

High interspecific variation in nutrient excretion within a guild of closely related caddisfly species

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Abstract. Understanding the amount of variation in functional traits between closely related species within guilds is critical for understanding links between community composition and ecosystem processes. Nutrient excretion is an important link between animals and their environments, and aquatic invertebrate communities can supply a considerable proportion of ecosystem nutrient demand via excretion. We quantified nitrogen (N) and phosphorus (P) excretion rates of 10 species of larval caddisflies that inhabit high-elevation ponds and wetlands to determine the magnitude of variation in nutrient excretion within this guild. We found considerable interspecific variation in biomass-specific excretion of nitrogen (eightfold differences), phosphorus (sevenfold differences), and the stoichiometric N:P ratios (fivefold differences). Through a meta-analysis, we compared the variation within this guild to the variation found in other family-level species assemblages to determine the overall range in the variation of nutrient excretion that could be expected across guilds and to determine whether the variation in this caddisfly guild is comparatively extreme, average, or low. The meta-analysis revealed a large range in variation among guilds, and comparatively, the variation within this caddisfly guild is high for N excretion and intermediate for P excretion. The considerable variation within guilds revealed by our meta-analysis suggests that functional redundancy among guild members is difficult to predict. Thus, some natural or human-caused species gains or losses within biological groupings such as guilds and trophic levels could have little or no effect on ecosystem processes, whereas others could have very large effects.

Key words: caddisflies; climate change; consumer-driven nutrient regeneration; ecological redundancy; ecological stoichiometry; excretion; functional traits; guilds; macroinvertebrates; trait-based variation; wetlands.

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INTRODUCTION

Combining taxonomically or functionally related species into groups, such as trophic levels, functional groups, and guilds, is often used to explore the relationships between biological diversity and ecosystem function (Tilman 1997, Boyero et al. 2007, Slade et al. 2007, O'Connor et al. 2017). Species richness, dominance,

evenness, and identity can all contribute to differences in functional diversity within and among groups that can in turn influence ecosystem functions (e.g., Hooper et al. 2005, Cardinale et al. 2006). Many studies have demonstrated that particular species (e.g., dominant, keystone, or foundational species, and ecosystem engineers) within groups can strongly regulate ecosystem processes (Symstad et al. 1998, Bruno

et al. 2005, Hooper et al. 2012, Schmitz et al. 2014), but few studies have considered the degree to which closely related species are functional equivalents in terms of their effects on basic ecosystem functions such as nutrient cycling and energy flow. Understanding the range of variation within taxonomic or functional groupings is important because human activities at local, regional, and global scales often lead to shifts in species relative abundances within guilds or cause species range shifts, introductions, or extinctions.

Numerous studies find that aquatic invertebrate communities can make significant contributions to ecosystem nutrient demand via excretion and closely related species are often assumed to make similar contributions (e.g., Grimm 1988, Vanni 2002, Hall et al. 2003, Alves et al. 2010, Ozersky et al. 2013). Vanni (2002) argues that of all the direct (excretion, ingestion, egestion, production) and indirect (competition and physical modification of the environment) effects that animals can have on nutrient cycling, excretion is the most important because dissolved inorganic forms of excreted nutrients (e.g., NH_4^+ and PO_4^{3-}) are readily assimilated by primary producers and microbial decomposers that form the trophic base of food webs. Perhaps because taxonomy so frequently succeeds at explaining interspecific variation in nutrient excretion (e.g., Allgeier et al. 2015), it is occasionally assumed that there will be redundancy in nutrient cycling among closely related species (Grimm 1995, Vanni et al. 2002). However, other studies have found that species are not functionally equivalent, and there are many examples of how the loss or addition of a single species can dramatically affect carbon flow and nutrient cycling (e.g., Hall et al. 2003, Taylor et al. 2006, McIntyre et al. 2007, Small et al. 2011). This suggests that within assemblages of closely related species, which are commonly grouped into ecological guilds and assumed to fulfill similar functional roles in ecosystems, there could be substantial variation in traits such as nutrient excretion. This variation could arise from underlying differences in body size, tissue stoichiometry, strength of stoichiometric homeostasis, elemental resource allocation, development rate, activity, and environmental factors such as

dietary resource quality and temperature, and/or a plethora of other factors, all of which could be magnified by differences in population-level biomass. But, this also implies that animal guilds that directly influence nutrient cycling in different ecosystems are not equal, and it is therefore reasonable to expect that the variation in nutrient excretion might differ among guilds and ecosystems. Thus, the purpose of this study was to compare the variation in nutrient excretion among species within guilds of aquatic animals to explore the range in variation in this particular functional trait.

Larval case-making caddisflies (Trichoptera: Limnephilidae) are aquatic insects whose detritivorous larvae are commonly found in shallow standing water habitats at temperate and boreal latitudes across the Northern Hemisphere. At our study sites near the Rocky Mountain Biological Laboratory (RMBL), Colorado, there are 10 species that occur in high-elevation ponds and wetlands. As a consequence of trade-offs between traits that facilitate coexistence with predators and those that facilitate the completion of larval development before ponds dry, the particular combinations of species vary among pond permanence types (temporary vs. permanent; Wissinger et al. 2003, 2006). These closely related caddisfly larvae (Ruiter et al. 2013) are the biomass-dominant processors of coarse detritus in these habitats (Wissinger et al. 1999) and are among the biomass-dominant prey resource for top predators in small lakes, ponds, and wetlands (salamanders or fish; Wissinger et al. 1999). Experimental studies that relate detritus processing to nutrient regeneration in this system have demonstrated that nutrients released during detritus processing by this caddisfly guild can stimulate algal growth (Klemmer et al. 2012). Further, because long-term survey data demonstrate climate change-related shifts in species composition along both a hydrologic permanence gradient and an elevational gradient (Wissinger et al. 2003, 2016; Appendix S1: Fig. S1), it is important to understand how changes in species composition will affect the rate at which nutrients are excreted by these detritivorous animals.

Our first objective was to quantify species-specific and mass-specific excretion rates for ten species of larval caddisflies and determine how

this key functional trait varied within this guild, especially across habitats (pond permanence) and along an elevational gradient. Over four years (2014–2017), we measured ammonium-nitrogen ($\text{NH}_4^+\text{-N}$), soluble reactive phosphorus (SRP), and total dissolved phosphorus (TDP) excretion rates for larvae of each species in the field. These data were used to test the following predictions: (1) Different species of larval caddisflies excrete different amounts of N and P, (2) species-specific excretion rates are consistent throughout the day and among sites that vary in hydrologic permanence and elevation, (3) species' mass-specific excretion rates scale predictably with larval instars, and (4) variation in excretion rates can be predicted by species identity as well as specific intrinsic species traits such as tissue stoichiometry, diet, and body size, as well as extrinsic factors such as water temperature, pond permanence classification, and elevation. We found considerable variation among species, but low variation within each species both within and among habitats, and this variation provides the opportunity to explore how intrinsic and extrinsic traits of these animals affect nutrient excretion.

These results stimulated our second objective, which was to compare the variation in nutrient excretion rates within this caddisfly guild to the variation in excretion rates found in other invertebrate and vertebrate guilds. Using the aquatic animal excretion database compiled by Vanni et al. (2017), we conducted a meta-analysis of the variation in ammonium and phosphorus excretion found in other aquatic animals. This meta-analysis allowed us to (1) predict the range in variation of excretion rates that could be expected across guilds and (2) determine whether the variation in nutrient excretion by this particular guild is typical or extreme. By exploring the variation in nutrient excretion rates found within and among guilds, we assess the extent to which species can be considered functionally redundant at the guild level. We found that differences among guilds can explain much of the variation in animal nutrient excretion, and that within a guild of caddisflies, variation in nutrient excretion was intermediate for P and high for N compared to the variation in excretion within guilds of other aquatic invertebrates and vertebrates.

METHODS

Site selection

Ponds and wetlands were selected across an elevational gradient from montane (~2900 m) to upper montane (~3200 m) to subalpine (~3500 m) in the upper East River valley near the RMBL in central Colorado (Appendix S1: Fig. S1; 38°6' N, 106°6' W) based on historical species distribution data (Wissinger et al. 2003, 2016). The subalpine sites were located in the Mexican Cut Nature Preserve (39°1' N, 107°4' W). At montane elevations, ponds have higher levels of nutrients, in part because of cattle grazing on public lands, whereas at subalpine elevations ponds are oligotrophic (nutrient-poor) and co-limited by phosphorus and nitrogen (Elser et al. 2009). At all elevations, pond permanence ranges from ephemeral (temporary) to semi-permanent to permanent (Wissinger et al. 1999, 2003). At all elevations, the available development time for temporary habitat species is very short, starting after snowmelt (e.g., late April–June depending on the elevation) and ending either when temporary ponds dry (e.g., June–July) or at the onset of new snowpack (e.g., October–November; Wissinger et al. 2003).

Annual censuses of the invertebrate communities in these habitats since 1990 indicate that cased caddisflies (nine species of Limnephilidae, one of Phryganeidae) dominate the animal biomass in these habitats, increasing from 30% to 70% of animal biomass along a gradient from permanent to semi-permanent to temporary lentic habitats (Wissinger et al. 1999, *unpublished data*). These caddisflies are the only taxa that are explicitly described as shredders of vascular plants (e.g., Merritt et al. 2008). Other taxa that are typically assumed to be biofilm grazers that might act as cryptic detritivores by breaking down CPOM as a result of scraping epidermal biofilm include the snail *Stagnicola elodes* and tadpoles of montane chorus frogs (*Pseudacris montanus*; Brady and Turner 2010, Stoler et al. 2016). The other biomass-dominant invertebrate taxa are either predators (dragonflies, damselflies, dytiscid beetles, water bugs) or consumers of fine particular organic matter (chironomid and culicid fly larvae; Wissinger et al. 1999).

All but two of the ten caddisfly species (*Nemotaulius hostilis*, *Agrypnia deflata*) overwinter in

high-elevation ponds as diapausing eggs that hatch in early spring when inundated with snowmelt water. Larval development (five instars) occurs from May to August with upslope delays within a species and temporary-pond species completing development before permanent pond species (Wissinger et al. 2003, Wissinger, unpublished data). *Agrypnia deflata* (family: Phryganeidae) complete development in late summer with adults immediately laying eggs in permanent habitats, where larval development begins in fall and finishes during the following summer. Similarly, adults of the recent upslope migrant, *N. hostilis*, emerge in early spring and exhibit a short adult diapause during summer before laying eggs that do not diapause during winter; rather larvae begin development in fall, overwintering under the ice as 2nd–4th instars, and completing development in late spring of the following year (Winterbourn 1971, Inkley et al. 2008). Because of these staggered life cycles, synoptic excretion measurements were not possible and we focused on final instars at appropriate times in the year based on interspecific differences in phenology.

Experimental design

We measured excretion rates of final (5th) instar larvae of each of the 10 species of caddisflies (summer 2014, *Asynarchus nigriculus*, *Grammotaulis lorettae*, *Limnephilus externus*, *Limnephilus picturatus*; 2015, *Limnephilus secludens*, *Limnephilus sublunatus*, *Limnephilus tarsalis*, *Hesperophylax occidentalis*, *Agrypnia deflata*; and 2017, *N. hostilis*). For each species, pond-side excretion incubations were conducted for one hour in the shade to maintain ambient temperature. This duration was selected as a compromise between capturing ambient excretion rate and minimizing stress or starvation effects on metabolic or excretion rates. For each incubation, five final instar caddisflies of a single species were placed into a Ziploc bag pre-filled with 315 mL of filtered pond water. To test for a time-of-day effect, triplicate incubations were repeated in the morning, midday, and early afternoon, yielding nine excretion measurements for each species. At the beginning of each time block, water and air temperature were recorded. Because four species have broad elevational ranges (*A. nigriculus*, *G. lorettae*, *L. externus*, and *L. picturatus*), we were also able to test for a site-specific or

elevational effect by repeating excretion measurements at three elevations; montane, upper montane, and subalpine, yielding 27 excretion measurements for each of these four species. The remaining six species are more narrowly distributed, with three species restricted to the montane (*L. secludens*, *L. sublunatus*, and *L. tarsalis*) and three to the subalpine (*A. deflata*, *H. occidentalis*, and *N. hostilis*). Thus, we collected nine excretion measurements for each of these six species.

In summer 2016 and 2017, we measured instar-specific excretion rates of several biomass-dominant species with broad distributions in elevation and pond hydroperiod (*A. nigriculus*, *L. externus*, and *L. picturatus*) to test whether excretion rates differed among instars within a species. Melt water below the snow in early spring triggers egg hatching in most of these species, and because 1st instar larvae last only a few days, larvae have typically reached the 2nd instar by the time ponds are ice free (Wissinger et al. 2003). Thus, instar-specific measurements were obtained for instars 2nd–5th. For each species, five replicate excretion measurements were conducted for one hour with five individuals of a given instar per bag. Additionally, for several species with large cases (*A. deflata*, *G. lorettae*, *L. externus*, and *L. picturatus*), we used five empty cases from 5th instar animals to test for empty-case microbe uptake or excretion during one-hour incubation periods.

To estimate species-specific excretion, we measured the differences in ammonium-nitrogen ($\text{NH}_4^+\text{-N}$), SRP, and TDP in filtered water collected before and after one hour of incubation. This technique has been used in previous studies on macroinvertebrates (e.g., Grimm 1988, Hall et al. 2003) as well as with fish (e.g., Brabrand et al. 1990, Wheeler et al. 2015) and amphibians (Whiles et al. 2009). All water samples were collected and filtered using a syringe and inline filter holder containing a 25-mm Gelman AE glass fiber filter. Following excretion measurements, caddisflies were retained to measure body mass and tissue stoichiometry (see *Caddisfly Body Mass and Body Stoichiometry Methods*).

Excretion nutrient analyses

Ammonium was measured for each excretion sample using quadruplicate 20 mL filtered water samples collected in amber Nalgene bottles. Samples were chilled in the field and frozen upon

returning to the laboratory. Samples were thawed and measured following a fluorometric method using a Turner Designs Trilogy Fluorometer (Sunnyvale, California, USA; Taylor et al. 2007). The method uses standard additions to determine a precise and matrix-corrected concentration of NH_4^+ -N (Taylor et al. 2007).

Phosphorus was measured using 20 mL filtered water samples collected in glass test tubes. Samples were chilled in the field and stored in a refrigerator upon returning to the laboratory. Soluble reactive phosphorus samples were analyzed as quickly as possible (immediately or the next day) to prevent sample degradation. Total dissolved phosphorus samples were digested with 0.2 g $\text{K}_2\text{S}_2\text{O}_8$ in a pressure cooker for one hour to oxidize all reactive P to PO_4^{3-} . Both SRP and TDP were measured following a standard colorimetric method (Ostrofsky and Rigler 1987) using a Thermo Scientific GENESYS 10S UV-VIS Spectrophotometer (Waltham, Massachusetts, USA). Because SRP was measured for only five species (*A. nigriculus*, *L. externus*, *L. picturatus*, *G. loettae*, and *N. hostilis*) and was $80 \pm 15\%$ of TDP excretion with paired differences not significantly different from zero ($T_{78} = -1.58$, $P = 0.12$), mass-specific P excretion was reported and analyzed as TDP.

Caddisfly body mass and body stoichiometry

In 2014 and 2015, caddisfly larvae were preserved in 95% EtOH and later oven dried for 48 h at 60°C to obtain dry mass. These samples could not be used for body stoichiometry analyses due to suspected lipid loss. In 2016 and 2017, all caddisfly larvae were preserved by freezing at -80°C. Because we did not conduct instar-specific or 5th instar case-only excretion measurements for all species, we collected representative 5th instars of *L. secludens*, *L. sublunatus*, and *L. tarsalis* for stoichiometric analysis. Body tissue samples were sent to UC Davis Stable Isotope Facility for C:N. Remaining tissue was combusted in a muffle furnace at 500°C for 2 h and used to measure body tissue P following the digestion and colorimetry protocols for TDP.

Calculation of mass and species-specific excretion rates

Rates of N or P excretion, in units of $\mu\text{g}\cdot\text{mg}^{-1}\cdot\text{d}^{-1}$, were calculated as the difference

between final and initial concentrations ($\mu\text{g/L}$) of nutrient in the bags divided by total caddisfly dry mass in bag (mg) and incubation time (h), and multiplied by 24 h and 0.315 L.

Statistical analyses: variation within a family

Factorial analysis of variance (ANOVA) was used to test for differences in excretion among the final (5th) instar of the 10 species across time of day and site/elevation. Simple linear regression was also used to test for an effect of water or air temperature on excretion rate. These analyses allowed us to generate mean final instar excretion rates with $n = 9$ or $n = 27$ replicates per species by combining measurements across temperature, time, and location (e.g., elevation). ANOVA was also used to compare body mass-specific excretion rates among species. Tukey's HSD was used to detect differences in mean excretion rates among species.

T-tests were used to determine whether mean nutrient excretion or uptake by empty caddisfly cases differed from zero. General linear models (GLM) were used to test for a relationship between developmental instar and mass-specific excretion for *A. nigriculus*, *L. externus*, and *L. picturatus*. Separate models tested for differences in the mass-specific excretion rates of N or P for each species, with developmental instar treated as an independent categorical variable in all models. General linear models were also used to test for an effect of temperature on excretion rates for each species and across all species.

A series of GLMs with single continuous predictors, including body size, average tissue stoichiometry, and previously collected dietary data (Wissinger et al. 1999), was used to test for relationships between species' intrinsic traits and excretion rates. Dietary data were collected using methods described in Wissinger et al. (1996); briefly, these included extracting the entire digestive tract, staining with Congo Red, and using a Sedgewick-Rafter cell to visually quantify the percent contribution of plant detritus, amorphous material, algal cells, and chitin-stained invertebrate exoskeletons. Predictors that were significantly related to either N or P excretion were subsequently used as fixed effects in trait-based GLMs to compare species traits and species identities as predictors of per-individual nutrient excretion. Trait models

also included species' ranked activity levels (most mobile to most sedentary), primary case material, and extrinsic traits (pond permanence and elevation) as fixed effect predictors (Appendix S1: Table S1). A correlation matrix was used to identify multicollinearity between traits and refine model selection; when two traits were highly correlated ($r > 0.5$), the trait that individually better predicted excretion was used. Variance inflation factors (VIFs) were also used to inform final trait model selection, with $VIF < 3.5$ considered acceptable. After minimizing model VIFs, the trait models with the lowest Akaike Information Criteria (AIC) were selected. Species-based models of N and P excretion only included species identity as a fixed effect predictor. We used AIC to compare species-based and trait-based models. All statistical analyses were performed using R Statistical Software (version 3.3.1; R Foundation for Statistical Computing, Vienna, Austria).

Meta-analysis: variation among families

We used the aquatic animal excretion database published by Vanni et al. (2017) to conduct a meta-analysis of the variation in nutrient excretion among families and feeding guilds. The database includes invertebrate and vertebrate species representing several feeding guilds (algavore/detritivore, invertivore, carnivore, piscivore, and omnivore) in freshwater and marine systems. Excretion measurements excluded from the analysis were those with unknown family-level taxonomic resolution and those that had fewer than four species per family guild. Species-level excretion measurements were grouped by their original data source, taxonomic family, and feeding guild before calculating the coefficient of variation (CV) for mass-specific N and P excretion rates and molar N:P excretion ratio. Original data source was used to restrict family and feeding guild groupings to specific systems. Coefficients of variation were calculated by family and feeding guild to separate the variation attributed to taxonomy among feeding guilds and to facilitate comparing the variation within the limnephilid caddisfly feeding guild to variation within guilds of other invertebrate and vertebrate families.

Coefficients of variation were used to quantify family-guild variation because SDs scaled

with mean family-guild excretion rates (N, $R^2 = 0.84$, $F_{1,25} = 141.8$, $P < 0.001$; P, $R^2 = 0.97$, $F_{1,24} = 858.2$, $P < 0.001$; N:P, $R^2 = 0.79$, $F_{1,24} = 95.34$, $P < 0.001$), whereas CVs did not scale with mean N excretion rates or N:P excretion ratios (N, $R^2 = 0.09$, $F_{1,25} = 3.48$, $P = 0.073$; N:P, $R^2 = 0.076$, $F_{1,24} = 1.98$, $P = 0.173$). Coefficients of variation did not scale with mean P excretion rates after removing four family guilds that were outliers in P excretion (outliers defined by 1.5 times interquartile range; $R^2 = 0.05$, $F_{1,20} = 1.09$, $P = 0.308$). SDs of log-transformed excretion data were not used because they were highly correlated with CVs of non-transformed excretion data (McArdle and Gaston 1995; N, $R^2 = 0.71$, $F_{1,25} = 66.14$, $P < 0.001$; P, $R^2 = 0.54$, $F_{1,24} = 30.3$, $P < 0.001$; N:P, $R^2 = 0.55$, $F_{1,24} = 29.16$, $P < 0.001$).

RESULTS

Final larval instar excretion

Excretion rates measured at three different times during the day were not different for any of the caddisfly species (Appendix S1: Table S2). For species with broad distributional ranges (*Asynarchus nigriculus*, *Limnephilus externus*, *Limnephilus picturatus*, *Grammotaulis lorettae*), excretion rates did not differ across elevations (Appendix S1: Table S2). Thus, for each species we combined excretion rates measured across different times during the day and across different elevations to calculate mean mass-specific excretion rates (N or P $\mu\text{g}\cdot\text{mg}^{-1}\cdot\text{d}^{-1}$). Mass-specific final instar excretion rates of nitrogen (N) and phosphorus (P) differed among species (N, $F_{9,177} = 2657$, $P < 0.01$, Fig. 1A; and P, $F_{9,176} = 756.6$, $P < 0.01$, Fig. 1B).

Patterns of excretion rates among species were different for N vs. P (Fig. 1A, B). For example, *Agrypnia deflata* had the 2nd highest N excretion but the lowest P excretion. In contrast, *Limnephilus tarsalis* and *Nemotaulius hostilis* had the highest P excretion, but moderate N excretion. *Limnephilus picturatus* had the highest N excretion rates and the 3rd highest P excretion. Differences among the other species in their rankings for N vs. P excretion rates were more moderate than those described above (Fig. 1A, B). Molar ratios of N:P excretion also differed among species (N:P, $F_{9,176} = 323.5$, $P < 0.01$, Fig. 1C).

Instar-specific and case-only excretion

Developmental instar explained some of the variation in mass-specific nutrient excretion for *A. nigriculus*, *L. externus*, and *L. picturatus*. For *A. nigriculus*, GLM models using instar as an independent categorical variable and either mass-specific N or P excretion as the dependent variable were significant with instar explaining 74% of the variation in mass-specific TDP excretion and 27% of the variation in mass-specific N excretion (Appendix S1: Fig. S2). For *L. externus*, both GLM models were significant with instar explaining 23% of the variation in mass-specific TDP excretion and 27% of the variation in mass-specific N excretion (Appendix S1: Fig. S2). For *L. picturatus*, only the GLM model for mass-specific N excretion was significant, with instar explaining 11.5% of the variation (Appendix S1: Fig. S2). Unlike mass-specific excretion, molar N:P excretion ratios did not vary with instar for any of the three species (*A. nigriculus*, $F_{3,16} = 3.19$, $P = 0.052$; *L. externus*, $F_{3,16} = 2.29$, $P = 0.117$; *L. picturatus*, $F_{3,16} = 0.255$, $P = 0.857$).

Nutrient release or uptake from caddisfly cases of four species with either large cases (*L. externus*, *G. lorettae*) and/or high excretion rates (*A. deflata*, *L. picturatus*) was not significantly different than $0 \mu\text{g}\cdot\text{mg}^{-1}\cdot\text{case d}^{-1}$ (Appendix S1: Table S3).

Stoichiometric theory and caddisfly nutrient excretion

Mean N excretion per individual increased as a function of mean body size (mass) within this guild of caddisflies ($R^2 = 0.36$, $F_{1,8} = 6.07$, $P = 0.04$; Appendix S1: Fig. S3A). However, there was no significant relationship between mean P excretion and body size ($R^2 = 0.15$, $F_{1,16} = 2.79$, $P = 0.11$). Mean body size also did not predict body tissue N or P content. Body tissue percent P was negatively associated with P excretion ($R^2 = -0.64$, $F_{1,7} = 15.29$, $P < 0.01$; Appendix S1: Fig. S3B). There was no relationship between body tissue percent N and N excretion ($R^2 = 0.05$, $F_{1,15} = 0.29$, $P = 0.60$).

Previously collected species-level dietary data (Wissinger et al. 1999) explained some of the variation in mass-specific P excretion, but not mass-specific N excretion or body tissue N and P. Although algae represents a small portion (0.7–6.2%) of this guild's diet, there is a strong positive

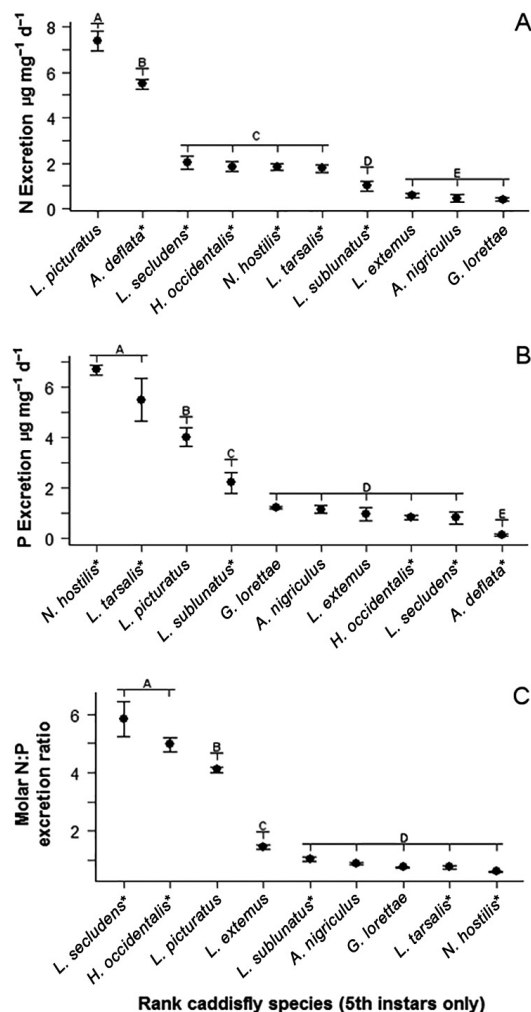


Fig. 1. Mean (± 1 SE) (A) nitrogen excretion rates and (B) phosphorus excretion rates of the final larval instars in a 10-species guild of lentic limnephilid caddisflies. Values are expressed as nutrient excreted (μg) per unit body mass (mg) per day (d) of each species. (C) Mean (± 1 SE) molar N:P excretion ratios for final larval instars of nine limnephilid caddisfly larvae. *Agrypnia deflata* is not shown; mean N:P excretion ratio of $81.07 (\pm 5.18)$. For species with broad distributions, $n = 27$, whereas for species studied at only one elevation $n = 9$ (denoted by *). Tukey groupings indicate no significant difference in mean excretion rates at the 95% confidence level. (A, $F_{9,177} = 2657$, $P < 0.01$; B, $F_{9,176} = 756.6$, $P < 0.01$; C, $F_{9,176} = 323.5$, $P < 0.01$).

relationship between percent filamentous algae in the diet and mass-specific P excretion ($R^2 = 0.86$, $F_{1,6} = 44.4$, $P < 0.01$; Appendix S1:

Fig. S4A). Similarly, percent total algae in the diet were positively related to mass-specific P excretion ($R^2 = 0.71$, $F_{1,6} = 18.44$, $P < 0.01$; Appendix S1: Fig. S4B). Detritus generally represented a much larger portion (68–94%) of this guild's diet, but did not significantly predict either N or P mass-specific excretion (N, $R^2 = 0.25$, $F_{1,6} = 3.29$, $P = 0.12$; P, $R^2 = 0.08$, $F_{1,6} = 0.44$, $P = 0.53$). However, the percent detritus in the diet did predict the molar N:P mass-specific excretion ratio ($R^2 = -0.68$, $F_{1,6} = 14.17$, $P < 0.01$; Appendix S1: Fig. S4C). None of the other dietary components explained significant amounts of variation in excretion or body tissue chemistry.

Ambient water temperature did not explain within-species variation in mass-specific N or P excretion for the broadly distributed taxa (*A. nigriculus*, *L. externus*, *L. picturatus*, or *G. lorettae*), and neither did ambient air temperature (Appendix S1: Table S4). Because excretion measurements were blocked by time of day, there were only three temperature measurements (one measurement per time block) for each of the narrowly distributed species (*Hesperophylax occidentalis*, *A. deflata*, *L. tarsalis*, *Limnephilus secludens*, *Limnephilus sublunatus*, and *Nemotaulius hostilis*); thus, we could not test for an effect of temperature within each of those species. Across all trials for all species, water and air temperature (ranging 18° and 16.1°C, respectively) did not significantly explain variation in mass-specific N or P excretion rates (N, $R^2 = 0.09$, $F_{1,25} = 2.37$, $P = 0.14$; P, $R^2 < 0.01$, $F_{1,24} = 0.49$, $P = 0.49$).

Traits vs. species identity as predictors of nutrient excretion

After identifying body size, tissue stoichiometry, and diet as significant individual predictors of excretion rates, we fitted GLMs for N and P excretion and molar N:P excretion ratio that combined several intrinsic traits including, species' ranked activity, primary case material, preferred pond permanence type, and pond elevation as fixed effect predictors. Following model selection, the final trait-based model of per-individual N excretion included body mass, body N:P, percent detritus in diet, activity, and pond permanence (AIC = 948.23, $F_{5,163} = 338.81$, $P < 0.001$). The final trait-based model of per-individual P excretion included body mass, body N:P, percent algae

in diet, activity, and pond permanence (AIC = 708.11, $F_{5,163} = 240.40$, $P < 0.001$), the same predictors as for N except percent algae in diet. The final trait-based model for molar N:P excretion included body N:P, percent detritus in diet, activity, and case material (AIC = 1257.91, $F_{5,163} = 173.49$, $P < 0.001$). These trait-based models were then compared to species-based models of excretion using AIC. For N excretion and molar N:P excretion, the species-based models (N, AIC = 813.14, $F_{9,163} = 737.51$, $P < 0.001$; Molar N:P, AIC = 1160.65, $F_{9,163} = 323.46$, $P < 0.001$) were superior to the trait-based models (N, $\Delta\text{AIC} = 134.83$; Molar N:P, $\Delta\text{AIC} = 97.26$), while for P excretion, the trait-based model was superior to the species-based model (AIC = 800.33, $F_{9,163} = 1040.34$, $P < 0.001$; $\Delta\text{AIC} = 92.22$).

Meta-analysis

Family-level CV for mass-specific N and P excretion rates and molar N:P excretion ratios did not scale with sample size (N, $R^2 = 0.03$, $F_{1,25} = 0.93$, $P = 0.34$; P, $R^2 = 0.02$, $F_{1,24} = 0.51$, $P = 0.48$; N:P, $R^2 = 0.03$, $F_{1,24} = 0.70$, $P = 0.41$). Coefficients of variation were not significantly different among trophic levels (N, $F_{3,23} = 1.16$, $P = 0.35$; P, $F_{3,22} = 0.755$, $P = 0.53$; N:P, $F_{3,23} = 0.21$, $P = 0.88$). Coefficients of variation for mass-specific N excretion ranged from 17.2 (Characidae) to 172.9 (Baetidae) and from 26.4 (Salpidae) to 300.1 (Baetidae) for mass-specific P excretion (Fig. 2A, B). Coefficients of variation for molar N:P excretion ratios ranged from 21.7 (Salpidae) to 265.1 (Leptophlebiidae; Fig. 2C). The CV for the limnephilid caddisflies' mass-specific N excretion ranked 5th out of 27 family guilds, while their CV for P excretion ranked 10th out of 26 families and 15th out of 26 for N:P excretion ratio.

DISCUSSION

Variation in nutrient excretion by detritivorous caddisflies

To explore the variation in nutrient excretion within a guild of closely related species, we quantified mass-specific N and P excretion rates of ten biomass-dominant detritivorous caddisfly species in high-elevation ponds. We found that (1) different species of larval caddisflies excrete different amounts of N and P (Fig. 1), (2)

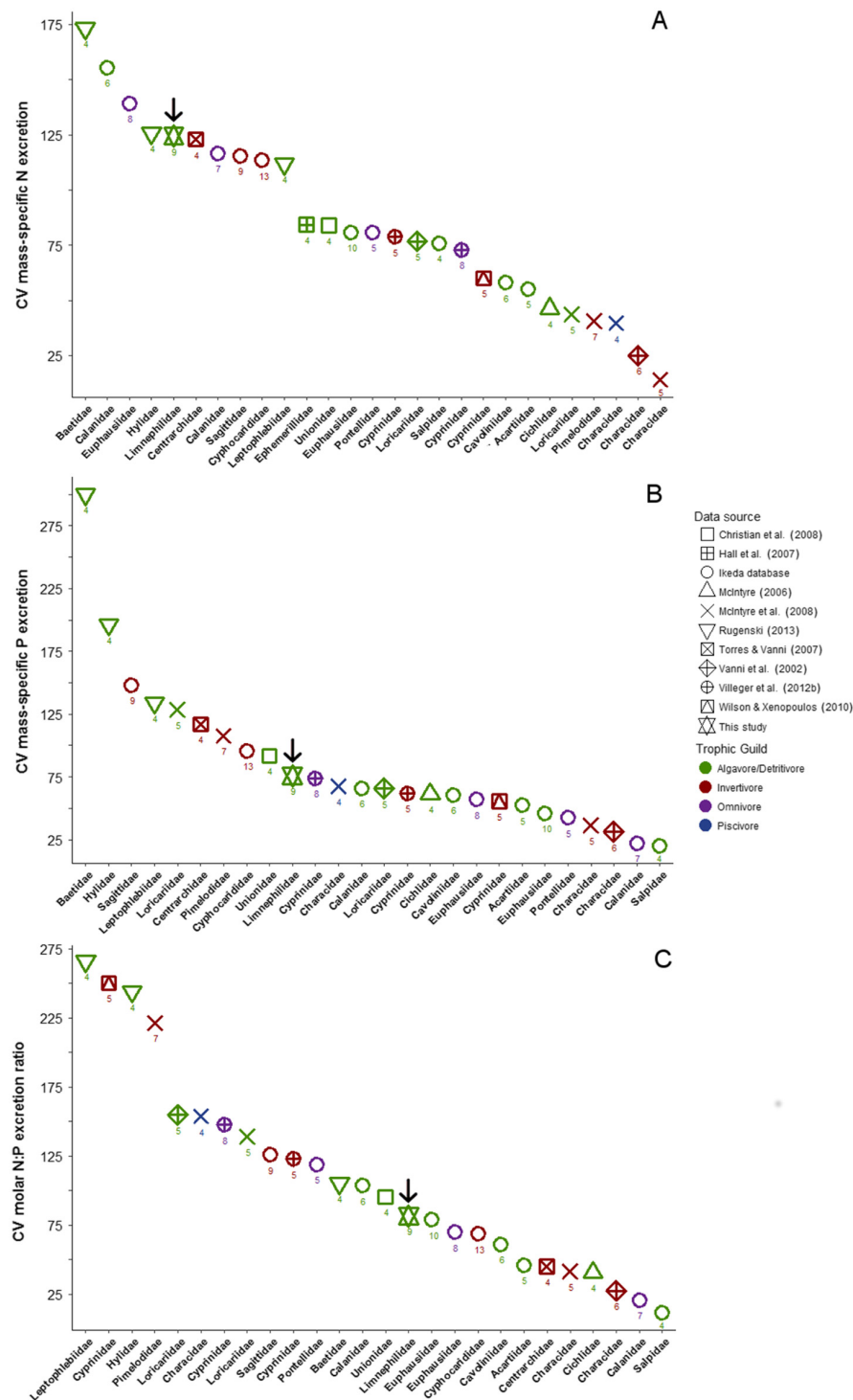


Fig. 2. (A) Coefficients of variation (CV) for family-level mass-specific nitrogen excretion, (B) for family-level mass-specific phosphorus excretion, and (C) for family-level molar N:P excretion ratio. Within each panel, families are ranked in order of decreasing CV. Color codifies data source and shape codifies trophic guild. Numbers below points correspond to number of species sampled in each guild.

species-specific excretion rates are not different throughout the day or among locations (Appendix S1: Table S2), and (3) species' mass-specific excretion rates tend to decline with instar (Appendix S1: Fig. S2). Differences across species-specific instars were small relative to the differences among species. Our excretion data were consistent with some, but not all predictions from ecological stoichiometry (e.g., Appendix S1: Fig. S3; see *Taxonomic, Size, and Trait-based Linkages with Ecological Stoichiometry*).

The consistency in species-specific excretion rates across habitats suggests that for these organisms, valid predictions can be made about the ecosystem consequences of species gains or losses that arise through range or distributional shifts, extinctions, or introductions. The large variation in nutrient excretion (e.g., $0.4\text{--}7.4\ \mu\text{g N}\cdot\text{mg}^{-1}\cdot\text{d}^{-1}$ and $0.2\text{--}6.6\ \mu\text{g P}\cdot\text{mg}^{-1}\cdot\text{d}^{-1}$) by these species reveals that they differ in key functional traits despite being closely related, with 9 of the 10 species from the same family. This guild has been shown to increase detritus decay rates and ambient water-column N and P concentrations, which have been posited to increase algal productivity (Klemmer et al. 2012). Therefore, if these excretion rates scale to the whole-pond level and supply a substantial percentage of nutrient demand, community reassembly associated with species gains or losses within this guild could change inorganic nutrient availability during the short growing season and consequently affect ecosystem productivity.

The differences in mass-specific excretion rates across instars were small relative to the differences in mass-specific excretion rates among species. This suggests that N and P allocation is nearly constant across larval development stages within a species, but is variable among closely related species. Within each species, the small differences in mass-specific excretion rates across instars of *Asynarchus nigriculus*, *Limnephilus externus*, and *Limnephilus picturatus* (except for P) generally adhered to the stoichiometric prediction that smaller and faster growing animals (here, earlier instars) should have higher mass-specific excretion rates (Sternern and Elser 2002). We suggest that *A. nigriculus* 5th instar N excretion is higher than expected given this prediction (Appendix S1: Fig. S2) because this species is highly aggressive and cannibalistic during the

final instar, and thus, they may be consuming more N-rich protein than their earlier instars (Wissinger et al. 1996, 2004).

Excretion measurements of empty *Agrypnia deflata*, *G. lorettae*, *L. externus*, and *L. picturatus* cases revealed no significant external microbial contribution (i.e., net nutrient release or uptake) to these caddisfly excretion estimates. While uptake by case-associated microbes is certainly a plausible nutrient flux and has been demonstrated in other systems (e.g., Kahlert and Baunsgaard 1999, Mooney et al. 2014), it appears to have negligible influence on the mass-specific excretion rates presented here.

Taxonomic, size, and trait-based linkages with ecological stoichiometry

Allometry or body size can exert a strong control on metabolism, which is closely related to excretion (Kleiber 1947). Because smaller animals have faster metabolism, smaller animals should generally have higher mass-specific excretion rates (Sternern and Elser 2002, Hall et al. 2007). Across species and instars, our data support the hypothesis that larger (heavier) animals excrete more N on a per-individual basis, but there was no significant relationship with P excretion. Allometric scaling for excretion of only one nutrient is not unusual, as Schindler and Eby (1997) also reported allometric scaling for N excretion of lentic fish, but not for P excretion. In that study, after resource quality was added to the model, allometric scaling of P excretion did become significant, demonstrating that consumer-driven nutrient recycling is a complex interaction of ecology and physiology.

The growth rate hypothesis (Elser et al. 1996) provides a possible stoichiometric explanation for why the generalized relationship between body size and P excretion might be confounded, and more broadly, why there is so much variation in N and P excretion among taxa. For instance, this guild includes taxa adapted to temporary pond habitats that consequently have very rapid larval growth rates (e.g., *A. nigriculus*, *Limnephilus tarsalis*) as well as taxa that are adapted to permanent ponds with much slower larval growth rates (e.g., *L. externus*, *L. picturatus*) (Wissinger et al. 2003). Organisms with higher growth rates should have high cellular ribosome concentrations, leading to greater

tissue rRNA content (Elser et al. 1996). Since RNA is P rich, organisms with higher growth rates should have lower tissue N:P ratios and higher P demand, and hence excrete less P. We do not have tissue rRNA data for these caddis, but body tissue elemental composition can also be used to predict an organisms' excretion rates (Frost et al. 2006). We could make that prediction for P body content and P excretion, but not for N.

Differences in life history strategies among these taxa may also explain variation in nutrient excretion. For example, Bärlocher and Porter (1986) reported that functional differences in feeding strategies and digestion among aquatic detritivores result in the differential incorporation of nutrients from detrital-microbial substrates, and Halvorson et al. (2016) recently demonstrated that different biofilm nutrient incorporation efficiencies can lead to species-specific differences in caddisfly body stoichiometry. Species-level digestive function and metabolic demand have not been studied within this guild, but previously collected dietary data (Wissinger et al. 1999) and our measurements of the stoichiometry of potential dietary sources have allowed us to identify several possible mechanistic drivers of this guild's nutrient excretion.

Dietary composition data did not predict body tissue chemistry and N excretion, but the percentage of filamentous algae and total algae in the diet predicted P excretion. This positive relationship is stoichiometrically intuitive because the nutritional value of detritus is attributed to microbial colonization, including epidermal algae (Bärlocher 1985, Moore et al. 2004, Kuehn 2016). For instance, biofilms on submerged sedge contained more P (87.8 $\mu\text{g}/\text{mg}$) and twice as much N (21.3 $\mu\text{g}/\text{mg}$) than the sedge itself (51.1 $\mu\text{g P}/\text{mg}$ and 9.52 $\mu\text{g N}/\text{mg}$). Notably, in this system both sedge detritus and their biofilms have extremely low N:P ratios (0.10 and 0.08, respectively). This provides a possible explanation for the absence of any relationship between caddis diet and N excretion or body N content. Additionally, the strong negative relationship between percent detritus consumed and caddisfly N:P molar excretion ratios further supports the hypothesis that caddisflies feeding primarily on detritus and detrital biofilms will excrete more P relative to N. However, an important caveat is that this mechanistic conclusion is being

made without diet and excretion data from the same individuals.

A complementary hypothesis for why we do not see any dietary or stoichiometric explanations for N excretion rates is that there could be species-specific differences in silk composition and production rates. The silk spun by larval caddisflies for case construction is composed of N-rich proteins and calcium (Ashton et al. 2013). The cases built by these particular caddisfly taxa are diverse in shape, structure, size (relative to body mass), and materials used (Wissinger et al. 2006), and therefore, it is reasonable to expect that their silk composition and production rates reflect that diversity. Therefore, it is possible that species-specific differences in silk composition and production could explain this guild's N excretion rates. For example, *L. picturatus*, which has the highest mean N excretion rate, builds relatively flimsy cases, while *L. externus* and *A. nigriculus*, which have the two lowest N excretion rates, both build very complex and robust cases. Thus, we posit that some taxa allocate more dietary N to silk, while others do not, thereby altering the relationship between body tissue N and N excretion. This kind of phenotypic variation in biomaterial could also be used to explain interspecific stoichiometric variation within other families, but to our knowledge has not been explored. For example, spiders (order: Araneae) also spin silk and have tremendous interspecific variation in rates, quantities, and qualities (Swanson et al. 2009). Many fish taxa secrete a glycoprotein mucus with antimicrobial and defensive properties, but the quantity of secretion gland cells and mucus chemistry vary among species (Sadovy et al. 2005). Likewise, bees (order: Hymenoptera) produce beeswax that exhibits considerable interspecific variation in its strength, which suggests underlying variation in chemistry (Buchwald et al. 2006). Thus, the production and export of biomaterials represents an understudied pool of stoichiometric resource allocation that could help explain the resource demands and nutrient excretion of many taxa.

It is well established that temperature influences metabolism (Gillooly et al. 2001), which could in turn influence excretion (Sternner and Elser 2002). In this study, however, ambient water temperature did not affect excretion rates.

During excretion measurements, ambient water temperatures varied by $<3^{\circ}\text{C}$ within each species except for *G. lorettae*, which varied by $<5^{\circ}\text{C}$. Temperature variation was small within each species because all experiments spanned the same six-hour period from morning to early afternoon and because we held animals in shaded areas of the pond to maintain ambient temperature during excretion measurements. Further, diel water temperature changes in these ponds are not much larger, averaging 6.5°C across the season, so it is unlikely that we did not detect an effect of temperature because of the time periods chosen for measuring excretion.

Ambient water temperatures varied by almost 18°C across excretion measurements for all species, but this variation in temperature did not explain variation in mass-specific excretion of N or P. Temperature variation across excretion measurements can be attributed to differences in elevation and time of year (i.e., early June vs. late July). For broadly distributed species, thermal adjustment could help explain consistent excretion rates among populations along an elevational gradient. A recent study with damselflies found that thermal adjustment could be explained by either genetic changes or phenotypic plasticity, or both (Stoks et al. 2014). Nevertheless, transplanting individuals across elevational (and therefore thermal) gradients has never been attempted with this guild of caddisflies, so the degree to which thermal adjustments might affect the ecosystem-level consequences associated with excretion is unclear. An understanding of the degree to which thermal adjustments impact excretion warrants further study given recent range shifts by some of these caddisflies and many other animals.

Our comparison of trait-based and species-based models of nutrient excretion suggests that traits can predict excretion as well as or better than species identity alone. Our best trait-based model for P excretion had a lower AIC ($\Delta\text{AIC} = 92.22$) and required fewer parameters ($\text{df} = 5$) than the species-based model ($\text{df} = 9$). The two models make similar predictions (trait-based $R^2 = 0.88$, species-based $R^2 = 0.98$), but the trait-based model is preferred because it is simpler. Further, the species-based model implicitly contains all species' traits in aggregate, even those traits that are not required to predict excretion. In our trait-based model, we identified and included

only those traits that were most important for predicting P excretion. Although the species-based model for N excretion had a much lower AIC ($\Delta\text{AIC} = 134.83$) than our best trait-based model, it again required more parameters ($\text{df} = 9$ for species-based model and $\text{df} = 5$ for trait-based model) and model fits were comparable (species-based $R^2 = 0.97$, trait-based $R^2 = 0.91$). While species identity provides a generalized linkage between organisms' functional traits and ecosystem processes (Vanni et al. 2002, Allgeier et al. 2015), our analysis suggests that trait-based models could also be highly successful at explaining variation in nutrient excretion and other functional traits among taxa.

These predictions were foreshadowed by our analysis of individual traits as predictors of excretion. Body chemistry and diet both successfully predicted P excretion, suggesting that we have identified several good traits for explaining variation in P excretion. Meanwhile, body size was the only successful predictor of N excretion. Thus, it follows that our trait-based model of P excretion is superior to the species model by comparison of AIC, whereas our trait-based model of N excretion is only simpler by number of parameters required. The simplest explanation for why our trait-based models for N excretion and molar N:P excretion do not perform as well as the species-based models is that we did not include the most important traits for predicting N excretion within this guild. For example, caddisfly silk is N rich, and as explained previously either the quantity or quality of silk produced by each species could be critical for predicting N excretion. Therefore, we would argue that even intrinsic traits linked to species' extended phenotype (Dawkins 1984) could be useful for explaining variation in functional traits.

Meta-analysis of variation in nutrient excretion among guilds

Our meta-analysis suggests that (1) there is considerable stoichiometric variation in nutrient excretion across family guilds, and (2) that relative to these metadata, the caddisfly guild we studied is highly variable in N excretion and moderately variable in P excretion and N:P excretion ratios. By extension, this suggests that while taxonomy (e.g., family groupings or species; Allgeier et al. 2015) can explain some of the

variation in nutrient excretion and other stoichiometric traits found across broad levels of biological organization, variation within groupings, such as guilds, might provide an inaccurate approximation of the functionally relevant variation in these traits.

The magnitude of variation in mass-specific excretion rates among these caddisfly species (eightfold for N and sevenfold for P) suggests there could be low functional redundancy within this guild in terms of their contribution to nutrient regeneration. Although differences in mass-specific excretion could be offset by differences in other species-specific traits, such as population density and larval development time (or how long they are excreting in a pond), it nonetheless reinforces the conclusion that species are not equal, even within a genus of organisms in the same feeding guild. We conclude that information on specific traits within a guild will be paramount for linking diversity and function, and thus for conservation or management of native or invading species.

Ranges in the variation of functional traits have been demonstrated previously among plant clades, functional groups, and growth forms (Diaz et al. 1998, Reich et al. 2003, Swenson and Enquist 2007, Wright et al. 2016). Some plant functional groups have low variation in % leaf N (e.g., conifers), whereas others have higher variation (e.g., woody climbers and herbs) (Reich et al. 2003). The ranges of variation in functional traits across plant groups are related to environmental conditions and are considered evidence of abiotic forces driving the evolution of plant form, function, and diversity. Environmental nutrient availability has been implicated as a potential driver of gene expression and animal evolution (Elser et al. 2000, Jeyasingh and Weider 2007); thus, abiotic forces might also play a role in the evolution of between-species variability in nutrient excretion by caddisflies that is mediated by their nutrient requirements.

By extension, the range in the variation of nutrient excretion rates among guilds revealed by the meta-analysis suggests that low functional redundancy in nutrient excretion might be the norm in other systems as well. An important caveat is that the ratio of nutrient demand to supply from consumer-driven nutrient recycling varies from system to system, and

therefore, the relevancy of this functional redundancy—and more generally, the variation in a guild's nutrient excretion—also varies from system to system. Ecological redundancy is known to vary from system to system and community to community (e.g., Walker 1992, Wellnitz and Poff 2001). Regardless, the within-guild variation among closely related species in functional traits like excretion has rarely been considered. Work by Post et al. (2008) demonstrated the importance of considering both intraspecific and interspecific variation in traits for ecological interactions and ecosystem function, and certainly variation within and among guilds is informative because guilds summarize their constituents' interspecific variation and facilitate comparisons to other systems. An among-guild perspective provides a foundation for identifying new questions about particular species, processes, and abiotic factors that could drive the evolution of functional trait variation.

A final caveat for interpreting this meta-analysis is that the number of species sampled within each family guild is not necessarily the total number of species in that family guild. In our study, we have comparative data for all caddisfly species that have been encountered in shallow lentic habitats in the region for the past 30 yr. Although there was not a significant relationship between the number of species sampled per family guild and the family-guild's CV, the variation within each could be larger or smaller than presented here if each family guild was sampled exhaustively.

CONCLUSIONS

Here, we have shown that there is substantial variation in nutrient excretion rates for ten closely related species of larval caddisflies that inhabit high-elevation ponds and wetlands. Within species, these excretion rates are consistent throughout the day, and for taxa with broad distributions, rates are consistent at different sites along an elevational gradient from montane (e.g., 2800 m) to subalpine (e.g., 3400 m). Although mass-specific excretion rates decline with instar, the variation among instars is trivial relative to the amount of variation among species. We were able to explain some, but not all, of the variation in nutrient excretion among taxa using ecological

stoichiometry theory. It is likely this variation is fundamentally trait-based, even if we are currently unable to ascribe it to particular traits. Variation within this guild is high for N excretion and intermediate for P excretion and N:P molar excretion ratio compared to that measured for other families and guilds of invertebrates and vertebrates. Our excretion data suggest climate-driven changes in caddisfly community composition could affect nutrient cycling and productivity in ponds if caddisfly driven nutrient recycling is relevant at the pond-scale. We think this is a reasonable prediction given that (1) this guild of caddisflies are the biomass-dominant detritivores in this system (Wissinger et al. 1999, 2003), (2) previous work has linked their detritus processing with algal biomass (Klemmer et al. 2012), and (3) in other systems, the biomass-dominant aquatic invertebrates have been shown to supply a significant proportion of an ecosystem's nutrient demand (e.g., Grimm 1988, Vanni 2002, Hall et al. 2003). Thus, as functional traits, these species-specific excretion rates provide the basis for a mechanistic understanding of the ecosystem consequences of range shifts and could have wide application for understanding how changes in community composition (from climate change, species introductions, extinctions, etc.) will affect ecosystem processes. Indeed, high-elevation species are particularly vulnerable to climate-induced range shifts, and some of these caddisfly species (e.g., *Nemotaulius hostilis*) have been recently found for the first time in high-elevation ponds and, hence, knowing the variation in functional traits within this guild could have direct application to understanding impacts of species range shifts on ecosystem processes in small ponds that are numerically dominant inland lentic habitats globally (Downing et al. 2006). More generally, these functional differences suggest that even among closely related taxa within the same guild, species replacements associated with any human stressor could drive changes in rates of ecosystem processes.

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