

## **ABSTRACT**

BALIK, JARED ANDREW. From Animals to Ecosystem Processes: Predicting Functional Outcomes of Climate-driven Changes in Animal Communities through Species Traits. (Under the direction of Dr. Bradley Taylor).

Animals can have large effects on ecosystem processes such as nutrient cycling or detritus processing, but these can be difficult to study directly in natural systems. Single species contributions are often particularly challenging to isolate and measure. However, species' functional traits provide mechanistic links between animals and their effects on ecosystem processes, and thus provide an indirect method of understanding species' functional roles and contributions to overall ecosystem processes. In this dissertation, I quantify key functional traits (nutrient excretion and detritus processing rates) of larval caddisflies that are biomass-dominant detritivores in subalpine ponds. I then develop a framework for making pond-level predictions of animal contributions to ecosystem processes and explore functional outcomes of differences in community composition among pond hydroperiod classifications and invasions by range-expanding caddisflies.

In chapter 1, I found considerable interspecific variation in biomass-specific excretion of nitrogen (eightfold differences) and phosphorus (sevenfold differences) among 10 larval caddisfly species. Through a meta-analysis, I compared the variation within this guild to the overall range in variation found among other family-level assemblages and found that, comparatively, the interspecific variation among caddisfly excretion rates was high for N and intermediate for P. This suggests that functional redundancy among species is difficult to predict, and consequently the functional outcomes of species gains or losses could be substantial.

In chapter 2, I found similarly high interspecific variation in larval caddisfly biomass-specific detritus processing rates (thirteenfold differences in coarse processing, eightfold for fine particulates). Furthermore, on a mass-specific basis, amphipods and chironomids processed a similar amount of detritus as caddisflies, suggesting that the importance of non-shredder detritivores may be greater than previously recognized. Finally, I show that processing by mixed caddisfly assemblages in natural ponds is strikingly similar to additive predictions based on individual species rates, suggesting that single-species rates may be useful for understanding functional outcomes of shifting assemblages.

In chapter 3, I combine nutrient excretion rates and biomasses of pelagic and benthic invertebrates and salamanders with nutrient uptake rates in a simulation model to estimate animal-driven nutrient supply and pond-level demand along a hydroperiod gradient of permanent ponds that never dry, semipermanent ponds that dry in some years, and temporary ponds that dry annually. I found that animal biomass increased with hydroperiod duration, and biomass predicted animal-driven supply contributions among hydroperiod classifications. Consequently, community-wide supply was greatest in permanent ponds. N supply exceeded demand in permanent and semi-permanent ponds, whereas P supply equaled demand in both. Conversely, temporary ponds had large deficits in both nutrients due to lower biomass and hydroperiod-induced constraints on dominant suppliers (oligochaetes and chironomids). Thus, as climate warming causes hydroperiods to become increasingly temporary and indirectly prompts biomass declines and compositional shifts, animal-driven nutrient supply will decrease and strong nutrient limitation may arise due to loss of animal-driven supply.

In Chapter 4, I adapted the simulation model framework to predict caddisfly nutrient supply and detritus processing throughout three upslope range expansions observed over a 30-

year long-term dataset. I found that the dominant resident regulated caddisfly nutrient supply and detritus processing until the third nonresident arrived, though total ecosystem process rates did not change. This suggests that processes regulated by dominant species may be more sensitive to invasion, and species' functional roles may change before overall ecosystem process rates.

Developmental phenology differences likely facilitated the third nonresident's numerical success and alteration of residents' functional roles. However, I also show that numerically unsuccessful range expansions could still contribute to greater among-habitat ecosystem process variability.

Thus, the body of work presented in this dissertation demonstrates that functional traits provide a novel approach to studying functional outcomes of changes in community compositions.

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From Animals to Ecosystem Processes: Predicting Functional Outcomes of Climate-driven  
Changes in Animal Communities through Species Traits

by  
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A dissertation submitted to the Graduate Faculty of  
North Carolina State University  
in partial fulfillment of the  
requirements for the degree of  
Doctor of Philosophy

Biology

Raleigh, North Carolina  
2021

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**DEDICATION**

This dissertation is dedicated to my parents, Karen and Matthew Balik

And to my wonderful and supportive partner, Susan Washko

And to my late undergraduate mentor and dear friend, Dr. Scott Wissinger

## BIOGRAPHY

I was born and raised in Darlington, Pennsylvania, the youngest of three siblings by 14 years. The lush rolling forests and diverse wildlife surrounding my home instilled a love of nature and outdoor recreation, particularly hiking, fishing, and camping. Meanwhile, observing my father repair seemingly anything mechanical caused me to develop a strong curiosity of how objects and systems worked. The transition away from dial-up internet allowed me to foster this curiosity by building computers and networks, and for a time I envisioned a future career in information technology. However, after witnessing sudden dramatic changes in the woods and streams behind my home after a logging operation, my interest in systems and love of nature combined into a conservation ethic as I wondered at the ecosystem's gradual recovery. Thus, after graduating high school I decided to major in biology and environmental science at Allegheny College, with the intention of learning how living things and ecosystems functioned.

Admittedly, I began my college experience thinking that as a backup plan my majors would also prepare me for a career in medicine. However, at the time I had little concept of what kinds of career possibilities were in the environmental field. That changed when I met Dr. Scott Wissinger in my second semester. Scott welcomed me into his lab, and soon thereafter I found myself conducting research with him in streams and wetlands in northwestern Pennsylvania. During an independent study course with Scott the following semester, he declared that I had a knack for analytical chemistry, which was largely thanks to the dedicated efforts of my high school advanced-placement chemistry teacher, Kim Baker. Scott then suggested that I might be a great candidate for his summer research program at a field station called the Rocky Mountain Biological Laboratory in Gothic, Colorado (RMBL). He gave me a nugget of an idea for an independent summer research project, which I developed into a research proposal for a

competitive but generous undergraduate research fellowship from the Arnold and Mabel Beckman Scholars Program. My proposal was funded, which provided financial support for two summers of independent research with Scott at RMBL.

Those first two summers at RMBL indeed changed my life. Allegheny was a small school, and Scott was one of five ecology professors, whereas hundreds of ecologists from across the USA migrated to RMBL every summer. Interacting with these diverse scientists and their graduate students helped me understand the breadth and flexibility of a career in science. Meanwhile, I had tremendous fun helping Scott with his long-term pond research program and working on my independent research, which later became my undergraduate senior thesis. These formative summers also introduced me to my future doctoral advisor, Dr. Brad Taylor, and deepened my bond with my partner and labmate, Susan Washko.

After completing my bachelors of science degree at Allegheny College in 2016, I began my PhD at North Carolina State University. Scott and Brad were co-PIs on an NSF grant, so I had the wonderful opportunity to continue collaborating on pond research with them as I began new stream research. Sadly, Scott passed away unexpectedly in October 2019. It has been a privilege to carry on his legacy with our science family, and to synthesize the foundations of the research career that I began with his guidance in this dissertation.

In my free time, I pursue diverse hobbies. Fitness is foremost among these, with emphases on bodybuilding-style weightlifting and swimming, as well as learning about nutritional biochemistry, exercise and sleep physiology, and conducting data-driven  $n=1$  fitness experiments to understand and improve my performance. Otherwise, I greatly enjoy baking breads, hiking, camping, kayaking, tinkering with electronics, reading, and strategy games.

## ACKNOWLEDGMENTS

I thank my dissertation committee for supporting my development as a scientist. In particular I thank my advisor Dr. Brad Taylor for his boundless generosity in supporting my intellectual growth and self-confidence in my ideas. I am continually impressed by the depth of his scientific thinking, his diverse knowledge of ecological literatures and statistics, along with the quickness of his mind in drawing broad connections to other ecosystems or ecological subdisciplines. I admire his attention to detail, dedication to the craft of scientific writing, and his ability to design expansive projects to pursue fundamental contemporary ecological questions. As a person, I also admire his considerable skill in woodworking, and most of all his loving dedication to his children. I also thank Drs. Marcelo Ardón, Jim Heffernan, and Stacy Nelson for their intellectual generosity and commitment to my success. Finally, I will always regret not thanking Dr. Scott Wissinger more profusely for all the doors he helped open and the personal and professional philosophies he shared with me prior to his passing.

I also thank my current labmates Sam Jordt and Dr. Lee “Mick” Demi, whose support, assistance, and feedback on my science has been almost as invaluable as their friendship, wonderful humor, and propensity for long coffee and tea breaks. I extend those same thanks to former labmate Dr. Amanda DeVecchia, whose sisterly guidance and uncanny ability to read my mind supported me throughout my graduate studies, despite several close encounters with large ungulates and apex predators. Likewise, I thank former labmates Drs. Andrew Sanders and Derek West for their continued willingness to share their statistical expertise and for helping me hone my ability to evaluate science during the early formative stages of my graduate training. I also thank Elin Bink, Jess Forbes, Jackie Fitzgerald, and Ben Swift, the exceptional roster of

research assistants who helped make my research possible and whose company and friendship made my work so much more fun.

Similarly, I thank my NCSU officemates Sean Buczek, Rubia Martin, Ani Popp, Drs. Megan Thoemmes and Jennifer Archambault, and cohort-mates Emily Reed, Andy Maurer, and Riley Gallagher for their friendship, support, and camaraderie. Additionally, I thank my former professors, mentors, and colleagues Drs. Hamish Greig, Howard Whiteman, Milt Ostrofsky, Casey Bradshaw-Wilson, Matt Venesky, Ron Mumme, Rich Bowden, Matt Ferrence, Jim Rice, Greg Cope, Martha Reiskind, Rob Dunn, Kevin Gross, Becky Irwin, Aranzazu Lascurain, Craig Layman, Bobbi Peckarsky, Nick Wasser, Mary Price, David Inouye, Dianne Campbell, Ken Williams, Jenny Reithel, Ian Billick, and Dave Dickey for the diverse, sometimes unexpected, and always invaluable ways in which they have supported and mentored me.

I also thank my friends and RMBLers Dr. Jackie Alpert, Billy Barr, Dave Beigel, Kelsey Brennan, Dr. Lauren Carley, Dr. Ross Conover, Elsa Cousins, Margot Cumming, Dr. Becky Dalton, Rachel Dickson, Marie Faust, Dr. Wilnelia Gonzalez-Recart, Dr. Sean Griffin, Dr. Jacob Heiling, Dr. Jeremiah Henning, Rachel Kanaziz, Connor Morozumi, Dr. Annika Nelson, Dr. Jane Ogilvie, Dewey Overholser, Dr. Gabby Pardee, Summer Schlageter, Kyle Spells, Dr. Rachel Steward, Mel Torres, Chelsea Wilmer, and Loy Xingwen for their wonderful company, support and motivation, diverse perspectives on science, hikes and rodeo adventures, Fourth of July shenanigans, potluck contributions, varied painting skills, late and loud nights of competitive chicken-hoof (alternatively, “chicken-foot dominoes” for the uninitiated), and generally for sharing such special memories in a deeply special place. Most of all, I thank my dear brother Isaac Shepard for our enumerable adventures and misadventures, our bouts of commiseration and celebration, our mutual love of Bon Iver, The National, and coffee, and for

continually inspiring me through his passions for poetry, literature, and music. Finally, I also thank the wonderful undergraduate students whom I had the privilege of mentoring at RMBL, including Austyn Long, Emily Jameson, Abby Wright, Cameron Leitz, Alexander Stogsdill, and Brittney Cleveland.

I'd also like to thank my former high school teachers Kim Baker, Ian Eastman, Theresa Adams, Jim Cox, and Jarrod McCowin for passing along their respective loves of chemistry, biology and natural history, environmental science, computer science, and writing. Their dedication to teaching undoubtedly prepared me for all of my subsequent successes.

I am also eternally grateful to my mother, Karen Balik, whose immense unconditional love and support made me the person I am, as well as my father, Matthew Balik, who taught me to work hard, efficiently, and with attention to detail. Similarly, I thank Susan Washko, whose love, support, and reminders to make time for myself have kept me grounded and optimistic. Lastly, I thank my late grandmother Leola Balik, whose good humor, wisdom, wit, and longevity will forever inspire me.

Finally, I am deeply grateful for the financial support I received from the National Science Foundation, North Carolina State University, The Southeast Climate Adaptation Science Center, The Colorado Mountain Club, The Rocky Mountain Biological Laboratory, Allegheny College, The Arnold and Mable Beckman Foundation, The Society for Freshwater Science, and The Ecological Society of America.

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## **CHAPTER 1: High interspecific variation in nutrient excretion within a guild of closely related caddisfly species**

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Balik, J. A., Taylor, B. W., Washko, S. E., & Wissinger, S. A. (2018). High interspecific variation in nutrient excretion within a guild of closely related caddisfly species. *Ecosphere*, 9(5), e02205.

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

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**Citation:** Balik, J. A., B. W. Taylor, S. E. Washko, and S. A. Wissinger. 2018. High interspecific variation in nutrient excretion within a guild of closely related caddisfly species. *Ecosphere* 9(5):e02205. 10.1002/ecs2.2205

**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.

**Received** 10 January 2018; revised 20 March 2018; accepted 23 March 2018. Corresponding Editor: Robert R. Parmenter.

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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.,  $\text{NH}_4^+$  and  $\text{PO}_4^{3-}$ ) 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  $\text{NH}_4^+$ -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  $\text{PO}_4^{3-}$ . 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. nigrificulus*, *L. externus*, *L. picturatus*, *G. lorettae*, 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. nigrificulus*, *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 (algivore/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. nigrificulus*, *L. externus*, and *L. picturatus*. For *A. nigrificulus*, 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. nigrificulus*,  $F_{3,16} = 3.19$ ,  $P = 0.052$ ; *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

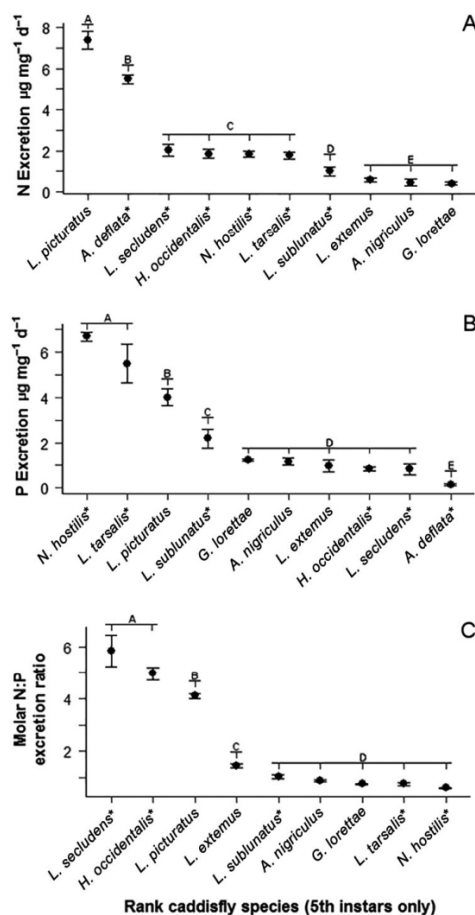


Fig. 1. Mean ( $\pm 1$  SE) (A) nitrogen excretion rates and (B) phosphorus excretion rates of the final larval instars in a 10-species guild of lentic limnephilid caddisflies. Values are expressed as nutrient excreted ( $\mu\text{g}$ ) per unit body mass ( $\text{mg}$ ) per day ( $\text{d}$ ) of each species. (C) Mean ( $\pm 1$  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)

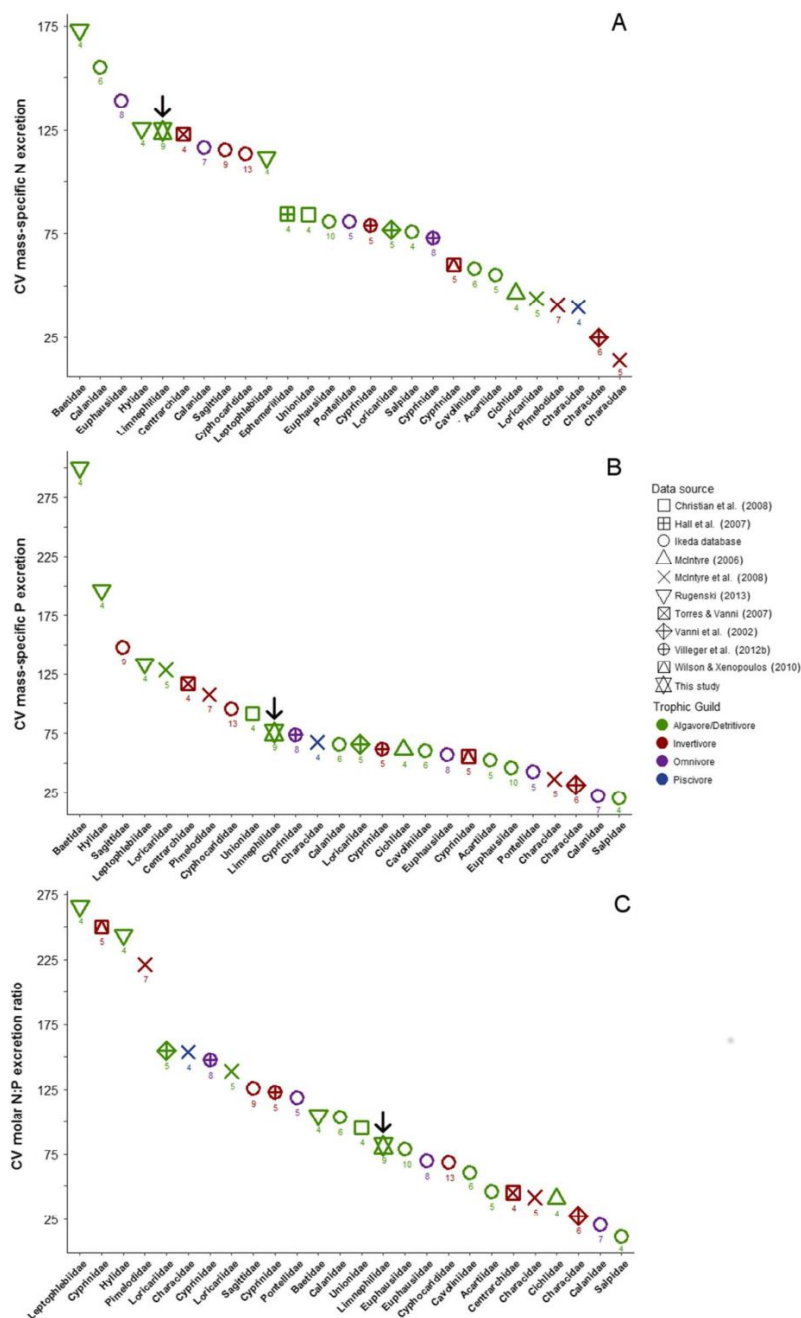


Fig. 2. (A) Coefficients of variation (CV) for family-level mass-specific nitrogen excretion, (B) for family-level mass-specific phosphorus excretion, and (C) for family-level molar N:P excretion ratio. Within each panel, families are ranked in order of decreasing CV. Color codifies data source and shape 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 (Sterner 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 (Sterner 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 epidetrital algae (Bärlocher 1985, Moore et al. 2004, Kuehn 2016). For instance, biofilms on submerged sedge contained more P (87.8 µg/mg) and twice as much N (21.3 µg/mg) than the sedge itself (51.1 µg P/mg and 9.52 µg N/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  $6.5^{\circ}\text{C}$  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  $18^{\circ}\text{C}$  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 lotic 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.

#### ACKNOWLEDGMENTS

We thank Amanda Klemmer, Hamish Greig, Jeffrey Marcus, Maria Smith, Brook Jeffery, Anna Rossow, Liana Leja, and Michelle Woods for field assistance. Milt Ostrofsky provided advice on

nutrient and statistical analysis. Amanda DelVecchia, Derek West, and two anonymous reviewers provided valuable comments for improving the manuscript. Thanks to Dave Ruitter for confirming species identifications from associated larval and adult material. The Nature Conservancy provided access to the Mexican Cut Nature Preserve and The Rocky Mountain Biological Laboratory facilitated access to other ponds on private lands. Balik was supported by a Beckman Scholar Fellowship from the Arnold and Mable Beckman Foundation, NSF Grants 125764 and 1556914 to Taylor, a NC State University Provost Fellowship, a NSF Graduate Research Fellowship, and a RMBL Graduate Fellowship. Washko was supported by a Harold State Fellowship from Allegheny College and NSF Grant 1557015 to Wissinger. This work was initially proposed and conducted as part of Balik's undergraduate senior thesis at Allegheny College.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online at: <http://onlinelibrary.wiley.com/doi/10.1002/ecs2.2205/full>

## **CHAPTER 2: Species-specific traits predict whole-assemblage detritus processing by pond invertebrates**

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## Abstract

Functional trait diversity within species assemblages ultimately determines whether or not ecosystem processes are sensitive to shifts in relative abundances or species composition. For example, trait variation suggests detritivores process detritus at different rates and make different relative contributions to whole-assemblage processing, which is therefore likely sensitive to global change-driven shifts in assemblage composition. Here, we address three main questions: 1) what is the magnitude of interspecific variation in detritus processing within the detritivore guild in high-elevation wetlands, 2) which traits drive interspecific variation in processing among larval caddisflies, 3) are larval caddisflies' single-species effects additive in multi-species assemblages? We used a series of microcosm experiments to quantify species-specific coarse and fine particulate organic matter (CPOM and FPOM) processing for ten closely-related larval caddisfly species and three non-caddisfly species. Next, we compared trait-based models including life history, dietary, and extrinsic trait predictors to determine which intrinsic and extrinsic traits best explain the interspecific variation among the larval caddisflies. Finally, we compared processing by mixed caddisfly assemblages in microcosms and in natural ponds to additive predictions based on individual species processing rates. We found considerable interspecific variation in biomass-specific CPOM (thirteen-fold differences) and FPOM (eight-fold differences) processing. Furthermore, on a mass-specific basis, amphipods and chironomids processed a similar amount of detritus as caddisflies, suggesting the importance of non-shredder taxa for processing CPOM may be greater than previously recognized. Next, the best trait model of CPOM processing included development rate, percent CPOM in the diet, and primary case material. Similarly, the best model for FPOM processing included development rate and percent of total detritus in the diet. Finally, additive predictions were strikingly similar to realized

processing in mixed caddisfly assemblages in both microcosms and natural ponds, with the largest difference being a 15% overestimate of CPOM processing in one microcosm experiment. Thus, as global change drives species range shifts, invasions, and extinctions, additivity of species-specific processing suggests that single-species rates may be useful for understanding functional consequences of shifting detritivore assemblages. Furthermore, a trait-based approach to predicting species-specific processing could support preparation of additive estimates of whole-assemblage processing.

## Introduction

The majority of vascular plant material is not consumed as live tissue; instead, it enters food webs as detritus (Cebrian 1999). Animal detritivores play an important role in determining the fate of detritus as they accelerate breakdown, generate fine particulates, enhance microbial colonization, and remineralize detrital nutrients through excretion and egestion (Gessner et al. 2010). In aquatic systems, and particularly in streams, the detritivore assemblage's effect on detritus processing is well-recognized (Wallace and Webster 1996), whereas there is considerably less known about species-specific processing beyond the effects of large- versus small-bodied detritivores (Bradford et al. 2002). However, some detritivores consume and process more detritus than others, suggesting low functional redundancy (Boyero et al. 2007, Dangles et al. 2011). Understanding species-specific contributions to detritus processing is important because whole-assemblage processing is likely to change due to compositional shifts in detritivore assemblages driven by global change (Wardle et al. 2011). Nevertheless, our understanding of how detritus processing rates vary among species, as well as which traits are associated with that variation, remains limited.

Linking detritivore traits to species-specific detritus processing could provide a powerful framework for predicting a taxon's contribution to whole-assemblage processing. For example, a taxon's processing could be influenced or controlled by intrinsic traits such as mouthpart morphology, physiology, diet composition (Merritt et al. 2017), body size (Tonin et al. 2018), growth rate and developmental phenology, or extrinsic traits such as preferred microhabitat. In turn, although functional traits of biomass-dominant species often regulate ecosystem processes (Grime 1998), functional trait diversity within species assemblages ultimately determines whether or not ecosystem processes are sensitive to shifts in relative abundance or assemblage

composition (Suding et al. 2008). Thus, subdominant taxa can make functional contributions disproportionate to their biomass (Tatarko and Knops 2018), and consequently diversity effects on ecosystem processes, including detritus processing, can only arise given interspecific variation in key traits (Gessner et al. 2010). Indeed, trait diversity is likely more useful than taxonomic diversity for identifying and studying the mechanisms responsible for diversity effects on ecosystem processes such as detritus processing (McKie et al. 2008, Schindler and Gessner 2009). By extension, establishing links between species' traits and their functional contributions offers generalizable predictive utility across ecoregions with different assemblages, in contrast to a species-based approach which would require new measurements for each new species (Fukami et al. 2005, Webb et al. 2010).

Although the relationship between detritivore traits and interspecific variation in detritus processing is well-recognized in streams (Frainer et al. 2014, Tonin et al. 2018), this link is less established in wetlands where species-specific processing rates have been quantified for fewer species. Although detritivore species classified as shredders are absent from many wetlands, species in other functional feeding groups such as collector-gatherers are often critical wetland detritivores (Batzner and Ruhí 2013). Nevertheless, when shredders are present they make large contributions; in montane Colorado wetlands, coarse (> 1mm) detritus breakdown is 2-3x faster in the presence of larval caddisflies (Wissinger et al. 2018). However, the extent to which individual caddisfly species and other detritivores, including those that are not classified as shredders, contribute to the breakdown of coarse detritus remains unclear.

A lack knowledge of the role of animal detritivores and potentially fewer detritivore species make wetlands ideal systems for exploring species- and trait-based effects on detritus processing. The Colorado wetland system studied by Wissinger et al. (2018) is an exemplar

because within the closely-related caddisfly guild (Ruiter et al. 2013) there is considerable interspecific variation in body size and life history strategies (Wissinger et al. 2003), diet (Wissinger et al. 1996, Wissinger et al. 2018), and stoichiometric traits (excretion, body C:N:P; Balik et al. 2018). Furthermore, closely-related organisms are often presumed to be functional equivalents, but this is not always the case (Balik et al. 2018). Thus, our primary objective was to quantify species- and mass-specific detritus processing (mass loss of coarse [ $> 1\text{mm}$ ] particulate organic matter, CPOM) for ten larval caddisfly taxa and identify which intrinsic and extrinsic traits best explain variation among taxa in detritus processing. From 2011-2020, we conducted five microcosm experiments to measure detritus processing of all ten taxa. These data were used to test the prediction that caddisfly species would process CPOM and FPOM at different rates. We also expected that trait-based models would better explain interspecific variation in detritus processing than species identity-based models because consumers with similar traits (i.e., dietary, life history, etc.) will presumably exhibit greater similarity in detritus processing.

Our secondary objective was to contextualize species-specific caddisfly detritus processing in pond communities. To compare caddisfly detritus processing to other detritus-associated taxa commonly found in the same habitats, we quantified processing by larval chironomids (mixed taxa in proportion to their relative abundance; see Wissinger et al. 1999), snails (Lymnaeidae), and amphipods (mixed; *Gammarus* and *Hyaella*). We expected that larval caddisflies would have greater mass-specific processing than the non-caddisfly taxa which are primarily not considered shredders. Chironomids can be collector-gatherers (Wissinger 1999, Merritt et al. 2017) and snails scrape epideutrital biofilms (Brady and Turner 2010, Stoler et al. 2016). Although amphipods are important shredders elsewhere (Little and Altermatt 2018), their diets can vary (MacNeil et al. 1997) and the extent to which they utilize detritus in this system is

unclear. Next, to further contextualize individual caddisfly taxa's processing, we measured processing in mixed caddisfly assemblages to explore how intraguild species interactions (e.g., facilitation, intraguild predation, etc.) could modulate processing. We expected that mixed-assemblage processing would exceed additive predictions (e.g., summing the products of each species' mass-specific processing and biomass) because positive species interactions tend to counteract negative interactions and result in net-positive effects on ecosystem function (Hooper et al. 2005). Finally, to determine if species-specific rates measured in microcosms provide additive predictions of processing by multi-species caddisfly assemblages in ponds, we compared additive predictions (using larval caddisfly abundance in natural ponds and species-specific microcosm processing rates) to previously collected *in-situ* measurements of caddisfly assemblage detritus processing (Wissinger et al. 2018).

## Materials and Methods

### *Study System*

Since 1990, annual censuses of the invertebrate communities in kettle-pond wetlands near the Rocky Mountain Biological Laboratory (RMBL; Gunnison, CO, USA) have indicated that mixed assemblages of case-making caddisflies (Limnephilidae and Phryganeidae) represent 30-70 % of aquatic animal biomass along a hydroperiod gradient from permanent ponds that never dry to vernal ponds that dry every summer (Wissinger et al. 1999; Wissinger et al. 2016). Previous work demonstrated that processing is 2-3x faster in their presence (Wissinger et al. 2018) and species-specific processing is density-dependent (Klemmer et al. 2012). *Carex aquatilis* is the dominant detrital source in all wetlands throughout the region, with annual inputs ranging from 50-1500 g dry mass /m<sup>2</sup> (Wissinger et al. 2018).

Beaver pond wetlands contain an additional limnephilid caddisfly genus, *Ecclisomyia*, and a high abundance of amphipods (*Gammarus* and *Hyaletella*), which are absent from most kettle-pond wetlands (Wissinger et al. 1999). Chironomids (e.g., *Paratanytarsus*, *Psectrocladius*, *Tanytarsus*, and others) are abundant in all wetlands, and can equal or exceed larval caddisfly biomass in permanent kettle-ponds. Other common taxa include omnivorous snails (Lymnaeidae) and tadpoles (*Pseudacris*) that can consume detritus, predators (Odonata, Dytiscidae, Hemiptera, Chironomidae), and collector-gatherers of fine particulate organic matter (FPOM) with low abundances (e.g., Culicidae larvae; see food webs in Wissinger et al. 1999).

### *Experimental Design*

We quantified invertebrate species-specific detritus processing using five microcosm experiments (Appendix S1: Table S1) conducted in a white plastic tent (WeatherPORT, Hansen WeatherPORT, Delta, CO) at the RMBL. All experiments were conducted mid-summer (i.e., late June through early August) and used 0.25 m<sup>2</sup> (51 x 36 x 13 cm) plastic bins as replicate microcosms. Experimental designs were similar across all five experiments (Appendix 2: Table S1). For all experiments, sedge detritus (*Carex aquatilis*) was collected from montane (2850 m elevation) kettle-pond wetlands near the USFS trail #401 south of the RMBL. Sedge was air-dried for 72 – 96 h, then 20 – 25 g were added to each microcosm along with ~ 9 L pond water. Microbial inoculum was collected by scrubbing submerged rocks, wood, and sedge from source ponds. All microcosms received equal volume aliquots of filtered (1 mm) inoculum and incubated for 4 - 5 d to encourage biofilm growth on re-hydrated sedge.

After incubation invertebrates collected from nearby ponds were added to microcosms. A treatment with *L. externus* was included in all experiments as a reference treatment (Appendix 2:

Table S1) because it was the most common caddisfly species and after the 2011 experiment it was suspected to be the most effective detritivore. The density of *L. externus* used in microcosms corresponds to the inflection point (100 individuals/m<sup>2</sup>) of an asymptotic detritus processing response reported in a previous in-situ experiment wherein *L. externus* density was manipulated in littoral cages (Klemmer et al. 2012). Similar densities were used when stocking caddisfly taxa with 5<sup>th</sup> (final) instar masses comparable to *L. externus*, but proportionally greater densities were used for taxa with smaller final instars (e.g., Table 2.1, Appendix 2: Table S1). Likewise, densities in mixed caddisfly assemblage treatments (2011, 2013, and 2015) were scaled to match total biomass of the *L. externus* treatment. Mixed caddisfly assemblage compositions included co-occurring taxa. When available, 3<sup>rd</sup> instar caddisflies were used to stock treatments. Otherwise, later instars were used. We did not match *L. externus* biomass in 2020 for amphipods, snails, or chironomids because we did not have prior average individual mass data for these taxa. Finally, a set of replicate microcosms without any invertebrates (no-invertebrate control) was used to quantify losses from ambient leaching and microbial processing.

Experiments or specific treatments ended when larval caddisflies began to pupate or if mortality was observed (11 – 42 days; Appendix 2: Table S1). Macroinvertebrates were removed, and in 2019 and 2020 were counted, dried at 60 °C for < 48 h, and weighed to measure average individual final mass. Detritus remaining in each microcosm was washed through a 1 mm net or sieve. Detritus > 1 mm was classified as CPOM and oven dried (60 °C for 72 h) to estimate CPOM dry mass. In 2013, 2019, and 2020, detritus < 1 mm – 63 µm was also collected, dried, and weighed to estimate fine particulate organic matter (FPOM) dry mass.

To compare invertebrate detritus processing to the no-invertebrate control in each experiment, we first calculated CPOM and FPOM daily rates as:

$$\text{mg CPOM microcosm}^{-1}\text{d}^{-1} = \frac{[\text{initial sedge (mg)} - \text{final CPOM (mg)}]}{\text{treatment duration(d)}}$$

$$\text{mg FPOM microcosm}^{-1}\text{d}^{-1} = \frac{[\text{FPOM (mg)}]}{\text{treatment duration (d)}}$$

Next, to compare invertebrate detritus processing among species and across experiments, we first subtracted the daily no-invertebrate control mean from all invertebrate treatment replicates to correct mass-specific invertebrate processing for leaching and microbial breakdown. We then standardized for invertebrate biomass (mg CPOM or FPOM  $\text{mg}^{-1}$  animal biomass  $\text{d}^{-1}$ ). Treatment biomass for each taxon was estimated for 2011, 2013, 2015, and for *L. picturatus* in 2019 as the product of stocking densities (Appendix 2: Table S1) and mean individual final instar masses from prior studies (Wissinger et al. 2003, Balik et al. 2018). For all other treatments in 2019 and 2020, final treatment biomass was measured, but not all individuals were recovered due to pupation or mortality near the end of the experiments. For these taxa, mean individual final body mass was calculated using the recovered animals, then multiplied by initial density to estimate treatment biomass prior to pupation or mortality (Appendix 2: Table S1). Thus, for all experiments, treatment biomass was estimated as a product of initial density and an average final individual mass. Species' per-capita detritus processing was also calculated using treatment initial density (mg CPOM or FPOM  $\text{individual}^{-1} \text{d}^{-1}$ ).

We first analyzed daily microcosm detritus processing within experiments to determine if invertebrate treatments differed from the no-invertebrate controls. Shapiro-Wilk normality tests indicated that processing rates were normally distributed in all experiments ( $p > 0.05$ ), so analyses were performed on untransformed data. All statistical analyses were conducted in R 4.0.2 (R Core Team, 2020). For experiments in which both CPOM and FPOM were measured, multivariate analysis of variance (MANOVA) was used to test for differences among treatments

in both response variables due to possible correlation. If the overall MANOVA was significant, univariate analysis of variance (ANOVA) was used to test response variables independently. Likewise, univariate ANOVA was used for experiments in which only CPOM was measured. Following significant univariate ANOVAs, Dunnett's multiple comparison was used to compare each invertebrate treatment to the no-invertebrate control within each experiment.

To compare mass-specific detritus processing among taxa and across experiments, we used linear models with terms for taxa and experiment-year as categorical variables, and their interaction term. Non-significant terms were dropped, then Tukey's honestly significant difference (HSD) was used to identify differences in mass-specific processing among invertebrate taxa. To demonstrate the influence of body size, we also compared per-capita processing among taxa using ANOVA (CPOM or FPOM = taxa). Next, to determine if caddisfly taxa convert CPOM to FPOM at different rates, we used the interaction term in an ANCOVA (FPOM = CPOM + taxa + CPOM\*taxa) to test for homogeneity of slopes among taxa CPOM and FPOM processing in mass-specific and per-capita units.

Null expectations for detritus processing in mixed caddisfly assemblage treatments were obtained using each species' mass-specific processing:

Predicted mixed assemblage processing ( $d^{-1}$ ) =

$$\sum_{\text{all member species}} \left[ \text{mg CPOM or FPOM } mg^{-1} \text{ animal tissue } d^{-1} * \text{density in mixed assemblage} * \text{average individual mass (mg)} \right]$$

Prior to comparing observed and predicted mixed assemblage processing with two-tailed one sample t-tests, we corrected observed values for leaching and microbial breakdown by subtracting no-invertebrate control means.

To determine if species-specific microcosm rates can provide additive predictions of processing by caddisfly assemblages in natural ponds, we made additive predictions for the three

ponds studied by Wissinger et al. (2018). Briefly, Wissinger et al. used in-situ litter trays with coarse and fine mesh enclosures to quantify larval caddisflies' effect on detritus processing in natural ponds. Fine mesh excluded caddisflies, whereas the change in detritus mass over time in coarse mesh treatments reflected caddisfly assemblage processing. Therefore, we used caddisfly species-specific microcosm processing rates and larval abundances in each pond (Supplemental Table 1 of Wissinger et al. 2018) to predict additive caddisfly assemblage processing:

$$\text{Predicted caddisfly assemblage processing (m}^2 \text{ d}^{-1}\text{)} = \sum_{\text{all larval caddis species}} [\text{mg CPOM individual}^{-1} \text{ d}^{-1} \text{ from microcosm}] * [\text{abundance per m}^2 \text{ in natural pond}]$$

We used standard errors of species' abundances and mean microcosm per-capita processing to estimate error of predicted caddisfly assemblage processing. We compared predicted to measured caddisfly assemblage processing (litter decay slopes; Figure 4 of Wissinger et al. 2018) with measured processing error expressed as litter decay slope standard error.

#### *Animal traits predicting detritus processing*

Diet composition data used in the traits analyses were collected previously for all taxa except *L. abbreviatus* and *A. bimaculata*, which we quantified following the same protocol (Appendix 2: Methods S1; Wissinger et al. 1996, Wissinger et al. 2018).

To explore which animal traits were most informative for predicting detritus processing, we used trait- and species identity-based models of larval caddisfly processing following methods used in other studies (e.g., Webb et al. 2010, Adler et al. 2018). Due to limited trait information, we excluded non-caddisfly taxa and *Ecclisomyia sp.* Because we included body mass as a predictor trait, we used per-capita rates as the response variable in this analysis. Trait-based models were prepared in two steps. First, a series of linear models with single continuous

predictors, including body mass, relative diet composition, tissue elemental stoichiometry, and molar N:P excretion ratio (Balik et al. 2018) were used to test for relationships between caddisfly intrinsic traits and per-capita processing. Second, continuous traits that individually explained large amounts of variation (defined by adjusted  $R^2 > 0.15$ ) in processing were used as fixed effects in subsequent trait-based linear models along with discrete categorical traits (Table 2.1). A correlation matrix identified multicollinearity between continuous traits and guided model selection (Appendix 2: Table S2). When two continuous traits were highly correlated (determined by Pearson's  $r > 0.5$ ), the trait that individually better predicted processing was used. Variance inflation factors (VIFs) also guided trait model selection, with  $VIF < 3.5$  considered acceptable. After minimizing VIFs, the trait models with the lowest Akaike Information Criteria (AIC) were selected. Species-based models included only species identity as a fixed effect predictor and were compared to the selected trait-based models using AIC.

## Results

### *Invertebrate-mediated detritus processing relative to no-invertebrate controls*

In all five experiments, there were significant differences in detritus processing among treatments (Appendix 2: Table S3; Appendix 2: Figure S1). In the 2011, 2013, and 2015 experiments, all invertebrate taxa processed more CPOM than their respective no-invertebrate control (Appendix 2: Table S4). In 2019, all taxa processed more CPOM and FPOM than the no-invertebrate control except *L. abbreviatus*, which did not differ (Appendix 2: Table S4). In 2020, CPOM processing by all invertebrate taxa was greater than the no-invertebrate control (Appendix 2: Table S4). FPOM in the amphipod, chironomid, and lymnaeid treatments did not differ from the no-invertebrate control, but FPOM was greater in the *L. externus* and *Ecclisomyia* sp. treatments (Appendix 2: Table S4). Although processing by a few taxa did not differ from

their corresponding no-invertebrate control, we calculated mass-specific processing for all taxa because comparing among taxa was central to our primary questions.

#### *Mass-specific and per-capita detritus processing among taxa*

Mass-specific invertebrate CPOM differed among taxa (Figure 2.1A;  $F_{12,68} = 27.27$ ,  $p < 0.001$ ), and *L. externus* processed 13.3x more than *L. abbreviatus*, which processed the least. CPOM differences among taxa were not dependent on experiment-year ( $F_{4,68} = 2.02$ ,  $p = 0.101$ ) or the interaction between experiment-year and taxon ( $F_{2,68} = 0.89$ ,  $p = 0.414$ ). Mass-specific invertebrate FPOM production also differed among taxa (Figure 2.2A;  $F_{8,44} = 3.27$ ,  $p = 0.005$ ), and *L. externus* processed 23.7x more than lymnaeid snails, which processed the least. FPOM differences among taxa were not dependent on experiment-year ( $F_{2,44} = 0.64$ ,  $p = 0.533$ ) or the interaction between experiment-year and taxon ( $F_{1,44} = 0.03$ ,  $p = 0.854$ ).

Per-capita CPOM also differed among taxa (Figure 2.1B;  $F_{12,74} = 62.87$ ,  $p < 0.001$ ), and in contrast to mass-specific processing, the larger *A. bimaculata* processed 1.3x more than *L. externus*. Furthermore, per-capita *A. bimaculata* processed 59.6x more than chironomids, which processed the least. Per-capita FPOM also differed (Figure 2.2B;  $F_{8,47} = 19.24$ ,  $p < 0.001$ ), and *L. externus* FPOM was 54.7x more than chironomids, which again processed the least.

Within the caddisfly guild, greater CPOM processing led to greater FPOM production (Appendix 2: Figure S2; mass-specific CPOM:  $F_{1,29} = 81.82$ ,  $p < 0.001$ ; per-capita CPOM:  $F_{1,29} = 100.51$ ,  $p < 0.001$ ), and this relationship did not differ among species (CPOM\*Species; mass-specific:  $F_{5,29} = 1.61$ ,  $p = 0.354$ ; per-capita:  $F_{5,29} = 1.05$ ,  $p = 0.407$ ). However, *L. externus* produced more FPOM than other caddisfly taxa when controlling for variation in CPOM

(Species; mass-specific:  $F_{5,29} = 2.81$ ,  $p = 0.035$ , *L. externus* intercept = 1.25,  $t = 2.19$ ,  $p = 0.037$ ; per-capita:  $F_{5,29} = 2.35$ ,  $p = 0.066$ , *L. externus* intercept = 10.24,  $t = 2.06$ ,  $p = 0.048$ ).

#### *Microcosm mixed assemblage treatment detritus processing*

Processing in mixed assemblages was generally similar to predictions calculated by summing the products of each species' mass-specific processing rates and biomass. The 2013 mixed assemblage containing *L. externus*, *A. nigriculus*, and *L. picturatus* processed only 15 % less CPOM than predicted (Appendix 2: Figure S3A;  $t_3 = 5.660$ ,  $p = 0.011$ ). Whereas, the 2011 assemblage containing *L. externus*, *L. sublunatus*, and *L. tarsalis* and the 2015 assemblage containing *L. externus*, *G. lorretae*, *L. picturatus*, and *L. secludens* both processed as much CPOM as predicted (Appendix 2: Figure S3A; 2011  $t_4 = 2.600$ ,  $p = 0.060$ ; 2015  $t_3 = 2.095$ ,  $p = 0.127$ ). The 2013 assemblage also produced as much FPOM as predicted (Appendix 2: Figure S3B;  $t_3 = 0.552$ ,  $p = 0.620$ ). Thus, additive predictions were statistically similar to realized processing in the mixed assemblages except for one experiment and response variable (i.e., 2013 CPOM) and this difference was less than 20 %.

#### *Predicting caddisfly assemblage processing with additive species-specific microcosm rates*

For all three ponds in which Wissinger et al. (2018) measured caddisfly detritus processing with litter trays, in-situ caddisfly assemblage processing was similar to additive predictions calculated by summing the products of species' per-capita processing rates and their abundances (Figure 2.3). Mean in-situ processing was only 8 % lower than predicted in Pond K1, 2 % higher in Pond K5, and 19 % higher in Pond K6. Moreover, standard error bars overlap

between in-situ and predicted processing in all three ponds, indicating additive predictions were statistically similar to realized processing in these ponds.

*Trait vs. species identity as predictors of larval caddisfly detritus processing*

Single trait models for per-capita caddisfly CPOM indicated that body mass, percent coarse or total detritus in the diet, and molar N:P excretion should be compared in trait-based models along with discrete categorical trait predictors (Appendix 2: Table S5). In particular, body mass alone explained 69 % of the variation in per-capita CPOM. Nonetheless, the best trait-based model of per-capita CPOM included larval development rate, primary case material, and percent coarse detritus in the diet (AIC = 374.42,  $F_{5,61} = 95.51$ ,  $p < 0.001$ , Adj.  $R^2 = 0.88$ ). The species-based model provided an AIC-better fit but explained a similar amount of variation (AIC = 366.27,  $F_{8,58} = 71.8$ ,  $p < 0.001$ , Adj.  $R^2 = 0.90$ ;  $\Delta AIC = 8.15$ ;  $\Delta R^2 \leq 0.02$ ).

Single trait models for per-capita larval caddisfly FPOM indicated that percent coarse, percent fine, or total detritus in the diet and body tissue C:P stoichiometry should be compared in trait-based models along with categorical trait predictors (Appendix 2: Table S5). Percent total detritus in the diet alone explained 72 % of the variation in per-capita FPOM. As such, the best trait-based model of per-capita FPOM included percent total detritus in the diet and larval development rate (AIC = 165.34,  $F_{3,32} = 24.82$ ,  $p < 0.001$ , Adj.  $R^2 = 0.67$ ). The corresponding species-based model provided an AIC-better but comparable fit (AIC = 166.88,  $F_{4,31} = 18.36$ ,  $p < 0.001$ , Adj.  $R^2 = 0.66$ ;  $\Delta AIC = 1.54$ ;  $\Delta R^2 \leq 0.01$ ). Thus, for both CPOM and FPOM, species-based models perform statistically better, but trait-based models provide a comparable fit.

## Discussion

*Larval caddisflies process detritus at different rates, but several non-caddisfly taxa match or exceed caddisfly processing*

Here, we show that closely-related larval caddisflies (Ruiter et al. 2013), the biomass-dominant detritivores in high elevation ponds (Wissinger et al. 1999), process CPOM at different rates in single-species experiments (Figure 2.1). Although mean FPOM production did not differ among taxa (Figure 2.2), controlling for covariation in CPOM processing indicates that *L. externus* generates more FPOM than other members of the guild (Appendix 2: Figure S2). This could suggest that *L. externus* is a comparatively sloppy feeder, or that it produces more feces. For *L. externus* and *L. picturatus*, CPOM and FPOM processing were consistent among experiment-years, suggesting that, for at least these taxa, detritus processing is consistent interannually. Furthermore, the magnitude of interspecific variation in mean mass-specific processing (thirteenfold for CPOM and eightfold for FPOM) is comparable to that of their nitrogen and phosphorus excretion (eightfold for N and sevenfold for P; Balik et al. 2018). Thus, there could be low functional redundancy among members of this guild in terms of their contributions to ecosystem functions associated with nutrient cycling and energy flow.

Surprisingly, although chironomids are generally regarded as primarily FPOM collector-gatherers (Wissinger 1999), their mass-specific CPOM processing was similar to that of *L. externus*, *G. lorretae*, *L. sublunatus* and *L. picturatus*, and exceeded that of six other caddisfly taxa (Figure 2.1A). Their mass-specific FPOM production was also similar to that of all caddisfly taxa (Figure 2.2A). Even lymnaeid snails, which predominantly scrape epidebital biofilm (Brady and Turner 2010, Stoler et al. 2016) had similar CPOM processing to five of ten caddisfly taxa, but processed less FPOM. Although amphipods are not found in many lentic

habitats within this system, they are abundant in some beaver ponds. Amphipods are important shredders elsewhere (Little and Altermatt 2018), but their diets are highly variable (MacNeil et al. 1997) and the extent to which they feed on sedge detritus in this system was unknown.

Amphipods had statistically similar mass-specific CPOM and FPOM processing to all caddisfly taxa except *L. externus*, which processed 3.4x more CPOM. However, 4 of 5 caddisfly taxa with statistically similar rates had means nearly double that of amphipods. These comparisons among shredder caddisfly taxa and other invertebrates classically organized into other functional feeding groups suggest that the importance of non-shredder taxa for processing CPOM may be greater than previously recognized in these ponds and perhaps other systems. However, sedge detritus was the only dietary resource in experimental microcosms; it is possible that in natural ponds shredders would outcompete non-shredder taxa for access to sedge, or that non-shredders would preferentially select other resources such as FPOM or biofilms.

#### *Life history and diet composition predicts detritus processing*

We expected that larval caddisfly diet composition, especially percent coarse detritus, would be a useful trait for predicting per-capita detritus processing. Indeed, percent coarse and total detritus in the diet individually explained 32-34 % and 62-74 % of interspecific variation in CPOM and FPOM processing, respectively. Trait-based model selection yielded models that included percent coarse (for CPOM) and total detritus (for FPOM) in the diet. Thus, relative to other traits we considered, coarse and total detritus in the diet were among the most useful for predicting processing. Indeed, diet often excels at predicting other functional traits such as nutrient excretion (Moody et al. 2015).

Likewise, larval development rate was in the best trait-based models of CPOM and FPOM processing. Smaller organisms often grow proportionally faster than larger organisms (Peters 1986); within this guild, taxa with the largest final instars indeed have slow or moderate growth rates. Although body mass individually explained 69 % of interspecific variation in CPOM processing, it was not included in the best trait-based model. Thus, differences in growth rate among taxa may encapsulate the influence of body size on detritus processing.

Primary case material was also in the best trait-based model for CPOM processing. Caddisflies often exhibit preference for case material types, and in this guild, material choice and case morphology are relatively consistent within species (Wissinger et al. 2006). Moreover, primary case material is often associated with preferred microhabitat, as it can provide camouflage from predators (Williams et al. 1987). Indeed, taxa with sedge-based cases are often abundant on or floating near submerged sedge, whereas taxa with stone or woody debris cases are abundant on the benthos (Wissinger et al. 1999). Consistent with these microhabitat preferences, relative to taxa with stone or woody debris cases, taxa with sedge-based cases process 5x more CPOM, possibly driven in part by case-building.

Comparisons with species-based models can evaluate how well trait-based models predict organisms' contributions to ecosystem functions. If species identity is interpreted as the aggregate of all known and unknown traits, model comparison suggests that the most important traits were included here. Trait-based model fit remains similar if primary case material is dropped to enhance model generality to other systems with non-caddisfly detritivores ( $AIC = 377.38$ ,  $F_{3,63} = 146.1$ ,  $p < 0.001$ ,  $Adj. R^2 = 0.87$ ;  $\Delta AIC = 11.1$ ;  $\Delta R^2 \leq 0.03$ ). For FPOM, trait- and identity-based models also have similar AIC and explain a similar amount of variation. Thus, trait-based models are preferred over species-based models for both CPOM and FPOM because

they potentially offer generalizable predictive utility across ecoregions with different species assemblages (Webb et al. 2010).

*Negative species interactions can exceed positive interactions in mixed assemblages*

Species interactions in mixed assemblages can cause realized ecosystem function to differ from additive predictions of species-specific contributions. Loreau (1998) refers to this difference as the diversity effect. Although classic diversity-function studies predict that the net-positive diversity effects due to niche or resource partitioning and facilitation counteract negative interactions (Hooper et al. 2005), we did not observe a net-positive diversity effect in any of our mixed assemblages. Instead, processing was consistently similar to our additive predictions. However, in the 2013 assemblage of *L. externus*, *L. picturatus*, and *A. nigriculus*, CPOM was 15 % lower than predicted but FPOM was as predicted. This small difference is not surprising because *A. nigriculus* engages in intraguild predation (IGP) of *Limnephilius* larvae as well as conspecific cannibalism (Wissinger et al. 1996, Wissinger et al. 2004, Wissinger et al. 2006). Here, antagonistic interactions likely counteracted and exceeded any positive effects of facilitation or resource partitioning.

Absence of positive diversity effects is not unprecedented (Maynard et al. 2017). First, positive effects of interspecific complementarity could take years to accumulate because ecosystem feedback effects such as microbial nutrient cycling develop interannually (Reich et al. 2012). Our mixed assemblages were short duration (23 – 42 days), and more importantly, microcosms were sterile prior to inoculation. Second, although each of the assemblages we tested occur in-situ (Wissinger et al. 2003, Wissinger et al. 2018), our microcosms may not be the correct venue to observe diversity effects because natural ponds have greater habitat and

resource heterogeneity, and therefore present greater opportunity for niche and resource partitioning, in addition to greater potential for ecosystem feedbacks. Consequently, positive diversity effects on detritus processing may be more likely to occur in-situ than microcosms.

*Species-specific microcosm rates provide accurate additive predictions of in-situ processing*

Although other studies have quantified species-specific detritus processing in laboratory microcosms (Bjelke and Herrmann 2005, Boyero et al. 2007, Dudgeon and Gao 2010), it is often unclear how these rates translate to natural systems (but see Boyero et al. 2006). However, here, prior studies provide points of comparison. First, for some taxa, single-species microcosm and in-situ processing are similar. Specifically, *L. externus* and *L. picturatus* microcosm per-capita CPOM processing were respectively only 10 % and 16 % lower than in littoral cages (Klemmer et al. 2012, Shepard et al. 2021). *A. nigrificulus* showed a similar pattern, with microcosm per-capita CPOM processing 42 % lower than in littoral cages (Shepard, unpublished data). Positive ecosystem feedbacks (e.g., epidermal biofilm resource quality, microbial conditioning, nutrient supply) absent from the microcosm venue may promote greater single-species processing in-situ.

Second, we demonstrate that species-specific microcosm detritus processing rates provide accurate additive predictions of caddisfly assemblage processing in ponds, measured previously by Wissinger et al. (2018). In-situ caddisfly assemblage processing slightly exceeded additive predictions only in Pond K6 (19 %), although overlapping standard errors suggest they are similar. Here, the natural pond context and greater species richness suggest that niche or resource partitioning and positive species interactions such as facilitation generally balance any effects of antagonistic species interactions. Consequently, species-specific microcosm processing rates and abundances in natural ponds could be useful for predicting functional consequences of changes

in detritivore assemblages, which could shift in many systems due to species invasions and local extinctions driven by global change (Wardle et al. 2011).

However, there are two key considerations regarding additive detritus processing predictions. First, similar to the mass-ratio hypothesis (Grime 1998), the nutrient excretion literature demonstrates that population-level biomass often determines species' contributions to community-wide supply despite considerable interspecific variation in mass-specific excretion (Atkinson et al. 2017). For example, caddisflies account for 30-70 % of animal biomass in subalpine ponds in the study area, but in some years and at particular times within each year (i.e., between emergence and egg hatching), chironomids attain similar or greater biomass (Wissinger unpublished data). Thus, chironomid contributions (i.e., mass-specific rate x biomass) to overall processing could occasionally exceed that of any single caddisfly species, including *L. externus*, which has the highest mass-specific processing and is the biomass dominant within the detritivore guild (Wissinger et al. 2003). However, it is unknown if chironomids readily utilize CPOM in this system when FPOM is available.

In addition to a taxon's biomass, time spent in the habitat also influences a taxon's contribution to functions such as detritus processing (i.e., mass-specific rate x biomass x time). For example, the larval development cycle of *L. externus* is at least three weeks longer than that of any other caddisfly in the guild (Wissinger et al. 2003, unpublished data), and consequently *L. externus* is likely the dominant detritivore in many habitats in this system, excluding beaver ponds where amphipods are more abundant. Thus, a taxon's realized effect on ecosystem processing is sensitive to their system-specific context (Wellnitz and Poff 2001), including all species interactions, their biomass, traits, and the amount of time they spend in the habitat.

Ecologists' understanding of the relationships between properties of species assemblages and their associated ecosystem functions is being tested as we try to anticipate the ecosystem outcomes of species range shifts, invasions, and extinctions in response to global change. Our results suggest that scaling direct measurements or estimates of species-specific processing from trait-based models to additive predictions of whole-assemblage processing is a fruitful path to understanding how shifts in assemblage composition alter key ecosystem functions.

### **Acknowledgements**

We thank the Rocky Mountain Biological Laboratory for logistic support and S. Jordt for comments on an early manuscript draft. JAB and MV were supported by the Arnold & Mabel Beckman Foundation. JAB was also supported by NSF GRFP and a RMBL graduate fellowship. CL was supported by an Inouye endowment RMBL Scholarship; SEW by an Allegheny College Harold State Fellowship; BC by NSF DEB 1457029 to Kailen Mooney; DMK by NSF DBI 0753774; MEP by the Allegheny College Shanbrom fund; AS by NSF DBI 1755522; OJW by an ROA supplement award to NSF DBI 1755522; BWT and LMD by NSF DEB 1556914, Jules Silverman, NCSU Department of Applied Ecology; HSG and IDS by NSF DEB 1556788 and USDA NIFA Project ME0-21607 through the Maine Agricultural and Forest Experiment Station, and RMBL Research Fellowships; and SAW by the RMBL, Schwartz Endowed Professorship in the Natural Sciences at Allegheny College, and NSF DEB RUI 1557015.

**Author Contributions:** *SAW conceived the experiments and designed methodology. JAB conceived the manuscript. With SAW's assistance, experiments were conducted by DMK and MEP in 2011, MV in 2013, SEW and JAB in 2015, and CL, IDS and HSG in 2019. The 2020*

*experiment was conceived and conducted by JAB, BWT, LMD, BC, AS, OJW. JAB processed and analyzed data and wrote the manuscript; all authors contributed critically to the drafts and gave final approval for publication.*

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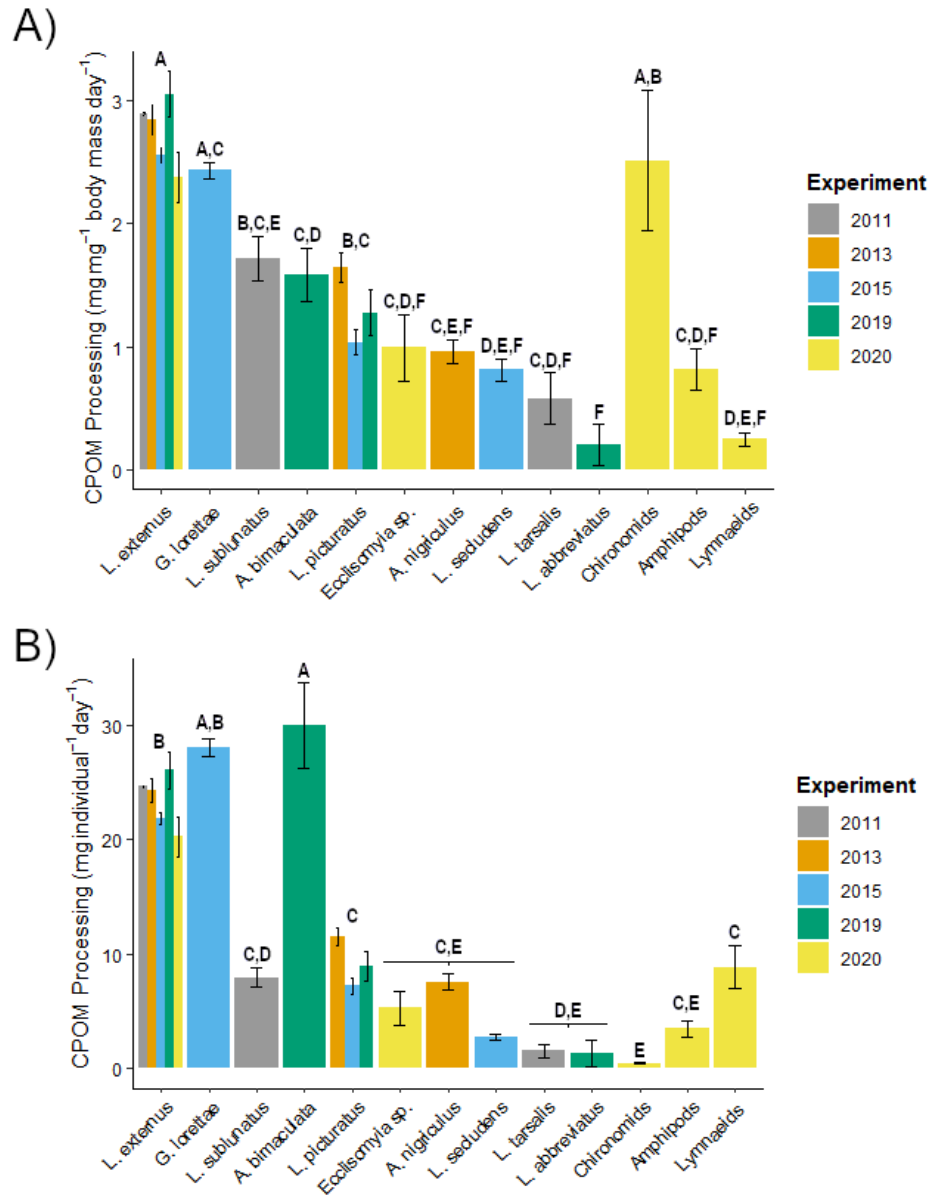
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**Table 2.1:** Summary of larval caddisfly species' traits used in trait-based models of detritus processing. Ranked development rate, case material, ranked mobility (1= highly mobile 9= slow mobility) from Wissinger et al. 2003, 2006, Balik et al. 2018, and unpublished data. Diet traits from Wissinger et al. 1996, 2018 for all taxa except *A. bimaculata* and *L. abbreviatus*, which were quantified here. We excluded *Ecclisomyia* sp. from all trait and species identity models due to limited trait information. Genus *L.* = *Limnephilus*, *A.* = *Asynarchus*, *An.* = *Anabolia*, *G.* = *Grammotallus*

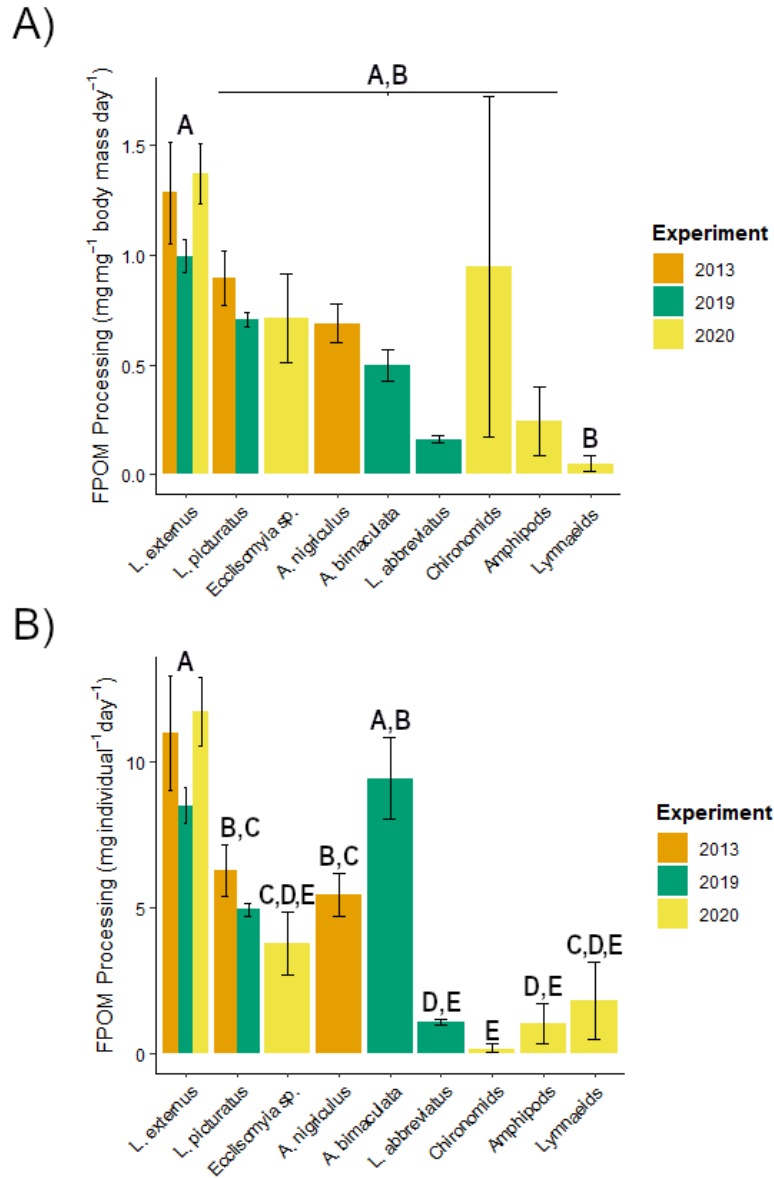
| Species               | Develop.<br>Rate | Final<br>Instar<br>Mass<br>(mg) | Hydroperiod                       | Elevational<br>Range | Case<br>Material | Ranked<br>Mobility | Tissue<br>C:P | Molar<br>N:P<br>Excretion | Percent of diet composition |                  |       |        |
|-----------------------|------------------|---------------------------------|-----------------------------------|----------------------|------------------|--------------------|---------------|---------------------------|-----------------------------|------------------|-------|--------|
|                       |                  |                                 |                                   |                      |                  |                    |               |                           | Coarse<br>Detritus          | Fine<br>Detritus | Algae | Chitin |
| <i>An. bimaculata</i> | Moderate         | 18.92                           | Permanent &<br>Semi-<br>permanent | Broad                | Sedge            | 6                  | 8.8           | 422.4                     | 40.5                        | 40.5             | 17.8  | 1.2    |
| <i>A. nigriculus</i>  | Fast             | 7.9                             | Vernal &<br>Semi-<br>permanent    | Broad                | Woody<br>debris  | 1                  | 25.5          | 0.9                       | 53.4                        | 39.5             | 3.5   | 3.6    |
| <i>G. lorettae</i>    | Moderate         | 11.8                            | Permanent &<br>Semi-<br>permanent | Broad                | Sedge            | 8                  | 32.3          | 0.7                       | 32.3                        | 59.6             | 3.8   | 4.6    |
| <i>L. abbreviatus</i> | Fast             | 6.8                             | Permanent &<br>Semi-<br>permanent | Broad                | Sedge            | 5                  | 39.9          | 0.6                       | 28.9                        | 48.6             | 21.9  | 0.6    |
| <i>L. externus</i>    | Slow             | 8.5                             | Permanent &<br>Semi-<br>permanent | Broad                | Sedge            | 4                  | 18.9          | 1.4                       | 74.6                        | 22.5             | 3.3   | 1.9    |
| <i>L. picturatus</i>  | Fast             | 7.0                             | Permanent &<br>Semi-<br>permanent | Broad                | Sedge            | 3                  | 60.9          | 4.1                       | 34.3                        | 56.2             | 5.2   | 8.4    |
| <i>L. secludens</i>   | Fast             | 3.4                             | Vernal &<br>Semi-<br>permanent    | Montane              | Stone            | 2                  | 26.0          | 5.8                       | 27.4                        | 50.6             | 0.8   | 21.4   |

**Table 2.1** (Continued):

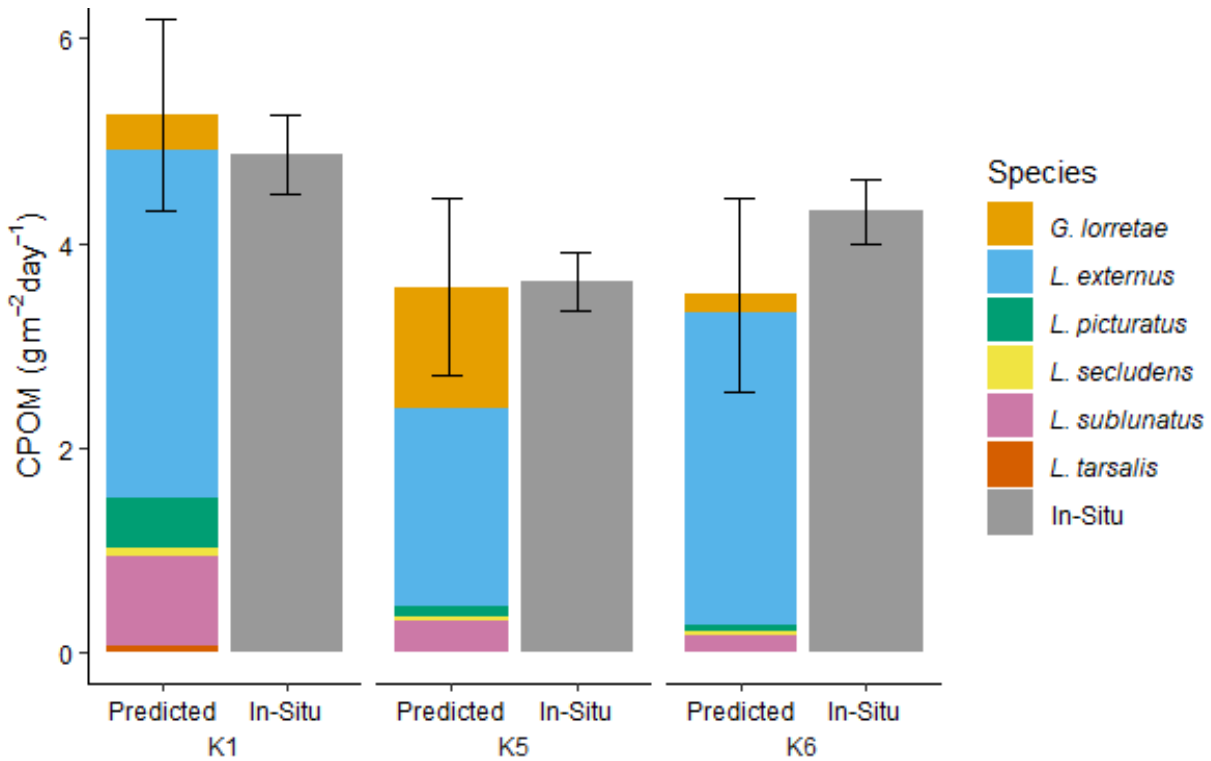
|                      |      |      |                                   |         |                 |   |      |     |      |      |     |     |
|----------------------|------|------|-----------------------------------|---------|-----------------|---|------|-----|------|------|-----|-----|
| <i>L. sublunatus</i> | Fast | 4.7  | Permanent &<br>Semi-<br>permanent | Montane | Sedge           | 7 | 29.5 | 1.0 | 40.0 | 51.0 | 4.2 | 2.0 |
| <i>L. tarsalis</i>   | Fast | 2.72 | Vernal &<br>Semi-<br>permanent    | Montane | Woody<br>debris | 9 | 33.5 | 0.7 | 23.3 | 64.4 | 6.2 | 6.0 |



**Figure 2.1:** Mean ( $\pm 1$  SE) invertebrate CPOM processing. Rates expressed in mass-specific (A) and per-capita (B) units. In both panels, larval caddisfly taxa are sorted by decreasing mass-specific CPOM, followed by non-caddisfly taxa (chironomids, amphipods, lymnaeids). Fill color indicates experiment-year, and Tukey's HSD letter groupings (grand mean for *L. externus* and *L. picturatus*) indicate no significant differences in CPOM processing at the 95% confidence level among species (A: Species:  $F_{12,70} = 29.11$ ,  $p < 0.001$ ; Experiment:  $F_{4,70} = 2.02$ ,  $p = 0.1$ ; B: Species:  $F_{12,74} = 62.87$ ,  $p < 0.001$ ).



**Figure 2.2:** Mean ( $\pm 1$  SE) invertebrate FPOM processing. Rates expressed in mass-specific (A) and per-capita (B) units. In both panels, larval caddisfly taxa are sorted by decreasing mass-specific FPOM, followed by non-caddisfly taxa (Chironomids, Amphipods, Lymnaeids). Fill color indicates experiment-year, and Tukey's HSD letter groupings (grand mean for *L. externus* and *L. picturatus*) indicate no significant differences in mean FPOM production rates at the 95% confidence level (A: Species:  $F_{8,45} = 3.339$ ,  $p = 0.004$ ; Experiment:  $F_{2,45} = 0.65$ ,  $p = 0.525$ ; B: Species:  $F_{8,47} = 19.24$ ,  $p < 0.001$ ).



**Figure 2.3:** Caddisfly assemblage detritus processing in ponds K1, K5, and K6, measured in-situ with litter trays by Wissinger et al. 2018 (grey fill; Figure 4 in Wissinger et al. 2018) and additive predictions calculated by summing products of species' mean per-capita microcosm processing and their abundance in natural ponds at the beginning of the litter tray experiment (Supplemental Table 1 in Wissinger et al. 2018). Bar height represents mean In-Situ and Predicted caddisfly assemblage processing. For In-Situ, error bars represent  $\pm 1$  SE of detritus decay slopes (Figure 4 of Wissinger et al. 2018). For Predicted, the products of species' abundance SE (Supplemental Table 1 of Wissinger et al. 2018) and mean per-capita microcosm processing rates were summed to estimate error.

**CHAPTER 3: Animal-driven nutrient supply declines relative to ecosystem nutrient demand along a pond hydroperiod gradient**

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## Abstract

In many lentic ecosystems, hydroperiod, or the duration of inundation, controls animal community composition and biomass. Although hydroperiod-imposed differences in wetland animal communities could cause differences in animal-driven nutrient supply, hydroperiod has not been considered as a template for investigating patterns of animal-driven nutrient cycling. Here, we use nutrient excretion rates ( $\text{NH}_4\text{-N}$  and SRP) and biomasses of pelagic and benthic invertebrates and salamanders, and nutrient uptake rates in a simulation model to estimate animal-driven nutrient supply and pond-level demand along a hydroperiod gradient of 12 subalpine ponds in the U.S. Rocky Mountains that are vulnerable to climate change. We found that animal biomass increased with hydroperiod duration, and biomass predicted animal-driven supply contributions among hydroperiod classifications (temporary-permanent). Consequently, community-wide supply was greatest in permanent ponds. Animal-driven N supply exceeded demand in permanent and semi-permanent ponds, whereas P supply equaled demand in both. Conversely, temporary ponds had large deficits in N and P supply due to lower community biomass and hydroperiod-induced constraints on dominant suppliers (oligochaetes and chironomids). The distribution of taxon-specific supply also differed among hydroperiods, with supply dominated by a few taxa in permanent ponds and supply more evenly distributed among temporary pond taxa. The absence or lower biomass of dominant suppliers in temporary ponds creates nutrient deficits and possibly limitation of productivity. Thus, as climate warming causes hydroperiods to become increasingly temporary and indirectly prompts biomass declines and compositional shifts, animal-driven nutrient supply will decrease and strong nutrient limitation may arise due to loss of animal-driven supply.

## Introduction

In small ponds and wetlands, hydroperiod is an important dimension of water availability that controls animal biomass and community composition (Wiggins et al., 1980; Schneider, 1999). Ponds with temporary hydroperiods that are inundated seasonally and dry annually, or periodically via rainfall, select for invertebrate communities shaped by dispersal and taxa capable of completing larval development before drying (Schneider and Frost, 1996; Wissinger et al., 1999a). In contrast, ponds with longer hydroperiods (semi-permanent ponds that dry occasionally and permanent ponds that never dry) allow for multiple trophic levels with invertebrate communities adapted to cohabitation with predators (Wissinger et al., 1999b; Stoks and McPeck, 2003). Furthermore, permanent ponds often have greater invertebrate densities (Schneider and Frost, 1996; Wissinger et al., 1999a) and species richness (Chase, 2007) than temporary ponds. Thus, hydroperiod maintains differences among ponds in community composition, biomass, and life history traits. However, it is unclear how differences in animal communities among hydroperiod classifications affect animal-driven nutrient cycling.

Higher animal biomass in permanent relative to temporary ponds (Schneider, 1999; Wissinger et al., 2016) suggests greater community-wide animal-driven nutrient supply in permanent ponds (Atkinson et al., 2017; Atkinson et al., 2019). Yet, contrasting life history strategies and differences in community composition promote variation in nutrient excretion rates among hydroperiods that could mediate or enhance the positive effect of biomass on community-wide supply. For example, in any hydroperiod, a taxon with high excretion could make supply contributions disproportionate to their biomass (Small et al., 2011). Alternatively, temporary pond taxa with rapid larval development may have lower excretion, especially of P (Elser et al., 2003). Numerically dominant taxa in permanent ponds are also smaller (Wissinger

et al., 1999a) which could promote higher excretion (Elser et al., 1996) and thus further enhance the positive effects of biomass. Although large-bodied vertebrate predators in permanent ponds could themselves make large supply contributions (Schindler and Eby, 1997; McIntyre et al., 2008), they also select for different invertebrate communities with lower biomass relative to temporary ponds (Wellborn et al., 1996; Wissinger et al., 1999a). Therefore, variation in excretion among differing communities and life history strategies complicates a first-principles prediction for greater community-wide supply in permanent ponds due to biomass alone.

Animal contributions to nutrient cycles can fulfill substantial proportions of ecosystem nutrient demand (Vanni, 2002; Hall et al., 2003; McIntyre et al., 2008), though few studies have estimated supply contributions of all benthic and pelagic animals in lentic systems, and little is known about how demand varies among hydroperiod classifications or how changes in animal communities will influence supply relative to demand (Elser et al., 1988). For example, demand could increase with growing season length, resulting in greater demand in permanent ponds that could match their supply from greater animal biomass. Although supply relative to demand could be similar in temporary ponds due to lower biomass and shorter growing seasons, mismatches between supply and demand could arise due to compositional differences in their animal communities. Furthermore, permanent and semi-permanent ponds that do not dry could have some overwinter nutrient demand and interannual microbial turnover, whereas nutrient demand in temporary ponds refilled by spring melt could be lower due to nascent microbial communities. Thus, evaluating animal-driven supply relative to ecosystem demand provides important context for comparing supply among systems, but it is unclear how community-wide animal supply relative to ecosystem demand varies among hydroperiod classifications.

Here, we explored differences in animal-driven nutrient supply relative to demand along a hydroperiod gradient in subalpine ponds using a resampling-based simulation model. The model was parameterized with survey data collected from four representative ponds in each of three hydroperiod classifications. Specifically, the model leverages observed variation in physical pond characteristics, nutrient uptake rates, community composition, species relative abundances, and their nutrient excretion rates to estimate nutrient supply and demand in permanent ponds that never dry, semi-permanent ponds that dry in some years, and temporary ponds that dry every summer. We used these estimates to explore where along the hydroperiod gradient animal-driven supply equaled, exceeded, or fell short of demand, and we support these inferences with empirical estimates from the twelve ponds we sampled.

Our specific predictions were 1) community-wide animal-driven nutrient supply would increase with hydroperiod because biomass increases with permanence (Wissinger et al., 2016) and is consistently a strong predictor of animal-driven supply (Carpenter et al., 1985; Atkinson et al., 2017). Likewise, 2) whole-pond seasonal demand would be greatest in permanent ponds due to greater area and longer hydroperiod durations. However, 3) supply would match demand in permanent and semi-permanent ponds but fall short in temporary ponds because of low biomass to drive supply. Next, we compared population-level biomass, nutrient excretion rate, time spent in the pond, and their interactions in linear models of taxon-specific supply to understand what best predicts a taxon's role as a dominant nutrient supplier across hydroperiod classifications. We expected that 4) population-level biomass would be the strongest driver of taxon-specific supply, and 5) salamanders would provide the largest supply contribution in permanent ponds because their biomass is comparable to the entire invertebrate community (Wissinger et al., 1999a) and vertebrates drive large nutrient supplies elsewhere (Schindler and Eby, 1997;

McIntyre et al., 2008). Finally, we predicted that 6) taxon-specific supply would be more evenly distributed in temporary ponds because drying selects for low-richness communities and taxa with similar life histories (Schneider and Frost, 1996; Chase, 2007) and functional roles due to niche complementarity (Loreau and Hector, 2001). In contrast, permanent hydroperiods with species-rich communities could have taxa that attain high biomass and dominate function (Grime, 1998).

## **Methods**

### *Site Selection and Animal Surveys*

Ponds were located within the Mexican Cut Nature Preserve, a pristine, subalpine (3560 m) wilderness area owned by The Nature Conservancy and managed by the Rocky Mountain Biological Laboratory (RMBL) in the Elk Mountains of central Colorado. The Mexican Cut is a glacial cirque with 60+ kettle-pond wetland habitats, all with similar basin substrate composition and geomorphology, emergent and riparian vegetation, and water chemistry (Wissinger et al., 1999a). Ponds in the Mexican Cut thaw and are supplemented or refill completely via snowmelt in June and are similar to high-elevation kettle-pond wetlands throughout the Rockies and other mountainous regions (Wissinger et al., 2016). For this study we selected four representative ponds in each of three hydroperiod classifications: permanent, semi-permanent, and temporary. Temporary ponds typically dry during August or earlier, and semi-permanent ponds hold water through winter freeze in most years but occasionally dry in August. Montane ponds and wetlands are ideal systems for the broader study questions because their snowmelt-driven hydroperiods are expected to become increasingly temporary due to rapid climate change at high elevations (Lee et al., 2015; Lund et al., 2016).

Large benthic invertebrates were surveyed with single 0.33 m<sup>2</sup> benthic D-net sweeps at the north, east, south, and west sides of each pond on 19 July 2018 following benthic census methods used for long-term population surveys in this system (Wissinger et al., 1999a). Small benthic invertebrates were sampled using a 0.02 m<sup>2</sup> benthic core. All individuals were identified to species-level (e.g., caddisfly sp.) or family-level (e.g., Gerridae, Hydrophilidae, Veliidae, etc.) according to previous census methods (Wissinger et al., 1999a). Average zooplankton density was estimated for each hydroperiod by pooling pond-level samples collected with a 80 µm mesh net and 2.2 L Van-Dorn following established methods for this system (Dodson, 1974).

Long-term monitoring of the Arizona tiger salamander (*Ambystoma mavortium nebulosum*) population started in 1988 and is censused with PIT tags or toe clips. The Mexican Cut salamanders are facultatively paedomorphic, and larvae develop into one of two adult morphs. Paedomorphs retain larval characteristics and attain sexual maturity in their natal ponds. In contrast, metamorphs transform and disperse into the terrestrial environment (Moore and Whiteman, 2016). Our sampling focused on larvae and paedomorphic adults because they spend their entire life in the ponds and are keystone predators (Wissinger et al., 1999b; Wissinger et al., 2006). Salamanders were captured using dipnets, and snout-vent length, total length, mass, capture date and pond were recorded. Sampling occurred daily between 15 June – 31 July. Pond-specific densities of 1+ year, 2+ year, and 3+ year larval age classes and 4+ year paedomorphic adults were estimated using continuous mark and recapture and Lincoln-Peterson estimates (Whiteman et al., 2012). Finally, pond-specific densities were averaged by hydroperiod for each age cohort.

### *Excretion and Uptake Measurements*

Excretion measurements were collected following established protocols for invertebrates (Hall et al., 2003; Balik et al., 2018) and amphibians (Whiles et al., 2009). Animals were collected and held for 0.5 to 1.5 h in plastic bags, which enabled us to measure ambient excretion rates while minimizing stress or starvation effects. All invertebrate excretion measurements were conducted by placing bagged animals in shaded areas of each pond to maintain ambient temperature. Ambient water temperatures varied by  $< 4^{\circ}\text{C}$  among replicates for each taxon. The number of individuals (1 - 80) and the volume of filtered pond water (100 – 200 mL) were adjusted per the taxon's size. Invertebrates used for excretion measurements were dried for 48 hours at  $60^{\circ}\text{C}$  to measure dry mass. Excretion measurements were opportunistically collected for each taxon from various ponds throughout the summer, and we assume that they were representative of each taxon's excretion in other ponds within the system across the season, which Balik and others (2018) previously demonstrated for the trichopteran (larval caddisfly) taxa. We collected 3-15 replicate excretion measurements for each taxon (223 total replicates across all taxa). During each sampling effort, we also included 2-4 controls without any animals to measure changes in ambient nutrient concentrations (52 total controls across all sample dates).

Salamanders used for excretion incubations were processed in a nearby shaded tent (Hansen Weatherport, Delta, Colorado, USA) to minimize handling, thermal, and oxygen stress. We collected 35 replicate salamander excretion measurements by placing individuals in 1 L of filtered pond water. Individual salamander dry mass was estimated using wet mass and a conversion factor of 0.233 (Hairston and Hairston, 1987).

To estimate taxon-specific excretion, we measured differences in ammonium-nitrogen ( $\text{NH}_4^{+}\text{-N}$ ) and soluble reactive phosphorus (SRP) in filtered water collected before and after

incubation (0.5 – 1.5 hr). All water samples were collected and filtered using a syringe and inline filter holder containing a 25 mm Gelman AE glass fiber filter, and kept cool in the field then stored in a refrigerator until analysis the following morning. For all excretion and uptake measurements, we analyzed  $\text{NH}_4^+$ -N following a standard fluorometric method on a Turner Designs Trilogy Fluorometer Model # 7200 (Sunnyvale, CA, USA; Taylor and others 2007). SRP samples were analyzed following standard methods on a Thermo Scientific GENESYS 10S UV-VIS Spectrophotometer (Waltham MA, USA; Ostrofsky and Rigler 1987).

We estimated pond-level nutrient demand by collecting nutrient uptake measurements from two compartments within the ponds. The first compartment included demand by the water column and benthos. Following a protocol adapted from Stanley and Ward (1997), paired open-bottom clear plastic cylinders (12.5 cm diameter, 1 mm wall thickness) were gently pressed into the benthos in three to four locations without sedge around each pond. Cylinder water depth was measured to calculate volume and estimate the nutrient addition required to increase concentration 7-10x above ambient by pipetting small volumes (200  $\mu\text{L}$  to 3 mL) of 100 mg/L  $\text{NH}_4$ -N and  $\text{PO}_4$ -P solutions made from  $\text{NH}_4\text{Cl}$  and  $\text{Na}_2\text{HPO}_4$ . Immediately after adding  $\text{NH}_4$  or  $\text{PO}_4$ , a meter stick was used to gently mix the added nutrients throughout the cylinder water column without disturbing the benthos. Samples were collected following 0, 15, 30, 60, and 120 minutes. Uptake rates were estimated by fitting exponential decay models to the decline in nutrient concentration over time. Slopes were expressed as  $\mu\text{g N or P m}^{-2} \text{ d}^{-1}$  in statistical analyses to test for differences among depths and hydroperiod classifications prior to use in the simulation model.

The second compartment included nutrient demand by microbial biofilms growing on submerged sedge (*Carex aquatilis*) in the littoral zone. Small amounts of submerged, senesced

sedge (<10 g) were transferred to plastic bags, and water samples were collected prior to, immediately after, and one hour after adding nutrients to increase concentrations 7-10x above ambient. Uptake was calculated as the decline in concentration during incubation divided by sedge mass ( $\mu\text{g N or P g sedge}^{-1} \text{ d}^{-1}$ ).

Physical, chemical, and biological uptake mechanisms have a nonlinear asymptotic response to elevated nutrient concentrations, and therefore this single-addition method may underestimate uptake (O'Brien and Dodds, 2007). To address this concern, we also collected uptake measurements in both compartments using higher additions (20-50x ambient) and found no difference in uptake (Water Column P:  $F_{1,42} = 0.018$ ,  $p = 0.895$ ; Water Column N:  $F_{1,42} = 0.423$ ,  $p = 0.519$ ; Sedge P:  $F_{1,10} = 1.4455$ ,  $p = 0.257$ ; Sedge N:  $F_{1,10} = 0.390$ ,  $p = 0.547$ ), suggesting that uptake kinetics were saturated at our lower nutrient additions. Therefore, these uptake rates are likely conservative estimates of demand.

### *Pond Parameters*

Characteristics of four ponds in each of the three hydroperiod classifications were used to parameterize hydroperiod-specific normal distributions of hydroperiod duration, pond area, habitable benthic area, sedge coverage, and sedge biomass for random sampling in the simulation model (Table 3.1). Hydroperiods were recorded with WT-HR Mark 3 data loggers (TrueTrack). The twelve ponds were mapped using Trimble GeoXT GPS units (< 50 cm accuracy) and areas were calculated using ESRI Arcmap 10 (DeVecchia et al., 2019). Most benthic invertebrates congregate within approximately 3 m of interior pond edges at the Mexican Cut, which are shallow habitats often characterized by live emergent sedge, senesced sedge detritus, and high light availability. This nearshore microhabitat area was quantified by creating

3 m inverse buffers within each pond (Figure 3.1A). Since pond shape varies, the inverse buffer areas were used to calculate habitable benthic area as a proportion of total area (Figure 3.1B). Finally, we combined measurements of sedge areal coverage and biomass to account for hydroperiod-imposed differences in pond-level sedge biomass. First, approximate total area of sedge within each pond was calculated by measuring area of 3-20 sedge patches (e.g., 1 m x 3 m) around pond perimeters, with the number of estimates depending on pond size and sedge contiguousness. Next, senesced sedge was clipped from 3-5 randomly selected 0.01 m<sup>2</sup> areas in each pond and massed separately after drying for 48 hrs at 60 °C to estimate senesced sedge biomass per m<sup>2</sup>.

#### *Simulation model framework for estimating community-wide excretion and demand*

We estimated average community-wide animal-driven nutrient supply and pond-level nutrient demand in permanent, semi-permanent, and temporary ponds using a simulation model (Figure 3.1). Each iteration simulates one pond in each of the three hydroperiod classifications, providing N and P supply estimates for all taxa in the community (Figure 3.1C), along with estimates of pond-level nutrient demand from the benthos and water column (Figure 3.1D) as well as from sedge biofilms (Figure 3.1E). The simulation operates by drawing random samples from hydroperiod-specific distributions parameterized by our physical pond parameter, animal survey, nutrient excretion and uptake rate datasets.

First, pond parameter distributions (Figure 3.1B, Table 3.1) were randomly sampled to simulate one pond of each hydroperiod. In summers when semi-permanent ponds do not dry, their hydroperiods are determined by spring melt and winter freeze, resulting in the same annual hydroperiod as permanent ponds. Thus, if the randomly sampled semi-permanent hydroperiod

was longer than that of the permanent pond, which was possible due to overlapping hydroperiod distributions, the permanent pond's hydroperiod was used for both hydroperiod classifications during that iteration.

Next, a set of taxon-specific parameters were randomly sampled for each taxon (Figure 3.1C). Each taxon's densities were drawn from unique distributions for each hydroperiod classification. These distributions were prepared by averaging invertebrate densities across the four census samples by pond, then averaging by hydroperiod to generate  $\lambda$  values (of hydroperiod-specific average density) that parameterize hydroperiod-specific Poisson distributions of invertebrate densities. In contrast, each taxon's mass-specific excretion rates were drawn from the same taxon-specific distributions for all three hydroperiods. Although excretion could vary among ponds and over time due to differences in diet or temperature, we collected replicate excretion measurements opportunistically from multiple ponds and often on different days. Furthermore, the trichopteran taxa have consistent excretion among ponds with differing hydroperiods from ~ 2900 – 3500 m elevation across the season (Balik et al., 2018). Thus, we assume that the taxon-specific variation we measured is representative across hydroperiods with potentially variable food resources over the season. We measured N and P excretion of 34 of 40 invertebrate taxa which comprise 98.7 % of invertebrate biomass across the three hydroperiods. The six taxa for which we did not measure excretion were omitted from the simulation. If a negative excretion value was randomly sampled, which is possible for some taxa with low mean excretion rates with high variance, their excretion was set to zero for that iteration. Taxon-specific distributions of the percent of hydroperiod spent in a pond represent how long each taxon spends in a permanent or semi-permanent pond relative to the total hydroperiod (Appendix 3: Table S1; mean values range from 35 to 100 %). These distributions

were generated from personal observations (J.A. Balik and B.W. Taylor) and literature searches (Dodson, 1975; Wissinger et al., 1999a; Babler et al., 2008). For temporary ponds, percent of hydroperiod spent in pond was always set to 100 because hydroperiod directly determines time spent in the pond for all taxa, as organisms generally emerge near pond drying or do not survive (Greig and Wissinger, 2010).

Each taxon's life history (e.g., benthic vs water column) and the pond hydroperiod classification determined how their supply contribution was calculated. For benthic invertebrates, which congregate in the ~3 m wide nearshore area surrounding the interior pond perimeter (Figure 3.1A), supply was calculated as:

$$\begin{aligned} & \text{Benthic invertebrate taxon} - \text{specific supply contribution} \left[ \frac{\text{ug N or P}}{\text{Pond} * \text{Summer}} \right] = \\ & \text{pond area} \left[ \frac{\text{m}^2}{\text{Pond}} \right] * \% \text{ benthic area} * \text{density} \left[ \frac{\text{individuals}}{\text{m}^2} \right] * \text{mean mass} \left[ \frac{\text{mg animal}}{\text{individual}} \right] \\ & * \text{hydroperiod} \left[ \frac{\text{d}}{\text{Summer}} \right] * \% \text{ hydroperiod spent in pond} * \text{excretion rate} \left[ \frac{\text{ug N or P}}{\text{mg animal} * \text{d}} \right] \end{aligned}$$

Here, densities are converted to biomass using taxon-specific mean mass constants previously used to estimate whole-pond biomass (Wissinger et al., 1999a).

Next, for chironomids and oligochaetes which are distributed throughout the pond basin, except for the deep center areas of permanent ponds (Figure 3.1A; Babler and others 2008), supply contributions were calculated as:

$$\begin{aligned} & \text{Chironomid and oligochaete supply contributions in permanent ponds} \left[ \frac{\text{ug N or P}}{\text{Pond} * \text{Summer}} \right] = \\ & \left( \text{pond area} \left[ \frac{\text{m}^2}{\text{Pond}} \right] * (1 - \% \text{ deep center}) \right) * \text{density} \left[ \frac{\text{individuals}}{\text{m}^2} \right] * \text{mean mass} \left[ \frac{\text{mg animal}}{\text{individual}} \right] \\ & * \text{hydroperiod} \left[ \frac{\text{d}}{\text{Summer}} \right] * \% \text{ hydroperiod spent in pond} * \text{excretion rate} \left[ \frac{\text{ug N or P}}{\text{mg animal} * \text{d}} \right] \end{aligned}$$

Where “% deep center” is a random sample from a distribution of percent permanent pond area deeper than 1.5 m generated from depth measurements (DelVecchia et al., 2019). Because semi-permanent and temporary ponds do not have center areas deeper than 1.5 m, chironomid and

oligochaete supply contributions in these hydroperiods were calculated in the same manner as for zooplankton and salamanders, which are evenly distributed throughout all ponds:

$$\begin{aligned} & \text{Chironomid and oligochete supply contributions in semipermanent and temporary ponds,} \\ & \text{and of zooplankton and salamanders in all hydroperiods} \left[ \frac{\text{ug N or P}}{\text{Pond} * \text{Summer}} \right] = \\ & \text{pond area} \left[ \frac{\text{m}^2}{\text{Pond}} \right] * \text{density} \left[ \frac{\text{individuals}}{\text{m}^2} \right] * \text{mean mass} \left[ \frac{\text{mg animal}}{\text{individual}} \right] \\ & * \text{hydroperiod} \left[ \frac{\text{d}}{\text{Summer}} \right] * \% \text{ hydroperiod spent in pond} * \text{excretion rate} \left[ \frac{\text{ug N or P}}{\text{mg animal} * \text{d}} \right] \end{aligned}$$

Finally, community-wide animal-driven supply was the sum of all taxon-specific contributions.

To estimate nutrient demand, random samples were drawn from normal distributions generated from our uptake rate measurements in the benthos plus water column (Figure 3.1D) and sedge compartments (Figure 3.1E). Uptake rates did not differ across the season (Benthos plus water column P:  $F_{1,42} = 0.45$ ,  $p = 0.51$ ; Benthos plus water column N:  $F_{1,43} = 0.02$ ,  $p = 0.89$ ; Sedge P:  $F_{1,10} = 1.13$ ,  $p = 0.31$ ; Sedge N uptake:  $F_{1,10} = 0.02$ ,  $p = 0.90$ ) or among depths (Benthos plus water column P:  $F_{1,42} < 0.01$ ,  $p = 0.99$ ; Benthos plus water column N:  $F_{1,43} = 1.99$ ,  $p = 0.17$ ), so measurements were pooled. However, benthos plus water column P uptake rates were 52 % slower in temporary ponds than in semi-permanent and permanent ponds ( $F_{2,5} = 7.04$ ,  $p = 0.04$ ), so temporary ponds used a unique distribution whereas semi-permanent and permanent ponds were pooled (Figure 3.1D). There were no differences among hydroperiods in benthos plus water column N, sedge N, and sedge P uptake rates, so all three hydroperiods drew from the same distributions (Water column N:  $F_{2,5} = 0.02$ ,  $p = 0.98$ ; Sedge N:  $F_{2,2} = 0.03$ ,  $p = 0.79$ ; Sedge P:  $F_{2,2} = 0.14$ ,  $p = 0.88$ ; Figure 3.1D, E). Benthos plus water column demand was estimated as:

$$\begin{aligned} & \text{Benthos plus water column demand} \left[ \frac{\text{ug N or P}}{\text{Pond} * \text{Summer}} \right] = \\ & \text{benthos plus water column uptake rate} \left[ \frac{\text{ug N or P}}{\text{m}^2 * \text{d}} \right] * \text{hydroperiod} \left[ \frac{\text{d}}{\text{Summer}} \right] * \text{pond area} \left[ \frac{\text{m}^2}{\text{Pond}} \right] \end{aligned}$$

Whereas, sedge biofilm demand was estimated as:

$$\text{Sedge demand} \left[ \frac{\text{ug N or P}}{\text{Pond} * \text{Summer}} \right] = \text{sedge uptake rate} \left[ \frac{\text{ug N or P}}{\text{g sedge} * \text{d}} \right] * \text{hydroperiod} \left[ \frac{\text{d}}{\text{Summer}} \right]$$

$$* \text{pond area} \left[ \frac{\text{m}^2}{\text{Pond}} \right] * \% \text{ coverage} * \text{sedge biomass} \left[ \frac{\text{g}}{\text{m}^2} \right]$$

The supply and demand estimates presented here were based on 100,000 iterations. Mean pond-level supply and demand estimates did not increase between 10,000 and 100,000 iterations, suggesting they had reached an asymptote (P flux means:  $t_{11} = -1.84$ ,  $p = 0.09$ ; N flux means:  $t_{11} = -1.49$ ,  $p = 0.17$ ). Supply and demand estimates are presented in two different units (predictions 1-3; Figure 3.2). First, areal daily estimates ( $\text{mg N or P m}^{-2} \text{ d}^{-1}$ ) standardize fluxes by pond area and hydroperiod duration (Figure 3.2A, B). Second, whole-pond seasonal estimates ( $\text{kg N or P pond}^{-1} \text{ season}^{-1}$ ) are conservative estimates of total fluxes that incorporate differences among hydroperiods in pond area and hydroperiod duration (Table 3.1; Figure 3.2C, D). Although nutrient turnover rates in each compartment could provide further context to our estimates, we do not have the nutrient storage data required to estimate mass balance. Because whole-pond seasonal estimates represent among-hydroperiod variation in a given taxon's contribution to supply, they were used to test drivers of taxon-specific contributions (prediction 4), and to compare taxon-specific contributions among taxa (prediction 5) and distributions of rank-order taxon-specific supply estimates among hydroperiods (prediction 6).

Although the simulation model is conceptually similar to a bootstrap, we sampled hydroperiod-specific distributions generated from our field data rather than resampling our pond-level datasets for three reasons. First, within a hydroperiod classification there is substantial variation in pond area and sedge coverage (Table 3.1). Simulating many ponds across the observed range of physical pond parameter distributions better characterized average supply and demand within each hydroperiod. Second, long-term invertebrate surveys demonstrate that dominant taxa are interannually consistent (e.g., chironomids, oligochaetes, larval caddisflies), but interannual variation in abundance of other taxa can be high (Wissinger et al., 2016). Thus,

the simulation model leverages variation in abundance among multiple ponds within each hydroperiod classification to predict nutrient supply of an “average” community in each hydroperiod. Third, invertebrate communities were surveyed for a subset of the ponds with physical datasets (10 of 12 ponds in Table 3.1). Only the overlapping subsets of animal and physical datasets could be resampled in a true bootstrap, whereas the simulation used the entirety of both datasets to estimate average supply and demand for each hydroperiod. Finally, to support inferences drawn from the simulation, the same calculations described above were used to estimate empirical supply and demand for each survey pond in areal daily units.

### *Statistical analyses*

The simulation model and all statistical analyses were performed in R 4.0.2 (R Core Team 2018). To guide preparation of water column and sedge uptake rate distributions for the simulation model, mixed models were used to test for differences in uptake among sample dates, depths, hydroperiod classifications, and nutrient spike volumes as fixed effects, and pond as a random effect (Pinhero et al., 2019). Empirical estimates of supply relative to demand were compared among hydroperiods with ANOVA and post-hoc Tukey’s HSD. Simulation model supply estimates were used in linear models to evaluate drivers of taxon-specific whole-pond seasonal supply across hydroperiods (Prediction 4) by comparing AIC scores among reduced models (Akaike 1998). Distributions of taxon-specific supply estimates from the simulation model were compared among hydroperiods (Prediction 6) with a Kolmogorov–Smirnov (KS) test, and multiple KS test p-values were Bonferroni adjusted. Lastly, to assess differences in how evenly supply was distributed among taxa, skewness of mean rank-order taxon-specific supply distributions were calculated for each hydroperiod (Meyer et al., 2019).

## Results

### *Nutrient supply and demand across a hydroperiod gradient*

Empirical estimates of supply relative to demand differed among hydroperiods (Table 3.2; N:  $F_{2,7} = 11.98$ ,  $p = 0.006$ ; P:  $F_{2,7} = 11.23$ ,  $p = 0.007$ ). Empirical estimates of N supply relative to demand did not differ between permanent and semi-permanent hydroperiods and averaged 188 % of demand, but N supply in temporary hydroperiods only met 18 % of demand ( $HSD < 0.05$ ). Likewise, empirical estimates of P supply equaled demand in permanent and semi-permanent hydroperiods, but only met 15 % of demand in temporary hydroperiods ( $HSD < 0.05$ ). Molar N:P of empirical supply and demand estimates did not differ among hydroperiods (Supply:  $F_{2,7} = 0.07$ ,  $p = 0.928$ ; Demand:  $F_{2,7} = 0.31$ ,  $p = 0.740$ ), and supply N:P was 187 % of demand N:P.

Mean areal daily estimates from the simulation model demonstrate that empirical patterns along the hydroperiod gradient prevail after considering each hydroperiod's various possible combinations and permutations of pond area, hydroperiod duration, and animal communities (Figure 3.2A, B). The simulation model's mean estimate of areal daily N supply was 221 % of demand in permanent and semi-permanent hydroperiods, but only 9 % of demand in temporary hydroperiods. Areal daily P supply was 101 % and 86 % of demand in permanent and semi-permanent hydroperiods but only 8 % of demand in temporary hydroperiods. Thus, when fluxes are standardized for pond area and hydroperiod duration, N and P supplies meet comparable proportions of their respective demand in permanent and semi-permanent ponds, whereas in temporary ponds both nutrients meet much lower proportions of demand. In turn, molar N:P of areal daily supply was 233 % of demand N:P in permanent and semi-permanent hydroperiods, but only 112 % of demand N:P in temporary (Table 3.2).

The simulation model's mean estimates of whole-pond seasonal N and P supply followed similar patterns after accounting for pond area and hydroperiod duration (Figure 3.2 C, D). Whole-pond seasonal N supply estimates were 203 % of demand in permanent and semi-permanent ponds and 25 % of demand in temporary ponds. Whole-pond seasonal P supply estimates were 85 %, 78 %, and 22 % of demand in permanent, semi-permanent, and temporary hydroperiods, respectively. Thus, whole-pond seasonal N and P supply and demand estimates decreased along the hydroperiod gradient from permanent to temporary.

#### *Predicting taxon-specific nutrient supply estimates across hydroperiod classifications*

Along the hydroperiod gradient from temporary to permanent, the variation in taxon-specific contributions to supply ranged from one to five orders of magnitude (Figures 3.3A, 3.4A, 3.5A). Taxonomic identity, population biomass, mass-specific excretion rate, and larval development length were all included in linear models of taxon-specific N and P supply among the three hydroperiod classifications (Appendix 3: Table S2). Relative to the selected models, reduced models with population biomass removed had the largest increases in AIC, indicating biomass was the strongest predictor of taxon-specific supply contributions among hydroperiods (Appendix 3: Table S3).

#### *Distributions of taxon-specific nutrient supply along a hydroperiod gradient*

The distributions of rank-order taxon-specific supply within each hydroperiod revealed the extent to which functionally dominant taxa drive community-wide supply (Figure 3.3A, 3.4A, 3.5A). Distributions of rank-order taxon-specific N supply differ between permanent and semi-permanent hydroperiods (KS Distance = 0.38,  $p = 0.043$ ) and between semi-permanent and

temporary (KS Distance = 0.52,  $p = 0.039$ ). Distributions of rank-order taxon-specific P supply did not differ between permanent and semi-permanent hydroperiods (KS Distance = 0.31,  $p = 0.191$ ) or between semi-permanent and temporary (KS Distance = 0.37,  $p = 0.349$ ). However, distributions for rank-order taxon-specific N and P supply both differ between permanent and temporary hydroperiods (N: KS Distance = 0.74,  $p < 0.001$ ; P: KS Distance = 0.62,  $p < 0.001$ ). N and P supply were more evenly distributed among taxa in temporary (Skewness: N = 0.437, P = 1.659) than in permanent (Skewness: N = 3.24, P = 4.40) or semi-permanent hydroperiods (Skewness: N = 3.39, P = 2.827).

## Discussion

### *Community-wide nutrient supply along a hydroperiod gradient*

Here, we show that ponds with longer hydroperiods (i.e. greater water availability) have larger animal-driven nutrient supply and greater demand. Specifically, whole-pond seasonal estimates demonstrate that cycling rates increase with pond size and hydroperiod duration; permanent pond cycling rates were 5-10x greater than those of semi-permanent and temporary ponds. In turn, areal daily estimates demonstrate that the N surpluses and P supply relative to demand were similar between permanent and semi-permanent ponds. However, temporary ponds had large supply deficits for both nutrients. These differences in supply relative to demand among hydroperiods (permanent and semipermanent vs temporary) are consistent with the absence of a dominant N and P supplier (oligochaetes) and a large reduction in the biomass of another (chironomids) in temporary ponds, rather than temporary pond taxa having overall lower excretion rates.

Population-level biomass predicted supply across hydroperiod classifications, but within

a hydroperiod, variation in excretion rates and time spent in the pond mediated the role of animal biomass in driving supply, and consequently taxonomic identity or functional traits improved supply predictions. For example, although salamanders had similar biomass to the invertebrate community in permanent ponds, they also had much lower excretion. Consequently, invertebrates supplied approximately  $157 \times$  more P and  $74 \times$  more N than salamanders. Finally, taxon-specific supply was distributed more evenly in temporary ponds, confirming that supply was dominated by a few key taxa in permanent ponds (oligochaetes and chironomids). This suggests that unexpected species losses are more likely to have a large impact on supply in temporary ponds if other taxa do not replace lost biomass. While as permanent hydroperiods become increasingly temporary due to climate driven declines in water availability (Lund and others, 2016), our estimates suggest that animal-driven supply will decrease relative to demand. Consequently, ponds with formerly permanent or semipermanent hydroperiods and surplus or adequate nutrient supplies will become increasingly nutrient limited.

*Biomass predicts supply across systems, and traits provide informative context within a system*

Consistent with our first and fourth predictions, population biomass was the best predictor of a taxon's contribution to community-wide supply. Consequently, Ssupply increased along the hydroperiod gradient from temporary to permanent as community biomass increased. There is ample precedent that animal biomass is a powerful predictor of supply in lakes (Carpenter et al., 1985; Schindler and Eby, 1997), streams (McIntyre et al., 2008; Benstead et al., 2010), and marine systems (Allgeier et al., 2017). Furthermore, because hydroperiod is functionally analogous to the amount or duration of water availability in terrestrial systems (Western, 1975; Newman et al., 2006), water availability could provide a generalizable

framework for predicting biomass-driven animal effects on nutrient cycling and identifying systems where climate-induced changes in water availability could impact nutrient cycles.

Within a given ecosystem (here, each hydroperiod classification), however, variation in functional traits such as mass-specific excretion rate can mediate the role of biomass as a driver of a taxon's contribution to supply. For example, a taxon with high biomass and slow excretion can contribute little to supply (e.g., Figure 3.4; paedomorphic and 3+ salamanders, *A. nigrificulus*, *L. externus*), or a taxon with low biomass and fast excretion can make a large contribution (e.g., Figure 3.5; zooplankton). This interaction explains a result that conflicts with our fifth prediction regarding the importance of salamanders in permanent pond nutrient cycling. We expected that salamanders would dominate permanent pond supply because of their high biomass, which on average is half of the entire invertebrate community (mean = 2.1 kg salamanders vs mean = 4.2 kg invertebrates). However, estimates of salamander N and P supplies were <1.5% of invertebrate supply. This is surprising because vertebrates are often assumed to contribute large fluxes due to their biomass dominance and because they tend to have higher mass-specific excretion relative to invertebrates (Vanni and McIntyre, 2016). Although vertebrate and invertebrate supply contributions are not often directly compared (Carpenter et al., 1992; Attayde and Hansson, 1999; Devine and Vanni, 2002), invertebrates can make larger contributions (Atkinson et al., 2019). Here, salamanders provide an example of how the interaction between high population biomass and low excretion produces a comparatively low supply contribution that would be greatly overestimated from biomass alone.

The taxon-specific nutrient supply contributions made by other biomass-dominant taxa provide further insight to the importance of the interaction between biomass and excretion. In permanent ponds, biomass-dominant chironomids supply 35 % of community-wide N and 55 %

of P supply. This adheres to the mass-ratio hypothesis from the plant productivity literature, which predicts that overall ecosystem processing or functioning is determined by a dominant taxon's functional trait (Grime, 1998). However, this is not always the case, as demonstrated by taxon-specific supply estimates in temporary ponds. Here, biomass dominant *A. nigriculus* contributes only 2 % and 10 % of community-wide N and P supply. In contrast, zooplankton, which have 57-fold less biomass than *A. nigriculus* in temporary ponds, contribute 20 % and 43 % of N and P supply owing to their comparatively higher excretion. Thus, predictions generated using the mass-ratio hypothesis are often useful, however, variation in a key functional trait (here, nutrient excretion) among taxa can ameliorate the importance of biomass dominance. Furthermore, among communities with low richness (e.g. temporary ponds with one-third as many taxa), dissimilarity in a key functional trait is likely more informative than species number for predicting each community's functional contributions (Heemsbergen et al., 2004).

In addition to functional traits like nutrient excretion, taxonomic identity and natural history provide valuable context for interpreting estimated contributions. For example, oligochaetes and chironomids drive large proportions of N supply in permanent and semi-permanent hydroperiods (35 % and 23 %). These taxa live in pond sediments, where their excreta likely enrich sediments and are subsequently buried, bound, or gradually released (Devine and Vanni, 2002; Hölker et al., 2015; Herren et al., 2017). This may reconcile the estimated N surplus with ambient water chemistry, as ponds in and near the Mexican Cut are considered oligotrophic (Wissinger et al., 1999a; Elser et al., 2009) with water column  $\text{NH}_4\text{-N}$  averaging 2.4  $\mu\text{g/L}$  and  $\text{NO}_3\text{-N}$  averaging 67.8  $\mu\text{g/L}$  over the summer. Likewise, chironomid and oligochaetes cumulatively contribute 62 % and 47 % of P supply in permanent and semi-permanent ponds. This large proportion of P supply entering the system slowly via bioturbation-

driven sediment release (Hölker et al., 2015) or binding to iron near the sediment-water interface under well-mixed aerobic conditions (B.W. Taylor unpub.) is also consistent with low water column SRP concentrations averaging 2.2 ug/L over the summer, and could contribute to P limitation previously measured in and around the Mexican Cut (Elser et al., 2009). Indeed, a greater relative demand for P was apparent in both empirical and simulation estimates, as molar N:P of community-wide supply was greater than demand N:P in all hydroperiod classifications.

Differences in chironomid and oligochaete abundance among hydroperiod classifications also explain the transition from N surplus in permanent and semi-permanent hydroperiods to a deficit in temporary hydroperiods. First, oligochaetes supplied the most N in permanent and semi-permanent ponds but are not present in temporary ponds. Second, chironomids in temporary ponds only attain 0.7 % and 5 % of their biomass in permanent and semi-permanent ponds, limiting their ability to contribute large amounts of nutrients. Although the ability of animal communities to contribute to nutrient cycling is well-recognized (Vanni, 2002), there are few examples of systems where one taxa dominates supply (but see Hall and others 2003, McIntyre and others 2007, Small and others 2011). Together with previous work quantifying their nutrient fluxes (Tatrai, 1986; Devine and Vanni, 2002; Hölker et al., 2015), this switch from N surplus to deficit along the hydroperiod gradient suggests that oligochaetes and chironomids could fulfill essential roles in driving ecosystem-level N supply.

#### *Climate change and animal driven nutrient supply in high elevation ponds*

Climate warming and associated changes in precipitation are causing hydroperiods of high-elevation wetlands to become increasingly temporary (Lee et al., 2015; Lund et al., 2016). Here, as ponds shift from permanent to temporary, they shift from an N supply surplus to a

deficit, and from sufficient P supply to a deficit. This occurs because hydroperiod directly controls community composition. Consistent with increasing species evenness from permanent to temporary ponds (Wissinger et al., 1999a), N and P supply are dominated by few taxa in permanent and semi-permanent ponds and are distributed more evenly in temporary ponds. Thus, if compositional changes result in reduced abundance of dominant taxa there could be large effects on community-wide supply as ponds transition towards temporary. Additionally, even though salamanders directly contribute very little to supply, their role in structuring the invertebrate community in permanent ponds (Wissinger et al., 1999a) is similar to that of fish in mountain lakes (Schindler et al., 2001), and could have large indirect effects on animal-driven supply that could be lost when permanent ponds begin to dry.

Here, our estimates suggest that as climate warming reduces water availability and causes biomass declines and compositional shifts in animal communities, nutrient limitation may arise due to loss of animal-driven supply. Furthermore, the framework of water availability determining animal biomass distribution and contributions to nutrient cycles across the landscape could be generalizable to other systems, allowing us to predict how climate-driven shifts in water availability could alter nutrient cycles. For example, droughts may reduce ungulate contributions to grassland nutrient cycles by reducing populations and altering dispersal (Augustine and McNaughton, 2007). These effects could even cross ecosystem boundaries, as wildebeest mass drownings at river crossings provide substantial energetic and nutrient subsidies to aquatic systems but do not commonly occur during low water (Subalusky et al., 2017). Likewise, droughts could disrupt nutrients transported by migratory taxa such as salmon or waterfowl (Greer et al., 2007; Isaak et al., 2007).

Furthermore, climate-driven changes in water availability are already commonly linked

to other ecosystem functions, particularly primary productivity. For example, changing water availability will impact primary productivity in agricultural systems, forests, and grasslands (Fay et al., 2008; Rosenzweig et al., 2014). In many of these studies, potential for consequent changes in energy and material flow are acknowledged but not quantified. Identifying systems where water availability is linked to animal-driven nutrient cycling could provide additional rationale for protecting species or water availability in systems threatened by climate change. Thus, there is precedent for using water availability to predict primary productivity and associated functions, but the potential of this currency for anticipating changes in animal driven N and P cycling remains underutilized.

## **Acknowledgments**

We thank the Rocky Mountain Biological Laboratory for logistic support and The Nature Conservancy for access to the Mexican Cut Nature Preserve. We thank Austyn Long for assistance sampling sedge, and Kevin Gross for helpful discussions on the simulation. Comments from Chelsea Little, Bobbi Peckarsky, and four anonymous reviewers greatly improved the manuscript. Balik was supported by an NSF Graduate Research Fellowship, a Southeast Climate Adaptation Science Center Global Change Fellowship, a Colorado Mountain Club Foundation Al Ossinger Fellowship, and a RMBL Graduate Fellowship from the Snyder Endowment. Wissinger was funded by NSF DEB 1557015. Whiteman was funded by NSF DEB 1354787. Jameson was funded by NSF REU supplement 1819108 to Taylor. Additional funding was provided by NSF DEB 1556914, REU supplement 1721812, and North Carolina State University.

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**Table 3.1:** Summaries of hydroperiod-specific parameters used in the simulation model.

Distributions are parameterized as normal distributions with (mean  $\pm$  1 standard deviation).

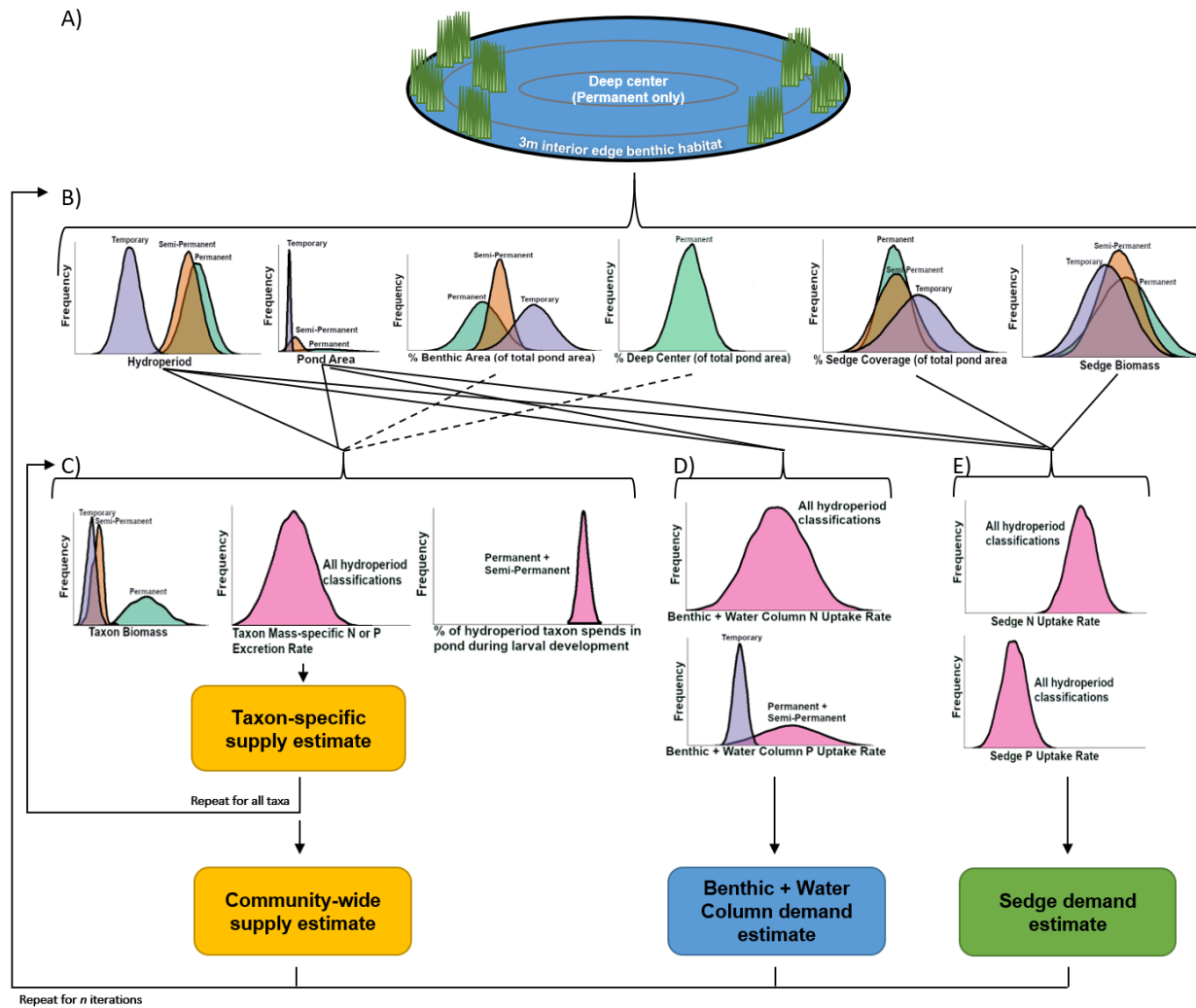
Permanent hydroperiod distributions were generated from ponds 1, 3, 5, and 12 at the Mexican Cut Preserve, Semi-permanent from ponds 6, 8, 10, and 44, and temporary from ponds 13, 15, 22, and 42.

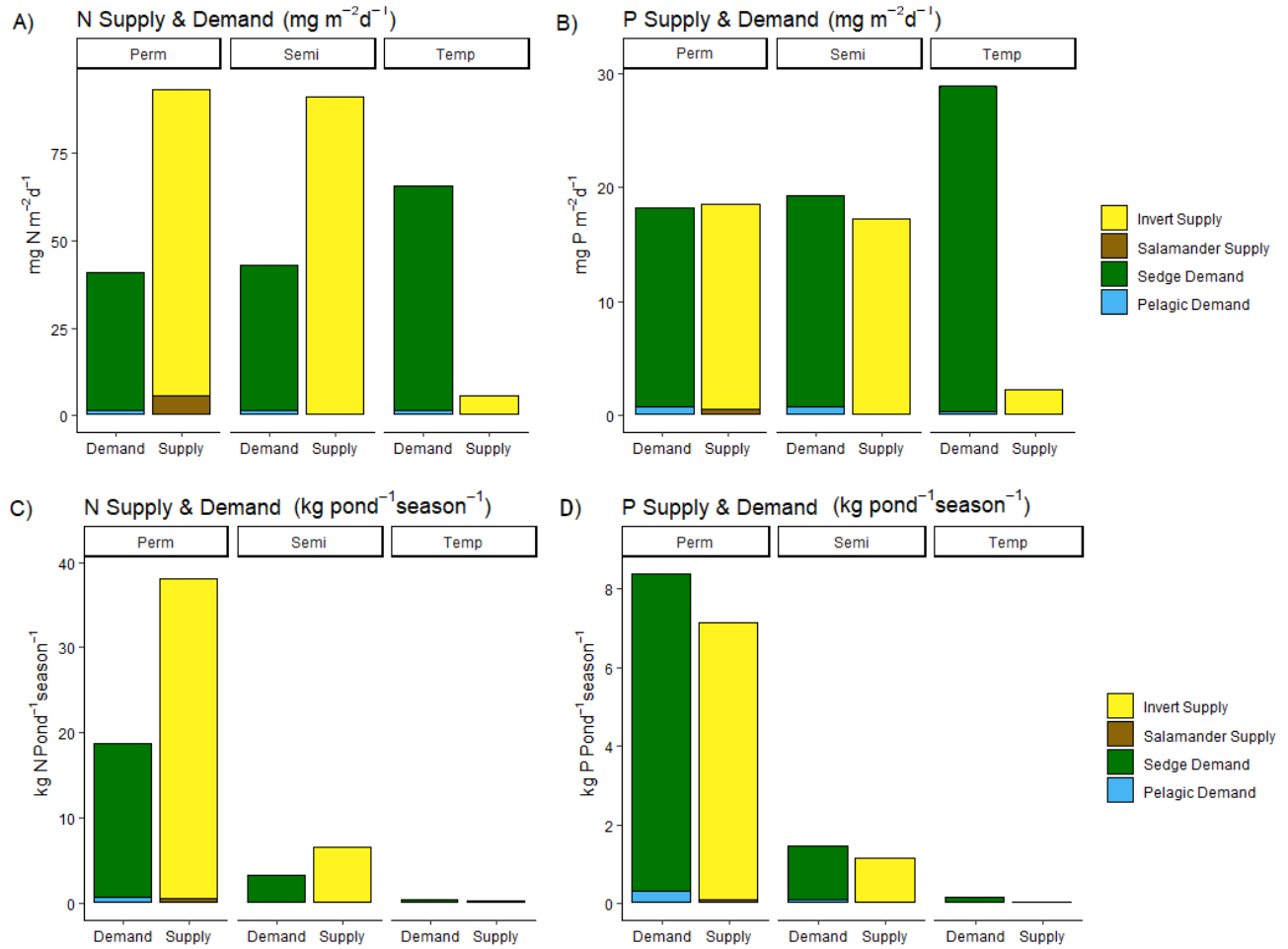
| <b>Hydroperiod</b> | <b>Hydroperiod<br/>(d)</b> | <b>Pond Area<br/>(m<sup>2</sup>)</b> | <b>Benthic Area<br/>(% total area)</b> | <b>Sedge Coverage<br/>(% total area)</b> | <b>Sedge Biomass<br/>(g/m<sup>2</sup>)</b> |
|--------------------|----------------------------|--------------------------------------|----------------------------------------|------------------------------------------|--------------------------------------------|
| Permanent          | (165 $\pm$ 22)             | (2147 $\pm$ 2167)                    | (0.28 $\pm$ 0.17)                      | (0.12 $\pm$ 0.14)                        | (167 $\pm$ 99)                             |
| Semi-Permanent     | (150 $\pm$ 20)             | (455 $\pm$ 360)                      | (0.44 $\pm$ 0.09)                      | (0.14 $\pm$ 0.19)                        | (146 $\pm$ 74)                             |
| Temporary          | (50 $\pm$ 19)              | (104 $\pm$ 83)                       | (0.76 $\pm$ 0.18)                      | (0.37 $\pm$ 0.26)                        | (100 $\pm$ 85)                             |

**Table 3.2:** Empirical pond supply and demand estimates in units of mg N or P m<sup>-2</sup> d<sup>-1</sup> and molar N:P ratios and for all ponds with 2018 invertebrate survey data. Estimates from the simulation model are also presented in the same units for comparison (Figure 3.2A, B).

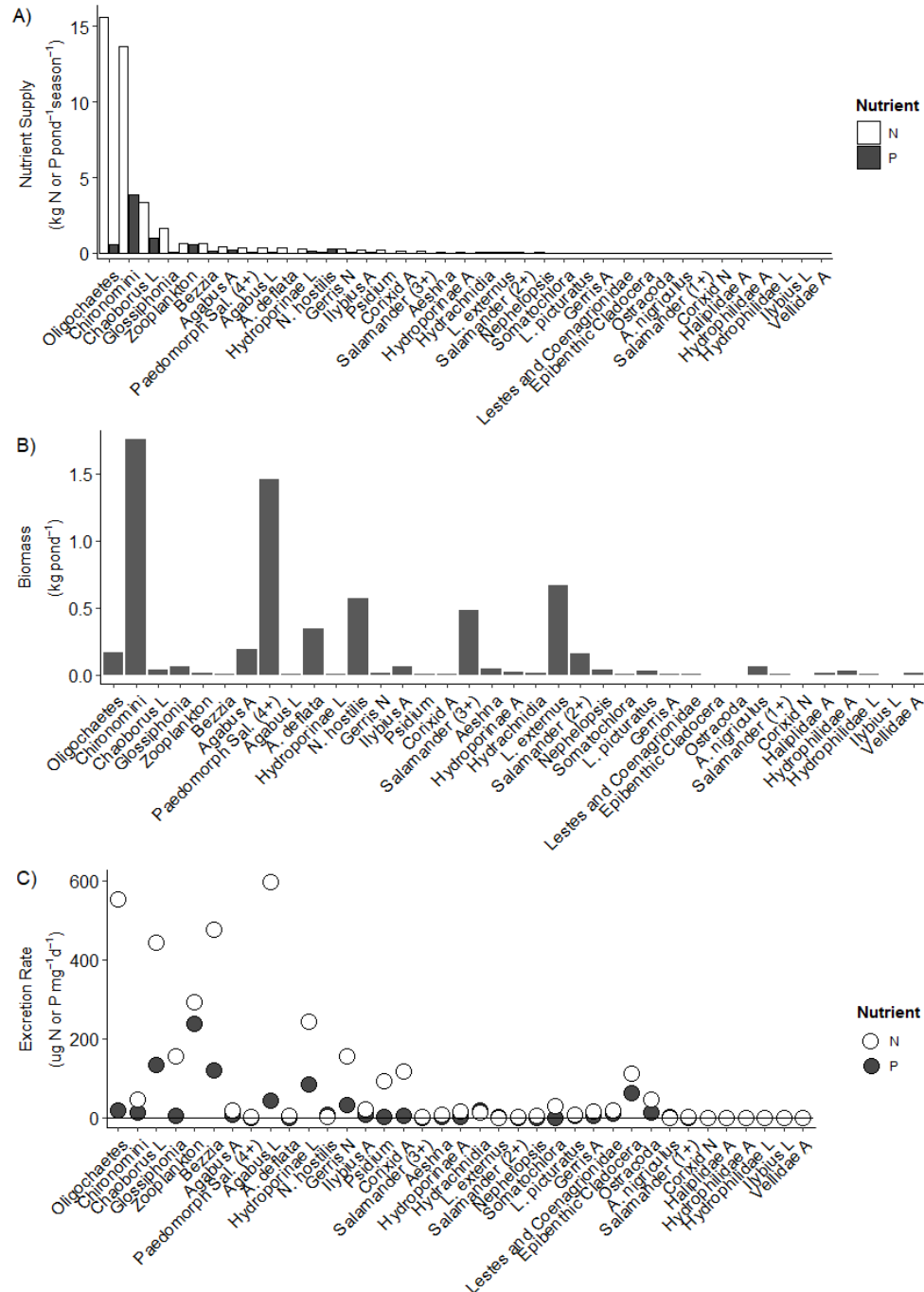
| Hydroperiod | Pond                          | N Supply<br>(mg N m <sup>-2</sup> d <sup>-1</sup> ) | N Demand<br>(mg N m <sup>-2</sup> d <sup>-1</sup> ) | P Supply<br>(mg P m <sup>-2</sup> d <sup>-1</sup> ) | P Demand<br>(mg P m <sup>-2</sup> d <sup>-1</sup> ) | N:P Supply<br>(molar) | N:P Demand<br>(molar) |
|-------------|-------------------------------|-----------------------------------------------------|-----------------------------------------------------|-----------------------------------------------------|-----------------------------------------------------|-----------------------|-----------------------|
| Perm        | MC1                           | 11.60                                               | 6.12                                                | 2.23                                                | 2.89                                                | 20.85                 | 4.68                  |
|             | MC3                           | 69.12                                               | 38.78                                               | 34.21                                               | 30.91                                               | 4.47                  | 2.77                  |
|             | MC5                           | 65.68                                               | 33.81                                               | 9.80                                                | 15.20                                               | 14.82                 | 4.92                  |
|             | MC12                          | 17.70                                               | 20.07                                               | 7.95                                                | 9.02                                                | 6.58                  | 4.92                  |
|             | <b>Empirical Mean ± 1 SE</b>  | <b>41.02 ± 15.29</b>                                | <b>24.69 ± 7.35</b>                                 | <b>13.5 ± 7.35</b>                                  | <b>14.5 ± 6.02</b>                                  | <b>11.68 ± 3.79</b>   | <b>4.32 ± 0.52</b>    |
|             | <b>Simulation Mean ± 1 SE</b> | <b>99.59 ± 0.73</b>                                 | <b>41.99 ± 0.11</b>                                 | <b>19.09 ± 0.09</b>                                 | <b>18.86 ± 0.05</b>                                 | <b>11.54 ± 0.02</b>   | <b>4.92 ± 0.01</b>    |
| Semi        | MC6                           | 52.51                                               | 16.83                                               | 6.76                                                | 6.62                                                | 17.18                 | 5.62                  |
|             | MC8                           | 22.40                                               | 9.38                                                | 26.51                                               | 27.00                                               | 1.87                  | 0.77                  |
|             | MC10                          | 50.34                                               | 31.64                                               | 10.40                                               | 6.92                                                | 10.70                 | 10.11                 |
|             | <b>Empirical Mean ± 1 SE</b>  | <b>41.75 ± 8.4</b>                                  | <b>19.28 ± 5.67</b>                                 | <b>14.56 ± 5.25</b>                                 | <b>13.51 ± 5.84</b>                                 | <b>9.92 ± 4.44</b>    | <b>5.50 ± 2.70</b>    |
|             | <b>Simulation Mean ± 1 SE</b> | <b>91.06 ± 0.21</b>                                 | <b>44.34 ± 0.11</b>                                 | <b>17.22 ± 0.04</b>                                 | <b>19.92 ± 0.05</b>                                 | <b>11.69 ± 0.01</b>   | <b>5.11 ± 0.01</b>    |
| Temp        | MC13                          | 5.39                                                | 11.01                                               | 2.34                                                | 4.33                                                | 3.57                  | 5.62                  |
|             | MC15                          | 10.44                                               | 57.41                                               | 2.02                                                | 19.86                                               | 11.43                 | 6.39                  |
|             | MC22                          | 9.54                                                | 70.99                                               | 2.65                                                | 29.48                                               | 7.96                  | 5.32                  |
|             | <b>Empirical Mean ± 1 SE</b>  | <b>8.46 ± 1.35</b>                                  | <b>46.47 ± 15.73</b>                                | <b>2.67 ± 0.33</b>                                  | <b>17.89 ± 6.34</b>                                 | <b>7.65 ± 2.27</b>    | <b>5.78 ± 0.32</b>    |
|             | <b>Simulation Mean ± 1 SE</b> | <b>5.8 ± 0.02</b>                                   | <b>66.78 ± 0.18</b>                                 | <b>2.27 ± 0.01</b>                                  | <b>29.2 ± 0.08</b>                                  | <b>5.65 ± 0.00</b>    | <b>5.06 ± 0.01</b>    |

**Figure 3.1:** Conceptual diagram of the simulation model. A) an idealized “average” pond at the Mexican Cut Nature Preserve. In all three hydroperiod classifications, benthic invertebrates typically inhabit a ~3m wide interior region around the pond perimeter. Most pond surface area is shallow depth (~0.5 m) in all hydroperiod classifications, but maximum depths of permanent ponds range from 1.5-3 m. During each iteration, B) hydroperiod-specific pond parameters (Table 3.1) are randomly sampled to simulate one pond of each hydroperiod classification. C) Taxon-specific supply estimates are calculated for each hydroperiod classification by multiplying randomly sampled hydroperiod, benthic area, areal biomass, and mass-specific excretion rates, and for permanent and semi-permanent hydroperiods, a taxon-specific percent of hydroperiod spent in larval development. Benthic area is determined by taxon life history, calculated for benthic invertebrates as the product of pond area and % benthic area. For chironomids and oligochaetes in permanent ponds, habitable area is pond area minus the product of pond area and % deep center area. For chironomids and oligochaetes in other hydroperiod classifications and zooplankton and salamanders in all hydroperiods, habitable area is pond area. D) Benthic and water column demand are the product of hydroperiod, area, and randomly sampled benthic and water column uptake rates. E) Senesced sedge demand is the product of hydroperiod, area, % sedge coverage, sedge areal biomass, and sedge uptake rates.

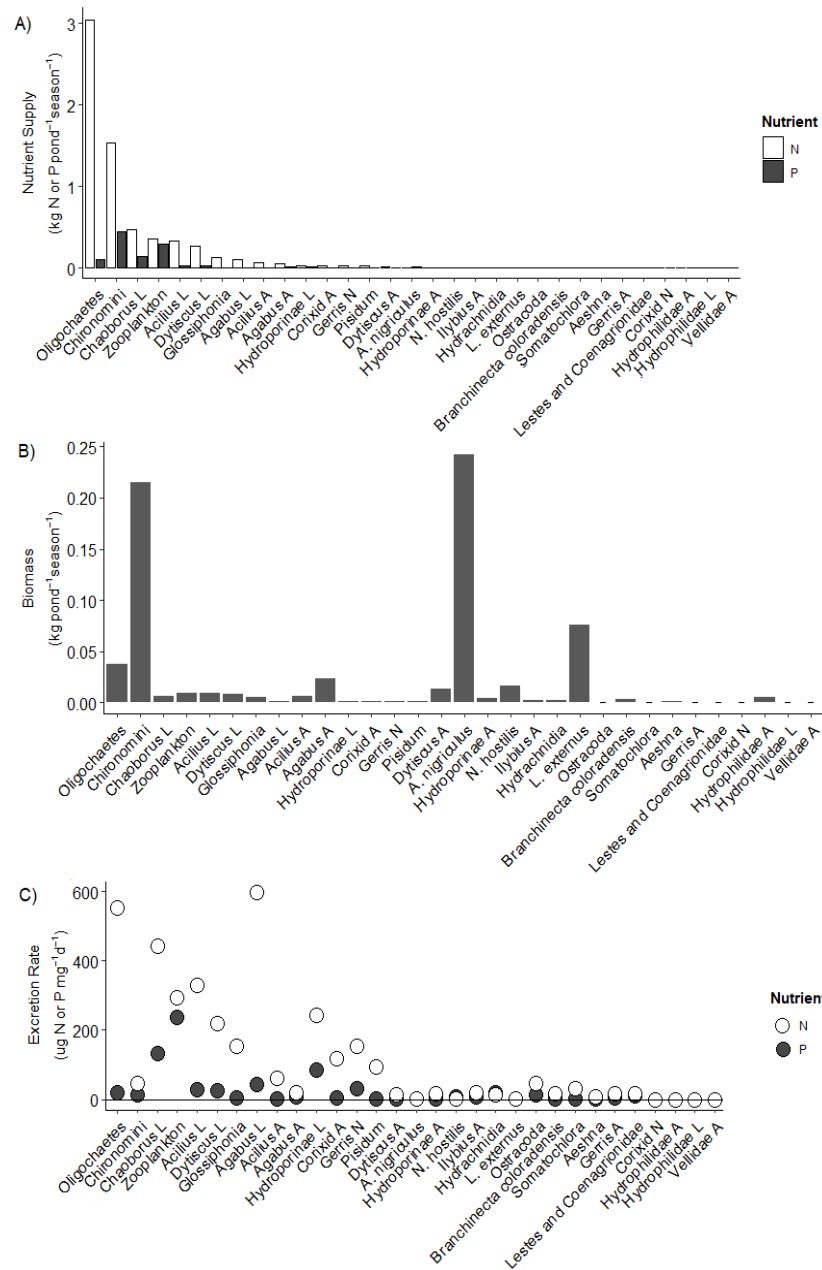




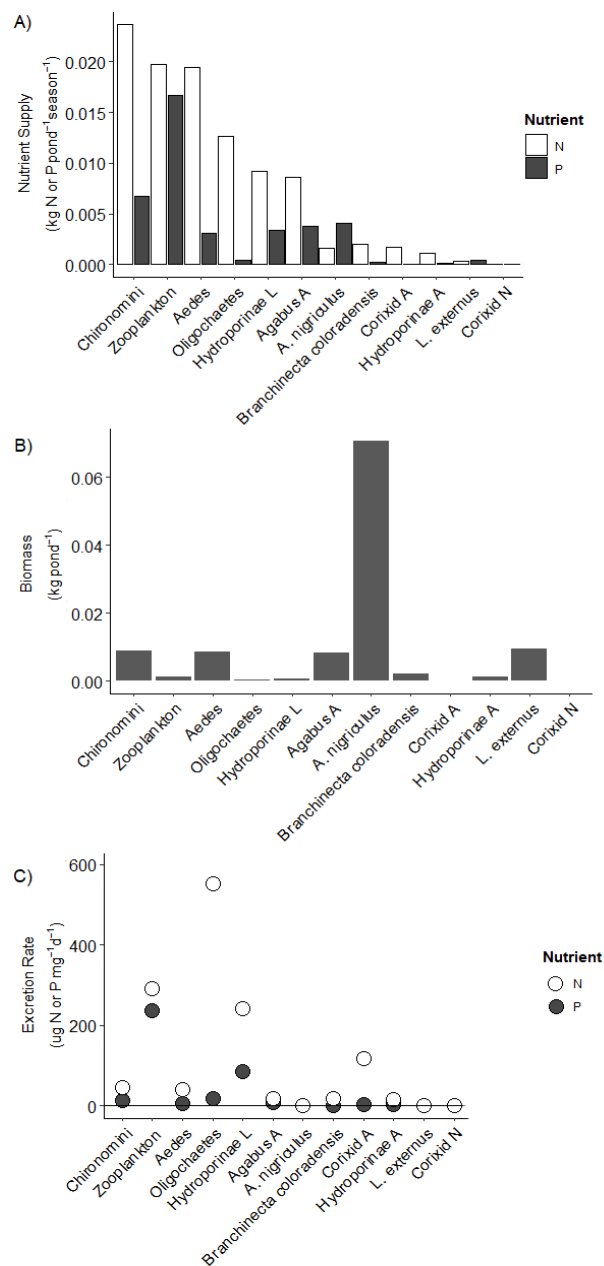
**Figure 3.2:** Mean estimates of N and P supply and demand from the simulation model. Panels A) and B) present estimates of each rate in units of  $\text{mg N or P m}^{-2} \text{d}^{-1}$ . Panels C) and D) scale estimates to seasonal whole-pond rates ( $\text{kg N or P pond}^{-1} \text{season}^{-1}$ ) to demonstrate effects of differences in pond area and season length among hydroperiod classifications. Error bars are omitted because the simulation ran until the mean supply and demand estimates were stable and thus variance was low.



**Figure 3.3:** Estimates of A) mean taxon-specific contributions to whole-pond seasonal nutrient supply in a permanent pond, with each taxon's B) biomass and C) excretion rates from 100,000 simulation iterations. Bar or point height gives mean estimate, and error bars are omitted because the simulation ran until mean supply estimates were stable and variance was low. Taxa sorted by rank-order contribution to N supply in each panel.



**Figure 3.4:** Estimates of A) taxon-specific contributions to whole-pond seasonal nutrient supply in a semi-permanent pond, with each taxon's B) biomass and C) excretion rates from 100,000 simulation iterations. Bar or point height gives mean estimate, and error bars are omitted because the simulation ran until mean supply estimates were stable and variance was low. Taxa sorted by rank-order contribution to N supply in each panel. Note: the different scale of the y axes compared to figure 3.3.



**Figure 3.5:** Estimates of A) taxon-specific contributions to whole-pond seasonal nutrient supply in a temporary pond, with each taxon's B) biomass and C) excretion rates from 100,000 simulation iterations. Bar or point height gives mean estimate, and error bars are omitted because the simulation ran until mean supply estimates were stable and variance was low. Taxa sorted by rank-order contribution to N supply in each panel. Note: the different scale of the y axes compared to figure 3.3.

## **CHAPTER 4: Consequences of climate-induced range expansions on multiple ecosystem functions**

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**Abstract:**

Climate-driven species range shifts and expansions prompt compositional changes in many communities, yet studying functional consequences in natural systems remains challenging. By combining a 30-year survey of subalpine pond larval caddisfly assemblages with species-specific functional traits (nitrogen and phosphorus excretion, and detritus processing rates), we predict three upslope caddisfly range expansions had limited consequences for nutrient supply and detritus processing. Subdominant resident *Ag. deflata* regulated nitrogen supply throughout all range expansions, whereas dominant resident *L. externus* regulated phosphorus and detritus processing until the third range expansion by *N. hostilis*, whose novel developmental phenology facilitated successful range expansion. However, total ecosystem process rates did not change throughout all range expansions. Thus, species' functional roles may change before overall ecosystem processes.

**One-Sentence Summary:** Species functional traits predict range expansions had small effects on ecosystem nutrient supply and detritus processing

## Main Text:

Species range shifts or expansions are some of the most frequent and conspicuous aspects of climate change and are a well-documented adaptation for withstanding changing species-level climate effects (1). However, range shifts or expansions also disrupt resident species compositions (2-4). Furthermore, this introduction of novel functional traits (5) and alteration of food web interactions (6) could alter ecosystem processes, though this has rarely been demonstrated as an outcome of range shifts or expansions driven by contemporary climate change. Although mesocosm experiments provide valuable insight towards functional outcomes of compositional shifts (7), predicting when and where species range expansions or other invasions will occur remains a barrier to studying outcomes in natural systems (8, 9). Thus, long-term community composition data present unique opportunities to predict how species range expansions, species losses, or other compositional shifts modulate ecosystem processes in natural systems. Indeed, the functional consequences of species range expansions can and likely will change over time, but they are rarely studied within such context (10). This long-term perspective is also valuable because over time ecosystems could receive multiple nonresidents expanding their ranges due to climate warming (1). Here, we leverage 30 years of annual censuses of larval caddisflies (Trichoptera; annual generations) that dominate detritivore biomass in subalpine permanent ponds (11) and their species-specific nutrient excretion and detritus processing rates (12, 13; *methods supplement*) to predict the functional consequences of sequential upslope range expansions by caddisfly species *Limnephilius picturatus* in 1998, *Grammotaulius lorretae* in 2006, and *Nemotaulius hostilis* in 2016. Specifically, we tested whether the relative contributions of dominant resident *L. externus* (14), subdominant residents, and range expanding nonresidents to nutrient recycling and detritus processing changed during

each range expansion. Finally, because we expected successive range expansions to increase detritivore evenness and provide greater redundancy in ecosystem processes, we tested for negative relationships between ecosystem process variability and evenness.

Although many montane insect populations are declining due to climate-driven changes in temperature and precipitation patterns (15, 16), caddisflies at the Mexican Cut Nature Preserve have not experienced systematic declines in abundance. Still, the assemblage has experienced the addition of three caddisfly species who have expanded their ranges, and thus relative abundances among species changed during range expansions (e.g., significant species:year:range expansion term in Appendix 4: Table S1; Figure 4.1A). In turn, relative contributions of the dominant resident *L. externus*, subdominant residents, and range expanding caddisflies to ecosystem processes also changed through time (Appendix 4: Table S2; Figure 4.1C, E, G). Although caddisflies total contributions have not changed (Figure 4.1B, D, F), current declines in P supply and detritus processing contributions by dominant resident *L. externus* and increases by nonresident *N. hostilis* (Appendix 4: Table S3; Figure 4.1C, G) suggest that future changes in total ecosystem processes are possible should *L. externus* continue to be numerically replaced (10).

The temporal dynamics of species' abundances and contributions to ecosystem processes provide important context for interpreting why range expansions may have occurred and the extent to which species' functional roles changed. For example, prior to any caddisfly range expansions over the past 30 years, the dominant resident *L. externus* and subdominant residents *Agrypnia deflata* and *Asynarchus nigriculus* populations declined by 1.7, 1.3, and 1.6 individuals/m<sup>2</sup>/year (Figure 4.1A; *L. externus*:  $p < 0.001$ , *Ag. deflata*  $p = 0.027$ , *An. nigriculus*  $p < 0.001$ ). Consequently, *L. externus*'s relative contributions to P supply and detritus processing

declined by 9.0 % annually (Figure 4.1C,  $p = 0.002$ ; G,  $p < 0.001$ ), though the subdominant residents' contributions did not change (P supply:  $p = 0.102$ ; detritus processing:  $p = 0.133$ ). The *L. externus* population decrease was likely caused by consecutive early autumn freezes in 1990 and 1991 that occurred 17.5 days earlier (1.6 standard deviations) than the 1989-2019 average and either killed adults during ovarian diapause or prevented successful oviposition by freezing pond surfaces (14, 17). It is unlikely that early freezes directly caused concurrent declines in subdominant resident populations, as *Ag. deflata* does not exhibit ovarian diapause, and although *An. nigriculus* does, its oviposition phenology occurs one month earlier than *L. externus* (14). However, it is likely that subdominant resident larvae were more vulnerable to salamander predation the following years due to lower *L. externus* abundance (18, 19). Regardless of why subdominant resident populations also declined, absence of a compensatory response may have opened niche space for invasion by range expanding caddisflies (20).

Indeed, perhaps due to lack of available niche space many invading or range expanding species fail to establish populations in new systems (9, 21). However, whether or not transient range expanding species or those attaining low population densities can usurp function remains unclear and likely depends on residents responses. Here, the *L. externus* population quickly recovered and increased by 1.3 individuals/m<sup>2</sup>/year during the 1<sup>st</sup> range expansion, and all other species' abundances were consistent over time, including the 1<sup>st</sup> nonresident *L. picturatus* (Figure 1A; all  $p > 0.05$ ). Thus, *L. externus*'s prompt return to numerical dominance may have arrested *L. picturatus* population growth by filling available niche space or through competitive interactions that reduce *L. picturatus* survival (22). Similarly, all species' abundances were constant during the 2<sup>nd</sup> range expansion, including the 2<sup>nd</sup> nonresident *G. lorretae* (all  $p > 0.05$ ). Furthermore, all caddisfly groups' relative contributions to ecosystem processes were constant

throughout the 1<sup>st</sup> and 2<sup>nd</sup> range expansions (Figure 4.1C, E, F; all  $p > 0.05$ ). However, during the 3<sup>rd</sup> range expansion *L. externus* and *An. nigriculus* abundances declined by  $\sim 1.9$  individuals/m<sup>2</sup>/year (Figure 4.1A;  $p = 0.012$ ;  $p = 0.054$ ) and the 3<sup>rd</sup> nonresident, *N. hostilis*, increased by  $\sim 1.4$  individuals/m<sup>2</sup>/year ( $p < 0.001$ ). Consequently, *L. externus*'s relative contributions to P supply and detritus processing declined by 11.2 % and 13.9 % annually (Figure 4.1C,  $p < 0.001$ ; G,  $p < 0.001$ ) while the nonresident group's relative contribution to P supply increased by 14.5 % (Figure 4.1C,  $p < 0.001$ ). The subdominant resident group's contributions to both ecosystem processes were consistently low (P:  $p = 0.917$ ; detritus:  $p = 0.464$ ). Because caddisflies' total contributions to all three ecosystem processes were temporally constant, and because *L. externus*'s large relative contributions were constant throughout the 1<sup>st</sup> and 2<sup>nd</sup> range expansions, it effectively regulated total P supply and detritus processing throughout sequential upslope range expansions. However, during the 3<sup>rd</sup> range expansion *N. hostilis* usurped *L. externus*'s dominant role in regulating total P supply. Thus, even if range expansions do not alter total ecosystem processes, they can alter resident species functional roles.

Furthermore, invaders may be more likely to usurp functional roles of dominant species than subdominants. Here, subdominant resident *Ag. deflata* consistently contributed large proportions of N supply throughout all three invasions (Figure 4.1D, E). Indeed, *Ag. deflata*'s N contribution matched or exceeded that of dominant resident *L. externus* in 75.6 % of pond-year observations despite only attaining equal or greater density in 22.8 % (Figure 4.2B). Thus, less common species with unique functional traits (here, high mass-specific N excretion) can make contributions to ecosystem processes disproportionate to their abundance (23). By extension, this suggests that if range expanding species have a unique functional trait, they could usurp function even at low abundance. However, because many organismal effects on ecosystem processes are

biomass driven, numerically successful range expansions are more likely to usurp residents' functional roles or alter ecosystem processes.

The most recent range expanding species *N. hostilis* and the dominant resident *L. externus* contributions to P supply were similar in magnitude, however, among the three range shifting species *N. hostilis* is the most likely to rise to numerical and functional prominence in permanent ponds. Specifically, *N. hostilis* matched or exceeded *L. externus*'s relative abundance ~19.4x more frequently than invader *L. picturatus* and ~24.2x more frequently than *G. lorretae* or *An. bimaculata*, though fewer ponds were surveyed since the 3<sup>rd</sup> range expansion began (e.g., 101 pond-years since 1<sup>st</sup> range expansion, 63 since 2<sup>nd</sup>, 13 since 3<sup>rd</sup>; Figure 4.2). Moreover, *N. hostilis* matched or exceeded *L. externus*'s contributions more frequently than the prior two nonresidents combined by factors of 7.1x for P supply, 4.5x for N, 6.9x for detritus processing. Indeed, many invasive species fail to establish in new ecosystems (21), and this long-term data also shows some range expansions not alter ecosystem processes. *N. hostilis* may have achieved rapid population growth and functional displacement of the dominant resident if their ability to disperse from lower elevation source habitats was greater than prior nonresidents (24, 25), but nonresidents source populations were at similar locations. Thus, a more likely hypothesis is that *N. hostilis*'s unique developmental phenology contributed to its numerically successful range expansion; as larvae hatch in the fall prior to pond freezing, they are able to complete initial stages of development when few other taxa are active, including salamander predators (11). Consequently, larvae have little competition or predation pressure at this vulnerable stage. Furthermore, later onset of winter (Appendix 4: Figure S1) likely enhanced these phenological advantages by extending the window for the autumnal component of *N. hostilis* development. Finally, this key difference in *N. hostilis* developmental phenology could also extend the

seasonal duration of caddisfly contributions to ecosystem processes beyond late summer when resident and other invaders pupate. Thus, although unique life histories, developmental phenologies, or other traits are often important determinants of range expanding species success at establishing in a new ecosystem (26), they likely also influence if and when non-resident species contribute to ecosystem processes and usurp residents functional roles.

In ecosystems where an assemblage's total contribution to an ecosystem process is regulated by a single species, whether abundant or uncommon, variation in that species' abundance among habitat patches (here, ponds) and population oscillations over time should cause high variation in the assemblage's contribution to that ecosystem process. Whereas, more even assemblages should have greater redundancy (27), and consequently lower aggregate variability in process contributions. Indeed, among pond aggregate variability in species' detritus processing, P supply, and N supply contributions declined by 10.3x, 9.9x, and 3.7x from the least to most even caddisfly assemblage (Figure 4.3). However, in contrast to our expectation caddisfly evenness declined over time (Figure 4.3A), a consequence of *L. externus* attaining greater numerical dominance following recovery from population decline in the early 1990s coupled with the arrival of range expanding nonresidents that generally attained low population sizes. The increase in *L. externus* numerical dominance, and by extension their functional dominance, was likely a consequence of salamander abundance and predation pressure declining as old, large paedomorphic salamanders are cannibalistic (28). Regardless, simultaneous increases in a dominant resident's abundance and arrival of range expanding nonresidents suggests that the first two range expansions were likely prompted by changing climatic drivers, not availability of suitable niche space. Whereas, *N. hostilis*'s range expansion and unique developmental phenology suggests that spatial or temporal niche availability may determine

whether or not range expansions are numerically successful (9, 29) or if they usurp resident's functional roles. However, even if range expanding species do not sustain large populations on average among habitat patches, their arrival in systems with increasing abundance of dominant resident may nonetheless promote variability in ecosystem processes through a negative effect on evenness.

In the broader context of range shifts and invasive species modulating ecosystem functioning, our results demonstrate that dominant and subdominant resident species can regulate ecosystem processes throughout sequential invasions by species with similar life histories and developmental phenologies. Throughout the range expansions summarized here, the complex long-term metapopulation dynamics of resident and range expanding taxa likely compound with species interactions, niche availability, and climate-driven fluctuations in habitat suitability to limit numeric or functional replacement of resident taxa (20, 30). In contrast, nonresidents with differing life histories and developmental strategies may circumvent this filter. Thus, in addition to functional traits that directly determine contributions to ecosystem processes, traits related to life history could be informative when attempting to predict or interpret functional consequences of species range expansions, shifts, invasions, or other compositional changes in species assemblages, particularly when considered in tandem with long-term natural history observations.

**Acknowledgments:** We thank the Rocky Mountain Biological Laboratory for logistical support and The Nature Conservancy for access to the Mexican Cut Nature Preserve. We also thank Wendy Brown and undergraduate students from Allegheny College for assistance with data collection. We are grateful to I. Billick, L.M. Demi, R.R. Dunn, A.J. Klemmer, S.E. Jordt, A.R. McIntosh, I.D. Shepard, S.E. Washko, and H.H. Whiteman for constructive discussions and

suggestions that greatly improved earlier versions of the manuscript. Finally, we thank Allegheny College, R.L. Mumme, M.D. Venesky, C. Wilson, E.L. Coates, and most importantly Sue Wissinger for securing and permitting access to S.A. Wissinger's data in January 2020.

**Funding:**

National Science Foundation grant 1556914 and 1641041 (BWT)

National Science Foundation grant 1557015 (SAW)

National Science Foundation grant 1556788 (HSG)

National Science Foundation Graduate Research Fellowship (JAB)

NCSU Provost Graduate Student Fellowship (JAB)

**Author contributions:**

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Investigation: JAB, SAW, BWT, HSG

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**Competing interests:** Authors declare that they have no competing interests.

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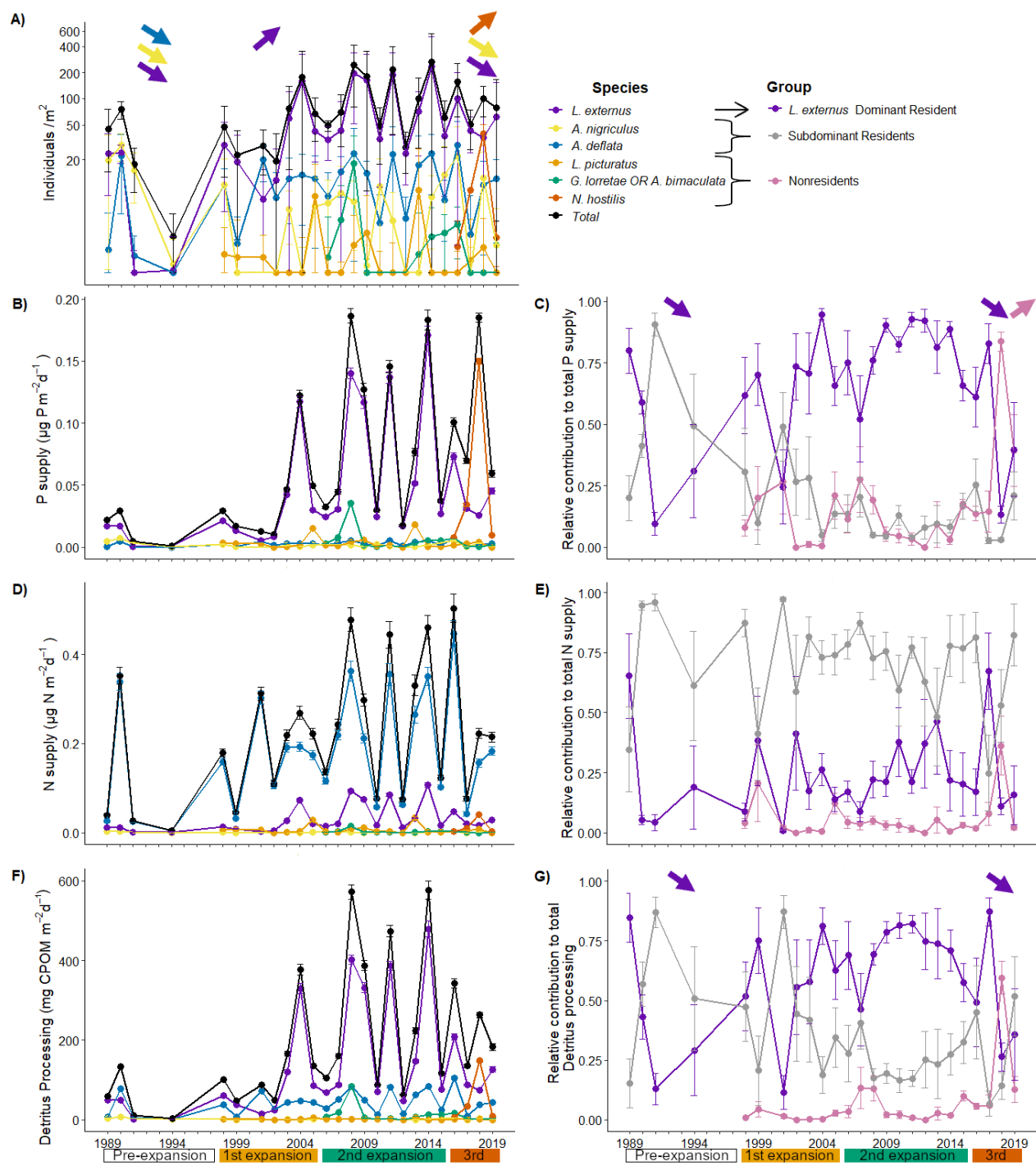
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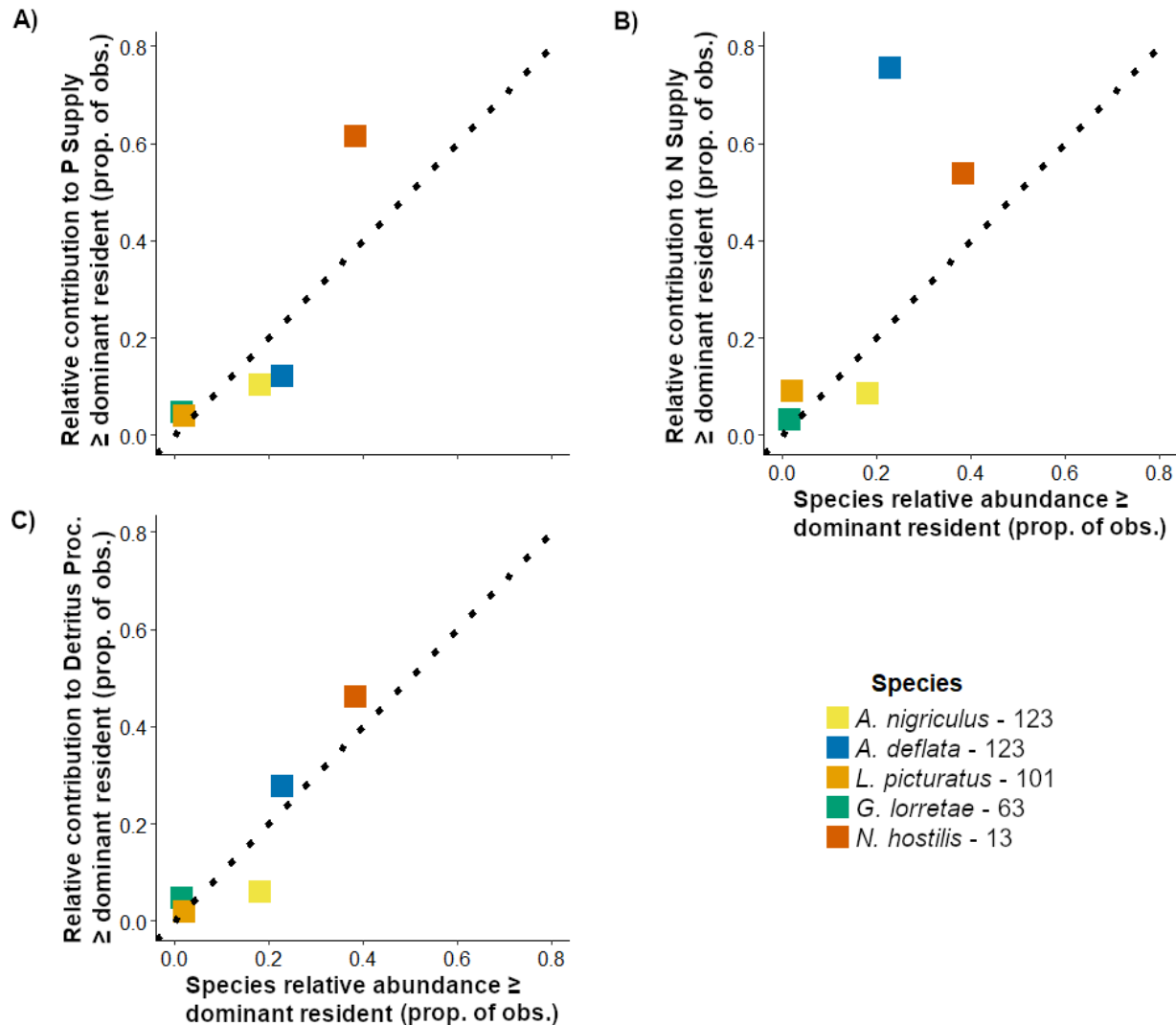
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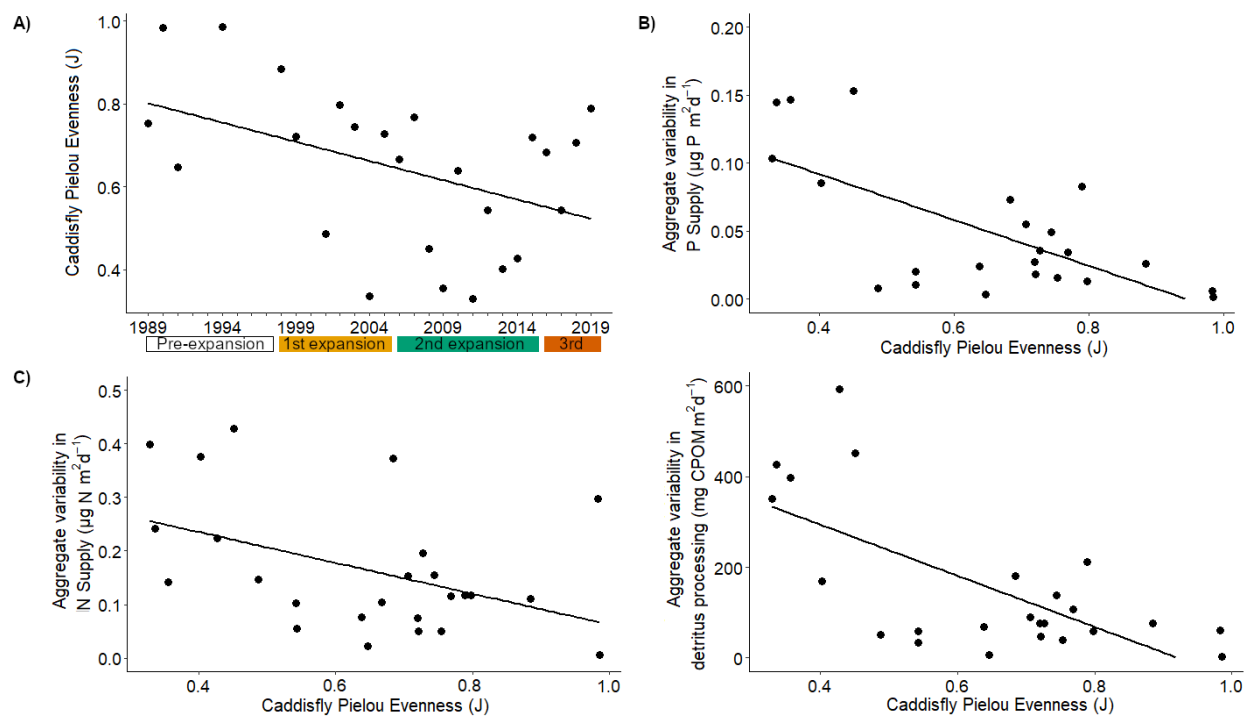
**Figure 4.1. Larval caddisfly abundance and predicted contributions to ecosystem processes in permanent ponds over 30 years at Mexican Cut, Colorado.** Natural log-transformed caddisfly densities [(A, note Y axis is backtransformed to original scale)] were averaged across all permanent ponds (range = 3 – 7 ponds per year), and error bars represent 95 % confidence intervals. Colored boxes under x-axes indicate range expansion periods defined by initial dates of upslope range expansion (e.g., orange 1<sup>st</sup> expansion box indicates *L. picturatus* arrived in 1998, green for *G. lorreatae* or *A. bimaculata* in 2006, and red for *N. hostilis* in 2016). Species' areal daily contributions to phosphorus and nitrogen supplies and detritus processing (as coarse particulate organic matter, CPOM) were predicted as the products of pond-level density, average final instar masses, and species-specific nutrient excretion or detritus processing rates. Although there were large intraspecific differences in excretion and detritus processing rates, within a species rates were temporally and spatially consistent (12, 13; *methods supplement*), allowing us to apply them across the long-term survey dataset. Still, we incorporated variation in species-specific rates by repeating pond-level calculations 1000 times with randomly sampled species-specific rates in each iteration. Nutrient supply and detritus processing were averaged across all pond-level iterations to estimate the mean  $\pm$  1 SE species-specific contributions to P supply [(B)], N supply [(D)], and detritus processing [(F)]. Black points and lines indicate total caddisfly abundance or total contribution to ecosystem processes. Species' relative contributions were calculated to standardize for interannual variation in the total. We grouped the relative contributions to P supply [(C)], N supply [(E)], and CPOM processing [(G)] of subdominant residents (*Ag. deflata* and *An. nigriculus*) and nonresident taxa (*L. picturatus*, *G. lorreatae* or *A. bimaculata*, *N. hostilis*) separately for comparison with the dominant resident (*L. externus*). Colored arrows within plot space indicate direction of statistically significant trends in corresponding species' or groups' relative contribution during each range expansion.





**Figure 4.2. Proportions of pond-year observations where species' relative abundance and contribution to an ecosystem process equaled or exceeded those of dominant resident *L. externus*.** Numeric labels in legend after species indicate total number of ponds sampled since the species was first observed at Mexican Cut (pond-years). Species above the dotted 1:1 lines can functionally surpass the dominant resident's contribution to ecosystem processes without matching their relative abundance, whereas species below infrequently exceed the dominant resident functionally despite greater abundance. Comparison of P supply contributions in [(A)], N supply in [(B)], and detritus processing in [(C)].

**Figure 4.3. Relationships between caddisfly assemblage evenness and aggregate variability in species contributions to ecosystem processes.** Pielou's Evenness (J) was calculated using average caddisfly densities across all permanent ponds at Mexican Cut (e.g., densities in Figure 1A; averages of = 3-7 ponds per year). Aggregate variability in species contributions to ecosystem processes (e.g., variability among ponds and species) was calculated as the sum of species' among-pond variance in process contribution and 2x the sum of contribution covariances among all species pairs. Aggregate variability was square root transformed to improve normality for statistical analysis, which also returns aggregate variability to the original data's scale. Colored boxes under **(A)** x-axis indicate range expansion periods defined by initial dates of upslope range expansions (e.g., orange 1<sup>st</sup> range expansion box indicates *L. picturatus* invaded in 1998, green for *G. lorreatae* or *A. bimaculata* in 2006, and red for *N. hostilis* in 2016). Evenness declined over time (**(A)**;  $F_{1,17} = 5.73$ ,  $p = 0.029$ ,  $R^2 = 0.20$ ) but did not differ among or change during range expansions (expansion:  $F_{3,17} = 1.74$ ,  $p = 0.20$ ; expansion\*Year:  $F_{3,17} = 0.72$ ,  $p = 0.55$ ). All three ecosystem processes' aggregate variability had negative relationships with evenness (P in **(B)**:  $F_{1,23} = 17.61$ ,  $p < 0.001$ ,  $R^2 = 0.42$ ; N in **(C)**:  $F_{1,23} = 5.74$ ,  $p = 0.025$ ,  $R^2 = 0.17$ ; detritus in **(D)**:  $F_{1,23} = 17.83$ ,  $p < 0.001$ ,  $R^2 = 0.43$ ).



**APPENDICES**

## APPENDIX 1

Available online at <https://esajournals.onlinelibrary.wiley.com/doi/full/10.1002/ecs2.2205>

**Appendix 1: Table S1.** Summary of species' traits used in trait-based models of excretion. Case material and behavior from Wissinger et al. 2006 and unpublished data.

| Species                | Habitat       | Elevation | Case Material    | Activity Rank |
|------------------------|---------------|-----------|------------------|---------------|
| <i>A. deflata</i>      | Permanent     | Subalpine | Pine             | 10            |
| <i>A. nigriculus</i>   | Vernal & Semi | Broad     | Stone            | 1             |
| <i>G. lorettae</i>     | Perm & Semi   | Broad     | Sedge            | 6             |
| <i>H. occidentalis</i> | Permanent     | Subalpine | Stone            | 9             |
| <i>L. externus</i>     | Perm & Semi   | Broad     | Sedge            | 4             |
| <i>L. picturatus</i>   | Perm & Semi   | Broad     | Sedge            | 3             |
| <i>L. secludens</i>    | Vernal & Semi | Montane   | Fine stones/silt | 2             |
| <i>L. sublunatus</i>   | Perm & Semi   | Montane   | Sedge            | 5             |
| <i>L. tarsalis</i>     | Vernal & Semi | Montane   | Woody debris     | 7             |
| <i>N. hostilis</i>     | Permanent     | Subalpine | Leaves           | 8             |

**Appendix 1: Table S2.** Summary of ANOVA tests for effects of time of day (blocking factor; morning, midday, or afternoon; n=3 excretion trials each) and elevation/site on species and mass-specific nitrogen (N) and phosphorus (P) excretion rates. For species with broad distributions, time of day trials at each site were modeled as separate blocks. For all nine species, none of the time of day blocks or sites/elevations are significantly different at  $\alpha=0.05$ . *N. hostilis* is present at all elevations, but excretion measurements were only collected at the subalpine sites.

| Species                                                                          | N (as [NH <sub>4</sub> <sup>+</sup> -N]) |        |      |           |        |      | P (as [TDP]) |        |      |           |        |      |
|----------------------------------------------------------------------------------|------------------------------------------|--------|------|-----------|--------|------|--------------|--------|------|-----------|--------|------|
|                                                                                  | Time of day                              |        |      | Elevation |        |      | Time of day  |        |      | Elevation |        |      |
|                                                                                  | F                                        | df     | p    | F         | df     | p    | F            | df     | p    | F         | df     | p    |
| <b>Broad Distributions<br/>(Lower &amp; Upper Montane,<br/>Subalpine) (n=27)</b> |                                          |        |      |           |        |      |              |        |      |           |        |      |
| <i>A. nigriculus</i>                                                             | 0.18                                     | (1,25) | 0.68 | 0.01      | (1,25) | 0.93 | 0.55         | (1,25) | 0.47 | 0.97      | (1,25) | 0.33 |
| <i>L. externus</i>                                                               | 2.7                                      | (1,25) | 0.12 | 0.88      | (1,25) | 0.36 | 0.37         | (1,24) | 0.55 | 2.12      | (1,24) | 0.16 |
| <i>L. picturatus</i>                                                             | 0.03                                     | (1,25) | 0.87 | 0.10      | (1,25) | 0.76 | 0.06         | (1,25) | 0.81 | 0.23      | (1,25) | 0.64 |
| <i>G. lorretae</i>                                                               | 0.84                                     | (1,25) | 0.84 | 0.05      | (1,25) | 0.82 | 0.00         | (1,25) | 0.96 | 3.67      | (1,25) | 0.07 |
| <b>Lower Montane Only (n=9)</b>                                                  |                                          |        |      |           |        |      |              |        |      |           |        |      |
| <i>L. tarsalis</i>                                                               | 0.22                                     | (1,7)  | 0.66 | --        | --     | --   | 5.27         | (1,7)  | 0.06 | --        | --     | --   |
| <i>L. sublunatus</i>                                                             | 0.51                                     | (1,7)  | 0.50 | --        | --     | --   | 2.19         | (1,7)  | 0.18 | --        | --     | --   |
| <i>L. secludens</i>                                                              | 0.06                                     | (1,7)  | 0.82 | --        | --     | --   | 5.45         | (1,7)  | 0.05 | --        | --     | --   |
| <b>Subalpine Only (n=9)</b>                                                      |                                          |        |      |           |        |      |              |        |      |           |        |      |
| <i>H. occidentalis</i>                                                           | 1.67                                     | (1,7)  | 0.24 | --        | --     | --   | 2.04         | (1,7)  | 0.20 | --        | --     | --   |
| <i>A. deflata</i>                                                                | 0.24                                     | (1,7)  | 0.64 | --        | --     | --   | 0.15         | (1,7)  | 0.72 | --        | --     | --   |
| <i>N. hostilis</i>                                                               | 0.02                                     | (1,7)  | 0.90 | --        | --     | --   | 0.02         | (1,7)  | 0.89 | --        | --     | --   |

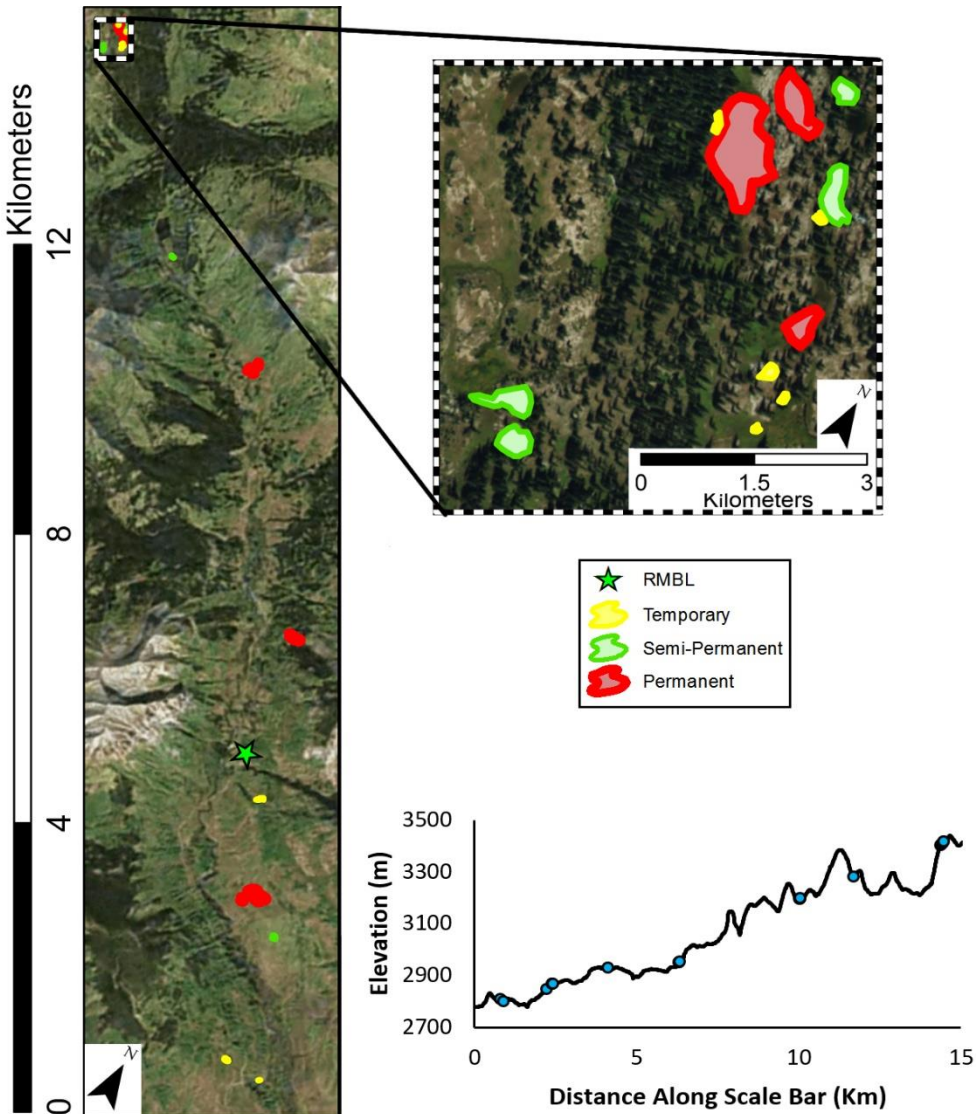
**Appendix 1: Table S3.** Summary of T tests for final instar empty case nutrient excretion or uptake. All tests compared mean excretion/uptake to hypothesized mean of 0 ug mg<sup>-1</sup> case d<sup>-1</sup>.

| Case Species         | NH <sub>4</sub> <sup>+</sup> -N excretion / uptake |    |      | TDP excretion / uptake |    |      |
|----------------------|----------------------------------------------------|----|------|------------------------|----|------|
|                      | t                                                  | df | p    | t                      | df | p    |
| <i>A. deflata</i>    | 0.36                                               | 4  | 0.73 | -0.31                  | 4  | 0.77 |
| <i>L. externus</i>   | 0.34                                               | 4  | 0.75 | 0.37                   | 4  | 0.75 |
| <i>L. picturatus</i> | 0.35                                               | 4  | 0.75 | -0.25                  | 4  | 0.61 |
| <i>G. lorrettae</i>  | 0.34                                               | 4  | 0.75 | 1.94                   | 4  | 0.12 |

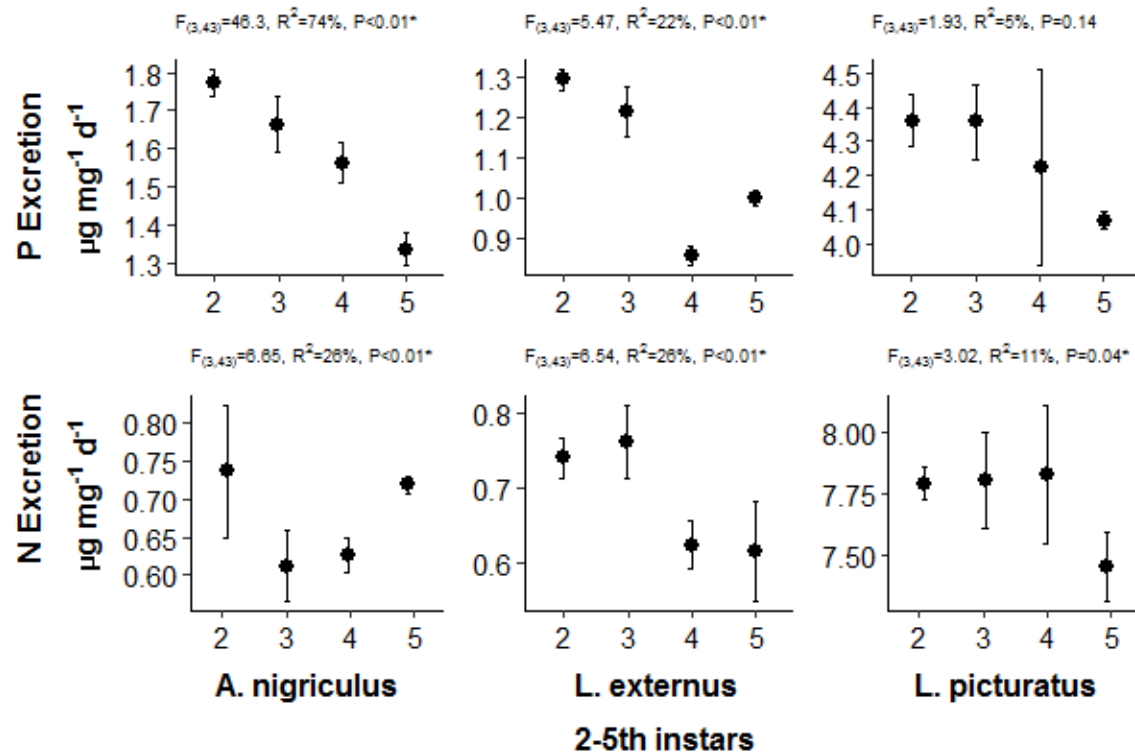
**Appendix 1: Table S4.** Summary of GLMs for within-species regressions of temperature and mass-specific excretion rates. No slopes were significantly different from zero.  $R^2$  not shown; all  $p < 0.05$ . Water and air temperatures ranged 3.5 °C and 4 °C respectively during *A. nigriculus* excretion trials, 6.5 °C and 5.5 °C during *L. externus* excretion trials, 9.2 °C and 16.1 °C during *L. picturatus* excretion trials, and 12.2 °C and 8.1 °C during *G. lorretae* excretion trials.

| Species                           | N (as [NH <sub>4</sub> <sup>+</sup> -N]) |        |      |                 |        |      | P (as [TDP])      |        |      |                 |        |      |
|-----------------------------------|------------------------------------------|--------|------|-----------------|--------|------|-------------------|--------|------|-----------------|--------|------|
|                                   | Water Temperature                        |        |      | Air Temperature |        |      | Water Temperature |        |      | Air Temperature |        |      |
|                                   | F                                        | df     | p    | F               | df     | p    | F                 | df     | p    | F               | df     | p    |
| <b>Broad Distributions (n=27)</b> |                                          |        |      |                 |        |      |                   |        |      |                 |        |      |
| <i>A. nigriculus</i>              | 2.91                                     | (1,25) | 0.13 | 0.36            | (1,25) | 0.55 | 2.01              | (1,25) | 0.19 | 1.42            | (1,25) | 0.24 |
| <i>L. externus</i>                | 0.25                                     | (1,25) | 0.62 | 1.14            | (1,25) | 0.29 | 0.14              | (1,24) | 0.72 | 0.93            | (1,24) | 0.34 |
| <i>L. picturatus</i>              | 0.02                                     | (1,25) | 0.88 | 1.26            | (1,25) | 0.27 | 0.34              | (1,25) | 0.56 | 0.01            | (1,25) | 0.56 |
| <i>G. lorretae</i>                | 0.03                                     | (1,25) | 0.86 | 0.05            | (1,25) | 0.82 | 3.52              | (1,25) | 0.07 | 1.03            | (1,25) | 0.32 |

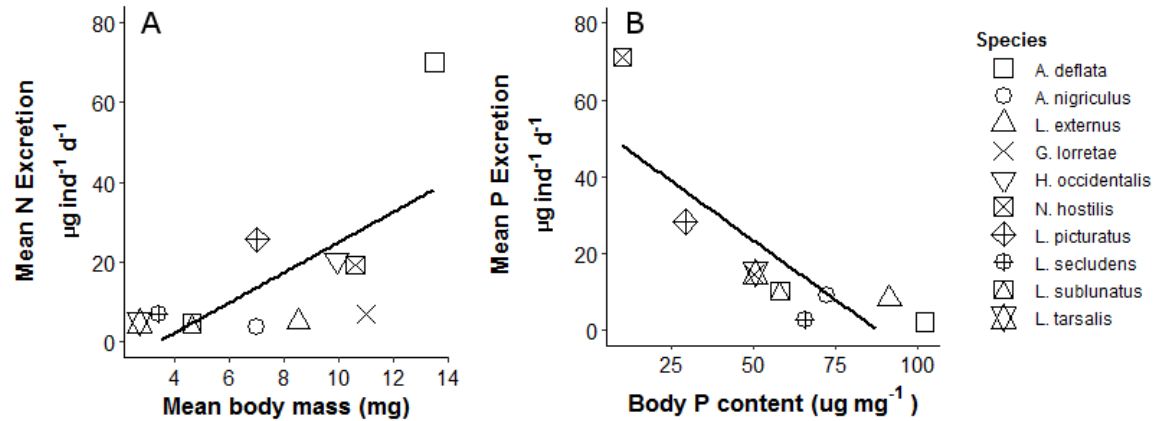
**Appendix 1: Figure S1.** Map shows locations and hydrologic permanence of ponds used to collect caddis for excretion measurements. Inset map magnifies Mexican Cut Preserve. Elevational profile travels northwest “up valley” from the bottom of the map, with blue points representing mapped ponds. Table gives elevational and habitat (hydrologic permanence) distribution of the 10 larval caddisfly species. \* indicates documented distributional shift upslope or into a new hydrologic permanence.



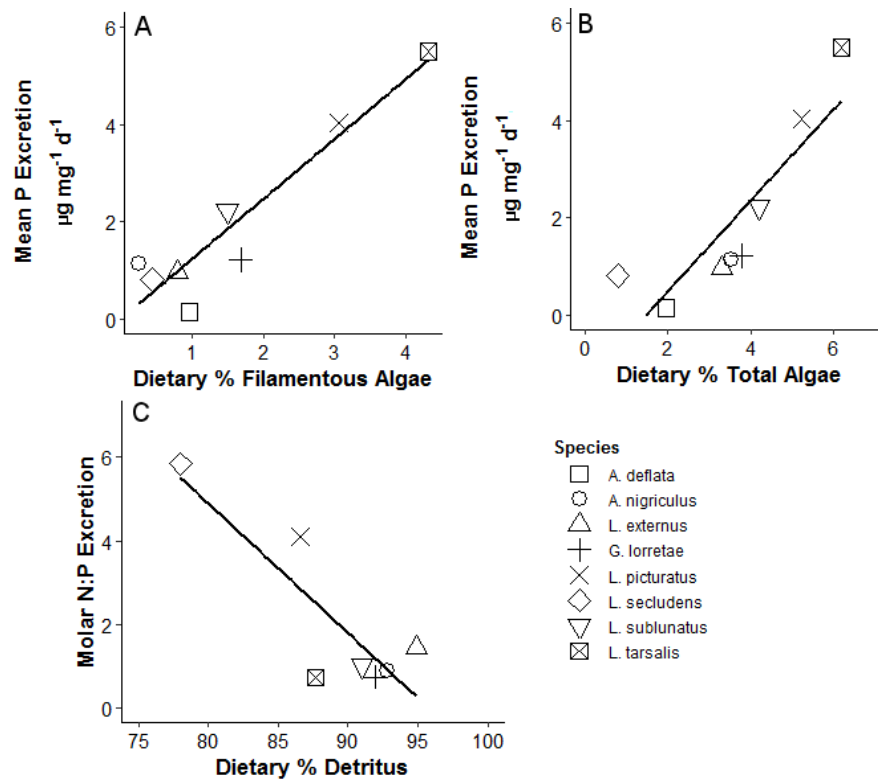
| Montane-Elevation Basins (2700m-3100m)    |                                                                                                                   |                                                                                            | Subalpine-Elevation Basins (3100m-3600m) |                                                                                                  |                                                                                                                                                          |
|-------------------------------------------|-------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------|------------------------------------------|--------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------|
| Temporary                                 | Semi-permanent                                                                                                    | Permanent w/ Predators                                                                     | Temporary                                | Semi-permanent                                                                                   | Permanent w/ Predators                                                                                                                                   |
| <i>L. tarsalis</i><br><i>L. secludens</i> | <i>L. externus</i><br>* <i>L. sublunatus</i><br><i>L. picturatus</i><br><i>G. lorretae</i><br><i>L. secludens</i> | <i>L. externus</i><br>* <i>L. sublunatus</i><br><i>L. picturatus</i><br><i>G. lorretae</i> | <i>A. nigriculus</i>                     | <i>L. externus</i><br><br>* <i>L. picturatus</i><br>* <i>G. lorretae</i><br><i>A. nigriculus</i> | <i>L. externus (LE)</i><br><br>* <i>L. picturatus</i><br>* <i>G. lorretae</i><br><br><i>H. occidentalis</i><br><i>A. deflata</i><br>* <i>N. hostilis</i> |



**Appendix 1: Figure S2.** Mean ( $\pm 1$  S.E.) instar-specific mass-specific nutrient excretion rates for *A. nigrificus*, *L. externus*, and *L. picturatus*. Units are nutrient excreted ( $\mu\text{g}$ ) per unit body mass ( $\text{mg}$ ) per day ( $\text{d}$ ). For all instars of all three species,  $n=5$  excretion measurements.



**Appendix 1: Figure S3. A)** Relationship between caddisfly body size and nitrogen excretion rates for all ten species. Excretion rates are expressed as a function of  $\mu\text{g N bug}^{-1} \text{ day}^{-1}$  by multiplying excretion rates in  $\mu\text{g mg}^{-1} \text{ d}^{-1}$  by each excretion measurement's respective mean caddisfly mass. The means of these species and instar-specific rates were paired and plotted with each species and instar's mean body mass. **B)** Relationship between caddisfly body size and phosphorus excretion rates for nine species (*H. occidentalis* omitted because body P content was unavailable). Mean instar-specific P excretion per bug (as TDP) paired with mean instar specific P body content (**A:**  $R^2=0.36$ ,  $F_{(1,8)}=6.07$ ,  $p=0.04$ ; **B:**  $R^2=-0.64$ ,  $F_{(1,7)}=15.29$ ,  $p<0.01$ ).



**Appendix 1: Figure S4.** Percent **A)** Filamentous algae and **B)** total algae in caddisfly diets predict mean mass-specific 5<sup>th</sup> instar P excretion rates. **C)** Percent detritus in caddisfly diets predict mean molar 5<sup>th</sup> instar N:P excretion ratios. *A. deflata* is not shown but is included in model. Two species (*H. occidentalis*, *N. hostilis*) are omitted from all panels and models because dietary data was unavailable (**A**:  $R^2=0.86$ ,  $F_{(1,6)}=44.4$ ,  $p<0.01$ ; **B**:  $R^2=0.71$ ,  $F_{(1,6)}=18.44$ ,  $p<0.01$ ; **C**:  $R^2=-0.68$ ,  $F_{(1,6)}=14.17$ ,  $p<0.01$ ).

## APPENDIX 2

**Appendix 2: Table S1.** Summary of detritus processing experimental designs, including response variables measured, species, densities, and treatment durations. Genus *L.* = *Limnephilus*, *A.* = *Asynarchus*, *An.* = *Anabolia*, *G.* = *Grammotaulius*

| Experiment-year | Response variables | <i>n</i> Replicate microcosms per treatment | Treatments              | Individuals per microcosm                                        | Treatment duration (days) |
|-----------------|--------------------|---------------------------------------------|-------------------------|------------------------------------------------------------------|---------------------------|
| 2011            | CPOM               | 5                                           | <i>L. externus</i>      | 24                                                               | 30                        |
|                 |                    |                                             | <i>L. sublunatus</i>    | 24                                                               | 30                        |
|                 |                    |                                             | <i>L. tarsalis</i>      | 48                                                               | 30                        |
|                 |                    |                                             | Mixed assemblage        | 8 <i>L.e.</i> , 8 <i>L.sub.</i> , 16 <i>L.t.</i>                 | 30                        |
|                 |                    |                                             | No-invertebrate control | 0                                                                | 30                        |
| 2013            | CPOM<br>FPOM       | 4                                           | <i>L. externus</i>      | 24                                                               | 42                        |
|                 |                    |                                             | <i>A. nigriculus</i>    | 24                                                               | 42                        |
|                 |                    |                                             | <i>L. picturatus</i>    | 24                                                               | 42                        |
|                 |                    |                                             | Mixed assemblage        | 8 <i>L.e.</i> , 8 <i>A.n.</i> , 8 <i>L.p.</i>                    | 42                        |
|                 |                    |                                             | No-invertebrate control | 0                                                                | 42                        |
| 2015            | CPOM               | 4                                           | <i>L. externus</i>      | 25                                                               | 36                        |
|                 |                    |                                             | <i>G. loettae</i>       | 20                                                               | 11                        |
|                 |                    |                                             | <i>L. picturatus</i>    | 30                                                               | 23                        |
|                 |                    |                                             | <i>L. secludens</i>     | 50                                                               | 23                        |
|                 |                    |                                             | Mixed assemblage        | 8 <i>L.e.</i> , 5 <i>G.l.</i> , 7 <i>L.p.</i> , 15 <i>L.sec.</i> | 23                        |
| 2019            | CPOM<br>FPOM       | 5                                           | No-invertebrate control | 0                                                                | 36                        |
|                 |                    |                                             | <i>L. externus</i>      | 24                                                               | 14                        |
|                 |                    |                                             | <i>An. bimaculata</i>   | 24                                                               | 14                        |
|                 |                    |                                             | <i>L. abbreviatus</i>   | 24                                                               | 39                        |
|                 |                    |                                             | <i>L. picturatus</i>    | 24                                                               | 20                        |
| 2020            | CPOM<br>FPOM       | 6                                           | No-invertebrate control | 0                                                                | 39                        |
|                 |                    |                                             | <i>L. externus</i>      | 20                                                               | 19                        |
|                 |                    |                                             | <i>Ecclisomyia</i> sp.  | 25                                                               | 18                        |
|                 |                    |                                             | Amphipods               | 25                                                               | 20                        |
|                 |                    |                                             | Chironomids             | 175                                                              | 12                        |
|                 |                    |                                             | Lymnaeidae              | 15                                                               | 19                        |
| 2020            | CPOM<br>FPOM       | 6                                           | No-invertebrate control | 0                                                                | 20                        |

**Appendix 2: Table S2.** Correlation Matrix used to identify multicollinearity between continuous traits and guide trait-based model selection. When two continuous traits were highly correlated (determined by Pearson's  $r > 0.5$ ), the trait that individually better predicted processing was used.

|                                | <b>Final Instar<br/>Mass</b> | <b>% Coarse<br/>Detritus</b> | <b>% Fine<br/>Detritus</b> | <b>% Algae</b> | <b>% Chitin</b> | <b>Tissue<br/>C:P</b> | <b>Molar N:P<br/>Excretion</b> |
|--------------------------------|------------------------------|------------------------------|----------------------------|----------------|-----------------|-----------------------|--------------------------------|
| <b>Final Instar<br/>Mass</b>   | 1.00                         | -0.05                        | -0.08                      | -0.28          | 0.28            | -0.23                 | 0.62                           |
| <b>% Coarse<br/>Detritus</b>   | -0.05                        | 1.00                         | -0.90                      | 0.07           | -0.68           | -0.18                 | -0.53                          |
| <b>% Fine Detritus</b>         | -0.08                        | -0.90                        | 1.00                       | 0.29           | 0.31            | 0.44                  | 0.20                           |
| <b>% Algae</b>                 | -0.28                        | 0.07                         | 0.29                       | 1.00           | -0.66           | 0.58                  | -0.43                          |
| <b>% Chitin</b>                | 0.28                         | -0.68                        | 0.31                       | -0.66          | 1.00            | -0.22                 | 0.82                           |
| <b>Tissue C:P</b>              | -0.23                        | -0.18                        | 0.44                       | 0.58           | -0.22           | 1.00                  | -0.33                          |
| <b>Molar N:P<br/>Excretion</b> | 0.62                         | -0.53                        | 0.20                       | -0.43          | 0.82            | -0.33                 | 1.00                           |

**Appendix 2: Table S3.** Summary of within-experiment models testing for differences in daily per-microcosm detritus processing rates among invertebrate taxa treatments including the no-invertebrate control treatment. MANOVAs were used when CPOM breakdown and FPOM production were both measured, followed by univariate ANOVAs for each response variable if overall MANOVAs were significant. Block effect and block\*treatment interaction terms were included when possible. Asterisks indicate a difference in detritus processing among one or more treatments.

| Experiment-<br>Year | Model                                                         | Term            | F     | df    | p       |
|---------------------|---------------------------------------------------------------|-----------------|-------|-------|---------|
| 2011                | ANOVA: CPOM $d^{-1}$ = Treatment                              | Treatment       | 65.97 | 3,16  | <0.001* |
| 2013                | MANOVA: (CPOM $d^{-1}$ and FPOM $d^{-1}$ ) = Treatment        | Treatment       | 6.26  | 6,24  | <0.001* |
|                     | ANOVA: CPOM $d^{-1}$ = Treatment                              | Treatment       | 143.5 | 3,12  | <0.001* |
|                     | ANOVA: FPOM $d^{-1}$ = Treatment                              | Treatment       | 13.99 | 3,12  | <0.001* |
| 2015                | ANOVA: CPOM $d^{-1}$ = Treatment                              | Treatment       | 279.7 | 4,15  | <0.001* |
| 2019                | MANOVA: (CPOM $d^{-1}$ and FPOM $d^{-1}$ ) = Treatment        | Treatment       | 6.23  | 8,40  | <0.001* |
|                     | ANOVA: CPOM $d^{-1}$ = Treatment                              | Treatment       | 133.6 | 4,20  | <0.001* |
|                     | ANOVA: FPOM $d^{-1}$ = Treatment                              | Treatment       | 74.2  | 4,20  | <0.001* |
|                     |                                                               |                 |       |       |         |
| 2020                | MANOVA: (CPOM $d^{-1}$ and FPOM $d^{-1}$ ) = Treatment *Block | Treatment       | 7.35  | 10,36 | <0.001* |
|                     |                                                               | Block           | 1.78  | 2,17  | 0.199   |
|                     |                                                               | Treatment*Block | 1.08  | 10,36 | 0.403   |
|                     | ANOVA: CPOM $d^{-1}$ = Treatment                              | Treatment       | 36.64 | 5,24  | <0.001* |
|                     | ANOVA: CPOM $d^{-1}$ = Treatment                              | Treatment       | 21.9  | 5,24  | <0.001* |
|                     |                                                               |                 |       |       |         |

**Appendix 2: Table S4.** Summary of within-experiment Dunnett's post-hoc multiple comparisons on daily per microcosm CPOM and FPOM ANOVA models presented in Table S3. The no-invertebrate control serves as the reference group for all contrasts within each experiment. Asterisks indicate a taxons' daily processing differs from the no-invertebrate control. Genus *L.* = *Limnephilus*, *A.* = *Asynarchus*, *An.* = *Anabolia*, *G.* = *Grammotallius*.

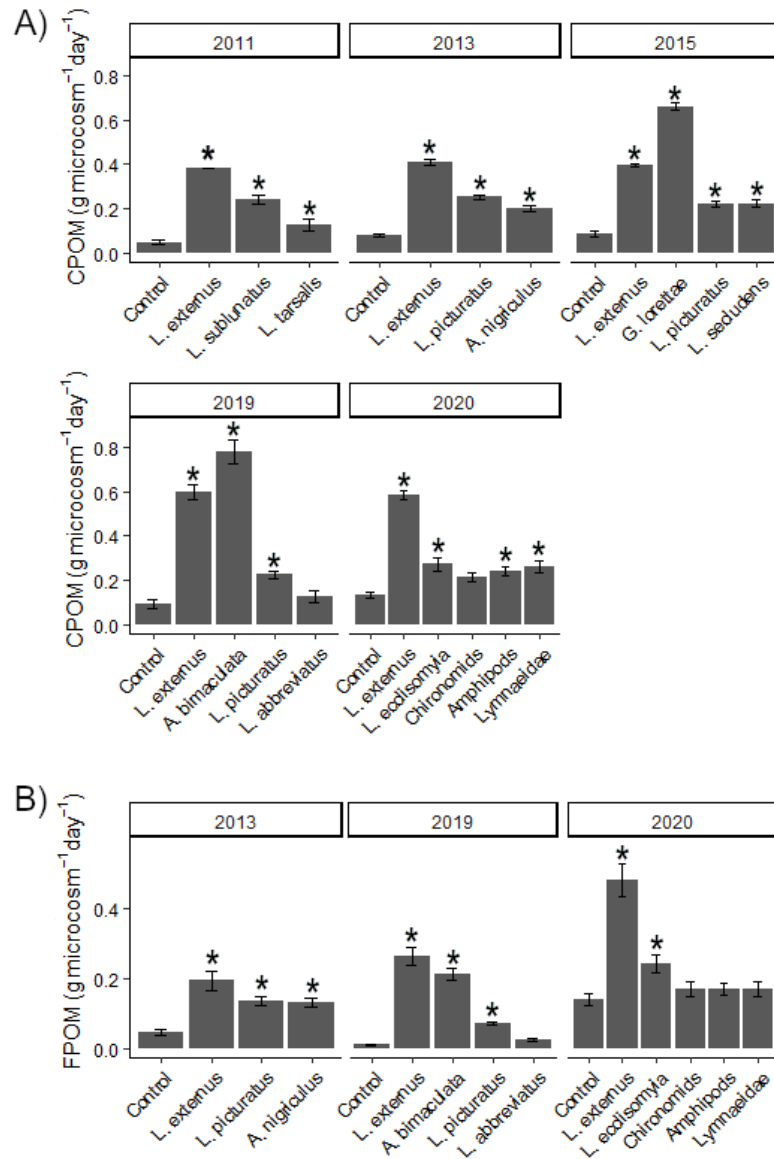
| Experiment | Response             | Taxa contrast with no-invertebrate control | t     | p       |
|------------|----------------------|--------------------------------------------|-------|---------|
| 2011       | CPOM d <sup>-1</sup> | <i>L. externus</i>                         | 13.18 | <0.001* |
|            |                      | <i>L. sublunatus</i>                       | 7.54  | <0.001* |
|            |                      | <i>L. tarsalis</i>                         | 2.99  | 0.023*  |
| 2013       | CPOM d <sup>-1</sup> | <i>A. nigriculus</i>                       | 7.46  | <0.001* |
|            |                      | <i>L. externus</i>                         | 20.44 | <0.001* |
|            |                      | <i>L. picturatus</i>                       | 10.54 | <0.001* |
|            | FPOM d <sup>-1</sup> | <i>A. nigriculus</i>                       | 3.72  | <0.001* |
|            |                      | <i>L. externus</i>                         | 6.41  | <0.001* |
|            |                      | <i>L. picturatus</i>                       | 4     | <0.001* |
| 2015       | CPOM d <sup>-1</sup> | <i>G. lorettae</i>                         | 30.73 | <0.001* |
|            |                      | <i>L. externus</i>                         | 16.51 | <0.001* |
|            |                      | <i>L. picturatus</i>                       | 7.13  | <0.001* |
|            |                      | <i>L. secludens</i>                        | 7.39  | <0.001* |
| 2019       | CPOM d <sup>-1</sup> | <i>An. bimaculata</i>                      | 14.66 | <0.001* |
|            |                      | <i>L. externus</i>                         | 17.12 | <0.001* |
|            |                      | <i>L. abbreviatus</i>                      | 0.71  | 0.887   |
|            |                      | <i>L. picturatus</i>                       | 2.81  | 0.036*  |
|            | FPOM d <sup>-1</sup> | <i>An. bimaculata</i>                      | 10.76 | <0.001* |
|            |                      | <i>L. externus</i>                         | 13.47 | <0.001* |
|            |                      | <i>L. abbreviatus</i>                      | 0.79  | 0.845   |
|            |                      | <i>L. picturatus</i>                       | 3.27  | 0.014*  |
| 2020       | CPOM d <sup>-1</sup> | Amphipods                                  | 3.13  | 0.020*  |
|            |                      | Chironomids                                | 2.5   | 0.079   |
|            |                      | <i>Ecclisomyia</i> sp.                     | 4.26  | 0.001*  |
|            |                      | <i>L. externus</i>                         | 13.03 | <0.001* |
|            |                      | Lymnaeidae                                 | 4.12  | 0.002*  |
|            | FPOM d <sup>-1</sup> | Amphipods                                  | 0.82  | 0.89    |
|            |                      | Chironomids                                | 0.88  | 0.86    |
|            |                      | <i>Ecclisomyia</i> sp.                     | 2.93  | 0.031*  |
|            |                      | <i>L. externus</i>                         | 9.33  | <0.001* |
|            |                      | Lymnaeidae                                 | 0.874 | 0.863   |

**Appendix 2: Table S5:** Summary of single trait linear models used to guide selection of traits to include in full trait-based linear models of CPOM and FPOM. Predictor traits with adjusted  $R^2 > 0.15$  were considered for inclusion in full trait-based linear models. When two predictor traits were highly correlated (Pearson's  $r > 0.5$ ; Appendix S1: Table S1), the trait that individually better predicted processing (e.g., greater adjusted  $R^2$ ) was used.

| <b>Single Trait Linear models: average mg CPOM indiv<sup>-1</sup> d<sup>-1</sup> = (Predictor Trait)</b> |          |           |          |                          |                                            |
|----------------------------------------------------------------------------------------------------------|----------|-----------|----------|--------------------------|--------------------------------------------|
| <b>Predictor Trait</b>                                                                                   | <b>F</b> | <b>df</b> | <b>p</b> | <b>adj R<sup>2</sup></b> | <b>Considered in full CPOM trait model</b> |
| Body Size (mg)                                                                                           | 18.52    | 1,7       | 0.004*   | 0.69                     | Yes                                        |
| Diet % Total Detritus                                                                                    | 4.74     | 1,7       | 0.066*   | 0.32                     | Yes                                        |
| Diet % Coarse Detritus                                                                                   | 5.16     | 1,7       | 0.057*   | 0.34                     | Yes                                        |
| Diet % Fine Detritus                                                                                     | 1.52     | 1,7       | 0.257    | 0.06                     | No                                         |
| Diet % Total Algae                                                                                       | 1.24     | 1,7       | 0.303    | 0.03                     | No                                         |
| Diet % Total Chitin                                                                                      | 1.09     | 1,7       | 0.331    | 0.01                     | No                                         |
| N:P Excretion Ratio                                                                                      | 3.18     | 1,7       | 0.118    | 0.21                     | Yes                                        |
| Body Stoichiometry C:P                                                                                   | 2.05     | 1,7       | 0.195    | 0.12                     | No                                         |

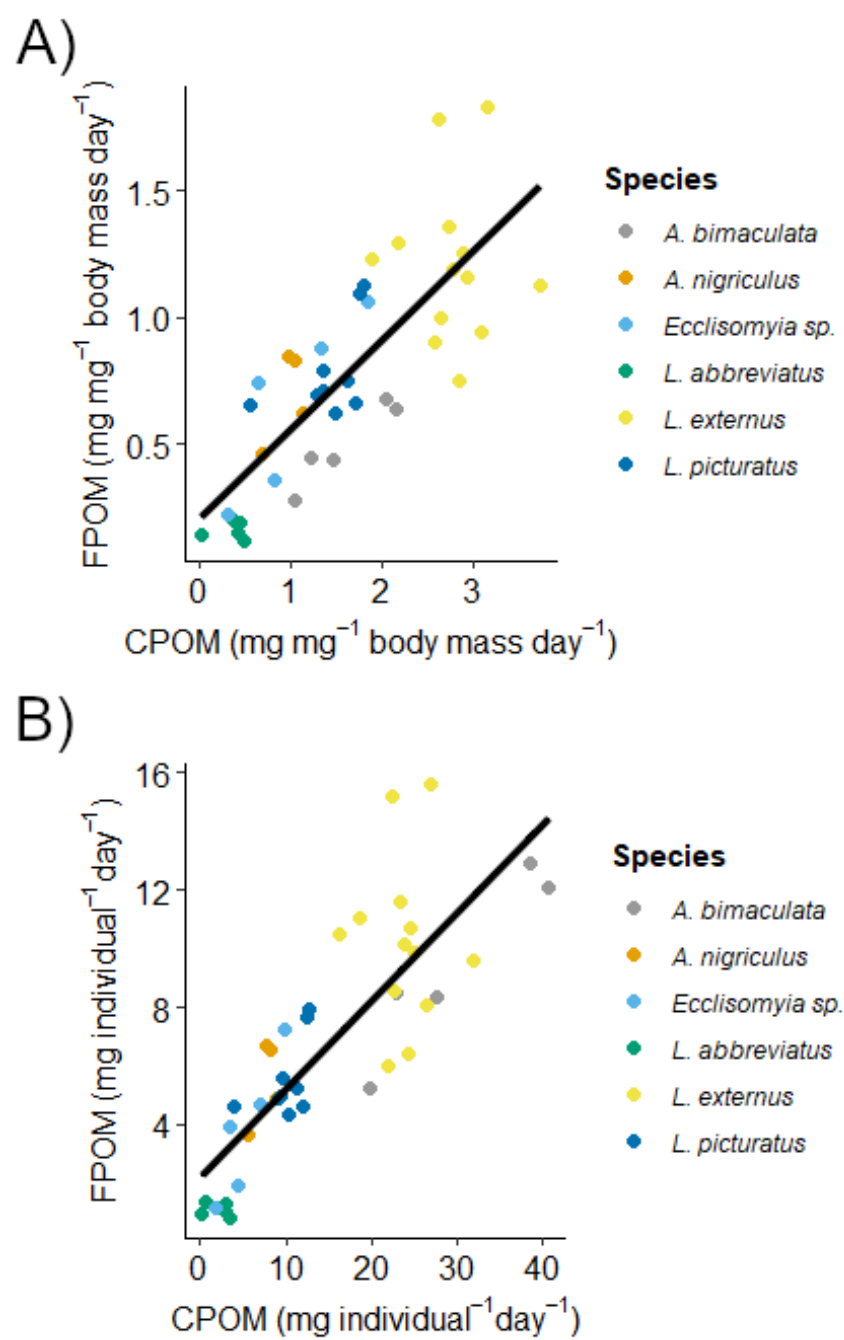
| <b>Single Trait Linear models: average mg FPOM indiv<sup>-1</sup> d<sup>-1</sup> = (Predictor Trait)</b> |          |           |          |                          |                                            |
|----------------------------------------------------------------------------------------------------------|----------|-----------|----------|--------------------------|--------------------------------------------|
| <b>Predictor Trait</b>                                                                                   | <b>F</b> | <b>df</b> | <b>p</b> | <b>adj R<sup>2</sup></b> | <b>Considered in full FPOM trait model</b> |
| Body Size (mg)                                                                                           | 1.45     | 1,3       | 0.315    | 0.1                      | No                                         |
| Diet % Total Detritus                                                                                    | 13.75    | 1,3       | 0.034*   | 0.76                     | Yes                                        |
| Diet % Coarse Detritus                                                                                   | 7.47     | 1,3       | 0.071*   | 0.62                     | Yes                                        |
| Diet % Fine Detritus                                                                                     | 1.81     | 1,3       | 0.27     | 0.16                     | Yes                                        |
| Diet % Total Algae                                                                                       | 0.67     | 1,3       | 0.47     | 0.09                     | No                                         |
| Diet % Total Chitin                                                                                      | 0        | 1,3       | 0.93     | 0.01                     | No                                         |
| N:P Excretion Ratio                                                                                      | 0.85     | 1,3       | 0.425    | 0.03                     | No                                         |
| Body Stoichiometry C:P                                                                                   | 1.78     | 1,3       | 0.27     | 0.16                     | Yes                                        |

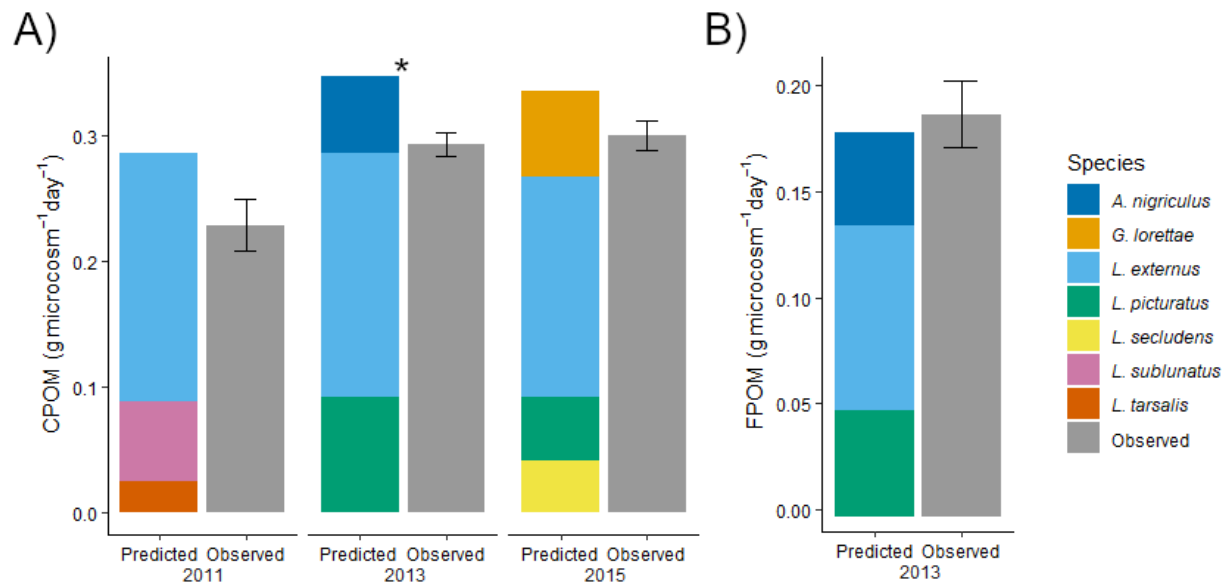


**Appendix 2: Figure S1.** Mean ( $\pm 1$  SE) per-microcosm daily CPOM (A) and FPOM (B)

processing rates. Asterisks indicate an invertebrate treatment is significantly different from the control within each experiment. (Dunnett's post-hoc multiple comparisons; Table 3 in main text).

**Appendix 2: Figure S2.** FPOM increases with CPOM processing within the larval caddisfly guild per unit body mass (A:  $F_{1,29} = 81.82$ ,  $p < 0.001$ ;  $FPOM = 0.319 \cdot CPOM - 0.0067$ ) and per-capita (B:  $F_{1,29} = 100.51$ ,  $p < 0.001$ ;  $FPOM = 0.319 \cdot CPOM - 0.127$ ). Color indicates species. Regression slopes are similar among species (CPOM\*Species, A:  $F_{5,29} = 1.16$ ,  $p = 0.354$ ; B:  $F_{5,29} = 1.06$ ,  $p = 0.407$ ). However, intercepts differ among species (Species, A:  $F_{5,29} = 2.81$ ,  $p = 0.035$ ; B:  $F_{5,29} = 2.35$ ,  $p = 0.066$ ); *L. externus* produces more FPOM than other caddisfly taxa (intercept coefficient, A: estimate = 1.25,  $t = 2.188$ ,  $p = 0.037$ ; B: estimate = 10.24,  $t = 2.061$ ,  $p = 0.048$ ). Values are from individual microcosms.





**Appendix 2: Figure S3.** Predicted and observed mixed assemblage microcosm processing for

CPOM (A) and FPOM (B). Predicted values were calculated by summing the products of microcosm estimated member species' mass-specific processing rates and their biomass in the mixed assemblage. Observed values (mean  $\pm$  1 SE) from mixed assemblage microcosms.

Asterisk indicates statistically significant difference between observed and expected values at  $p < 0.05$  (two-tailed one sample t-tests; 2011 df = 4 , 2013 & 2015 df = 3).

## Appendix 2: Supplemental Methods

*Animal traits predicting detritus processing: diet composition protocol used here and previously by (Wissinger et al. 1996, Wissinger et al. 2018)*

We collected ten individuals of each species from the same source habitats used to stock the 2019 experiment. Their digestive tracts were removed, homogenized, and preserved in 95% ethanol with Congo Red stain. After 4 days, 1 mL aliquots were transferred to Sedgewick–Rafter counting chambers (Wildlife Supply Company, Yulee, Florida) to separate particulate matter and quantify relative amounts of 1) coarse plant detritus >1 mm), 2) amorphous fine detritus, 3) algal cells, and 4) invertebrate exoskeletons stained by the dye. The percent contribution of each diet category was calculated as the area covered by each category divided by the total area covered by all diet items.

Wissinger, S. A., M. E. Perchik, and A. J. Klemmer. 2018. Role of animal detritivores in the breakdown of emergent plant detritus in temporary ponds. *Freshwater Science* **37**:826-835.

Wissinger, S. A., G. B. Sparks, G. L. Rouse, W. S. Brown, and H. Steltzer. 1996. Intraguild predation and cannibalism among larvae of detritivorous caddisflies in subalpine wetlands. *Ecology* **77**:2421-2430.

## APPENDIX 3

**Appendix 3: Table S1:** Taxon-specific distributions for percent of permanent pond hydroperiod spent in larval development.

| <b>Taxon</b>                            | <b>Mean</b> | <b>SD</b> |
|-----------------------------------------|-------------|-----------|
| <i>Acilius</i> A                        | 0.9         | 0.01      |
| <i>Acilius</i> L                        | 0.6         | 0.05      |
| <i>Aeshnid</i>                          | 0.7         | 0.025     |
| <i>Agabus</i> A                         | 0.7         | 0.05      |
| <i>Agabus</i> L                         | 1           | 0.05      |
| <i>A. deflata</i>                       | 0.51        | 0.05      |
| <i>A. nigriculus</i>                    | 0.34        | 0.05      |
| <i>Bezzia</i>                           | 0.9         | 0.01      |
| <i>Chaoborus</i> L                      | 1           | 0         |
| <i>Chironomini</i>                      | 0.9         | 0.01      |
| <i>Clams</i>                            | 1           | 0         |
| <i>Corixid</i> A                        | 0.9         | 0.01      |
| <i>Corixid</i> N                        | 0.9         | 0.01      |
| <i>Aedes</i>                            | 0.9         | 0.1       |
| <i>Lestes</i> and <i>Coenagrionidae</i> | 1           | 0.1       |
| <i>Dytiscus</i> A                       | 0.6         | 0.05      |
| <i>Dytiscus</i> L                       | 1           | 0.05      |
| Epibenthic <i>Cladocera</i>             | 0.7         | 0.1       |
| <i>L. externus</i>                      | 0.5         | 0.025     |
| <i>Branchinecta coloradensis</i>        | 0.4         | 0.05      |
| <i>Gerris</i> A                         | 1           | 0.05      |
| <i>Gerris</i> N                         | 1           | 0.05      |
| <i>Glossiphonia</i>                     | 1           | 0         |
| <i>G. lorretae</i>                      | 0.47        | 0.025     |
| <i>Haliplidae</i> A                     | 0.9         | 0.01      |
| <i>Hydrophilidae</i> A                  | 0.8         | 0.05      |
| <i>Hydrophilidae</i> L                  | 1           | 0.05      |
| <i>Hydroporinae</i> A                   | 0.8         | 0.05      |
| <i>Hydroporinae</i> L                   | 1           | 0.05      |
| <i>Ilybius</i> A                        | 0.8         | 0.05      |
| <i>Ilybius</i> L                        | 1           | 0.05      |
| <i>Hydrachnidia</i>                     | 1           | 0         |
| <i>N. hostilis</i>                      | 0.47        | 0.05      |
| <i>Nephelopsis</i>                      | 1           | 0         |
| <i>Oligochaetes</i>                     | 1           | 0         |
| <i>Ostracoda</i>                        | 0.9         | 0.025     |

**Appendix 3: Table S1** (continued)

|                      |      |       |
|----------------------|------|-------|
| <i>L. picturatus</i> | 0.47 | 0.05  |
| <i>Somatochlora</i>  | 1    | 0.025 |
| <i>Vellidae</i> A    | 1    | 0.1   |
| Zooplankton          | 0.7  | 0.03  |

**Appendix 3: Table S2:** Linear models of mean taxon-specific contributions to whole-pond seasonal N and P supply across hydroperiod classifications. All parameters in the selected models (bold) were significant in ANOVA tests (Appendix 3: Table S3, F tests  $p < 0.05$ ). Individual parameters were evaluated by removal and calculating the  $\Delta$ AIC scores relative to the selected models. Reduced models (*italics*) are listed in order of decreasing  $\Delta$ AIC to present the rank-order of each parameter's strength as a predictor of supply. \*\*\* denotes AIC-best predictor of supply. Units for each parameter are as follows: N or P supply =  $\mu\text{g N or P pond}^{-1} \text{ season}^{-1}$ ; Taxon = taxonomic identity; Biomass =  $\mu\text{g taxon pond}^{-1}$ , Excretion =  $\mu\text{g N or P mg taxon}^{-1} \text{ day}^{-1}$ ; Development length = days.

| Model                                                         |                         | AIC            | $\Delta$ AIC   | R <sup>2</sup><br>(adjusted) |
|---------------------------------------------------------------|-------------------------|----------------|----------------|------------------------------|
| N Supply ~ Taxon + Biomass + N Excretion + Development Length | (Full Model)            | 5979.83        |                | 0.3851                       |
| <b>N Supply ~ Taxon + Biomass + N Excretion</b>               | <b>(Selected Model)</b> | <b>5979.14</b> | <b>-0.69</b>   | <b>0.3862</b>                |
| <i>N Supply ~ Taxon + N Excretion</i>                         | <i>(-Biomass)***</i>    | <i>6016.21</i> | <i>37.07</i>   | <i>0.1843</i>                |
| <i>N Supply ~ Taxon + Biomass</i>                             | <i>(-N Excretion)</i>   | <i>5986.12</i> | <i>6.98</i>    | <i>0.3505</i>                |
| <i>N Supply ~ Biomass + N Excretion</i>                       | <i>(-Taxon)</i>         | <i>5961.58</i> | <i>-17.56</i>  | <i>0.3128</i>                |
| P Supply ~ Taxon + Biomass + P Excretion + Development Length | (Full Model)            | 5516.56        |                | 0.5118                       |
| <b>P Supply ~ Taxon + Biomass</b>                             | <b>(Selected Model)</b> | <b>5514.67</b> | <b>-1.89</b>   | <b>0.5153</b>                |
| <i>P Supply ~ Taxon</i>                                       | <i>(-Biomass)***</i>    | <i>5595.31</i> | <i>80.64</i>   | <i>0.1039</i>                |
| <i>P Supply ~ Biomass</i>                                     | <i>(-Taxon)</i>         | <i>5393.1</i>  | <i>-121.57</i> | <i>0.4641</i>                |

**Appendix 3: Table S3:** F Tests for full and selected linear models of among hydroperiod taxon-specific supply presented in Appendix 3: Table S2.

| <b>Full Model: N Supply ~ Taxon + Biomass + N Excretion + Development Length</b> |    |          |          |         |        |
|----------------------------------------------------------------------------------|----|----------|----------|---------|--------|
|                                                                                  | df | Sum Sq   | Mean Sq  | F       | p      |
| Taxon                                                                            | 43 | 1.84E+20 | 4.29E+18 | 2.0621  | 0.002  |
| Biomass                                                                          | 1  | 6.69E+19 | 6.69E+19 | 32.1694 | <0.001 |
| Dev Length                                                                       | 1  | 3.45E+18 | 3.46E+18 | 1.662   | 0.2    |
| N Excretion                                                                      | 1  | 1.03E+19 | 1.03E+19 | 4.9899  | 0.028  |
| Residuals                                                                        | 85 | 1.77E+20 | 2.08E+18 |         |        |
| <b>Selected Model: N Supply ~ Taxon + Biomass + N Excretion</b>                  |    |          |          |         |        |
|                                                                                  | df | Sum Sq   | Mean Sq  | F       | p      |
| Taxon                                                                            | 43 | 1.84E+20 | 4.29E+18 | 2.0711  | 0.002  |
| Biomass                                                                          | 1  | 6.69E+19 | 6.69E+19 | 32.3108 | <0.001 |
| N Excretion                                                                      | 1  | 1.25E+19 | 1.25E+19 | 6.0548  | 0.016  |
| Residuals                                                                        | 86 | 1.78E+20 | 2.08E+18 |         |        |
| <b>Full Model: P Supply ~ Taxon + Biomass + P Excretion + Development Length</b> |    |          |          |         |        |
|                                                                                  | df | Sum Sq   | Mean Sq  | F       | p      |
| Taxon                                                                            | 43 | 6.63E+18 | 1.54E+17 | 2.4809  | <0.001 |
| Biomass                                                                          | 1  | 4.67E+18 | 4.67E+18 | 74.0685 | <0.001 |
| Dev Length                                                                       | 1  | 1.27E+16 | 1.27E+16 | 0.2038  | 0.6528 |
| P Excretion                                                                      | 1  | 6.67E+16 | 6.67E+16 | 1.0721  | 0.3034 |
| Residuals                                                                        | 85 | 5.28E+18 | 6.21E+16 |         |        |
| <b>Selected Model: P Supply ~ Taxon + Biomass</b>                                |    |          |          |         |        |
|                                                                                  | df | Sum Sq   | Mean Sq  | F       | p      |
| Taxon                                                                            | 43 | 6.63E+18 | 1.54E+17 | 2.4809  | <0.001 |
| Biomass                                                                          | 1  | 4.67E+18 | 4.67E+18 | 74.0685 | <0.001 |
| Residuals                                                                        | 87 | 5.36E+18 | 6.16E+16 |         |        |

## APPENDIX 4

**Appendix 4: Table S1. Mixed model F test of species relative abundance in permanent ponds.** Year, species, range expansion period, and their interactions were modeled as fixed effects. Model also included pond as a random effect and a third-order autocorrelation function (ACF) of year nested within pond. Bold p\* denote model term is statistically significant at  $\alpha=0.05$ .

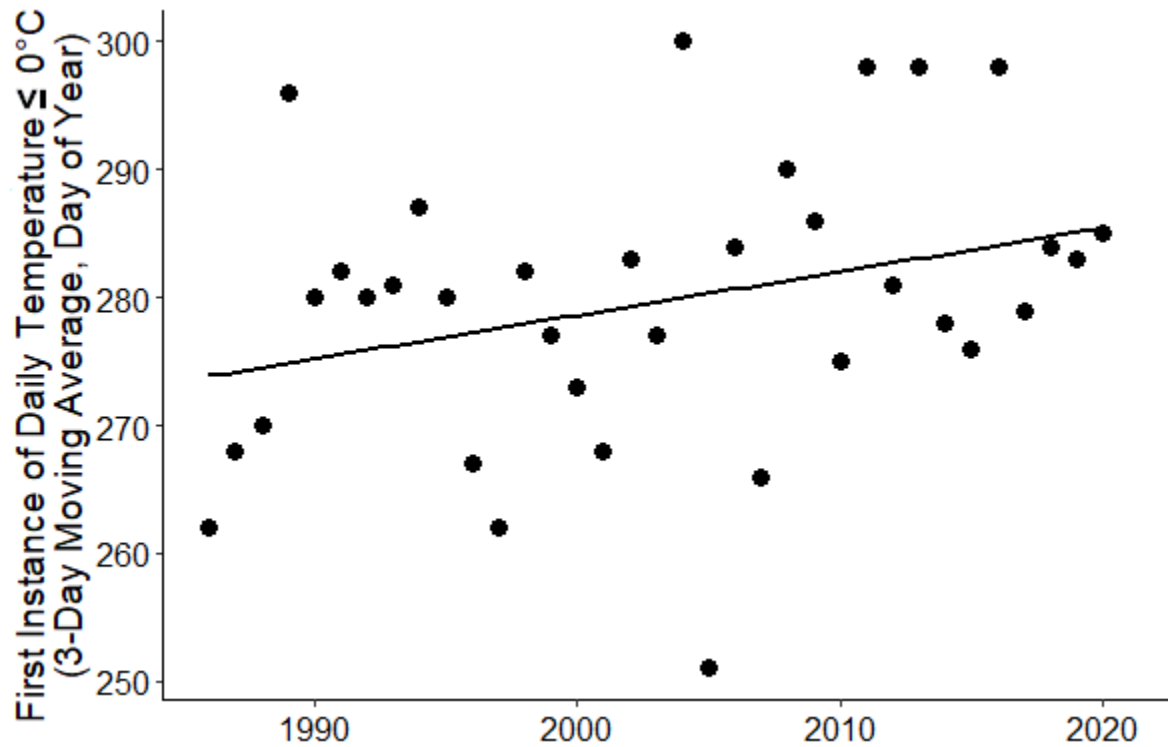
| <b>term</b>                  | <b>df (num,den)</b> | <b>F</b> | <b>p</b>          |
|------------------------------|---------------------|----------|-------------------|
| (Intercept)                  | 1,684               | 1.69     | 0.195             |
| Year                         | 1,684               | 1.73     | 0.189             |
| Species                      | 5,684               | 1.73     | 0.126             |
| Range Expansion              | 3,684               | 2.30     | <b>0.076*</b>     |
| Year:Species                 | 5,684               | 1.75     | 0.122             |
| Year:Range Expansion         | 3,684               | 2.31     | <b>0.075*</b>     |
| Species:Range Expansion      | 15,684              | 3.25     | <b>&lt;0.001*</b> |
| Year:Species:Range Expansion | 15,684              | 3.25     | <b>&lt;0.001*</b> |

**Appendix 4: Table S2. Mixed model F tests of species groups' relative contributions to ecosystem processes in permanent ponds.** Year, species group (i.e., dominant resident, subdominant resident, invader), invasion period, and their interactions were modeled as fixed effects. Models also included pond as a random effect, and a third-order autocorrelation function (ACF) of year nested within pond. Bold p\* denote model term is statistically significant at  $\alpha=0.05$

| term                       | <u>P Supply</u> |       |                   | <u>N Supply</u> |       |       | <u>CPOM Processing</u> |       |               |
|----------------------------|-----------------|-------|-------------------|-----------------|-------|-------|------------------------|-------|---------------|
|                            | df<br>(num,den) | F     | p                 | df<br>(num,den) | F     | p     | df<br>(num,den)        | F     | p             |
| (Intercept)                | 1,339           | 0.212 | 0.645             | 1,339           | 0.156 | 0.693 | 1,339                  | 0.008 | 0.931         |
| Year                       | 1,339           | 0.209 | 0.648             | 1,339           | 0.154 | 0.695 | 1,339                  | 0.007 | 0.931         |
| Group                      | 2,339           | 1.951 | 0.144             | 2,339           | 0.133 | 0.876 | 2,339                  | 1.051 | 0.351         |
| Range Expansion            | 3,339           | 2.982 | <b>0.031*</b>     | 3,339           | 0.104 | 0.958 | 3,339                  | 0.614 | 0.606         |
| Year:Group                 | 2,339           | 1.972 | 0.141             | 2,339           | 0.139 | 0.870 | 2,339                  | 1.056 | 0.349         |
| Year:Range Expansion       | 3,339           | 2.988 | <b>0.031*</b>     | 3,339           | 0.104 | 0.958 | 3,339                  | 0.616 | 0.605         |
| Group:Range Expansion      | 6,339           | 5.494 | <b>&lt;0.001*</b> | 6,339           | 1.440 | 0.199 | 6,339                  | 3.114 | <b>0.006*</b> |
| Year:Group:Range Expansion | 6,339           | 5.507 | <b>&lt;0.001*</b> | 6,339           | 1.439 | 0.199 | 6,339                  | 3.120 | <b>0.006*</b> |

**Appendix 4: Table S3. Mixed model F tests of caddisfly assemblage-total contributions to ecosystem processes in permanent ponds.** Year, invasion period, and their interaction were modeled as fixed effects. Models also included pond as a random effect, and a third-order autocorrelation function (ACF) of year nested within pond. Bold p\* denote model term is statistically significant at  $\alpha=0.05$ .

| term                 | <u>P Supply</u> |      |       | <u>N Supply</u> |      |       | <u>CPOM Processing</u> |      |       |
|----------------------|-----------------|------|-------|-----------------|------|-------|------------------------|------|-------|
|                      | df<br>(num,den) | F    | p     | df<br>(num,den) | F    | p     | df<br>(num,den)        | F    | p     |
| (Intercept)          | 1,109           | 2.41 | 0.124 | 1,109           | 1.26 | 0.265 | 1,109                  | 2.56 | 0.112 |
| Year                 | 1,109           | 2.42 | 0.123 | 1,109           | 1.27 | 0.262 | 1,109                  | 2.58 | 0.111 |
| Range Expansion      | 3,109           | 0.72 | 0.541 | 3,109           | 1.47 | 0.227 | 3,109                  | 0.96 | 0.413 |
| Year:Range Expansion | 3,109           | 0.72 | 0.542 | 3,109           | 1.47 | 0.227 | 3,109                  | 0.96 | 0.413 |



**Appendix 4: Figure S1. Onset of autumn freeze is occurring later at Schofield Pass (USDA NRCS SNOTEL Site 737).** A 3-day moving average daily temperature was used to determine the first date of subfreezing ( $\leq 0^{\circ}\text{C}$ ) autumn temperature at the Schofield Pass SNOTEL Site 737 (data retrieved from (15); ( $F_{1,33} = 3.56$ ,  $p = 0.068$ ,  $\text{adj } R^2 = 0.07$ )). Relative to the Mexican Cut, this weatherstation is approximately 2 km southeast and 300 m lower in elevation.

## Appendix 4: Materials and Methods

### *Study sites and larval caddisfly natural history*

Ponds were located within the Mexican Cut Nature Preserve, a pristine, subalpine (3560 m) wilderness area owned by The Nature Conservancy and managed by the Rocky Mountain Biological Laboratory (RMBL) in the Elk Mountains of central Colorado. The Mexican Cut is a glacial cirque comprised of two shelves with 60+ kettle-pond wetland habitats, all with similar basin substrate composition and geomorphology, emergent and riparian vegetation, and water chemistry (1). Ponds at the Mexican Cut are ecologically representative of high-elevation kettle-pond wetlands throughout the Rockies and other mountainous regions (2).

Annual censuses of pond communities since 1989 indicate that larval cased caddisflies (five species of Limnephilidae, one of Phryganeidae) dominate animal biomass, increasing from 30 to 70 % of animal biomass from permanent to temporary ponds (1). An additional Limnephilid caddisfly species, *Hesperophylax occidentalis*, is present at the Mexican Cut, but is restricted to the intermittent streams draining an alpine lake above the cirque's upper shelf. Larval caddisflies in lentic habitats were surveyed annually for 25/30 years between 1989 and 2019 with single 0.33 m<sup>2</sup> benthic D-net sweeps at the north, east, south, and west sides of 3-7 permanent, 2-11 semi-permanent, and 1-7 temporary ponds. Samples were collected between late June and mid-July each year, approximately 2-3 weeks after pond ice-out. This seasonal timing provides the best annual snapshot of the caddisfly assemblage given their staggered life histories. For example, four taxa overwinter as diapausing eggs that hatch when inundated with spring snowmelt. Among these, the temporary pond specialist *Asynarchus nigriculus* generally completes larval development and pupates before species adapted to permanent hydroperiods (*Limnephilius externus*, *L. picturatus*, *Grammotaulius lorretae* or *Anabolia bimaculata*).

Whereas, *Agrypinia deflata* hatch in the fall, overwinter as larvae, and complete development in mid-late summer around the same time as the Limnephilids. Finally, the most recent upslope invader, *Nemotaulius hostilis*, has a similar life history strategy as *Ag. deflata*, though it typically completes larval development earlier in the summer.

### *Predicting caddis assemblage nutrient supply and detritus processing*

Previous work demonstrated that although there is high interspecific variation among the larval caddisflies' species-specific nutrient excretion, within a species excretion is strikingly consistent throughout the day and among pond habitats along an elevational gradient from montane to subalpine (3). Furthermore, excretion declines predictably with developmental instar, though differences among species-specific instars are small relative to interspecific differences. Additionally, there is comparable interspecific variation in detritus processing rates measured in laboratory microcosms over the course of larval development (4), and species' microcosm processing rates are generally within 20 % of species-specific processing rates measured in-situ with littoral cages (5, 6). Furthermore, this previous work demonstrates that additive predictions of caddisfly assemblage detritus processing calculated by summing the products of species' mass-specific processing rate and biomass provide accurate estimates of in-situ assemblage-total detritus processing (4). Thus, we predicted caddisfly species' contribution to ecosystem processes over time as the products of average density, final instar mass, larval development rate, and either nutrient excretion rate or detritus processing rate.

To incorporate variation in species-specific detritus processing and nutrient excretion rates into our additive predictions of caddisfly contributions to ecosystem processes, we adapted a random sampling framework from (7). Specifically, pond-level calculations of species' nutrient

supply and detritus processing contributions across the long-term survey dataset were repeated 1000 times with randomly sampled species-specific rates in each iteration. Rather than estimating the caddisfly assemblage's contribution to ecosystem processes for the empirical survey ponds, we instead use our pond-year caddisfly assemblage densities to estimate their contributions in an average pond of each hydroperiod classification following the methods of (7). This approach is advantageous for three reasons. First, larval caddisflies tend to congregate in the shallow littoral habitats of pond perimeters near emergent sedge vegetation and are not distributed evenly across deeper pond centers. Thus, our census samples collected near pond shorelines likely over-estimate caddisfly population density in an average square meter of pond benthos. The random sampling framework corrects for this by simulating a random pond of the appropriate hydroperiod classification for each empirical pond-year caddis assemblage. Specifically, random samples from hydroperiod-specific normal distributions of pond area and % of pond area habitable to larval caddisflies are used to rescale empirical caddisfly assemblage densities to a square meter of total pond area. Second, we do not have data describing total pond area or area habitable to larval caddisflies for all survey ponds. Because we cannot appropriately rescale caddisfly population densities for all empirical ponds, simulating a random pond for each pond-year caddisfly assemblage 1000 times allows us to utilize all pond-year survey samples to predict the caddisfly assemblage's hydroperiod-average contributions to ecosystem processes. Likewise, third, although some permanent ponds were surveyed annually ( $n = 2$  of 7), others were surveyed opportunistically. The simulation framework thus leverages all pond-year surveys to estimate the caddisfly assemblage's hydroperiod-year average contributions.

In addition to the three confirmed upslope invasions, we suspect that individuals of a fourth invading species *Anabolia bimaculata* were occasionally misidentified as the 2<sup>nd</sup> upslope

invader *G. lorreatae*. However, the two species' N excretion and detritus processing rates are not statistically different (3, 4), and thus we pooled both taxa's excretion and detritus processing rates and used a grand mean final instar mass to predict *G. lorreatae*'s contribution. Lastly, because detritus processing was not directly measured for *A. deflata* or *N. hostilis*, their processing rates were predicted using the trait-based model in (4) that explained 88 % of interspecific variation in processing rates.

Predicted species-specific ecosystem process contributions for each pond-year were averaged across all iterations to estimate species' contributions in an average pond of the appropriate hydroperiod classification. Thus, within each hydroperiod classification, among-pond variation in species-specific predicted contributions is due to within-hydroperiod variation in species' density, standardized for known within-hydroperiod variation in pond size and area habitable to larval caddisflies. Species-specific contributions were then summed within pond-years to estimate the caddisfly assemblage's total contribution. To compare species' contributions in subsequent statistical analyses and standardize for interannual variation in the assemblage-total, species' relative contributions to the assemblage-totals were calculated. Finally, we grouped subdominant species' and upslope invader species' relative contributions separately for comparison with the presumed dominant resident *L. externus* (8, 9).

Species average pond-specific contributions (e.g., species average contribution across all simulation iterations within a given pond) were used estimate aggregate variability of caddisflies contributions to ecosystem processes among ponds following methods of (10, 11). Specifically, each species' among-pond variance in process contribution were summed and added to 2x the sum of covariances among all species pairs within each pond-year. We then square-root transformed aggregate variability to improve normality for statistical analysis. Notably, this

transformation also returns aggregate variability to the original data's scale, as untransformed aggregate variability shares the squared units of its variance and covariance components.

### *Statistical analyses*

To determine if species groups' relative contributions differed among or changed during time periods that correspond with upslope invasions, we created a categorical variable called "Invasion" to group years between sequential upslope invasions. The category "pre-invasion" was applied to 1997 and earlier, whereas years between 1998 – 2005 were categorized as "1<sup>st</sup> invasion", 2006 – 2015 as "2<sup>nd</sup> invasion", and 2016 – 2019 as "3<sup>rd</sup> invasion".

All statistical analyses were completed in R 4.0.2 (12). The package "lme4" was used to fit mixed effects models to caddisfly relative abundances, assemblage-total predicted ecosystem processes, and species' relative contributions over time within each hydroperiod classification (13). For mixed models with caddisflies total predicted ecosystem process as the response variable, Year and Invasion were modeled as fixed effects along with their interaction. Whereas, when relative contributions or abundance were the response variable, Year, Invasion, Group (for relative contributions, i.e., dominant resident, subdominant residents, invaders) or Species (for relative abundance), and their interactions were modeled as fixed effects. All mixed models also included Pond as a random effect, and a third-order autocorrelation function (ACF) of year nested within pond. Inclusion of and appropriate ACF order was determined by examining ACF plots for dominant taxon relative abundance; the ACF function for *L. externus* relative abundance exceeded the  $\alpha = 0.05$  confidence region at lag = 3.

Following mixed effects models of relative contributions with significant interactions (e.g., Year\*Invasion\*Species, Year\*Invasion\*Group, Year\*Species, or Year\*Group) the R package “emmeans” (14) was used to compare the slope estimates of each Group’s relative contribution over time (Year) within each Invasion. Slope estimates were compared against a null hypothesis of slope = 0 using linear contrasts.

Finally, we compared aggregate variability to caddisfly evenness (J, Pielou) with simple linear models (model: square-root transformed aggregate variability in N or P supply or detritus processing = evenness).