Selective Feeding Determines Patterns of Nutrient Release by Stream Invertebrates

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Selective feeding determines patterns of nutrient release by stream invertebrates

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Abstract: One common stoichiometric approach to predicting patterns of nutrient release (excretion + egestion) by animals in aquatic ecosystems is to base predictions on elemental mass-balance constrained by homeostatic maintenance. An easily measured resource composite (i.e., seston, epilithon, or leaf litter) often is used to represent ingested stoichiometry, but whether such a composite is a good indicator of food actually ingested is a relatively unexplored assumption. We examined the application of a stoichiometric model to the diets of 4 generalist stream invertebrates. We fed 3 trichopteran and 1 amphipod taxa rations consisting of cultured algae, stream epilithon, and several species of conditioned leaf litter. The rations ranged widely in C : N from 10 to 69 (molar) and in C : P from 165 to 3500. After a 2-d feeding period, we measured NH_4^+ and PO_4^{3-} excretion, and C, N, and P egestion rates. The relationships observed between the stoichiometries of release and ration were unexpected. Total N : P release rates conformed to stoichiometric predictions for only 1 taxon. Excretion and egestion rates and ratios were generally similar across diets and rarely varied with ration stoichiometry. These patterns were the result of smaller-than-expected responses to leaf-litter rations, which were the most imbalanced relative to body stoichiometry. Analysis of the C : N stoichiometry of foregut material for 2 taxa showed selective ingestion of an N-rich fraction of leaf litter, in 1 case reducing an apparent 8.4 : 1 C : N imbalance between diet and body composition to 1.5 : 1. Our results show that selective feeding can reduce potential stoichiometric imbalances, altering patterns of nutrient release relative to expectations based on bulk-diet stoichiometry. Assuming that stream invertebrates consume materials stoichiometrically similar to a resource composite can obscure understanding of stoichiometric imbalances and the role of invertebrates in nutrient cycles.
Key words: consumer-driven nutrient recycling, ecological stoichiometry, streams, excretion, egestion, selective feeding

Animals play many important roles in freshwater nutrient cycles (Vanni et al. 2002). One important pathway involves the release of nutrients in solid and soluble waste products. In benthic habitats, nutrient excretion by animals can influence soluble N : P ratios and can be an important source of nutrient regeneration (Grimm 1988, Hall et al. 2003, McIntyre et al. 2008, Liess and Kahlert 2009, Benstead et al. 2010). Fecal particles also can constitute an important contribution to benthic fine particulate organic matter (FPOM) pools (Grimm 1988, Wallace et al. 1991, Mulholland et al. 1995), creating a high-quality substrate for animals and microbes. Animal waste products link animals, basal resources, and nutrient cycles. Therefore, predicting nutrient release rates and ratios has been a long-standing goal of ecologists (Peters and Rigler 1973, Olsen and Ostgaard 1985, Sterner 1990, Elser and Urabe 1999).

Nutrient-release models vary from empirical (Sereda et al. 2008) to relatively simple mass-balance models (Sterner 1990, Olsen and Ostgaard 1985) to physiologically explicit (Darchambeau 2005). A mass-balance model formalized by Sterner (1990; homeostatic consumer model) has been applied widely as a framework for predicting and understanding nutrient release rates (Elser and Urabe 1999, Vanni et al. 2002, James et al. 2007, Rothlisberger et al. 2008, McManamay et al. 2011). The homeostatic consumer model uses a mass-balance approach (i.e., excretion + egestion = ingestion – growth) and an assumption of strict homeostatic maintenance (no change in animal tissue nutrient content in response to variation in diet nutrient content) to predict how diet and body stoichiometry influence total release ratios (excretion + egestion). Predicting either excretion or egestion alone requires a different approach (e.g., Darchambeau 2005) or additional assumptions (e.g., excretion dominates

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total release rates; McManamay et al. 2011). When body elemental content is tightly regulated, total nutrient release ratios reflect consumer nutrient stoichiometry and diet nutrient content. In the range of diet stoichiometry where X : Y indicates relative deficiency of Y, a positive relationship with slope > 1 is predicted (Fig. 1). These predicted relationships between nutrient release and body and diet stoichiometry provide one framework for interpreting the role of animals in nutrient cycles (e.g., McCarthy et al. 2006, Taylor et al. 2012). The validity of these predictions and their ramifications have been examined mainly in pelagic (Andersen and Hessen 1991, Elser and Urabe 1999) and benthic systems (Vanni et al. 2002, Frost and Tuchman 2005, Evans-White and Lamberti 2006, James et al. 2007, Rothlisberger et al. 2008). However, successful application of the homeostatic consumer model may still be hindered by 2 underexamined simplifications related to selective feeding and how homeostatic regulation is expressed in egestion and excretion.

The 1st simplification concerns the stoichiometry of the ingested material. The mass-balance model requires as input the stoichiometry of the material entering the gut of the animal. Users of this framework (e.g., Elser and Urabe 1999, Balseiro and Albariño 2006, McManamay et al. 2011) frequently make the simplifying assumption that animals ingest material stoichiometrically similar to an easily measured resource composite (e.g., seston, epilithon, or leaf litter). A large literature exists concerning selective feeding by freshwater invertebrates (e.g., Cummins and Klug 1979, Kleppel 1993, Graça 2001), but few stoichiometric applications to field situations include careful measurement of the ingested food material or an evaluation of this assumption (but see Vanni 1996, Higgins et al. 2006).

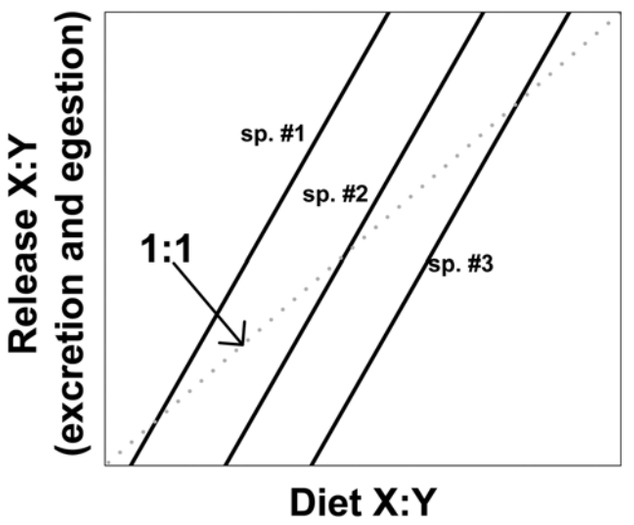

Figure 1. The homeostatic consumer model predicts that if animals maintain strong elemental homeostasis the relationship between total release X : Y (excretion + egestion) and food X : Y should be positive and nonlinear. This relationship has 2 regions demarcated by the threshold X : Y ratio separating X and Y deficiency. When X is deficient, X : Y release is predicted to be low and not to vary with diet X : Y (not shown). When Y is deficient, X : Y release increases linearly with diet X : Y with a slope > 1. Solid lines represent 3 species, consuming a diet deficient in Y, differing in body X : Y stoichiometry (sp. 1 X : Y < sp. 2 X : Y < sp. 3 X : Y). The dotted gray line is the 1 : 1 line. The influence of body X : Y on release X : Y is shown for completeness. We did not examine the influence of body X : Y or make interspecific comparisons. X and Y are any element. Figure adapted from Sterner and George (2000).

The assumption of similarity between bulk diets and ingested material holds reasonably well for some invertebrates in pelagic systems. Composite or bulk seston stoichiometry often appears to be a reliable predictor of the stoichiometry of material ingested by certain zooplankton because release ratios of these organisms relate to diet stoichiometry as predicted (Sterner 1990, Elser and Urabe 1999). However, even in these environments, many zooplankton species selectively ingest narrow particle-size fractions or even individual particles (Kleppel 1993). Animal diets in pelagic systems are stoichiometrically variable, but often not as structurally complex as benthic organic matter sources (i.e., benthic epilithon, leaves, or detritus) where food selection may play an even larger role in influencing stoichiometric imbalances.

In benthic systems, correspondence between the stoichiometry of a measured composite resource and ingested material may be weak because of the complex, structured nature of benthic basal substrates (Cummins and Klug 1979, Lock et al. 1984). This situation is more analogous to that found in terrestrial systems than in pelagic systems. Terrestrial systems contain multiple potential diets items that are stoichiometrically variable (e.g., bark, leaf shoots, phloem, and microbial communities). Just as some invertebrates select specific resources in a forest, some benthic grazers (scrapers) often selectively ingest just a portion of the epilithic matrix (Hall et al. 1998, Mulholland et al. 2000, Tank et al. 2000, Parkyn et al. 2005, McNeely et al. 2006). Stream detritivores (shredders) often selectively ingest or assimilate the microbes growing on leaf litter instead of the leaves (Arsuffi and Suberkropp 1989, Graça et al. 1993, Chung and Suberkropp 2009a). The potential stoichiometric implications of selective feeding for benthic nutrient cycles have been noted (Cross et al. 2005, Evans-White and Lamberti 2005, 2006), but the influence of selective feeding on stoichiometric imbalances or nutrient release have received little attention (but see Higgins et al. 2006).

A 2nd challenge in the application of this framework to nutrient release involves our understanding of how egestion and excretion are separately influenced by homeostatic regulation, food digestibility, and other factors influencing nutrient assimilation, use, and loss. In spite of the fact that the homeostatic consumer model is based on to-

tal nutrient input and output from consumers, most freshwater uses of this framework have examined excretion and not egestion (but see Elser et al. 1995, Sterner and George 2000, Hood et al. 2005, Balseiro and Albariño 2006). This simplification raises a number of questions. Does homeostatic regulation occur both in the gut and postassimilation so that egestion and excretion, respectively, relate to food stoichiometry as predicted by Sterner (1990)? Are there differences among elements (i.e., C, N, or P) in the expression of homeostatic regulation in egestion and excretion? Variation in the influence of homeostatic regulation on excretion and egestion will influence the role animals play in freshwater nutrient cycles. This makes it important to fully examine how both excretion and egestion are influenced by food stoichiometry.

We examined experimentally the relationships between nutrient release and resource stoichiometry for 4 common stream shredders. We offered these 4 taxa several stoichiometrically contrasting rations (algae, epilithon, and leaf litter) and measured N and P egestion and excretion rates and the C : N stoichiometry of a subset of the material ingested. We used these data to test 3 stoichiometric predictions, developed by Olsen and Ostgaard (1985) and Sterner (1990), concerning nutrient release and food stoichiometry. 1) Variation in the stoichiometry of nutrient release should be greater than the stoichiometric variation of the ingested material (Sterner 1990). 2) Release and ingested material stoichiometries scale positively with a slope > 1, but this relationship may be curvilinear at the low range of the data (Fig. 1; Sterner 1990). 3) Release rates of N and P decline with C : nutrient stoichiometry of ingested material (Olsen and Ostgaard 1985). To test these predictions, we used and assessed the simplifying assumption that bulk ration stoichiometry is an accurate representation for the stoichiometry of ingested material.

METHODS

Study sites

We measured N and P release (excretion and egestion) by 2 common shredder taxa in Elder Creek (Mendocino County, California) and 2 taxa from Valley Creek (Washington County, Minnesota). Both streams are cool-water, moderately shaded, and have stable base flow during the summer (McNeely and Power 2007, Zimmerman and Vondracek 2007). Elder Creek is a 3rd-order steam in the Angelo Coast Range Reserve (watershed area = 17 km^2 at our study site). The region's climate is Mediterranean with warm, dry summers and cool, wet winters. The riparian community includes bay (*Umbellularia californica*), madrone (*Arbutus menziesii*), Douglas fir (*Pseudotsuga menziesii*), alder (*Alnus rhombifolia*), and maple (*Acer macrophyllum*). A thin, heavily grazed layer of diatoms dominates epilithic communities during the summer months (McNeely and Power 2007).

The Elder Creek experiments focused on individuals in the genera *Lepidostoma* and *Psychoglypha*, common benthic invertebrates in this system. Ongoing taxonomic research suggests that multiple species of both *Lepidostoma* (CM, unpublished data) and *Psychoglypha* (*Psychoglypha bella* and *Psychoglypha leechi*) can be found in the Angelo Coast Range Reserve. Unfortunately, larval *Lepidostoma* and *Psychoglypha* individuals cannot be identified to species in the field. We took care to use only the dominant morphotypes of both taxa. Stable C and N isotopes suggest that *Psychoglypha* primarily consumes epilithon in Elder Creek, but it consumes allochthonous detritus at other locations in this system (JMH, unpublished data). Isotopic evidence also suggests that the diet of *Lepidostoma* in Elder Creek shifts ontogenetically from epilithon to allochthonous detritus (JMH, unpublished data).

Valley Creek is a 1st-order, groundwater-fed stream (watershed area = 161 km^2; Zimmerman and Vondracek 2007). We collected samples within the Belwin Reserve (www.belwin.org), where a narrow riparian zone and a grass lawn bound the stream. Riparian trees are primarily willow (*Salix* sp.) and eastern cottonwood (*Populus deltoides*), but few trees are found in the riparian area. *Gammarus pseudolimnaeus* and *Lepidostoma* (VC [Valley Creek] *Lepidostoma*) are common shredders in Valley Creek (Ruetz et al. 2002). As for the Elder Creek site, we could not visually distinguish among *Lepidostoma* species present at Valley Creek. Therefore, it is possible that experiments at both sites involved closely related *Lepidostoma* species rather than a single taxon, although all larvae used were very similar in appearance.

Elder Creek experiments

Feeding trials for Elder Creek experiments used Elder Creek epilithon, South Fork Eel River epilithon (*Psychoglypha* sp. experiment only), and conditioned bay, madrone, maple, and oak (*Quercus wislizenii*) litter (Table 1). We collected freshly fallen litter and conditioned it in flow-through chambers (0.3 × 0.6 × 0.4 m, 2-mm nylon screen on all sides) incubated in a riffle in Elder Creek for ≥1 mo before experiments. We collected epilithon just before initiation of feeding experiments from typical habitats for Elder *Lepidostoma* and *Psychoglypha* in Elder Creek or the South Fork Eel River.

We conducted *Psychoglypha* and Elder *Lepidostoma* experiments in July and early September 2007, respectively. Immediately prior to each experiment, we collected mid-sized *Psychoglypha* (3.5 ± 0.4 mg [SD]) or Elder *Lepidostoma* (4.0 ± 0.5 mg) from Elder Creek and took care to minimize the range in animal size used in the experiment. We distributed animals to 24 flow-through chambers containing 1 of the 5 (Elder *Lepidostoma*) or 6 (*Psychoglypha*) ration types. We placed 10 Elder *Lepidostoma* or 8 *Psychoglypha* in each container. Epilithon treatments were

Table 1. Mean (±1 SE) ration C : N, C : P, and N : P stoichiometry. NA = not available.

Species	Ration	C : N	C : P	N : P
Elder *Lepidostoma*	Range	11.0–54.5	362.8–1654.0	28.2–33.2
	Bay	34.1 (0.5)	1159.3 (17.8)	31.5 (1.0)
	Madrone	54.5 (1.8)	1654.0 (25.0)	28.2 (0.6)
	Maple	21.2 (0.4)	651.8 (77.0)	28.4 (2.8)
	Oak	53.3 (0.6)	1574.1 (111.3)	27.3 (2.2)
	Elder epilithon	11.0 (0.4)	362.8 (7.5)	33.2 (0.9)
Psychoglypha	Range	11.1–59.5	287.2–3020.6	24.8–156.5
	Bay	47.0 (3.4)	3020.6 (399.8)	65.2 (13.2)
	S. Fork Eel epilithon	11.1 (0.2)	326.8 (13.9)	29.5 (1.6)
	Elder epilithon	11.6 (0.4)	287.2 (23.6)	24.8 (1.8)
	Madrone	59.5 (3.7)	2804.0 (40.9)	47.3 (3.6)
	Maple	19.2 (NA)	3010.4 (NA)	156.5 (NA)
	Oak	53.6 (0.3)	1640.7 (505.2)	30.6 (9.2)
Valley Creek experiments	Range	10.8–69.1	165.7–3513.4	15.4–49.8
	Maple	54.8 (3.4)	2014.4 (514.3)	36.3 (7.1)
	Oak	45.8 (0.4)	1387.2 (111.5)	30.3 (2.5)
	Pine	69.1 (5.2)	3513.4 (788.5)	49.8 (7.1)
	Scenedesmus obliquus	10.8 (1.0)	165.7 (13.4)	15.4 (0.5)
	Willow	38.1 (1.1)	1338.7 (75.0)	35.3 (2.5)

two 5- to 10-cm-diameter rocks placed in each chamber. Flow-through chambers were 1.9-L rectangular, Gladware® containers with the narrow ends replaced with 2-mm nylon screen. We placed chambers in Elder Creek in a pool with intermediate flow that ran through the chambers for a 48-h acclimation period prior to nutrient-release measurements. We considered the 48-h acclimation period long enough for the organisms to begin feeding on the new rations and for that material to move through their guts several times before nutrient-release measurements were made.

All Elder Creek nutrient release measurements followed the same protocol adapted from Vanni et al. (2002). After the 48-h acclimation period, we distributed animals in flow-through containers as follows. We rinsed 6 *Psychoglypha* or 8 Elder *Lepidostoma* in stream water and transferred them to containers with no food and 60 or 80 mL of filtered (Whatman GF/F, average pore size = 0.7 μm; Whatman, Maidstone, UK) Elder Creek water. We took initial water chemistry samples immediately from each container, filtered them (GF/F filter), and later analyzed them for SRP and NH_4^+. We rinsed 2 additional animals, which we placed in a glass vial on ice, and froze upon return to the laboratory (~2–3 h later), and subsequently used for foregut analysis. We made control containers with filtered stream water and empty caddisfly cases to account for nonexcretory changes in nutrient concentrations. Incubations lasted ~30 min for *Psychoglypha* and 60 min for Elder *Lepidostoma*. Consumption of fecal material may have occurred during these incubations and potentially could have led to underestimation of egestion rates. However, we think it unlikely that fecal particles ingested during these 30- or 60-min incubations could pass through the gut in time to influence the stoichiometry of fecal material. Gastric evacuation rates for these taxa are not available, but gut clearance times for aquatic invertebrates are generally >1 h (Cowan and Peckarsky 1990, Cristo 2001).

Incubation durations often must be tailored to each species based on feeding and nutrient-release rates. Incubation durations represent a tradeoff between competing objectives: maximizing the signal-to-noise ratio for nutrient-release measurements and minimizing the period of starvation (Whiles et al. 2009). We conducted pilot experiments with the Elder Creek taxa to optimize incubation durations and the number of individuals/container. Differences in incubation times among taxa do not influence our conclusions because statistical comparisons were not made between taxa.

Following the incubation, we gently mixed the water and fecal material in the excretion chamber and filtered all of the water and particles in the excretion chamber (Whatman GF/F in a Gelman syringe filter holder; Gelman, Cortland, New York). We filtered water from the excretion chambers into glass test tubes and immediately analyzed it for SRP and NH_4^+ in duplicate. We dried the filter at 60°C and retained it to estimate particulate C, N, and P egestion. We

removed leaf rations from the feeding chambers, consolidated them into 2 replicate samples, dried them (60°C), and stored them for C, N, and P analysis. We used a wire brush to remove biofilm surrounding a 4.2-cm^2-diameter sample area on the top of each stone. We used a hard toothbrush to collect the epilithon sample, which was filtered onto 2 preweighed and precombusted (550°C) GF/F filters for C, N, and P analysis. We dried filters at 60°C and stored them for analysis. Litter and epilithic stoichiometry could change during the acclimation period because of uptake of excreted nutrients, consumption of algae and microbes, or the inclusion of fecal material. Therefore, we used the ration stoichiometry at the end of the feeding period in all analyses. This measure best reflects the material available to the animals immediately prior to the excretion measurements.

Valley Creek experiments

The methods for Valley Creek (VC) measurements differed somewhat from Elder Creek methods because the laboratory and field resources available in Minnesota differed. We collected *G. pseudolimnaeus* (4.9 ± 1.0 mg) and VC *Lepidostoma* spp. (16 ± 6 mg) from Valley Creek in October, returned them to the laboratory, and placed them in aerated aquaria containing 10 L of Valley Creek water and 1 of 5 diet treatments: oak (*Quercus* sp.), willow (*Salix* sp.), maple (*Acer* sp.), pine (*Pinus strobus*), or *Scenedesmus obliquus*, a planktonic green alga. *Scenedesmus obliquus* was cultured in chemostats under N-limited conditions, as described by Hood and Sterner (2010). We did not use Valley Creek epilithon because the structure of these communities (often a thick layer of bryophytes, epilithic algae, and detritus) made isolating a single resource impractical. *Scenedesmus obliquus* settled to the bottom of the aquaria within a few hours and was observed in the guts of both taxa. We used separate aquaria for each consumer × resource combination. We kept aquaria in an environmental chamber at 10°C during a 48- (*G. pseudolimnaeus*) or 72-h (VC *Lepidostoma*) acclimation period. Following acclimation, we conducted nutrient-release measurements as described for the Elder Creek experiments except that all nutrient release incubations were ~60 min. In the *G. pseudolimnaeus* experiments, controls contained only filtered water. We preserved and analyzed leaf-litter ration stoichiometry samples as described for the Elder Creek measurements. We siphoned *S. obliquus* from the aquaria and filtered the material for C, N, and P analysis as described previously.

Foregut and hindgut material

We dissected animals from all experiments to extract foregut samples. Elder *Lepidostoma* and *Psychoglypha* larvae have simple, cylindrical guts. We classified the first ⅓ as the foregut. We gently removed material removed from this section, placed it in a preweighed tin capsule and dried it at 60°C. We weighed the material to the nearest 0.1 μg then analyzed for C and N as described below. Adequate mass for analysis was collected from the Elder Creek experiments for C and N analysis, but the mass was insufficient to measure P. We composited samples across replicates, resulting in ~2 foregut samples per treatment.

Chemical and statistical analyses

We analyzed filtrate samples for PO_4^{3-} with the acid-molybdate method on a spectrophotometer and for NH_4^+ with the fluorometric method (Holmes et al. 1999, Taylor et al. 2007). We created NH_4^+ standard curves with filtered stream water (Elder Creek experiments) or sample water (i.e., filtered water from each excretion chamber; Valley Creek experiments). Excretory products create matrix effects, which lead to underestimation of NH_4^+ when stream and not sample water is used for standard curves (Whiles et al. 2009). In 2008, we conducted Elder *Lepidostoma* and *Psychoglypha* NH_4^+-excretion experiments using both approaches (JMH and CM, unpublished data). NH_4^+ concentrations calculated through these 2 approaches were strongly correlated ($\mu g\ NH_4^+/L_{sample} = 1.554[\mu g\ NH_4^+/L_{stream}] - 13.62$; $R^2 = 0.982$, $p < 0.001$). The relationship did not differ between species (standardized major axis test for similar slopes: $n = 12$, 2 species, $p = 0.723$). Therefore, we used this linear equation to correct all Elder Creek NH_4^+ data originally analyzed with stream-water standard curves.

We dried (60°C), weighed, and cut the filters with fecal samples in half. We reweighed each half and analyzed them for C and N or P. To calculate the mass of material on each half, we assumed that the initial mass of each half was 50% of the whole filter mass. The small fecal particles of these taxa were distributed relatively evenly around the edge of the filter (JMH, personal observation). We dried (60°C), homogenized with a Wiley mill (Thomas Wiley Mills, Swedesboro, New Jersey), and subsampled leaf-litter samples for C, N, and P analysis. We combusted (550°C) particulate P samples and hydrolyzed them in HCl before measuring PO_4^{3-} colorimetrically as molybdenum blue (DeMott et al. 1998). We analyzed particulate C and N samples with a Perkin–Elmer 2400 CHNS analyzer (Waltham, Massachusetts).

We calculated mass-specific N and P excretion rates as the change in NH_4-N or PO_4-P per unit time divided by the dry mass (DM) of animals. Mass-specific C, N, and P egestion rates were calculated as the mass of C, N, or P on the half-filter × 2, then divided by the DM of animals and length of incubation. We estimated total nutrient release as the sum of nutrient excretion and nutrient egestion. We can fully examine our predictions only with total release N : P and not C : nutrient because we did not measure respiration or C excretion. However, we examined the relationships between excretion and egestion and ration stoichiometry to gain a better understanding of how

these types of waste elimination might relate to diet stoichiometry. We examined the influence of ration type on release rates and ratios with 1-way analyses of variance (ANOVAs). We compared bulk rations to foregut material, expressed in molar ratios, with a 2-way ANOVA and Tukey Honestly Significant Difference (HSD) post hoc tests. We assessed prediction 1 by comparing ranges between release and ration stoichiometry. We used standard least-squares regression to test for the predicted relationships (predictions 2 and 3; Fig. 1) between nutrient release and ration stoichiometry. We made no statistical comparisons among taxa. All statistical tests were conducted in Statistica 9.0 (StatSoft, Tulsa, Oklahoma).

RESULTS

Ration C, N, and P stoichiometry

The appropriateness of the tests we performed depends on using rations representing a wide stoichiometric range that encompasses values where the element in the denominator is relatively deficient. In each experiment, a strong stoichiometric contrast among the rations and ration stoichiometry often exceeded by many-fold the stoichiometry of most stream invertebrates (Table 1). For example, Valley Creek experiment rations ranged in C : P from ~166 (algae) to ~3513 (pine) and in C : N from ~11 (algae) to ~69 (pine). A large C : nutrient range also was observed in the Elder Creek experiments. As expected, *S. obliquus* and epilithon were more nutrient rich than conditioned leaf litter (Table 1).

A smaller range was observed in ration N : P, which reflected N-rich material (Table 1). For instance, Valley Creek ration N : P ranged from 15.4 (algae) to 49.8 (pine), whereas Elder *Lepidostoma* experiment ration N : P ranged from only ~27 (oak) to ~33 (epilithon). C : N, C : P, and N : P differed significantly among rations ($p < 0.05$) in all cases except for Elder *Lepidostoma* ration N : P ($p = 0.053$).

Predicting nutrient release using ration stoichiometry

The homeostatic consumer model predicts a greater range in element ratios of material released than in the food (prediction 1). In spite of the wide stoichiometric contrast across rations, particularly in C : nutrient, release stoichiometry had a range similar to or less than ration stoichiometry in 16 of 20 cases (Fig. 2). A case constitutes each release ratio that can be compared to stoichiometric predictions. For example, for prediction 1 we can compare variation in N : P total release, N : P excretion, and C : N, C : P, and N : P egestion (5 cases) for 4 species (20 cases total). We did not present all possible cases (e.g., total C : P release) because we did not measure respiration or C excretion rates.

The 4 instances in which the range of release stoichiometry was greater than ration stoichiometry all involved

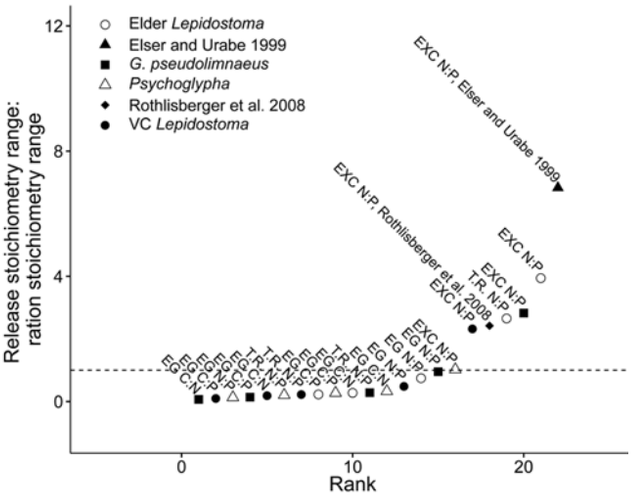

Figure 2. The ratio of the range of release stoichiometry (e.g., maximum release N : P − minimum release N : P) to the range of ration stoichiometry (e.g., maximum ration N : P − minimum ration N : P) varies widely among taxa, nutrient ratios, and waste products (total release = T.R., excretion = EXC, and egestion = EG). The homeostatic consumer model predicts that the range of release stoichiometry will be greater than the range of ration stoichiometry. Only patterns of excretion N : P are consistent with these predictions. Published ratios for zooplankton (Elser and Urabe 1999) and mayflies (Rothlisberger et al. 2008) are shown for comparison. VC = Valley Creek.

N : P excretion (Elder *Lepidostoma*, VC *Lepidostoma*, *G. pseudolimnaeus*) or total N : P release (Elder *Lepidostoma*). *Psychoglypha* exhibited a range of excretion N : P ≈ ration N : P. Thus, these taxa magnified the range of available N : P through excretion but diminished the range of available N : P, C : N, and C : P through egestion.

For large ranges of diet X : Y, theory predicts a positive relationship between release and ration stoichiometry with a slope > 1 (prediction 2; Fig. 1). This prediction was supported in only 3 of 20 possible cases, and each supporting case involved N : P stoichiometry (Table 2). Total N : P release by Elder *Lepidostoma* was related to ration N : P as predicted (Fig. 3A), and N : P excretion by Elder and Valley Creek *Lepidostoma* was positively related to ration N : P with a slope > 1 (Fig. 3B). N : P excretion ratios of both *G. pseudolimnaeus* and *Psychoglypha* were variable relative to ration N : P (Fig. 2), but did not exhibit the predicted relationship (Table 2, Fig. 3B). The relationship between release and ration ratios was not significant in 12 of 20 cases (Table 2) and overall, data clearly indicated slopes < 1 (Fig. 4A–C).

Last, theory predicts a negative relationship between nutrient release rates and ration C : nutrient (prediction 3). Our results did not conform to this prediction in most cases (Fig. 5A–F). Nutrient release rates and ration C : nutrient were unrelated in 23 of 24 possible cases. P excretion by

Table 2. Evaluation of prediction 2. Least-squared slopes (R^2, p-value) from the relationship between nutrient release and ration stoichiometry. Least-squared regressions used all data points and not treatment means. † = $p < 0.1$, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Variable	*Gammarus pseudolimnaeus*	Elder *Lepidostoma*	VC *Lepidostoma*	*Psychoglypha*
Total release				
N : P vs ration N : P	0.21 (0.05, 0.192)	1.66* (0.16, 0.015)	0.18* (0.26, 0.026)	0.04 (0.01, 0.710)
Excretion				
N : P vs ration N : P	–0.41 (0.00, 0.703)	2.62** (0.17, 0.009)	2.42*** (0.67, <0.001)	–0.33 (0.08, 0.230)
Egestion				
C : N vs ration C : N	0.06† (0.09, 0.077)	0.18*** (0.35, <0.001)	0.00 (0.00, 0.93)	0.20** (0.45, 0.002)
C : P vs ration C : P	0.09** (0.24, 0.002)	0.12** (0.20, 0.005)	0.03 (0.03, 0.513)	0.07 (0.08, 0.266)
N : P vs ration N : P	0.56† (0.10, 0.053)	0.28 (0.02, 0.394)	0.25 (0.10, 0.191)	0.04 (0.03, 0.477)

Valley Creek *Lepidostoma* was negatively related to ration C : P as predicted (Fig. 5B, inset).

Taken together, our results do not consistently conform to stoichiometric predictions. Results can be separated into 2 groups. On the one hand, we found little match to theory for predictions involving egestion C : nutrient. On the other hand, we found mixed support for predictions involving N : P excretion (but not egestion) and ration N : P. In comparisons involving N : P excretion, we found complete support for prediction 1 and partial support for prediction 2 (2 of 4 cases). As noted earlier, tests involving total N : P release were the only tests we can make of prediction 2 without making additional assumptions. In this restricted subset of cases, only total N : P release by 1 of the 4 taxa (Elder *Lepidostoma*) conformed to theoretical predictions.

Balance between excretion and egestion

The balance between nutrient excretion and egestion provides information on how homeostatic regulation influences excretion and egestion and on the relative contribution of these animals to soluble and particulate nutrient cycling. Nutrient excretion : egestion ranged from ~0.3 to ~2.9 (Appendix). In most cases, egestion was the dominant form of nutrient release. However, Elder *Lepidostoma* excreted more nutrients than it egested. Ration identity influenced the balance between nutrient excretion and egestion in 3 of 8 cases (Appendix).

Comparison between ration and foregut stoichiometry

The paradoxical nature of high stoichiometric contrasts in rations (Table 1) compared with constrained stoichiometric variability of wastes (Figs 3–5) may indicate that bulk C : nutrient measurements were poor estimates of the actual diets. We tested for selective ingestion by analyzing the C and N content of Elder *Lepidostoma* and *Psychoglypha* foregut material. We assumed that material in the foregut was subject to little assimilation and, therefore, represented the stoichiometry of ingested material. We could not acquire enough material to measure the P content of foregut material for any taxon or the C and N content of VC *Lepidostoma* and *G. pseudolimnaeus* foregut material.

The foregut materials of Elder *Lepidostoma* and *Psychoglypha* were N rich relative to the rations (Fig. 6A–F). Elder *Lepidostoma* foregut material was higher in N (2-way ANOVA: $F_{1,16} = 591.0$, $p < 0.001$; Fig. 6C) and sometimes lower in C (2-way ANOVA: $F_{1,16} = 4.9$, $p = 0.041$; Fig. 6A) than the rations. The only significant difference between foregut and ration C content occurred in the maple treatment. *Psychoglypha* foregut material was significantly higher in N than all rations except Elder epilithon (2-way ANOVA: $F_{1,17} = 202.1$, $p < 0.001$, Fig. 6D). *Psychoglypha* foregut material differed from the rations in terms of C content (2-way ANOVA: $F_{1,17} = 4.6$, $p = 0.048$; Fig. 6B), but post hoc tests indicated that the contrasts were not significant (Tukey HSD, $p > 0.05$). Selective feeding greatly reduced stoichiometric variation among ration types (Fig. 6E, F).

DISCUSSION

Nutrient release and bulk ration stoichiometry relationships were unexpected relative to our predictions. Our results suggest that the assumption of correspondence between bulk ration stoichiometry and the stoichiometry of ingested material was violated. At least 2, and probably all 4, taxa selectively consumed a nutrient-rich portion of the leaf-litter rations (Fig. 6A–F). Investigators commonly use the stoichiometry of bulk food items (e.g., ration stoichiometry) as a proxy for the stoichiometry of ingested material (e.g., Elser and Urabe 1999, Balseiro and Albariño 2006, McManamay et al. 2011). Thus, our results highlight the importance of accurate model parameterization (i.e., direct measurements of the stoichiometry of ingested material) and the potential implications of selective feeding for stoi-

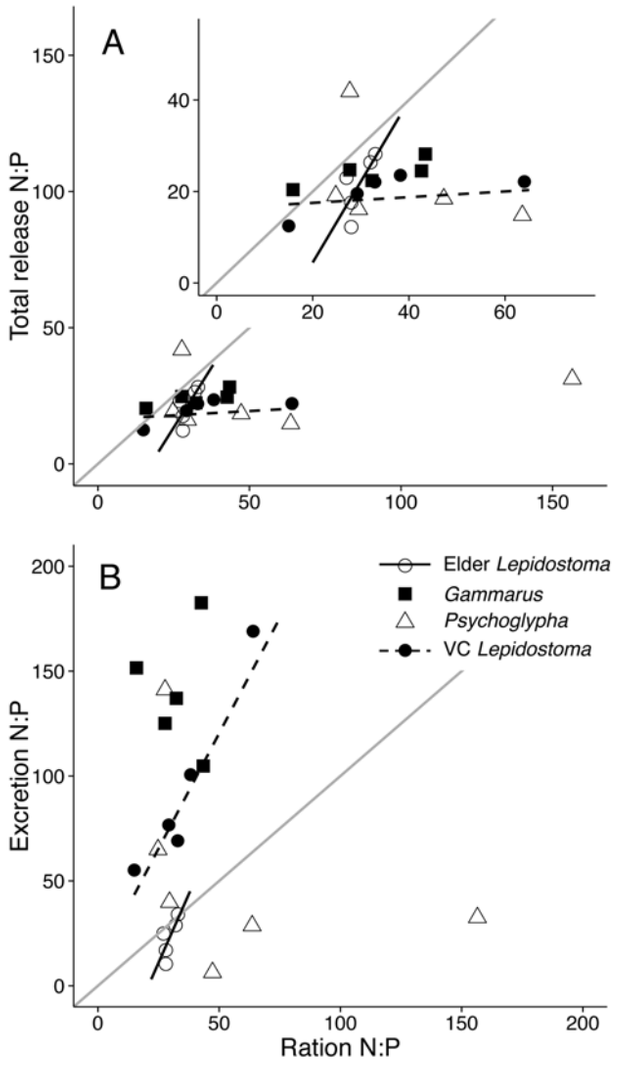

Figure 3. The influence of ration N : P on total release N : P (inset includes only ration N : P treatments <80) (A), and excretion N : P (B). The solid gray line is the 1 : 1 line. Significant ($p < 0.05$) least-squares fits are shown.

chiometric models, animal–diet stoichiometric imbalances, and the role of organisms in aquatic nutrient cycles. Although previously recognized in reviews (Cross et al. 2005, Frost et al. 2005), the influence of selective feeding on nutrient release has received little attention (but see Higgins et al. 2006). Below we explore 3 lines of evidence suggesting that selective feeding explains our unexpected patterns of nutrient release, and then we explore the potential influence of selective feeding on stoichiometric imbalances and aquatic nutrient cycles.

Explaining nutrient release patterns: foregut–ration comparisons

Comparisons of foregut and ration N contents indicate that Elder *Lepidostoma* and *Psychoglypha* ingested mate-

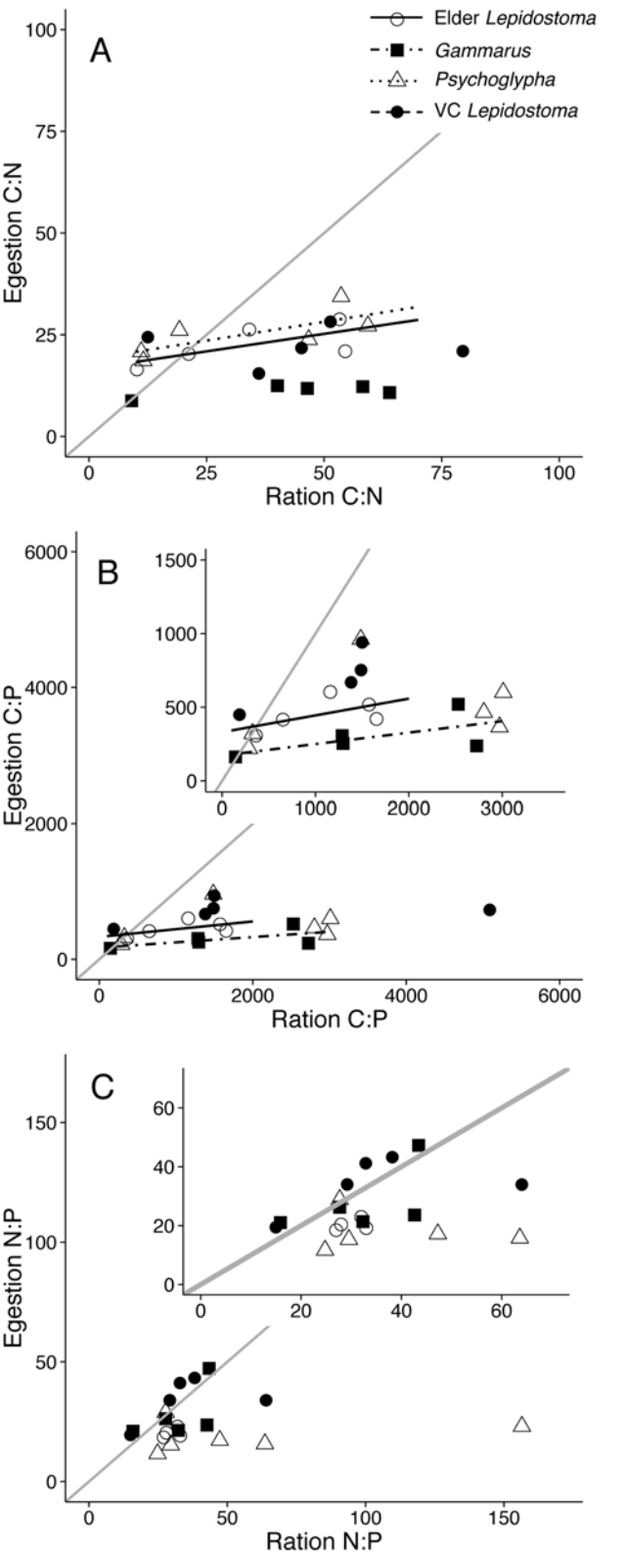

Figure 4. The influence of ration stoichiometry on egestion C : N (A), C : P (inset includes only ration C : P treatments <3500) (B), and N : P (inset includes only ration N : P treatments <75) (C). The gray line shows unity. Significant ($p < 0.05$) least-squares fits are shown. VC = Valley Creek.

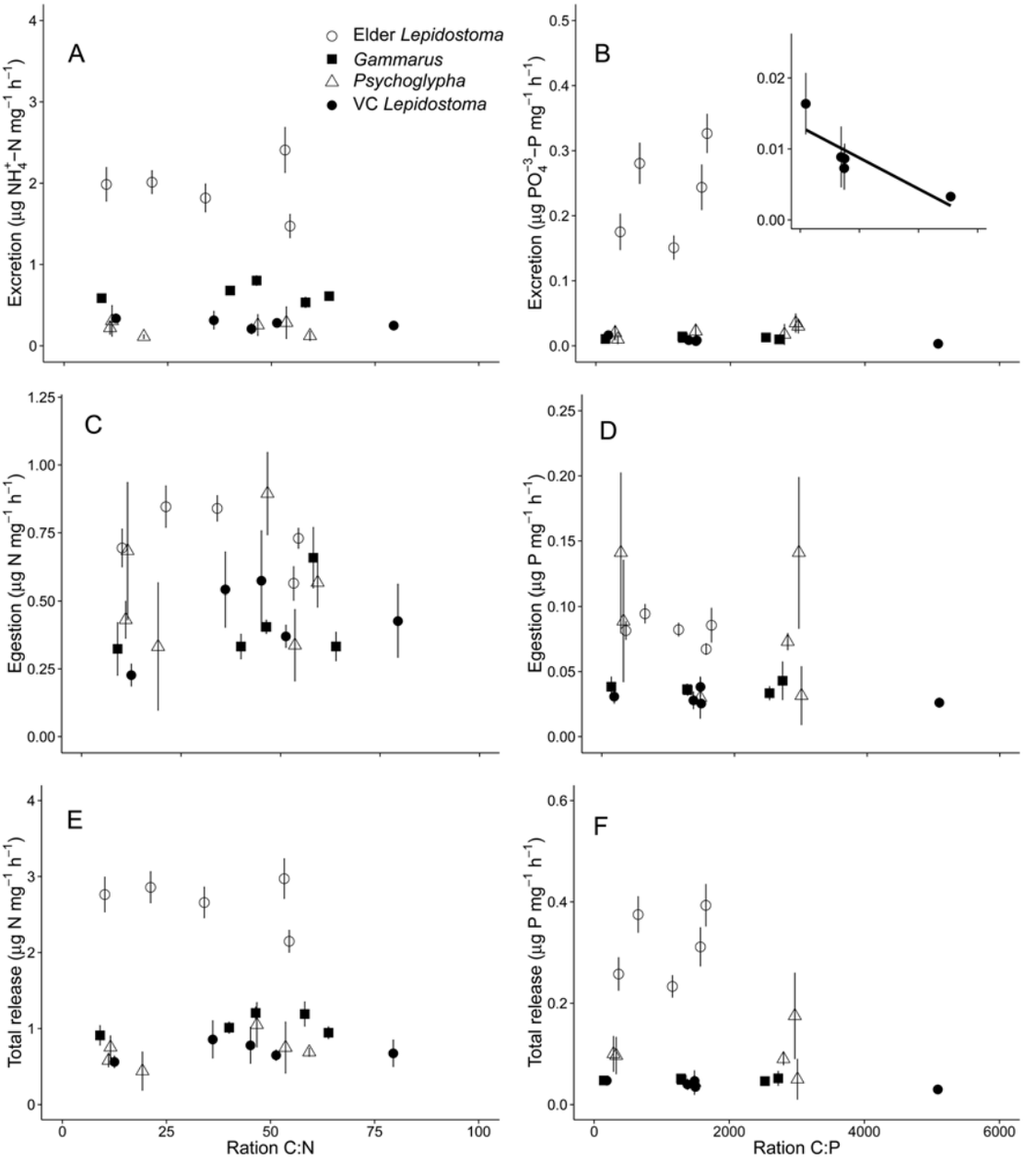

Figure 5. Mean (±1 SE) excretion (A, B), egestion (C, D), and total release (E, F) of N (A, C, E) and P (B, D, F) as a function of ration C : nutrient ratios. Release declined with ration as predicted in only 1 case (B, inset). VC = Valley Creek.

rial which was 2 to 4× higher in N than the leaf-litter rations offered (Fig. 6C, D). No other food source was available during the ration acclimation period, so foregut nutrient concentrations probably are indicative of selective feeding within the leaf litter, a matrix containing leaf material, fungus, and bacteria. Microbes, often an order of magnitude higher in N content than leaves (Cross et al. 2005), probably constitute a substantial fraction of the material ingested by these taxa when consuming leaf litter.

To estimate the microbial contribution required to account for the differences between ration and foregut N content, we used a 2-compartment (microbial N + leaf N) mixing model. We did not measure N partitioning between microbial and leaf pools. Therefore, we bracket our estimates with a range of microbial N values. If microbial N content were 5% (Ooijkaas et al. 2000), the ingested material would be predicted to be 75 to 90% microbial. If microbes colonizing this leaf litter were 10% N, close to the

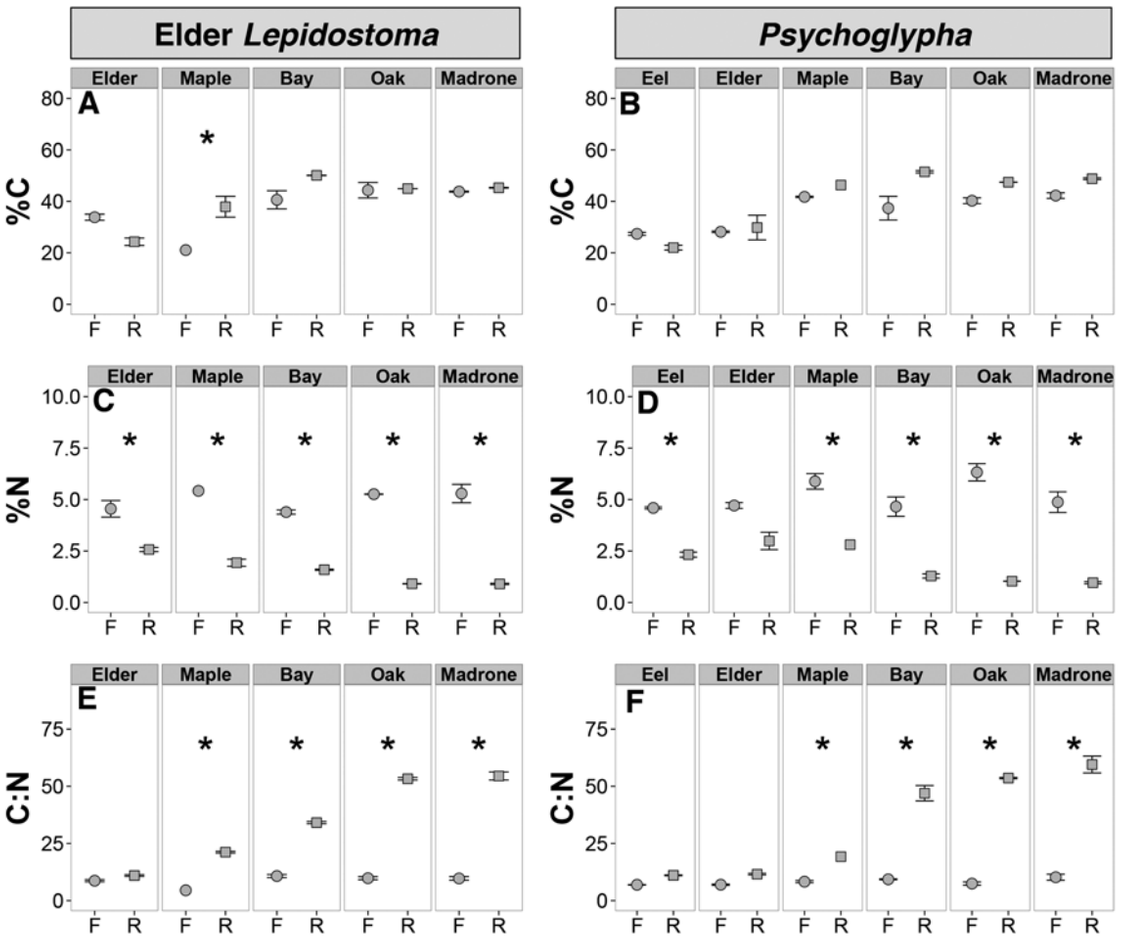

Figure 6. Mean (±1 SE) %C (A, B), %N (C, D), and C : N (E, F) of material from the foregut (F) and rations (R) of Elder *Lepidostoma* (A, C, E) and *Pyschoglypha* (B, D, F). Eel and Elder refer to the epilithic material collected from those rivers for rations. Asterisks indicate significant differences between foregut material and rations (Tukey Honestly Significant Difference, $p < 0.05$).

maximum N content of fungus (Levi and Cowling 1969), the material ingested by this species would be predicted to be 40% microbial. In the absence of selective feeding, the high N content of the foregut material samples might indicate that the foregut material was contaminated with caddisfly tissue (mean = 6.3% N; JMH unpublished data), but mixing models (caddisfly N + leaf N) predict that the foregut material would have to be 55 to 68% caddisfly. We think contamination of foregut material was a minor issue because ingested material was collected without damaging the gut. Taken together, the mixing model estimates indicate the importance of nutrient-rich microbial resources to Elder *Lepidostoma* and *Psychoglypha*.

Explaining nutrient release patterns: comparisons of release–ration relationships

The relationships between nutrient release and ration stoichiometry were generally similar among all 4 taxa in spite of differences in litter species and conditioning. The similarity of these patterns strongly suggests that *G. pseudolimnaeus* and VC *Lepidostoma* also selectively consumed a nutrient-rich fraction of the leaf-litter rations. The patterns of nutrient release we report are similar to what the homeostatic consumer model would predict given a relatively narrow stoichiometric range of ingested material (Fig. 6E, F); i.e., a small range in nutrient release rates and weak relationships between release rates and ration C : N (Figs 3, 4). However, N : P excretion did increase with ration N : P as predicted in 2 of 4 cases (Table 2). Such a correspondence might occur if the N : P of the selectively ingested fraction were correlated with ration N : P, perhaps because microbial N : P strongly influenced litter N : P.

Explaining nutrient release patterns: selective feeding in benthic systems

The generality of selective feeding by shredder taxa lends additional support to our hypothesis that the unpredicted nutrient release–ration relationships for all 4 taxa can be

attributed to selective feeding. Shredder taxa exhibit 3 types of selectivity, and the mode of selection has implications for the application of the homeostatic consumer model. 1) Many shredder taxa ingest leaf-litter species or patches based on characteristics, such as degree of decomposition, leaf toughness, nutrient content, and fungal species composition (Graça 2001). Many of these diets are probably stoichiometrically different from bulk litter. 2) Shredder taxa use several distinct feeding techniques to consume litter, resulting in selectivity relative to the bulk ration. Some taxa consume the entire leaf, whereas others scrape the surface of the leaf, ingesting more microbes than leaf (Graça et al. 1993). 3) Some shredders assimilate only an easily digestible litter fraction, probably dominated by microbes (Arsuffi and Suberkropp 1985, Chung and Suberkropp 2009a), whereas others are capable of assimilating microbial and leaf C and nutrients with the help of microbes (Sinsabaugh et al. 1985). Selective ingestion or assimilation of microbes has been observed for both Trichoptera and *Gammarus* (Bärlocher and Kendrick 1975, Arsuffi and Suberkropp 1985, 1989, Chung and Suberkropp 2009a). Knowledge of the identity and nutrient content of ingested material will help clarify the nutritional environment of aquatic organisms and their role in nutrient cycles.

Explaining nutrient release patterns: caveats

Selective consumption of a nutrient-rich fraction of the leaf-litter ration is the most likely explanation for the unexpected nutrient release–ration stoichiometry relationships we report. However, at least 2 caveats merit discussion. 1) We sorted each taxon to the lowest possible taxonomic group, but multiple species may have been involved in some of our experiments. Poor taxonomic resolution may add to our unexplained variation. However, differences in excretion among closely related species probably is minimal relative to the influence of ration stoichiometry or body size (Elser and Urabe 1999, Hall et al. 2007). 2) We made 2 assumptions in our analysis that, if violated, might explain the observed patterns of nutrient release. First, we assumed that the range of ration stoichiometry was wide enough to encompasses diets that were nutrient deficient and, therefore, that we were examining the linear portion of the release–ration relationship described by Sterner (1990). It is reasonable to assume that the leaf litter diets are nutrient deficient when adequate food is provided and in the absence of selective feeding (Iversen 1974, Frost et al. 2006). Second, the homeostatic consumer model assumes strict homeostasis of consumer tissue. This model is probably robust to some stoichiometric flexibility, but organisms with very weak homeostasis might use nutrient stores to buffer growth and maintain high nutrient release rates on low-nutrient diets. Large violations of either assumption seem unlikely, but full evaluation of either requires additional information.

Influence of selective feeding on stoichiometric imbalances

Many benthic invertebrates consume resources that are, in bulk, stoichiometrically out of balance with their nutritional demands (Cross et al. 2003, 2005), but selective feeding within these bulk resources may reduce stoichiometric imbalances. For example, selective feeding by Elder *Lepidostoma* (mean C : N = 6.3; JMH, JCF, RWS unpublished data) consuming oak leaves reduced an apparent 8.4 : 1 C : N imbalance (diet : animal) to 1.5. Mitigation of apparent stoichiometric imbalances by diet selection may be common. For instance, many aquatic invertebrates consume a fraction of the bulk organic matter pool with rapid turnover rates (Hall et al. 1998, Mulholland et al. 2000, Tank et al. 2000, Parkyn et al. 2005, Fellows et al. 2006, McNeely et al. 2006). Rapid growth requires large nutrient investments (Sterner and Elser 2002), so selective feeding by these aquatic invertebrates on compartments with high turnover may diminish stoichiometric imbalances.

Influence of selective feeding on nutrient flux pathways

The shredders examined in our study appear to consume a nutrient-rich fraction of the nutrient-poor leaf-litter pool. This feeding behavior probably influences stream nutrient cycles in 3 ways that are unexpected based on bulk leaf-litter stoichiometry. First, these shredders exhibit much higher nutrient excretion rates than expected in the absence of selective feeding. This high excretion rate increases the availability of PO_4^{3-} and NH_4^+ and may stimulate algal or microbial growth in nutrient-poor streams. Second, the fecal particles produced by these animals are richer in nutrients than the bulk diets. These particles enter the FPOM pool and provide a nutrient-rich substrate for microbes and collector gatherers. Last, the leaf-litter fraction not consumed by these animals must, by mass balance, be lower in nutrients than the bulk leaf litter. This unconsumed material probably is stripped of microbes and may be the most recalcitrant portion of the litter rations. Whether this material remains a part of the leaf litter (as skeletonized leaves) or enters the FPOM pool is not clear. We encourage future research on how this resource partitioning influences decomposition, organic matter budgets, and nutrient cycles.

Significance

Selective feeding is common in aquatic ecosystems (Arsuffi and Suberkropp 1989, Hall and Meyer 1998, Mulholland et al. 2000, Parkyn et al. 2005, Chung and Suberkropp 2009b), but many stoichiometric studies assume that animals consume a resource nonselectively (e.g., Elser and Urabe 1999, Balseiro and Albariño 2006, McManamay et al. 2011). Our results and those of Higgins et al. (2006) show that selective feeding shapes both animal nutrition and the role of animals in aquatic nutrient cycles. We suggest that future studies include careful measurements of the stoichi-

ometry of ingested material. While challenging, such efforts will provide more accurate stoichiometric models and a better understanding of both animal–diet stoichiometric imbalances and the role of animals in freshwater nutrient cycles.

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APPENDIX

Mean (±1 SE) release rates and ratios and the influence of ration identity. DM = dry mass. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Variable	*Gammarus pseudolimnaeus*	Elder *Lepidostoma*	VC *Lepidostoma*	*Psychoglypha*
Total release				
N (μg N mg^{-1} DM h^{-1})	1.09 (0.06)	2.69 (0.10)	0.70 (0.07)	0.70 (0.08)
P (μg P mg^{-1} DM h^{-1})	0.05 (0.00)	0.31 (0.02)**	0.04 (0.00)	0.09 (0.02)
N : P (molar)	24.02 (1.61)	21.78 (1.70)*	19.05 (1.40)	23.34 (3.77)
Excretion				
N (μg N mg^{-1} DM h^{-11})	0.67 (0.04)	1.94 (0.10)*	0.28 (0.03)	0.21 (0.05)
P (μg P mg^{-1} DM h^{-1})	0.01 (0.00)	0.24 (0.02)**	0.01 (0.00)	0.02 (0.00)
N : P (molar)	140.61 (11.46)	23.05 (2.48)*	95.44 (11.43)	52.40 (13.54)
Egestion				
C (μg C mg^{-1} DM h^{-1})	3.77 (0.30)***	14.09 (0.66)**	7.88 (0.96)	10.89 (1.34)**
N (μg N mg^{-1} DM h^{-1})	0.42 (0.04)*	0.74 (0.03)*	0.42 (0.05)	0.54 (0.08)
P (μg P mg^{-1} DM h^{-1})	0.04 (0.00)	0.08 (0.00)	0.03 (0.00)	0.09 (0.02)*
C : N (molar)	11.21 (0.62)	22.78 (0.84)***	22.86 (1.62)	24.92 (1.50)**
C : P (molar)	295.88 (29.06)***	459.24 (22.27)***	714.59 (75.32)	473.14 (72.88)*
N : P (molar)	27.93 (3.00)*	20.31 (0.78)	32.90 (3.13)	18.08 (2.09)
Excretion : egestion				
N	2.08 (0.22)	2.94 (0.29)**	0.82 (0.13)*	0.55 (0.15)
P	0.41 (0.06)	2.88 (0.21)**	0.32 (0.06)	0.28 (0.07)