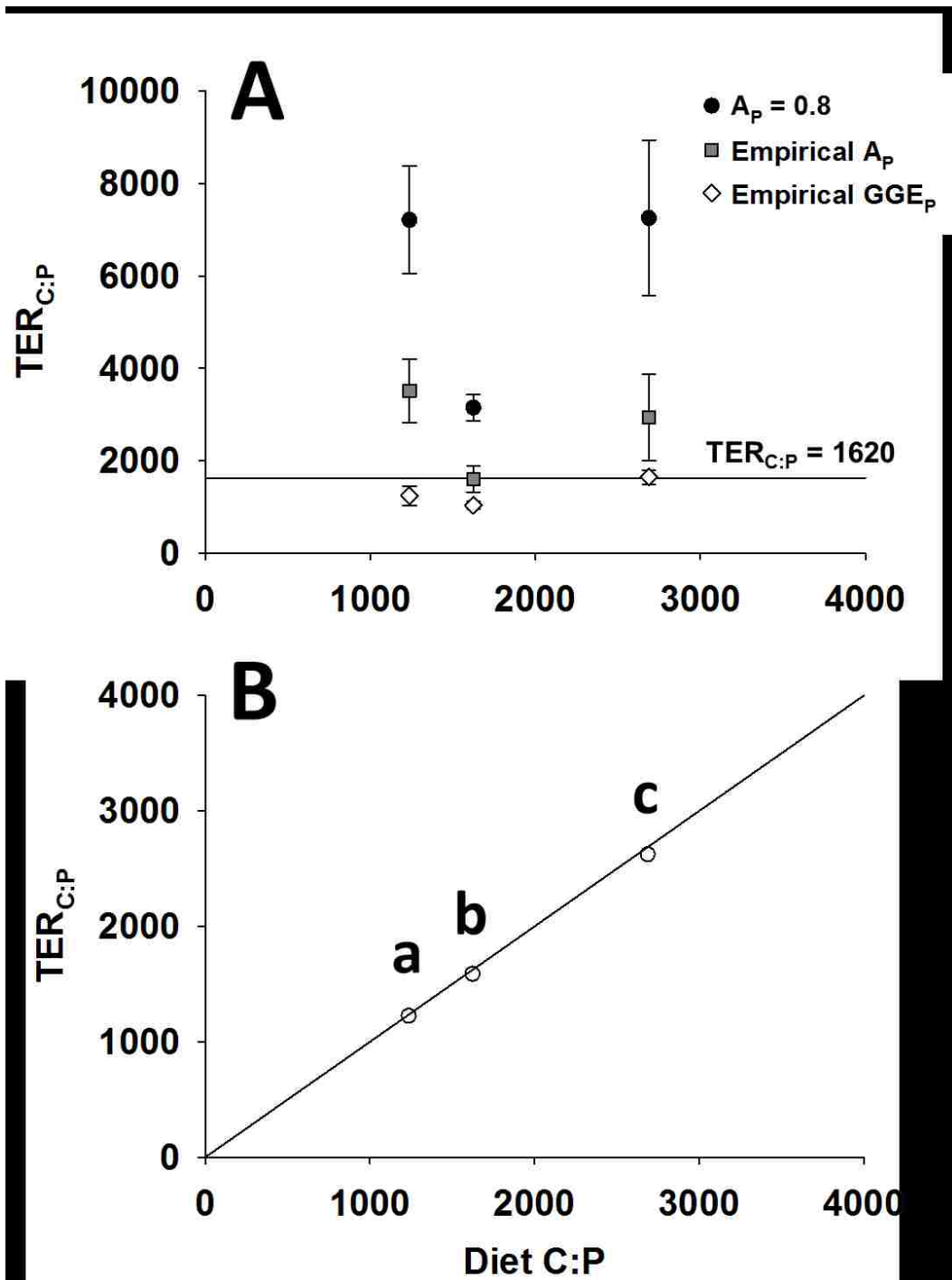


Figure 4. Predicted molar C:P threshold elemental ratios ($TER_{C:P}$) of divergently growing *Pycnopsyche lepida* fed oak litter of varying C:P content. A.—Mean (± 1 SE) $TER_{C:P}$ estimates from bioenergetics models using empirical C gross growth efficiency (GGE_C), assuming fixed initial *P. lepida* C:P, and using P assimilation efficiency (A_P) = 0.8, empirical A_P (Eq. 5), or empirical GGE_P (Eq. 3). The $TER_{C:P} = 1620$ line designates the diet C:P of peak *P. lepida* growth in the present study. B.— $TER_{C:P}$ estimates from empirical GGE_P , GGE_C , and C:P of *P. lepida* growth (Eq. 2). The line designates $TER_{C:P} = \text{diet C:P}$. Letters designate statistically different groups (Tukey's Honestly Significant Difference, $p < 0.05$).

APPENDICES

Table S1. Blocked 2-way analysis of variance table for leaf-litter stoichiometry terms. [P] = leaf-litter incubation dissolved P concentration, Litter = maple or oak, Date = feeding date. Bold indicates significant effects ($p < 0.05$).

Variable	Factor	<i>F</i> -ratio	<i>p</i> -value	Variable	Factor	<i>F</i> -ratio	<i>P</i> -value
% C	[P]	3.8	0.013	C:P ^a	[P]	397.4	<0.001
	Litter	57.4	<0.001		Litter	16.9	<0.001
	[P] × litter	10.2	<0.001		[P] × litter	5.2	0.002
	Date	5.7	<0.001		Date	2.2	0.013
% N	[P]	31.4	<0.001	C:N	[P]	28.7	<0.001
	Litter	3.4	0.069		Litter	10.5	0.002
	[P] × litter	0.3	0.816		[P] × litter	1.0	0.412
	Date	1.5	0.136		Date	2.0	0.028
% P ^a	[P]	404.5	<0.001	N:P ^a	[P]	469.9	<0.001
	Litter	24.1	<0.001		Litter	60.7	<0.001
	[P] × litter	4.0	0.009		[P] × litter	5.8	0.001
	Date	2.4	0.007		Date	4.1	<0.001

^alog(x)-transformed prior to analysis

Table S2. One-way analysis of variance table for final case dry mass (DM) and case:insect DM of *Pycnopsyche lepida* fed maple or oak litter in the laboratory.

Variable	Factor	<i>F</i> -ratio	<i>P</i> -value
Case DM	Litter	33.2	<0.001
Case:insect DM	Litter	0.498	0.483

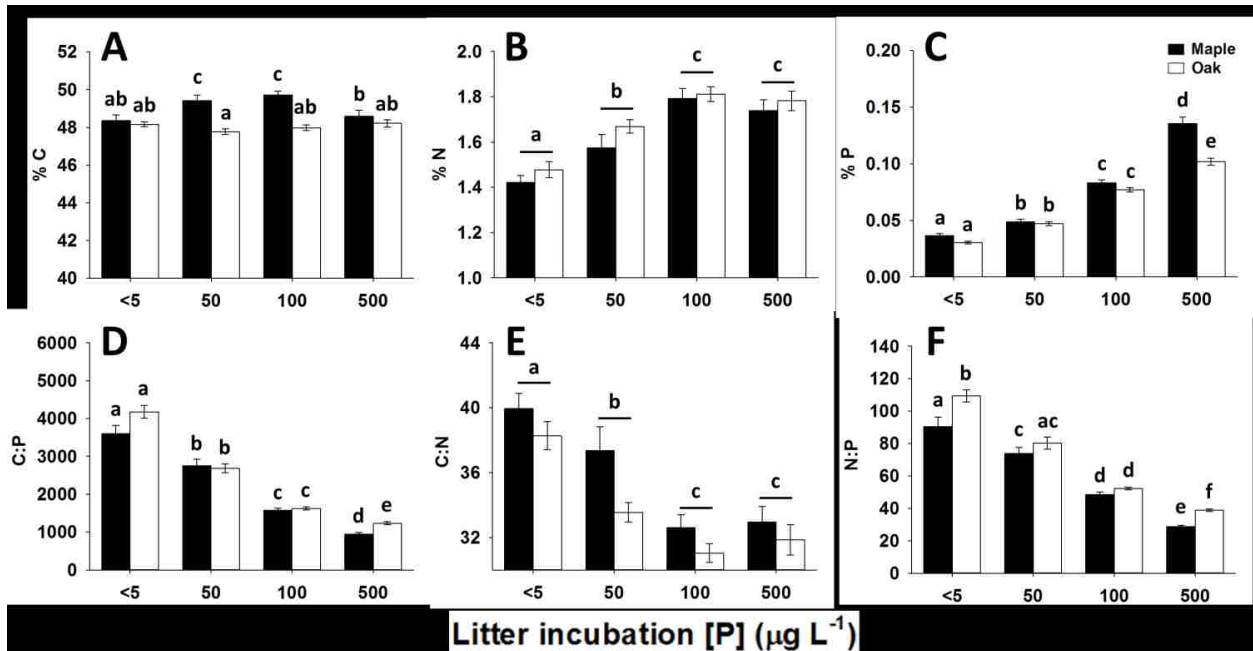


Figure S1. Mean (± 1 SE) elemental content of oak and maple litter conditioned under 4 P concentrations for 11 wk before to feeding to *Pycnopsyche lepida*. %C (A), %N (B), and %P (C) are per unit litter dry mass, whereas C:N (D), C:P (E), and N:P (F) are molar ratios. Bars with the same letter are not significantly different ($p > 0.05$).

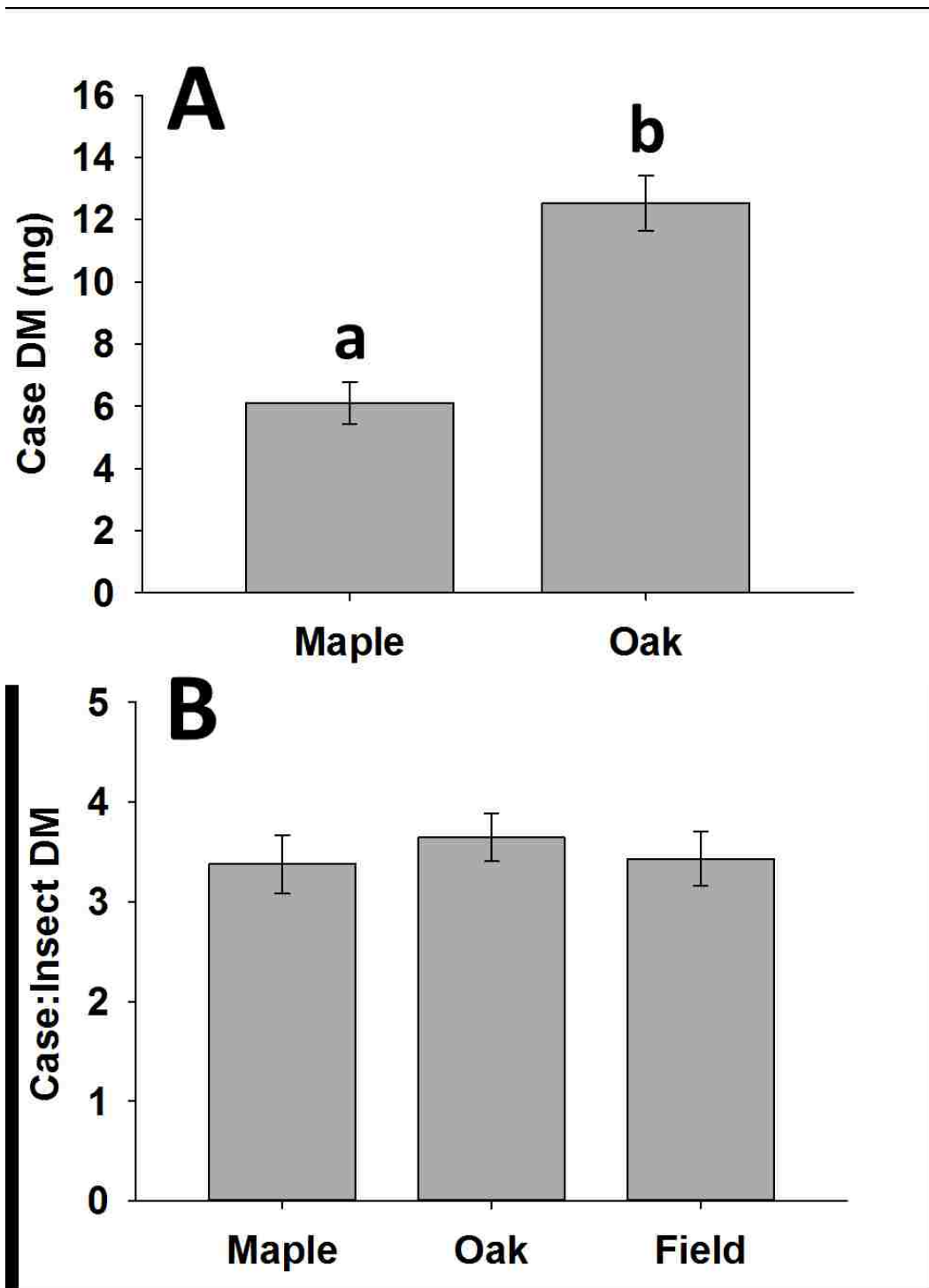


Figure S2. Mean (± 1 SE) final case dry mass (DM) of 4th- and 5th-instar *Pycnopsyche lepida* fed oak or maple diets in the laboratory over a 5-wk period (A) and final case:insect DM ratio for similar instars of *P. lepida* fed maple or oak diets in the laboratory or collected from the same population in December 2013 (B). Bars with the same letters are not significantly different ($p > 0.05$).

CHAPTER II

Dietary and taxonomic controls on incorporation of microbial carbon and phosphorus by
detritivorous caddisflies²

²Halvorson HM, White G, Scott JT, Evans-White MA (2016) Dietary and taxonomic controls on incorporation of microbial carbon and phosphorus by detritivorous caddisflies. *Oecologia* 180:567-679. doi: 10.1007/s00442-015-3464-6

ABSTRACT

Heterotrophic microbes on detritus play critical roles in the nutrition of detritivorous animals, yet few studies have examined factors controlling the acquisition of microbial nutrients toward detritivore growth, termed incorporation. Here, I assessed effects of detrital substrate identity (leaf type), background nutrients, and detritivore species identity on detritivore incorporation of microbial carbon (C) and phosphorus (P) in leaf litter diets. I fed oak and maple litter conditioned under two nutrient concentrations (50 or 500 $\mu\text{g L}^{-1}$ P) to the detritivorous caddisfly larvae *Isonychia* spp., *Lepidostoma* spp., and *Pycnopsyche lepida* and used the radioisotopes ^{14}C as glucose and ^{33}P as phosphate to dually trace incorporation of microbial C and P by caddisflies. Incorporation efficiencies of microbial C (mean \pm SE = $12.3 \pm 1.3\%$) were one order of magnitude higher than gross growth efficiencies for bulk detrital C from recent studies ($1.05 \pm 0.08\%$). Litter type did not affect incorporation of microbial nutrients; however, caddisflies incorporated microbial P 11% less efficiently when fed litter from the higher P concentration. Two lower body C:P species (*Pycnopsyche* and *Isonychia*) exhibited 9.9 and 7.1% greater microbial C and 19.0 and 17.7% greater microbial P incorporation efficiencies, respectively, than the higher body C:P species (*Lepidostoma*). These findings support ecological stoichiometry theory on post-ingestive regulation that animals fed lower C:P diets should reduce P incorporation efficiency due to excess diet P or alleviation of P-limited growth, and that lower C:P species must incorporate dietary C and P more efficiently to support fast growth of P-rich tissues.

INTRODUCTION

In a diversity of ecosystems, heterotrophic microbes such as bacteria and fungi provision a substantial proportion of detritivorous animal growth (Chung and Suberkropp 2009; Pollierer et al. 2012). Heterotrophic microbes serve as a valuable source of nutrients including labile carbohydrates, protein, and mineral phosphorus (P) that can constrain secondary production and are relatively depleted in the detrital substrate itself (Bärlocher 1985). Indeed, studies suggest that the guts of detritivores such as aquatic macroinvertebrates and terrestrial fungus-feeding beetles are specially adapted to digest microbial biomass in detritus (Martin et al. 1980; 1981). The contributions of microbial carbon (C) to growth and energetic demands of aquatic detritivores are particularly well-studied; for example, radiolabel experiments suggest that leaf litter fungal C can meet 50-100% of growth requirements of the caddisfly *Pycnopsyche gentilis* (Chung and Suberkropp 2009). It is critical to understand the nutritional importance of microbes to detritivores because microbes link detrital energy and nutrients, as well as mobile background nutrients, with larger structure and function of detritus-based ecosystems (Mann 1988; Hall and Meyer 1998; Moore et al. 2004).

Detritivore assimilation and allocation of nutrients for the production of new tissues, termed incorporation, may differ between microbial biomass and detrital substrates both within and across taxa (Sinsabaugh et al. 1985, Bärlocher and Porter 1986). Aquatic detritivores differ in their capacity to incorporate detrital substrate versus microbial nutrients due to differences in feeding strategies and digestive capabilities (Bärlocher and Porter 1986; Arsuffi and Suberkropp 1989). For example, although many detritivores can digest the plant polymer cellulose through reliance on gut microbes (Sinsabaugh et al. 1985), gut pH and proteolytic activity as well as the ability to incorporate unconditioned detritus can vary taxonomically (Bärlocher and Porter 1986).

Radiolabel studies indicate bacterial and fungal C provide an important, though not majority, contribution to respiratory and growth demands of several detritivores (Findlay et al. 1986a,b). But a more recent radiolabel study suggests fungi contribute the most to C growth of the aquatic detritivore *Pycnopsyche gentilis* (Chung and Suberkropp 2009), highlighting disagreement across studies in the role of microbial versus substrate C in detritivore nutrition. Because leaf litter microbial biomass and litter chemistry strongly affect gut enzymatic activity and incorporation of microbial C (van Frankenhuyzen and Geen 1985; Canhoto and Graça 2006), some of the observed variation across studies and taxa may be attributable to dietary and not taxonomic factors. This variation must be discerned to predict bottom-up controls on energy and nutrient dynamics such as secondary production and nutrient cycling as well as community composition in detrital food webs.

The importance of detrital microbes to detritivore nutrition likely varies with factors that control microbial biomass and nutrient content on detritus, such as detrital substrate identity. Detrital substrates vary widely in nutrient content and recalcitrance according to the source plant species and tissue type (Enriquez et al. 1993). Variation across substrates can directly affect microbial biomass and nutrient content. Recalcitrant, nutrient-poor substrates often support lower microbial biomass with lower nutrient content compared to labile substrates with higher nutrient content (Gulis and Suberkropp 2003; Fanin et al. 2013), which may explain why detritivore growth is often diminished on recalcitrant detritus (Ward and Cummins 1979; Cothran et al. 2014). However, extended microbial conditioning can diminish substrate differences, in some cases permitting faster detritivore growth on well-conditioned recalcitrant leaf litter such as oak relative to labile leaf litter such as birch or maple (Hutchens et al. 1997, Fuller et al. 2015, Halvorson et al. 2015b). Substrate identity can also affect incorporation of

microbial nutrients by detritivores by constraining digestive ability or shifting digestion to favor detrital versus microbial components. As an example of these more direct effects of substrate identity on digestion, oils in eucalyptus leaves may inhibit gut enzyme activity in *Tipula* (Canhoto and Graça 2006). The multiple effects of substrate identity on detritivore nutrition are often difficult to separate, and it remains unclear whether substrate identity directly alters detritivore incorporation of microbial nutrients independent of differences in microbial biomass or nutrient content.

A second factor controlling incorporation of detrital microbes by detritivores is background nutrient availability because microbes can assimilate mobile nutrients such as dissolved N and P (Cheever et al. 2012; Cheever et al. 2013; Pastor et al. 2014). Wide evidence suggests nutrient addition increases fungal biomass on detritus (Gulis and Suberkropp 2003; Tant et al. 2013) as well as the nutrient composition of detrital microbes (Danger and Chauvet 2013; Scott et al. 2013), thus enhancing detritivore growth (Danger et al. 2013; Fuller et al. 2015). In this way, microbes serve as the link between increased background nutrient concentrations and profound alterations of detritus-based ecosystems such as increased secondary production in headwater streams (Cross et al. 2006). Although nutrient enrichment enhances the magnitude of C and P entering detrital stream food webs through microbial pathways (Cross et al. 2007), enrichment may simultaneously reduce the efficiency with which microbial P is incorporated by detritivores due to alleviation of detritivore P-limitation (Sterner and Elser 2002). Indeed, detritivores fed high-nutrient diets often increase rates of P release as egesta and excreta (Halvorson et al. 2015a), presumably due to excess P in microbial biomass. We must know the efficiency of detritivore incorporation of microbial C and P under low versus high nutrient

availability to understand how nutrient enrichment affects the larger fate of energy and nutrients assimilated by microbes from detritus and the water column.

Taxonomic variation in nutritional requirements may serve as a third factor influencing detritivore incorporation of microbial nutrients. Ecological stoichiometry theory predicts consumer growth requirements to vary in relation to body elemental composition (Sterner and Elser 2002); for example, detritivores of higher body P content should exhibit lower C:P threshold elemental ratios ($TER_{C:P}$), or higher diet P requirements, than detritivores of lower body P content (Frost et al. 2006). Because body P content is positively related to organismal growth rates through allocation to ribosomal RNA (the growth rate hypothesis; Elser et al. 2003), detritivores with higher body P content may exhibit greater incorporation of microbial C and P to support fast growth (Hood and Sterner 2014). Evidence suggests that microbial P content, independent of microbial biomass, may constrain growth of the aquatic detritivore *Gammarus* (Danger et al. 2013). However, no studies have tested the ability of ecological stoichiometry theory to predict interspecific variation in detritivore incorporation of microbial nutrients. An examination of taxonomic variation in incorporation of microbial nutrients could help explain why certain taxa such as detritivores of low body C:P become dominant under nutrient enrichment (Evans-White et al. 2009).

The objective of this study was to examine dietary and taxonomic variation in incorporation efficiency (IE) of microbial C and P by three aquatic detritivorous caddisfly larvae. Caddisflies were fed oak or maple litter conditioned under low or high P concentrations and I used radioisotopes of phosphorus (^{33}P) as orthophosphate and carbon (^{14}C) as glucose to trace microbial C and P incorporation by caddisflies. Because I used well-conditioned leaf litter among which oak litter, compared to maple litter, has previously conferred better growth for the

aquatic detritivores *Pycnopsyche lepida* and *Tipula abdominalis* (Fuller et al., 2015, Halvorson et al. 2015b), I hypothesized that (1) detritivores would exhibit more efficient incorporation of microbial C and P on oak than on maple litter. I also hypothesized that (2) detritivores would incorporate microbial P more efficiently on low-P diets, due to increased egestion and excretion of excess microbial P on high-P diets potentially below detritivore $TER_{C:P}$ (Halvorson et al. 2015a). Finally, I expected that detritivores with higher body P content would display greater microbial C and P IEs than detritivores with low body P content, because high body P content is associated with faster growth rates that would require more efficient incorporation of microbial nutrients by detritivores.

METHODS

Leaf litter incubation

Leaf litter diets were incubated in the laboratory following methods described previously (Halvorson et al. 2015a,b). In brief, 13.5 mm leaf disks of sugar maple (*Acer saccharum*) and post oak (*Quercus stellata*) were conditioned in dechlorinated tap water amended with stock solution of KNO_3 and Na_2HPO_4 to attain final concentrations of $1000 \mu g L^{-1}$ N and either 50 or $500 \mu g L^{-1}$ P. The high N concentrations were chosen to prevent N-limitation of microbial conditioning. Water was changed and nutrients amended every 2-3 d. Leaf disks were incubated for 77 d at ambient room temperature prior to feeding, with the exception that radiolabeled disks were incubated for 70 d prior to dual labeling with ^{33}P and ^{14}C (see radiolabeling methods below). During incubations, the amount of leaf litter biomass per volume medium per unit time was $0.25 g \text{ litter } L^{-1} \text{ wk}^{-1}$. I chose the two leaf species, incubation P concentrations, and incubation durations to permit comparison to results from similar diets indicating strong effects on detritivore growth (Fuller et al. 2015); for example, the two P concentrations were expected to

result in leaf litter diets above and below the estimated $TER_{C:P}$ of 1620 for *Pycnopsyche lepida* (Halvorson et al. 2015b).

Caddisfly collection and acclimation

I used field-caught caddisflies (Trichoptera) from three different genera for radiolabeling trials in the laboratory. Individual *Pycnopsyche lepida* (Limnephilidae) were collected from Chamber Springs in Benton County, Arkansas in March 2013 whereas both *Ironoquia* spp. (Limnephilidae) and *Lepidostoma* spp. (Lepidostomatidae) were collected from an unnamed headwater stream in Madison County, Arkansas in April and May 2013, respectively. All caddisflies were in the terminal instar prior to pupation and emergence. Experiments for each genus were conducted separately under similar acclimation and radiolabeling conditions. For each genus, 32 individuals were randomly assigned acclimation chambers in an environmental room at the University of Arkansas set to 10°C and a 12/12 light/dark cycle. A subset of individuals from each genus was also collected to determine initial elemental content. Caddisflies were twice fed 3-5 leaf disks from one of the four diets (maple or oak; 50 or 500 $\mu\text{g L}^{-1}$ P incubation; n=8 per diet) over a 6 d acclimation period. Acclimation chambers were equipped with a 1 mm mesh to separate caddisflies from particulate wastes and prevent coprophagy.

Radiolabel experiments

Leaf disks incubated for 70 days were radiolabeled for 6 d prior to feeding to each chamber-acclimated caddisfly genus. Thirty leaf disks from each diet treatment were separated by treatment and placed in beakers containing 50 mL stream water spiked with stock solutions of ^{14}C as glucose (specific activity = 1.67 mCi mg^{-1}) and ^{33}P as orthophosphate (specific activity = 155.8 Ci mg^{-1}) to attain final activities of 0.01 $\mu\text{Ci mL}^{-1}$ for each radioisotope. Three days after

initial spiking, stream water was given a second aliquot of radioisotopes to raise specific activities to the original experimental level. This extended labeling period and second spike helped ensure thorough labeling of bacterial and fungal C and P pools with ^{14}C and ^{33}P prior to feeding (Findlay et al. 1986a, Chung and Suberkropp 2009). After the labeling period, radioactive leaf disks were each rinsed 5 times with unlabeled stream water and placed in radiation chambers (3-4 disks per chamber, depending on genus) immediately prior to introduction of caddisflies. Five radiolabeled and rinsed disks from each diet type were also subsampled and frozen for determination of mean initial ^{33}P and ^{14}C content per leaf disk.

A random subset of 20 of the 32 caddisflies from each acclimation trial (n=5 per diet) was chosen for the radiolabel feeding experiments. The remaining 12 individuals were allowed to clear their guts for 24 h and frozen for elemental analysis. The 20 radiolabel individuals were randomly assigned radiation chambers with the appropriate leaf disk diet from the acclimation period. Radiation chambers were designed similar to acclimation chambers and were kept at 10°C and a 12/12 light/dark cycle and contained 100 mL stream water as well as the 1 mm mesh insert. Radiation chambers were kept in a low-temperature incubator and were designed to prevent release of ^{14}C into the atmosphere using potassium hydroxide as a trap based on methods used to measure soil microbial respiration because radiation chambers could not be kept at experimental temperature in the fume hood (Wolf et al. 1994; Fig. S1).

Caddisflies were given a 36 h period to feed on leaf disks; I ended the feeding period for *Isonychia* after 25 h because one individual had ingested all leaf disks at that time point. At the end of the feeding period, all remaining leaf litter was removed and frozen for eventual determination of ^{14}C and ^{33}P remaining. This uneaten litter did not include substantial feces or other small particulate wastes because particulate wastes fell through the mesh inserts. Although

the ingestion measures would include some losses of radiolabel to fragmentation by shredding, the measures may be more ecologically relevant, by accounting for all material lost or broken down toward incorporation into new biomass, at the cost of physiological relevance (i.e. measurement of strictly ingested radiolabel). After removal of labeled leaf litter, all caddisflies were subsequently given 100 mL fresh stream water and 5 unlabeled leaf disks of the appropriate diet for a 24 h period to clear their guts of radioactive material and allow a period of assimilation and incorporation of ingested radiolabel (Chung and Suberkropp 2009). Caddisflies were subsequently frozen for determination of ^{14}C and ^{33}P content.

Measurement of ^{33}P and ^{14}C

All radioactive samples were thawed and caddisflies were rinsed with unlabeled water and removed from their cases for determination of ^{33}P and ^{14}C content. The process of freezing and thawing, especially in liquid, may cause some leakage of incorporated radioisotopes and would reduce incorporation efficiencies. I kept the rinsing time to <1 hr prior to digestion in solubilizer; however, I recommend that future investigators employ rinsing while caddisflies are living, prior to freezing. Leaf disks were digested in 0.5 mL NCS Tissue Solubilizer (MP Biomedicals, Santa Ana, CA) for 36 hours at 45°C. Caddisflies were digested similarly in 1.6 mL NCS Tissue Solubilizer following maceration. Samples were then allowed to cool and each given 0.3 mL of 30% H_2O_2 and heated for 1 hour at 45°C to reduce quenching due to coloration. After another cooling period, all samples were suspended in Ecolume scintillation fluid (MP Biomedicals, Santa Ana, CA) to attain a 1:10 solubilizer:scintillant volumetric ratio across samples. All scintillation vials were permitted to settle with occasional mixing for 3 d in the dark and subsequently measured for radioactivity using liquid scintillation counting.

I followed the dual-label methods of Duhamel et al. (2006) to calculate disintegrations per minute (dpm) of ^{33}P and ^{14}C for each sample at the time of freezing. All samples were measured for total counts per minute (cpm) using a Beckman Coulter LS 6500 (Beckman Coulter, Brea, CA) at approximately 2 wk intervals for a minimum of 90 d, a period of over 3 half-lives of ^{33}P . This method measures total cpm in the emission spectrum of 0 to 250 keV, spanning the energy window of beta particles emitted from decaying ^{33}P and ^{14}C , and uses the shorter half-life of ^{33}P (25.4 d) relative to the half-life of ^{14}C (5,730 yr) to distinguish cpm attributable to ^{14}C versus ^{33}P over time. A linear regression of natural log-transformed ^{33}P cpm in each sample over time was used to extrapolate to initial ^{33}P at the time of freezing, whereas ^{14}C cpm for each sample were calculated as mean ^{14}C cpm over the period of decay (Fig. 1). I accounted for 3-4% decay of ^{33}P in labeled leaf litter during the ingestion period that would cause post-ingestion labeled litter to be lower in ^{33}P relative to pre-ingestion labeled litter. I used quench curves for each radioisotope to convert cpm to dpm for all samples (Scott et al. unpublished data).

Elemental Analysis

All pre-experiment and acclimation but not radiolabeled insects were analyzed for body elemental content. Caddisflies were homogenized into fine powder using a spatula. Leaf disks fed during acclimation trials, as well as a subset of post-leaching but pre-conditioning oak and maple disks, were homogenized using a ball-bearing grinder (Wig-L-Bug; Crescent Dental Manufacturing, Elgin, IL). All samples were dried at 48°C overnight prior to weighing out to the nearest 10 µg using a microbalance (Mettler Toledo, Columbus, OH). For P analysis, samples were digested in 1 N hydrochloric acid at 85°C for 30 minutes, diluted, and analyzed for soluble reactive phosphorus using the Ascorbic Acid method (APHA 2005). For C/N analysis, samples

were analyzed using a CHN analyzer (Thermo Scientific, Waltham, MA). I corrected for elemental recovery efficiencies (106%, 105%, and 92% mean recovery for C, N, and P respectively) using peach leaf standards (NIST SRM 1547).

Calculations and Statistical Analyses

Total ingestion for each radioisotope was calculated as the difference between total dpm of leaf litter fed to each caddisfly (determined for each diet from mean ^{14}C or ^{33}P dpm per leaf disk multiplied by number of leaf disks fed) and measured dpm of leaf litter post-ingestion in each chamber. The ^{14}C and ^{33}P dpm of each caddisfly were used to indicate total radioisotope incorporated over the labeling period. I calculated incorporation efficiencies (IEs) for ^{14}C and ^{33}P by dividing insect radioisotope incorporation by radioisotope ingestion and I defined relative use efficiencies (RUEs) as the ratio of IE_C relative to IE_P .

I used a preliminary three-way ANOVA to compare leaf litter stoichiometry (% C, N, and P as well as molar C:P, C:N, and N:P) between litter types, P treatment, and experiments (i.e. caddisfly genera). Because there were no differences in leaf litter stoichiometry across experiments ($P > 0.05$), I pooled data across experiments to analyze diet stoichiometry and was able to statistically compare caddisfly genera with respect to radioisotope IEs. Due to concerns about the statistical properties of ratios that can impose isometry on allometric data, I statistically analyzed IEs with utilization plots and ANCOVA that treated radioisotope ingestion as the covariate (Raubenheimer 1992; Raubenheimer and Simpson 1994) using three separate ANCOVA models that separately examined each main effect (litter type, nutrient levels, or caddisfly genus). I broke up the ANCOVA in this way because the fully-factorial ANCOVA model exhibited heterogeneous slopes at several levels of interaction that violated ANCOVA assumptions, and I had more statistical power to detect heterogeneous slopes by examining only

main effects. I adjusted experimental α within related response variables to correct for experiment-wise error using Bonferroni correction based on the number of ANOVA or ANCOVA tests performed. All statistical analyses were conducted using R version 3.1.2 (2014, R Foundation for Statistical Computing).

RESULTS

Leaf litter and caddisfly body stoichiometry

Leaf litter microbial conditioning increased the N and P content of leaf litter, as did greater incubation dissolved P concentrations. Prior to conditioning, maple and oak litter were similar in %C and %P content; however oak litter was of higher %N (1.05 ± 0.03) than maple litter (0.69 ± 0.12), resulting in divergent initial C:N and N:P between litter types (Table S1). Conditioning reduced initial differences between maple and oak %N and more than doubled %N of both leaf types (Tables S1,S2). Leaf litter responded strongly to incubation P concentration, exhibiting significant decreases in molar C:N, C:P, and N:P and increases in %N and %P from 50 to 500 $\mu\text{g L}^{-1}$ P concentrations. Conditioned oak and maple litter differed only in %C, with maple higher in %C than oak litter, and there were no interactive effects of leaf litter type with nutrient treatment (Table 1; Fig. 2). In particular, leaf litter doubled from approximately 0.05 %P to greater than 0.10 %P for both leaf types from 50 to 500 $\mu\text{g L}^{-1}$ P incubations, resulting in two distinct levels of dietary P fed to caddisflies within each leaf type. Limited differences in C:P content of leaf litter prior to conditioning, as well as post-conditioning within each nutrient level, suggest that subsequent statistical comparisons of nutrient treatment and litter type effects may not be confounded by differential effects of incubation P concentration on each litter type.

Caddisfly genera varied in %N and %P content, resulting in a wide range of body molar ratios of C:N, C:P, and N:P (Table 2). Body %N and %P were positively associated with one

another such that caddisflies were arranged, from smallest to largest mean body %N and %P, *Lepidostoma*, *Ironoquia*, and *Pycnopsyche*. *Pycnopsyche* exhibited the lowest body N:P (14) and *Lepidostoma* exhibited the highest body N:P (31) , indicating the former genus was P-rich relative to N whereas the latter was N-rich relative to P (Table 2).

Radiolabel incorporation efficiencies

The radiolabel data revealed reduced IE_P in response to litter P enrichment as well as strong differences in IE_C and IE_P across caddisfly genera. There were no effects of litter type or incubation P concentration on IE_C ; however, caddisfly genera differed such that *Lepidostoma* exhibited $7.10 \pm 1.31\%$ and $9.92 \pm 2.01\%$ lower IE_C compared to *Pycnopsyche* and *Lepidostoma*, respectively (Table 3, Fig. 3). Caddisfly IE_P similarly did not differ between litter types; however IE_P was significantly lower by $11.0 \pm 4.5\%$ on higher-nutrient leaf litter (Table 3, Fig. 4). Differences in incorporation of microbial C across caddisfly genera were also consistent with those for microbial P such that *Lepidostoma* exhibited $17.7 \pm 5.1\%$ and $19.0 \pm 5.1\%$ lower IE_P than *Ironoquia* and *Pycnopsyche*, respectively (Table 3, Fig. 4).

Ratio-based IEs indicated that caddisflies incorporated microbial P with greater efficiency than they incorporated microbial C. Across diets and taxa, ratio-based IEs were averaged for oak and maple within each nutrient level because IEs from ANCOVA did not differ between litter species (Table 3). Caddisfly genera varied widely in IE_C (mean ± 1 SE range = 0.031 to ± 0.004 to 0.196 ± 0.042) and IE_P (0.11 ± 0.08 to 0.47 ± 0.13). Lower IE_C relative to IE_P resulted in mean C:P RUE less than 1 for all caddisflies and diets, ranging from 0.22 ± 0.10 to 0.66 ± 0.17 (Fig. 5; Table S3).

DISCUSSION

My study revealed dietary P and caddisfly taxonomic identity influenced the incorporation of microbial nutrients by caddisflies, supporting two of my three original hypotheses. I did not observe the predicted effects of leaf litter type on incorporation of microbial C and P by caddisflies, indicating substrate identity may not always affect the nutritional value of detrital microbes for detritivore growth. Caddisflies fed diets of lower P content did incorporate microbial P more efficiently than caddisflies fed diets of higher P content, as predicted. Although I did not measure growth or calculate $TER_{C:P}$ in these experiments, the decline in IE_P would be consistent with stronger P-limitation of growth on the low P diet or increased release of excess P on higher-P diets below caddisfly $TER_{C:P}$ (Halvorson et al. 2015b). Finally, the detritivore genus with the lowest body P content (*Lepidostoma*) exhibited lower IE of microbial C and P compared to two genera of higher body P content (*Ironoquia* and *Pycnopsyche*). These findings suggest that ecological stoichiometry theory can predict incorporation of heterotrophic microbe nutrients by detritivores, similar to better-studied incorporation of autotroph nutrients by herbivores (Frost et al. 2002; Ferrão-Filho et al. 2007).

My data do not support a strong effect of substrate identity (leaf litter type) on detritivore incorporation of microbial C and P, at least for the two leaf species used in this study. Although substrate identity could act through several mechanisms to affect incorporation of microbial nutrients by detritivores, none of these effects appeared important in this study. One reason that I observed little differences between oak and maple litter in IEs may have been the extended period of leaf litter conditioning prior to feeding; 77 days may be sufficient to reduce substrate-associated differences, such as substrate recalcitrance, fungal or bacterial biomass, or microbial stoichiometry, between oak and maple litter (Hutchens et al. 1997; Hieber and Gessner 2003, Scott et al. 2013). Indeed, oak and maple only differed in %C in this study, suggesting microbial

stoichiometry was relatively similar between leaf litter types within each nutrient treatment. Because my study did reveal strong effects of background nutrient concentrations on incorporation of microbial nutrients, it is still possible that substrate identity may indirectly affect detritivore nutrition when microbial biomass or stoichiometry differ between substrates (Fanin et al. 2013). Common observations of leaf type effects on detritivore growth, especially on well-conditioned leaf litter (Graça et al. 2001, Fuller et al. 2015, Halvorson et al. 2015b), may be more likely driven by differences in detritivore incorporation of substrate and not microbial nutrients.

Ecological stoichiometry theory predicts that detritivores fed P-limiting diets above the $TER_{C:P}$ should increase efficiency of incorporation of dietary P due to P-limitation of growth (Sternner and Elser 2002). The low-P and high-P diets were above and below estimated $TER_{C:P}=1620$ for *Pycnopsyche lepida* (Halvorson et al. 2015b), suggesting diets spanned a range from P limitation to excess for caddisfly growth. Consistent with P-limitation of growth above the $TER_{C:P}$, caddisflies incorporated microbial P 11% more efficiently when leaf litter was conditioned under lower P concentrations. Microbial P content can constrain growth of the aquatic detritivore *Gammarus* (Danger et al. 2013), and my study further suggests that incorporation of microbial P by detritivores may depend on microbial P content that can vary with environmental conditions such as stream water P availability. While $50 \mu\text{g P L}^{-1}$ is a high P concentration for stream water to serve as a low P treatment, leaf litter C:P at $50 \mu\text{g P L}^{-1}$ (mean \pm SE = 3270 ± 480) was above that of mixed litter from Ozark streams below $20 \mu\text{g P L}^{-1}$ (Scott et al. 2013), indicating the low P diet represented conditions of low P availability in-stream. Litter C:P was relatively high in my experiments at both P concentrations due to methodological differences such as continuous addition and 4-fold greater concentrations of leaf litter in my

incubations relative to those of Scott et al. (2013). These data add to evidence that background P concentrations strongly affect detritivore growth and functional roles in aquatic ecosystems (González et al. 2014, Fuller et al. 2015). One recent study found a 6.3% reduction in gross growth efficiency (GGE) of bulk detrital P by *Pycnopsyche lepida* fed oak litter conditioned similarly at 500 $\mu\text{g P L}^{-1}$ relative to those fed oak litter conditioned at 50 $\mu\text{g P L}^{-1}$ (Halvorson et al. 2015b). The larger decline in IE of microbial P (11%) than GGE of bulk detrital P (6.3%) suggests that reductions in incorporation of microbial P, not of substrate P, drive lower P growth efficiencies. Lower IE_P of detritivores would reduce the proportion of available microbial P that enters the food web, strengthening animal-mediated transformations of P into wastes (excreta and egesta) that would impact downstream ecosystems (Halvorson et al. 2015a).

Consumer body stoichiometry is often used to predict taxonomic variation in nutrient demands for growth (Frost et al. 2006). Here, I found that the caddisfly species with the highest body C:P (*Lepidostoma*) displayed lower IE_C and IE_P than two caddisfly species with lower body C:P (*Pycnopsyche* and *Ironoquia*). The difference in incorporation of microbial P makes sense in light of stoichiometric constraints that would require *Pycnopsyche* and *Ironoquia* to incorporate dietary P more efficiently to support greater P demands for growth. The similar taxonomic differences in incorporation of microbial C are not direct predictions of ecological stoichiometry; however, body C:P may be negatively related to caddisfly growth rates (Elser et al. 2003), necessitating more efficient incorporation of microbial C by *Pycnopsyche* and *Ironoquia* to support fast growth compared to *Lepidostoma*. Although instantaneous measurement of body C:P may not be the most accurate predictor of nutrient demands throughout organism ontogeny (Back and King 2013, Halvorson et al. 2015b), it may still serve to explain coarse differences in demands for microbial nutrients across detritivore taxa because fast growth requires investment

in low-C:P tissues during animal growth (Hood and Sterner 2014). Further, laboratory growth data for caddisflies fed maple and oak litter across a wide nutrient gradient indicate consistently faster growth of *Pycnopsyche* (mean \pm SE = 0.016 ± 0.002 d⁻¹; Halvorson et al. 2015b) compared to *Lepidostoma* (mean \pm SE = 0.006 ± 0.002 d⁻¹; Halvorson et al. unpublished data). These data point to a credible link between taxonomic variation in detritivore reliance on microbial biomass (Arsuffi and Suberkropp 1989) and detritivore body stoichiometry through a connection to detritivore growth rates and nutritional requirements.

A comparison of detritivore IEs for microbial C and P to GGEs for bulk detritus further indicates that microbes are an important source of dietary C and P for detritivores. Microbial C was incorporated at least one order of magnitude more efficiently (IE_C mean \pm SE = $12.3 \pm 1.3\%$) than bulk detrital C based on growth experiments for other detritivores (GGE_C mean \pm SE = $1.05 \pm 0.08\%$; Fuller et al. 2015, Halvorson et al. 2015b), suggesting microbial C is much more easily assimilated and retained compared to substrate C. Unlike for C, IEs for microbial P were similar to GGEs for bulk dietary P (Fuller et al. 2015, Halvorson et al. 2015b). Bulk GGE_P and microbial IE_P were most likely similar because microbes form a major pool of dietary P on detritus, whereas microbes often compose 10% or less of total detrital C (Gessner and Chauvet 1994; Findlay et al. 2002), causing bulk detritus GGE_C to deviate from IE_C specific to the microbial pool. Although IEs are calculated over shorter time periods compared to GGEs, these two metrics should be comparable because they both address dietary contributions to new tissue production and account for losses such as to egestion and respiration. Given mean bulk GGE_C of 1.05%, mean microbial biomass from similar incubation conditions of 6.8% (Halvorson unpublished data), mean microbe-specific IE_C of 12.3%, and assuming identical %C between leaf litter and microbial biomass, I estimate GGE specific to substrate C to be 0.0023 (0.23%).

This rough estimate suggests detritivores incorporate microbial C >50 times more efficiently than substrate C.

Relative use efficiencies (RUEs) were consistently below 1 on all diets, indicating further that microbial P was incorporated more efficiently than microbial C, probably due to substantial losses of ingested microbial C to egestion and respiration (van Frankenhuyzen and Geen 1985). Interestingly, RUEs were higher among caddisflies with lower body C:P, suggesting microbial C was used more efficiently relative to microbial P by caddisflies with higher P requirements. This pattern is opposite to predictions of ecological stoichiometry theory that would predict lower C:P RUE among caddisflies of lower body C:P, but it does support a connection between body C:P and fast growth that would require efficient acquisition of microbial C by detritivores (Frost et al. 2006). Indeed, it is possible that efficient incorporation of microbial C, not of microbial P, is the stronger constraint on fast growth across detritivore species. Although microbes compose a small pool of detrital biomass, my data agree with conceptions that the high quality of microbial nutrients relative to substrate nutrients provisions substantial growth requirements of detritivorous animals (Kaushik and Hynes 1971, Cummins 1973, Chung and Suberkropp 2009, Pollierer et al. 2012).

My approach to assessing detritivore incorporation of microbial C and P carries a few caveats. I employed dissolved tracers, meaning I could not accurately trace microbial C and P originally derived from the leaf litter substrate itself. Although this means my methods were not representative of all microbial nutrients available to detritivores, recent isotope studies do suggest that microbes derive increasing proportions of C and N (c. 35% or more) from the water column instead of the leaf litter substrate later into decomposition (Cheever et al. 2013, Pastor et al. 2014). Dissolved tracers may be well-representative of microbial C and P available to

detritivores because I used leaf litter conditioned for 77 d, and dissolved tracers are the best way to ensure labeling of microbial and not substrate nutrients. While tracing substrate C and P into microbes and subsequently detritivores would be valuable, there are significant challenges such as the labeling timeframe required as well as the difficulty of distinguishing detritivore incorporation of labeled substrate versus microbes when both sources contain the tracer. I also used an extended incubation period of 6 d to label microbial pools of C and P with slow turnover rates (Chung and Suberkropp 2009); for example, this ensured a labeling timeframe for bacteria exhibiting fast turnover and fungi exhibiting slow turnover, though bacteria may have been labeled more thoroughly than fungi (Baldy et al. 2002). Disproportionate labeling of labile, fast-turnover pools such as bacteria instead of recalcitrant pools such as fungi could cause overestimation of incorporation of microbial nutrients (Dodds et al. 2014). My method of measuring radioisotope ingestion should be robust to a related concern of selective feeding on leaf litter microbes (Hood et al. 2014), because I determined ingestion as the difference between estimated initial radioisotope content and final measured radioisotope content that would account for any selective removal of microbial biofilm. However, I could not estimate radioisotope ingestion with complete accuracy because of variability in the amount of ^{14}C and ^{33}P uptake across leaf disks (mean coefficients of variation in dpm disk^{-1} for each diet = 0.42 and 0.38 for ^{14}C and ^{33}P , respectively). A combination of this inter-disk variation in radioactivity and low rates of ingestion by some individuals probably explains negative ^{33}P ingestion by 3 *Lepidostoma* individuals (Fig. 5) and further justifies use of ANCOVA instead of ratios to statistically compare IEs (Raubenheimer 1992; Raubenheimer and Simpson 1994).

My data permit summary and comparison of how each of three prominent factors – detrital substrate type, background nutrient availability, and detritivore taxonomic identity – may

act through various mechanisms to control the incorporation of microbial C and P by detritivores (Fig. 6). I found limited roles of leaf litter type, probably because this factor exerts reduced control on substrate recalcitrance, microbial biomass, and nutrient content on well-conditioned detritus. In contrast, dissolved P availability strongly affects leaf litter C:P, shifting the degree of P limiting or in excess relative to detritivore requirements to affect incorporation of microbial P. Detritivore taxonomic identity finally exerts the greatest control over incorporation of microbial P and especially microbial C, possibly due to variable nutritional requirements ($TER_{C:P}$) or growth rates in relation to body C:P. My study has the advantage of using three caddisfly taxa of similar life stages from two closely related families (Kjer et al. 2002). This finding suggests differences between taxa were less likely driven by inherent phylogenetic differences such as in mouthpart or gut morphology and are more likely attributable to plastic traits such as caddisfly gut conditions, growth rates, or nutrient requirements. Because heterotrophic microbes serve as the link of detrital resources and nutrient availability to detritivore growth and production (Hall and Meyer 1998), they play critical roles in energy and nutrient flow through detritus-based food webs. Higher IEs of microbial C and P by low-C:P detritivore taxa, along with reductions in IE of microbial P under P enrichment, could explain widespread alteration of structure and function of detritus-based ecosystems subject to nutrient enrichment (Cross et al. 2006, Evans-White et al. 2009, Halvorson et al. 2015a).

ACKNOWLEDGEMENTS

The National Science Foundation (DEB 1020722; BIO REU 1063067 and 1359188) and the University of Arkansas Honors College provided funding for this research. I thank Brad Austin, Ayla Smartt, Kayla Sayre, Erin Scott, Erin Grantz, Jason Ramey, Delaney Hall, Amanda Eddy, Katharine Stewart, Andrew Sanders, Sally Entrekin, Chris Fuller, Dan Magoulick, Steve Beaupre, Duane Wolf, Ralph Henry, Julia Tchakhalian, and two anonymous reviewers for input and assistance. Halvor Halvorson was supported by a Doctoral Academy Fellowship at the University of Arkansas.

LITERATURE CITED

- American Public Health Association (2005) Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington, DC
- Arsuffi TL, Suberkropp K (1989) Selective feeding by shredders on leaf-colonizing stream fungi: comparison of macroinvertebrate taxa. *Oecologia* 79:30-37. doi: 10.1007/Bf00378236
- Back JA, King RS (2013) Sex and size matter: ontogenetic patterns of nutrient content of aquatic insects. *Freshw Sci* 32:837-848. doi: 10.1899/12-181.1
- Baldy V, Chauvet E, Charcosset JY, Gessner MO (2002) Microbial dynamics associated with leaves decomposing in the mainstem and floodplain pond of a large river. *Aquat Microb Ecol* 28:25-36. doi 10.3354/Ame028025
- Bärlocher F (1985) The role of fungi in the nutrition of stream invertebrates. *Bot J Linn Soc* 91:83-94. doi: 10.1111/j.1095-8339.1985.tb01137.x
- Bärlocher F, Porter CW (1986) Digestive enzymes and feeding strategies of three stream invertebrates. *J N Am Benthol Soc* 5:58-66. doi: 10.2307/1467747
- Canhoto C, Graça MAS (2006) Digestive tract and leaf processing capacity of the stream invertebrate *Tipula lateralis*. *Can J Zool* 84:1087-1095. doi: 10.1139/Z06-092
- Cheever BM, Kratzer EB, Webster JR (2012) Immobilization and mineralization of N and P by heterotrophic microbes during leaf decomposition. *Freshw Sci* 31:133-147. doi: 10.1899/11-060.1
- Cheever BM, Webster JR, Bilger EE, Thomas SA (2013) The relative importance of exogenous and substrate-derived nitrogen for microbial growth during leaf decomposition. *Ecology* 94:1614-1625. doi: 10.1890/12-1339.1
- Chung N, Suberkropp K (2009) Contribution of fungal biomass to the growth of the shredder, *Pycnopsyche gentilis* (Trichoptera: Limnephilidae). *Freshw Biol* 54:2212-2224. doi: 10.1111/j.1365-2427.2009.02260.x
- Cothran RD, Stoler AB, Relyea RA (2014) Leaves and litterbugs: how litter quality affects amphipod life-history and sexually selected traits. *Freshw Sci* 33:812-819. doi: 10.1086/677214
- Cross WF, Wallace JB, Rosemond AD (2007) Nutrient enrichment reduces constraints on material flows in a detritus-based food web. *Ecology* 88:2563-2575. doi: 10.1890/06-1348.1
- Cross WF, Wallace JB, Rosemond AD, Eggert SL (2006) Whole-system nutrient enrichment increases secondary production in a detritus-based ecosystem. *Ecology* 87:1556-1565. doi: 10.1890/0012-9658(2006)87[1556:WNEISP]2.0.CO;2

- Cummins KW (1973) Trophic relations of aquatic insects. *Annu Rev Entomol* 18:183-206. doi: 10.1146/annurev.en.18.010173.001151
- Danger M, Chauvet E (2013) Elemental composition and degree of homeostasis of fungi: are aquatic hyphomycetes more like metazoans, bacteria or plants? *Fungal Ecol* 6:453-457. doi: 10.1016/j.funeco.2013.05.007
- Danger M, Funck JA, Devin S, Heberle J, Felten V (2013) Phosphorus content in detritus controls life-history traits of a detritivore. *Funct Ecol* 27:807-815. doi: 10.1111/1365-2435.12079
- Dodds WK, Collins SM, Hamilton SK et al. (2014) You are not always what we think you eat: selective assimilation across multiple whole-stream isotopic tracer studies. *Ecology* 95:2757-2767. doi: 10.1890/13-2276.1
- Duhamel S, Zeman F, Moutin T (2006) A dual-labeling method for the simultaneous measurement of dissolved inorganic carbon and phosphate uptake by marine planktonic species. *Limnol Oceanogr-Meth* 4:416-425. doi: 10.4319/lom.2006.4.416
- Elser JJ, Acharya K, Kyle M et al. (2003) Growth rate-stoichiometry couplings in diverse biota. *Ecol Lett* 6:936-943. doi: 10.1046/j.1461-0248.2003.00518.x
- Enriquez S, Duarte CM, Sandjensen K (1993) Patterns in decomposition rates among photosynthetic organisms: the importance of detritus C:N:P Content. *Oecologia* 94:457-471. doi: 10.1007/Bf00566960
- Evans-White MA, Dodds WK, Huggins DG, Baker DS (2009) Thresholds in macroinvertebrate biodiversity and stoichiometry across water-quality gradients in Central Plains (USA) streams. *J N Am Benthol Soc* 28:855-868. doi: 10.1899/08-113.1
- Fanin N, Fromin N, Buatois B, Hattenschwiler S (2013) An experimental test of the hypothesis of non-homeostatic consumer stoichiometry in a plant litter-microbe system. *Ecol Lett* 16:764-772. doi: 10.1111/Ele.12108
- Ferrão-Filho AD, Tessier AJ, DeMott WR (2007) Sensitivity of herbivorous zooplankton to phosphorus-deficient diets: Testing stoichiometric theory and the growth rate hypothesis. *Limnol Oceanogr* 52:407-415. doi: 10.4319/lo.2007.52.1.0407
- Findlay S, Meyer JL, Smith PJ (1986a) Contribution of fungal biomass to the diet of a freshwater isopod (*Lirceus* sp). *Freshw Biol* 16:377-385. doi: 10.1111/j.1365-2427.1986.tb00978.x
- Findlay S, Meyer JL, Smith PJ (1986b) Incorporation of microbial biomass by *Peltoperla* sp. (Plecoptera) and *Tipula* sp. (Diptera). *J N Am Benthol Soc* 5:306-310. doi: 10.2307/1467483
- Findlay S, Tank J, Dye S et al. (2002) A cross-system comparison of bacterial and fungal biomass in detritus pools of headwater streams. *Microbial Ecol* 43:55-66. doi: 10.1007/10.1007/S00248-001-1020-X

- Frost PC, Benstead JP, Cross WF et al. (2006) Threshold elemental ratios of carbon and phosphorus in aquatic consumers. *Ecol Lett* 9:774-779. doi: 10.1111/j.1461-0248.2006.00919.x
- Frost PC, Elser JJ (2002) Growth responses of littoral mayflies to the phosphorus content of their food. *Ecol Lett* 5:232-240. doi: 10.1046/j.1461-0248.2002.00307.x
- Fuller CL, Evans-White MA, Entrekin SA (2014) Growth and stoichiometry of a common aquatic detritivore respond to changes in resource stoichiometry. *Oecologia* 177: 837-848. doi: 10.1007/s00442-014-3154-9
- Gessner MO, Chauvet E (1994) Importance of stream microfungi in controlling breakdown rates of leaf-litter. *Ecology* 75:1807-1817. doi: 10.2307/1939639
- González AL, Romero GQ, Srivastava DS (2014) Detrital nutrient content determines growth rate and elemental composition of bromeliad-dwelling insects. *Freshw Biol* 59:737-747. doi: 10.1111/fwb.12300
- Graça MAS, Cressa C, Gessner MO, Feio MJ, Callies KA, Barrios C (2001) Food quality, feeding preferences, survival and growth of shredders from temperate and tropical streams. *Freshw Biol* 46:947-957. doi: 10.1046/j.1365-2427.2001.00729.x
- Gulis V, Suberkropp K (2003) Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. *Freshw Biol* 48:123-134. doi:10.1046/j.1365-2427.2003.00985.x
- Hall RO, Meyer JL (1998) The trophic significance of bacteria in a detritus-based stream food web. *Ecology* 79:1995-2012. doi: 10.1890/0012-9658(1998)079[1995:Ttsobi]2.0.Co;2
- Halvorson HM, Fuller C, Entrekin SA, Evans-White MA (2015a) Dietary influences on production, stoichiometry and decomposition of particulate wastes from shredders. *Freshw Biol* 60: 466-478. doi: 10.1111/fwb.12462
- Halvorson HM, Scott JT, Sanders AJ, Evans-White MA (2015b) A stream insect detritivore violates common assumptions of threshold elemental ratio bioenergetics models. *Freshw Sci* 34: 508-518. doi: 10.1086/680724
- Hieber M, Gessner MO (2002) Contribution of stream detritivores, fungi, and bacteria to leaf breakdown based on biomass estimates. *Ecology* 83:1026-1038. doi: 10.2307/3071911
- Hood JM, McNeely C, Finlay JC, Sterner RW (2014) Selective feeding determines patterns of nutrient release by stream invertebrates. *Freshw Sci* 33:1093-1107. doi: 10.1086/678693
- Hood JM, Sterner RW (2014) Carbon and phosphorus linkages in *Daphnia* growth are determined by growth rate, not species or diet. *Funct Ecol* 28:1156-1165. doi: 10.1111/1365-2435.12243
- Hutchens JJ, Benfield EF, Webster JR (1997) Diet and growth of a leaf-shredding caddisfly in southern Appalachian streams of contrasting disturbance history. *Hydrobiologia* 346:193-201. doi: 10.1023/A:1002930419317

- Kaushik NK, Hynes HBN (1971) Fate of dead leaves that fall into streams. *Arch Hydrobiol* 68:465-515.
- Kjer KM, Blahnik RJ, Holzenthal RW (2002) Phylogeny of caddisflies (Insecta, Trichoptera). *Zool Scr* 31:83-91. doi: 10.1046/j.0300-3256.2001.00079.x
- Mann KH (1988) Production and use of detritus in various freshwater, estuarine, and coastal marine ecosystems. *Limnol Oceanogr* 33:910-930. doi: 10.4319/lo.1988.33.4part2.0910
- Martin MM, Kukor JJ, Martin JS, Otoole TE, Johnson MW (1981) Digestive enzymes of fungus-feeding beetles. *Physiol Zool* 54:137-145
- Martin MM, Martin JS, Kukor JJ, Merritt RW (1980) The digestion of protein and carbohydrate by the stream detritivore, *Tipula abdominalis* (Diptera, Tipulidae). *Oecologia* 46:360-364. doi: 10.1007/BF00346265
- Moore JC, Berlow EL, Coleman DC et al. (2004) Detritus, trophic dynamics and biodiversity. *Ecol Lett* 7:584-600. doi: 10.1111/j.1461-0248.2004.00606.x
- Pastor A, Compson ZG, Dijkstra P et al. (2014) Stream carbon and nitrogen supplements during leaf litter decomposition: contrasting patterns for two foundation species. *Oecologia* 176:1111-1121. doi: 10.1007/s00442-014-3063-y
- Pollierer MM, Dyckmans J, Scheu S, Haubert D (2012) Carbon flux through fungi and bacteria into the forest soil animal food web as indicated by compound-specific ¹³C fatty acid analysis. *Funct Ecol* 26:978-990. doi: 10.1111/j.1365-2435.2012.02005.x
- Raubenheimer D, Simpson SJ (1992) Analysis of covariance - an alternative to nutritional indices. *Entomol Exp Appl* 62:221-231. doi: 10.1111/j.1570-7458.1992.tb00662.x
- Raubenheimer D, Simpson SJ (1994) The analysis of nutrient budgets. *Funct Ecol* 8:783-791. doi: 10.2307/2390238
- Scott EE, Prater C, Norman E, Baker BC, Evans-White M, Scott JT (2013) Leaf-litter stoichiometry is affected by streamwater phosphorus concentrations and litter type. *Freshw Sci* 32:753-761. doi: 10.1899/12-215.1
- Sinsabaugh RL, Linkins AE, Benfield EF (1985) Cellulose digestion and assimilation by 3 leaf-shredding aquatic insects. *Ecology* 66:1464-1471. doi: 10.2307/1938009
- Sterner RW, Elser JJ (2002) *Ecological Stoichiometry*. Princeton University Press, Princeton
- Tant CJ, Rosemond AD, First MR (2013) Stream nutrient enrichment has a greater effect on coarse than on fine benthic organic matter. *Freshw Sci* 32:1111-1121. doi: 10.1899/12-049.1
- Van Frankenhuyzen K, Geen GH, Koivisto C (1985) Direct and indirect effects of low pH on the transformation of detrital energy by the shredding caddisfly, *Clistoronia magnifica* (Banks) (Limnephilidae). *Can J Zool* 63:2298-2304. doi: 10.1139/z85-341

Ward GM, Cummins KW (1979) Effects of food quality on growth of a stream detritivore, *Paratendipes albimanus* (Meigen) (Diptera, Chironomidae). *Ecology* 60:57-64. doi: 10.2307/1936468

Wolf DC, Legg JO, Boutton TW (1994) Isotopic methods for the study of soil organic matter dynamics. In: Weaver RW, Scott A, Bottomley P et al. (eds) *Methods of soil analysis. Part 2. Microbiological and biochemical properties*. The Soil Science Society of America, Madison, pp 865-906

Table 1. Analysis of variance for stoichiometry of leaf litter diets. Response variables include percent carbon (%C), nitrogen (%N), and phosphorus (%P) and molar ratios of carbon:nitrogen (C:N), carbon:phosphorus (C:P), and nitrogen:phosphorus (N:P).

Variable ^a	Predictor ^b	df	F-value	P-value ^c	Variable ^a	Predictor ^b	df	F-value	P-value ^c
% C	Litter	1	22.40	<0.001	C:N	Litter	1	0.57	0.453
	Nutrient	1	4.99	0.032		Nutrient	1	12.73	0.001
	Interaction	1	0.08	0.779		Interaction	1	0.10	0.755
% N	Litter	1	0.03	0.864	C:P	Litter	1	1.38	0.248
	Nutrient	1	12.31	0.001		Nutrient	1	45.15	<0.001
	Interaction	1	0.29	0.594		Interaction	1	0.31	0.581
% P	Litter	1	2.27	0.141	N:P	Litter	1	1.47	0.233
	Nutrient	1	50.07	<0.001		Nutrient	1	22.36	<0.001
	Interaction	1	0.01	0.940		Interaction	1	0.44	0.511

^a %C, %N, C:P, and N:P were log-transformed; %P was square-root transformed.

^b Litter = maple or oak; Nutrient = leaf litter incubation P concentration.

^c Values in bold indicate significant Bonferonni corrected P-values (significant $\alpha = 0.008$).

Table 2. Mean (\pm SE) dry mass (DM; mg) and percent elemental content and molar ratios of carbon (C), nitrogen (N), and phosphorus (P) of three caddisfly genera used in acclimation and radiolabel experiments. Genera are arranged from top to bottom in order of increasing body P content.

Genus	DM (mg)	% C	% N	% P	C:N	C:P	N:P
<i>Lepidostoma</i>	2.99 (0.21)	48.3 (0.3)	6.96 (0.14)	0.69 (0.05)	8.1 (0.2)	247 (59)	31 (8)
<i>Isonychia</i>	23.8 (3.0)	48.4 (0.6)	7.84 (0.21)	0.90 (0.10)	7.3 (0.2)	161 (17)	22 (2)
<i>Pycnopsyche</i>	19.1 (2.0)	45.2 (0.3)	9.17 (0.16)	1.59 (0.11)	5.8 (0.1)	81 (6)	14 (1)

Table 3. Analysis of covariance for caddisfly radioisotope incorporation efficiencies. Effects of each main factor on ¹⁴C or ³³P incorporated were examined in a separate ANCOVA model using ingested ¹⁴C or ³³P as covariates. Slopes were homogeneous in all preliminary models (factor*covariate interaction $P > 0.05$). Note that incorporated ¹⁴C and ³³P dpm (response variables) were natural-log transformed to achieve homoscedasticity. Global model coefficients and effect sizes for levels within each main factor are described for each model on the right-hand side of the table.

Response	Predictor ^a	F-value	P-value ^b	Model Term ^c	Model Coefficient	Coefficient Std Error	Level ^a	Effect ^d
Incorp ¹⁴ C	Ingest ¹⁴ C	0.14	0.710	Intercept	8.34	0.28	Maple	0.175
	Litter type	2.65	0.110	Slope	1.78 x 10 ⁻⁶	4.69 x 10 ⁻⁶	Oak	-0.173
Incorp ¹⁴ C	Ingest ¹⁴ C	0.27	0.604	Intercept	8.30	0.27	50	0.207
	P level	4.00	0.051	Slope	2.32 x 10 ⁻⁶	4.44 x 10 ⁻⁶	500	-0.198
Incorp ¹⁴ C	Ingest ¹⁴ C Genus	3.48	0.068	Intercept	8.06	0.20	Lep	-0.734
		25.54	<0.001	Slope	6.04 x 10 ⁻⁶	3.24 x 10 ⁻⁶	Iro	0.307
							Pyc	0.435
Incorp ³³ P	Ingest ³³ P	5.43	0.024	Intercept	9.37	0.19	Maple	0.096
	Litter type	1.02	0.317	Slope	4.94 x 10 ⁻⁶	2.12 x 10 ⁻⁶	Oak	-0.098
Incorp ³³ P	Ingest ³³ P	5.75	0.020	Intercept	9.39	0.17	50	0.252
	P level	8.25	0.006	Slope	4.62 x 10 ⁻⁶	1.93 x 10 ⁻⁶	500	-0.250
Incorp ³³ P	Ingest ³³ P Genus	3.77	0.058	Intercept	9.46	0.16	Lep	-0.546
		10.75	<0.001	Slope	3.47 x 10 ⁻⁶	1.79 x 10 ⁻⁶	Iro	0.236
							Pyc	0.305

^a Ingest = total ingested ³³P or ¹⁴C; Litter type = oak or maple; P level= 50 or 500 µg L⁻¹ incubation phosphorus concentration; Genus = *Ironoquia* (Iro), *Lepidostoma* (Lep), or *Pycnopsyche* (Pyc).

^b Values in bold indicate significant Bonferonni corrected P-values ($\alpha = 0.008$).

^c Global ANCOVA model fits for log-normal transformed incorporated ¹⁴C and ³³P.

^d Describes mean observed differences (intercepts) of each factor level from global ANCOVA intercepts

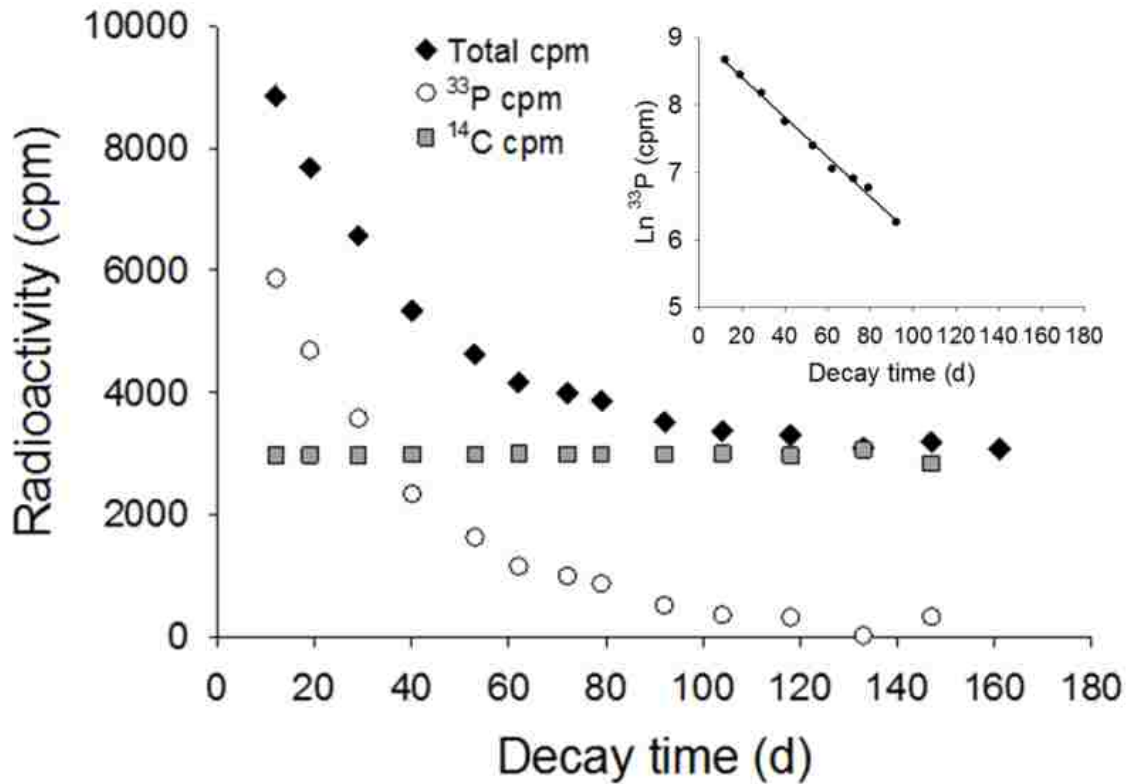


Figure 1. Radioactive decay as indicated by the decline in total and ³³P counts per minute (cpm) in the body of a *Pycnopsyche* individual. In the inset graph, the linear regression of natural log-transformed ³³P cpm was used to extrapolate to ³³P cpm at time zero (time of freezing). Where Ln (³³P cpm) < 6, data became nonlinear and were excluded from linear regression. Data were corrected for quench to estimate disintegrations per minute (dpm). See Duhamel et al. (2006) for further details.

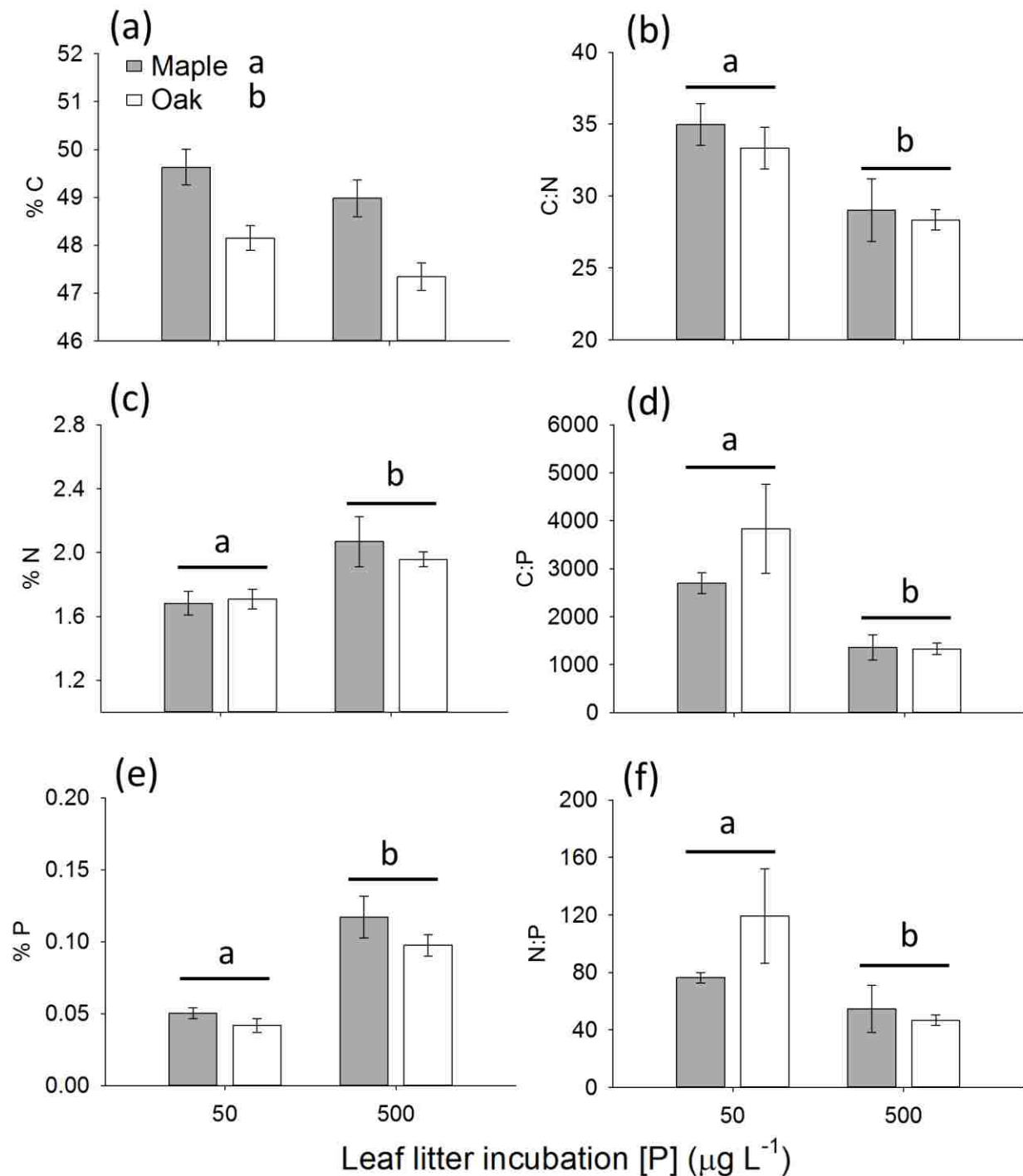


Figure 2. Maple and oak litter diet stoichiometry after 77 days' incubation at 50 or 500 $\mu\text{g L}^{-1}$ phosphorus (P) concentrations prior to feeding ($n=40$). Mean percent carbon (C), nitrogen (N), and P are expressed as percent of dry mass in (a), (c), and (e) respectively. Mean molar ratios are of C:N, C:P, and N:P in (b), (d), and (f) respectively. Letters represent statistically different groups (Bonferroni corrected $P < 0.008$). Error bars represent ± 1 SE. For associated statistics, see Table 1.

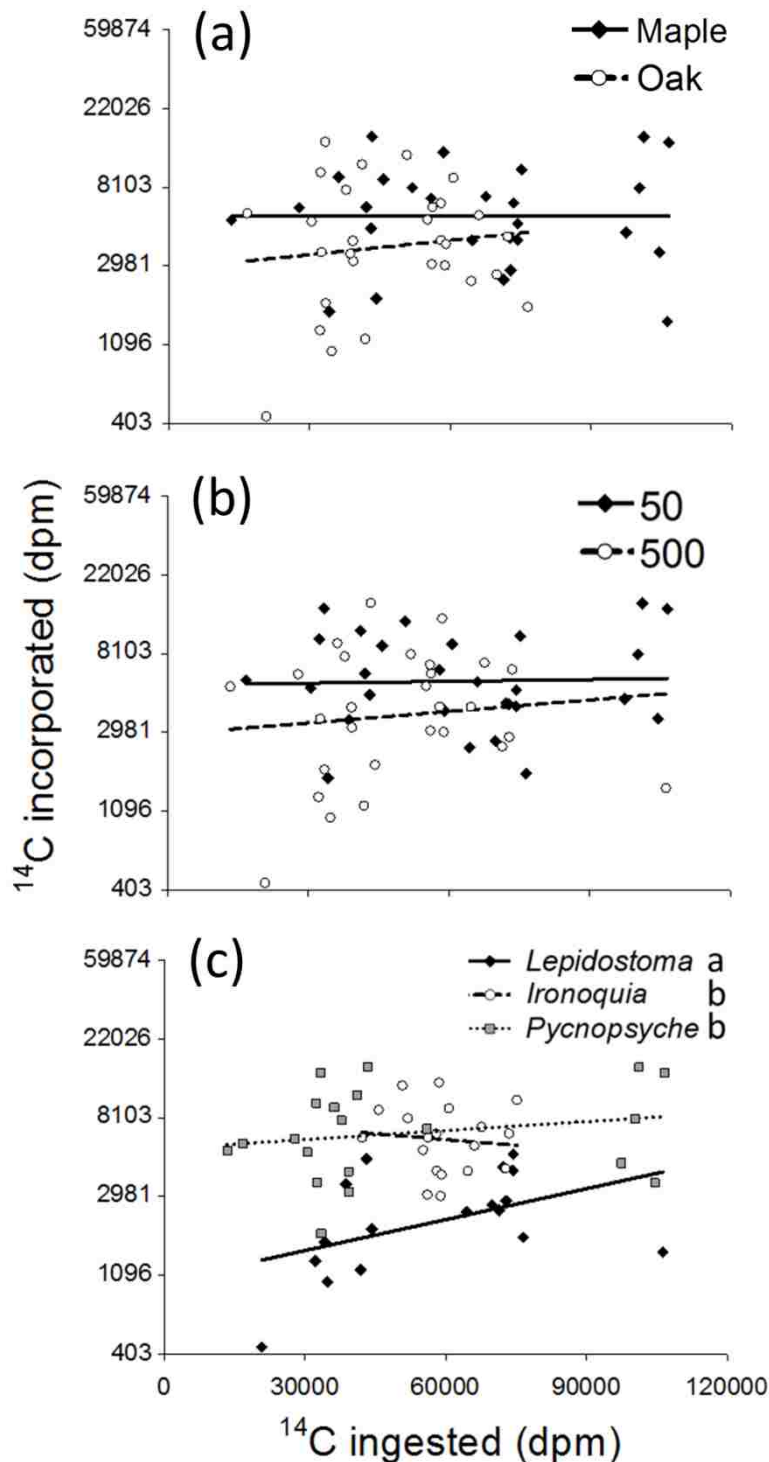


Figure 3. Total ^{14}C disintegrations per minute (dpm) of incorporated and ingested material from all experiments ($n=56$). The data are plotted according to one of three different ANCOVA models testing differences across (a) litter types, (b) litter phosphorus incubation concentrations ($\mu\text{g L}^{-1}$), or (c) caddisfly genera. Letters in the legend indicate groups significantly different from one another (Tukey's HSD, $P<0.05$). Note the logarithmic scale of the y-axes. For associated statistics, see Table 3.

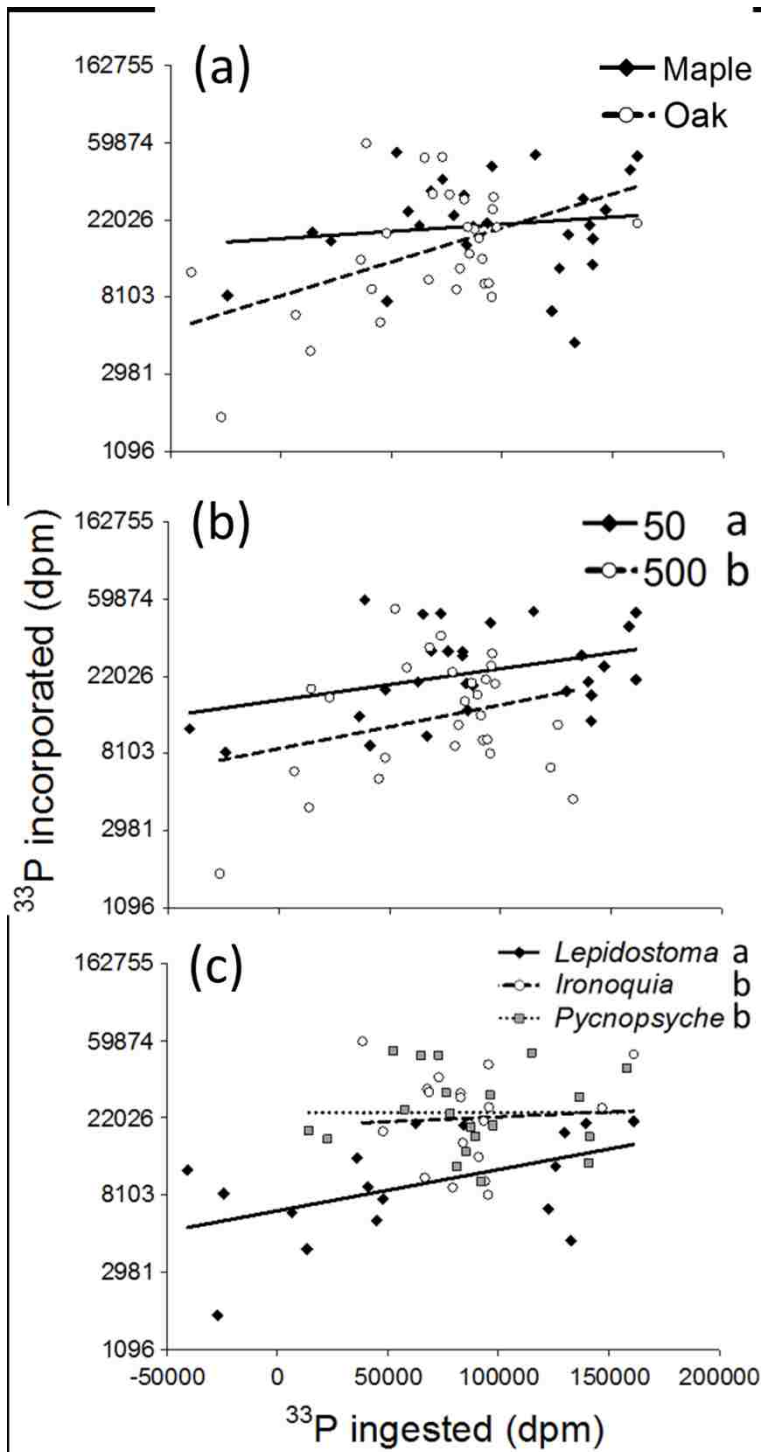


Figure 4. Total ^{33}P disintegrations per minute (dpm) of incorporated and ingested material from all experiments ($n=56$). The data are plotted according to one of three different ANCOVA models testing differences across (a) litter types, (b) litter phosphorus incubation concentrations ($\mu\text{g L}^{-1}$), or (c) caddisfly genera. Letters in the legend indicate groups significantly different from one another (ANCOVA, $P<0.008$ or Tukey's HSD, $P<0.05$). Note the logarithmic scale of the y-axis. For associated statistics, see Table 3.

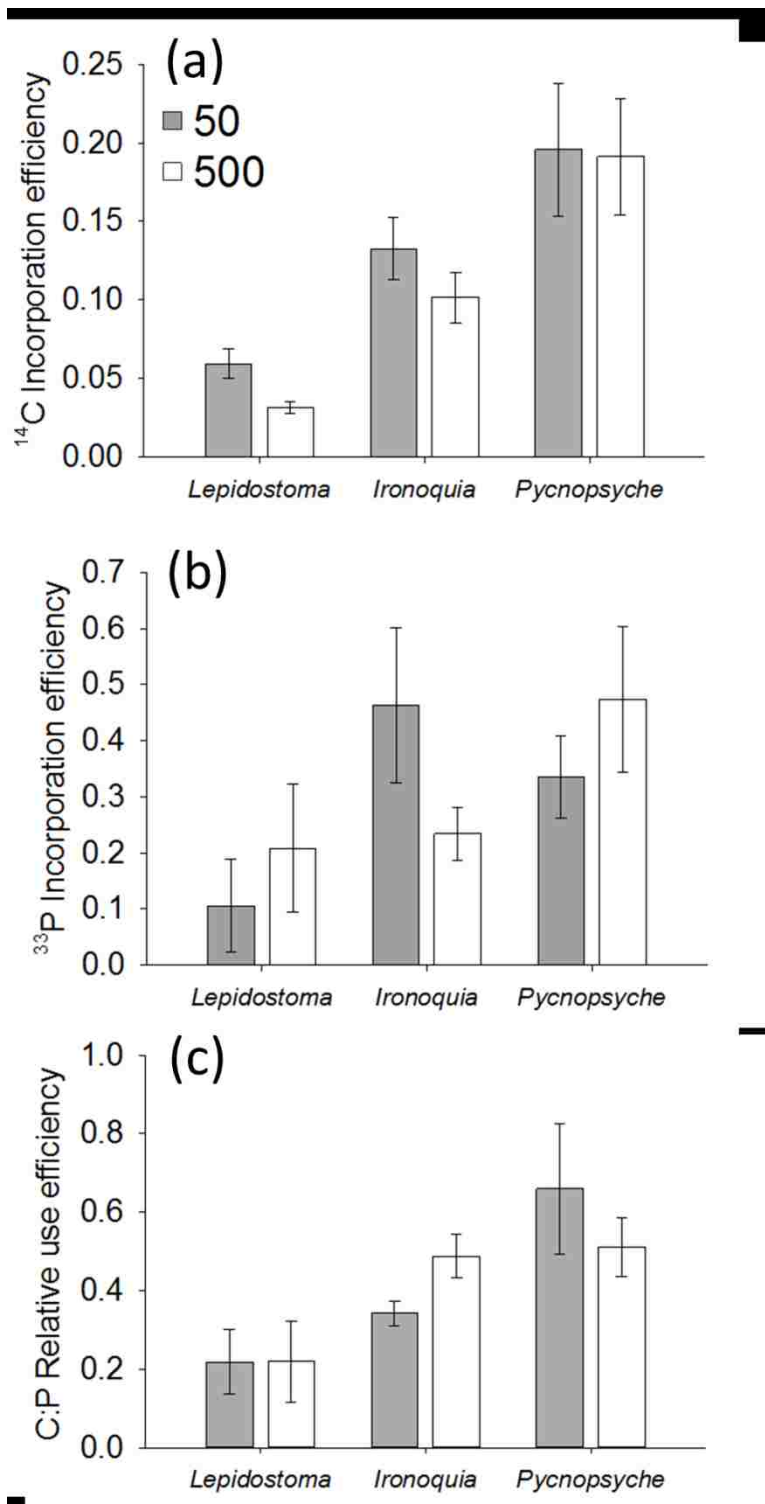


Figure 5. Mean \pm SE ratio-based incorporation efficiencies of microbial carbon (a) and phosphorus (b) and relative use efficiencies (c) of detritivorous caddisflies fed leaf litter incubated under concentrations of 50 or 500 $\mu\text{g L}^{-1}$ P. Data were pooled across leaf species because there were no statistical differences in incorporation efficiencies between oak and maple litter (Table 3), however ratio indices were not compared statistically because ratios are prone to error by imposing isometry on allometric data. See Supplemental 4 for a table summarizing data.

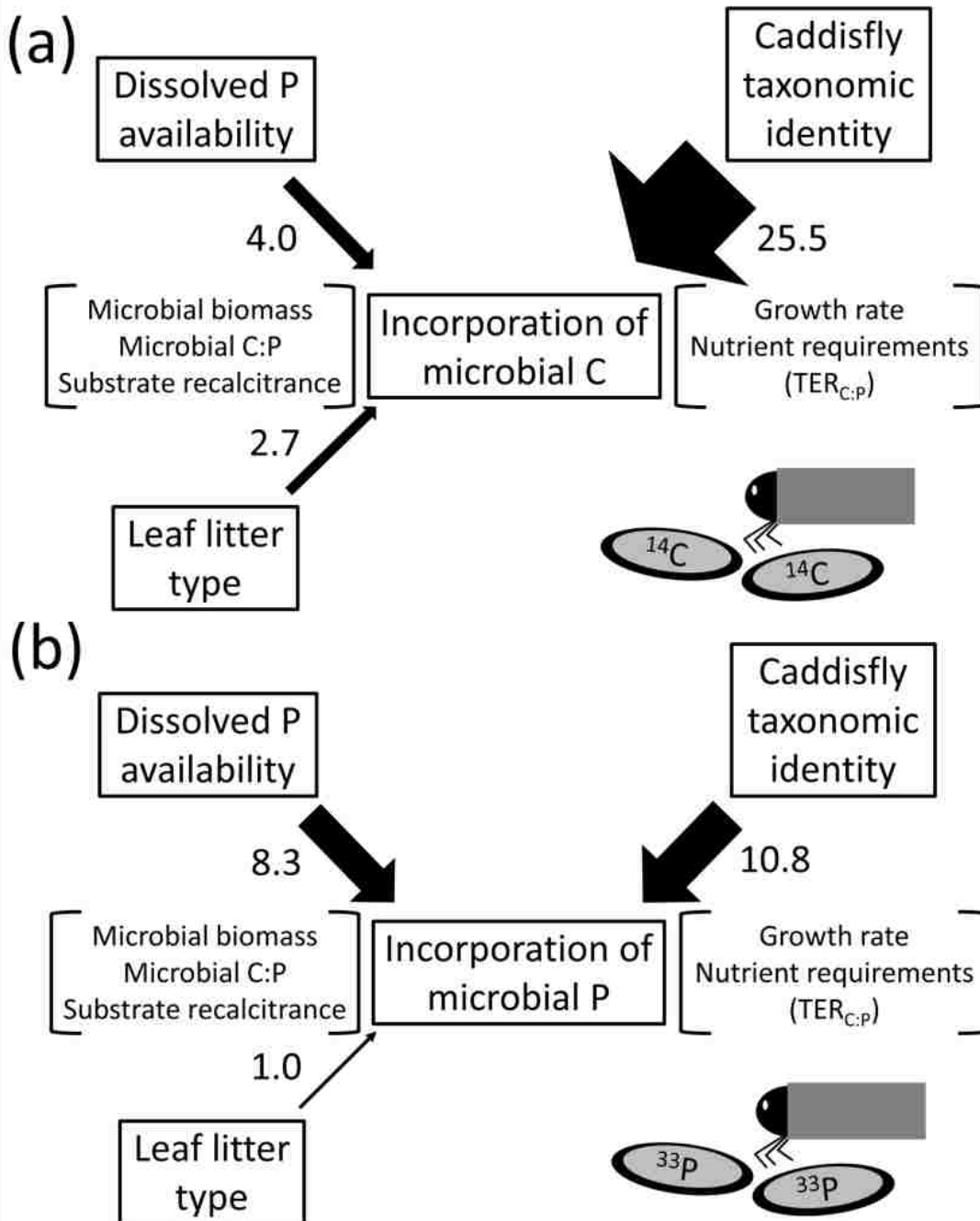


Figure 6. Conceptual diagram summarizing the relative effects of dissolved phosphorus (P) availability, leaf litter type, and caddisfly taxonomic identity on incorporation of detrital microbial (a) carbon (C) and (b) phosphorus. Arrows designate the effect (as indicated by F-values; Table 3) of each factor on total incorporation after removing effects of ingestion using ANCOVA, with arrow thickness proportional to the magnitude of effects. Brackets designate mechanisms of dietary or taxonomic factors that drive variable incorporation of microbial C and P.

APPENDICES

Table S1. Mean (± 1 SE) elemental content (percent (%) carbon (C), nitrogen (N), and phosphorus (P) as well as molar ratios of C:N, C:P, and N:P) of maple and oak litter after 3 d of leaching but prior to microbial conditioning, as an indicator of initial detrital substrate stoichiometry.

Leaf	% C	% N	% P	C:N	C:P	N:P
Maple	46.7 (0.1)	0.69 (0.12)	0.031 (0.002)	79.2 (5.9)	3950 (340)	49.9 (3.3)
Oak	48.9 (0.2)	1.05 (0.03)	0.031 (0.002)	55.7 (3.5)	4170 (270)	75.4 (3.2)

Table S2. Mean (± 1 SE) elemental content (percent (%)) carbon (C), nitrogen (N), and phosphorus (P) as well as molar ratios of C:N, C:P, and N:P of maple and oak litter conditioned for 77 d under 50 or 500 $\mu\text{g P L}^{-1}$ prior to feeding to caddisflies.

Leaf	[P]	% C	% N	% P	C:N	C:P	N:P
Maple	50	49.6 (0.4)	1.68 (0.07)	0.050 (0.004)	35.0 (1.5)	2700 (210)	76.4 (3.7)
	500	49.0 (0.4)	2.07 (0.16)	0.117 (0.015)	29.0 (2.2)	1360 (260)	54.6 (16.5)
Oak	50	48.2 (0.3)	1.71 (0.06)	0.042 (0.005)	33.3 (1.4)	3830 (930)	119 (33)
	500	47.3 (0.3)	1.96 (0.05)	0.098 (0.007)	28.3 (0.7)	1330 (120)	46.8 (3.7)

Table S3. Mean (\pm 1 SE) incorporation efficiencies of microbial carbon (IE_C) and microbial phosphorus (IE_P) and relative use efficiencies of microbial C and P (C:P RUE) by caddisflies using ^{14}C and ^{33}P as radiotracers. Caddisfly genera are arranged from top to bottom by order of increasing body P content.

Genus	[P] ^a	IE_C	IE_P	C:P RUE
<i>Lepidostoma</i>	50	0.059 (0.009)	0.11 (0.08)	0.22 (0.08)
	500	0.031 (0.004)	0.21 (0.11)	0.22 (0.10)
<i>Ironoquia</i>	50	0.133 (0.020)	0.46 (0.14)	0.34 (0.03)
	500	0.102 (0.016)	0.23 (0.05)	0.49 (0.06)
<i>Pycnopsyche</i>	50	0.196 (0.042)	0.34 (0.07)	0.66 (0.17)
	500	0.191 (0.037)	0.47 (0.13)	0.51 (0.07)

^a Leaf litter incubation phosphorus concentration ($\mu g L^{-1}$)

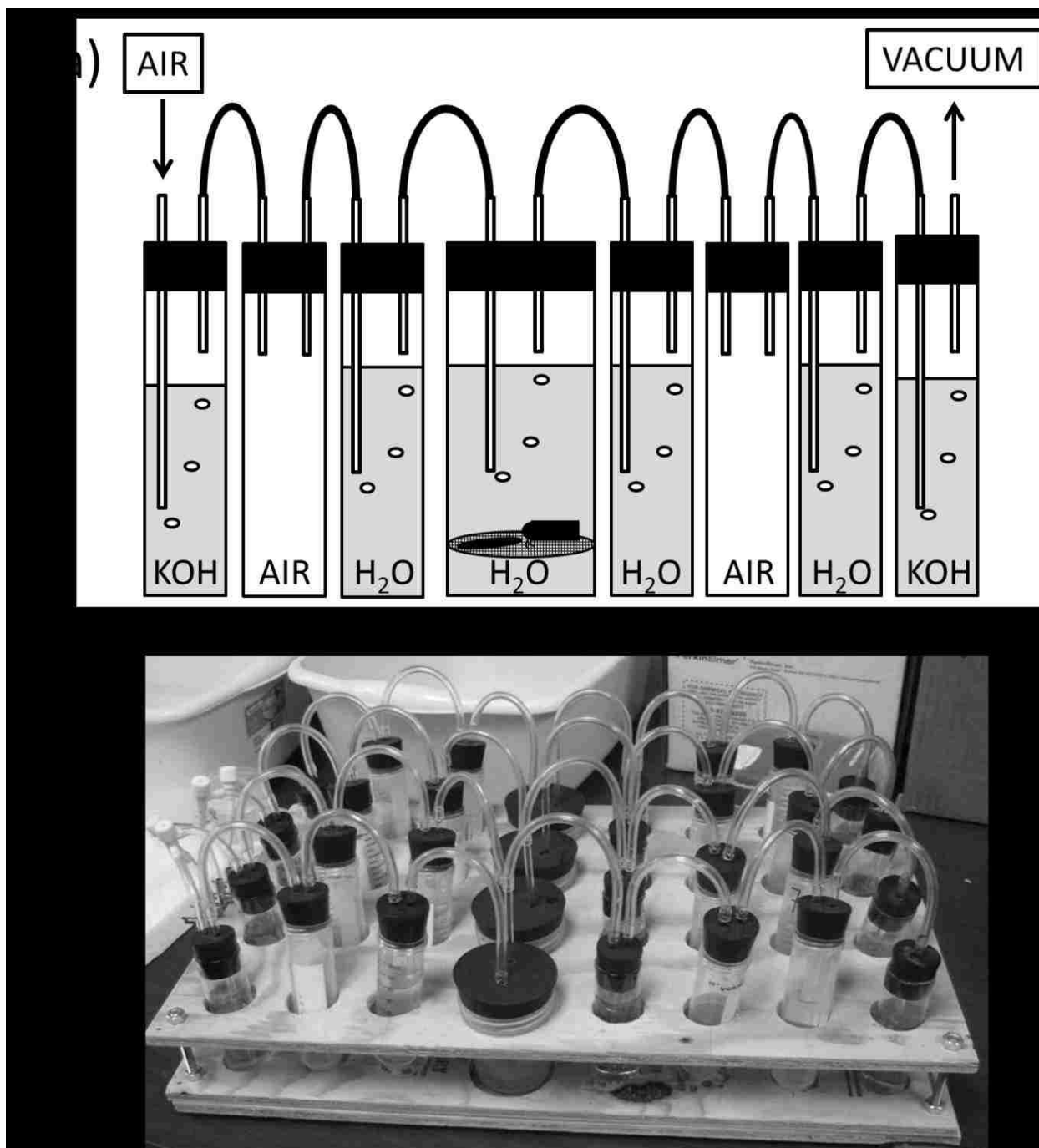


Figure S1. (a) Design of radiation chambers used in caddisfly radiolabel experiments. Potassium hydroxide (KOH) served as a trap for CO_2 entering and leaving the system to prevent release of $^{14}\text{C-CO}_2$, whereas air and water (H_2O) chambers served as safety traps and buffer chambers respectively to prevent transmittance of KOH to the insect chamber (fourth from left). Each series of tubes was replicated to permit radiolabeling of 20 individuals from each genus. (b) Final constructed version of chambers (1 rack containing tubes for 4 total labeling chambers) used in experiments.

Chapter III

Dietary influences on production, stoichiometry and decomposition of particulate wastes from shredders³

³ Halvorson H.M., Fuller C., Entekin S.A. & Evans-White M.A. (2015) Dietary influences on production, stoichiometry and decomposition of particulate wastes from shredders. *Freshwater Biology* **60**, 466-478.

ABSTRACT

Aquatic shredders produce large quantities of fine particulate organic matter (FPOM) as fragments and egesta, but the significance of shredder FPOM in carbon (C), nitrogen (N), and phosphorus (P) spiraling in streams remains understudied and could represent an important form of consumer-mediated nutrient cycling. I fed the stream shredders *Pycnopsyche lepida*, *Lepidostoma* sp. and *Tipula abdominalis* oak or maple litter conditioned under contrasting phosphorus concentrations to produce gradients in dietary carbon:phosphorus (C:P) and carbon:nitrogen (C:N) content (range=850-4480 and 30-49 by moles, respectively). I measured total FPOM production and stoichiometry to estimate particulate N and P release rates, compared resultant rates to those of P excretion and measured microbial decomposition of FPOM. FPOM production was greater for *Tipula* and *Pycnopsyche* compared to *Lepidostoma*; FPOM production by *Tipula* increased on higher-nutrient diets. The C:P, C:N and N:P of FPOM from *Pycnopsyche* and *Tipula* often diverged from diet stoichiometry depending on litter type, and rates of particulate N and P release by shredders were greater with increasing nutrient content of the diet. Shredders fed high-nutrient diets produced FPOM with greater microbial decomposition rates, although these trends differed between litter types. These findings indicate bottom-up changes in litter type and nutrient content may modify production, stoichiometry and decomposition of FPOM from shredders and shredder-mediated nutrient transformations may differ across shredder species.

INTRODUCTION

Ecological stoichiometry theory predicts that consumers will produce nutrient wastes such as nitrogen (N) and phosphorus (P) in a diet-dependent manner to maintain consistent body elemental content or homeostasis (Elser & Urabe, 1999; Sterner & Elser, 2002). This theory of diet-dependent consumer nutrient recycling (CNR) is well-tested among herbivorous zooplankton (DeMott, Gulati & Siewertsen, 1998; Elser & Urabe, 1999), although traits such as feeding mode, body size and body stoichiometry also contribute to variation in CNR among diverse consumer species (Vanni *et al.*, 2002; McManamay *et al.*, 2011). Most studies of inter- and intra-specific variation in CNR have focused on dissolved wastes produced via excretion, under the central premises that dissolved wastes are (1) ecologically important because they complete a feedback between consumers and their resources (Elser & Urabe, 1999), and (2) the dominant means for consumers to produce nutrient wastes (Zanotto, Simpson & Raubenheimer, 1993; DeMott *et al.*, 1998).

Although consumers can significantly control freshwater nutrient dynamics through recycling of dissolved wastes (Evans-White & Lamberti, 2006; McIntyre *et al.*, 2008; Atkinson *et al.*, 2013), consumers may also modify nutrient pools and fluxes by producing particulate wastes such as egesta (Fig. 1; Strayer 2014). For example, extended exposure to snail fecal pellets in the laboratory caused periphyton to display lower N:P content than periphyton exposed to either ambient conditions or snail excreta (Liess & Haglund, 2007). The authors postulated that these differential effects were driven by high P content of egesta, although this mechanism was not tested and surprisingly few studies have examined the stoichiometry of consumer egesta (but see Balseiro & Albariño, 2006 and Villanueva, Albariño & Canhoto, 2011). Although the stoichiometry of particulate wastes may be distinct from that of dissolved wastes, the two forms

of waste may also differ in their ecological significance. Indeed, particulate wastes represent a matrix of carbon (C) and nutrients subject to fates different than excreta, including passive leaching into the water column, mineralization by microbes, transportation or deposition downstream or ingestion by other animals such as collectors (Fig. 1; Wotton & Malmqvist, 2001). Given the diverse fates of particulate wastes, the effects of consumer wastes on ecosystem nutrient dynamics may occur beyond the direct consumer-resource feedbacks via excretion that are considered in many studies of CNR.

Egestion represents the release of material ingested but not assimilated by a consumer. This form of waste should be especially important among consumers that have low assimilation efficiencies, such as shredders (Grafius & Anderson, 1979; Wotton & Malmqvist, 2001). In streams, shredders fulfill a key functional role of converting coarse particulate organic matter (CPOM) such as terrestrially derived leaf litter into egesta as well as fragments of fine particulate organic matter (FPOM), thereby facilitating the mineralization and downstream transport of allochthonous C (Cuffney, Wallace & Lugthart, 1990). Additionally, shredders may excrete nutrients at lower rates than taxa using other feeding modes (McManamay *et al.*, 2011) possibly because of shredders' low assimilation efficiencies or the low nutrient content of detritus (Cross *et al.*, 2003). Thus, shredder waste production as particulates may be as large as or larger than nutrient release as excreta, which implies that the focus of CNR theory on dissolved wastes is in need of expansion.

Shredders may alter elemental pools and fluxes of POM during the conversion of CPOM to FPOM through diet-dependent changes in consumption and assimilation. At ingestion, litter type may influence FPOM production because shredders often selectively ingest conditioned over unconditioned and labile over recalcitrant detritus to reduce their intake of toxic secondary

plant metabolites (Kaushik & Hynes, 1971; Graça *et al.*, 2001). Shredders may also ingest litter of higher biofilm nutrient content at faster rates (Kendrick & Benstead, 2013). These trends suggest that FPOM production by shredders may slow on recalcitrant, nutrient-poor diets. Because shredders face significant consumer-resource imbalances (Cross *et al.*, 2003), shredders likely also employ selective N and P assimilation, such as by altering gut conditions to enhance protein digestion (Martin *et al.*, 1980), that would reduce the N and P content of egesta relative to that of food. However, some leaf diets may include forms of N that are too recalcitrant for assimilation, resulting in lower shredder particulate waste C:N than expected based on preferential assimilation of limiting dietary N (Balseiro & Albariño, 2006). Previous studies thus indicate potentially complex interactive effects of litter type and nutrient content on transformation of CPOM to FPOM by shredders.

The degree to which shredders modify FPOM stoichiometry from CPOM stoichiometry may also vary across shredder species depending on traits such as nutritional requirements or feeding behaviour. For example, stoichiometric theory would predict low C:P shredders to produce P-poor wastes compared to high C:P shredders when given diets of similar C:P content, much as has been predicted and observed for excretion by herbivorous zooplankton (Elser & Urabe, 1999). Threshold elemental ratios (TERs) also suggest that high C:P shredders with high $TER_{C:P}$ should release more P due to alleviation of P-limitation on higher C:P diets compared to low C:P shredders with low $TER_{C:P}$ (Frost *et al.*, 2006). Additionally, it is likely that shredder taxa differ in their selective consumption of detrital biofilm versus leaf components (Arsuffi & Suberkropp, 1989). Because shredders rely strongly on nutrient-rich biofilm for growth (Chung & Suberkropp 2009), they may produce wastes richer in nutrients than expected from bulk CPOM nutrient content. Conversely, shredder FPOM consists of both fragments and egesta

(Cummins & Klug 1979), and because fragments should resemble CPOM, shredder FPOM may more closely reflect diet stoichiometry and be less influenced by taxon-specific nutritional requirements than egesta or excreta that are expelled after physiological processing.

My objective was to investigate how dietary and taxonomic variation control waste production and thus potential CNR effects of shredders in streams. I hypothesized that shredders would increase FPOM production on high-nutrient diets, due to increased consumption rates, as well as on labile maple compared to recalcitrant oak diets. I also tested the hypothesis that shredders would produce FPOM with lower C:P, C:N and N:P as a result of lower diet C:P, C:N and N:P. In addition to this, I expected that preferential assimilation of limiting N and P especially on high C:N and C:P diets would lower N and P content of shredder egesta, causing C:N and C:P of FPOM to be greater than diet C:N and C:P. I also expected that, given greater nutrient demands, shredders having low C:N and C:P would produce FPOM poor in N and P compared to shredders having high C:N and C:P. I predicted that increased dietary N and P content would result in greater overall rates of N and P waste production as FPOM, as well as greater P excretion. Because detritivores produce large quantities of FPOM, I expected that P release rates via excretion would be eclipsed by P release rates via FPOM production. Finally, I expected that FPOM from shredders fed higher-nutrient diets would decompose more quickly.

METHODS

Leaf litter incubations

I produced a resource-quality gradient in the laboratory by incubating sugar maple (*Acer saccharum*) and post oak (*Quercus stellata*) litter under contrasting nutrient conditions. Recently senesced leaves were collected from the same site and season in Washington County, AR (U.S.A.) for each experiment, air-dried in a greenhouse and stored in bags. For the *Pycnopsyche*

and *Tipula* experiments, leaves were cut into 13.5 mm diameter disks, added to polypropylene mesh bags, leached in tap water for 3 d, transferred to one of four incubation chambers every 2-3 d and incubated for 75-88 d (late stage litter; see Table S1) prior to feeding. Protocols were similar for *Lepidostoma*, with the exception that I used whole leaves added in mesh bags weekly and incubated for 62 d prior to feeding (early stage litter; Table S1). Incubation chambers contained 20 L dechlorinated tap water, and every 2-3 d water was changed and amended with Na_2HPO_4 to achieve concentrations of <5 (ambient tap water), 50, 100 or 500 $\mu\text{g L}^{-1}$ P; all chambers received 1 mg L^{-1} N- NO_3 as KNO_3 . The incubation chambers were initially inoculated with subsamples of leaf litter slurry from Mullins Creek in Fayetteville, AR.

Growth experiments

Larvae of the detritivorous stream insects *Pycnopsyche lepida* (Trichoptera: Limnephilidae), *Lepidostoma* sp. (Trichoptera: Lepidostomatidae) and *Tipula abdominalis* (Diptera: Tipulidae) were collected from headwater streams in the Ozark Highlands and Boston Mountains ecoregions of Arkansas in the winter of 2012-2013. For each experiment, larvae were collected within the same stream reach and returned to an environmental chamber (12 L: 12 D light cycle; 10°C for *Pycnopsyche* and *Lepidostoma*, 15°C for *Tipula*). I estimated initial larval dry mass using head capsule width regression or blotted-dry mass regression (*Pycnopsyche* and *Tipula*, respectively). Because *Lepidostoma* were of similar size and head capsule width, I subsampled the collected population to estimate initial masses. Individual larvae were randomly distributed among continuously aerated chambers containing 100 mL of stream water assigned to one of six (*Tipula*, n=90) or eight (*Pycnopsyche*, n=80; *Lepidostoma*, n=40) diet treatments. Nutrient concentrations in stream water of the growth experiments for *Lepidostoma*, *Tipula* and *Pycnopsyche* respectively were <5, <5 and 9.3 $\mu\text{g L}^{-1}$ soluble reactive P (SRP) and 8.3, 51.9 and

10.1 $\mu\text{g L}^{-1}$ N-NH₄. I did not directly measure N-NO₃ of the stream water during the growth experiments; however, concentrations from the same streams on a later date were 5.3, 250 and 2900 $\mu\text{g L}^{-1}$ N-NO₃ for *Lepidostoma*, *Tipula* and *Pycnopsyche* respectively. I assume that the potentially high N-NO₃ concentrations in the *Pycnopsyche* experiment minimally affected nutrient content of FPOM over production trials; different nutrient concentrations across experiments also bolster the decision not to statistically compare taxa (see discussion for further details).

Chambers were given 1 mm mesh inserts to prevent coprophagy and collect FPOM for measurement of FPOM production rates. Old leaves were removed and fresh leaves fed to larvae *ad libitum* every 2-3 d (*Pycnopsyche*, *Tipula*) or weekly (*Lepidostoma*); water was changed at least every 5 d. Subsets of approximately 10 disks or 2-3 whole leaves from each treatment were sampled on each feeding date and oven dried before elemental analysis. Feeding experiments lasted 33, 28 and 27 d for *Pycnopsyche*, *Tipula* and *Lepidostoma* respectively. At the end of each experiment, individuals undertook 24 h gut clearance and were frozen. Insects were then thawed, oven dried at 48°C for 24 h, desiccated for 30 m and weighed to the nearest μg on a microbalance (Mettler Toledo, Columbus, OH).

Shredder FPOM production and P excretion

Trials to measure FPOM production were conducted in larval growth chambers over the final 2-3 d of each experiment. At the end of each trial, I removed insects and leaf litter; for *Pycnopsyche* and *Lepidostoma*, all particles were filtered onto muffled and pre-weighed 25 mm 1 μm pore glass-fiber filters (GFFs; Pall Inc., Port Washington, NY) whereas for *Tipula*, FPOM was filtered on pre-weighed GFFs and subsamples were collected for stoichiometry on muffled and pre-weighed tins. Filters and tins were oven dried at 48°C for 48 h, desiccated for 30 m and

weighed to the nearest 0.1 mg. FPOM filters from *Pycnopsyche* and *Lepidostoma* were cut in half and both pieces were re-weighed and analysed for either P or C/N content (see elemental analysis). For analyses using filters, I corrected for background filter and leaf contributions to FPOM measures by subtracting total C, N and P on filters from a set of 32 control chambers (n=4 per treatment) given only leaf litter. FPOM measures captured production of both fragments and egesta by shredders, although I believe most particles (>90%) consisted of egested material.

After FPOM production trials, I commenced gut clearance periods during which I measured shredder P excretion rates. Larvae were rinsed in filtered stream water and transferred to cups (caddisfly cases were not removed) containing 30 mL of filtered stream water. Five cups containing only filtered stream water served as controls. After 3 h, larvae were removed and excreta filtered through muffled 25-mm 0.7 µm pore GFFs (Whatman Inc., Kent, UK). Filtered excreta were kept at 4⁰C until SRP was analysed within 48 h using the ascorbic acid method (APHA 2005). I used individual growth rates (Halvorson *et al.*, 2015, Fuller *et al.*, 2015) to estimate insect mass (Benke & Huryn, 2006) and thereby obtain mass-specific rates of total, N and P particulate waste production and P excretion for each insect.

FPOM respiration trials

Prior to FPOM production trials, FPOM from *Pycnopsyche* and *Lepidostoma* accrued over a 2 d period were collected on 25 mm 0.7 µm pore GFFs (Whatman Inc. Kent, UK) for measurement of microbial respiration. Filters were immediately transferred to 24 mL vials containing filtered and well-aerated stream water. Vials were sealed with septa lids to prevent atmospheric gas exchange and placed in the dark at 10⁰C. After 22 h of incubation, vials were measured for oxygen:argon ratios using a membrane-inlet mass spectrometer equipped with a Prisma mass spectrometer (Pfeiffer Vacuum Technology AG, Asslar, Germany) and a DGA

membrane inlet S-25-75 (Bay Instruments, Easton, MD). I subtracted for background respiration from leaf particles and stream water by running a series of control vials containing filtered material from one insect-absent, leaf-present chamber for each diet treatment. After MIMS analysis, filters and FPOM were transferred to individual pre-weighed tins, oven dried at 48°C for 48 h, desiccated and weighed to the nearest 0.01 mg. Tins were combusted for 2 h at 500°C, desiccated and re-weighed to estimate FPOM ash-free dry mass (AFDM).

Elemental analysis

Dried initial insect larvae and leaf litter were homogenized prior to elemental analysis. All leaf samples were homogenized using a ball bearing grinder (Wig-L-Bug; Crescent Dental Manufacturing, Elgin, IL); insect larvae were ground to a fine powder using a spatula. Samples were oven dried at 48°C overnight and desiccated prior to weighing for P or C/N analysis. Samples for P content were combusted at 500°C for 2 h, digested in hot hydrochloric acid, diluted and measured for SRP using the ascorbic acid method (APHA 2005). Samples for C/N content were analysed using a CHN analyser (Thermo Scientific, Waltham, MA). All elemental analyses were corrected for recovery efficiencies using a ground peach leaves standard (NIST SRM 1547).

Statistical analysis

Mass-specific rates of total, N and P particulate waste production were compared statistically using a two-way ANOVA design testing litter species and nutrient treatment effects for each shredder. I used ANCOVA to test effects of litter type on FPOM C:P, C:N and N:P using diet C:P, C:N or N:P as respective covariates. Heterogeneity of FPOM/diet stoichiometry slopes between litter diets was indicated by a significant litter*diet stoichiometry interaction ($\alpha < 0.05$) in a preliminary ANCOVA. When slopes were homogeneous, I tested the null

hypothesis that FPOM stoichiometry changed linearly with diet stoichiometry (i.e. slope =1) using Student's *t*-tests; where slopes were heterogeneous, I conducted *t*-tests of slope=1 within each litter type independently. I used a two-way ANCOVA model to test litter and nutrient effects on FPOM respiration using FPOM AFDM as a covariate; because there was little group overlap in the covariate for the *Pycnopsyche* experiment, I instead pursued a two-way ANOVA on AFDM-specific FPOM respiration rates (Quinn & Keough, 2002). Significant main nutrient or interaction effects were examined across groups using Tukey's Honestly Significant Difference (HSD) test. Statistical analyses were performed only within and not across taxa because of different rearing conditions. I used box and residual plots to assess assumptions of ANOVA, and employed logarithmic transformation as necessary to satisfy assumptions. All statistical analyses were conducted using SYSTAT (SYSTAT Software, Inc., Chicago, IL).

RESULTS

Leaf litter and shredder stoichiometry

Both early-stage litter fed to *Lepidostoma* and late-stage litter fed to *Pycnopsyche* and *Tipula* increased in N and P content with greater concentrations of dissolved P in litter incubations. This resulted in three- to five-fold range in molar C:P and N:P content of litter fed to shredders (overall range = 850 to 4880 and 28 to 109 for C:P and N:P respectively) and a smaller range in litter molar C:N content (range = 30 to 49). There were distinct levels of diet nutrient content fed to each shredder within each litter type (see Table S1 for leaf litter stoichiometry).

Shredder taxa displayed divergent body stoichiometry prior to the experiments, though I did not test for statistical differences. *Pycnopsyche* and *Tipula* were of similar mean body N content (9.22 and 9.73 %N, respectively) that was greater than that of *Lepidostoma* (6.55 %N). *Pycnopsyche* had the highest body P content (1.41 %P), followed by *Lepidostoma* (0.92 %P) and

Tipula (0.62 %P). These trends also contributed to differences in shredder body molar ratios of C:N, C:P and N:P (Table 1).

FPOM production and P excretion

Litter type and nutrient incubation interacted to affect total FPOM production rates by *Tipula* and *Pycnopsyche*, whereas FPOM production rates by *Lepidostoma* were not affected by diet treatment (Table 2). Taxa varied in absolute FPOM production rates; *Lepidostoma* produced FPOM at slower rates compared to *Tipula* and *Pycnopsyche* and the latter two exhibited similar FPOM production rates. *Pycnopsyche* FPOM production differed only marginally across diets. *Tipula* FPOM production increased as much as two-fold on diets incubated under higher P concentrations only on oak litter (Fig. 2).

Molar elemental ratios of FPOM generally resembled those in the diet of *Lepidostoma* and did not differ between litter types (Table 3), although *Lepidostoma* FPOM C:N was consistently below diet C:N (Fig. 3). This was in contrast to stoichiometry of FPOM from *Tipula* and *Pycnopsyche*, which often differed from diet stoichiometry and depended on litter type (Fig. 3). The linear effects of diet C:P and C:N on *Tipula* FPOM C:P and C:N, respectively, differed between oak and maple litter (regression slopes of FPOM and diet stoichiometry were >1 for maple but ≤ 1 for oak litter; Table 3). N:P of FPOM from *Tipula* was similar between oak and maple litter, with a slope >1 (Table 3). For *Pycnopsyche*, both C:P and N:P of FPOM were greater on oak litter than maple litter after accounting for parallel effects of diet C:P and N:P respectively as covariates; on the contrary, the slopes of FPOM C:N on diet C:N were different between oak and maple litter (Table 3). With the exception of FPOM C:N on maple diets, regression slopes of *Pycnopsyche* FPOM and diet stoichiometry were <1 (Table 3; Fig. 3).

Rates of particulate N production by *Tipula* and *Pycnopsyche* was affected by a litter type and nutrient incubation interaction, whereas particulate N production by *Lepidostoma* did not change with diet treatment (Table 2). Particulate N production by *Tipula* increased on litter that was incubated at higher P concentrations for both litter types, and effects were stronger on maple diets (Fig. 4). Particulate N production by *Pycnopsyche* similarly increased with incubation P concentration on maple diets but not on oak diets; on oak diets, rates peaked on the 100 $\mu\text{g L}^{-1}$ P treatment, whereas on maple diets, rates peaked on the 500 $\mu\text{g L}^{-1}$ P treatment.

Rates of particulate P production by all three shredders increased for litter incubated under greater P concentrations. In the *Tipula* and *Pycnopsyche* experiments, there were interactive effects of litter type and P treatment (Table 2) driven by stronger nutrient effects for maple diets. Particulate P production rates by *Tipula* and *Pycnopsyche* increased two- to more than five-fold with increasing P incubation treatment (Fig. 4). Nutrients but not litter type affected particulate P production by *Lepidostoma* (Table 2, Fig. 4).

Rates of P excretion increased for higher-nutrient diets for both *Pycnopsyche* and *Tipula*. *Pycnopsyche* switched from a net sink on $<5 \mu\text{g L}^{-1}$ P diets (e.g. no measurable P excretion) to a net source of dissolved P on 100 and 500 $\mu\text{g L}^{-1}$ P diets, and rates did not differ between maple and oak litter (Fig. 5). *Tipula* had consistently positive and greater P excretion rates than *Pycnopsyche* on all diets. P excretion by *Tipula* was lower on the maple $<5 \mu\text{g L}^{-1}$ P diet than all others (Fig. 5). I did not calculate P excretion rates by *Lepidostoma* because P concentrations in excreta from *Lepidostoma* were not measurably different from controls.

FPOM decomposition rates

In both the *Lepidostoma* and *Pycnopsyche* experiments, FPOM respiration rates were affected by diet nutrient content depending on litter type. Oak-fed *Pycnopsyche* produced FPOM

with lower respiration rates than maple-fed *Pycnopsyche* ($F_{1,51}=103.10$, $P<0.001$), but respiration rates of FPOM from oak diets did not vary with nutrient treatment, which resulted in a litter and P treatment interaction ($F_{3,51}=12.21$; $P<0.001$); on maple diets, the highest-nutrient treatment resulted in higher FPOM respiration rates than all other treatments (Fig. 6). Respiration rates of FPOM from *Lepidostoma* did not differ between litter diets ($F_{1,31}=3.03$, $P=0.092$) but there was an interaction between litter type and P treatment ($F_{3,31}=4.19$, $P=0.013$) and *Lepidostoma* fed the highest-nutrient oak and maple diets produced FPOM with greater respiration rates than those fed a lower-nutrient oak diet (Fig. 6).

DISCUSSION

This study exposes a suite of diet- and species-dependent changes in particulate consumer nutrient recycling (CNR) of shredders in streams. Consistent with the predictions of ecological stoichiometry theory (Elser & Urabe, 1999; Sterner & Elser, 2002), all three taxa increased rates of nutrient waste production on nutrient-rich diets. Nutrients often interacted with litter type or differed between maple and oak litter in their effects on shredder FPOM production. Shredder taxa also varied in magnitude and directionality of responses to dietary nutrients – in many cases, shredders altered the stoichiometry of FPOM from that of CPOM, and N and P particulate waste production increased on higher-nutrient diets. Finally, *Tipula* and *Pycnopsyche* differed in rates of P release as particles vs. excreta. Together with evidence of dietary effects on microbial decomposition of FPOM, my findings indicate strong bottom-up effects of litter type and nutrient content on shredder-mediated C, N and P transformation that can vary across shredder species.

Shredder taxa also varied in absolute rates of FPOM production. Contrary to my hypotheses, FPOM production by *Pycnopsyche* and *Lepidostoma* did not differ strongly across litter types or nutrient levels; however, FPOM production by *Tipula* increased with diet nutrient

content (Fig. 2), suggesting that conversion of CPOM to FPOM by *Tipula* may increase with nutrient enrichment. *Pycnopsyche* and *Tipula* exhibited more than two times greater rates of FPOM production than *Lepidostoma*. This is consistent with previous studies describing *Pycnopsyche* as a functionally dominant shredder (Creed *et al.*, 2009), and although *Pycnopsyche* can exhibit higher litter consumption rates than *Tipula* (Eggert & Wallace, 2007), the higher rearing temperature of *Tipula* may have resulted in FPOM production rates similar to those of *Pycnopsyche*. It is possible that relatively N- rich *Pycnopsyche* and *Tipula* employed faster (e.g. compensatory) feeding to obtain needed N relative to N-poor *Lepidostoma*, which could explain greater FPOM production rates. I did not conduct interspecific statistical comparisons in this study because of different rearing conditions.

All three shredders modified FPOM stoichiometry across the diet gradient as expected; however, not all predictions from ecological stoichiometry were supported. The stoichiometry of *Lepidostoma* FPOM generally resembled diet stoichiometry, whereas both *Tipula* and *Pycnopsyche* modified FPOM significantly from diet stoichiometry (Fig. 3). Because simple fragmentation should produce FPOM that closely resembles diets, deviation of FPOM C:N:P from diet C:N:P can be attributed to shifts strictly in the stoichiometry of egesta. *Pycnopsyche* produced FPOM at molar C:P and N:P ratios below diet C:P and N:P whereas *Tipula* produced FPOM at C:P and N:P ratios above diet C:P and N:P, suggesting the former shredder produced relatively P-rich egesta whereas the latter produced P-poor egesta. These trends were opposite those expected from ecological stoichiometry theory, which predicts that P-rich *Pycnopsyche* would produce low-P waste and P-poor *Tipula* would produce high-P waste on any given diet C:P or N:P (Elser & Urabe, 1999). It is possible that initial body stoichiometry does not relate to nutritional requirements, especially given I measured FPOM production after four weeks of

growth and macroinvertebrate body P content can decline throughout larval development (Back & King, 2013), leading to reductions in P growth requirements that could vary across species.

Counterintuitive trends in FPOM stoichiometry may also be attributable to variable shredder feeding habits, such as the relative importance of selective feeding on P-rich biofilm. Given that FPOM from *Pycnopsyche* was more P-rich than bulk diets, selective feeding on either litter biofilm or among better-conditioned leaf disks may be especially important in this taxon, causing actual ingestion C:P to be lower than diet C:P and outweighing effects of preferential assimilation of limiting P that would raise FPOM C:P. Indeed, selective feeding may be a critical means of obtaining nutrients for low C:P and C:N shredders such as *Pycnopsyche* when fed high C:P or C:N diets. On the other hand, *Tipula* produced FPOM that was less P-rich than bulk diets, indicating that in this species preferential assimilation of P may outweigh potential effects of selective feeding. Overall, taxonomic differences in reliance on pre-ingestive regulation (selective feeding; Arsuffi & Suberkropp 1989) versus post-ingestive regulation (selective assimilation; Clissold *et al.*, 2010) for obtaining dietary nutrients could drive variation of particulate CNR by shredders and other taxa.

Leaf litter type may additionally play a key role in mediating the effects of altered resource stoichiometry on shredder CNR in streams by modifying patterns in FPOM stoichiometry. Slopes between diet C:N and FPOM C:N for *Tipula* and *Pycnopsyche* were negative on oak but positive on maple diets (Table 3; Fig. 3). This counterintuitive result from oak litter suggests enhanced N relative to C assimilation on low C:N diets, especially for *Tipula* that displayed a negative relationship. It is plausible that oak-fed *Tipula* were N-limited in growth, given that *Tipula* did not respond strongly to additional P content of oak litter (Fuller *et al.*, 2015). Under N-limitation, C:N of egesta would not decrease on diets that are lower in C:N. I

also found that contrary to patterns in C:N of FPOM from *Tipula* and *Pycnopsyche*, there were parallel linear effects of diet C:P and N:P on *Pycnopsyche* FPOM C:P and N:P between the two litter types (Table 3). However, FPOM from *Pycnopsyche* was higher in P content from maple diets than from oak diets (Fig. 3). *Pycnopsyche* growth and P assimilation efficiencies were greater on oak diets than on maple diets (Halvorson *et al.*, unpublished data), which could explain lower P content of FPOM from oak litter than maple litter.

A combination of shifts in FPOM production and stoichiometry significantly changed shredder N and P waste production, with rates often increasing on high-nutrient diets within each litter type. *Pycnopsyche* had greater rates especially of particulate P release than either *Tipula* or *Lepidostoma* (Fig. 4). Because FPOM contains both fragments and egesta, these trends may have been driven by egestion of excess ingested nutrients (Clissold *et al.*, 2010), such as to maintain homeostasis (Persson *et al.*, 2010), as well as greater nutrient content of CPOM fragments produced by messy feeding. Rates of P excretion by both *Pycnopsyche* and *Tipula* similarly increased on higher-nutrient diets; however, P excretion by *Tipula* rose significantly on higher-C:P diets compared to P excretion by *Pycnopsyche*. This result is consistent with predictions of threshold elemental ratios (TERs) that low-C:P *Pycnopsyche* should remain P-limited at lower diet C:P than high-C:P *Tipula* (Frost *et al.*, 2006). Because excreta undergo more physiological processing prior to release than fragments or egesta, it is probable that body stoichiometry better predicts variation of dissolved waste production than particulate waste production by consumers.

Rates of particulate P production by *Pycnopsyche* exceeded those of excretion, whereas those for *Tipula* were consistently below excretion. The relative importance of pre-assimilatory P waste (egestion and fragmentation) versus post-assimilatory P waste (excretion) may thus differ among shredder taxa. Very low or unmeasurable P excretion by *Pycnopsyche* and *Lepidostoma*

may be in part attributable to uptake by microbes on leaf cases that may diminish or outpace excretion rates on low-P diets. Rates of N and P release as particles, especially by *Pycnopsyche*, were upwards of one order of magnitude greater than those of N and P excretion reported for other insect shredders at slightly higher temperatures and higher C:P diets (N excretion ≈ 0.17 to $0.81 \mu\text{g N mg DM}^{-1} \text{d}^{-1}$; P excretion ≈ 0.07 to $0.08 \mu\text{g P mg DM}^{-1} \text{d}^{-1}$ at $14\text{-}20^{\circ}\text{C}$; McManamay *et al.*, 2011, Villanueva *et al.*, 2011). Rates of P excretion by *Tipula* and *Pycnopsyche* in this study may have surpassed these literature values because many diets I used were P-rich relative to those in previous studies.

Unlike excreta, particulate wastes represent a biologically active matrix that may vary depending on consumer feeding habits as well as diet (Wotton & Malmqvist, 2001). I found that the microbial decomposition of FPOM, measured as respiration, was positively associated with diet nutrient content for *Pycnopsyche* and *Lepidostoma*. Ash free dry mass (AFDM)-specific rates of *Pycnopsyche* FPOM respiration ($0.8 - 8.6 \text{ mg O}_2 \text{ g AFDM}^{-1} \text{ d}^{-1}$) spanned much of the published range of FPOM respiration ($0.2 - 10.6 \text{ mg O}_2 \text{ g AFDM}^{-1} \text{ d}^{-1}$) in streams (Webster *et al.*, 1999), implying that variability in resource quality may explain natural variation in FPOM decomposition. The high FPOM respiration rates from nutrient-rich diets may also indicate high nutritional quality for invertebrate consumers such as chironomids (Ward & Cummins, 1979). Thus, shredders may facilitate positive nutrient enrichment effects on downstream collector food webs, similar to how addition of sewage-derived particles can enhance production of collector-gatherers in streams (Singer & Battin, 2007). These effects could arise from differences in FPOM carbon quality, nutrient content or surviving microbial biomass and could speed turnover and respiration of FPOM in nutrient-rich streams (Benstead *et al.*, 2009; Tant *et al.*, 2013).

One limitation of this study is that FPOM was subject to leaching and microbial colonization, uptake or mineralization over the 2-3 d duration of production trials. I extended production trials to ensure measurable FPOM production by all three shredder taxa given the constraint that only one insect could be assigned per chamber to prevent antagonism among coexisting individuals. The extended trials may be more ecologically realistic than an hour-long trial because they allow for initial leaching of nutrients from egesta that would normally occur in the stream. Therefore, the 2-3 d trials may better represent longer-term shredder effects on FPOM stoichiometry. While uptake or mineralization of nutrients by microbes is possible, previous studies suggest that these effects may not be significant over a 2-3 d period. FPOM exhibits far lower mass-specific rates of P uptake than CPOM and algae (Newbold *et al.*, 1983), and FPOM stoichiometry may not respond as flexibly to dissolved nutrient amendment as does CPOM stoichiometry, possibly because bacteria that dominate FPOM are not as capable of storing nutrients (especially P) compared to fungi that dominate CPOM (Tant *et al.*, 2013). The duration of trials represents a trade-off between methodological constraints and multiple confounding variables. Future work should address leaching, uptake and mineralization of FPOM to determine the appropriate duration of FPOM production trials and better track the fate of FPOM in streams.

Increased nutrient waste production on high nutrient diets may be used to assess shredder growth limitation. TERs predict higher nutrient release when diet C:P or C:N decline below the $TER_{C:P}$ or $TER_{C:N}$ (Sterner & Elser, 2002); of the taxa in this study, only *Lepidostoma* has a published $TER_{C:P}$ estimate (3086; Frost *et al.*, 2006). On diets below C:P=3086, *Lepidostoma* should significantly increase P release. Contrary to this prediction, I observed very low rates of particulate P production even at oak C:P=1720 by *Lepidostoma* (Fig. 3; Table S1). These low

rates suggest strong P-limitation of growth at diet C:P well below the estimated $TER_{C:P}$. Current $TER_{C:P}$ models for many consumers assume a P assimilation efficiency (A_P) of 80% (Frost *et al.* 2006), but this may be high for *Lepidostoma*, given that this insect has a reported maximum bulk assimilation efficiency of 33% (Grafius & Anderson, 1979) and recent studies suggest A_P of other shredders may be below 80% (Villanueva *et al.*, 2011; Halvorson *et al.* unpublished data; Fuller *et al.* 2015). Shredder assimilation efficiencies may be difficult to measure accurately due to selective consumption and messy feeding; however, a plausibly low A_P would result in $TER_{C:P}$ closer to 1720. Rates of nutrient release as both excreta and egesta could serve as valuable tools for assessing TERs and may complement other approaches to estimate nutrient limitation (Wagner *et al.*, 2013).

These data indicate that CNR effects in detritus-based systems may be mediated through particulate pathways similar to better-known dissolved pathways in autotrophic systems. The conversion of CPOM to FPOM by stream shredders represents a transformation of dietary carbon and nutrients that clearly depends on diet and consumer species, much like excretion (Elser & Urabe, 1999; Vanni, 2002). However, shredder FPOM production could have direct and indirect consequences for stream nutrient dynamics that are spatially and temporally expressed differently than those of excretion. Altered N and P content of FPOM could affect growth of downstream collectors (Singer & Battin, 2007; Veldbloom & Haro, 2011), and transport dynamics of FPOM could differ from those of excreta. For example, FPOM could accrue and persist in depositional areas (Joyce & Wotton, 2008) to create slow-release hotspots of nutrient remineralization. Depending on feeding habits and diet characteristics such as recalcitrance and nutrient content, consumer taxa may vary in the relative importance of CNR via dissolved versus particulate wastes. Future studies should consider the magnitude and consequences of both

particulate and dissolved CNR to further discern the importance of consumer wastes in aquatic ecosystems.

ACKNOWLEDGEMENTS

Thank you to J. Gilbert, E. Grantz, E. Scott, D. Hall, A. Sanders, J. Ramey, G. White, L. Abel and A. Eddy for laboratory assistance. J.T. Scott, B. Austin, A. Smartt and two anonymous reviewers provided valuable discussion and input on earlier versions of the manuscript. This research was funded by the National Science Foundation (DEB 1020722). H.M. Halvorson was supported by a Doctoral Academy Fellowship at the University of Arkansas.

LITERATURE CITED

- American Public Health Association (2005) *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, Washington, DC.
- Arsuffi T.L. & Suberkropp K. (1989) Selective feeding by shredders on leaf-colonizing stream fungi: comparisons of macroinvertebrate taxa. *Oecologia*, **79**, 30-37.
- Atkinson C.L., Vaughn C.C., Forshay K.J. & Cooper J.T. (2013) Aggregated filter-feeding consumers alter nutrient limitation – consequences for ecosystem and nutrient dynamics. *Ecology*, **94**, 1359-1369.
- Back J.A. & King R.S. (2013) Sex and size matter: ontogenetic patterns of nutrient content of aquatic insects. *Freshwater Science*, **32**, 837-848.
- Balseiro E. & Albariño R. (2006) C-N mismatch in the leaf litter-shredder relationship of an Andean Patagonian stream detritivore. *Journal of the North American Benthological Society*, **25**, 607-615.
- Benke A.C. & Huryn A.D. (2006) Secondary production of macroinvertebrates. In: *Methods in Stream Ecology*, 2nd edn. (Eds. F.R. Hauer & G.A. Lamberti), pp. 691-710. Academic Press, Burlington, MA, USA.
- Benstead J.P., Rosemond A.D., Cross W.F., Wallace J.B., Eggert S.L., Suberkropp K. *et al.* (2009) Nutrient enrichment alters storage and fluxes of detritus in a headwater stream ecosystem. *Ecology*, **90**, 2556-2566.
- Clissold F.J., Tedder B.J., Conigrave A.D. & Simpson S.J. (2010) The gastrointestinal tract as a nutrient-balancing organ. *Proceedings of the Royal Society B*, **277**, 1751-1759.
- Creed R.P., Cherry R.P., Pflaum J.R. & Wood C.J. (2009) Dominant species can produce negative relationship between species diversity and ecosystem function. *Oikos*, **118**, 723-732.
- Cross W.F., Benstead, J.B., Rosemond, A.D., & Wallace, J.B. (2003) Consumer-resource stoichiometry in detritus-based streams. *Ecology Letters*, **6**, 721-732.
- Cuffney T.F., Wallace J.B. & Lughart J.G. (1990) Experimental evidence quantifying the role of benthic invertebrates in organic matter dynamics of headwater streams. *Freshwater Biology*, **23**, 281-299.
- Cummins, K.W. & Klug M.J. (1979) Feeding ecology of stream invertebrates. *Annual Review of Ecology and Systematics*, **10**, 147-172.
- DeMott W.R., Gulati R.D. & Siewertsen K. (1998) Effects of phosphorus-deficient diets on the carbon and phosphorus balance of *Daphnia magna*. *Limnology & Oceanography*, **43**, 1147-1161.

- Eggert S.L. & Wallace J.B. (2007) Wood biofilm as a food resource for stream detritivores. *Limnology & Oceanography*, **52**, 1239-1245.
- Elser J.J. & Urabe J. (1999) The stoichiometry of consumer-driven nutrient recycling: theory, observations, and consequences. *Ecology*, **80**, 735-751.
- Evans-White M.A. & Lamberti G.A. (2006) Stoichiometry of consumer-driven nutrient recycling across nutrient regimes in streams. *Ecology Letters*, **9**, 1186-1197.
- Frost P.C., Benstead J.P., Cross W.F., Hillebrand H., Larson J.H., Xenopoulos M.A. *et al.* (2006) Threshold elemental ratios of carbon and phosphorus in aquatic consumers. *Ecology Letters*, **9**, 774-779.
- Fuller C.L., Evans-White M.A. & Entekin S.A. (2015) Growth and stoichiometry of a common aquatic detritivore respond to changes in resource stoichiometry. *Oecologia*, **177**, 837-848.
- Graça M.A.S., Cressa C., Gessner M.O., Feio M.J., Callies K.A. & Barrios C. (2001) Food quality, feeding preferences, survival and growth of shredders from temperate and tropical streams. *Freshwater Biology*, **46**, 947-957.
- Grafius E. & Anderson N.H. (1979) Population dynamics, bioenergetics, and role of *Lepidostoma quercina* Ross (Trichoptera: Lepidostomatidae) in an Oregon woodland stream. *Ecology*, **60**, 433-441.
- Halvorson H.M., Scott J.T., Sanders A.J. & Evans-White M.A. (2015) A stream insect detritivore violates common assumptions of threshold elemental ratio bioenergetics models. *Freshwater Science*, **34**, 508-518.
- Joyce P. & Wotton R.S. (2008) Shredder fecal pellets as stores of allochthonous organic matter in streams. *Journal of the North American Benthological Society*, **27**, 521-528.
- Kaushik N.K. & Hynes H.B.N. (1971) The fate of the dead leaves that fall into streams. *Archiv für Hydrobiologie*, **68**, 465-515.
- Kendrick M.R. & Benstead J.P. (2013) Temperature and nutrient availability interact to mediate growth and body stoichiometry of a detritivorous stream insect. *Freshwater Biology*, **58**, 1820-1830.
- Liess A. & Haglund A. (2007) Periphyton responds differentially to nutrients recycled in dissolved or faecal pellet form by the snail grazer *Theodoxus fluviatilis*. *Freshwater Biology*, **52**, 1997-2008.
- Martin M.M., Martin J.S., Kukor J.J. & Merritt R.W. (1980) The digestion of protein and carbohydrate by the stream detritivore, *Tipula abdominalis* (Diptera, Tipulidae). *Oecologia*, **46**, 360-364.

- McIntyre P.B., Flecker A.S., Vanni M.J., Hood J.M., Taylor B.W. & Thomas S.A. (2008) Fish distributions and nutrient cycling in streams: can fish create biogeochemical hotspots? *Ecology*, **89**, 2335-2346.
- McManamay R.A., Webster J.R., Valett H.M. & Dolloff C.A. (2011) Does diet influence consumer nutrient cycling? Macroinvertebrate and fish excretion in streams. *Journal of the North American Benthological Society*, **30**, 84-102.
- Newbold, J.D., Elwood J.W., O'Neill R.V. & Sheldon A.L. (1983) Phosphorus dynamics in a woodland stream ecosystem: a study of nutrient spiraling. *Ecology* **64**: 1249-1265.
- Persson J., Fink P., Goto A., Hood J.M., Jonas J. & Kato S. (2010) To be or not to be what you eat: regulation of stoichiometric homeostasis among autotrophs and heterotrophs. *Oikos*, **119**, 741-751.
- Quinn G.P. & Keough M.J. (2002) *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, Cambridge, UK.
- Singer G.A. & Battin T.J. (2007) Anthropogenic subsidies alter stream consumer-resource stoichiometry, biodiversity, and food chains. *Ecological Applications*, **17**, 376-389.
- Sterner R.W. & Elser J.J. (2002) *Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere*. Princeton University Press, Princeton, NJ, USA.
- Strayer, D.L. (2014) Understanding how nutrient cycles and freshwater mussels (Unionoida) affect one another. *Hydrobiologia*, **735**: 277-292.
- Tant C.J., Rosemond A.D. & First M.R. (2013) Stream nutrient enrichment has a greater effect on coarse than on fine benthic organic matter. *Freshwater Science*, **32**, 1111-1121.
- Vanni M.J. (2002) Nutrient recycling by animals in freshwater ecosystems. *Annual Review of Ecology and Systematics*, **33**, 341-370.
- Vanni M.J., Flecker A.S., Hood J.M. & Headworth J.L. (2002) Stoichiometry of nutrient recycling by vertebrates in a tropical stream: linking species identity and ecosystem processes. *Ecology Letters*, **5**, 285-293.
- Veldbloom J.A. & Haro R.J. (2011) Stoichiometric relationship between suspension-feeding caddisfly (Trichoptera: Brachycentridae) and seston. *Hydrobiologia*, **675**, 129-141.
- Villanueva V.D., Albariño R. & Canhoto C. (2011) Detritivores feeding on poor quality food are more sensitive to increased temperatures. *Hydrobiologia*, **678**, 155-165.
- Wagner N.D., Hillebrand H., Wacker A. & Frost P.C. (2013) Nutritional indicators and their uses in ecology. *Ecology Letters* **16**, 535-544.
- Ward G.M. & Cummins K.W. (1979) Effects of food quality on growth of a stream detritivore, *Paratendipes albimanus* (Meigen) (Diptera: Chironomidae). *Ecology*, **60**, 57-64.

- Webster J.R., Benfield E.F., Ehrman T.P., Shaeffer M.A., Tank J.L. , Hutchens J.J. *et al.* (1999) What happens to allochthonous material that falls into streams? A synthesis of new and published information from Coweeta. *Freshwater Biology*, **41**, 687-705.
- Wotton R.S. & Malmqvist B. (2001) Feces in aquatic ecosystems. *BioScience*, **51**, 537-544.
- Zanotto F.P., Simpson S.J. & Raubenheimer D. (1993) The regulation of growth by locusts through post-ingestive compensation for variation in the levels of dietary protein and carbohydrate. *Physiological Entomology*, **18**, 425-434.

Table 1. Mean (\pm 1 SE) body elemental content of subsets of initial larvae used for growth experiments. Percent carbon (%C), nitrogen (%N) and phosphorus (%P) are per unit dry mass, whereas C:N, C:P and N:P are molar ratios.

Taxon	% C	% N	% P	C:N	C:P	N:P
<i>Lepidostoma</i>	46.8 (0.2)	6.55 (0.11)	0.92 (0.03)	8.4 (0.2)	141 (5)	16.8 (0.4)
<i>Tipula</i>	42.1 (1.0)	9.73 (0.31)	0.62 (0.08)	5.1 (0.2)	190 (29)	37.1 (4.8)
<i>Pycnopsyche</i>	44.7 (0.2)	9.22 (0.14)	1.41 (0.04)	5.7 (0.1)	82 (3)	14.5 (0.3)

Table 2. Two-way analysis of variance for rates of total FPOM production, particulate nitrogen (N) production, particulate phosphorus (P) production and P excretion. Boldface indicates statistical significance ($P < 0.05$). See Tables S2 and S3 for full ANOVA results.

Variable	Factor*	P-value		
		<i>Lepidostoma</i>	<i>Tipula</i>	<i>Pycnopsyche</i>
Total FPOM production	Litter	0.187†	0.784	0.740
	[P]	0.675†	<0.001	0.055
	Interaction	0.767†	0.010	0.017
Particulate N production	Litter	0.096	0.643†	0.321†
	[P]	0.245	<0.001 †	0.020 †
	Interaction	0.321	0.007 †	0.001 †
Particulate P production	Litter	0.115	0.094	<0.001
	[P]	0.015	<0.001	<0.001
	Interaction	0.568	0.002	0.009
P excretion	Litter	N/A	0.022	0.110
	[P]	N/A	0.052	<0.001
	Interaction	N/A	0.001	0.217

*Litter = maple or oak; [P] = dissolved phosphorus concentration during litter incubation
†indicates analysis where the response variable was log-transformed.

Table 3. Analysis of covariance for molar ratios of carbon:phosphorus (C:P), carbon:nitrogen (C:N) and nitrogen:phosphorus (N:P) of FPOM produced by three shredders fed diets of maple and oak litter that differed in stoichiometry. Homogeneity of slopes between litter diets was tested by an interaction ($P < 0.05$) in ANCOVA. Interactions were removed from ANCOVA models when $P > 0.05$ (indicated by N/A). Where slopes were homogeneous, t -tests assessed the null hypothesis that FPOM stoichiometry changed with diet stoichiometry in a 1:1 manner (slope=1) among both litter types; where slopes were heterogeneous, t -tests assessed slope=1 within each litter type independently. Boldface indicates statistical significance ($P < 0.05$). See Table S4 for full ANCOVA and t -test results

Variable Factor*	<i>Lepidostoma</i>				<i>Tipula</i>				<i>Pycnopsyche</i>			
	<i>P</i> -value	Litter	Slope	<i>P</i> -value	<i>P</i> -value	Litter	Slope	<i>P</i> -value	<i>P</i> -value	Litter	Slope	<i>P</i> -value
FPOM Litter	0.916	Both	1.34	0.138	0.001	Maple	2.04	<0.001	<0.001	Both	0.57	<0.001
C:P Diet C:P	<0.001				<0.001	Oak	0.70	0.188	<0.001			
Interaction	N/A				<0.001				N/A			
FPOM Litter	0.069	Both	0.70	0.239	<0.001	Maple	3.20	<0.001	0.019	Maple	1.10	0.754
C:N Diet C:N	0.008				<0.001	Oak	-1.10	<0.001	0.105	Oak	-0.16	0.006
Interaction	N/A				<0.001				0.030			
FPOM Litter	0.088	Both	2.07	0.191	0.905	Both	1.42	0.015	<0.001	Both	0.59	<0.001
N:P Diet N:P	0.015				<0.001				0.016			
Interaction	N/A				N/A				N/A			

*Litter = maple or oak

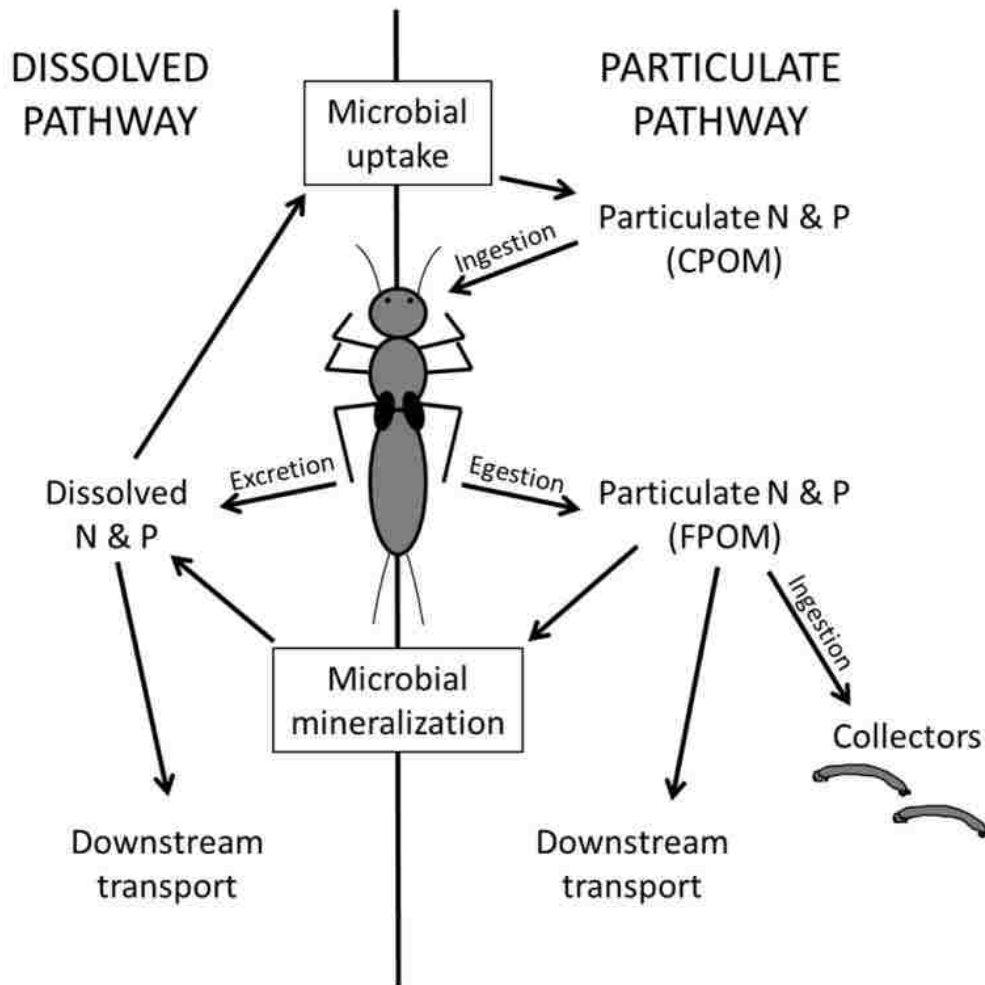


Figure 1. Conceptual diagram of shredder-mediated nitrogen (N) and phosphorus (P) cycling via dissolved and particulate pathways. Shredders ingest N and P as coarse particulate organic matter (CPOM), which undergoes transformation to dissolved N and P excreta that may be subject to microbial uptake or transport. Alternatively, N and P in CPOM may be transformed to fine particulate organic matter (FPOM) via fragmentation or egestion. FPOM may then be mineralized, transported or ingested by other organisms such as collectors.

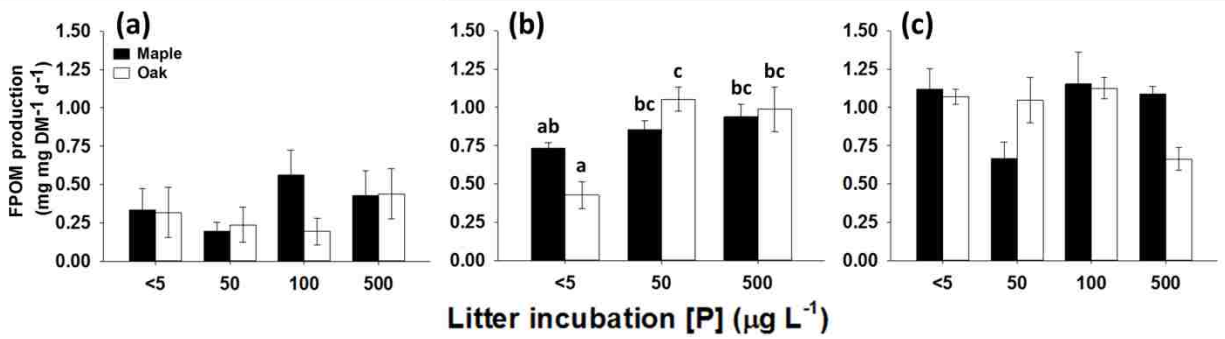


Figure 2. Mean dry mass (DM)-specific FPOM production rates by (a) *Lepidostoma* (b) *Tipula* and (c) *Pycnopsyche* fed maple and oak litter incubated under contrasting levels of dissolved phosphorus (P). Letters designate statistically different groups (Tukey's HSD, $P < 0.05$). Error bars represent ± 1 SE.

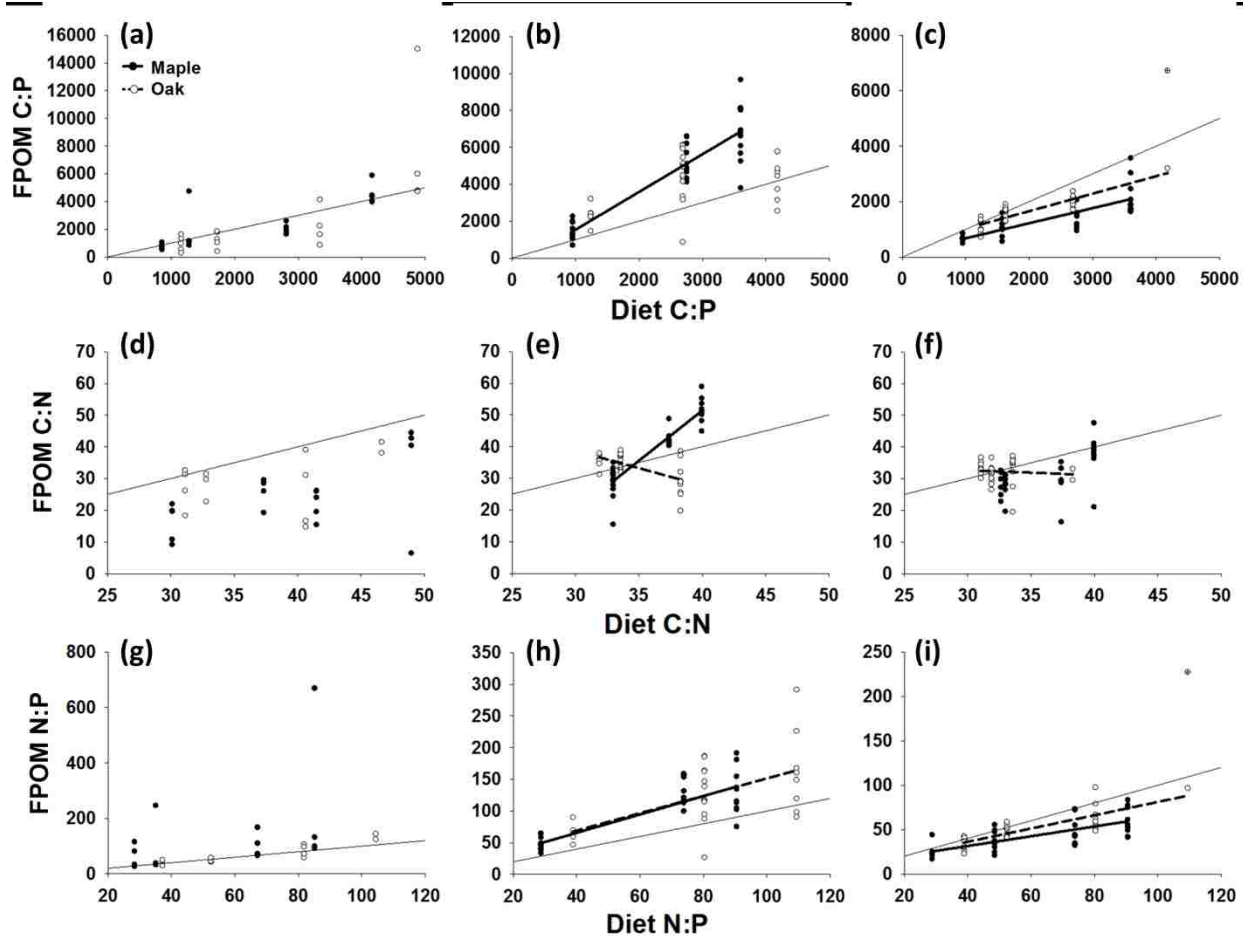


Figure 3. Molar carbon:phosphorus (C:P, a-c), carbon:nitrogen (C:N, d-f) and nitrogen:phosphorus (N:P, g-i) ratios of FPOM produced by *Lepidostoma* (a, d, g), *Tipula* (b, e, h) and *Pycnopsyche* (c, f, i) fed diets of maple and oak litter that differed in stoichiometry. Thin black lines designate FPOM=diet stoichiometry; thicker lines are regressions with slope significantly different from the 1:1 line (t -test, $P < 0.05$). In (c) and (i), cross-hatched symbols were not included in analyses due to high Cook's Distance (Cook's $D > 2.50$). Note different scales among y-axes of C:P and N:P graphs.

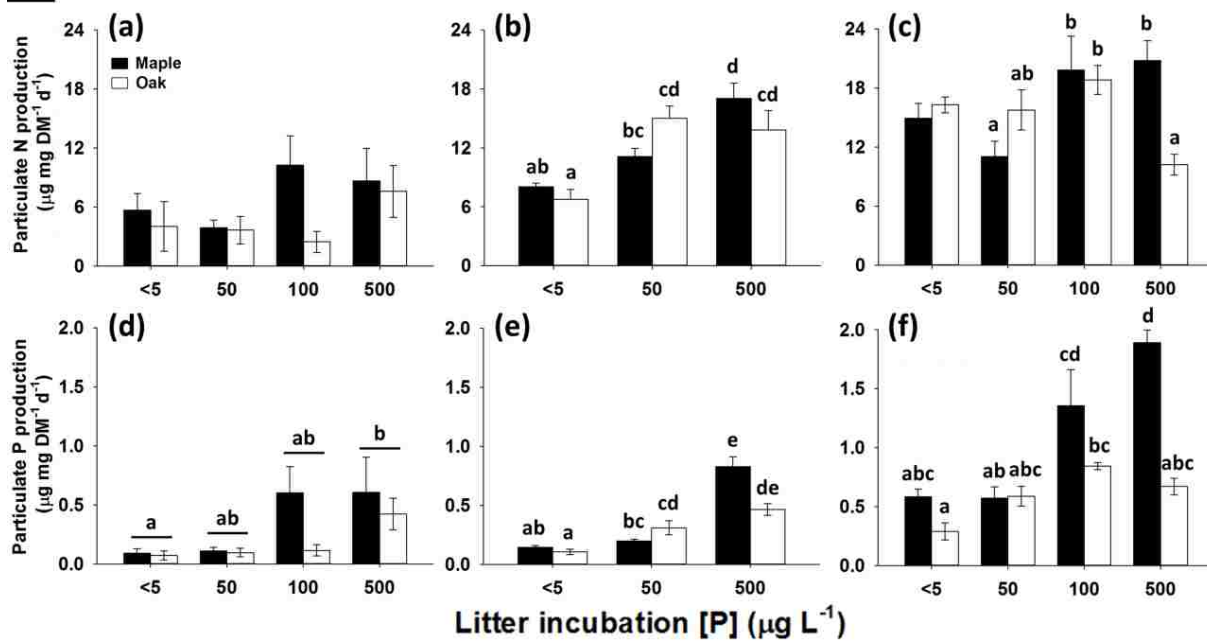


Figure 4. Mean dry mass (DM)-specific rates of particulate nitrogen (N; a-c) and phosphorus (P; d-f) production by *Lepidostoma* (a, d), *Tipula* (b, e) and *Pycnopsyche* (c, f) fed maple and oak litter incubated under contrasting levels of dissolved P. Data for N particulate production by *Pycnopsyche* fed $<5 \mu\text{g L}^{-1}$ P diets in (c) were excluded from ANOVA due to low sample sizes ($n=2$ on oak litter). Letters designate statistically different groups (Tukey's HSD, $P<0.05$) with lines above bars representing nutrient groups pooled for both leaf types where there was no interaction. Error bars represent ± 1 SE.

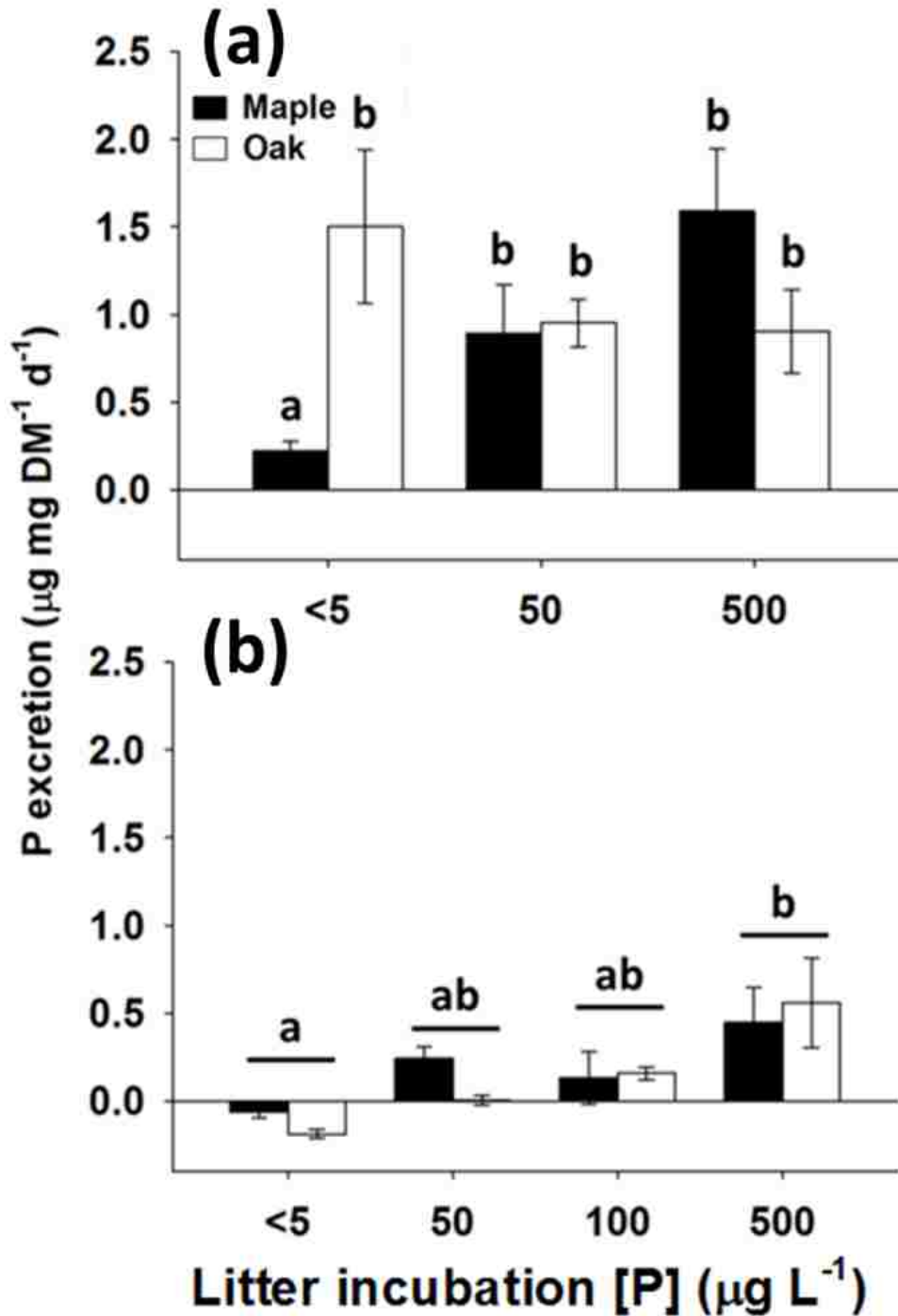


Figure 5. Mean dry mass (DM)-specific rates of phosphorus (P) excretion by (a) *Tipula* and (b) *Pycnopsyche* fed maple and oak litter incubated under contrasting levels of dissolved P. Letters designate statistically different groups (Tukey's HSD, $P < 0.05$) with lines above bars representing nutrient groups pooled for both leaf types where there was no interaction. Error bars represent ± 1 SE.

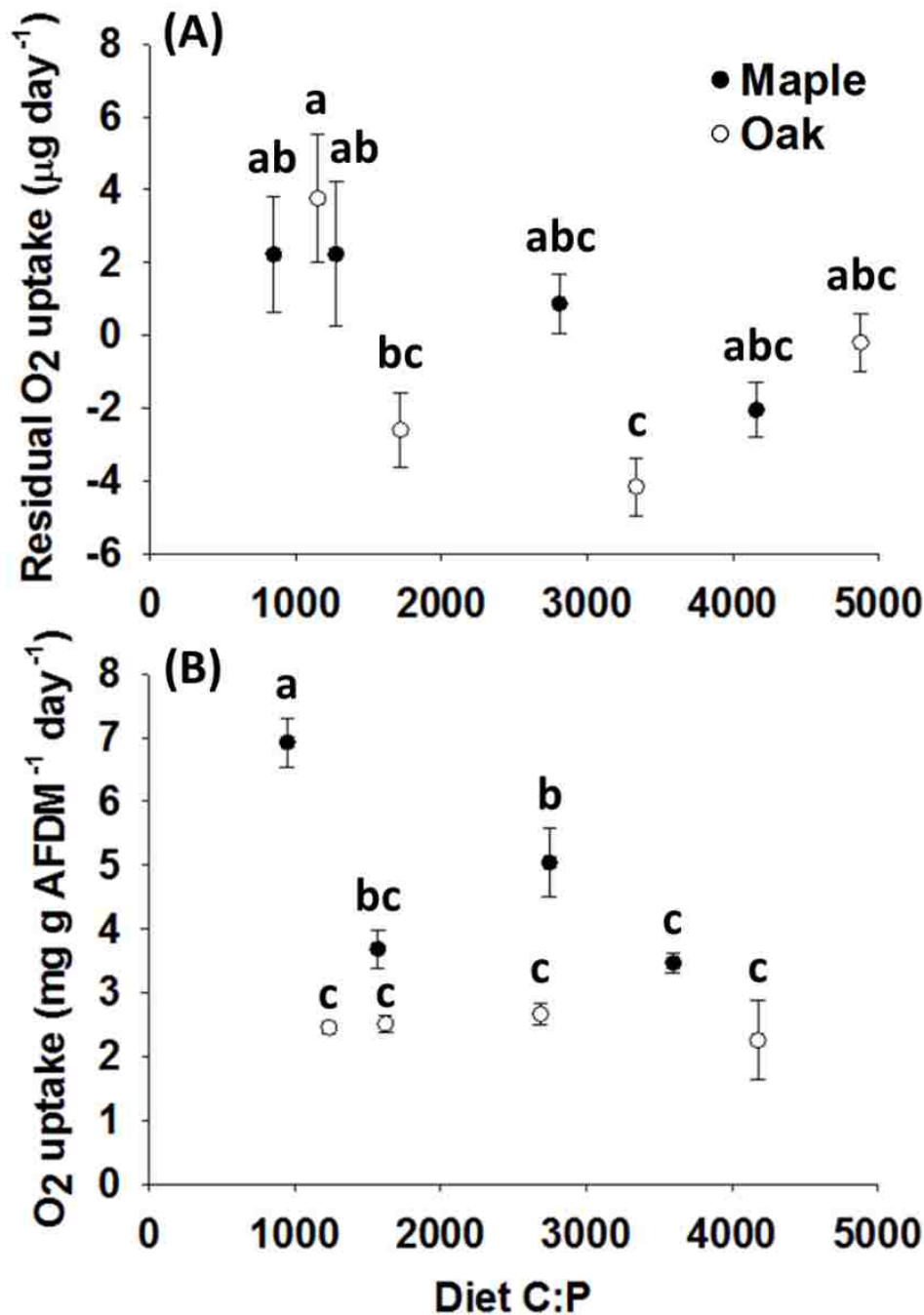


Figure 6. Microbial respiration rates of FPOM produced by (a) *Lepidostoma* and (b) *Pycnopsyche* fed diets of maple and oak litter differing in carbon:phosphorus (C:P) ratio. In (a), rates were analysed using ANCOVA; here, the residuals after accounting for FPOM AFDM are plotted by group to illustrate treatment effects. In (b), rates were rendered mass-specific by dividing by AFDM of FPOM and analysed using ANOVA. Letters designate statistically different groups (Tukey's HSD, $P < 0.05$). Error bars represent ± 1 SE. For full ANOVA and ANCOVA results, see Tables S2-S5.

APPENDICES

Table S1. Mean (± 1 SE) elemental content of diets of maple or oak leaf litter conditioned for 62 (early stage; fed to *Lepidostoma*) or 77 (late stage; fed to *Tipula* and *Pycnopsyche*) days under one of four dissolved P concentrations. Percent carbon (%C), nitrogen (%N) and phosphorus (%P) are per unit leaf dry mass, whereas C:N, C:P and N:P are molar ratios.

Leaf	[P] ($\mu\text{g L}^{-1}$)	% C	% N	% P	C:N	C:P	N:P
Early Stage Maple	< 5	47.8 (0.4)	1.14 (0.01)	0.030 (0.001)	49.0 (0.7)	4160 (100)	85.1 (2.5)
	50	46.8 (0.2)	1.33 (0.07)	0.046 (0.006)	41.5 (2.1)	2810 (350)	67.1 (6.3)
	100	47.8 (0.5)	1.56 (0.17)	0.098 (0.006)	37.3 (4.0)	1280 (70)	35.0 (2.1)
	500	46.9 (0.4)	1.85 (0.12)	0.144 (0.008)	30.1 (1.9)	850 (89)	28.3 (0.7)
Early Stage Oak	< 5	48.0 (0.2)	1.23 (0.09)	0.026 (0.002)	46.6 (3.2)	4880 (420)	104 (4)
	50	47.8 (0.2)	1.39 (0.07)	0.038 (0.003)	40.6 (2.1)	3340 (270)	81.8 (2.8)
	100	47.6 (0.1)	1.71 (0.07)	0.072 (0.004)	32.8 (1.3)	1720 (100)	52.4 (1.7)
	500	47.7 (0.1)	1.82 (0.12)	0.110 (0.008)	31.1 (1.8)	1150 (90)	37.1 (2.4)
Late Stage Maple	< 5	48.4 (0.3)	1.42 (0.03)	0.036 (0.002)	39.9 (1.0)	3600 (220)	90.4 (5.8)
	50	49.4 (0.3)	1.57 (0.06)	0.049 (0.002)	37.4 (1.5)	2750 (170)	73.8 (3.8)
	100	49.7 (0.2)	1.79 (0.04)	0.083 (0.003)	32.6 (0.8)	1570 (60)	48.4 (1.8)
	500	48.6 (0.3)	1.74 (0.05)	0.136 (0.006)	33.0 (1.0)	949 (42)	28.7 (0.8)
Late Stage Oak	< 5	48.2 (0.1)	1.48 (0.04)	0.030 (0.001)	38.3 (0.9)	4180 (160)	110 (4)
	50	47.8 (0.2)	1.67 (0.03)	0.047 (0.002)	33.5 (0.6)	2690 (120)	80.3 (3.7)
	100	48.0 (0.2)	1.81 (0.03)	0.077 (0.002)	31.0 (0.6)	1620 (40)	52.3 (0.9)
	500	48.2 (0.2)	1.78 (0.04)	0.102 (0.003)	31.9 (0.9)	1240 (40)	38.8 (0.8)

Table S2. Analysis of variance for rates of total FPOM, particulate nitrogen (N) and phosphorus (P) particulate waste production by shredders. Boldface indicates statistical significance ($P < 0.05$).

Variable	Shredder	Factor*	df	F-ratio	P-value
FPOM production	<i>Lepidostoma</i>	Litter	1	1.82	0.187
		[P]	3	0.52	0.675
		Litter*[P]	3	0.38	0.767
		Error	32		
	<i>Tipula</i>	Litter	1	0.08	0.784
		[P]	2	13.62	<0.001
		Litter*[P]	2	4.96	0.01
		Error	58		
	<i>Pycnopsyche</i>	Litter	1	0.11	0.740
		[P]	3	2.69	0.055
		Litter*[P]	3	3.70	0.017
		Error	56		
Particulate P production	<i>Lepidostoma</i>	Litter	1	2.63	0.115
		[P]	3	4.08	0.015
		Litter*[P]	3	0.69	0.568
		Error	32		
	<i>Tipula</i>	Litter	1	2.89	0.094
		[P]	2	74.98	<0.001
		Litter*[P]	2	7.27	0.002
		Error	58		
	<i>Pycnopsyche</i>	Litter	1	14.77	<0.001
		[P]	3	14.54	<0.001
		Litter*[P]	3	4.20	0.009
		Error	56		
Particulate N production	<i>Lepidostoma</i>	Litter	1	2.94	0.096
		[P]	3	1.46	0.245
		Litter*[P]	3	1.21	0.321
		Error	32		
	<i>Tipula</i>	Litter	1	0.22	0.643
		[P]	2	29.94	<0.001
		Litter*[P]	2	5.33	0.007
		Error	58		
	<i>Pycnopsyche</i> †	Litter	1	1.01	0.321
		[P]	2	4.25	0.020
		Litter*[P]	2	8.43	0.001
		Error	44		

*Litter = maple or oak; [P] = phosphorus concentration during leaf litter incubation

†Lowest-[P] diets removed due to low sample size

Table S3. Analysis of variance for rates of phosphorus (P) excretion by shredders. Boldface indicates statistical significance ($P < 0.05$).

Variable	Shredder	Factor*	df	F-ratio	P-value
P Excretion	<i>Tipula</i>	Litter	1	5.59	0.022
		[P]	2	3.11	0.052
		Litter*[P]	2	8.43	0.001
		Error	57		
	<i>Pycnopsyche</i>	Litter	1	2.64	0.11
		[P]	3	12.47	<0.001
		Litter*[P]	3	1.53	0.217
		Error	55		

* Litter = maple or oak; [P] = phosphorus concentration during leaf litter incubation

Table S4. Analysis of covariance for molar ratios of carbon:phosphorus (C:P), carbon:nitrogen (C:N) and nitrogen:phosphorus (N:P) of FPOM produced by three shredders fed a diet stoichiometry gradient of maple and oak litter. Homogeneity of slopes between litter diets was tested by interaction ($P < 0.05$) in ANCOVA. Interactions were removed from ANCOVA models when $P > 0.05$. Where slopes were homogeneous, t -tests assessed the null hypothesis that FPOM stoichiometry changed with diet stoichiometry in a 1:1 manner (slope=1) among both litter types; where slopes were heterogeneous, t -tests assessed slope=1 within each litter type independently. Boldface indicates statistical significance ($P < 0.05$).

Variable	Shredder	Factor*	df	F-ratio	P-value	Litter	Slope	df	t-stat	P-value
FPOM C:P	<i>Lepidostoma</i>	Diet C:P	1	35.07	<0.001	Both	1.34	35	1.52	0.138
		Litter	1	0.01	0.916					
		Error	35							
	<i>Tipula</i>	Diet C:P	1	109.15	<0.001	Maple	2.04	33	6.86	<0.001
		Litter	1	11.69	0.001	Oak	0.70	27	1.35	0.188
		Diet C:P*Litter	1	26.23	<0.001					
		Error	60							
		Diet C:P	1	117.90	<0.001	Both	0.57	57	8.23	<0.001
	<i>Pycnopsyche</i>	Litter	1	19.95	<0.001					
		Error	57							
	FPOM C:N	<i>Lepidostoma</i>	Diet C:N	1	8.10	0.008	Both	0.70	29	1.20
Litter			1	3.56	0.069					
Error			29							
<i>Tipula</i>		Diet C:N	1	35.21	<0.001	Maple	3.20	33	9.95	<0.001
		Litter	1	138.84	<0.001	Oak	-1.10	27	7.59	<0.001
		Diet C:N*Litter	1	146.54	<0.001					
		Error	60							
		Diet C:N	1	5.78	0.019	Maple	1.10	31	0.32	0.754
<i>Pycnopsyche</i>		Litter	1	2.72	0.105	Oak	-0.16	26	2.96	0.006
		Diet C:N*Litter	1	4.93	0.030					
		Error	57							

Table S4 continued. Analysis of covariance for molar ratios of carbon:phosphorus (C:P), carbon:nitrogen (C:N) and nitrogen:phosphorus (N:P) of FPOM produced by three shredders fed a diet stoichiometry gradient of maple and oak litter. Homogeneity of slopes between litter diets was tested by interaction ($P < 0.05$) in ANCOVA. Interactions were removed from ANCOVA models when $P > 0.05$. Where slopes were homogeneous, t -tests assessed the null hypothesis that FPOM stoichiometry changed with diet stoichiometry in a 1:1 manner (slope=1) among both litter types; where slopes were heterogeneous, t -tests assessed slope=1 within each litter type independently. Boldface indicates statistical significance ($P < 0.05$).

Variable	Shredder	Factor*	df	F-ratio	P-value	Litter	Slope	df	t-stat	P-value
FPOM N:P	<i>Lepidostoma</i>	Diet N:P	1	6.73	0.015	Both	2.07	29	1.34	0.191
		Litter	1	3.13	0.088					
		Error	29							
	<i>Tipula</i>	Diet N:P	1	72.20	<0.001	Both	1.42	61	2.50	0.015
		Litter	1	0.01	0.905					
		Error	61							
	<i>Pycnopsyche</i>	Diet N:P	1	62.80	<0.001	Both	0.59	56	5.44	<0.001
		Litter	1	6.20	0.016					
		Error	56							

*Leaf=maple or oak

Table S5. Analysis of variance (*Pycnopsyche*) or analysis of covariance (*Lepidostoma*) for FPOM respiration rates. Boldface indicates statistical significance ($P < 0.05$).

Variable	Shredder	Factor*	df	F-ratio	P-value
FPOM Respiration	<i>Lepidostoma</i>	FPOM AFDM	1	206.75	<0.001
		Litter	1	3.03	0.092
		[P]	3	5.09	0.006
		Litter*[P]	3	4.19	0.013
		Error	31		
	<i>Pycnopsyche</i>	Litter	1	103.10	<0.001
		[P]	3	13.35	<0.001
		Litter*[P]	3	12.21	<0.001
		Error	51		

*Litter = maple or oak; [P] = phosphorus concentration during leaf litter incubation; FPOM AFDM = fine particulate organic matter ash free dry mass

CHAPTER IV

Diet and source animal affect carbon and nutrient dynamics of decomposing egesta from aquatic invertebrate shredders⁴

⁴Halvorson, H.M. D.J. Hall, and M.A. Evans-White. Formatted for submission to *Biogeochemistry*.

ABSTRACT

Animal egestion is a pronounced transformation of particulate organic matter in ecosystems, but roles of egestion as a transformation of nutrients remain understudied, constraining understanding of the significance of animals in ecosystem nutrient dynamics. Here, I investigated patterns of carbon (C), nitrogen (N), and phosphorus (P) dynamics during microbial decomposition of animal egesta in the laboratory. I collected egesta from the aquatic invertebrate shredder genera *Allocapnia*, *Lirceus*, and *Tipula* fed American Sycamore litter conditioned under low- or high-P concentrations. I measured egesta microbial respiration over 7 days and mass loss over 107 days as indicators of decomposition, and repeatedly measured egesta C/N/P content and uptake and release of dissolved organic C, ammonium, nitrate+nitrite, total nitrogen, and soluble reactive P over long-term decomposition. *Tipula* produced N- and P-deplete egesta compared to *Lirceus* and *Allocapnia*, and both decomposition measures indicated faster decomposition of *Tipula* and *Allocapnia* egesta compared to *Lirceus* egesta. Egesta exhibited consistent temporal shifts between release and uptake of dissolved constituents, regardless of diet or source animal, likely due to leaching and uptake/mineralization regulated by decomposer microbes. *Allocapnia* and *Lirceus* egesta exhibited greater nitrate+nitrite uptake than *Tipula* egesta, and egesta from low-P litter exhibited lower uptake of total N and net release of ammonium compared to net uptake of ammonium by egesta from high-P litter. My study supports pronounced links of animals to ecosystem carbon and nutrient dynamics via particulate egesta, conceptually analogous but biologically distinct from links via dissolved excreta.

INTRODUCTION

Across terrestrial, marine, and freshwater settings, animals can strongly affect ecosystem structure and function through the transformation of food resources into particulate feces, henceforth termed egestion (Malmqvist et al. 2001, Sitters et al. 2014, Cavan et al. 2015). In freshwaters, animal egestion plays significant roles in organic matter budgets, with benthic macroinvertebrates responsible for as much as two-thirds of fine particulate organic matter (FPOM) export from headwater streams (Cuffney et al. 1990) and the annual sedimentation of black fly fecal pellets approaching or exceeding magnitudes of annual leaf litter inputs in some boreal streams (Malmqvist et al. 2001). Animal egestion also provides valuable food resources for collector macroinvertebrates (Short and Maslin 1977, Heard and Richardson 1995, Bundschuh and McKie *in press*). Despite their potential importance in streams, however, contributions of egesta to ecosystem nutrient cycles remain poorly understood because nitrogen (N) and phosphorus (P) associated with egesta are often considered recalcitrant and not ecologically impactful compared to highly bioavailable animal excreta such as ammonium and phosphate (Vanni 2002, Liess and Haglund 2007).

Fine particulate organic matter is among the most diverse and significant but least understood forms of organic matter in streams, of which animal egesta form a part (Findlay et al. 2002, Tank et al. 2010). Aquatic FPOM is classified as material falling in the size range of 0.45 μm to 1 mm and, beyond animal egestion, can originate from processes as diverse as dissolved organic matter flocculation, terrestrial soil run-off, atmospheric deposition of dust, and physical fragmentation of organic matter such as senescent algae and leaf litter (Ward et al. 1994, Wallace et al. 2006). FPOM is ecologically significant as a substrate for bacterial biomass and activity (Findlay et al. 2002), as a dominant form of organic matter exported from stream ecosystems

(Webster et al. 1999, Benstead et al. 2009), and as a link between upstream and downstream ecosystems in the detrital processing chain (Short and Maslin 1977, Heard and Richardson 1995, Navel et al. 2011). While studies have also shown potential biogeochemical roles of FPOM in aquatic ecosystems, such as in uptake of dissolved nutrients (Newbold et al. 1982, Bonin et al. 2003) and denitrification (Arango et al. 2007), roles specific to animal-derived FPOM (egesta) as a subset of the wide diversity of FPOM remain poorly studied. Evidence does indicate animal-derived FPOM can be significant sources of dissolved nitrogen (Le et al. 2016) and dissolved organic carbon (Yoshimura et al. 2010). However, previous studies remain mostly limited to egesta from the amphipod shredder *Gammarus* spp., track fates of egesta over short timeframes of days to weeks, and have not yet tracked simultaneous dynamics of multiple elements (carbon (C), N, and P) during microbial decomposition. Long-term patterns of egesta carbon and nutrient dynamics may be especially important to scale the significance of animal egestion up to ecosystem levels, considering egesta accumulate in depositional areas and exhibit slow decomposition over months to years (Joyce et al. 2007, Joyce and Wotton 2008, Yoshimura et al. 2008).

Given their shared form as particulate organic matter, egesta may undergo similar processes of leaching, microbial conditioning, and fragmentation of better-known plant litter during decomposition (Webster and Benfield 1986, Gessner et al. 1999). Though there are scarce data regarding long-term, coupled carbon and nutrient dynamics of FPOM during decomposition, sequential processes of decomposition will affect whether egesta serve as sources or sinks of carbon and nutrients to the water column over time (Mulholland 2004, Cheever et al. 2013, Pastor et al. 2014). Much like plant litter, egesta exhibit wide physical and chemical characteristics, driven by diet and the source animal, which likely affect short- and long-term

microbial decomposition (Joyce et al. 2007, Halvorson et al. 2015a). However, egesta are unique from coarse particulate organic matter in their size, shape, and chemical binding (Ladle and Griffiths 1980, Wotton and Malmqvist 2001, Yoshimura et al. 2008), their probable dominance by bacterial and not fungal decomposers (Findlay et al. 2002, Jing et al. 2012), their initial inoculation by microbes from the source animal additional to colonization by microbes in the environment (Joyce et al. 2007, Jing et al. 2012), and unique biochemistry and nutrient composition associated with feeding and assimilation by the source animal (Clissold et al. 2010, Hood et al. 2014, Halvorson et al. 2015a). Together, these differences may drive diverse, unique roles of animal egesta in the biogeochemistry of freshwaters, apart from those of plant litter and other forms of organic matter.

Diet and the source animal both control initial egesta characteristics and may therefore affect long-term fates of animal egesta in ecosystems. To achieve nutritional homeostasis, animals fed higher-nutrient diets will tend to produce higher-nutrient wastes, because assimilation even of limiting nutrients can be as low as 40% efficient and animals can regulate assimilation to egest excess nutrients (Clissold et al. 2010, Hood et al. 2014, Fuller et al. 2015, Halvorson et al. 2015a,b). Elevated N or P content of egesta, in turn, may support microbial growth and stimulate decomposition (Enriquez et al. 1993, Yoshimura et al. 2008). Because source animals also vary in particle size, chemical composition, and binding of egesta (Joyce et al. 2007 Patrick 2013, Halvorson et al. 2015a), animal identity may similarly affect decomposition and nutrient dynamics over decomposition. For example, larger-bodied *Limnephilid* caddisflies produce larger fecal pellets compared to the isopod *Caecidotea* and the amphipod *Hyaella* (Patrick 2013), and larger fecal pellets provide lower substrate surface area: volume ratios that may slow microbial decomposition (Hargrave 1972, Atkinson et al. 1992).

Lower surface area:volume ratios may additionally prevent microbial access to endogenous (particulate) nutrients and increase relative microbial reliance on exogenous (dissolved) nutrients for growth, causing greater overall microbial uptake of dissolved nutrients from the water column during decomposition (Cheever et al. 2013). Furthermore, microbial decomposition associated with larger particle sizes or low N and P content may decrease or delay mineralization of egesta nutrients (Manzoni et al. 2010). Given their potential complexity, characteristics controlling long-term fates of egested carbon and nutrients must be investigated to link food resources and animal community composition to ecosystem structure and function (Vanni 2002, Patrick 2013, Bundschuh and McKie *in press*).

The objective of this study was to investigate short-term (daily) to long-term (monthly) patterns of carbon and nutrient leaching, uptake, and mineralization during microbial decomposition of egesta from three distinct but common aquatic shredder-detritivore invertebrate taxa (*Allocapnia* sp., *Lirceus* sp., and *Tipula* sp.) fed low- versus high-nutrient leaf litter in the laboratory. Preliminary findings (Halvorson et al. 2015a; Halvorson, unpublished data) led to hypotheses that *Lirceus* would produce the highest-P egesta, followed respectively by *Allocapnia* and *Tipula*, whereas *Allocapnia* would produce the highest-N egesta, followed respectively by *Lirceus* and *Tipula*, and that higher-P diets would result in greater egesta N and P content. I also hypothesized higher-nutrient egesta would exhibit greater rates of nutrient leaching and mineralization as well as greater rates of microbial decomposition, measured short-term as microbial O₂ uptake and long-term as mass loss.

METHODS

Leaf litter conditioning and incubation

On November 4, 2015 I collected recently-senesced leaves of American Sycamore (*Platanus occidentalis*), a dominant riparian tree in the Ozark Highlands ecoregion (Sagers and Lyon 1997), from the dry streambed of London Creek, a third-order tributary of the West Fork of the White River in Washington County, Arkansas. Leaves were returned to the laboratory, removed of stems, cut into approximate 5 cm x 5 cm pieces, dried at 48°C for 2 d, and divided among 30 leaf bags each containing 20 leaf pieces. Bags were then split evenly among 2 tubs containing 20 L dechlorinated tap water to begin leaching. Water in tubs was flushed and replaced twice over a 3 d leaching period.

After the leaching period, a small subset of leaf pieces was collected to determine substrate elemental content prior to microbial conditioning. Leaves were then given 20 L dechlorinated tap water and amended with either 20 $\mu\text{g L}^{-1}$ or 200 $\mu\text{g L}^{-1}$ P as Na_2HPO_4 , designated low- and high-P tubs, respectively. Each tub also received 1000 $\mu\text{g L}^{-1}$ N- NO_3 as KNO_3 . These nutrient levels were chosen to produce contrasting N and P content of litter prior to feeding to shredders (Scott et al. 2013, Halvorson et al. 2015a). Leaves were inoculated with 50 mL mixed litter slurry from London Creek. Tub s were constantly aerated and water was flushed and replenished with 20 L dechlorinated tap water with appropriate nutrient amendments every 2-3 d over a period of 25 days prior to feeding to shredders.

Field collection and feeding of aquatic invertebrate shredders

In early December 2015, I collected individuals of the taxa *Allocapnia* sp. (Plecoptera: Capniidae), *Lirceus* sp. (Isopoda: Asellidae), and *Tipula* sp. (Diptera: Tipulidae) over a 3 d period at London Creek. These genera were chosen because they were the dominant shredders in London Creek at the time of sampling and because they represented distinct evolutionary lineages across Arthropoda (Carapelli et al. 2007). Animals were returned to an environmental

chamber at the University of Arkansas set to a 10:14 light:dark cycle and 10°C. All animals were kept in aerated stream water and were given plentiful mixed litter from London Creek prior to assignment to aquaria.

I filled 30 plastic aquaria each with 1.5 L 250 µm-sieved stream water. Aquaria were constantly aerated and equipped with a 500 µm mesh insert. Aquaria were randomly assigned one of the 3 shredder genera (*Allocapnia*, *Lirceus*, or *Tipula*) and one of the 2 leaf litter P treatments (high or low) for 5 replicated aquaria in each treatment combination. Each aquarium received all conditioned litter pieces from a leaf bag of the assigned P level, with the exception of 2 pieces from each bag subsampled to determine initial elemental content (see below). I placed known numbers of 25-36 *Lirceus*, 30-45 *Allocapnia*, and 6-9 *Tipula* individuals in each appropriate aquarium and ensured equal total numbers of individuals across high-nutrient and low-nutrient aquaria. Because I did not control for body size, animals assigned represented the range in body size of each taxon at the time of field collection. Fewer *Tipula* were used because they were of higher biomass. I purposefully varied numbers of individuals in aquaria to provide variable egesta dry mass (DM) across sample units, permitting use of egesta DM as a covariate during statistical analyses (see below).

Long-term egesta decomposition: Set-up

After 2 days of animal feeding and acclimation, water was changed in all aquaria and replaced with fresh, 37-µm sieved stream water to begin an egesta accrual period. After 5 days, leaf litter and animals were removed by extracting the mesh insert of each aquarium. Accrued egesta in a given aquarium were collected on a 37 µm mesh, and the mesh was then inverted to resuspend egesta in 40 mL filtered (1 µm pore size) stream water. The suspension was gently shaken to homogenize egesta into a slurry, and 7 separate 5 mL subsamples were collected and

disbursed among 7 plastic specimen cups containing 55 mL filtered (1 μm pore size) stream water. Cups were given plastic lids drilled with 2 small (6 mm diameter) holes to permit atmospheric gas exchange, placed randomly in the environmental chamber, and randomly assigned a sampling day (2, 9, 23, 37, 51, 79, or 107 days). After disbursing egesta for a given aquarium, leaf litter and animals were transferred back to the aquarium and allowed to continue a second period of egesta accrual in 1.5 L fresh 37 μm sieved stream water. During disbursement of egesta, an additional 21 control cups (3 sets of 7) containing 60 mL stream water but no egesta were also interspersed randomly among egesta cups in the environmental chamber and randomly assigned among the 7 sampling days. Given 30 aquaria plus 3 sets of controls and 7 cups each, this totaled to 231 decomposition cups.

Short-term egesta decomposition

After 4 additional days of accrual in aquaria, I collected egesta for short-term microbial respiration trials as an indicator of decomposition. For a given aquarium, egesta were collected on a 37 μm mesh and resuspended in 25 mL filtered (1 μm pore size) stream water. I collected 3 separate 5 mL aliquots of the suspension, filtered the egesta onto pre-weighed and pre-combusted 1 μm glass fiber filters (Pall Inc., Port Washington, NY), and placed each filter into a 23 mL scintillation vial filled with vigorously aerated, filtered stream water to measure microbial respiration as oxygen (O_2) uptake over time. I also collected 5 mL of egesta suspension from each aquarium for particle size measures (see below). Vials were sealed with septa caps to prevent gas exchange with the atmosphere, checked to ensure there were no air bubbles, and placed in the dark at 10°C. Each of 3 vials used for a given aquarium was randomly assigned a respiration duration of 2, 4, or 7 d prior to measurement of dissolved O_2 . A subset of 9 vials (3 sets of 3 vials) was also started with 23 mL filtered stream water and a pre-combusted filter but

no egesta to control for background microbial respiration of stream water. All vials were inverted once daily during respiration trials to enhance homogeneity of dissolved gases.

On a given sampling day, water in vials was measured for dissolved O₂ concentrations using a membrane inlet mass spectrometer (MIMS; Kana et al. 1994). The MIMS determines the ratio of dissolved O₂ : Argon, and uses the ratio and concentration of inert Argon to determine the concentration of O₂ dissolved in water. On a given sampling day, I determined total mg O₂ uptake as the difference between total experimental vial O₂ content and average total O₂ content of control vials. After measurement of O₂ using the MIMS, vials were kept at 5°C in the dark until all samples had been measured for O₂ concentration, after which all filters and egesta were transferred to pre-weighed tins, dried at 50°C, desiccated and re-weighed to determine egesta DM.

Once all vials had been sealed for respiration trials, I removed leaf litter from aquaria, counted the number of surviving individuals, and allowed animals to clear their guts overnight. After gut clearance, animals were frozen. After 10 days in aquaria, mean survivorship was 93%, 92%, and 96% for *Allocapnia*, *Lirceus*, and *Tipula*, respectively.

Long-term egesta decomposition: Sampling

Shortly after disbursing egesta among cups for the long-term decomposition experiment, I began sampling cups at their designated sampling day into the decomposition experiment. On a given day, egesta were filtered onto pre-weighed, pre-combusted 1 µm glass fiber filters (Pall Inc., Port Washington, NY). Filters were oven dried at 48°C for 24 h, desiccated, and weighed to determine egesta DM. Filters were stored dry at room temperature until elemental analysis (see below). I used egesta DM to calculate a mass loss coefficient k (d⁻¹) of egesta across all 7 cups

from each aquarium based on the negative slope between $\log_{10}(\text{egesta DM})$ and sampling date over the 107 d study (Benfield 2006).

Aliquots of the associated filtrate were collected and kept on ice for a maximum of 12 hours prior to determination of concentrations of N as ammonium (N-NH₄) and P as soluble reactive phosphorus (SRP). Aliquots were also collected and kept on ice until freezing to determine concentrations of N as nitrate+nitrite (N-(NO₃ + NO₂)), total N (TN) and dissolved organic C (DOC). Vials frozen for TN/DOC determination were acidified with 1 N hydrochloric acid to achieve pH<2 prior to freezing. Beginning on day 23, I noticed measurable water loss to evaporation and, prior to sampling, I weighed each cup to the nearest 0.01 g and subtracted mean mass of dry plastic cups to determine water volume.

After day 23 and subsequently every 3 weeks over decomposition, I amended remaining control and experimental cups with stock nutrient solution to replace water lost to evaporation and supply initial P-SRP and N-NH₄ concentrations measured in filtered stream water at the beginning of the decomposition experiment. Nutrient amendments consisted of 1, 5, or 10 mL of stock solution at appropriate stock concentrations to amend equal absolute amounts of SRP and N-NH₄ (target concentrations: 6 $\mu\text{g P-SRP L}^{-1}$ and 10 $\mu\text{g N-NH}_4 \text{ L}^{-1}$) in 60 mL water for each cup. I varied the volume added across cups according to the extent of evaporation across cups; volumes were chosen to achieve approximately 60 mL water in each cup after amendment.

Filtrate collected during the decomposition study was analyzed for N-NH₄ and P-SRP concentrations using the phenate and ascorbic acid methods, respectively (APHA 2005). Acidified frozen samples were thawed and analyzed to determine DOC and TN concentrations using a Shimadzu TOC-V CSH equipped with a TNM-1 analyzer (Shimadzu Scientific Instruments, Kyoto, Japan). Frozen but not acidified samples were also thawed and analyzed for

N-(NO₃+NO₂) concentrations using the cadmium reduction method on a Lachat QuickChem 8500 Autoanalyzer (Lachat Instruments, Hach Company, Loveland, CO). Some samples exhibited constituent concentrations below the minimum detection limit (MDL) for a given analysis; concentrations of these samples were assumed to be half the MDL. The MDLs were determined as concentrations distinguishable two standard deviations above zero and were 1.85 µg L⁻¹ P-SRP, 4.73 µg L⁻¹ N-NH₄, 8.44 µg L⁻¹ N-NO₃, 23 µg L⁻¹ TN, and 223 µg L⁻¹ DOC. For each sample, total dissolved N-NH₄, P-SRP, DOC, TN, and N-(NO₃ + NO₂) were calculated as the concentration multiplied by cup water volume; total exchange of each constituent from egesta to the water column was determined as the difference between total dissolved constituent in experimental cups minus the average of three control cups on a given sampling date. Negative total exchange thus indicates net uptake whereas positive total exchange indicates net release of dissolved constituents by egesta.

Measurement of litter and egesta elemental content

Leaf litter and egesta filters collected over the decomposition experiment were analyzed for total C, N, and P content. Litter pieces were oven-dried and homogenized into fine powder using a wig-1-bug (Crescent Dental Manufacturing, Elgin, IL). Each egesta filter was cut in half, each half weighed to the nearest 0.1 mg, and each half was assigned analysis for either P or C/N content. Filters with low egesta DM (<2 mg) were either cut into one-quarter and three-quarter pieces for P and C/N content, respectively, or analyzed only for C/N to ensure detectable C and N content. Leaf powder and filter pieces assigned for P content analysis were combusted at 500°C for 3 h, digested in 1 N hydrochloric acid at 85°C, and diluted in 50 mL water prior to analysis for SRP using the ascorbic acid method (APHA 2005). Leaf powder and filter pieces assigned for C and N content analysis were folded into tins and analyzed using a Flash 2000

CHN analyzer (Thermo Scientific, Waltham, MA). Measured P and C/N content for each filter piece were divided by the piece's mass proportion of total filter mass to calculate total filter C, N, and P content. Total P contents of each egesta filter were subtracted from that of control filters from control (no egesta) cups collected over the experiment to determine total P content of egesta; because control filters had low (<0.1 mg) dry mass below instrument detection limits, they were not measured for C/N content. Total measured C, N, and P contents of egesta were divided by egesta DM to determine % C, N, and P and determine molar C:N, C:P, and N:P ratios of egesta.

Egesta particle size measurement

Particle sizes of egesta were determined from 5 mL subsamples collected at the outset of microbial respiration trials. One day after collection, I used an ocular micrometer to measure length and width dimensions of 10 random particles (fecal pellets or large, clumped debris) to the nearest 30 μm under 35X magnification. I also noted the characteristic shape and binding of particles. I calculated mean size of each particle as the average length and width, and for each aquarium I determined mean particle size (μm) across all measured particles. Particle size samples were subsequently returned to vials and placed in the environmental chamber.

Ninety days later, I wet-sieved the same particle size samples to determine the distribution of particle sizes further into decomposition. Samples were passed through subsequently smaller mesh sizes of 250, 120, 64, and 37 μm . Particles that did not pass through a given mesh were filtered onto pre-weighed 1 μm filters and filters were oven dried and weighed to determine the mass proportion of particles within the following size classes: >250, 120-250, 64-120, 37-64, or 1-37 μm .

Statistical analysis

I used two-way analysis of variance (ANOVA) to test effects of shredder genus, diet P level, and their interaction on initial mean particle size, mass loss coefficients k , and egesta elemental contents (%C, %N, %P) and molar ratios of C:N, C:P, and N:P across aquaria. I used repeated-measures analysis of covariance (rmANCOVA) to examine effects of the factors time, detritivore genus, and diet nutrient level, along with the covariate egesta DM (mg), on total O₂ uptake over the short-term decomposition study and total exchange of dissolved constituents (N-NO₃, N-NH₄, TN, P-SRP, and DOC) over the long-term decomposition study. In the rmANCOVA, effects of time and its interaction with genus or litter P level were examined within-subjects (aquaria) using vials or cups as the repeated sample unit, whereas effects of egesta DM (covariate), genus, and nutrient level were examined across-subjects (aquaria). In the across-aquaria ANCOVA, I checked for factor*covariate interactions to test homogeneous slopes. Where slopes were heterogeneous (factor*covariate interaction $P < 0.05$), I broke up the ANCOVA into each main effect to investigate which slopes differed across factor levels. I Bonferroni-adjusted significant α within each analysis type (ANOVA or rmANCOVA). Response variables were log-transformed where necessary to achieve homogeneity of variances. All statistical analyses were conducted in R version 3.1.2 (2014, R Foundation for Statistical Computing).

RESULTS

Leaf litter diet and egesta elemental content

Microbial conditioning of leaf litter did not change %C content, but increased N content from mean 1.36 %N to 1.63 and 1.68 %N in the low- and high-P treatments, respectively (Table 1). Conditioning did not change litter P content from initial litter in the low-P treatment, but elevated litter mean %P content from 0.095 to 0.129 %P in the high-P treatment. Strong effects

of P concentrations on conditioned litter %P resulted in divergent molar C:P and N:P between diet P levels (Table 1).

Differences between diet P treatments and across shredder genera produced a wide range of egesta N and P content across treatments (Table 2, Fig. 1). Egesta %N was not affected by diet P level, but indicated greater N content of egesta from *Lirceus* and *Allocapnia* (mean \pm SE = 2.16 ± 0.12 %N) compared to egesta from *Tipula* (1.56 ± 0.09 %N; Fig. 1C). Across treatments, there was a nearly three-fold range of egesta P content, from mean \pm SE 0.078 ± 0.003 %P (*Tipula* fed low-P litter) to 0.212 ± 0.009 %P (*Allocapnia* fed high-P litter). Egesta from low-P diets exhibited significantly lower %P content than egesta from high-P diets, and *Tipula* produced egesta of significantly lower %P content than *Lirceus* or *Allocapnia* (Table 2, Fig. 1E). Molar C:N and C:P ratios of egesta were not affected by diet P level, but were significantly higher among egesta from *Tipula* compared to egesta from *Allocapnia* or *Lirceus* (Fig. 1B,D). Egesta molar N:P contents were affected by both diet P level and shredder genus, with N:P ratios lowest among *Allocapnia* fed high-P litter and highest among *Tipula* fed low-P litter (Fig. 1F). *Lirceus* and *Allocapnia* consistently increased whereas *Tipula* consistently decreased egesta N and P content relative to N and P content of litter diets (Table 1, Fig. 1).

Particle size measurements

The size of initial egested particles did not differ between diet P levels, but ranged from diameter of mean \pm SE 212 ± 9 μ m (*Tipula*) to 326 ± 7 μ m (*Allocapnia*) to 350 ± 11 μ m (*Lirceus*), with particles from the latter two genera significantly larger than those of *Tipula* (Fig. 2; Table 2). Egesta from *Allocapnia* and *Lirceus* took the form of cylindrical, bound fecal pellets, interspersed with small fragments and debris, whereas egesta from *Tipula* consisted of diffuse clumps of amorphous detritus intermingled with small debris and occasional leaf fragments.

After 90 days' decomposition, fecal pellets of both *Allocapnia* and *Lirceus* had mostly disintegrated into very small particles, given 40-52% mass proportions in the smallest 1-37 μm size class (Fig. 3) that was excluded during initial disbursement of samples. In contrast, egesta from *Tipula* retained their large size, with the dominant size class (64-66% by mass) in the 120-250 μm size class that overlapped with mean diameter of initial particles (Figs. 2,3).

Dissolved constituent exchange during decomposition

Egesta shifted from low release or uptake of DOC starting on day 23 to consistently strong uptake of DOC, especially on day 79, during long-term decomposition (Table 3, Fig. 4A,B). The ANCOVA indicated total exchange of DOC was affected by an interaction of egesta DM and diet P level (Table 3), signifying heterogeneous slopes. The positive relationship between egesta DM and DOC exchange across aquaria was stronger among egesta from low-P litter than egesta from high-P litter diets (Fig. S1). The relationship between egesta DM and DOC exchange was weaker and did not differ between groups when broken up among shredder genera (Table 3).

Total N-NH₄ exchange was mostly negative over the first 37 days of decomposition, indicating net uptake, and switched to weakly positive starting on day 51, indicating net release by egesta (Fig. 4C,D). Egesta from low-P diets exhibited greater, net average release of N-NH₄ (mean \pm SE exchange = $0.148 \pm 0.078 \mu\text{g N-NH}_4$) compared to egesta from high-P diets that exhibited lower, net average uptake of N-NH₄ ($-0.059 \pm 0.018 \mu\text{g N-NH}_4$; Table 3, Fig. 4C,D). Egesta from *Allocapnia* also exhibited marginally higher N-NH₄ exchange (net release) than egesta from other genera, with notably high release late into decomposition (Table 2, Fig. 4C,D).

Total N-(NO₃+NO₂) exchange was consistently negative over the duration of the study, from outset and leading up to a strong negative peak on day 23, indicating net uptake by egesta.

Uptake became negligible by day 51 (Fig. 4E,F), after which N-(NO₃+NO₂) concentrations were below MDL in all samples. The ANCOVA indicated a significant negative relationship between egesta DM and N-(NO₃+NO₂) exchange (Table 3; Fig. S2), but slopes did not differ between treatments (P>0.05). Egesta from *Allocapnia* and *Lirceus* exhibited significantly greater N-(NO₃+NO₂) uptake compared to egesta from *Tipula*, after removing effects of egesta DM using ANCOVA (Table 3; Figs. 4E,F, S2).

Egesta exhibited significant temporal variation in total exchange of TN over the study, generally shifting from high uptake at the beginning to low uptake or, in some cases, net release later into the experiment (Table 3, Fig. 4G,H). Total exchange of TN was not related to egesta DM, but similar to N-NH₄ exchange, was significantly higher among egesta from low-P diets (-0.665 ± 0.467 µg TN) compared to egesta from high-P diets (-2.137 ± 0.366 µg TN; Table 3, Fig. 4G,H).

Total P-SRP exchange also varied significantly over time, exhibiting a consistent shift from net release on days 2 and 9, to net uptake on day 23, back to net release from day 37 forward in all treatments (Fig. 4I,J). Notably, P-SRP exchange was positively associated with egesta DM across all aquaria, but did not differ across diet P treatments or shredder genera (Table 3, Fig. S3).

Short- and long-term egesta decomposition

Over short-term trials, concentrations of O₂ in egesta respiration vials were consistently below those of control vials, indicating net O₂ uptake by egesta. Total O₂ uptake by egesta over short-term respiration trials increased over time, consistent with increasing cumulative microbial respiration over the 7 d period (Table 3, Fig. 5). Total O₂ uptake was not related to egesta DM,

and did not differ between diet P levels, but was significantly greater for egesta from the genera *Allocapnia* and *Tipula* compared to egesta from *Lirceus* (Table 3, Fig. 5).

Long-term egesta decomposition rates, measured as dry mass loss coefficients k over 107 days, were immeasurable among some treatments, given negative mass loss coefficients of some egesta (Fig. 6). Two-way ANOVA revealed significant differences in decomposition across shredder genera (Table 2), with egesta from *Tipula* and *Allocapnia* exhibiting measurable, faster mass loss (mean \pm SE $k = 0.00021 \pm 0.00008 \text{ d}^{-1}$) than egesta from *Lirceus* exhibiting immeasurable, slower mass loss ($k = -0.00052 \pm 0.00031$; Fig. 6).

To examine the relationship between short- and long-term measurements of egesta decomposition, I conducted a subsequent linear regression of egesta mass loss coefficients against DM-specific O_2 uptake rates. Across all treatments, there was a significant positive relationship ($P < 0.05$) between log-transformed DM-specific egesta O_2 uptake rates and long-term egesta mass loss rates (Fig. 7).

DISCUSSION

My data indicate strong effects of diet and source animal characteristics on short- and long-term fates of egesta carbon and nutrients, providing data necessary to understand extended roles of animal egesta in freshwater ecosystems. Previous studies have indicated significant potential for both diet and animal taxonomy to affect animal-derived FPOM nutrient content and release (Patrick 2013, Hood et al. 2014, Halvorson et al. 2015a, Le et al. 2016), but there are sustained calls for further empirical work to understand links to ecosystem function (Tank et al. 2010, Bundschuh and McKie *in press*). My findings improve upon those previous in their wide, robust inter-taxonomic comparison of shredders from the same ecosystem, simultaneously fed one of the same two diets. Furthermore, my work expands on previous studies of egesta nutrient

dynamics and decomposition by extending the duration of measurements and tracking uptake/release dynamics of multiple elements during microbial decomposition of egesta.

Egesta from *Tipula* versus *Lirceus* and *Allocapnia* exhibited consistently divergent N and P contents, likely due to taxon-specific patterns of selective feeding and assimilation prior to egestion. As a relatively immobile organism, *Tipula* may not employ selective feeding and may instead use post-ingestive strategies (selective assimilation) to incorporate growth-limiting nutrients, resulting in nutrient-deplete egesta. Studies suggest *Tipula* strongly relies on microbial symbionts for digestion, especially of cellulose (Sinsabaugh et al. 1985, Canhoto and Graça 2006), and is well-adapted for digestion of microbial protein (Martin et al. 1980). My study reinforces evidence that *Tipula* may be unique among diverse shredder taxa in its production of N- and P-deplete egesta relative to litter diets (Hood et al. 2014, Halvorson et al. 2015a). In contrast, both *Lirceus* and *Allocapnia* are highly mobile and may be selective feeders, such as of better-conditioned patches of litter (Arsuffi and Suberkropp 1989) or of nutrient-rich biofilm over nutrient-deplete litter substrate, increasing N and P ingestion and producing N- and P-rich egesta relative to bulk litter diets (Hood et al. 2014, Halvorson et al. 2015a). Taxon-specific signatures of egesta stoichiometry could modify the stoichiometry of bulk FPOM in stream ecosystems to affect growth of collector species (Veldboom and Haro 2011, Callisto and Graça 2013) as well as particulate nutrient standing stocks and export fluxes downstream (Vanni et al. 2001, Inamdar et al. 2015).

Nutrient contents of particulate organic matter, including animal-derived FPOM, are often positively associated with microbial decomposition (Enriquez et al. 1993, Yoshimura et al. 2008, Halvorson et al. 2015a). I expected greater egesta N and P content would increase microbial decomposition measured as both short-term respiration and long-term mass loss.

However, while *Lirceus* egesta were relatively high in N and P content, they consistently decomposed more slowly, based on respiration and mass loss, than egesta from *Tipula* and *Allocapnia*. These findings are inconsistent with my hypotheses and suggest strong roles of the source animal in controlling microbial decomposition of egesta. Both *Allocapnia* and *Lirceus* appear to produce similar-sized fecal pellets, bound by peritrophic membrane that may constrain microbial colonization in the first days of decomposition (Joyce et al. 2007, Jing et al. 2012). Moreover, *Allocapnia* and *Lirceus* exhibited similar patterns of particle fragmentation into very small sizes toward the end of my study (Fig. 3). Greater microbial respiration of *Allocapnia* egesta may indicate greater initial microbial activity compared to *Lirceus* egesta, leading to long-term differences in mass loss. Taxonomic differences in egesta microbial activity, especially during early stages, could reflect divergent presence/absence or community composition of animal gut flora, suggesting derived or resident invertebrate gut microbes may affect roles of egesta in ecosystem structure and function (Harris 1993).

Existing theory and evidence suggest that animal egesta, like leaf litter (Gessner et al. 1999, Wallace et al. 2008), are net sources of dissolved organic carbon and nutrients immediately after release by the source animal, due to physical leaching of soluble constituents (Yoshimura et al. 2010, Le et al. 2016). Egesta were sources of DOC and P-SRP, but not consistently sources of dissolved N immediately after release by the animal, given net uptake of N-NH₄, TN, and N-(NO₃+NO₂) in most treatments during the first three weeks of decomposition. Although egesta likely exhibit leaching of some digested but not assimilated nutrients, such as amino acids measurable as dissolved organic N (Le et al. 2016), microbes colonizing egesta from the beginning of decomposition appear to assimilate inorganic N, outpacing leaching and driving net N uptake. Egesta may have exhibited P-SRP release over the first two weeks of the

experiment due to leaching of soluble P or microbial mineralization outpacing uptake. Microbes were likely N-limited over the experiment, given high P and low N:P contents of leaf litter and egesta, as well as drawdown of N-NH₄ and N-(NO₃+NO₂) concentrations, signifying microbial demand for dissolved N (Cheever et al. 2013). As decomposition advanced, egesta began to net uptake both P-SRP (day 23) and DOC (day 51), perhaps due to colonization and subsequent growth of microbes from the water column (Jing et al. 2012). A pronounced shift back to net release of P-SRP after day 23 would be consistent with microbial P mineralization, perhaps stoichiometrically regulated as a consequence of low substrate N:P (Frost et al. 2005, Manzoni et al. 2010), whereas continued net uptake of DOC may signify C-limitation of microbial growth late into decomposition, perhaps due to depletion of labile C in egesta. Given significant standing stocks of FPOM in stream ecosystems (Findlay et al. 2002), these temporal patterns of dissolved constituent release/uptake over decomposition may strongly affect long-term freshwater carbon and nutrient dynamics. My findings should be compared to those from other forms of FPOM to discern whether animal egesta are the norm or the exception among diverse biogeochemical roles of FPOM in ecosystems (Bundschuh and McKie *in press*).

Although the temporal trends in dissolved constituent exchange were similar across all egesta, my study revealed marked differences in total N-NH₄, and N-(NO₃+NO₂) uptake/release over decomposition, depending on the source animal. Despite their greater N content, egesta from *Allocapnia* and *Lirceus* exhibited significantly higher N-(NO₃+NO₂) uptake than egesta from *Tipula*. These trends are opposite to predictions of ecological stoichiometry theory, given microbes should exhibit greater demand for dissolved N to decompose lower-N egesta from *Tipula* (Manzoni et al. 2010). Bacteria colonizing *Tipula* egesta may be derived from the *Tipula* gut, where, compared to bacteria colonizing *Allocapnia* and *Lirceus* egesta, they are adapted to

“mine” organic N efficiently (Martin et al. 1980, Mooshammer et al. 2012), reducing reliance on inorganic N throughout decomposition. Furthermore, smaller sizes and comparatively irregular and diffuse binding of *Tipula* egesta would increase egesta surface area:volume ratios, permitting better microbial access to organic N contained in egesta early into decomposition. Larger fecal pellet size, in addition to the peritrophic membrane of *Allocapnia* and *Lirceus* egesta (Joyce et al. 2007), may comparatively reduce microbial access to organic N contained within fecal pellets until fragmentation, necessitating microbial reliance on dissolved inorganic N during the first days and weeks of decomposition. After pellet fragmentation, microbes may gain access to egesta organic N and, especially in the case of high-N content *Allocapnia* egesta, mineralize excess N as NH_4 late into decomposition. Similar to divergent, relatively short-term animal contributions to dissolved N and P availability due to taxonomically variable excretion (Vanni et al. 2002, Evans-White and Lamberti 2006, Capps et al. 2015), my data show potential for taxonomically-variable egesta to exert divergent, long-term effects on dissolved N availability in streams.

The two leaf litter diets also differed in exchange of N- NH_4 and TN between egesta and the water column during decomposition, expanding evidence that background nutrient concentrations alter roles of animals in freshwater ecosystems (Evans-White and Lamberti 2006, Wilson and Xenopolous 2011). Lower net average uptake of TN of egesta from low-P litter, in addition to net average release instead of uptake of N- NH_4 over decomposition, would indicate microbes used and retained dissolved N more strongly when decomposing egesta from high-P litter. Given high-P litter egesta were higher in %P but similar in %N content, driving lower N:P content relative to egesta from low-P litter, dietary differences were likely associated with stronger microbial N demands due to a greater relative degree of N-limitation on high-P litter

egesta (Mooshammer et al. 2012). Together with trends across taxa, my findings suggest higher-P egesta broadly serve as greater sinks of dissolved N in stream ecosystems compared to lower-P egesta. *Platanus occidentalis* litter used in the present study was quite high in P content, resulting in high-P content of egesta; future studies would benefit from comparison to N dynamics of egesta from shredders fed higher-N and lower-P content litter such as alder, maple, or oak species (Kendrick and Benstead 2013, Danger et al. 2013, Halvorson et al. 2015a), where egesta microbes may exhibit P-limitation.

My study reveals a positive relationship between short-term microbial respiration and long-term mass loss as measures of egesta microbial decomposition, suggesting the former measure may provide a useful indicator of FPOM breakdown in streams (Webster et al. 1999, Callisto and Graça 2013). Microbial respiration measures have distinct advantages, such as the possibility of field measurements over short time frames, and are not as labor-intensive as long-term mass loss measurements. The linear regression indicated net positive and measurable egesta mass loss above microbial respiration rates of $5.53 \text{ mg O}_2 \text{ mg}^{-1} \text{ DM d}^{-1}$ (Fig. 7). Respiration rates below this cut-off may represent background respiration by microbes not directly associated with egesta mass loss, attributable to maintenance respiration or indirect stimulation of respiration by water column microbes due to presence of egesta (e.g. respiration of leached DOC; Yoshimura et al. 2010). Negative or immeasurable long-term mass loss in some treatments reflects methodological error during initial disbursement of egesta and small losses of particles during filtering; these samples were mostly those of lowest overall DM ($<2 \text{ mg}$) where measurement error would have the greatest proportional effect on mass loss calculations. Future mass loss studies would benefit from greater initial DM of egesta, where possible. Among egesta exhibiting measurable mass loss, mean \pm SE mass loss rate k was $0.00033 \pm 0.00006 \text{ d}^{-1}$,

approximately one third of FPOM mass loss rates reported in field studies (Webster et al. 1999, Jackson and Vallaire 2007, Yoshimura et al. 2008). Lower mass loss rates in my study may be attributable to the onset of nutrient limitation (especially drawdown of nitrate) during decomposition, initial exclusion of very fine particles (<37 μm) that may exhibit faster microbial decomposition (Jackson and Vallaire 2007), and exclusion of macroinvertebrate collectors that would facilitate breakdown of egesta *in situ* (Joyce et al. 2007).

Though there are artifacts of conducting this study in a laboratory setting, my study tests novel pathways for resource characteristics and animal taxonomic identity to affect ecosystem nutrient cycles (Urabe and Elser 1999, Vanni 2002) and elucidates contributions of animals to the diverse attributes and functional roles of FPOM in stream ecosystems (Tank et al. 2010, Bundschuh and McKie *in press*). Future studies would benefit from more frequent measurement of particle size as an indicator of surface area:volume of particles over decomposition (Joyce et al. 2007, Joyce and Wotton 2008), as well as measurement of bacterial community composition, biomass, and production to examine differences across shredder taxa and subsequent effects on microbial decomposition. My findings indicate anthropogenic forces that alter resource nutrient content and/or animal community composition, such as nutrient enrichment or species invasions and extirpations (Cross et al. 2003, Evans-White et al. 2009, Capps et al. 2015), may indirectly affect stream ecosystem function by changing attributes and fates of animal egesta (Bundschuh and McKie *in press*). Future studies should further investigate variable controls and fates of animal egesta in ecosystems and scale my findings up to whole-ecosystem levels, especially considering long-term effects of egested carbon and nutrients on stream ecosystem structure and function.

ACKNOWLEDGEMENTS

Thank you to Liz Moore, Ross Shierry, Travis Edwards, Brad Austin, Erin Grantz, Sally Entekin, Dan Magoulick, Thad Scott, Steve Beaupre, Allyn Dodd, Brooke Howard-Parker, and Kayla Sayre for providing laboratory assistance and feedback. This project was supported by the U.S. National Science Foundation (grants DEB 1020722 and 1501703), the University of Arkansas Delbert Swartz Endowed Graduate Fellowship, and the Society for Freshwater Science Endowment Fund.

LITERATURE CITED

- American Public Health Association (2005) Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington, DC
- Arango CP, Tank JL, Schaller JL, Royer TV, Bernot MJ, David MB (2007) Benthic organic carbon influences denitrification in streams with high nitrate concentration. *Freshwater Biol* 52(7): 1210-1222
- Arsuffi TL, Suberkropp K (1989) Selective feeding by shredders on leaf-colonizing stream fungi - comparison of macroinvertebrate taxa. *Oecologia* 79(1): 30-37
- Atkinson CF, Aumen NG, Miller GL, Ward GM (1992) Influence of particle shapes and size distributions on fine particulate organic-matter surface area in streams. *J N Am Benthol Soc* 11(3): 261-268
- Benfield EF (2006) Decomposition of leaf material. In: Hauer FR, Lamberti GA (eds) *Methods in Stream Ecology*. Academic Press, San Diego, pp 711-720
- Benstead JP, Rosemond AD, Cross WF, Wallace JB, Eggert SL, Suberkropp K, Gulis V, Greenwood JL, Tant CJ (2009) Nutrient enrichment alters storage and fluxes of detritus in a headwater stream ecosystem. *Ecology* 90(9): 2556-2566
- Bonin HL, Griffiths RP, Caldwell BA (2003) Nutrient and microbiological characteristics of fine benthic organic matter in sediment settling ponds. *Freshwater Biol* 48(6): 1117-1126
- Bundschuh M, McKie BG (In press) An ecological and ecotoxicological perspective on fine particulate organic matter in streams. *Freshwater Biol*
- Callisto M, Graca MAS (2013) The quality and availability of fine particulate organic matter for collector species in headwater streams. *Int Rev Hydrobiol* 98(3): 132-140
- Canhoto C, Graca MAS (2006) Digestive tract and leaf processing capacity of the stream invertebrate *Tipula lateralis*. *Can J Zool* 84(8): 1087-1095
- Capps KA, Atkinson CL, Rugenski AT (2015) Implications of species addition and decline for nutrient dynamics in fresh waters. *Freshw Sci* 34(2): 485-496
- Carapelli A, Lio P, Nardi F, van der Wath E, Frati F (2007) Phylogenetic analysis of mitochondrial protein coding genes confirms the reciprocal parphyly of Hexapoda and Crustacea. *Bmc Evol Biol* 7 (Suppl 2):S8
- Cavan EL, Le Moigne FAC, Poulton AJ, Tarling GA, Ward P, Daniels CJ, Fragoso GM, Sanders RJ (2015) Attenuation of particulate organic carbon flux in the Scotia Sea, Southern Ocean, is controlled by zooplankton fecal pellets. *Geophys Res Lett* 42(3): 821-830

- Cheever BM, Webster JR, Bilger EE, Thomas SA (2013) The relative importance of exogenous and substrate-derived nitrogen for microbial growth during leaf decomposition. *Ecology* 94(7): 1614-1625
- Clissold FJ, Tedder BJ, Conigrave AD, Simpson SJ (2010) The gastrointestinal tract as a nutrient-balancing organ. *P Roy Soc B-Biol Sci* 277(1688): 1751-1759
- Cross WF, Benstead JP, Rosemond AD, Wallace JB (2003) Consumer-resource stoichiometry in detritus-based streams. *Ecol Lett* 6(8): 721-732
- Cuffney TF, Wallace JB, Lugthart GJ (1990) Experimental evidence quantifying the role of benthic invertebrates in organic matter dynamics of headwater streams. *Freshwater Biol* 23(2): 281-299
- Danger M, Funck JA, Devin S, Heberle J, Felten V (2013) Phosphorus content in detritus controls life-history traits of a detritivore. *Funct Ecol* 27(3): 807-815
- Elser JJ, Urabe J (1999) The stoichiometry of consumer-driven nutrient recycling: Theory, observations, and consequences. *Ecology* 80(3): 735-751
- Enriquez S, Duarte CM, Sandjensen K (1993) Patterns in decomposition rates among photosynthetic organisms - the importance of detritus C-N-P content. *Oecologia* 94(4): 457-471
- Evans-White MA, Dodds WK, Huggins DG, Baker DS (2009) Thresholds in macroinvertebrate biodiversity and stoichiometry across water-quality gradients in Central Plains (USA) streams. *J N Am Benthol Soc* 28(4): 855-868
- Evans-White MA, Lamberti GA (2006) Stoichiometry of consumer-driven nutrient recycling across nutrient regimes in streams. *Ecol Lett* 9(11): 1186-1197
- Findlay S, Tank J, Dye S, Valett HM, Mulholland PJ, McDowell WH, Johnson SL, Hamilton SK, Edmonds J, Dodds WK, Bowden WB (2002) A cross-system comparison of bacterial and fungal biomass in detritus pools of headwater streams. *Microbial Ecol* 43(1): 55-66
- Frost PC, Evans-White MA, Finkel ZV, Jensen TC, Matzek V (2005) Are you what you eat? Physiological constraints on organismal stoichiometry in an elementally imbalanced world. *Oikos* 109(1): 18-28
- Gessner MO, Chauvet E, Dobson M (1999) A perspective on leaf litter breakdown in streams. *Oikos* 85(2): 377-384
- Halvorson HM, Fuller C, Entekin SA, Evans-White MA (2015a) Dietary influences on production, stoichiometry and decomposition of particulate wastes from shredders. *Freshwater Biol* 60(3): 466-478

- Halvorson HM, Scott JT, Sanders AJ, Evans-White MA (2015b) A stream insect detritivore violates common assumptions of threshold elemental ratio bioenergetics models. *Freshw Sci* 34(2): 508-518
- Hargrave BT (1972) Aerobic decomposition of sediment and detritus as a function of particle surface area and organic content. *Limnol Oceanogr* 17(4): 583-586
- Harris JM (1993) The presence, nature, and role of gut microflora in aquatic invertebrates - a synthesis. *Microbial Ecol* 25(3): 195-231
- Heard SB, Richardson JS (1995) Shredder-collector facilitation in stream detrital food webs - is there enough evidence? *Oikos* 72(3): 359-366
- Hood JM, McNeely C, Finlay JC, Sterner RW (2014) Selective feeding determines patterns of nutrient release by stream invertebrates. *Freshw Sci* 33(4): 1093-1107
- Inamdar S, Dhillon G, Singh S, Parr T, Qin ZX (2015) Particulate nitrogen exports in stream runoff exceed dissolved nitrogen forms during large tropical storms in a temperate, headwater, forested watershed. *J Geophys Res-Bioge* 120(8): 1548-1566
- Jackson CR, Vallaire SC (2007) Microbial activity and decomposition of fine particulate organic matter in a Louisiana cypress swamp. *J N Am Benthol Soc* 26(4): 743-753
- Jing HM, Shek L, Yung WY, Jin XW, Liu HB (2012) Dynamics of bacterial community composition during degradation of copepod fecal pellets. *J Plankton Res* 34(8): 700-710
- Joyce P, Warren LL, Wotton RS (2007) Faecal pellets in streams: their binding, breakdown and utilization. *Freshwater Biol* 52(10): 1868-1880
- Joyce P, Wotton RS (2008) Shredder fecal pellets as stores of allochthonous organic matter in streams. *J N Am Benthol Soc* 27(3): 521-528
- Kana TM, Darkangelo C, Hunt MD, Oldham JB, Bennett GE, Cornwell JC (1994) Membrane inlet mass-spectrometer for rapid high-precision determination of N₂, O₂, and Ar in environmental water samples. *Anal Chem* 66(23): 4166-4170
- Kendrick MR, Benstead JP (2013) Temperature and nutrient availability interact to mediate growth and body stoichiometry in a detritivorous stream insect. *Freshwater Biol* 58(9): 1820-1830
- Ladle M, Griffiths BS (1980) A study on the feces of some chalk stream invertebrates. *Hydrobiologia* 74(2): 161-171
- Le QN, Fujii M, Yoshimura C, Tockner K (2016) Dissolved nitrogen release from coarse and amphipod-produced fine particulate organic matter in freshwater column. *Limnology* 17(1): 33-46

- Liess A, Haglund AL (2007) Periphyton responds differentially to nutrients recycled in dissolved or faecal pellet form by the snail grazer *Theodoxus fluviatilis*. *Freshwater Biol* 52(10): 1997-2008
- Malmqvist B, Wotton RS, Zhang YX (2001) Suspension feeders transform massive amounts of seston in large northern rivers. *Oikos* 92(1): 35-43
- Manzoni S, Trofymow JA, Jackson RB, Porporato A (2010) Stoichiometric controls on carbon, nitrogen, and phosphorus dynamics in decomposing litter. *Ecol Monogr* 80(1): 89-106
- Martin MM, Martin JS, Kukor JJ, Merritt RW (1980) The digestion of protein and carbohydrate by the stream detritivore, *Tipula Abdominalis* (Diptera, Tipulidae). *Oecologia* 46(3): 360-364
- Mooshammer M, Wanek W, Schneckler J, Wild B, Leitner S, Hofhansl F, Blochl A, Hammerle I, Frank AH, Fuchslueger L, Keiblinger KM, Zechmeister-Boltenstern S, Richter A (2012) Stoichiometric controls of nitrogen and phosphorus cycling in decomposing beech leaf litter. *Ecology* 93(4): 770-782
- Mulholland PJ (2004) The importance of in-stream uptake for regulating stream concentrations and outputs of N and P from a forested watershed: evidence from long-term chemistry records for Walker Branch Watershed. *Biogeochemistry* 70(3): 403-426
- Navel S, Simon L, Lecuyer C, Fourel F, Mermillod-Blondin F (2011) The shredding activity of gammarids facilitates the processing of organic matter by the subterranean amphipod *Niphargus rhenorhodanensis*. *Freshwater Biol* 56(3): 481-490
- Newbold JD, Oneill RV, Elwood JW, Vanwinkle W (1982) Nutrient spiralling in streams - implications for nutrient limitation and invertebrate activity. *Am Nat* 120(5): 628-652
- Pastor A, Compson ZG, Dijkstra P, Riera JL, Marti E, Sabater F, Hungate BA, Marks JC (2014) Stream carbon and nitrogen supplements during leaf litter decomposition: contrasting patterns for two foundation species. *Oecologia* 176(4): 1111-1121
- Patrick CJ (2013) The effect of shredder community composition on the production and quality of fine particulate organic matter. *Freshw Sci* 32(3): 1026-1035
- Scott EE, Prater C, Norman E, Baker BC, Evans-White M, Scott JT (2013) Leaf-litter stoichiometry is affected by streamwater phosphorus concentrations and litter type. *Freshw Sci* 32(3): 753-761
- Short RA, Maslin PE (1977) Processing of leaf litter by a stream detritivore - effect on nutrient availability to collectors. *Ecology* 58(4): 935-938

- Sinsabaugh RL, Linkins AE, Benfield EF (1985) Cellulose digestion and assimilation by 3 leaf-shredding aquatic insects. *Ecology* 66(5): 1464-1471
- Sitters J, Maechler MJ, Edwards PJ, Suter W, Venterink HO (2014) Interactions between C:N:P stoichiometry and soil macrofauna control dung decomposition of savanna herbivores. *Funct Ecol* 28(3): 776-786
- Tank JL, Rosi-Marshall EJ, Griffiths NA, Entekin SA, Stephen ML (2010) A review of allochthonous organic matter dynamics and metabolism in streams. *J N Am Benthol Soc* 29(1): 118-146
- Vanni MJ (2002) Nutrient cycling by animals in freshwater ecosystems. *Annu Rev Ecol Syst* 33: 341-370
- Vanni MJ, Renwick WH, Headworth JL, Auch JD, Schaus MH (2001) Dissolved and particulate nutrient flux from three adjacent agricultural watersheds: A five-year study. *Biogeochemistry* 54(1): 85-114
- Veldboom JA, Haro RJ (2011) Stoichiometric relationship between suspension-feeding caddisfly (Trichoptera: Brachycentridae) and seston. *Hydrobiologia* 675(1): 129-141
- Wallace JB, Hutchens JJ, Grubaugh JW (2006) Transport and storage of FPOM. In: Hauer FR, Lamberti GA (eds) *Methods in Stream Ecology*. Academic Press, San Diego, pp 249-271
- Wallace TA, Ganf GG, Brookes JD (2008) A comparison of phosphorus and DOC leachates from different types of leaf litter in an urban environment. *Freshwater Biol* 53(9): 1902-1913
- Ward GM, Ward AK, Dahm CN, Aumen NG (1994) Origin and formation of organic and inorganic particles in aquatic systems. In: Wotton RS (ed) *The Biology of Particles in Aquatic Systems*. Lewis Publishers, Ann Arbor, pp 45-73
- Webster JR, Benfield EF (1986) Vascular plant breakdown in freshwater ecosystems. *Annu Rev Ecol Syst* 17: 567-594
- Webster JR, Benfield EF, Ehrman TP, Schaeffer MA, Tank JL, Hutchens JJ, D'Angelo DJ (1999) What happens to allochthonous material that falls into streams? A synthesis of new and published information from Coweeta. *Freshwater Biol* 41(4): 687-705
- Wilson HF, Xenopoulos MA (2011) Nutrient recycling by fish in streams along a gradient of agricultural land use. *Global Change Biol* 17(1): 130-139
- Wotton RS, Malmqvist B (2001) Feces in aquatic ecosystems. *Bioscience* 51(7): 537-544
- Yoshimura C, Fujii M, Omura T, Tockner K (2010) Instream release of dissolved organic matter from coarse and fine particulate organic matter of different origins. *Biogeochemistry* 100(1-3): 151-165

Yoshimura C, Gessner MO, Tockner K, Furumai H (2008) Chemical properties, microbial respiration, and decomposition of coarse and fine particulate organic matter. *J N Am Benthol Soc* 27(3): 664-673

Table 1. Mean (\pm SE) dry mass percent carbon (C), nitrogen (N), phosphorus (P) and molar ratios of *Platanus occidentalis* litter after 3 d of leaching prior to microbial conditioning (Leached) or after microbial conditioning for 25 d under contrasting P concentrations (Low P or High P).

Treatment	%C	%N	%P	C:N	C:P	N:P
Leached	49.7 (0.2)	1.36 (0.07)	0.095 (0.010)	43.1 (2.2)	1390 (160)	33.1 (1.9)
Low P	49.7 (0.1)	1.63 (0.03)	0.096 (0.003)	35.7 (0.7)	1360 (60)	37.8 (1.0)
High P	49.7 (0.1)	1.68 (0.04)	0.129 (0.004)	34.7 (0.8)	1010 (30)	29.1 (0.5)

Table 2. Two-way analysis of variance results testing effects of detritivore genus and diet phosphorus level on initial mean particle size, egesta percent carbon, nitrogen, and phosphorus content, molar ratios of C:N, C:P, and N:P, and egesta mass loss rates k .

Response	Predictor ^a	<i>F</i> -value	<i>P</i> -value ^b
Initial mean particle size	G	55.1	< 0.001
	P	<0.1	0.950
	G x P	0.5	0.625
% carbon	G	4.5	0.021
	P	0.9	0.349
	G x P	0.6	0.582
% nitrogen ^c	G	9.4	< 0.001
	P	0.6	0.463
	G x P	2.5	0.107
% phosphorus	G	156.5	< 0.001
	P	75.7	< 0.001
	G x P	1.3	0.289
molar C:N ^c	G	35.3	< 0.001
	P	4.7	0.040
	G x P	2.3	0.127
molar C:P ^c	G	57.9	< 0.001
	P	8.4	0.008
	G x P	0.1	0.867
molar N:P ^c	G	15.9	< 0.001
	P	18.2	< 0.001
	G x P	1.5	0.248
Mass loss rates (k)	G	8.4	0.002
	P	3.1	0.091
	G x P	3.6	0.043

^aG=Detritivore genus, P=Diet phosphorus level

^bBoldface values indicate statistical significance after Bonferroni correction ($P < 0.006$)

^cLog-transformed prior to analysis

Table 3. Repeated-measures analysis of covariance testing effects of the factors time, detritivore genus, diet phosphorus level, and the covariate egesta dry mass (mg) on total O₂ uptake and total exchange of the dissolved constituents dissolved organic carbon (DOC), ammonium (N-NH₄), nitrate-nitrite (N-(NO₃+NO₂)), total nitrogen (TN), and soluble reactive phosphorus (P-SRP) from egesta to the water column. The across-subjects ANCOVA of DOC exchange was split into separate analyses within each main effect because of heterogeneous slopes (covariate*factor interaction; $P < 0.001$). See Figs. S1-S3 for associated ANCOVA plots where covariate effects were significant ($P < 0.008$).

Response	Within-subjects (aquaria)			Across-subjects (aquaria)		
	Predictor ^a	F-value	P-value ^b	Predictor ^a	F-value	P-value ^b
O ₂ uptake ^c	T	239.5	<0.001	DM	1.0	0.343
	T x G	2.7	0.040	G	6.7	0.005
	T x P	4.9	0.012	P	1.8	0.198
	T x G x P	1.1	0.351	G x P	0.5	0.623
DOC exchange	T	49.0	<0.001	DM	6.6	0.017
	T x G	1.1	0.364	G	3.2	0.059
	T x P	1.0	0.422	DM x G	1.2	0.312
	T x G x P	0.3	0.957			
				DM	52.4	<0.001
				P	2.5	0.126
				DM x P	14.4	<0.001
N-NH ₄ exchange ^c	T	17.0	<0.001	DM	<0.1	0.907
	T x G	1.7	0.067	G	4.9	0.018
	T x P	0.5	0.840	P	8.7	0.007
	T x G x P	1.7	0.081	G x P	1.6	0.219
N-(NO ₃ + NO ₂) exchange ^c	T	219.6	<0.001	DM	19.4	<0.001
	T x G	1.5	0.164	G	11.1	<0.001
	T x P	1.4	0.243	P	0.2	0.675
	T x G x P	1.5	0.142	G x P	<0.1	0.978
TN exchange	T	7.2	<0.001	DM	2.4	0.135
	T x G	1.6	0.088	G	0.1	0.881
	T x P	0.7	0.664	P	9.1	0.006
	T x G x P	1.5	0.116	G x P	2.5	0.101
P-SRP exchange ^c	T	13.2	<0.001	DM	12.2	0.002
	T x G	1.3	0.202	G	0.1	0.887
	T x P	0.7	0.682	P	1.4	0.250
	T x G x P	1.0	0.461	G x P	1.1	0.360

^aT=Time, G=Detritivore Genus, P=Diet phosphorus level, DM=Egesta dry mass

^bBoldface values indicate statistical significance after Bonferroni correct ($P < 0.008$)

^cLog-transformed prior to analysis

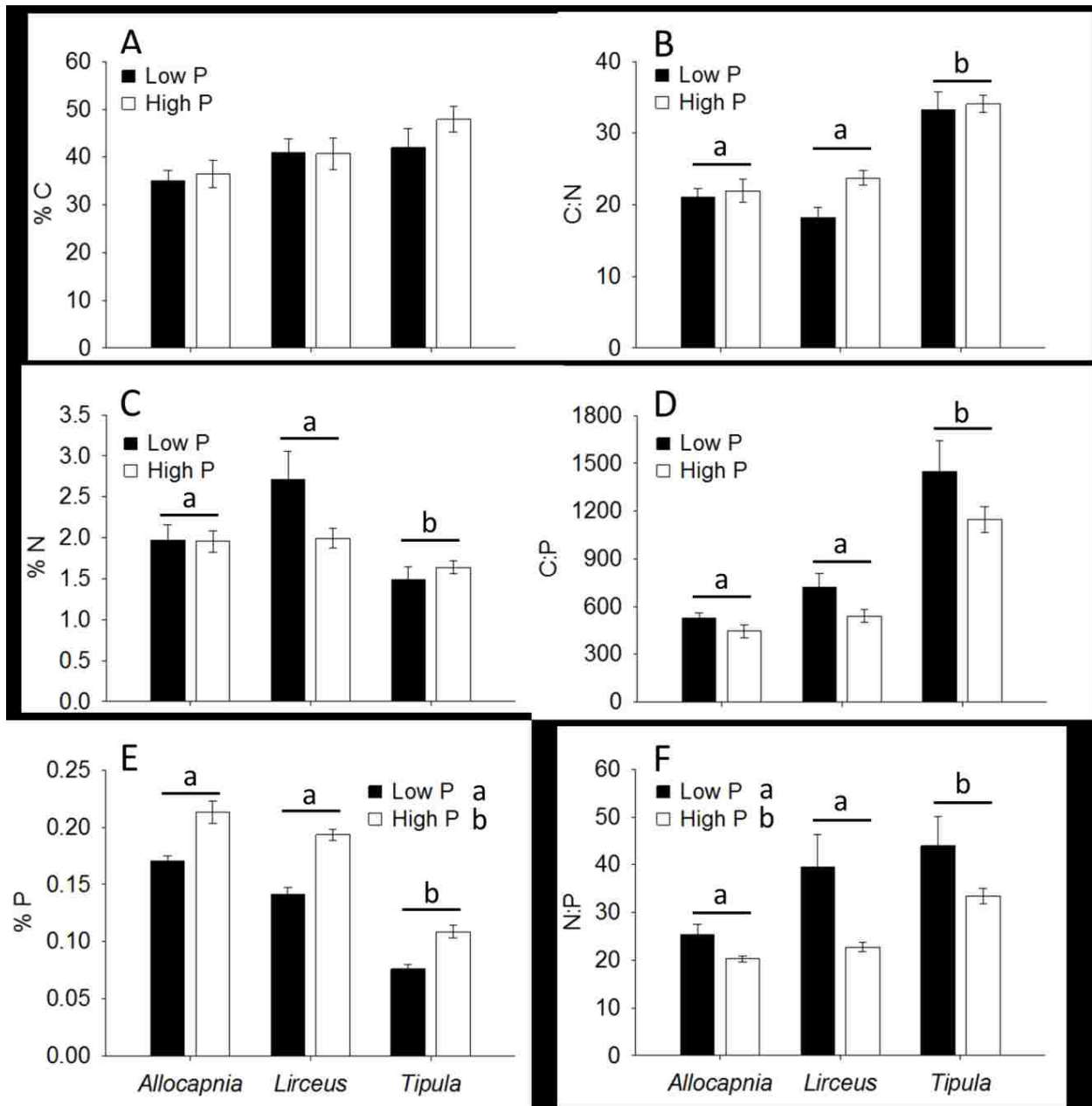


Figure 1. Mean (\pm SE) egesta percent elemental content (A, C, E) and molar ratios (B, D, F) from three shredder genera fed low- or high-P leaf litter. Panels are arranged by order of (A) percent carbon (B), molar C:N, (C) percent nitrogen, (D) molar C:P, (E), percent phosphorus, and (F) molar N:P. Lower-case letters designate statistically different groups across genera (letters above horizontal bars) or diet P levels (letters to right of legends). See Table 2 for associated statistics.

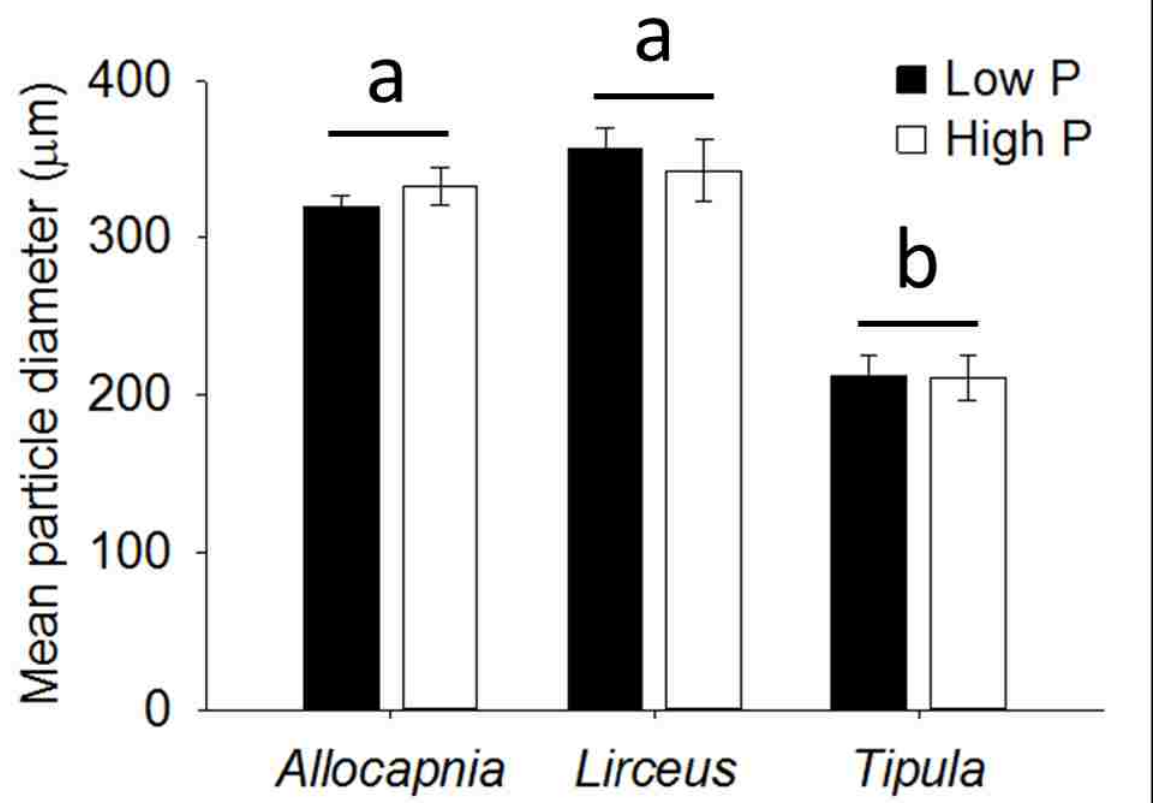


Figure 2. Mean (\pm SE) initial sizes of particles egested by the shredder genera *Allocapnia*, *Lirceus*, and *Tipula* fed low- or high-P leaf litter. Lower-case letters designate statistically distinct particle sizes across genera (Tukey's HSD, $P < 0.05$). See Table 2 for associated statistics.

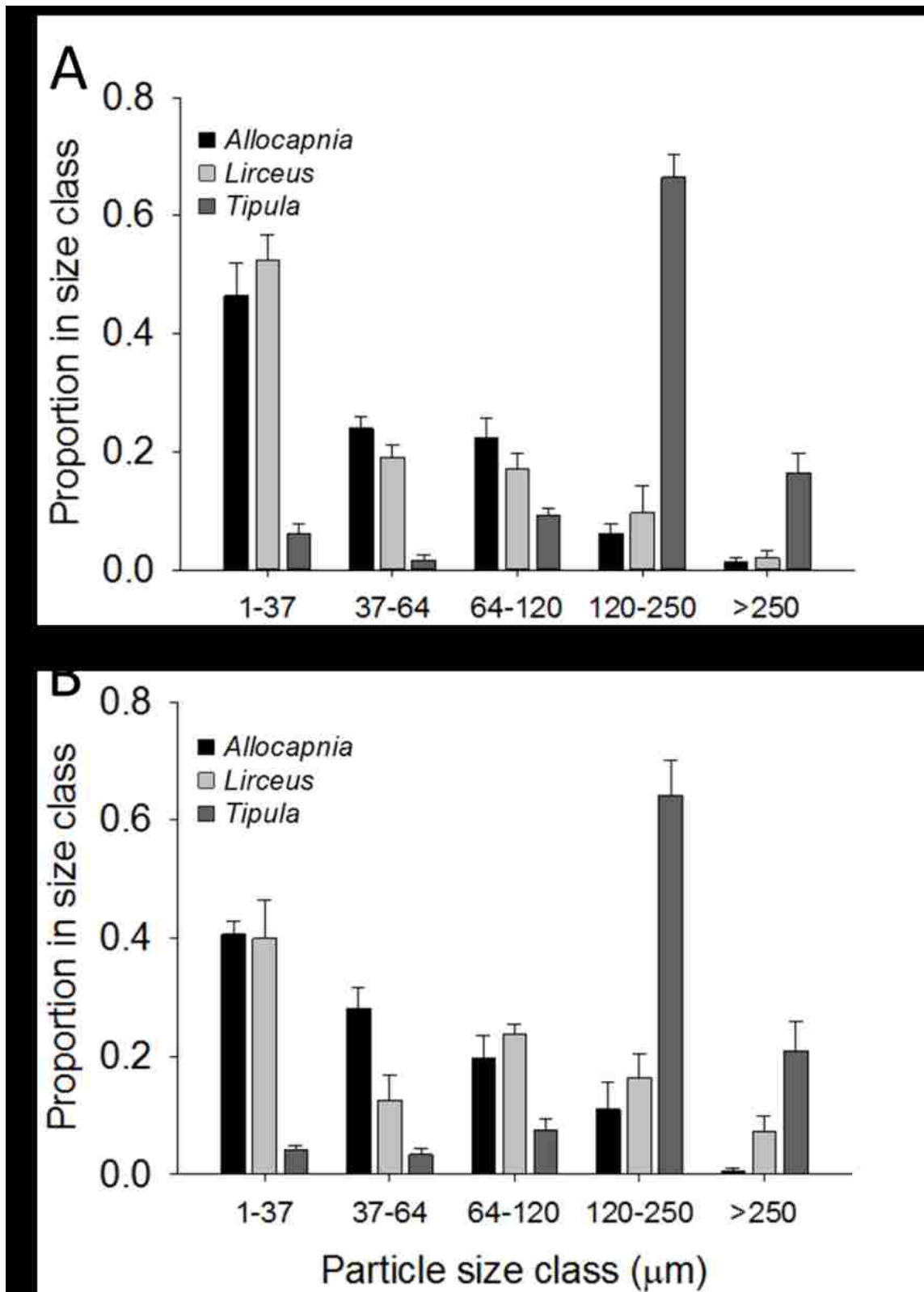


Figure 3. Mean (\pm SE) mass proportions of particles among five size classes, derived from egesta of three shredder genera fed (A) low-P and (B) high-P leaf litter. Proportions were determined from dry mass in each size class 90 d into decomposition.

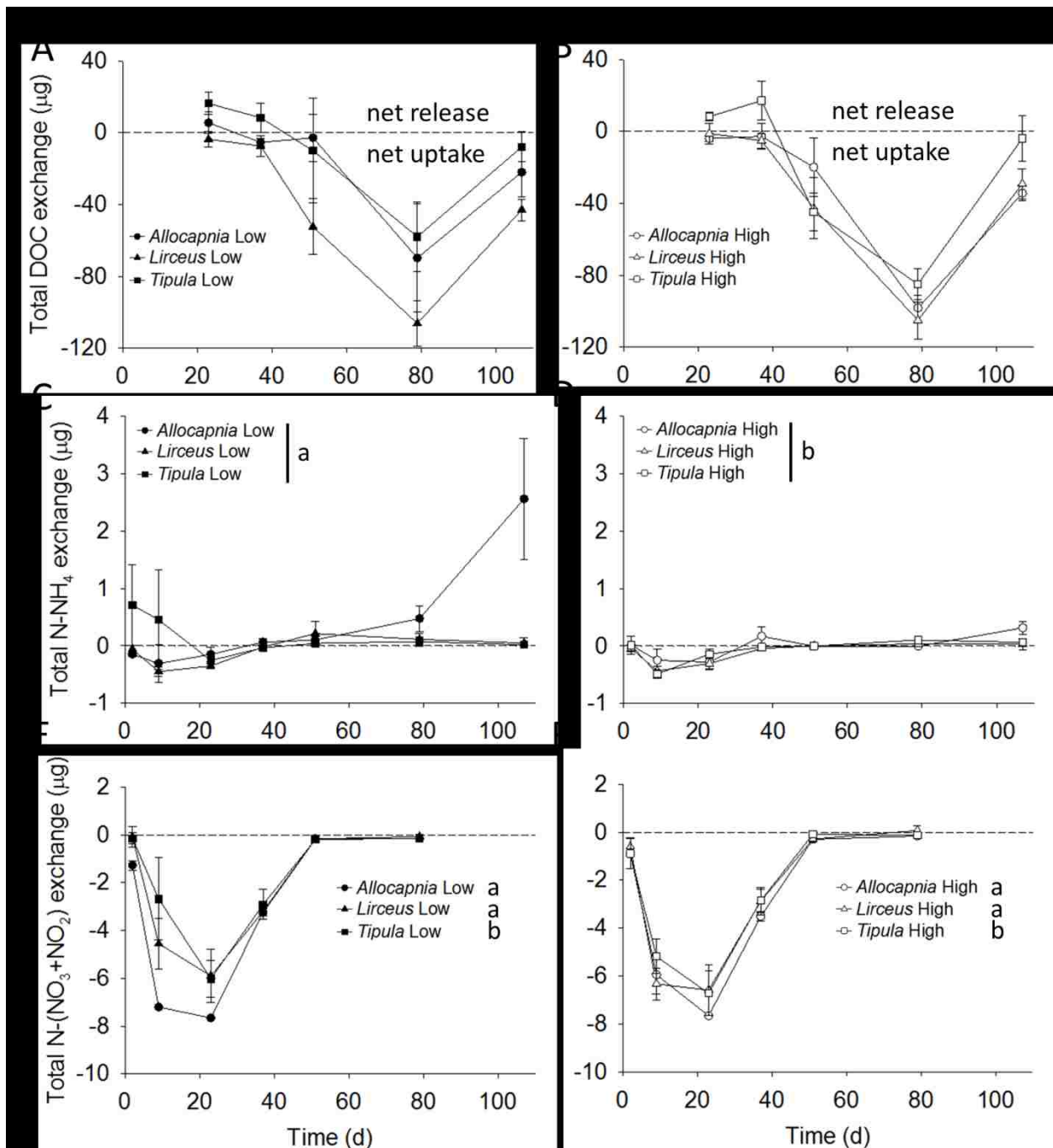


Figure 4. Mean (\pm SE) total exchange of dissolved constituents from egesta to the water column over long-term decomposition. Positive exchange indicates net release, whereas negative exchange indicates net uptake of the constituent by egesta at a given time. Panels are arranged by low- (A, C, E) and high-P (B, D, F) leaf litter treatments for exchange of dissolved organic carbon (DOC; A,B), ammonium (N-NH₄; C,D), and nitrate+nitrite (N-(NO₃+NO₂); E,F). Lowercase letters designate statistically distinct groups across genera or between diet P levels (Tukey's HSD, $P < 0.05$). See Table 3 for associated statistics.

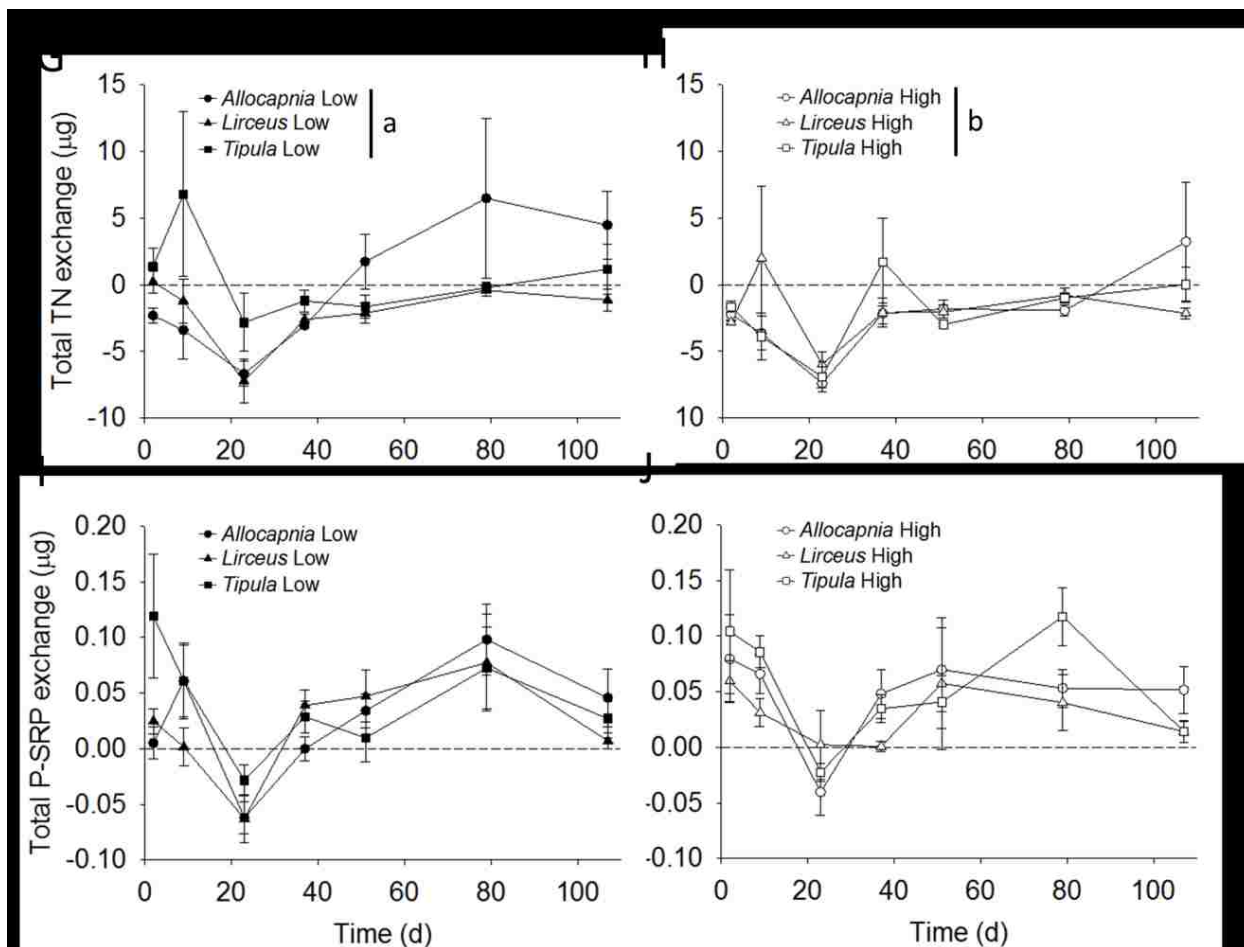


Figure 4 continued. Mean (\pm SE) total exchange of dissolved constituents from egesta to the water column over long-term decomposition. Positive exchange indicates net release, whereas negative exchange indicates net uptake of the constituent by egesta at a given time. Panels are arranged by low- (G, I) and high-P (H, J) leaf litter treatments for exchange of total nitrogen (TN; G,H), and soluble reactive phosphorus (P-SRP; I,J). Lower-case letters designate statistically distinct groups across genera or between diet P levels (Tukey's HSD, $P < 0.05$). See Table 3 for associated statistics.

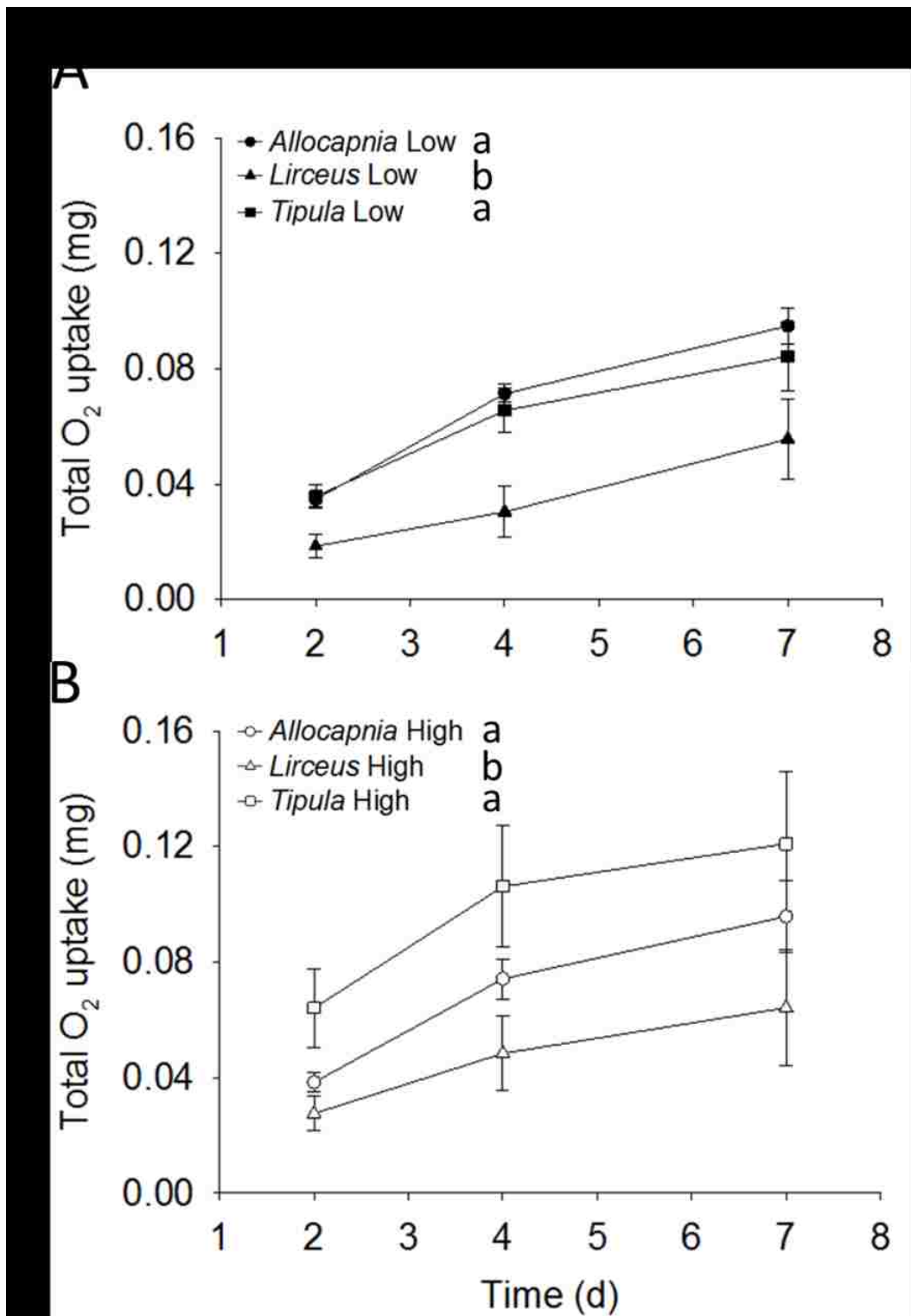


Figure 5. Mean (\pm SE) total O₂ uptake over time of egesta from the shredder genera *Allocapnia*, *Lirceus*, and *Tipula* fed either low-P (A) or high-P (B) leaf litter. Diet treatments were split into separate panels to ease visual comparison, but lower-case letters designate statistically distinct genera across both diet treatments (Tukey's HSD, $P < 0.05$). See Table 3 for associated statistics.

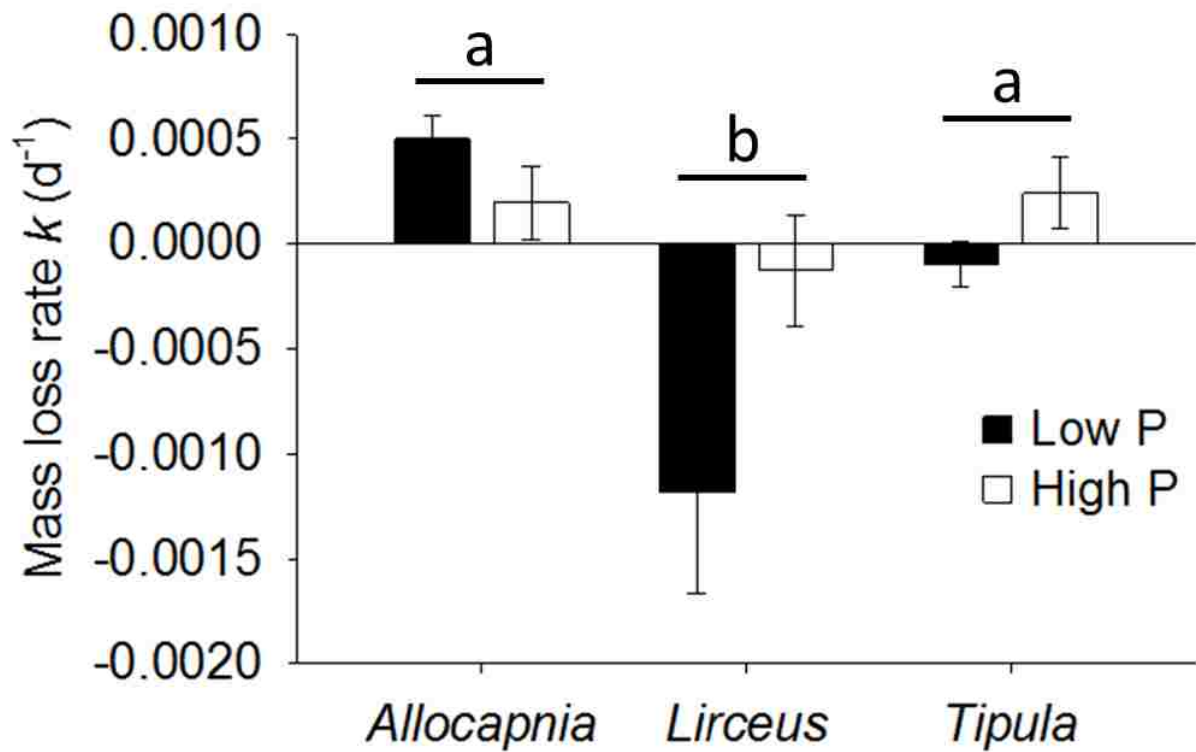


Figure 6. Mean (\pm SE) mass loss rates k of egesta derived from three shredder genera fed low-P or high-P leaf litter. Lower-case letters designate statistically different groups across shredder genera (Tukey's HSD, $P < 0.05$). See Table 2 for associated statistics.

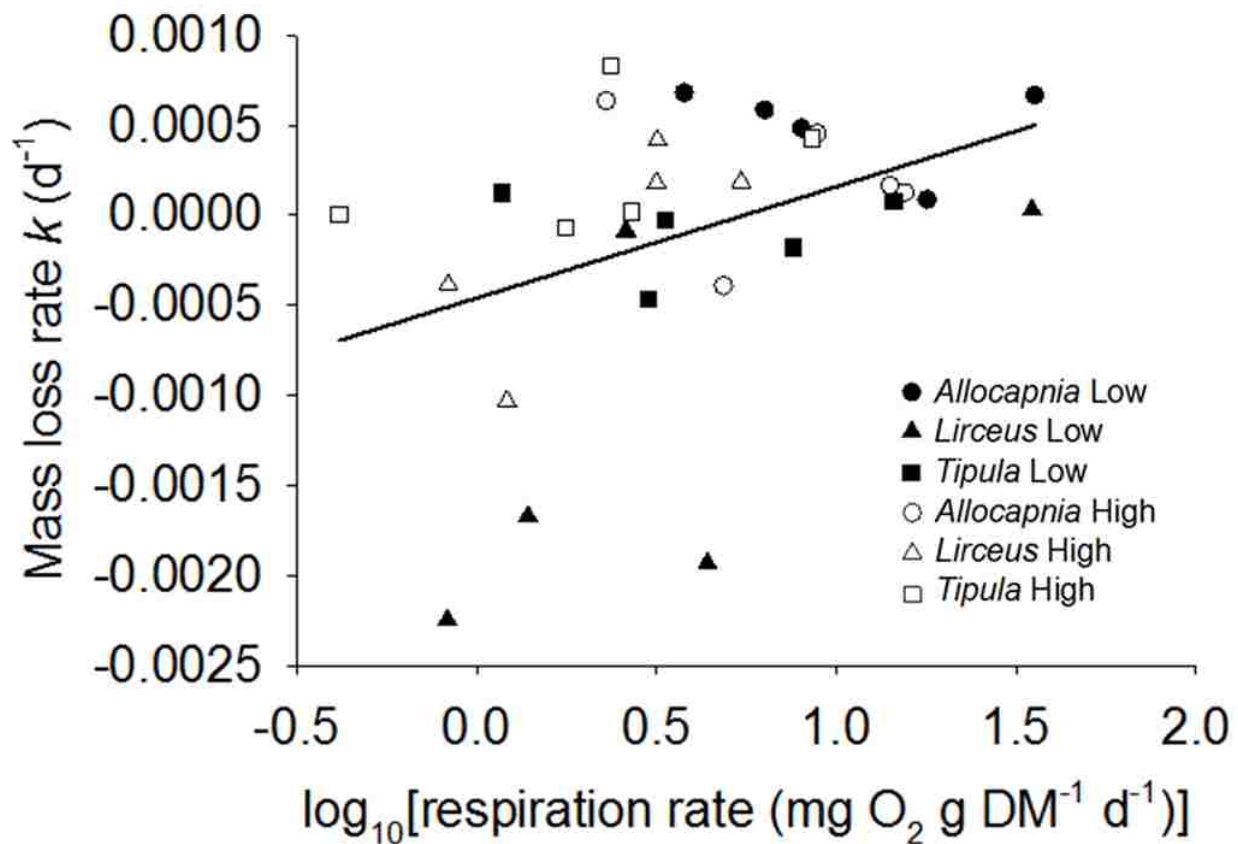


Figure 7. Scatterplot of long-term egesta mass loss rates k plotted against mean log-transformed short-term respiration rates of egesta from three shredder genera fed low- or high-P leaf litter. Each datapoint designates measurements of egesta from an individual aquarium. The solid black line represents a positive linear regression fit across all aquaria (intercept= -0.000459; slope= 0.000618; slope $P < 0.05$).

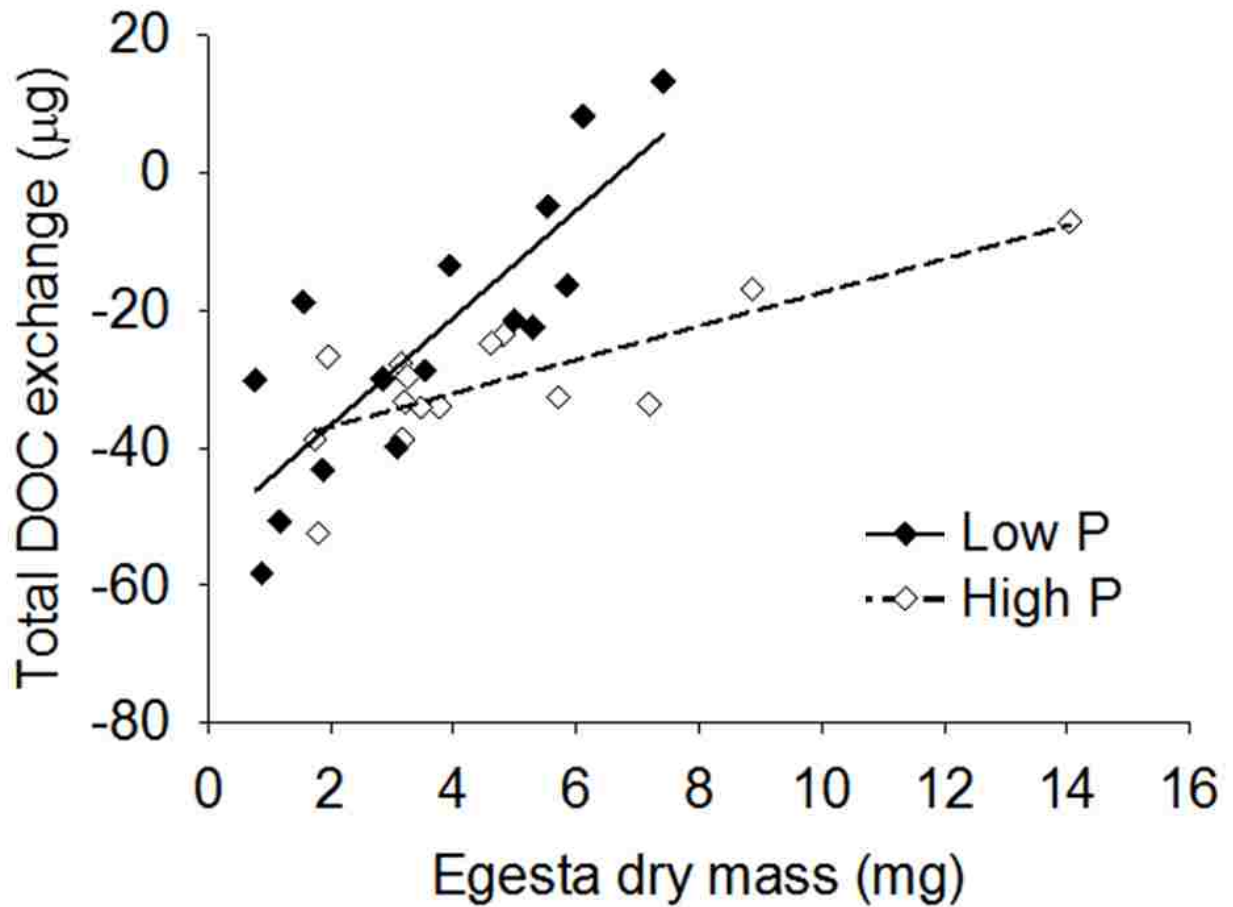


Figure S1. Scatterplot of total exchange of dissolved organic carbon (DOC) from egesta to the water column and egesta dry mass among egesta from low- or high-P diets produced by three shredder genera. There was a significant difference between slopes fit to each diet P level (ANCOVA, $P < 0.001$). See also Table 3.

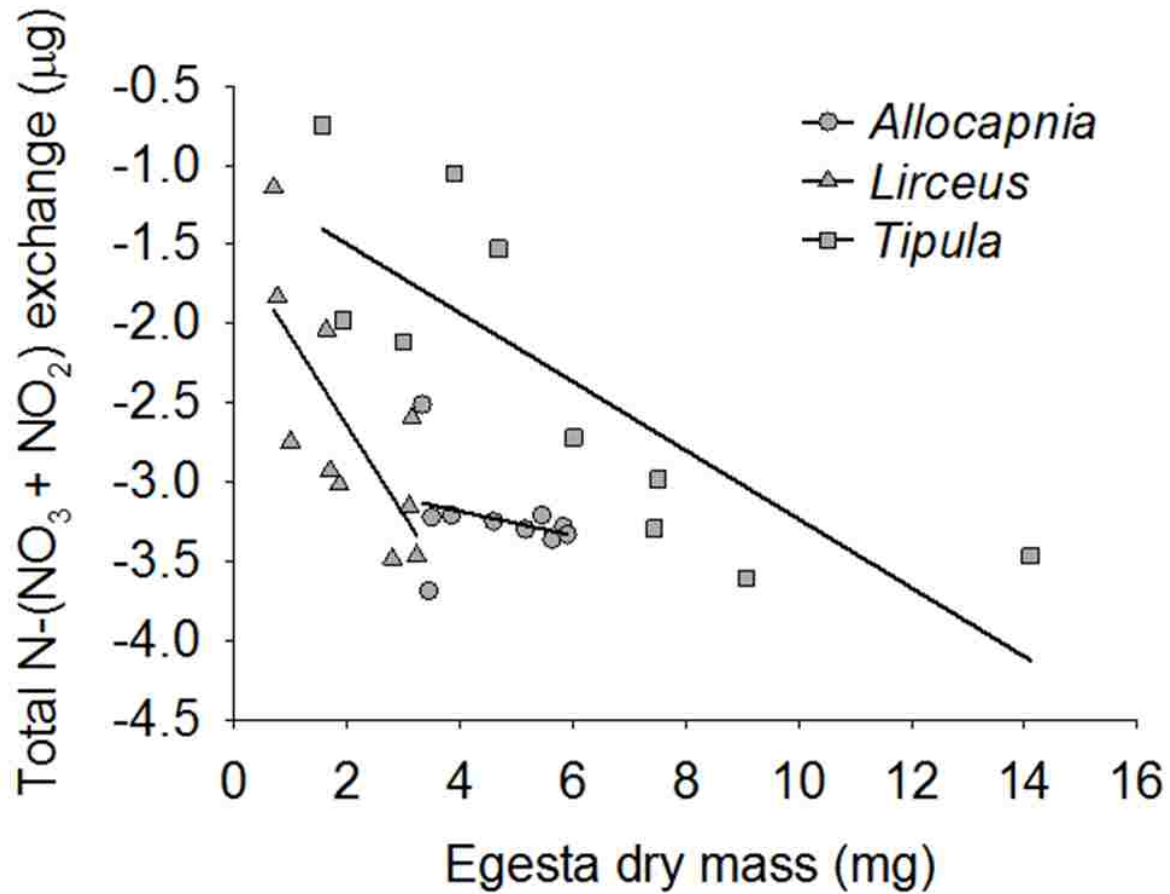


Figure S2. Scatterplot of total exchange of nitrate+nitrite (N-(NO₃+NO₂)) from egesta to the water column and egesta dry mass among egesta from three different shredder genera fed low- and high-P leaf litter. The ANCOVA indicated slopes do not differ between genera ($P>0.05$) but *Allocapnia* and *Lirceus* egesta exhibited lower total N-(NO₃+NO₂) exchange than egesta from *Tipula* ($P=0.007$). See also Table 3.

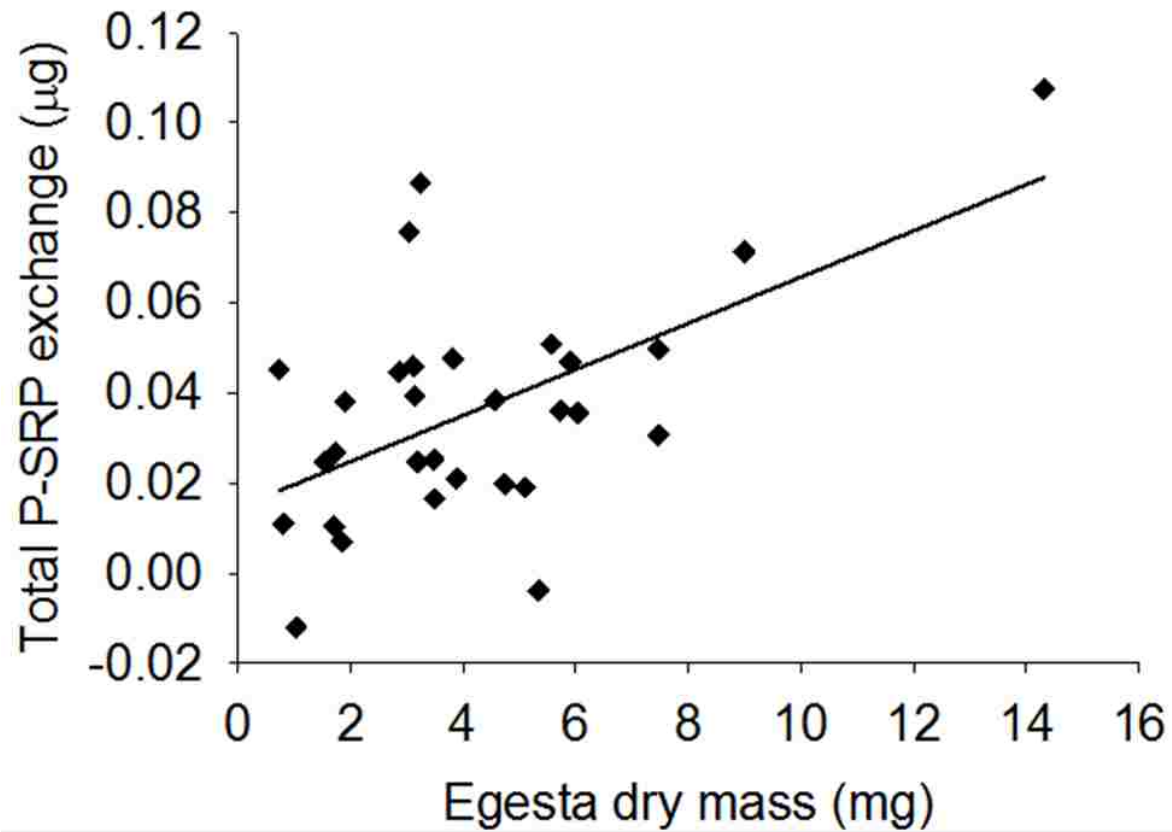


Figure S3. Scatterplot of total exchange of soluble reactive phosphorus (P-SRP) from egesta to the water column and egesta dry mass among egesta from three shredders fed low- and high-P leaf litter. There is a significant positive relationship between egesta dry mass and P-SRP exchange (ANCOVA, $P=0.002$), but this relationship did not differ between treatments and P-SRP exchange did not differ across shredder genera or diet P treatments ($P>0.05$). See also Table 3.

CONCLUSIONS

Diet leaf type and stoichiometry drive variable stoichiometry of growth rates, post-ingestive regulation, and waste production across stream insect detritivores. In this dissertation, I empirically tested predictions of ecological stoichiometry (ES) theory among detritivorous animals, providing mechanistic insights of how taxa respond to nutrient pollution associated with anthropogenic activity. Laboratory experiments suggested growth of some species may respond negatively to excess diet P, and increased diet P relative to C content can alter assimilation and incorporation of bulk detrital and microbe-specific elements by detritivorous taxa. Because leaf species and nutrient content of leaf litter diets alter growth and stoichiometric regulation of detritivores, and detritivores appear to be largely homeostatic in elemental content (Halvorson, unpublished data), diet characteristics also drive variable waste production and stoichiometry, potentially altering freshwater nutrient dynamics. Short- and long-term measurements of C and nutrient dynamics during decomposition suggested diet nutrient content and source animal identity can modify potential roles of shredder-detritivore egesta in stream ecosystem, especially the cycling of N, providing an additional link of anthropogenic nutrient enrichment and community composition to altered functional roles of detritivores in Ozark headwater streams.

Reduced oak litter C:P associated with P enrichment can stimulate growth of *P. lepidus*, but diet C:P below C:P=1620 significantly reduced *P. lepidus* growth, supporting a $TER_{C:P}$ of 1620 for *P. lepidus* fed oak diets (Chapter 1; Halvorson et al. 2015b). Although I provide the first empirical evidence of a “stoichiometric knife edge” (Elser et al. 2005) for a detritivorous animal, the mechanisms of reduced growth below $TER_{C:P}=1620$ are not clear. *P. lepidus* slightly reduced mass-specific rates of consumption and elevated excretion from 8% at peak growth to 24% of its P budget on the lowest-C:P diet, indicating a combination of reduced consumption and

physiological costs of excreting excess P may explain reduced growth on P-enriched diets (Plath and Boersma 2001, Boersma and Elser 2006). Further TER estimates among other detritivorous animals, grounded in empirical growth data, will help discern mechanisms and may provide a basis of predicting community responses to nutrient enrichment (Evans-White et al. 2009, Woodward et al. 2012).

Microbial C and P both contribute significantly to detritivore nutrition, with detritivore incorporation efficiencies of microbial C more than one order of magnitude greater than growth efficiencies for bulk detrital C (Chapter 2; Halvorson et al. 2016). Although microbial biomass may not consist of the majority of bulk detrital dry mass (Findlay et al. 2002), microbes strongly affect detritivore growth; my study suggests caddisfly detritivores use microbial C 50 times more efficiently than detrital substrate C. Incorporation efficiencies of both microbial C and P were inversely related to body C:P across taxa, with *Lepidostoma* (mean \pm SE body C:P=247 \pm 59) exhibiting lower incorporation efficiencies than *Isonychia* and *Pycnopsyche* (body C:P=161 \pm 17 and 81 \pm 6, respectively). These taxonomic differences support a connection between detritivore elemental growth requirements (e.g. $TER_{C:P}$ or growth rate) and reliance on detrital microbial biomass. Given elevated P concentrations also significantly reduced efficiency of incorporation of microbial P by detritivorous caddisflies, stoichiometric relationships between detritivores and detrital microbes may explain broad alterations of detritivore growth and waste stoichiometry under nutrient enrichment.

In streams, detritivores play key functional roles of converting coarse particulate organic matter into fine particulate wastes via egestion and fragmentation (Cummins and Klug 1979, Cuffney et al. 1990). In Chapter 3 (Halvorson et al. 2015a), I show how the stoichiometry of detritivore particulate wastes reflects a combination of diet (leaf type and stoichiometry) and

taxonomy (species-specific selective feeding and assimilation). Across detritivore taxa, elevated diet N and P content consistently increased the N and P content of particulate wastes. In the examples of both *Lepidostoma sp.* and *Pycnopsyche lepida*, particulate wastes were more N- and P-rich than their diets, indicating selective feeding on nutrient-rich microbial biofilm may eclipse selective assimilation of growth-limiting N and P (Hood et al. 2014). In contrast, *Tipula abdominalis* reduced N and P content of particulate wastes compared to their diets, indicating stronger roles of selective assimilation than of selective feeding on microbial biofilm. Species-specific patterns of waste stoichiometry may translate to the ecosystem level, signifying links between community composition and broader cycling of dissolved and particulate nutrients in stream ecosystems (Vanni et al. 2002, Evans-White and Lamberti 2006).

Though animal egesta can be a significant component of stream fine particulate organic matter (FPOM) budgets (Malmqvist et al. 2001), their role in stream nutrient cycles remains understudied, constraining understanding of the role of animals in stream ecosystems. The results in Chapter 4 provide crucial data to understand the role animal egesta play in stream C, N, and P cycles, especially microbial uptake versus release of dissolved constituents during long-term decomposition. I found egesta from the genera *Allocapnia* and *Tipula* decompose faster than egesta from *Lirceus*, and I attribute these taxonomic differences to divergent egesta nutrient content, particle size, chemical binding, and initial microbial biomass and activity controlled by the source animal (Harris 1993, Wotton and Malmqvist 2001, Joyce et al. 2007). Dietary and taxonomic differences affected long-term nitrate+nitrite, total nitrogen, and ammonium uptake versus release by decomposing egesta, with lower-N:P egesta generally exhibiting greater uptake and retention of dissolved N than higher-N:P egesta. These trends suggest microbes decomposing egesta rely on dissolved N sources when they are limited by substrate N content

(Mooshammer et al. 2012, Cheever et al. 2013). My study provides empirical evidence to link food resources and animals to diverse attributes, fates, and long-term roles of FPOM in stream ecosystems (Bundschuh and McKie, *in press*).

LITERATURE CITED

- Boersma, M. and J. J. Elser. 2006. Too much of a good thing: On stoichiometrically balanced diets and maximal growth. *Ecology* 87:1325-1330.
- Bundschuh, M. and B. G. McKie. In press. An ecological and ecotoxicological perspective on fine particulate organic matter in streams. *Freshwater Biology*.
- Cheever, B. M., J. R. Webster, E. E. Bilger, and S. A. Thomas. 2013. The relative importance of exogenous and substrate-derived nitrogen for microbial growth during leaf decomposition. *Ecology* 94:1614-1625.
- Cuffney, T. F., J. B. Wallace, and G. J. Lughart. 1990. Experimental evidence quantifying the role of benthic invertebrates in organic matter dynamics of headwater streams. *Freshwater Biology* 23:281-299.
- Cummins, K. W. and M. J. Klug. 1979. Feeding ecology of stream invertebrates. *Annual Review of Ecology and Systematics* 10:147-172.
- Elser, J. J., J. H. Schampel, M. Kyle, J. Watts, E. W. Carson, T. E. Dowling, C. Tang, and P. D. Roopnarine. 2005. Response of grazing snails to phosphorus enrichment of modern stromatolitic microbial communities. *Freshwater Biology* 50:1826-1835.
- Evans-White, M. A., W. K. Dodds, D. G. Huggins, and D. S. Baker. 2009. Thresholds in macroinvertebrate biodiversity and stoichiometry across water-quality gradients in Central Plains (USA) streams. *Journal of the North American Benthological Society* 28:855-868.
- Evans-White, M. A. and G. A. Lamberti. 2006. Stoichiometry of consumer-driven nutrient recycling across nutrient regimes in streams. *Ecology Letters* 9:1186-1197.
- Findlay, S., J. Tank, S. Dye, H. M. Valett, P. J. Mulholland, W. H. McDowell, S. L. Johnson, S. K. Hamilton, J. Edmonds, W. K. Dodds, and W. B. Bowden. 2002. A cross-system comparison of bacterial and fungal biomass in detritus pools of headwater streams. *Microbial Ecology* 43:55-66.
- Halvorson, H. M., C. Fuller, S. A. Entrekin, and M. A. Evans-White. 2015a. Dietary influences on production, stoichiometry and decomposition of particulate wastes from shredders. *Freshwater Biology* 60:466-478.
- Halvorson, H. M., J. T. Scott, A. J. Sanders, and M. A. Evans-White. 2015b. A stream insect detritivore violates common assumptions of threshold elemental ratio bioenergetics models. *Freshwater Science* 34:508-518.

- Halvorson, H. M., G. White, J. T. Scott, and M. A. Evans-White. 2016. Dietary and taxonomic controls on incorporation of microbial carbon and phosphorus by detritivorous caddisflies. *Oecologia* 180:567-579.
- Harris, J. M. 1993. The presence, nature, and role of gut microflora in aquatic invertebrates - a synthesis. *Microbial Ecology* 25:195-231.
- Hood, J. M., C. McNeely, J. C. Finlay, and R. W. Sterner. 2014. Selective feeding determines patterns of nutrient release by stream invertebrates. *Freshwater Science* 33:1093-1107.
- Joyce, P., L. L. Warren, and R. S. Wotton. 2007. Faecal pellets in streams: their binding, breakdown and utilization. *Freshwater Biology* 52:1868-1880.
- Mooshammer, M., W. Wanek, J. Schnecker, B. Wild, S. Leitner, F. Hofhansl, A. Blochl, I. Hammerle, A. H. Frank, L. Fuchslueger, K. M. Keiblinger, S. Zechmeister-Boltenstern, and A. Richter. 2012. Stoichiometric controls of nitrogen and phosphorus cycling in decomposing beech leaf litter. *Ecology* 93:770-782.
- Plath, K. and M. Boersma. 2001. Mineral limitation of zooplankton: Stoichiometric constraints and optimal foraging. *Ecology* 82:1260-1269.
- Vanni, M. J., A. S. Flecker, J. M. Hood, and J. L. Headworth. 2002. Stoichiometry of nutrient recycling by vertebrates in a tropical stream: linking species identity and ecosystem processes. *Ecology Letters* 5:285-293.
- Woodward, G., M. O. Gessner, P. S. Giller, V. Gulis, S. Hladyz, A. Lecerf, B. Malmqvist, B. G. McKie, S. D. Tiegs, H. Cariss, M. Dobson, A. Eloegi, V. Ferreira, M. A. S. Graca, T. Fleituch, J. O. Lacoursiere, M. Nistorescu, J. Pozo, G. Risnoveanu, M. Schindler, A. Vadineanu, L. B. M. Vought, and E. Chauvet. 2012. Continental-scale effects of nutrient pollution on stream ecosystem functioning. *Science* 336:1438-1440.
- Wotton, R. S. and B. Malmqvist. 2001. Feces in aquatic ecosystems. *Bioscience* 51:537-544.