

Looks good to me!

The role of non-caddisfly taxa on detrital processing in montane ponds

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Abstract

Understanding the processing of detritus by different macroinvertebrates in montane ponds is important because either directly or indirectly most energy and nutrients flow through the detrital pathway. Understanding the degree to which macroinvertebrate species vary in detrital processing is central to predicting how climate- or other environmentally-induced shifts in species abundance and distribution will effect detritus processing and subsequent energy flow and nutrient cycling. Thus, our main question is, what is the role of non-caddisfly taxa on detrital processing in montane ponds? We tested three hypotheses 1) Compared to *L. externus*, chironomids (non-biting midges) effects may be small because of their small body size. Alternatively, because metabolic activity is inversely related to body size, midge per unit body mass effects may be equal or greater than *L. externus*. Size can play a large factor in how much an animal can consume. Next, we hypothesize that 2) non-caddisfly taxa, such as lymnaeidae and amphipods, will have lower detrital breakdown rates than caddisfly taxa in microcosm. Non-caddisfly taxa spend their entire life cycle in the ponds, they may have higher processing rates in natural ponds. Finally, we hypothesize that 3) caddisflies (*L. externus*) dominate detrital processing because they are processing during their period of maximum growth. Using a randomized block experimental design, we tested the effects on detrital sedge processing of five different species of aquatic invertebrates that inhabit high elevation ponds near Gothic, Colorado, USA. We compared differences in the five species' ability to breakdown detritus from coarse to fine particles to one another and to a control treatment with no animals.. We found that two species, a caddisfly *L. externus*, used in previous experiments and used here as a reference, and chironomids processed the most sedge detritus, and detritus processing per biomass effects of chironomids were the highest. Thus, in addition to caddisflies, which have been considered the dominant detritivores in these montane ponds, chironomids may be important detritivores especially if their high per biomass effects are combined with high population biomass in natural ponds.

Introduction

Detritus, or dead plant material, is an overlooked source of energy that supplies nutrients to a large population of consumers such as microbes and arthropods (Solomon et al. 2008). Recent studies demonstrate how pivotal this source of energy is as a basal resource for many animals and for carbon cycling in ecosystems. This “brown side” of the foodweb based on detrital energy pathways also influences the autotrophic “green side” of the foodweb by recycling carbon and other nutrients from detritus back to autotrophs and other higher trophic levels (Wolkovich. 2010). Thus, detritus and detritivores can change food web structure and ecosystem function by controlling nutrient cycling.

Detrital processing or decomposition is the process of detritus breakdown often from leaves (or coarse particulate organic matter, CPOM) to fine particulates (or fine particulate organic matter, FPOM). Animals or physical processes, such as stream flow or waves, are critical for CPOM to FPOM conversion. Detritivorous animals consume larger particles but can be “sloppy feeders”, and thus some fine particles fall to the bottom or drift away and are available to other organisms. A consequence of CPOM to FPOM conversion is an increase in the surface area to volume ratio, which enables more bacteria, algae, and fungi to colonize the detritus and further enhances processing. Microbial conditioning also indirectly accelerates breakdown by improving detritus resource quality (e.g nutritional value) for animal consumers.

Lentic ecosystems like montane ponds make up more fresh water surface area than large lakes world wide (Downing et al. 2006). Thus, despite their small size, montane ponds cumulatively play a large role in carbon dynamics. In addition to breakdown of coarse organic matter into finer particles that provides food and energy sources for microbes and animals, detritus processing in montane ponds also releases readily available inorganic forms of nutrients such as N & P back into the water column. This makes montane ponds hot spots for carbon cycling (DeVecchia 2019).

Montane ponds have been studied at RMBL to better understand the role of animals in the processing detritus, including the release of nutrients and linkages between the brown and green food webs and as a food source for invertebrates. Previous work conducted at RMBL suggests that the larval caddisfly, *Limnephilus externus*, is the dominant detritivore because it dominates benthic invertebrates biomass in most ponds around RMBL and has high detrital processing rates (Balik et al. in prep). During their larval stage, caddisflies use silk to weave detritus into a shelter or case (hence the name caddisfly) but they also consume and process detritus by converting large fragments of detrital sedge into finer particles. Caddisfly larvae inhabit these ponds from April to September after overwintering as eggs. In contrast, non-caddisfly invertebrates such as amphipods, isopods, and oligochaete worms spend their entire life cycle in the aquatic environment. These non-caddisfly invertebrates are known to play a role in detritus processing in other ecosystems (Anderson 1979), but the magnitude of their impacts on detritus processing relative to that of larval caddisflies is unclear.

Thus, our main question is, what is the role of non-caddisfly taxa on detrital processing in montane ponds? Because not all non-caddisfly taxa were abundant enough in ponds to warrant

inclusion in the experiment, we included a caddisfly, *Ecclisomyia* sp., for which detrital processing had not been measured. We hypothesize that 1) Compared to *L. externus*, chironomids (non-biting midges) effects may be small because of their small body size. Alternatively, because metabolic activity is inversely related to body size, midge per unit body mass effects may be equal or greater than *L. externus*. Size can play a large factor in how much an animal can consume. Next, we hypothesize that 2) non-caddisfly taxa, such as lymnaeidae and amphipods, will have lower detrital breakdown rates than caddisfly taxa in microcosm. Non-caddisfly taxa spend their entire life cycle in the ponds, they may have higher processing rates in natural ponds. We hypothesized that the caddisfly *Ecclisomyia* sp. would process less detritus than *L. externus* because they are known to prefer algae in their diet. Finally, we hypothesize that 3) caddisflies (*L. externus*) dominate detrital processing because they are processing during their period of maximum growth.

Methods

Our study system was the montane ponds and organisms that feed on detritus. These ponds receive the majority of their detrital inputs as senesced sedge, *Carex aquatilis*, around the edges of most and extending into the center of some ponds. Sedge senesces in the fall and remains in the ponds as intact leaves until the following summer. Aquatic invertebrates are the main detritivores in these ponds, though there are snails and tadpole larvae, which can enhance detrital processing in other systems (Wissinger et al 1999). We performed the experiment in a weather port located at the Rocky Mountain Biological Laboratory in Gothic, Colorado. We used 36 plastic rectangular tanks 51 x 38 x 13 cm as the experimental units. Treatments were distributed throughout the weatherport in a randomized block design, so that each block (i.e., tables located in the center, and N, E, W sides) contained an equal number of replicates of each treatment to control for any spatial positioning effects on the tanks, such as light, temperature, doorway, etc. We added 14.75 liters of pond water filtered to remove invertebrates to each microcosm, along with senesced (brown, non-living) sedge collected from ponds M1 and M2. Sedge was air dried and 20 g $\pm$ 0.1 g added to each tank on June 29th and allowed to become rehydrated with water and re-conditioned with microbes such as algae, bacteria and fungi for 3 days prior to adding animals to tanks. Filtered water was added as needed to tanks during the experiment to maintain this volume.

We quantified species-specific detrital processing using five different treatments with similar biomasses of individual invertebrate species, with one set of 6 replicate tanks as the control with no animals added. The control measured microbially mediated sedge detritus breakdown in the absence of animals. The five additional treatments included: 1) 20 individual *Limnephilus externus*, which is the dominant detritivorous invertebrate by biomass in these ponds and has been used as a reference in previous experiments that measured detrital processing rates of nine larval caddisfly taxa. Thus, we included *L. externus* to compare our estimates with experiments conducted in previous years. 2) 25 individual *Ecclisomyia* sp., a caddisfly found

primarily in flow-through beaver ponds that is recognized as a detrital processor in other systems (Cowan 1983). The remaining three treatments were non-caddisfly taxa that we hypothesized may equal or surpass caddisfly detrital processing rates based on their impacts on detritus dynamics in other ecosystems, the duration of time they are in the ponds relative to caddisflies, their feeding preferences and mouthparts, or some combinations of these characteristics. 3) 25 amphipods, an invertebrate that is a common detritivore in streams and ponds worldwide. 4) Lymnaeidae, a common freshwater gastropod known for inhabiting ponds with vegetation. 5) Chironomids, the most abundant invertebrate in these ponds (Balik et al in prep) and can be an important detrital processor (Henriques-Oliveira 2003).

The primary response variable was the mass of sedge lost based on the difference between the mass added at the start of the experiment and the mass remaining at the end (g dry sedge start - g dry sedge end). To measure the sedge remaining in each tank at the end of the experiment, we removed, identified, and counted the animals in each tank, then removed the coarse and fine sedge from each tank by hand and by poured the water out of the tanks through a nested set of sieves containing a 1 mm and 0.063 mm sieve. Sedge collected on the 1 mm sieve was considered coarse particulate matter (CPOM) and includes both intact sedge as well as larger sedge particles processed by the detritivores. Whereas, sedge that passes through the 1 mm sieve onto the 0.063 mm sieve was considered fine particulate matter (FPOM) and included only sedge processed into finer particles by detritivores. The amount of CPOM converted to FPOM was the estimated based on the mass remaining divided by the number of days animals processed sedge (i.e., before pupating or experiment end), and FPOM was standardized for differences in animal biomass among treatments by dividing by animal biomass in each tank. We then calculated the mass loss of coarse particulate sedge due to both consumption or conversion to FPOM as:

$$\text{g sedge day}^{-1} = (\text{g sedge start} - \text{g sedge end})/\text{days}$$

To compare differences in the CPOM consumed and converted to FPOM among treatments, we standardized by animal biomass in each tank and the number of days animals processed sedge as follows:

$$\text{g sedge g animal}^{-1} \text{ day}^{-1} = ((\text{g dry sedge start} - \text{g dry sedge end})/\text{g animal tank}^{-1})/\text{days}.$$

We tested for differences among any of the treatments using a one-way multivariate analysis of variance (MANOVA) to control for type II error associated with the potential intercorrelation of the coarse and fine particulate sedge response variables. We proceeded with individual ANOVAs for fine and coarse sedge since the MANOVA was significant. To determine whether any of the five treatments with animals differed from the control with no animals, we performed a one-sided Dunnett's post hoc test to explore differences in daily processing rates among each of the animal treatments and the no-animal control. Next, we used a

two-sided Dunnett's test to explore differences in per unit biomass processing rates among each of the non-caddisfly treatments and the reference treatment with the caddisfly *L. externus* by specifying *L. externus* as the "control". To assess differences among the *Ecclisomyia* and the three non-caddisfly treatments, we performed a Tukey's post hoc multiple comparisons test.

Results

The MANOVA on daily CPOM breakdown and FPOM production rates revealed a significant effect of treatment ($F_{5,24}=7.75$, $p<0.001$). There was no block effect ($F_{1,24}=0.78$, $p=0.471$) and no block by treatment interaction effect ($F_{5,24}=0.74$, $p=0.680$). Subsequent univariate ANOVAs on daily CPOM breakdown and FPOM production indicated significant differences among treatments for both response variables (CPOM: $F_{5,24}=13.09$, $p<0.001$; FPOM: $F_{5,24}=14.67$, $p<0.001$), and there were no block or block by treatment interaction effects in either ANOVA (block: $F_{5,24}=0.355$, $p=0.874$; block×treatment: $F_{5,24}=0.664$, $p=0.654$).

Dunnett's post hoc tests' indicated that only *L. externus* differed from the control in daily CPOM breakdown rates (Fig.1, $P=0.0333$) and FPOM production rates (Fig.2, $P=0.126$). *L. externus* daily CPOM breakdown was nearly 3X more than the control and FPOM production was 2X more than the control. Tukey's post hoc tests indicated no differences in daily CPOM and FPOM among *L. externus* and the other four taxa ($P>0.05$).

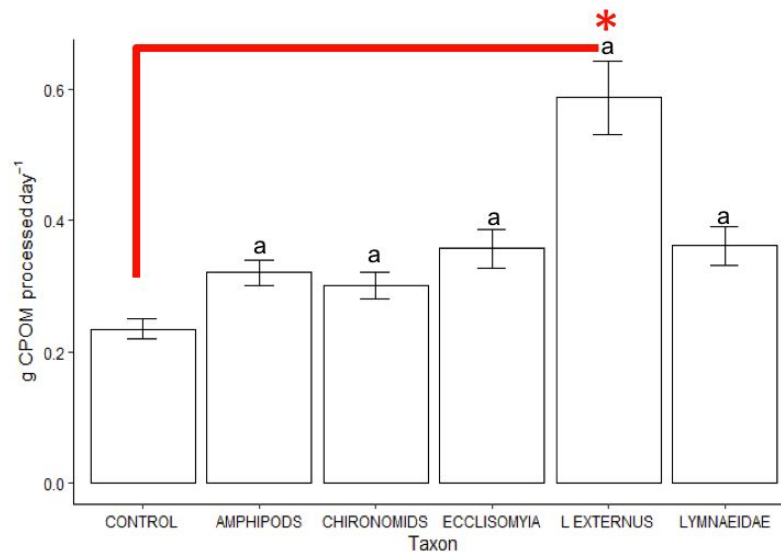


Figure 1. The daily breakdown rate of coarse particulate organic matter, or detritus > 1 mm was highest in the *L. externus* treatment compared to the control with no animals. The four other treatments (including *Ecclisomyia* sp. A caddisfly) were not different from the control. Bars show the mean and error bars are 1 standard error of the mean. The control contained sedge but no macroinvertebrates. Asterisk shows significant results of Dunnett's comparison with the control. Letters show results of Tukey's comparisons among treatments with animals.

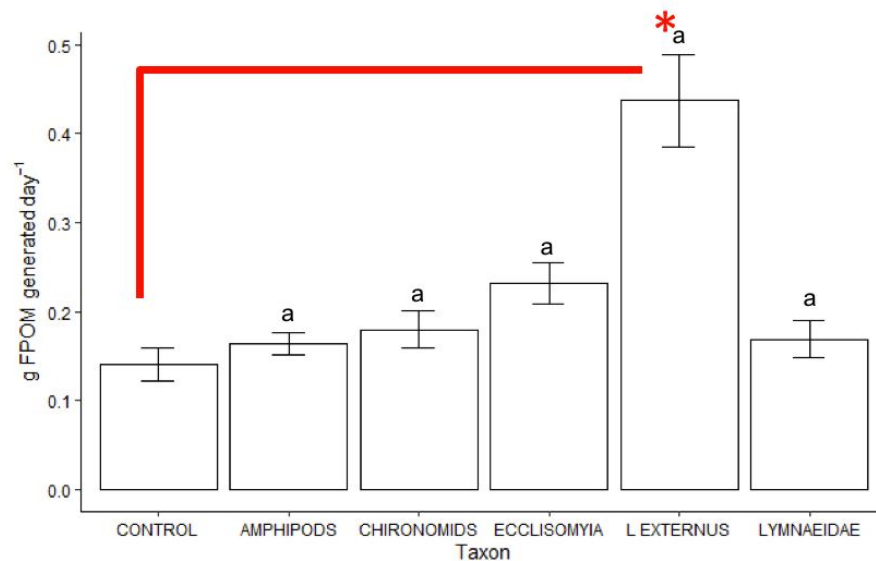


Figure 2. The daily production of fine particulate organic matter, or detritus $1 < \text{mm}$ was highest in the *L. externus* treatment compared to the control with no animals. The four other treatments were not different from the control. Bars show the mean and error bars are 1 standard error of the mean. The control contained sedge but no macroinvertebrates. Asterisk shows significant results of Dunnett's comparison with the control. Letters show results of Tukey's comparisons among treatments with animals.

Dunnett's test also revealed that daily rates of breakdown of CPOM and production of FPOM by the other four taxa were not different from the control (Fig. 1 and 2, lymnaeid snails: CPOM, $P = 0.443$; FPOM $P = 0.448$; amphipods: CPOM, $P = 0.321$, FPOM, $P = 0.5$; *Ecclisomyia*: CPOM, $P = 0.335$; FPOM, $P = 0.487$ chironomid midges: CPOM, $P = 0.483$; FPOM, $P = 0.495$).

The MANOVA of daily CPOM breakdown and FPOM production rates standardized for invertebrate biomass in each treatment revealed a significant effect of treatment ($F_{5,21} = 25.799$, $p < 0.001$), and no block ($F_{1,21} = 0.129$, $p = 0.723$) and no block by treatment interaction effects ($F_{5,21} = 0.504$, $p = 0.770$). Subsequent univariate ANOVAs on daily CPOM breakdown and FPOM production per g invertebrate biomass indicated significant differences among treatments for both response variables (CPOM: $F_{5,21} = 34.381$, $p < 0.001$; FPOM: $F_{5,21} = 25.799$, $p < 0.001$). Dunnett's post hoc tests indicated that only chironomids differed from the control in daily CPOM breakdown rates (Fig. 3, $P = 0.0024$) and FPOM production rates (Fig. 4, $P = < 0.001$) per g invertebrate biomass.

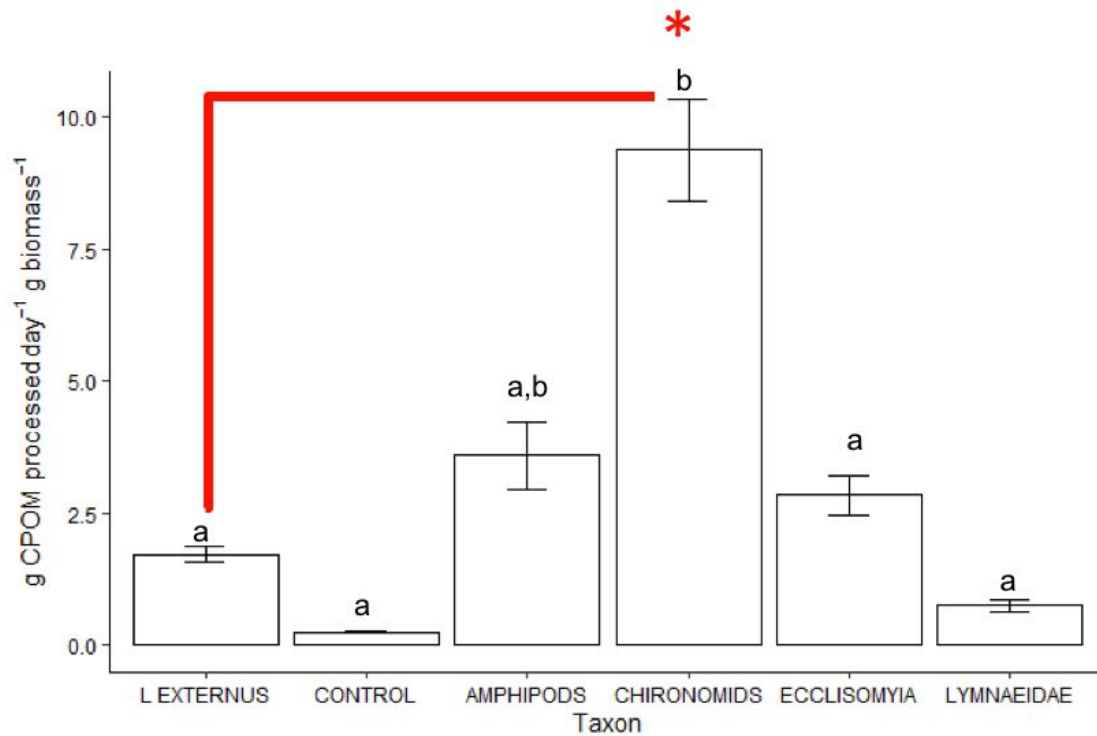


Figure 3. The daily breakdown rate of coarse particulate organic matter, or detritus > 1 mm per g invertebrate biomass in the chironomid treatment was significantly higher than the control with no animals, reference taxon *L. externus* when compared with the animal's biomass in grams. The four other treatments were not different from the control. Bars show the mean and error bars are 1 standard error of the mean. The control contained sedge but no macroinvertebrates. Asterisk shows significant results of Dunnett's comparison with the control. Letters show results of Tukey's comparisons among treatments with animals.

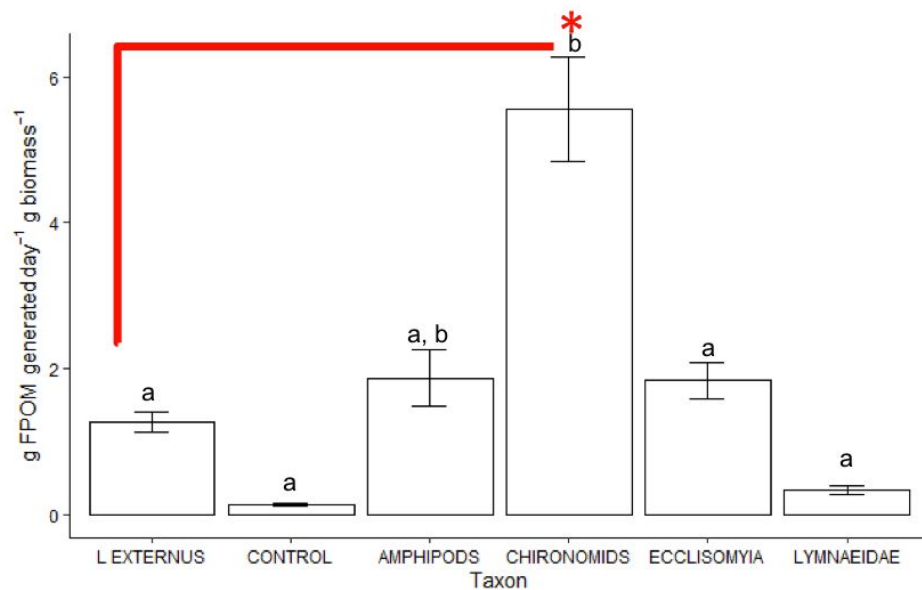


Figure 4. The daily production of fine particulate organic matter, or detritus < 1 mm per g invertebrate biomass in the chironomid treatment was significantly higher than the control with non animals. The four other treatments were not different from the control. Bars show the mean and error bars are 1 standard error of the mean. The control contained sedge but no macroinvertebrates.

Discussion

Here, we show that non-caddisfly taxa can process as much as or more detritus than detritivorous caddisflies near the RMBL. Specifically, we demonstrate that (1), relative to *L. externus*, chironomids have a greater per unit biomass effect on detritus processing, yet caddisflies are not dominant detrital processors compared to chironomids based on per biomass per capita effects. (2) other non-caddisfly taxa, such as amphipods, *Ecclisomyia*, and Lymnaeidae have lower processing rate, and amphipods effects were similar to the control with no animals. *Ecclisomyia* consumed less than half of what the *L. externus* consumed, and the chironomids and lymnaeidae consumed less than the *Ecclisomyia*. (3) *L. externus* did not dominate detritus processing in montane ponds when rates are expressed per unit biomass.

Processing rates may be underestimated because some species like *L. externus* and especially the chironomids began pupating during the second week of the experiment. Just prior to and during pupation invertebrates stop feeding. To prevent underestimating detrital processing the duration of each treatment was ended when pupae were observed; however, chironomids pupated much earlier than expected and almost synchronously. Thus estimates of daily detrital processing by chironomids may be underestimated as the number of days of processing by all

the individuals added to the tanks was likely much shorter than 12 days. Future experiments should be ended when the first chironomid pupae are observed because of their synchronous and rapid development. Despite possibly underestimating the effect of chironomids, they processed the most detritus per unit biomass of the species tested, an effect likely due to their rapid growth and metabolism. Thus, chironomids may be as or more important than caddisflies as detritivores especially if their high per biomass effects are combined with high population biomass in natural ponds. Indeed, data from Balik et al (Balik et al. in review) shows that chironomid biomass is 3X more than *L. externus* in ponds at Mexican Cut. However, for detritus processing not standardized for invertebrate biomass, *L. externus*' daily breakdown rate of sedge was greater than all other species, likely because their biomass was 2-10X greater than other species except snails. Despite significant chironomid per biomass effects, daily estimates of detrital processing (both CPOM and FPOM) by chironomids were not different from the controls.

Reconciling the effects of chironomids in light of previous research showing the importance of caddisflies. Caddisflies make up 50% biomass and are thought to consume more coarse detritus than other invertebrates in montane ponds because sedge is their main food source (Wissinger et al. 2018). Whereas, chironomids have greater biomass than caddisflies, it is unclear if their main food is coarse detritus or if they prefer fine detritus. A previous study by Klemmer et al. (2018) found substantial effects of *L. externus* on detrital processing using 1-mm mesh cages placed in natural ponds. The 1-mm mesh cages were stocked with different densities of *L. externus* but the control cages had no caddisflies and should have allowed access by small invertebrates, such as chironomids. *L. externus*' effects on detritus processing were significantly greater than the control, suggesting weak effects of chironomids in natural ponds despite their large per biomass effects observed in this study and their high population biomass reported in Balik et al. (in review). However, we do not know the biomass of chironomids in the control cages in Klemmer et al. (2018), and it is possible the mesh restricted chironomid's access thereby resulting in low chironomid biomass in the control cages. A experiment by Wissinger et al. (2018), used a different type of *in situ* cage design with 20-mm mesh allowing the naturally occurring caddisfly assemblage access to cages with detritus and a control with 2-mm mesh restricting caddisflies but allowing other invertebrates access to cages with detritus, and also found strong effects of caddisflies relative to the control. However, no chironomids, or other invertebrates, were present in any of the controls despite the larger mesh size, either because the timing of the experiment did not overlap with their presence in the ponds (Wissinger et al. 2018) or chironomids could not swim or crawl into cages, which were constructed of 12-cm high plastic trays covered with mesh. We can not rule out that Klemmer et al.'s (2012) and Wissinger et al.'s (2018) experiments included a realistic effect of chironomids and their effects on detritus processing are weak because they do not prefer to eat coarse sedge, which they were forced to eat in our experiment, or because they inhabit fine benthic sediment habitats in ponds and thus despite their high biomass most chironomids are not in habitats with access to coarse sedge. Taken together, our results and those of previous studies suggest the effects of chironomids on

detrital processing in natural ponds need to be reconciled with experiments in natural ponds comparing effects of the natural biomass of chironomids and caddisflies. Future questions should address how these findings scale to natural ponds where differences in population biomass, feeding choices, habitat use, predators, and variation in other natural history characteristics among species may ultimately influence detrital processing of sedge. Moreover, follow up experiments could test the interactive effects of detritivores by combining, for example, *L. externus* and chironomids to test the synergistic or facilitation effects on detrital processing, animal growth, and nutrient cycling. Determining whether the dominant detritivores are chironomid or *L. externus* is important because their abundances and distribution may be differentially affected by climate change or other environmental factors, such as fluctuations in predatory salamanders. With global warming increasing, changes in ponds drying can differentially affect detritivore abundance, which can alter carbon cycling in montane ponds.

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