TEMPERATURE EFFECTS ON STREAM ORGANIC MATTER PROCESSING ACROSS SCALES FROM ORGANISMS TO ECOSYSTEMS

by

CAROLYN SIOBHAN CUMMINS

(Under the Direction of Amy D. Rosemond)

ABSTRACT

Temperature is an important control on biological processes and affects ecosystem structure and function across scales. The Metabolic Theory of Ecology (MTE) provides a framework for predicting how temperature effects on metabolism scale up to ecosystem processes, but the conformity of biological rates to MTE predictions varies due to system-specific characteristics, antecedent conditions, and species' thermal preferences. Headwater streams, where detritivorous invertebrates (detritivores) and microorganisms contribute to carbon (C) storage, transport, and emissions, are hotspots of organic matter processing and thus, essential to study in the context of global change. Here, I examined temperature effects on (1) leaf litter breakdown mediated by detritivores versus microorganisms, (2) invertebrate community structure, and (3) detritivore thermal physiology in streams across a 2185-hectare basin in the southern Appalachian mountains (North Carolina, USA). I also quantified how leaf characteristics (namely carbon-to-nutrient (C:N) ratios) modulate temperature effects on leaf breakdown and detritivore physiology. I found that the temperature dependence of leaf breakdown mediated by detritivores was higher than both MTE predictions and the temperature

dependence of microbial breakdown. In accordance with theoretical predictions, the breakdown of higher-C:N leaf litter had a higher temperature dependence than the breakdown of lower C:N litter. When examining temperature effects on aquatic invertebrate community structure across a landscape gradient, I found that temperature was a significant predictor of community dissimilarity in the summer, but not in the winter. Finally, measuring thermal responses of the stonefly *Tallaperla* (Plecoptera: Peltoperlidae), I found that survival probability declined with temperature, development rate increased with temperature, growth rate was highest at intermediate temperatures (n.s.), and temperature effects on consumption rate depended on leaf characteristics. These results imply that C routing to detritivores may increase relative to current conditions as stream temperatures rise. However, summer aquatic invertebrate communities, particularly long-lived detritivore taxa, may be vulnerable in the face of global change due to periods of increasingly high temperatures, increases in detrital C:N, and depletion of detrital resources earlier in the year.

INDEX WORDS: Leaf litter breakdown, Metabolic Theory of Ecology, Headwater stream, Macroinvertebrate, Microorganism, Consumer, Thermal performance, Community structure, Coweeta

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DEDICATION

To my grandmother, Sally Rynne.

Thank you for inspiring me,

and for always nurturing my curiosity, enthusiasm, and strength.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Human-caused climate change is altering global weather and climate conditions, leading to adverse impacts on the world's ecosystems (IPCC 2023). Given predicted increases in temperature of 4°C or more within the next century (IPCC 2023), there is widespread interest in how rising environmental temperatures will shift ecological processes at individual, population, community, and ecosystem scales. Of particular interest in the context of rising temperatures is the rate of organic matter (OM) breakdown, which may increase at a faster rate than primary production with warming, leading to reduced carbon (C) sequestration (Yvon-Durocher et al. 2010). Understanding temperature effects on the organisms responsible for OM processing, shifts in their community structure, and resultant changes in C storage, transport, and emissions is therefore essential in the context of global change.

Approximately 5.1 petagrams of terrestrially derived organic matter enter stream and river ecosystems each year (Drake et al. 2018), and the processing of this OM plays a substantial role in the global C cycle (Aufdenkampe et al. 2011). Headwater streams, where large quantities of terrestrial OM directly enter streams from the terrestrial environment, are particular hotspots of C processing and emissions (Hotchkiss et al. 2015). In these systems, biological OM breakdown is driven by aquatic microorganisms and detritivorous macroinvertebrates (detritivores), including insects and macroconsumers such as crayfish (Webster et al. 1999, Hieber and Gessner 2002, Yang

et al. 2020). These organismal groups contribute to different fates of stream detrital C. Microorganisms (primarily aquatic fungi) initially colonize detrital material, leading to maceration, microbial biomass production, and CO_2 emissions (Webster et al. 1999, Hieber and Gessner 2002). Detritivores, which feed on both fungal biomass and the OM itself (Marks 2019), contribute to coarse- and fine-particle generation and invertebrate biomass production (Webster et al. 1999, Hieber and Gessner 2002). These organisms are also responsible for the transfer of large quantities of stream detrital C across aquatic and terrestrial food webs (Baxter et al. 2005, Wallace et al. 2015). The relative thermal responses of microorganisms and detritivores will scale up to affect the rate of OM breakdown in headwater streams, as well as the proportion of detrital C that is routed to microbial fates (maceration, microbial biomass, CO_2 evasion) versus detritivore fates (particle generation, invertebrate biomass, storage, and transport) (Boyero et al. 2011, Follstad Shah et al. 2017).

Ecological theory aids predictions about how changing thermal regimes will alter the rate of OM breakdown in stream ecosystems. The Metabolic Theory of Ecology (MTE, Brown et al. 2004) predicts that organismal metabolic rates scale with body size and temperature across temperatures suitable for biological activity (approximately 0-40°C) according to ¾ scaling relationships (body size) and Arrhenius-Boltzmann enzyme kinetics (temperature) (Eq. 1).

$$B = B_0 M^{-\frac{3}{4}} e^{-\frac{E_a}{k_B T}} \qquad \textbf{Equation 1}$$

Where B is metabolic rate, B_0 is a scaling coefficient, M is organism mass, E_a is activation energy (or temperature dependence), k_B is Boltzmann's constant, and T is temperature in Kelvin. Linearizing Equation 1 allows for predictions about the slope of

the relationship between biological rates and temperature, which is equal to the activation energy or E_a. According to MTE, heterotrophic processes consistently exhibit an E_a of approximately 0.65 electronvolts (eV), and this temperature dependence scales predictably from individual metabolism to consumer-mediated ecosystem processes (e.g., decomposition rate) (Brown et al. 2004).

Variation in thermal responses across the organisms responsible for stream OM processing may lead to deviations from MTE predictions at the individual, community, and ecosystem scales. For example, ectotherm physiological rates (such as those for stream macroinvertebrates) generally follow thermal performance curves, where rates increase to a thermal optimum, then decline to a critical thermal maximum at which performance is zero (Huey and Kingsolver 1989). While the MTE is thought to accurately describe the ascending portion of thermal performance curves, thermal optima and critical thermal maxima differ interspecifically (Chown et al. 2015). Thermal responses may also vary intraspecifically across different physiological parameters (e.g., development and growth, Sweeney et al. 2018). This variation in thermal responses across ectothermic detritivore taxa may imply shifts in their stream C processing with warming that differ from MTE predictions, with responses depending on species' thermal niches, the magnitude of warming, and antecedent temperature conditions. In addition, thermal responses may differ between organismal groups, such as detritivores and microorganisms. While aquatic fungi exhibit interspecific differences in thermal preference (Dang et al. 2009, Tomczyk et al. 2023), stream microbial communities generally encompass high functional redundancy and turnover, implying predictable increases in microbial C processing with warming based on the MTE (Ferreira et al.

2014). On the other hand, detritivores are thought to be particularly sensitive to warming compared to other aquatic insect functional groups and have been demonstrated to be particularly vulnerable to extirpation in the face of climate change-induced stream warming and drying (Pyne and Poff 2017, Jourdan et al. 2018). Due to the higher proportion of microbially-processed C that is rapidly evaded as CO_2, increased microbial processing of stream OM coupled with decreased processing by detritivores has been put forth as a potential positive feedback to climate change. Indeed, Boyero et al. (2011) observed that leaf litter breakdown mediated by microbes increased with temperature while detritivore-mediated breakdown decreased with temperature across a global-scale latitudinal temperature gradient.

Variation in organismal thermal responses is not the only factor that may lead to deviations from MTE predictions in the face of global change. Another essential factor that determines the temperature dependence of OM breakdown is the chemical composition of detrital material. Biologically recalcitrant OM is characterized by high carbon-to-nutrient (C:N) ratios, a high concentration of C-rich compounds (e.g., lignin and tannins), and slower breakdown rates. On the other hand, labile organic matter is characterized by lower C:N and faster breakdown rates (Marks 2019). According to enzyme kinematics, the enzymes required to breakdown recalcitrant organic matter have higher activation energies, thus requiring higher temperatures to facilitate breakdown (Sinsabaugh and Follstad Shah 2012). This leads to a higher temperature dependence for recalcitrant OM breakdown relative to labile OM breakdown (Fierer et al. 2005). OM chemical composition also affects palatability and nutritional quality for detritivores (Marks et al. 2019). Thus, detritivores may exhibit different responses to temperature

depending on resource characteristics. For example, detritivores feeding on higher-C:N leaves may increase feeding rate with temperature more when fed recalcitrant OM than when fed labile OM, a phenomenon termed "compensatory feeding" (Flores et al. 2014). Feeding on higher-C:N food may also impact other physiological rates for detritivores (e.g., growth, Halvorson et al. 2017), and the costs of feeding on high-C:N detritus may lead to increased temperature sensitivity for these organisms (Díaz Villanueva et al. 2011).

Many studies have examined the temperature dependence of organic matter breakdown in streams (Boyero et al. 2011, Benstead and Huryn 2011, Follstad-Shah et al. 2017, Tiegs et al. 2019 Wilmot et al. 2021) and temperature effects on stream invertebrate physiology (Sweeney et al. 1986, 2018, Shah et al. 2017, Kim et al. 2017, Birrell et al. 2020) and community structure (Dossena et al. 2012, Pyne and Poff 2017, Nelson et al. 2017, Jourdan et al. 2018). However, questions remain about how temperature will affect detritus-based headwater stream ecosystems in the context of climate change. Studies across large geographic temperature gradients suggest a lower temperature dependence for stream OM breakdown than predicted by MTE and a lower E_a for detritivore breakdown than for microbial breakdown (Boyero et al. 2011, Follstad-Shah et al. 2017, Wilmot et al. 2021). However, landscape-scale OM breakdown studies are needed to understand temperature effects across streams with similar organismal assemblages and leaf litter inputs. In addition, studies of temperature effects on macroinvertebrate community structure have commonly employed large geographic temperature gradients (Boyero et al. 2012) or experimental studies (Jonsson et al. 2015), but less is known about the intra-annual vulnerability of stream macroinvertebrate

communities to changing temperature regimes (e.g., summer versus winter communities). Finally, at the individual level, previous studies have quantified aquatic macroinvertebrate critical thermal limits (Chown et al. 2015) and estimated their thermal niches based on current distributions (Pyne and Poff 2017, Twardochleb et al. 2021). However, fewer studies have examined thermal physiology in longer-lived taxa, simultaneously measured multiple thermal responses, or considered how detrital resource quality mediates detritivore thermal physiology.

Dissertation overview

In this dissertation, I examined the effects of temperature on leaf litter breakdown, aquatic macroinvertebrate community structure, and detritivore physiology in southern Appalachian headwater streams. These projects took place as part of the "CREWS" (Carbon Response to Experimental Warming in Streams) project, in which a collaborative team of researchers used experimental manipulations, observational studies, and a network modeling approach to understand how C processing in detritus-based stream ecosystems may shift with projected increases in stream temperatures due to climate change. This research was conducted at the USDA Forest Service Southern Research Station - Coweeta Hydrologic Laboratory (Coweeta), a 2185-hectare basin encompassing an extensive network of forested headwater streams. Collectively, this work aids predictions about the role of temperate headwater streams in the C cycle in the face of a changing climate.

Chapter 2: Temperature dependence of leaf litter breakdown across leaf species and organismal groups

In this study, I conducted leaf litter incubations of two different leaf species (*Rhododendron maximum* and *Acer rubrum*) in 20 streams at Coweeta monthly for two years. Using litterbags of two different mesh sizes (5mm and 250µm), I quantified the temperature dependence (as MTE activation energy, E_a) of breakdown mediated by detritivores versus microorganisms for both leaf species. Additionally, I examined temperature effects on fungal biomass accrual for a subset of the litterbags to examine the mechanisms behind temperature effects on microbial and detritivore breakdown. Further, I quantified the implications of the microbial and detritivore E_a values for absolute versus relative shifts in C breakdown mediated by these two organismal groups with warming, a previously underappreciated aspect of studies that quantify shifts in stream detrital C routing to detritivores versus microbes. This study sheds light on the relative roles of two important organismal groups in an essential ecosystem process and informs our understanding of stream detrital C fates in a warmer world.

Chapter 3: Changes in macroinvertebrate community structure across a landscape-scale temperature gradient in winter versus summer streams

Based on invertebrate samples collected from the litterbags in Chapter 1, I examined the effects of temperature and other environmental parameters on macroinvertebrate community structure in winter versus summer streams across a landscape-scale temperature gradient. I classified invertebrates into functional feeding groups and identified all specimens to the family (non-detritivore taxa) or genus

(detritivore taxa) level. Using non-metric multidimensional scaling, I identified significant environmental and invertebrate predictors of community dissimilarity across three different scales of taxonomic resolution (family, functional feeding group, and genus). While previous studies have pointed out that macroinvertebrate communities in warmer streams may be differentially vulnerable to warming than those in cooler streams (Boyero et al. 2012), fewer have considered how these dynamics may play out on a seasonal basis. This study provides evidence that temperature effects on aquatic invertebrate community structure may vary on a seasonal basis.

Chapter 4: Temperature and leaf species effects on detritivore survival, development, growth, and consumption

In my final dissertation chapter, I quantified thermal responses in a common and functionally important stream insect detritivore (Plecoptera: Peltoperlidae *Tallaperla*) in an artificial streams experiment. Over the course of this five-week experiment, I quantified *Tallaperla* survival probability, development rates, growth rates, and feeding rates across four temperature treatments (ambient, +1°C, +2°C, +3°C, and +4°C) and two leaf species treatments (*Rhododendron maximum* - higher C:N, *Acer rubrum* - lower C:N). By simultaneously measuring multiple physiological and functional responses in *Tallaperla*, we gained a clearer picture of how temperature may affect detritivore-mediated C processing in streams such as ours. This study also highlights the potential for thermal responses to vary across physiological parameters and the role of leaf litter characteristics in driving thermal responses.

Overall, this dissertation provides a detailed assessment of how rising temperatures may alter stream OM breakdown at the organismal, community, and ecosystem scales. My results suggest that factors like seasonality and leaf litter characteristics may play particularly important roles in determining temperature effects on stream OM processing and highlight the importance of landscape-scale studies for understanding temperature effects on complex ecosystem processes.

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CHAPTER 2

TEMPERATURE DEPENDENCE OF LEAF BREAKDOWN DIFFERS BETWEEN MICROBIAL AND INVERTEBRATE PATHWAYS AND LEAF SPECIES IN STREAMS[1]

[1]Cummins, C.S., Rosemond, A.D., Tomczyk, N.J., Wenger, S.J., Bumpers, P.M., Gulis, V., Helton, A.M., and Benstead, J.P. Submitted to *Ecology* 5/8/23.

Abstract

Increased temperatures are altering rates of primary production and organic matter (OM) breakdown in stream ecosystems with implications for carbon (C) cycling in the face of global change. The Metabolic Theory of Ecology (MTE) provides a framework for predicting temperature effects on OM breakdown, but differences in the temperature dependence of breakdown driven by different organismal groups (i.e., microorganisms vs. invertebrate detritivores) and litter species remain unresolved. We conducted twelve 60-d litterbag incubations in each of 20 headwater streams across seasonal and landscape temperature gradients in the southern Appalachian Mountains (USA). We compared temperature dependence (as activation energy, E_a) between microbial and detritivore breakdown and between a recalcitrant (*Rhododendron maximum*) and a labile (*Acer rubrum*) leaf species. Detritivore breakdown had a higher E_a than microbial breakdown for both leaf species (*Rhododendron*: 1.49 eV > 0.56 eV; *Acer*: 0.97 eV > 0.29 eV), and *Rhododendron* breakdown had a higher E_a than *Acer* breakdown for both organismal groups. Similarly, the E_a of total (coarse-mesh) *Rhododendron* breakdown (0.89 eV) was higher than the E_a of total *Acer* breakdown (0.52 eV). These effects for total breakdown were large, implying a 40% (*Rhododendron*) and 26% (*Acer*) decline in days to 95% leaf mass loss between 12°C (our mean temperature value) and 16°C (+4°C, reflecting projected increases in global surface temperature due to climate change). Despite these patterns in E_a, overall breakdown rates were higher for microbes than detritivores and for *Acer vs. Rhododendron* over most of our temperature gradient. The E_a for a subset of the microbial breakdown data declined from 0.40 eV to 0.22 eV when fungal biomass was included as a model predictor, highlighting the key role of fungi in determining the

temperature dependence of litter breakdown. Our results imply that, as streams warm, routing of leaf litter C to detritivore-mediated fates will increase faster than predicted by previous studies and MTE, especially for labile litter. As temperatures rise, earlier depletion of autumn-shed, labile leaf litter combined with rapid breakdown rates of year-round, recalcitrant litter could exacerbate seasonal resource limitation and alter storage and transport dynamics in temperate headwater stream networks.

Introduction

Consumers play important roles in ecosystem processes through their assimilation, transformation, and dissipation of energy and matter in basal resources (Reiners 1986). In forested headwater streams, heterotrophic microorganisms (microbes, e.g., fungi and bacteria) and detritivorous macroinvertebrates (detritivores) consume and transform the billions of tonnes of terrestrially derived organic carbon (C) that enter these systems annually (Webster et al. 1999, Hieber and Gessner 2002, Drake et al. 2018). As stream temperatures rise, differences in the thermal responses of detritivores and microbes may shift the proportions of C routed to invertebrate production, coarse and fine particle generation, and storage (detritivore-mediated fates) vs. microbial production, maceration, and CO_2 evasion (microbial fates; Webster et al. 1999, Hieber and Gessner 2002, Boyero et al. 2011, Marks 2019). Understanding how temperature dependence varies for different components of stream C breakdown will improve predictions about how warming alters the role of inland waters in the global C cycle.

The Metabolic Theory of Ecology (MTE) provides a framework for predicting temperature effects on ecological processes across scales from cells to ecosystems. MTE

predicts that the slope between temperature and mass-corrected organismal metabolic rates for heterotrophic consumers (e.g., growth and development) falls between 0.6-0.7 eV, reflecting the canonical activation energy (E_a) of cellular respiration (Brown et al. 2004). The rates of many ecosystem processes depend on organismal processing of C and nutrients, so MTE further predicts that the canonical E_a scales to processes such as organic matter decomposition. However, temperature effects on metabolism may vary across organismal groups due to differences in thermal performance. For example, aquatic insect detritivores may be particularly cold-adapted relative to other functional groups (e.g., grazers or predators, Pyne and Poff 2017, Tomczyk et al. 2022a) with implications for their contributions to stream C processing at high temperatures. Aquatic fungi also exhibit variation in thermal performance and associated shifts in community structure across temperatures (Dang et al. 2009, Ferreira et al. 2014, Tomczyk et al. 2023). However, fungi also exhibit high functional redundancy and turnover rates (Ferreira et al. 2014), so temperature-induced shifts in fungal communities may not result in decreased microbial breakdown over temperature ranges generally observed in stream ecosystems (Ferreira et al. 2014). Decreased detritivore contributions at high temperatures could lead to a higher proportion of stream C routed to microbial fates as streams warm (Boyero et al. 2011).

Several studies have estimated the temperature dependence of consumer-mediated litter breakdown in streams and found that 1) microbial decomposition typically dominates the breakdown process and 2) the E_a of microbial breakdown is greater than or similar to that of detritivore breakdown (Boyero et al. 2011, Follstad Shah et al. 2017, Wilmot et al. 2021). These findings imply that the proportion of C routed to microbial

fates will increase as stream temperatures rise. However, E_a estimates from global-scale temperature gradients (e.g., Boyero et al. 2011; Follstad Shah et al. 2017) may not be predictive of warming effects at smaller spatial scales because changes in temperature co-occur with detritivore community shifts across latitudinal gradients (Boyero et al. 2012). Even at the regional scale, changes in community structure can complicate inference about temperature effects on detritivore breakdown (Wilmot et al. 2021). Local-scale studies in streams with similar consumer assemblages are therefore essential for predicting temperature effects on stream C cycling.

The temperature dependence of leaf litter breakdown may also be affected by litter characteristics, especially the structural complexity of leaf components. The prevailing explanation for differences in temperature dependence for different leaf species is based on enzyme kinetics: the multi-step reactions required to break down complex lignocellulose compounds in recalcitrant litter have higher activation energies than those required for the breakdown of labile litter components (Sinsabaugh and Follstad Shah 2012). Thus, recalcitrant litter breakdown is expected to have a higher inherent temperature dependence than labile litter breakdown (Davidson and Janssens 2006, Sinsabaugh and Follstad Shah 2012), a pattern that has been demonstrated in soil C decomposition studies (Fierer et al. 2005, Davidson and Janssens 2006, but see Liáng et al. 2023). Follstad Shah et al. (2017) found some evidence for a similar relationship between E_a and leaf species in streams, but other studies in streams have found no difference in the temperature dependence of breakdown across leaf species (Griffiths and Tiegs 2016). Therefore, while there is a theoretical basis for expecting differences,

variation in E_a based on leaf species remains unresolved in stream ecosystems, including whether patterns vary for microbial vs. detritivore breakdown.

To examine the temperature dependence of OM breakdown across consumer groups and leaf species, we conducted litter incubations in 20 forested headwater streams across a seasonal and landscape temperature gradient. We compared E_a between detritivore and microbial breakdown, hypothesizing that the E_a of microbial breakdown would be greater than the E_a of detritivore breakdown due to the relative temperature sensitivity of aquatic insect detritivores. Based on previous studies (Boyero et al. 2011, Follstad-Shah et al. 2017, Wilmot et al. 2021), we also predicted that the E_a for both microbial and detritivore-mediated breakdown would be lower than MTE predictions (0.6-0.7 eV). Additionally, we compared E_a for recalcitrant and labile litter breakdown, hypothesizing that the E_a of recalcitrant litter breakdown would be higher than that of labile litter breakdown for both microbes and detritivores. Recognizing that the E_a of litter breakdown integrates temperature effects on many consumer parameters, we investigated the degree to which fungal biomass explained the E_a of microbial breakdown in a subset of the data. Finally, we explored the implications of the E_a values we observed for relative (i.e., proportional) vs. absolute (i.e., magnitude) increases in breakdown rate for different consumer groups (microbes and detritivores) and leaf species with warming.

Methods

Study site

This study took place at the U.S. Department of Agriculture Forest Service (USFS) Southern Research Station - Coweeta Hydrologic Laboratory, a 2185-ha forested

basin in Macon County, North Carolina, USA that encompasses a network of first- to third-order streams. Forest composition at Coweeta is dominated by maple, oak, and tulip poplar and an understory of *Rhododendron maximum* (Swank and Crossley 1988). Our study was carried out over two years (YR1: 21 September 2017 - 28 September 2018; YR2: 31 August 2018 - 30 August 2019) and included a total of 20 streams (11 streams in YR1, 9 new streams in YR2) across a temperature gradient generated by differences in elevation and aspect (Appendix A: Table A1).

Physicochemical data

We measured temperature at 15-min intervals with HOBO pendant temperature loggers (Onset Computer Corp., MA, USA) in each stream near the location of the litterbags. Daily discharge and weekly nutrient concentrations (dissolved inorganic nitrogen [DIN] as $NH_4^+ + NO_3^-$ and soluble reactive phosphorus [SRP] as PO_4^{3-} for 15 of our study streams were provided by the USFS or the Coweeta Long-Term Ecological Research Program (LTER) (Appendix A, Table A1-A3). For the other five streams, we measured stage height using pressure transducers at the downstream terminus of our study reaches and converted to discharge using a rating curve (Appendix A: Table A4). We also collected water samples for DIN and SRP from these five streams monthly (Appendix A). Due to equipment malfunctions, there were some time periods in which temperature data were lost (an average of 6% of the data record for each stream). We modeled missing data using linear regressions based on (a) the closest, most similar stream, or (b) air temperature data from Climate Station 1 at Coweeta (Appendix A: Table A5).

Litterbag preparation, deployment, and processing

We collected and air-dried *Rhododendron maximum* (*Rhododendron*, recalcitrant) and *Acer rubrum* (*Acer*, labile) leaves from Coweeta during autumn 2017 (YR1) and autumn 2018 (YR2). *Rhododendron* has a slower breakdown rate than *Acer* due to its waxy cuticle, greater thickness, and high-C:N compounds such as lignin (Webster and Benfield 1986, Manning et al. 2015, 2016). We sewed fine-mesh bags from 250-µm nylon mesh (*Rhododendron*: 6 × 23 cm, *Acer*: 14 × 12 cm, Industrial Netting, Maple Grove, MN, USA). Coarse-mesh bags were 5-mm polypropylene mesh (22 × 40 cm, Cady Bag, Incorporated, Pearson, Georgia, USA). *Acer* petioles have variable lengths which can contribute substantially to leaf mass, so we trimmed them to 25 mm. We filled coarse-mesh bags with 5.0 ± 0.1g and fine-mesh bags with 1.5 ± 0.1g of *Rhododendron* or *Acer* leaves. We tethered one fine-mesh bag to each coarse-mesh bag and secured pairs of the same leaf species together in sets of four. We deployed one set of litterbags per leaf species in each stream monthly and incubated them for ~60 d, creating overlapping "serial" litter incubations (*sensu* Benstead and Huryn 2011, Wilmot et al. 2021). At the end of each incubation, we transported litterbags back to the laboratory, washed the remaining leaf material in each bag, and dried it for at least 48 h at 60°C. We weighed the dried material, combusted a subsample at 500°C for 5.5 h, and determined the ash-free dry mass (AFDM) remaining in each litterbag (Benfield et al. 2017).

At the start of YR1, we transported 10 coarse-mesh bags per leaf species to the field, placed them in a stream for less than five seconds, and immediately transported them back to the laboratory for processing. We quantified mass loss from these bags and subtracted leaf species-specific averages from the initial mass in each coarse-mesh bag to correct for handling loss (Benfield et al. 2017). Fine-mesh bags had small openings, so

we assumed no handling loss from them. We multiplied mean leaf species-specific %AFDM in handling loss bags by the initial dry mass in each litterbag to estimate initial AFDM. We calculated breakdown rate, k (d^{-1}), as the absolute value of the difference between $\log_e$-transformed final %AFDM and $\log_e$-transformed initial % AFDM ($\log_e$ (100%)) divided by days incubated (Eq. 1):

$$k = abs\left(\frac{log_e(Final\ \%AFDM) - log_e(Initial\ \%AFDM)}{Days\ incubated}\right) \quad \textbf{Eq. 1}$$

Fungal biomass

In YR2, we estimated fungal biomass (mg g^{-1} AFDM) in fine-mesh *Rhododendron* litterbags by quantifying ergosterol, a specific biomarker found in fungal cell membranes. In each YR2 stream and deployment, we cut six to eight leaf fragments from *Rhododendron* leaves in three of our four fine-mesh bags. We placed leaf fragments in pre-weighed scintillation vials and stored them frozen, then freeze-dried them to obtain dry mass. We extracted lipids and calculated ergosterol concentrations following the methods of Gulis and Bärlocher (2017; Appendix A). After excluding samples with quality control issues that arose during sample processing, we were left with n = 248 samples for data analysis.

Data analysis

i. Data processing: For each stream and deployment, we averaged temperature (°C) and discharge (L s^{-1}) and averaged k across the four coarse- or fine-mesh litterbags in each leaf species-specific set. We separated mean k values into six categories for analysis (*Rhododendron* coarse-mesh, *Rhododendron* microbes, *Rhododendron* detritivores, *Acer* coarse-mesh, *Acer* microbes, and *Acer* detritivores). Mean k estimates based on fewer than three litterbag k values were excluded from analyses. Coarse-mesh k values included

all components of breakdown, including leaching, abrasion, microbial and detritivore processing. Microbial *k* values reflected leaching and microbial breakdown in fine-mesh bags, which exclude most detritivores and are less prone to abrasion than coarse-mesh bags (Tomczyk et al. 2022b). To estimate detritivore breakdown, we calculated the litter fragmentation rate (λ_f) following Lecerf (2017; Eq. 2):

$$\lambda_f = k_c - \frac{k_f - k_c}{log_e(k_f) - log_e(k_c)} \quad \textbf{Eq. 2}$$

where k_c and k_f are the breakdown rates in coarse- and fine-mesh bags. This method is intended to produce a more accurate detritivore *k* estimate than subtracting fine-mesh *k* from coarse-mesh *k*. Here, λ_f represents the likelihood of litter fragmentation at any point during breakdown, explicitly incorporating the relationship between fragmentation and microbial decomposition.

ii. Linear mixed-effects models: For each stream, deployment, and breakdown category, we $\log_e$-transformed the absolute values of mean *k* and calculated the inverse Boltzmann temperature ($1/k_BT$, where k_B – not to be confused with *k,* the breakdown rate coefficient – is Boltzmann's constant, 8.617×10^{-5} eV K^{-1}, and *T* is temperature in kelvin). We fit linear mixed-effects models for each of the six breakdown categories in which we regressed $\log_e$-transformed mean *k* against $1/k_BT$ (mean-centered) and *z*-scored discharge (Appendix A: Table A6). From our models, we obtained the E_a according to the linearized Arrhenius-Boltzmann equation, the discharge parameter estimate (β), and the 95% confidence intervals around these estimates for each breakdown category (Eq. 3; Appendix A: Table A6):

$$log_e(k) = log_e(k_0) + \left[\frac{1}{k_BT} - \frac{1}{k_BT_0}\right] * (-E_a) + Q_z * \beta, \quad \textbf{Eq. 3}$$

where k_0 is the reference breakdown rate at the mean $1/k_BT$ (i.e., $1/k_BT_0$) and z-scored discharge (Q_z), and β is the discharge parameter estimate (see $\log_e(k_0)$ and $1/k_BT_0$ values in Appendix A: Table A7). We then fit a second set of models to compare E_a between organismal groups (i.e., microbes and detritivores) and leaf species (*Rhododendron* and *Acer*). In these models, centered $1/k_BT$ and z-scored discharge included a consumer group or leaf species interaction (Appendix A: Table A6).

To determine the degree of alignment between our E_a values and MTE, we calculated the probability that each E_a value fell within the MTE-predicted range of 0.6-0.7 eV (MTE probability). We calculated MTE probability values using *pnorm* in R as the relative area under the probability density curve between 0.6 and 0.7 given the E_a and standard error from each model. Lower MTE probability values indicate a lower probability that the true E_a for a given breakdown category falls between 0.6-0.7 eV. Additionally, we used our E_a values and model intercepts to compare relative (i.e., percent) increases in k to absolute (i.e., magnitude) increases in k with increased temperatures. Results of such comparisons depend on the temperatures considered. For this analysis, we compared k values at 12°C, our approximate median and mean temperature value, and 16°C, an increase that reflects projected increases in global surface temperature due to climate change (~+4°C, IPCC 2023).

To investigate the role of fungal biomass in determining the E_a of microbial breakdown, we regressed fine-mesh k against fungal biomass and fungal biomass against temperature (°C) in a subset of the data (*Rhododendron*, fine-mesh, YR2). We estimated E_a for this data subset in a model with additive terms for $1/k_BT$ (mean-centered) and discharge (z-scored). Then, we estimated E_a for this same data subset in a second model,

which included a term for fungal biomass (mg g^{-1} AFDM, centered on the mean) in addition to centered $1/k_BT$ and z-scored discharge (Appendix A: Table A6).

We accounted for seasonal and stream-level variation by including random intercepts for stream and deployment date in all our linear mixed-effects models. Early models included nutrient concentrations, but we ultimately excluded nutrients after finding no significant effects on breakdown (nutrient concentrations are consistently low in Coweeta streams; see Appendix A: Table A2). We conducted all analyses and made all figures in R using the *lme4* and *ggplot2* packages (Bates et al. 2015, Wickham 2016, R Core Team 2021)

Results

Physicochemical parameters

The landscape temperature gradient (across streams, within deployments) resulted in a mean temperature difference of 2.5°C across the 11 YR1 streams and 3.1°C across the 9 YR2 streams. Nonetheless, the greatest temperature gradient in our study arose due to seasonal temperature changes. The warmest deployments were on average 12°C warmer than the coldest deployments in the YR1 streams and 10°C warmer in the YR2 streams. In contrast, differences in size among the 20 streams meant that the landscape discharge gradient was greater than the seasonal discharge gradient. Across deployments, the highest-flow landscape stream had a discharge value that was an average of 142× the discharge value in the lowest-flow landscape stream in YR1 (mean range = 324 L s^{-1}) and 900× in YR2 (mean range = 898 L s^{-1}). However, discharge values in individual streams during their highest-flow deployments were only an average of 4.4× those measured

during their lowest-flow deployments in YR1 (mean range = 9.4 L s^{-1}) and 4.9× in YR2 (mean range = 231 L s^{-1}; Appendix A: Table A1, Figure A1, A2).

Temperature dependence of leaf litter breakdown

Across models, we found that the detritivore breakdown E_a was higher than the microbial breakdown E_a and that the *Rhododendron* breakdown E_a was higher than the *Acer* breakdown E_a (Figure 2.1, Table 2.1). For *Rhododendron*, the detritivore breakdown E_a (1.49 eV, 95% CI 1.06–1.93) was significantly higher than the microbial breakdown E_a (0.56 eV, 95% CI 0.43–0.66; p<<0.001 Figure 2.1b, Table 2.1). For *Acer*, the detritivore breakdown E_a (0.97 eV, 95% CI 0.45–1.44) was also significantly higher than the microbial breakdown E_a (0.29 eV, 95% CI 0.20–0.36; p<<0.001 Figure 2.1a, Table 2.1). The *Rhododendron* breakdown E_a was significantly higher than the *Acer* breakdown E_a in coarse-mesh bags (0.89 eV, 95% CI 0.69–1.08 > 0.52 eV, 95% CI 0.33–0.69; p <<0.001) and for microbial breakdown (0.56 eV > 0.29 eV, p <<0.001). The *Rhododendron* E_a was also higher than the *Acer* E_a for detritivore breakdown, but this difference was only marginally significant (1.49 eV > 0.97 eV; p = 0.08; Table 2.1; Appendix A: Figure A3). Most E_a values were substantially lower or higher than the canonical MTE-predicted range of 0.6-0.7 eV; consequently, MTE probability values were generally low (ranging from *Rhododendron* microbes, 21%, to *Acer* microbes, << 1%; Table 2.1). Therefore, the "true" E_a values for each breakdown category in our study likely fell outside of MTE predictions, but the direction (higher or lower than MTE) depended on consumer group and leaf species.

Absolute versus relative increases in leaf litter breakdown with temperature

Though the E_a of litter breakdown was greater for detritivores than for microbes and for *Rhododendron* than for *Acer* (Figure 2.2a), microbial and *Acer* breakdown rates were higher than detritivore and *Rhododendron* breakdown rates across most of our temperature gradient (Figure 2.1a-b; Appendix A: Figure A3). Consequently, microbial breakdown made up a higher proportion of total breakdown than did detritivore breakdown. In line with the observed patterns in E_a, percent increases in k from 12°C to 16°C were greater for detritivores than for microbes (*Rhododendron*: 131% > 37%, *Acer*: 73% > 18%, Figure 2.2c). Percent increases in k from 12°C to 16°C were also greater for *Rhododendron* than for *Acer* (Figure 2.2c). However, due to differences in the intercept of the temperature dependence relationship, absolute increases in k from 12°C to 16°C were similar for microbes and detritivores and were greater for *Acer* than *Rhododendron* (Figure 2.2b, 2.2d). For *Rhododendron*, the absolute increase in k from 12°C to 16°C was the same for detritivores and microbes (0.0014 d^{-1}), while for *Acer*, this increase was similar but slightly higher for detritivores (0.0034 d^{-1}) than for microbes (0.0023 d^{-1}; Figure 2.2b, 2.2d).

Discharge effects on leaf litter breakdown

For *Rhododendron*, the slope between breakdown and discharge was significantly greater for detritivores (0.29, 95% CI 0.11-0.48) than for microbes (0.12, 95% CI 0.07–0.16; $p = 0.0085$, Table 2.1). This pattern was similar for *Acer* (0.25, 95% CI 0.09–0.42 > 0.05, 95% CI 0.02–0.08; $p << 0.001$, Table 2.1). For microbes, discharge effects on breakdown were significantly greater for *Rhododendron* (0.12, 95% CI 0.07–0.16) than for *Acer* (0.05, 95% CI 0.02–0.08; $p = 0.0001$). However, discharge effects were not significantly different between leaf species for coarse-mesh or detritivore breakdown.

Fungal biomass and the temperature dependence of leaf litter breakdown

In YR2 *Rhododendron* fine-mesh litterbags, fungal biomass was positively correlated with both temperature and microbial breakdown (Figure 2.3a-b, Table 2.2). In a model with fixed effects for centered $1/k_BT$ and z-scored mean discharge (L s^{-1}), the E_a of microbial breakdown for this data subset was 0.40 eV (95% CI 0.25–0.53, $p<<0.001$, Table 2.2). When a term for fungal biomass (mg g^{-1} AFDM, mean-centered) was added to this model, E_a was reduced to 0.22 eV (95% CI: 0.10–0.34, $p = 0.0015$, Table 2.2).

Discussion

Implications for temperature effects on stream C fates at local scales

We found that the E_a of detritivore breakdown exceeded the E_a of microbial breakdown, the MTE-predicted canonical E_a of cellular respiration (0.6-0.7 eV), and the E_a values estimated in previous studies of OM breakdown in streams (Boyero et al. 2011, Follstad Shah et al. 2017, Tiegs et al. 2019, Wilmot et al. 2021). In a published OM budget from Coweeta, ~49% of CPOM inputs (232 g C m^{-2}) was routed to microbial metabolism on an annual basis, while only ~8% was routed to macroinvertebrates (35 g C m^{-2}; Benstead et al. 2021). Though detritivore breakdown rates were variable in our study, the high E_a for detritivore breakdown indicates that detritivore-mediated routing of stream OM (e.g., to secondary production, particle generation, and transport) will play an increasingly important role in stream C cycling as stream temperatures rise. Despite the higher E_a of detritivore breakdown, microbial k was higher than detritivore k over most of our temperature gradient. Thus, while the increase in detritivore-mediated stream C fates is most sensitive to warming, our results suggest that the overall amount of C routed to

microbes will be higher than the amount routed to detritivores except at temperatures exceeding 20°C, where our data suggest that microbial and detritivore *k* values approximately converge.

Our higher observed E_a for detritivore breakdown compared to microbial breakdown differs from studies conducted across large spatial gradients. Global-scale studies have implied that warming would increase the proportion of C routed to microbial fates (Boyero et al. 2011) or that the proportions of C routed to detritivore vs. microbial fates would remain unchanged with warming (Follstad Shah et al. 2017). However, E_a estimates obtained from large spatial temperature gradients may have been driven by detritivore community shifts. Aquatic insect detritivore diversity, abundance, and biomass have all been shown to increase with latitude, suggesting that these organisms play a larger role in OM processing in cooler, temperate streams (Boyero et al. 2012, 2021). This could explain low detritivore E_a values when measured across latitudinal temperature gradients because breakdown mediated by insect detritivores would be lower than expected based on MTE in warm, tropical streams. Even in a smaller-scale study across the southeast USA, regional shifts in detritivore communities likely affected the observed temperature dependence of detritivore breakdown (Wilmot et al. 2021). We conducted our study within a single 2185-ha basin, where variation in detritivore assemblages among streams is greatly reduced compared to global- and regional-scale studies. Our results shed light on how discrete detritivore communities respond to temperature and improve predictions about warming-induced shifts in C fates at the local scale.

Effects of leaf species on temperature dependence and resource quantity

The E_a of total (coarse-mesh) breakdown was close to the MTE predicted range of 0.6-0.7 for our labile litter species (*Acer*), but the E_a of total breakdown for our recalcitrant species (*Rhododendron*) was higher than predicted by MTE. We also observed higher E_a values for recalcitrant litter breakdown than for labile litter breakdown for both microbes and detritivores. These patterns are consistent with theoretical predictions and previous studies in soils (Fierer et al. 2005, Yvon-Durocher et al. 2010), though a recent article challenged the notion that recalcitrant litter has a higher inherent E_a than labile litter (Liáng et al. 2023). The higher E_a of *Rhododendron* breakdown implies a greater proportional increase in recalcitrant breakdown compared to labile breakdown as temperatures rise; however, the magnitude of the increase in breakdown rate with temperature was greater for labile *Acer* leaves than for recalcitrant *Rhododendron* leaves. In deciduous forested headwater streams, labile OM derived from autumn-shed leaves becomes largely depleted in the summer while recalcitrant OM like *Rhododendron* leaves can be available throughout the year. Thus, year-round inputs combined with a slow breakdown rate makes recalcitrant OM an important resource for consumers during summer months (Eggert and Wallace 2003, Greenwood et al. 2007). As streams warm, earlier depletion of labile litter (e.g., *Acer*) combined with rapid increases in breakdown with temperature for recalcitrant litter (e.g., *Rhododendron*) may exacerbate seasonal resource limitation, especially for detritivores. Illustrating this, our E_a values for total (coarse-mesh) breakdown suggest that, between 12°C and 16°C, the time to 95% mass loss (calculated as $\log_e(0.05)/k$) would be reduced from 130 to 97 days for *Acer* (a reduction of approximately one month) and from 505 to 306 days for *Rhododendron* (a reduction of approximately seven months). These dynamics, in addition

to climate-driven changes in leaf characteristics and phenology (Knott et al. 2019), could alter the timing and magnitude of downstream C transport.

The role of fungal biomass in determining E_a

Understanding the importance of temperature-mediated shifts in organismal biomass vs. metabolic rates is important for predicting the effects of warming on ecosystem processes like OM breakdown. Our results suggest that temperature-mediated increases in fungal biomass accrual were important for determining the E_a of microbial OM breakdown. The additional variation in the microbial E_a that was not accounted for by correcting for fungal biomass may have been associated with temperature effects on microbial physiology (e.g., respiration rate; Manning et al. 2018) or bacterial biomass/activity (Hieber and Gessner 2002), though litter-associated bacterial biomass is negligible in southern Appalachian headwater streams (~0.02-1.3% of fungal biomass, Gulis et al. 2003, Suberkropp et al. 2010, V. Gulis, *unpublished data*). Differences in the temperature dependence of microbial parameters like biomass accrual, sporulation, respiration, and carbon use efficiency (Dang et al. 2009, Ferreira et al. 2014, Tomczyk et al. 2023) may impact higher trophic levels. For example, aquatic fungi are both a resource and a competitor for detritivores, so differences in the temperature dependence of, for example, fungal growth rate/ production vs. respiration, could determine temperature effects on the quantity and quality of litter resources available for detritivores in warmer streams (Chung and Suberkropp 2009, Marks 2019).

Seasonal effects of resource quantity, detritivore phenology, and physicochemical variables

Biological OM breakdown is a function of consumer abundance and biomass, OM quantity and quality, and factors that influence per-capita consumption rates, including species identity and development stage (Hieber and Gessner 2002). Many of these parameters shift seasonally in temperate headwater streams, which may have affected our E_a values given that our temperature gradient was mostly seasonal. As part of concurrent studies, we quantified (1) leaf litter standing stock (LLSS) in each stream in January, April, July, and October of YR1 and YR2, and (2) detritivore biomass and abundance in *Rhododendron* litterbags at the end of two winter and two summer YR1 deployments (collection dates: 11 December 2017, 5 January 2018, 5 July 2018, and 31 August 2018). Average LLSS was higher in January (~87 g m^{-2}) relative to July (~12 g m^{-2}; Appendix A: Table A8), and detritivore abundance and biomass were higher on the summer collection dates relative to the winter collection dates (Appendix A: Table A10, Figure A4). These patterns suggest that our litterbags may have provided an attractive resource for detritivores during summer months when background LLSS was low. While previous Coweeta studies suggest that both total invertebrate and detritivore biomass are higher in the winter on a per m^2 basis (P. A. Rogers, *unpublished data*, Cross et al. 2006, Wallace et al. 2015), several important Coweeta detritivores are also active in the summer (Huryn and Wallace 1987). Thus, leaf mass-specific detritivore biomass (and thus, breakdown rate) may be higher in summer months when LLSS is low. Despite these patterns, the random effect for deployment month in our models accounted for a substantial amount of variation in breakdown rates, much of which was likely relevant to the dynamics we have discussed here. Even after accounting for seasonality in our models, we still observed

clear effects of temperature on detritivore breakdown that exceeded predictions based on metabolic theory.

Other physicochemical factors also varied across seasons and streams in our study and may have impacted our E_a estimates. Physical abrasion from water flow can affect mass loss from coarse-mesh litterbags more than from fine-mesh litterbags (Tomczyk et al. 2022b). We observed higher discharge effects on detritivore breakdown than on microbial breakdown and variation in detritivore breakdown rates was high relative to microbial decomposition, suggesting that abrasion may have played a larger role in coarse-mesh than in fine-mesh breakdown in our study. However, differences in discharge across our study streams far surpassed seasonal differences within streams and detritivore breakdown rates were highest in warm summer months when discharge is at a seasonal low. Though water velocity at the litterbag surface would have been an ideal metric to account for abrasive leaf mass losses in our litterbags, the fixed effect for stream discharge in all our models allowed us to account for stream flow effects on breakdown rate that were independent of temperature. Thus, we are confident that our results with respect to temperature are robust to seasonal stream flow dynamics.

Numerous studies have made use of spatial (latitudinal; Guittar et al. 2016, De Frenne et al. 2013) or temporal (seasonal; Lipson 2007, Mofu et al. 2019) gradients to examine and predict the effects of temperature on population, community, and ecosystem processes, including organic matter breakdown. Just as large spatial gradients have limitations as a proxy for the local effects of warming due to climate change (e.g., due to shifts in organismal communities that co-occur with latitude), so too do seasonal gradients such as the one used in our study. Many factors co-vary with temperature

seasonally, and it is difficult to fully isolate one variable of interest (in our case, temperature; see Wilmot et al. 2021). We accounted for sources of variation other than temperature by including a fixed effect for stream discharge and random effects for deployment date and stream identity in each of our linear mixed-effects models. Including the random effect for deployment date is particularly important as it allows the model to account for seasonal effects. The fact that we still observed substantial temperature effects after accounting for these factors gives us confidence in the parameter estimates associated with temperature.

Conclusions

Our results shed new light on the importance of detritivores and microbes in controlling the temperature dependence of leaf litter breakdown in headwater streams. Detritivore breakdown rates were on average 2× lower than microbial breakdown rates but increased at a higher rate as temperatures rise, suggesting that C routing to detritivore-mediated fates such as invertebrate production, particle generation, and transport may increase to a greater degree than predicted by MTE as streams warm. Under a warming climate, earlier depletion of labile leaf litter and rapid breakdown of recalcitrant litter may exacerbate seasonal resource limitation for stream organisms and processes that depend on terrestrially-derived OM. Our study informs predictions about the thermal responses of stream consumers, how litter quality affects these responses, and how stream C fates will change in a warmer world.

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Table 2.1. Results of linear mixed-effects models for each breakdown category in our study. The "MTE probability" value is the probability (%) that the "true" E_a for a given breakdown category falls within the MTE-predicted range of 0.6-0.7 eV. These values were calculated as the relative area under the probability density curve between 0.6 and 0.7 given the E_a and standard error from each model.

	$1/k_BT - 1/k_BT_0$			**Discharge (L s^{-1})**		
	Slope (E_a)	95% CI	MTE probability	Slope	95% CI	*p*
Acer coarse (n = 209)	0.52	0.33 - 0.69	15%	0.09	0.02 - 0.15	0.0084
Acer detritivores (n = 184)	0.97	0.45 - 1.44	7%	0.25	0.09 - 0.42	0.0028
Acer microbes (n = 217)	0.29	0.20 - 0.36	<<1%	0.05	0.02 - 0.08	0.0026
Rhododendron coarse (n = 214)	0.89	0.69 - 1.08	2%	0.22	0.14 - 0.31	1.45×10^{-6}
Rhododendron detritivores (n = 185)	1.49	1.06 - 1.93	0.01%	0.29	0.11 - 0.48	0.0034
Rhododendron microbes (n = 217)	0.56	0.43 - 0.66	21%	0.12	0.07 - 0.16	9.81×10^{-7}

Note: All linear mixed-effects models included random effects for stream and deployment date.

Table 2.2. Results of linear mixed-effects models exploring the effect of fungal biomass on the relationships between *Rhododendron* fine-mesh breakdown and temperature/discharge in year 2 of our study.

Model 1:

$\text{lmer}(\log_e(\text{fine-mesh breakdown rate}) \sim (1/k_BT - 1/k_BT_0)$ + discharge (*z*-scored) + (1|stream) + (1|deployment date))

	Slope	**95% CI**	***p***
$1/k_BT - 1/k_BT_0$	0.40	0.25 - 0.53	4.39×10^{-5}
Discharge (L s^{-1}) (*z*-scored)	0.12	0.07 - 0.18	1.24×10^{-5}

Model 2:

$\text{lmer}(\log_e(\text{fine-mesh breakdown rate}) \sim \text{fungal biomass (centered)} + (1/k_BT - 1/k_BT_0)$ + discharge (*z*-scored) + (1|stream) + (1|deployment date))

	Slope	**95% CI**	***p***
Fungal biomass (centered)	0.01	0.008 - 0.013	$< 2 \times 10^{-16}$
$1/k_BT - 1/k_BT_0$	0.22	0.10 - 0.34	0.0015
Discharge (L s^{-1}) (*z*-scored)	0.11	0.06 - 0.15	3.12×10^{-6}

Notes: Model 1 included only temperature and discharge as fixed effects, while model 2 included an additional fixed effect for fungal biomass. Both models included random effects for stream and deployment date.

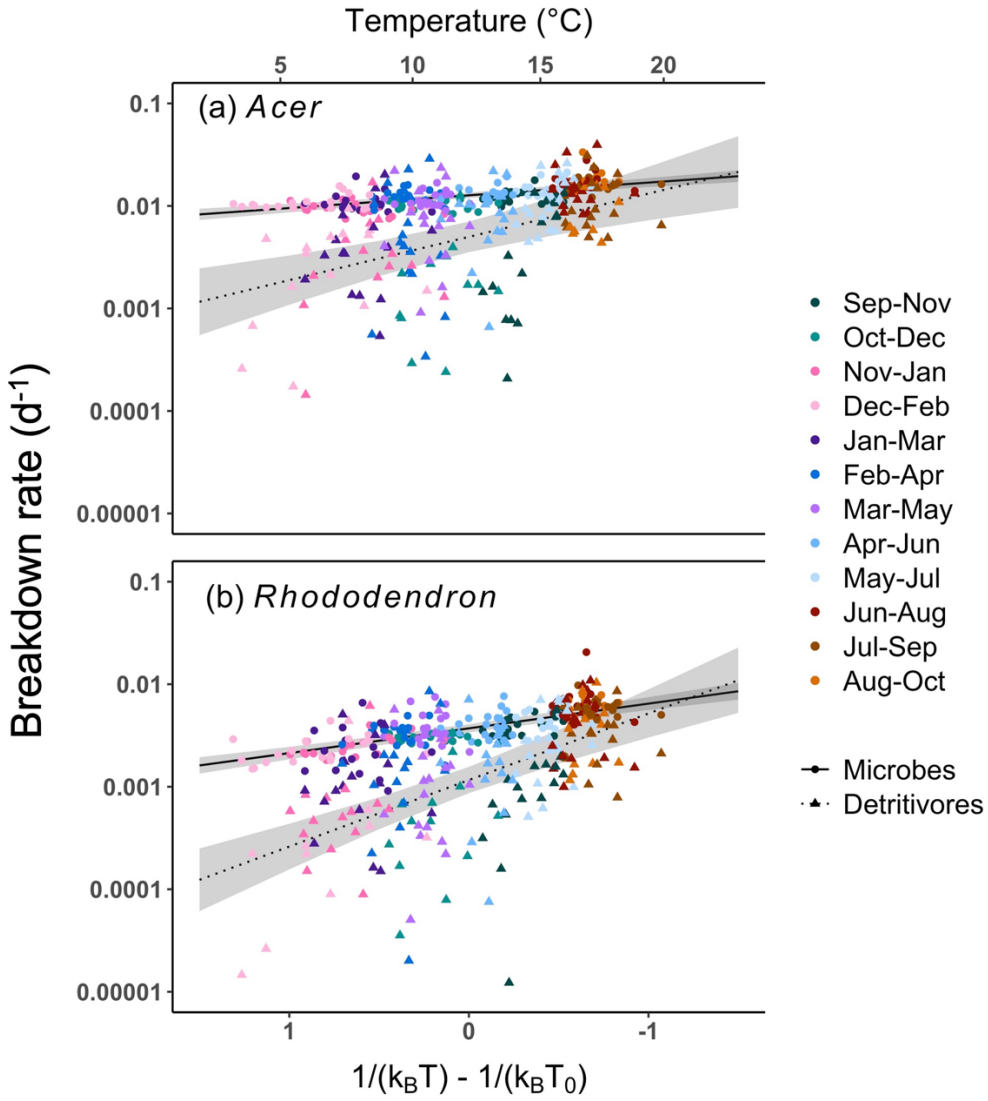

Figure 2.1. Breakdown rate (k, d^{-1}) vs. centered inverse Boltzmann temperature for *Acer* (a, detritivore $E_a = 0.97$ eV, microbe $E_a = 0.29$ eV) and *Rhododendron* (b, detritivore $E_a = 1.49$ eV, microbe $E_a = 0.56$ eV). Trendlines were generated using 'ggpredict' in the R package *ggeffects* (Lüdecke 2018) and reflect all fixed and random effects (Table 2.1). Shaded areas represent the 95% confidence intervals. Note the y-axis is $\log_e$-transformed and the bottom x-axis is reversed.

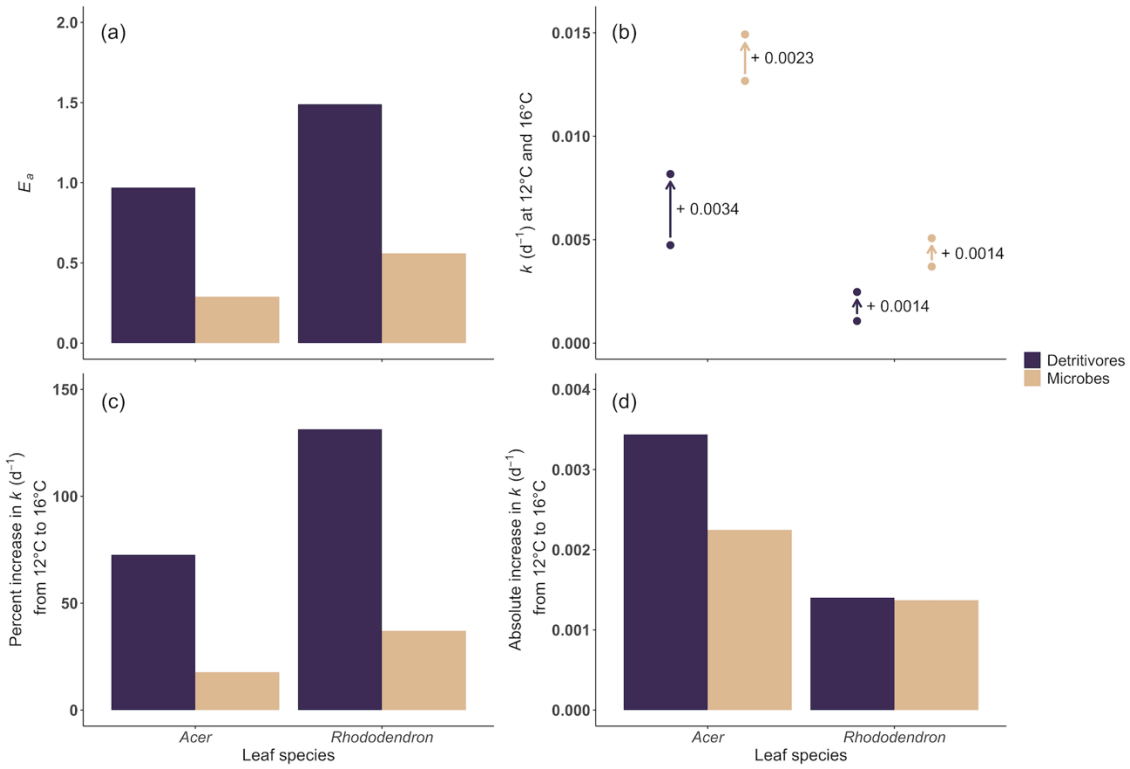

Figure 2.2. For detritivore and microbial breakdown of *Rhododendron* and *Acer* leaves: (a) E_a values, (b) k at 12°C and 16°C, (c) percent increases in k from 12°C to 16°C, and (d) absolute increases in k from 12°C to 16°C. k values were calculated according to the linearized Boltzmann-Arrhenius equation (Eq. 3) given our model E_a, $1/k_B T_0$, and $\log_e$-transformed k_0 values for each breakdown category.

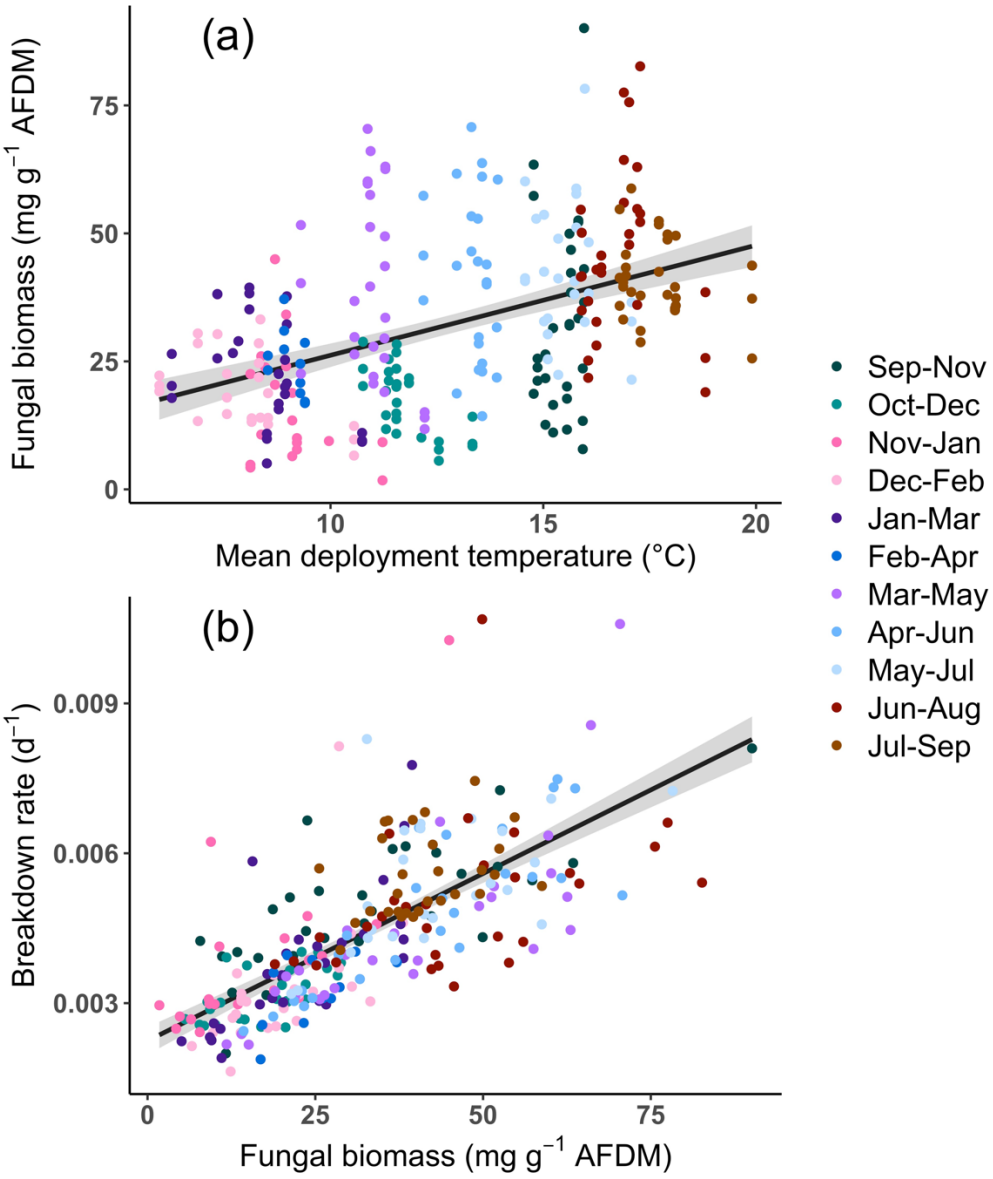

Figure 2.3. For YR2 *Rhododendron* fine-mesh breakdown: (a) fungal biomass vs. mean deployment temperature (°C) and (b) breakdown rate vs. fungal biomass. Solid lines represent least squares regression lines, shaded areas depict 95% confidence intervals.

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CHAPTER 3

SEASONAL DIFFERENCES IN AQUATIC INSECT COMMUNITY COMPOSITION ACROSS A LANDSCAPE-SCALE STREAM TEMPERATURE GRADIENT[2]

[2]Cummins, C.S., Rosemond, A.D., Freeman, M.C., Wenger, S.J., Bumpers, P.M., Rugenski, A.T., Tomczyk, N.J., and Benstead, J.P. To be submitted to *Freshwater Biology*.

Abstract

Temperature plays an important role in determining organismal physiology and community structure and, in turn, determining the rates of ecosystem processes. As global climate change progresses, there is considerable interest in understanding how temperature affects aquatic insect community structure, as these organisms play essential roles in basal resource processing and food web dynamics in riverine ecosystems. We identified invertebrates that we collected from leaf litterbags incubated for two two-month periods in the winter (October - December 2017, November - January 2018) and two two-month periods in the summer (May - July and July - late August 2018) in streams across a landscape-scale temperature gradient in the southern Appalachian mountains. We performed NMDS ordinations on aquatic invertebrate biomass data at the family, functional feeding group (FFG), and genus (detritivores only) scales to determine the role of temperature versus other environmental variables (stream discharge, nutrient concentrations, and leaf mass remaining in associated litterbags) in structuring aquatic invertebrate communities. We found that temperature was significantly related to community dissimilarity in summer months, but not winter months, and that stream temperature was correlated with stream discharge in the summer. Contrary to our expectations, we found few significant relationships between temperature and the biomasses or individual body sizes of invertebrate families and FFG's that were significantly related to community dissimilarity. These results suggest that temperature-associated shifts in aquatic invertebrate community structure are related to shifts in community composition and not changes in the biomass or body size of individual taxa. Our results also highlight that invertebrate communities in smaller, lower-order streams

may be particularly vulnerable to shifts in community composition as summer stream temperatures rise.

Introduction

Aquatic invertebrates play important roles in stream ecosystems through their processing of basal resources (Rosi-Marshall and Wallace 2002, Wallace et al. 2015) and generation of biomass and fine particles which fuel aquatic and terrestrial food webs (O'Hop et al. 1984, Webster et al. 1999, Baxter et al. 2005). Given that these organisms are restricted to the aquatic environment for all or part of their life cycles, stream temperature plays an essential role in determining their metabolism, physiological rates, and contributions to ecosystem structure and function (Bonacina et al. 2023). As stream temperatures rise due to global climate change, invertebrate community assembly may shift due to differences in thermal responses across taxonomic and functional groups. These temperature-mediated shifts may also depend on site-specific antecedent temperature conditions, species interactions, and resource dynamics. Understanding how temperature structures aquatic invertebrate communities, as well as how these shifts scale up to affect consumer-mediated ecosystem processes, is a central question in stream ecology.

Previous studies of temperature effects on aquatic invertebrate community structure have revealed that thermal preferences and responses vary according to species traits. For example, theoretical and empirical work suggests that warming may favor smaller-bodied taxa across scales of biological organization (Brown et al. 2004, Daufresne et al. 2009, Gardner et al. 2011). However, temperature effects on body size

may also depend on site-specific factors. In a stream warming experiment in Iceland, (Nelson et al. 2017) observed that warming increased the biomass and production of large-bodied aquatic invertebrate taxa while decreasing the biomass and production of small-bodied taxa. Unexpected patterns such as these may reflect site-specific species thermal preferences, species interactions, or effects of resource dynamics. Thermal responses may also vary according to the functional roles that aquatic invertebrates play in streams. For example, aquatic invertebrates in the grazer, scraper, and detritivore functional feeding groups have been suggested by several studies to be particularly cool-adapted and vulnerable to extirpation based on their current distributions and measured thermal limits (Pyne and Poff 2017, Jourdan et al. 2018, Tomczyk et al. 2022). On the other hand, the thermal responses for these taxonomic groups at the community scale may vary from expected patterns due to plasticity in thermal responses (Weaving et al. 2022) or differences in the thermal limits for survival versus other physiological traits (e.g., growth, development, and reproduction, Sweeney and Vannote 1984, Sweeney et al. 2018).

Antecedent, site-specific temperature conditions may interact with species traits to determine shifts in aquatic invertebrate community structure with warming. For example, invertebrate taxa living in cool, alpine streams may be particularly vulnerable to climate change due to cold adaptation of resident taxa and the likelihood that these ecosystems will undergo large changes in temperature and flow regimes as climate change progresses (Lencioni 2018, Birrell et al. 2020). Aquatic invertebrate communities in warm, tropical streams may also be particularly threatened by increasing temperatures, as resident taxa

adapted in streams with stable annual thermal regimes (Shah et al. 2017) and may already be living close to their thermal limits (Boyero et al. 2012, Shah et al. 2017).

While many studies have examined temperature-induced shifts in aquatic invertebrate community structure at global (Boyero et al. 2012), regional (Arai et al. 2015), stream reach (Nelson et al. 2017) and mesocosm scales (Jonsson et al. 2015), fewer have considered how temperature structures aquatic invertebrate communities across landscape-scale temperature gradients, as well as whether these effects differ seasonally. To examine the importance of temperature in structuring headwater stream invertebrate communities, we collected and identified invertebrates from leaf litterbags incubated for four winter months and four summer months across a landscape-scale stream temperature gradient generated by differences in elevation and aspect. We conducted non-metric multidimensional scaling (NMDS) analyses to determine the extent to which temperature and other factors (discharge, nutrient concentrations, and leaf mass remaining in associated litterbags) were related to invertebrate community dissimilarity at three different levels of taxonomic resolution (family, functional feeding group (FFG) and genus). We also used NMDS to examine correlations between community dissimilarity and changes in the biomass of invertebrate families, functional groups, and detritivore genera in winter and summer months. Since aquatic insect detritivores are thought to be more temperature-sensitive than other insect functional groups (Pyne and Poff 2017, Tomczyk et al. 2022), we hypothesized that community dissimilarity across the landscape temperature gradient would be related to changes in detritivore biomass, and specifically, that detritivore biomass would decline with temperature. Further, in line with previous studies and theoretical predictions (Brown et al. 2004, Daufresne et al.

2009, Gardner et al. 2011), we hypothesized that differences in temperature across the landscape gradient would be associated with differences taxon-specific body size.

Methods

Invertebrate sampling

We sampled invertebrates from leaf litterbags as part of a complimentary study (Cummins et al. *in review)* in which we measured leaf breakdown rates across a seasonal and landscape temperature gradient at the U.S. Department of Agriculture Forest Service Southern Research Station – Coweeta Hydrologic Laboratory (CHL). The CHL encompasses a 2185-ha basin with an extensive network of first-to third-order streams, which exist along a natural temperature gradient generated by changes in elevation and aspect. CHL forests are primarily composed of maple, oak, and tulip poplar trees and an understory dominated by *Rhododendron maximum* (Swank and Crossley 1988).

We incubated four pairs of coarse- (5 mm) and fine- (250 μm) mesh litterbags containing *Rhododendron* leaf litter (coarse-mesh: 5 ± 0.1g, fine-mesh: 1.5 ± 0.1g) in each of eleven first-order Coweeta streams for two two-month periods in the winter (19 October - 11 or 18 December 2017, 15 November 2017 - 5 January 2018) and two two-month periods in the summer (9 May - 5 July 2018, 5 July - 31 August 2018). Due to weather-related sampling constraints, there were two collection dates for the first winter incubation from October-December of 2017 such that we collected litterbags from 6 streams on 11 Dec and from the other 4 streams on 18 Dec. At the end of each incubation, we collected the litterbags, placed each one in a separate plastic bag, and transported them on ice back to the laboratory. We washed each pair of litterbags over a

1-mm sieve, then placed all the material that was left on the sieve after washing both bags (including invertebrates and miscellaneous detritus) into a plastic cup filled with 70% ethanol to preserve insect specimens. We randomly selected three litterbag pairs per stream and sampling event for invertebrate measurement and identification. For these samples, we sorted the material in each plastic cup under a dissecting microscope, isolating invertebrates from detrital material. We saved all invertebrates in scintillation vials filled with 70% ethanol, and we subsequently identified each individual and measured their lengths in millimeters (Merritt et al. 2019). To examine finer-scale community shifts within the detritivore functional group, we identified detritivore taxa to the genus level. We identified non-detritivore taxa to the family level.

Physicochemical data

As part of the leaf breakdown study, we deployed HOBO pendant temperature loggers (Onset Computer Corp., MA, USA) in each stream near the location of the litterbags. These loggers recorded temperature data at 15-minute intervals for the duration of each leaf litter incubation. We obtained daily discharge and weekly nutrient concentrations (dissolved inorganic nitrogen [DIN] as $NH_4^+ + NO_3^-$ and soluble reactive phosphorus [SRP] as PO_4^{3-}) for each of our study streams and litter incubation periods from the USFS and the Coweeta Long-Term Ecological Research Program (LTER). Additionally, as part of the leaf breakdown study, we determined the ash-free dry mass (AFDM) remaining in each litterbag at the end of each deployment (for detailed methods, please see Cummins et al. *in review*).

Data analysis

Physicochemical data

We determined mean daily temperature (°C), mean daily discharge (L s^{-1}), mean weekly nutrient concentrations (DIN and SRP, mg L^{-1}), and mean ash-free dry mass remaining in the litterbags (g) at the level of each season (winter or summer), averaging values across both winter incubation periods and both summer incubation periods (winter: 19 October 2017 - 5 January 2018, summer: 9 May - 31 August 2018). This resulted in season-specific temperature, discharge, nutrient concentration, and leaf AFDM remaining values for each stream, allowing us to examine shifts in invertebrate community structure according to these variables across the landscape gradient and determine whether patterns were consistent between winter and summer.

Invertebrate data

We constrained our analysis to invertebrate larvae, excluding other life stages (i.e., pupae and adults). After excluding one third-order stream from the dataset which was much larger than the other streams (WS08 - Shope Fork), as well as one incubation in which litterbags were collected one month too late, we were left with 113 invertebrate samples and a total of 7243 individual invertebrates across ten streams and four sampling events for community analysis. We assigned insects to functional feeding groups (FFGs) at the family level based on Merritt et al. (2019). We calculated taxon-specific insect mass (mg) in each sample based on previously-published order, family, or genus-level length-mass regressions (Benke et al. 1999). When calculating masses, we used the length-mass regression for the lowest available taxonomic resolution for each individual invertebrate specimen (order, family, or genus).

We calculated mean family- and FFG-level biomass (mg) at the level of each sampling event based on the three selected samples in each stream and incubation period.

To examine shifts in detritivore community structure, we filtered the whole community dataset for detritivores only, then calculated mean genus-level biomass for detritivores at the level of each stream and sampling event. This data carpentry resulted in two observations of invertebrate community structure based on averaged data from six litterbags (three in each sampling month) per stream per season for our NMDS analyses.

Data visualization and analysis

To examine changes in invertebrate community structure across the landscape gradient, we performed NMDS ordinations using a Bray-Curtis distribution based on $\log_e$-transformed family, FFG, and genus-level biomass values in winter and summer months. To minimize the influence of large, rare taxa, we first added 1 to each of the biomass values in the dataset before performing log transformations and ordinations. Using the function "envfit," we fit vectors onto each ordination for each of our environmental predictors (temperature, discharge, nutrient concentrations, and leaf AFDM remaining) and for each invertebrate family, FFG, and genus, depending on the data resolution for that particular ordination. We extracted the weighted average scores for each environmental and invertebrate predictor using the function "wascores" and plotted significant vectors ($p<0.05$) on NMDS ordination plots. To determine the extent to which invertebrate community structure across the landscape gradient depended on stream site or sampling date, we also ran permutational analyses of variance (PERMANOVA) for these factors for both winter and summer data. All analyses were performed in R using the package "vegan" (Oksanen et al. 2022)

For family- and FFG-level ordinations where temperature was significantly related to community dissimilarity, we performed exploratory linear regression analyses

to determine whether the biomass (mg) of each significant family or FFG was related to mean seasonal stream temperature (°C) across the landscape gradient. Each season-specific regression contained biomass data from two sampling events per stream in winter (December and January) and summer (July and August) months. To avoid erroneous conclusions based on the coincidental occurrence of rare taxa in warm or cool streams, we limited these exploratory regressions between temperature and the biomass of specific families to families that occurred in >10% of the litterbag invertebrate samples. There were a total of 57 samples in the winter and 56 in the summer, so we performed the family-level regressions on invertebrate families that occurred in >5.7 samples in the winter and >5.6 samples in the summer. Thus, in the winter, we performed family-level linear regressions for 24 out of 49 families, and in the summer, we performed family-level linear regressions for 28 out of 40 families. There were no detritivore families with more than one significant genus in genus-level analyses. Thus, we did not run linear regressions at the detritivore genus level.

To assess the degree to which invertebrate body size changed in response to temperature across the landscape gradient, we filtered the whole-community dataset for invertebrate families that occurred in >50% of the samples in each season. For these taxa, we ran season-specific linear regressions between mean season-specific stream temperature (°C) and average taxon-specific body size (mg) in each sample (n = 2 samples per stream per season). The invertebrate and physicochemical data associated with this project are publicly available on Zenodo (Cummins 2024).

Results

Physicochemical data

In winter months (19 October 2017 - 5 January 2018), mean daily temperature ranged from 7.4°C to 10.0°C across the landscape gradient (Table 3.1). In summer months (9 May - 31 August 2018), temperatures were higher on average and the landscape gradient slightly narrower (15.1°C - 17.2°C, Table 3.1). In the winter, mean daily discharge ranged from 1.1 L s^{-1} to 21.1 L s^{-1} (Table 3.1). The discharge gradient spanned nearly twice this range in summer months (3.4 L s^{-1} - 41.5 L s^{-1}) (Table 3.1). Mean weekly nutrient concentrations in our study streams were generally low and spanned similar ranges in both seasons for both DIN (winter: 2.7 - 142.3 µg L^{-1}; summer: 3.2 - 196.1 µg L^{-1}) and PO_4^{2-} (winter: 2.0 - 7.6 µg L^{-1}, summer: 2.0 - 6.9 µg L^{-1}; Table 3.1). Finally, mean AFDM remaining in coarse-mesh bags ranged from 3.9 g to 4.4 g in the winter and from 1.73 g - 3.2 g in the summer (Table 3.1). In checking for correlations among environmental variables, we found that temperature and DIN were correlated in winter months (Pearson correlation coefficient = -0.58, $p = 0.007$, Figure B4). In summer months, we found correlations between AFDM remaining and DIN (Pearson correlation coefficient = 0.66, $p = 0.002$, Figure B4), temperature and DIN (Pearson correlation coefficient = 0.52, $p = 0.02$, Figure B4), and temperature and discharge (Pearson correlation coefficient = -0.75, $p < 0.01$, Figure B4).

Family-level invertebrate community analysis

In the family-level, winter NMDS ordination, mean daily discharge (L s^{-1}) was the only significant environmental predictor of community dissimilarity (p=0.002, Table 3.5, Figure 3.1A), and there were eight significant family predictors (Table 3.6, Figure 3.1B). In the family-level, summer NMDS ordination, mean daily temperature (p=0.001), mean

daily discharge (p=0.005), and mean leaf AFDM remaining in associated litterbags (p=0.03) were all significant environmental predictors of community dissimilarity (Table 3.5, Figure 3.2A), and there were 15 significant invertebrate family predictors (Table 3.6, Figure 3.2B). Of the significant summer invertebrate family predictors that occurred in >10% of our samples, two exhibited significant, positive relationships between mean biomass and temperature according to linear regression models (Ephemeroptera: Heptageniidae, p=0.004; Megaloptera: Corydalidae, p=0.01, Table 3.2). One additional family exhibited a marginally significant, positive relationship between mean biomass and temperature (Plecoptera: Leuctridae, p=0.09, Table 3.2).

According to PERMANOVA, winter communities did not differ by sampling date (11 or 18 December, 5 January) but did differ among streams (p=0.01) at the family level. On the other hand, summer communities differed by both sampling date (5 July vs. 31 August, p=0.03) and stream (p=0.02) at the family level.

Functional feeding group-level invertebrate community analysis

In the FFG-level, winter NMDS ordination, mean daily discharge (L s^{-1}) was the only significant environmental predictor of community dissimilarity (p = 0.014, Table 3.5, Figure 3.3A) and there were three significant FFG predictors (Table 3.6, Figure 3.3B). In the summer, mean daily temperature (°C) and mean weekly DIN concentrations (mg L^{-1}) were significant environmental predictors of community dissimilarity (Table 3.5, Figure 3.4A), and there were five significant FFG predictors (Table 3.6, Figure 3.4B). Detritivores were a marginally significant FFG predictor in the summer NMDS (Table 3.6, p=0.06). Scrapers were the only summer FFG that exhibited a significant relationship between mean biomass (mg) and temperature (p=0.04, Table 3.3).

PERMANOVA tests at the FFG level indicated that invertebrate community structure did not differ among sampling dates but did differ among streams (p=0.02) in winter months. In contrast, summer FFG community structure exhibited a marginally significant difference among sampling dates (p=0.09), but no difference among streams.

Genus-level detritivore community analysis

In the detritivore-only, genus-level, winter NMDS ordination, there were no significant environmental predictors of community dissimilarity (Table 3.5); however, there were two significant detritivore genus predictors (Table 3.6, Figure 3.5). In the summer, mean daily temperature (°C), mean daily discharge (L s^{-1}) and mean AFDM remaining in litterbags (g) were all significant predictors of community dissimilarity at the genus level for detritivores (Table 3.5, Figure 3.6A), and there were seven significant detritivore genus predictors (Table 3.6, Figure 3.6B).

In PERMANOVA tests for detritivore, genus-level data, community structure did not differ by sample date or stream in winter months. However, in the summer, detritivore communities differed by both sample date (p=0.03) and stream (p=0.02).

Family-specific body size and temperature

We generally found no relationship between temperature and taxon-specific body mass for insect families that occurred in >50% of samples in winter and summer, respectively. The one exception to this was Leuctridae (Plecoptera), which exhibited a marginally significant, negative relationship between body mass and temperature in the summer (p=0.06, Table 3.4).

Discussion

We found that environmental and invertebrate predictors of community dissimilarity differed between winter and summer months across the landscape-scale stream temperature gradient, which spanned approximately 2 - 2.5 °C within each season. Temperature was a significant predictor of community dissimilarity in summer, but not in winter, indicating that temperature may play a more important role in structuring invertebrate communities at the landscape scale when overall temperatures are relatively high. In addition, temperature and discharge were correlated along our stream temperature gradient in the summer such that smaller, low-flow streams were also relatively warmer than larger, higher-flow streams. This may imply differences in the temperature sensitivity of invertebrate communities in lower- versus higher-order headwater streams.

With few exceptions, the biomass of the families and FFGs that were significantly correlated with community dissimilarity in NMDS ordinations were not related to among-stream temperature differences. This implies that temperature-associated shifts in summer community structure may be driven by changes in community composition rather than changes in the biomass of specific taxa. Further, we did not observe significant relationships between temperature and invertebrate body size with the exception of one detritivore family (Plecoptera: Leuctridae), which highlights that the relationship between temperature and invertebrate body size may depend on the magnitude and spatial extent of stream temperature gradients.

Variation in the importance of environmental factors across seasons

Temperature was not related to community dissimilarity in the winter, but arose as a significant environmental predictor of community dissimilarity at all three of the

taxonomic scales we tested (family, FFG, and genus) in the summer. Importantly, temperature was also correlated with stream discharge across our summer streams such that the lowest-flow streams were also the highest in temperature. Previous studies at the global scale have suggested that invertebrate communities in warmer streams may be more vulnerable to increases in temperature (Boyero et al. 2012). Our findings suggest that these dynamics could also play out at small spatial scales such that summer invertebrate communities may shift more with increasing temperature than winter communities, and that small, low-order streams may be particularly vulnerable to changes in temperature. Though we cannot say definitively whether changes in invertebrate community structure among streams were related to temperature, discharge, or a combination of these and other environmental variables in summer months, considering how temperature and discharge simultaneously structure invertebrate communities is essential. For example, Pyne and Poff (2017) highlighted that extirpation risk for aquatic invertebrate taxa across the western United States was related to both projected stream warming and stream drying as a result of global change. Increases in summer stream temperatures may be particularly consequential for aquatic invertebrate detritivores in small headwater streams, as these organisms face additional stressors related to resource supply with global change. These include increases in detrital carbon-to-nutrient ratios (Tuchman et al. 2002) and earlier annual depletion of detrital resources (Hare et al. *in review*).

Influence of invertebrate predictors across scales of taxonomic resolution

Though both temperature and multiple invertebrate vectors were significantly related to community dissimilarity in the summer, we observed very few significant

correlations between temperature and the biomass of specific invertebrate families or FFGs. The exceptions to this were Heptageniidae biomass and scraper biomass in the summer, both of which were positively related to temperature. Heptageniidae is an abundant scraper family in our study streams, and unsurprisingly, scraper biomass was significantly correlated with Heptageniidae biomass in the summer (estimate = 0.74, p = 0.003). Thus, it is likely that the significant correlation between scraper biomass and temperature in summer months was driven primarily by increases in Heptageniidae biomass. At first glance, these relationships appear contradictory to previous studies at regional scales, which have suggested that scrapers are particularly vulnerable to increasing temperatures (Pyne and Poff 2017, Jourdan et al. 2018). Our temperature gradients were relatively narrow in both seasons, and even the higher summer stream temperatures were likely not outside the thermal envelopes of most taxa in our study streams. Indeed, even taxa that are considered cool-adapted (thermal envelope ≈ 0 - 15°C based on current distributions, Twardochleb et al. 2021) were found in high abundance and biomass in streams across the landscape gradient where mean daily temperature reached >16°C (Figure B3). Aquatic invertebrate thermal limits are often measured in the lab using short-term thermal ramping experiments (Chown et al. 2015) or estimated from current distributions (Twardochleb et al. 2021), but other lines of evidence suggests that these organisms may persist well outside of measured thermal limits depending on the timing, duration, and intensity of temperature increases (Kendrick and Benstead 2013, Kim et al. 2017). Temperature effects on organismal community structure likely reflect multiple interdependent factors, including species interactions (e.g., predation, competition, and facilitation) and basal resource dynamics. These factors may play a

larger role in community-scale thermal responses across small spatial gradients than the thermal responses of individual taxa.

Temperature and body size across a landscape-scale temperature gradient

Many previous studies have observed declines in both taxon-specific and whole-community aquatic insect body size at emergence at higher temperatures (Jonsson et al. 2015, Sweeney et al. 2018), in line with theoretical predictions and observations from other organismal groups (Brown et al. 2004, Daufresne et al. 2009, Gardner et al. 2011). Of the five invertebrate taxa we tested in the winter and the eight taxa we tested in the summer, only one (Plecoptera: Leuctridae) exhibited a marginally significant, negative relationship between temperature and individual body size. Our gradient in mean temperature spanned approximately 2.1°C in the summer and 2.6°C in the winter, but the standard deviation in mean daily temperature for any given stream was often on the scale of or greater than this temperature gradient, particularly in the winter. In addition to mean temperatures, thermal variation is also known to predict insect thermal physiology and performance (Colinet et al. 2015); thus, despite differences in mean temperature, it is possible that within-stream temperature variation led to similar invertebrate thermal responses in the communities across our study streams. Indeed, aquatic invertebrate thermal niches are hypothesized to overlap more across altitudes in temperate streams than in tropical streams, as temperate systems exhibit high annual temperature variation and organisms likely experience a similar range in temperatures throughout the year (Shah et al. 2021)

Conclusions

We found that temperature was correlated with changes in aquatic invertebrate community structure among streams in summer months, but not winter months across a small-scale, landscape temperature gradient. Though many invertebrate families and functional feeding groups were correlated with community dissimilarity along the same axis as temperature in the summer, only one (Heptageniidae) exhibited a positive relationship between biomass and temperature across the summer landscape gradient. We also generally observed no relationship between body size and temperature for the most common invertebrates in our samples. Our results suggest seasonal variation in the biotic and abiotic factors that are most important for structuring aquatic invertebrate communities at the landscape scale and suggest that invertebrate communities in warm, low-order streams may shift with increases in summer stream temperatures.

Table 3.1. Mean daily temperature (°C), mean daily discharge (L s^{-1}), mean weekly DIN (mg L^{-1}), mean weekly PO_4^{2-} (mg L^{-1}), and mean AFDM remaining in litterbags (g) (± 1 SD for all parameters) in our 10 study streams during focal winter (19 October 2017 - 5 January 2018) and summer (9 May - 31 August 2018) time periods.

Stream	Elevation (m)	Temperature		Discharge		DIN		PO_4^{2-}		AFDM rem.	
		Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
WS06	690	7.4 ± 3.8	17.2 ± 1.2	1.1 ± 0.6	3.4 ± 2.5	142.3 ± 76.2	196.1 ± 26.3	5.9 ± 5.5	2.0 ± 0.1	4.0 ± 0.1	4.4 ± 4.6
WS14	695	7.9 ± 3.6	16.5 ± 1.1	9.1 ± 4.2	30.5 ± 22.2	11.6 ± 4.7	20.3 ± 11.7	2.7 ± 2.4	2.7 ± 1.4	4.1 ± 0.1	2.1 ± 0.9
WS13	701	8.4 ± 3.3	16.1 ± 1.0	2.1 ± 0.9	7.6 ± 5.2	5.1 ± 0.9	9.2 ± 2.7	2.0 ± 0.1	2.1 ± 0.5	4.0 ± 0.2	2.5 ± 0.6
WS07	716	9.2 ± 3.1	15.9 ± 0.8	7.8 ± 2.1	28.8 ± 15.5	22.4 ± 15.2	69.4 ± 11.5	3.7 ± 3.6	3.3 ± 1.4	3.9 ± 0.2	1.7 ± 0.5
WS18	717	7.8 ± 3.6	16.3 ± 1.4	1.7 ± 0.7	6.9 ± 5.9	8.6 ± 2.9	18.7 ± 10.5	2.7 ± 2.4	2.2 ± 0.6	4.0 ± 0.2	1.9 ± 0.6
WS02	721	10.0 ± 2.6	16.3 ± 0.9	1.1 ± 0.4	5.1 ± 3.9	5.4 ± 2.5	11.1 ± 2.6	7.6 ± 5.7	6.9 ± 3.7	4.0 ± 0.3	2.5 ± 0.6
WS31	863	9.2 ± 2.8	15.6 ± 0.9	8.4 ± 2.0	22.5 ± 15.9	2.7 ± 1.5	3.2 ± 0.7	4.7 ± 4.8	2.0 ± 0.05	4.2 ± 0.1	3.1 ± 0.5
WS32	921	8.6 ± 3.2	15.3 ± 0.9	11.8 ± 3.5	30.7 ± 24.0	3.0 ± 1.4	4.6 ± 1.5	2.7 ± 2.4	2.0 ± 0.1	4.1 ± 0.1	3.2 ± 0.4
WS36	1037	8.7 ± 2.7	15.2 ± 1.0	21.1 ± 14.3	41.5 ± 61.0	13.8 ± 11.4	33.0 ± 13.1	2.8 ± 2.7	2.2 ± 0.7	4.0 ± 0.1	2.1 ± 0.6
WS27	1078	7.5 ± 3.1	15.1 ± 1.3	15.3 ± 13.2	31.8 ± 54.2	60.4 ± 39.3	56.6 ± 18.6	4.9 ± 5.1	2.2 ± 0.8	4.1 ± 0.1	2.8 ± 0.3

Table 3.2. Results of linear regression models testing the relationship between temperature and the biomass (mg) of each invertebrate family that (1) was a significant predictor of community dissimilarity in the summer, family-level NMDS ordination, and (2) occurred in >10% of summer invertebrate samples.

Order: Family	DF	Intercept	Estimate (slope)	Std. Error	*p*	R^2
Diptera: Empididae	1, 17	1.8	-0.1	0.1	0.4	0.04
Diptera: Tipulidae	1, 17	-188.1	12.5	7.9	0.1	0.13
Ephemeroptera: Ephemerellidae	1, 17	-1.6	0.1	0.1	0.4	0.04
Ephemeroptera: Heptageniidae	1, 17	-21.2	1.4	0.4	0.004*	0.40
Megaloptera: Corydalidae	1, 17	-66.6	4.3	1.5	0.01*	0.31
Odonata: Gomphidae	1, 17	-31.6	2.4	4.2	0.6	0.02
Plecoptera: Leuctridae	1, 17	-5.2	0.4	0.2	0.09.	0.16
Plecoptera: Peltoperlidae	1, 17	183.6	-9.8	6.5	0.15	0.12
Plecoptera: Perlidae	1, 17	32.3	-1.7	3.7	0.66	0.01
Plecoptera: Perlodidae	1, 17	-6.1	0.5	1.2	0.7	0.01
Trichoptera: Hydropsychidae	1,17	-32.8	2.8	7.0	0.7	0.01
Trichoptera: Lepidostomatidae	1, 17	10.5	-0.3	1.9	0.9	0.001

Table 3.3. Results of linear regression models testing the relationship between temperature and the biomass (mg) of each FFG that was a significant predictor of community dissimilarity according to the summer, FFG-level NMDS ordination.

FFG	DF	Intercept	Estimate (slope)	Std. Error	*p*	R^2
Scrapers	1, 17	-17.8	1.2	0.6	0.04*	0.23
Predators	1, 17	-92.1	6.5	3.7	0.10	0.15
Collector-Gatherers	1, 17	-8.7	0.7	0.5	0.15	0.12
Collector-Filterers	1, 17	-35.1	3.0	7.0	0.7	0.01
Shredders (marginally significant, p=0.06)	1, 17	102.9	-4.1	6.7	0.55	0.02

Table 3.4. Results of linear regression models testing the relationship between temperature and taxon-specific body mass (mg) for insect families that occurred in >50% of samples in winter and in summer, respectively.

Season	Order-Family	DF	Intercept	Estimate (slope)	Std. Error	*p*	R^2
Winter	Diptera: Chironomidae	1, 18	0.05	0.001	0.006	0.84	0.002
Winter	Ephemeroptera: Leptophlebiidae	1, 18	0.9	-0.06	0.04	0.16	0.15
Winter	Plecoptera: Nemouridae	1, 18	0.6	-0.01	0.09	0.87	0.002
Winter	Plecoptera: Perlodidae	1, 18	6.0	-0.5	0.77	0.53	0.03
Winter	Trichoptera: Hydropsychidae	1, 18	1.7	0.03	0.24	0.90	0.001
Summer	Diptera: Chironomidae	1, 17	-0.1	0.009	0.006	0.19	0.10
Summer	Ephemeroptera: Heptageniidae	1, 17	-11.3	0.8	0.50	0.14	0.15
Summer	Plecoptera: Leuctridae	1, 17	1.3	-0.06	0.03	0.06.	0.25
Summer	Plecoptera: Nemouridae	1, 17	1.1	-0.1	0.07	0.41	0.05
Summer	Plecoptera: Peltoperlidae	1, 17	-0.3	0.1	0.23	0.66	0.01
Summer	Plecoptera: Perlidae	1, 17	5.3	-0.1	2.75	0.97	8.4e-05
Summer	Trichoptera: Hydropsychidae	1, 17	0.7	-0.004	0.1	0.97	7.9e-05
Summer	Trichoptera: Lepidostomatidae	1, 17	5.7	-0.3	0.4	0.55	0.02

Table 3.5. Environmental variables significantly associated with invertebrate community dissimilarity ($p<0.05$) in summer and winter months for family, FFG, and genus-level NMDS ordinations. Environmental variables were stream- and season-specific discharge (L s^{-1}), temperature (°C), dissolved inorganic nitrogen (DIN, mg L^{-1}), PO_4^{2-} (mg L^{-1}) and ash-free dry mass (AFDM) remaining in associated litterbags (g). Ordinations were based on biomass data calculated at each level of invertebrate community resolution. We added 1 and log-transformed each biomass value before performing ordinations. Community analyses were performed in the R package *vegan* (Oksanen et al. 2022).

	Winter	**Summer**
Family	Discharge	Discharge, Temperature, AFDM remaining
FFG	Discharge	Temperature, DIN
Genus (detritivores)	None	Discharge, Temperature, AFDM remaining

Table 3.6. Invertebrate variables significantly associated with invertebrate community dissimilarity ($p<0.05$) in summer and winter months for family, FFG, and genus-level NMDS ordinations. Ordinations were based on biomass data calculated at each level of invertebrate community resolution. We added 1 and log-transformed each biomass value before performing ordinations. Community analyses were performed in the R package *vegan* (Oksanen et al. 2022).

	Winter	Summer
Family	**Ephemeroptera:** Ephemeridae, Heptageniidae, Leptophlebiidae, Baetidae **Odonata:** Gomphidae **Plecoptera:** Peltoperlidae **Trichoptera:** Hydropsychidae, Philopotamidae	**Diptera:** Empididae, Tipulidae **Ephemeroptera:** Ephemerellidae, Heptageniidae, "unknown" **Megaloptera:** Corydalidae **Odonata:** Cordulegastridae, Gomphidae **Plecoptera:** Leuctridae, Peltoperlidae, Perlidae, Perlodidae **Trichoptera:** Hydropsychidae, Lepidostomatidae, Odontoceridae
FFG	Collector-Filterers, Scrapers, Shredders	Collector-Filterers, Collector-Gatherers, Predators, Scrapers, Shredders
Genus (detritivores)	**Diptera:** Tipulidae *Tipula* **Plecoptera:** Peltoperlidae *Tallaperla*	**Coleoptera:** Ptilodactylidae *Anchytarsus* **Diptera:** Tipulidae *Tipula* **Plecoptera:** Leuctridae *Leuctra*, Peltoperlidae *Tallaperla* **Trichoptera:** Lepidostomatidae *Lepidostoma*, Odontoceridae *Psilotreta*, Sericostomatidae *Fattigia*

Table 3.7. Unique invertebrate families, functional feeding groups (FFGs), and genera (detritivores only) from our winter litterbag samples based on the filtered invertebrate data used for our NMDS analyses. Orders with associated "unk" families indicate that the order had at least some specimens that were unidentifiable to family, but the order-only data were nonetheless included in the NMDS.

Families (Order: Family)		FFGs	Genera (Order: Family *Genus*)
Amphipoda:	(unk family)	Collector-Gatherers	**Coleoptera:**
Coleoptera:	Elmidae	Collector-Filterers	Ptilodactylidae *Anchytarsus*
	Psephenidae	Predators	
	Ptilodactylidae	Scrapers	**Diptera:**
Collembola:	(unk family)	Shredders	Tipulidae *Tipula*
Diptera:	Athericidae	Unknown	
	Cecidomyiidae		**Plecoptera:**
	Ceratopogonidae		Capniidae *Paracapnia*
	Chironomidae		Leuctridae *Leuctra*
	Dixidae		Peltoperlidae *Tallaperla*
	Empididae		Peltoperlidae *Viehoperla*
	Limoniidae		Taeniopterygidae *Taeniopteryx*
	Pediciidae		
	Psychodidae		**Trichoptera:**
	Sciaridae		Lepidostomatidae *Lepidostoma*
	Simuliidae		Limnephilidae *Hydatophylax*
	Tipulidae		Limnephilidae *Pycnopsyche*
	(unk family)		Odontoceridae *Psilotreta*
Ephemeroptera:	Baetidae		Sericostomatidae *Fattigia*
	Ephemerellidae		
	Ephemeridae		
	Heptageniidae		
	Leptophlebiidae		
	(unk family)		
Odonata:	Cordulegastridae		
	Gomphidae		
	(unk family)		
Oligochaeta:	(unk family)		
Plecoptera:	Capniidae		
	Chloroperlidae		
	Leuctridae		
	Nemouridae		
	Peltoperlidae		
	Perlidae		
	Perlodidae		
	Taeniopterygidae		
	(unk family)		
Trichoptera:	Brachycentridae		
	Hydropsychidae		
	Lepidostomatidae		
	Leptoceridae		
	Limnephilidae		
	Odontoceridae		
	Philopotamidae		
	Polycentropodidae		
	Psychomyiidae		
	Rhyacophilidae		
	Sericostomatidae		
	(unk family)		
Total family richness: **49**		Total FFG richness: **6**	Total detritivore genus richness: **12**

Table 3.8. Unique invertebrate families, functional feeding groups (FFGs), and genera (detritivores only) found in our litterbag samples in summer months, as well as the total richness for each, based on the filtered invertebrate data used for our NMDS analyses. Orders with associated "unk" families indicate that the order had at least some specimens that were unidentifiable to family, but the order-only data were nonetheless included in the NMDS.

Families (Order: Family)		FFGs	Genera (Order: Family *Genus*)
Amphipoda:	(unk family)	Collector-Gatherers	**Coleoptera:**
Coleoptera:	Carabidae	Collector-Filterers	Ptilodactylidae *Anchytarsus*
	Elmidae	Predators	
	Psephenidae	Scrapers	**Diptera:**
	Ptilodactylidae	Shredders	Tipulidae *Tipula*
Collembola:	(unk family)	Unknown	
Diptera:	Ceratopogonidae		**Plecoptera:**
	Chironomidae		Leuctridae *Leuctra*
	Dixidae		Peltoperlidae *Tallaperla*
	Empididae		Peltoperlidae *Viehoperla*
	Limoniidae		Pteronarcyidae *Pteronarcys*
	Pediciidae		
	Psychodidae		**Trichoptera:**
	Simuliidae		Lepidostomatidae *Lepidostoma*
	Tipulidae		Odontoceridae *Psilotreta*
Ephemeroptera:	Ameletidae		Sericostomatidae *Fattigia*
	Baetidae		
	Ephemerellidae		
	Ephemeridae		
	Heptageniidae		
	Leptophlebiidae		
	(unk family)		
Megaloptera:	Corydalidae		
	(unk family)		
Odonata:	Cordulegastridae		
	Gomphidae		
Oligochaeta:	(unk family)		
Plecoptera:	Leuctridae		
	Nemouridae		
	Peltoperlidae		
	Perlidae		
	Perlodidae		
	Pteronarcyidae		
Trichoptera:	Hydropsychidae		
	Lepidostomatidae		
	Odontoceridae		
	Philopotamidae		
	Psychomyiidae		
	Rhyacophilidae		
	Sericostomatidae		
Total family richness: **40**		Total FFG richness: **6**	Total detritivore genus richness: **9**

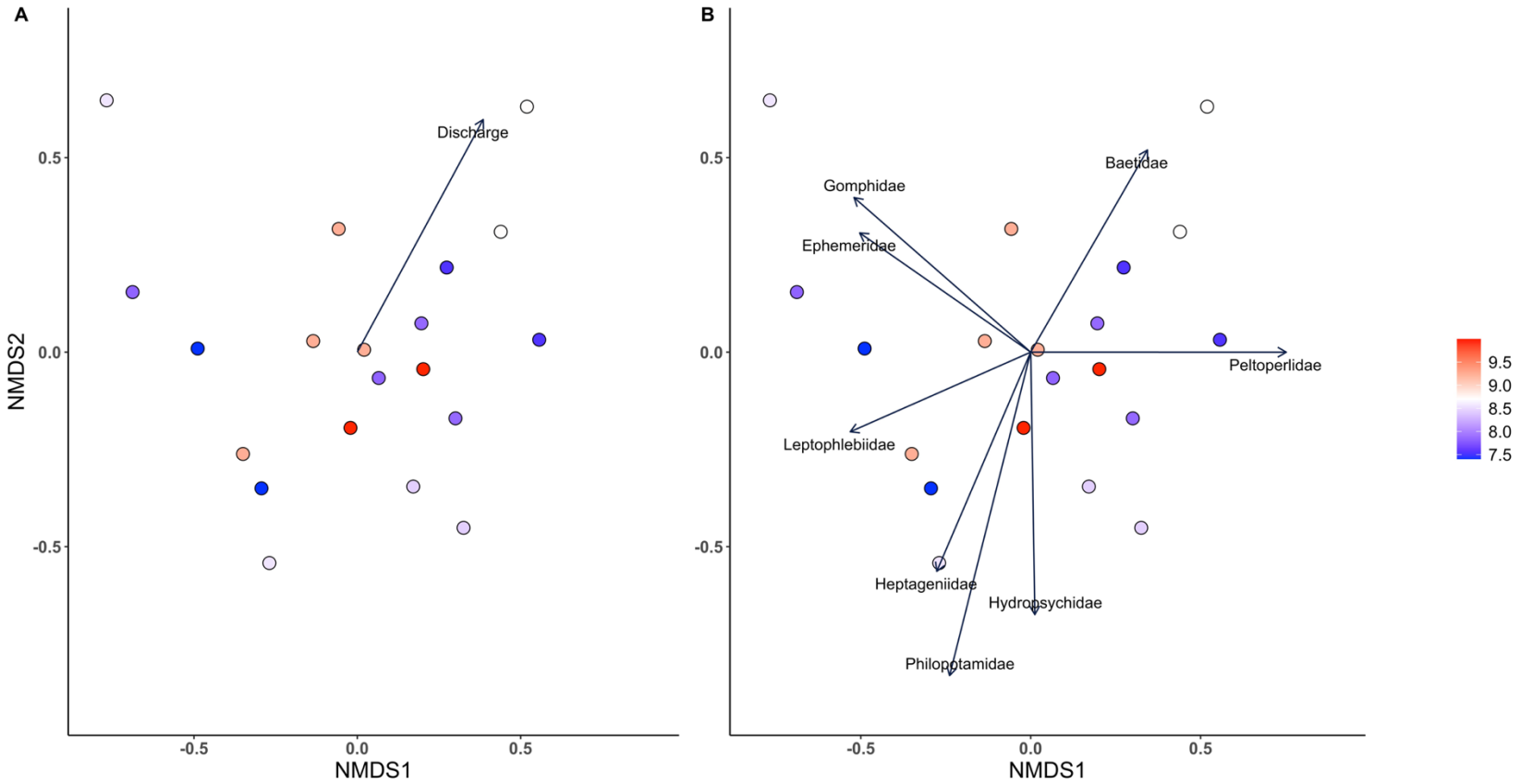

Figure 3.1. Winter invertebrate community differences based on family-level biomass data, visualized using NMDS. Arrows represent significant ($p < 0.05$) environmental (A) and family (B) correlations with sample position in ordination space. Points represent the invertebrate community in a given stream on a given sampling date (n = 2 dates per stream; 11 or 18 December 2017, 5 January 2018) based on mean family biomass data averaged across three litterbags. Points are colored according to the mean daily temperature in each stream during the winter litterbag incubations (19 October 2017 - 5 January 2018)

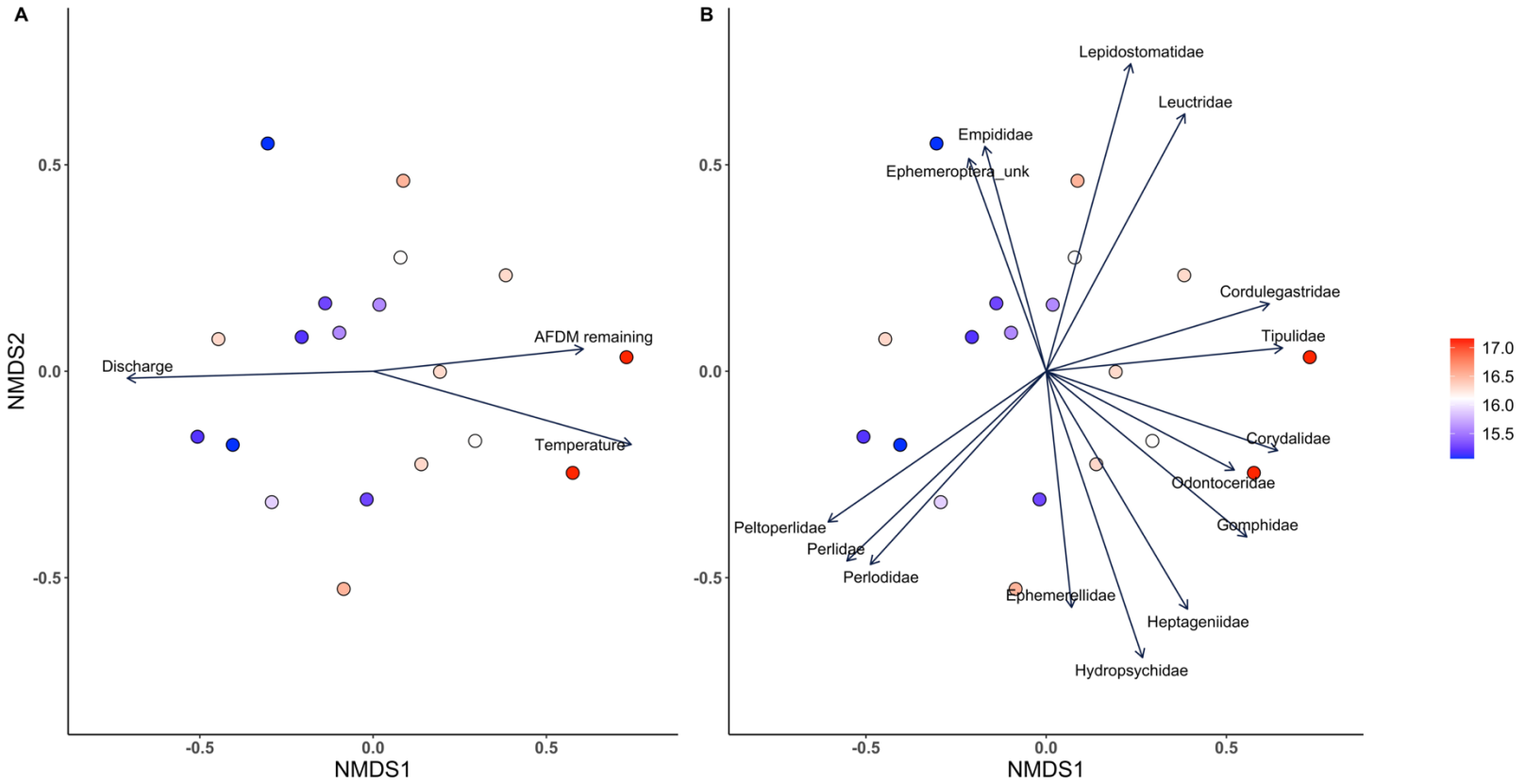

Figure 3.2. Summer invertebrate community differences based on family-level biomass data, visualized using NMDS. Arrows represent significant ($p < 0.05$) environmental (A) and family (B) correlations with sample position in ordination space. Points represent the invertebrate community in a given stream on a given sampling date (n = 2 dates per stream; 5 July 2018, 31 August 2018) based on mean family biomass data averaged across three litterbags. Points are colored according to the mean daily temperature in each stream during the summer litterbag incubations (9 May - 31 August 2018).

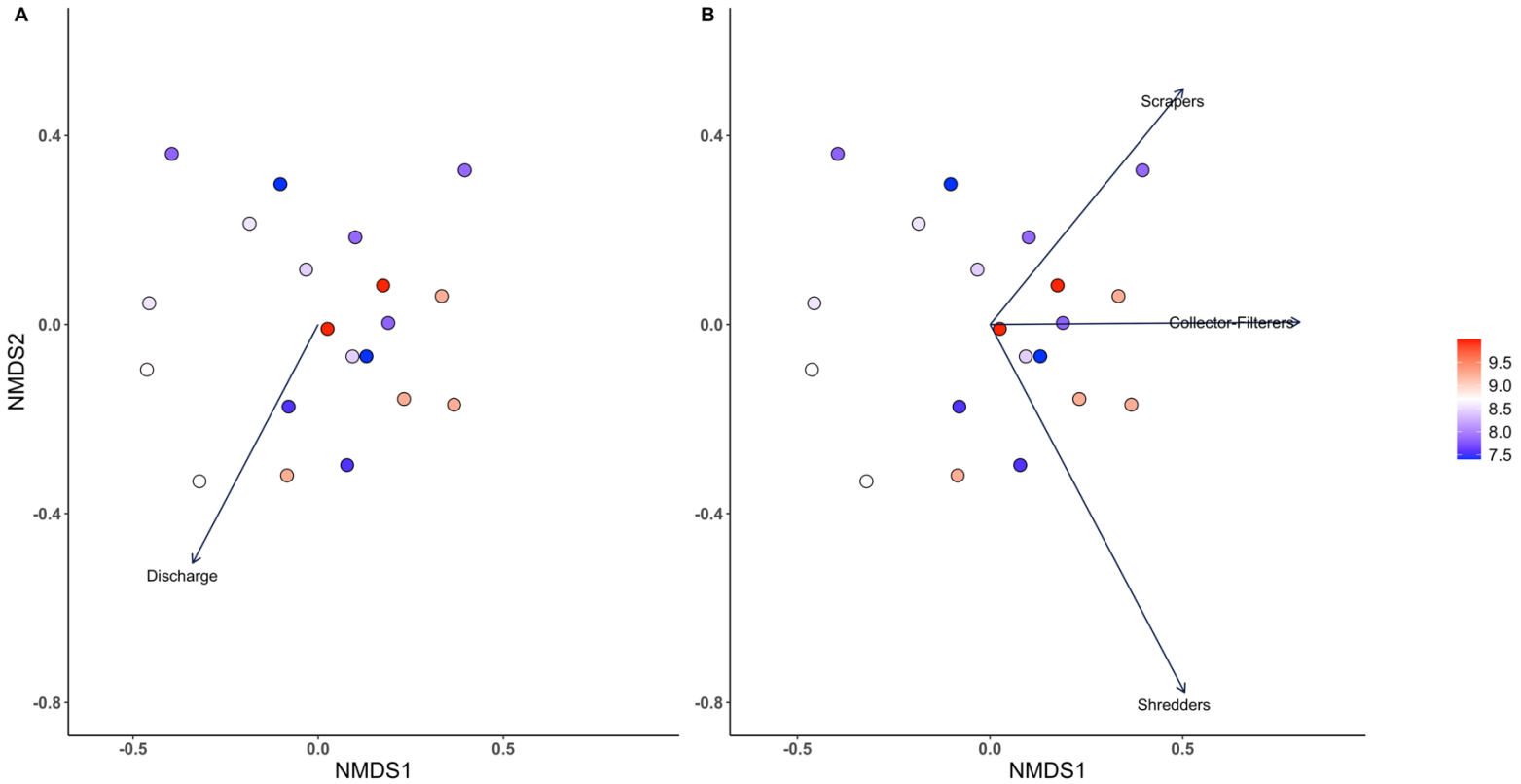

Figure 3.3. Winter invertebrate community differences based on functional feeding group-level biomass data, visualized using NMDS. Arrows represent significant ($p < 0.05$) environmental (A) and FFG (B) correlations with sample position in ordination space. Points represent the invertebrate community in a given stream on a given sampling date (n = 2 dates per stream; 11 or 18 December 2017, 5 January 2018) based on mean FFG biomass data averaged across three litterbags. Points are colored according to the mean daily temperature in each stream during the winter litterbag incubations (19 October 2017 - 5 January 2018).

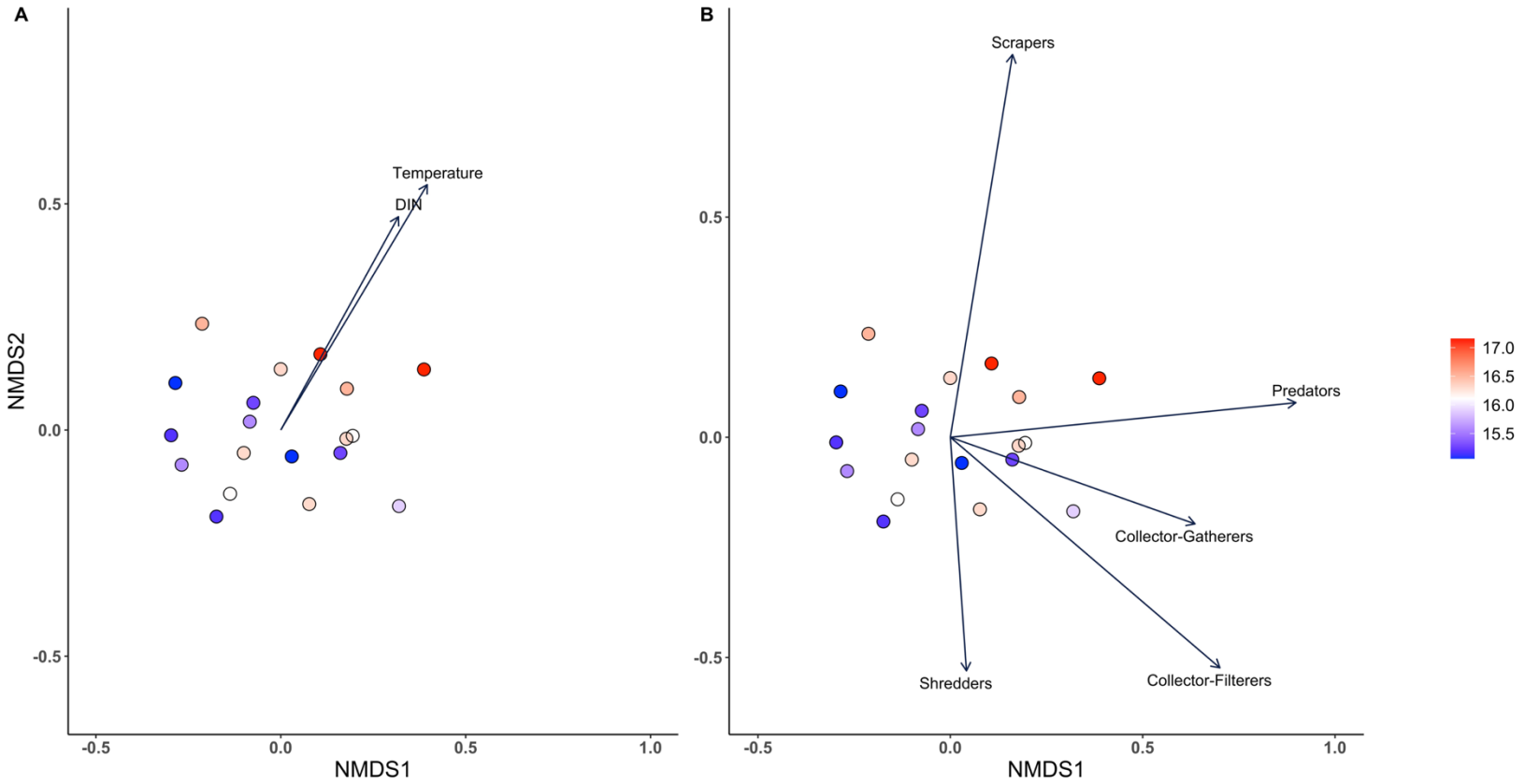

Figure 3.4. Summer invertebrate community differences based on functional feeding group-level biomass data, visualized using NMDS. Arrows represent significant ($p < 0.05$) environmental (A) and FFG (B) correlations with sample position in ordination space. The marginally significant "shredders" arrow ($p=0.06$) is also displayed in panel B. Points represent the invertebrate community in a given stream on a given sampling date (n = 2 dates per stream; 5 July 2018, 31 August 2018) based on mean FFG biomass data averaged across three litterbags. Points are colored according to the mean daily temperature in each stream during the summer litterbag incubations (9 May - 31 August 2018).

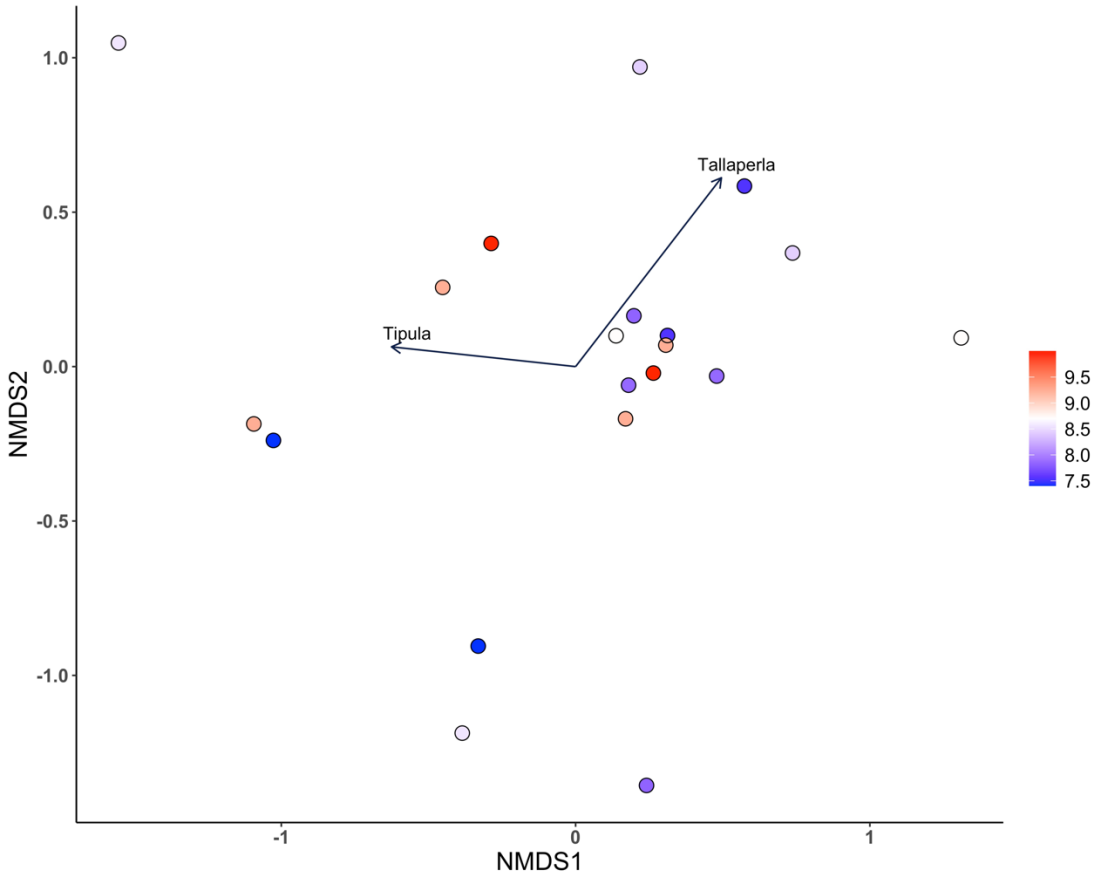

Figure 3.5. Winter invertebrate community differences based on detritivore genus-level biomass data, visualized using NMDS. Arrows represent significant ($p < 0.05$) genus correlations with sample position in ordination space. Points represent the detritivore community in a given stream on a given sampling date (n = 2 dates per stream; 11 or 18 December 2017, 5 January 2018) based on mean genus biomass data averaged across three litterbags. Points are colored according to the mean daily temperature in each stream during the winter litterbag incubations (19 October 2017 - 5 January 2018). There were no significant environmental vectors in the winter for the genus-level detritivore community analysis.

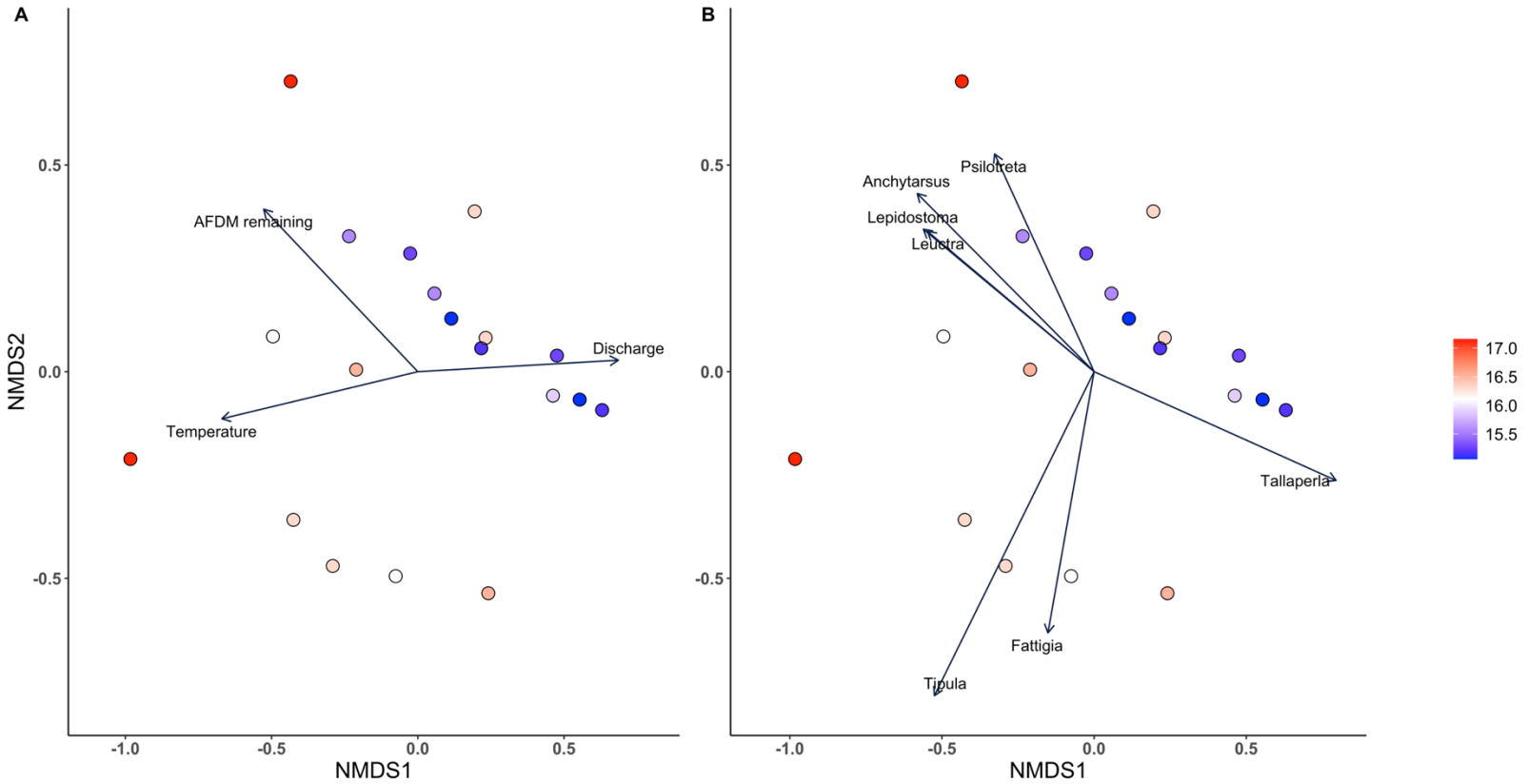

Figure 3.6. Summer invertebrate community differences based on detritivore genus-level biomass data, visualized using NMDS. Arrows represent significant ($p < 0.05$) environmental (A) and genus (B) correlations with sample position in ordination space. Points represent the detritivore community in a given stream on a given sampling date (n = 2 dates per stream; 5 July 2017, 31 August 2018) based on mean genus biomass data averaged across three litterbags. Points are colored according to the mean daily temperature in each stream during the summer litterbag incubations (9 May - 31 August 2018)

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CHAPTER 4

AQUATIC INSECT THERMAL RESPONSES VARY ACROSS PHYSIOLOGICAL PARAMETERS AND DIETS[3]

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Abstract

Temperature is an important control on metabolic rate and organismal contribution to ecosystem processes. While many studies have measured aquatic insect thermal performance, fewer have simultaneously measured thermal responses for multiple physiological parameters and examined how different parameters are modulated by diet. We quantified multiple thermal responses (survival probability, development stage, growth rate, and consumption rate) of a detritivorous stream insect across five temperature treatments (ambient (mean = 10.4°C), +1°C, +2°C, +3°C, +4°C) and two leaf litter types that differ in nutritional quality (*Rhododendron maximum* = high carbon-to-nutrient [C:N] ratio; *Acer rubrum* = low C:N ratio). Our five-week experiment took place in outdoor streamside channels (n = 20) with food resources and aquatic insects (nymphs of *Tallaperla* spp., Plecoptera: Peltoperlidae) collected from nearby streams, Survival probability declined with temperature, particularly later in the experiment, and was lowest in the +3°C and +4°C treatments. Development stage was more advanced for insects in higher (+3°C and +4°C) vs lower (ambient, +1°C, and +2°C) temperature treatments. Growth rates were not predictably related to temperature, but were highest in intermediate temperature treatments. Though temperature treatment effects did not differ based on food quality for survival, development, or growth rate, temperature effects on consumption rate depended on diet. Consumption rates increased with temperature for insects fed higher-C:N *Rhododendron*, but there was no relationship between temperature and consumption rate for insects fed lower-C:N *Acer*. Our results highlight differential thermal sensitivity of different physiological parameters in a detritivorous stream insect, as well as the mechanisms (e.g., compensatory feeding) that these organisms may use to

cope with thermal stress. These findings have implications for detritivore population dynamics and detritivore-mediated ecosystem processes in a warmer world.

Introduction

Temperature plays an essential role in determining organismal metabolic rates (Huey and Kingsolver 1989, Brown et al. 2004), species interactions (Magnuson et al. 1979), and community structure (Stillman 2002, Sunday et al. 2012). Organismal physiological responses to stressors like increased temperature can scale up to determine consumer-mediated ecosystem processes, with consequences for energy and nutrient cycling under warmer conditions (Reiners 1986, Atkinson et al. 2017). According to the Metabolic Theory of Ecology (MTE), organismal metabolic rates increase with temperature in line with the canonical temperature dependence of cellular respiration (0.6-0.7 eV; Brown et al. 2004); however, thermal responses may depend on species traits, proximity to thermal limits, and the ability to acclimate physiologically or adapt genetically to changes in the thermal environment (Somero 2010, Michaletz and Garen 2024). Thermal responses are known to vary among species and individuals, but the degree to which variation in thermal responses reflects differential temperature effects on different physiological parameters (e.g., growth, development, or feeding rates) warrants further exploration. As ecosystems warm due to climate change, understanding variation in thermal responses across physiological parameters is essential for predicting species distributions and activity, as well as the rates of associated ecosystem processes.

Headwater streams are hotspots for processing of terrestrial organic carbon (C; Hotchkiss et al. 2015), which in these systems is driven by microbes (primarily aquatic

fungi) and detritivorous macroinvertebrates (hereafter detritivores; Webster et al. 1999, Hieber and Gessner 2002). Both of these organismal groups are sensitive to temperature, but detritivores are thought to be particularly sensitive given that they are primarily restricted to cool, shaded streams and often belong to taxonomic groups that possess temperature-sensitive traits (e.g., external gills; Pyne and Poff 2017, Tomczyk et al. 2022). Detritivores play important roles in converting terrestrially-derived detrital C into smaller particles and invertebrate biomass, fueling terrestrial and aquatic food webs (Rosi-Marshall and Wallace 2002, Baxter et al. 2005). Aquatic fungi play an essential role in this food web, as microbial biomass on leaf litter helps support detritivore growth despite the low nutrient content of detrital resources (Chung and Suberkropp 2009, Marks 2019). Concurrent thermal responses of detritivores and aquatic fungi will determine shifts in both the rate of stream C processing and the fates of detrital C as temperatures rise.

Aquatic insect physiological rates generally increase with temperature, but thermal responses vary across parameters, species, and environmental contexts. For example, both aquatic insect growth and development rates have been consistently shown to increase with temperature, but increases in development rates tend to outpace increases in growth rate (Sweeney and Vannote 1978, Sweeney et al. 2018, Bonacina et al. 2023). As a consequence of these mismatched thermal responses, insects reared at higher temperatures often exhibit smaller adult body sizes and reduced fecundity compared to those reared at lower temperatures (Sweeney et al. 2018). Insect consumption rate may also increase with temperature due to higher energetic demand (Vannote and Sweeney 1980), but this response may be modulated by food resource quality and quantity. For

example, insects feeding on “poor-quality” food (i.e., high-C, low-nutrient resources) may compensate for nutritional deficiency by increasing consumption rates at high temperatures more than their counterparts feeding on higher-quality food (Flores et al. 2014). A lower-quality diet may also affect other physiological processes, increasing insect thermal sensitivity (Díaz Villanueva et al. 2011, Kendrick and Benstead 2013). Understanding aquatic insect thermal responses in the face of changing detrital chemistry is essential, as global change is expected to result in higher leaf C:N in concert with rising temperatures (Tuchman et al. 2002, Knott et al. 2019).

Many studies have measured aquatic insect thermal responses and considered interactions between temperature and other factors (e.g., food quality, CO_2 concentrations, and species interactions; Bonacina et al. 2023). However, studies aimed at quantifying aquatic insect thermal physiology have been primarily conducted in laboratory environments, exposing insects to a gradient of constant temperatures over relatively short timescales (e.g., hours) (Kendrick and Benstead 2013, Kim et al. 2017, Bonacina et al. 2023). Further, while many studies measure thermal responses for one or two parameters, fewer have measured simultaneous responses for a broader suite of physiological and functional parameters. For a better understanding of the thermal responses, climate vulnerability, and future functional role of aquatic insects, studies are needed that (1) mimic natural environmental conditions (e.g., flowing water, diel temperature fluctuation), (2) simultaneously measure thermal responses for multiple parameters, and (3) examine the extent to which food resource quality modulates multiple thermal responses.

We conducted an experiment in which we measured survival, development, growth rates, and consumption rates of a representative aquatic insect detritivore (nymphs of *Tallaperla* spp., Plecoptera: Peltoperlidae) in 20 artificial flow-through stream channels. *Tallaperla* is widely distributed in headwater streams across eastern North America (Merritt et al. 2019) and its shredding activity is an important contributor to instream C processing (O'Hop et al. 1984, Wallace et al. 1999, Cross et al. 2005). Our experiment encompassed five temperature treatments (ambient, +1°C, +2°C, +3°C, and +4°C) and two litter species: *Acer rubrum* (hereafter *Acer* = lower C:N ratio) and *Rhododendron maximum* (hereafter *Rhododendron* = higher C:N ratio; Manning et al. 2015). For an additional metric of leaf litter quality, we quantified fungal biomass on a subset of leaves fed to *Tallaperla* nymphs. We predicted that *Tallaperla* in higher temperature treatments would generally exhibit higher consumption, growth, and development rates due to temperature-mediated increases in individual metabolic rate. However, we hypothesized that thermal responses would be modulated by litter characteristics. Specifically, we predicted that, if the low nutritional value of *Rhododendron* litter exacerbated thermal stress, insects fed higher-nutrient *Acer* litter would exhibit higher growth, development, and survival rates at high temperatures than insects fed *Rhododendron*. Finally, we predicted that insects in higher temperature treatments would have a lower survival probability due to thermal stress.

Methods

Study site and experimental design

We conducted our experiment in artificial stream channels at the U.S. Department of Agriculture Forest Service (USFS) Coweeta Hydrologic Laboratory (CHL; Macon County, North Carolina, USA) from 13 March to 17 April 2019. The CHL encompasses a network of first- to third-order forest streams with high biomass and productivity of detritivorous insects (Wallace et al. 2015). In-stream productivity of Coweeta streams is driven by leaf inputs dominated by maple (*Acer*), oak (*Quercus*), and tulip poplar (*Liriodendron*), as well as *Rhododendron*, which makes up much of the forest understory (Swank and Crossley 1988). We pumped water from a fourth-order stream (Shope Fork) into a 1500-L tank and diverted it into five header tanks. One header tank (378 L) held stream water at the ambient stream temperature, while the remaining four tanks (189 L) were fitted with recirculating heaters that warmed the water to 1, 2, 3, and 4°C above ambient. Water from header tanks flowed through adjustable spouts at a rate of 0.1 L s^{-1} into channels (height = 60 mm, width = 75 mm [bottom] and 115 mm [top], length = 3 m), which were arranged in two randomized blocks, each with two channels per temperature treatment (n = 20, Appendix C, Figure C2).

Growth rate and development stage measurements

On 11 March 2019, we collected *Tallaperla* nymphs from naturally occurring leaf packs in a first-order stream at the CHL (Carpenter Branch). We sorted insects into size-classes and selected individuals from the "medium" class for the experiment (range in initial body lengths = 6.3 - 10.4 mm). On 12 March 2019, we placed each *Tallaperla* individual in a Petri dish fitted with 1-mm grid paper and took photos to measure initial lengths. We then transferred each individual to an experimental chamber, which were halves of Toby TeaBoy™ plastic and nylon-mesh tea infusers (diameter = 56 mm, height

= 32 mm, mesh size ≈ 250 µm) sealed on top with 500-µm nylon window screen secured by a rubber band. After we had taken photos of all insects assigned to a given channel, we submerged those chambers into the assigned channel in random order (n = 16 insect chambers per channel, 8 per leaf species). Due to logistical constraints, we set up 15 of 20 channels on 12 March 2019 (240 insects). Thus, to ensure similar initial conditions for all *Tallaperla* in the experiment, we collected 80 new nymphs from Carpenter Branch at the end of the day on 12 March 2019 for setup of the remaining five channels on 13 March 2019. Insects were first placed in empty chambers for 8-12 hours to allow for gut clearance. At the end of the experiment (17 Apr 2019), we took photos of all remaining live insects, following the same channel order and methods used during experimental setup.

Insect feeding and consumption rate measurements

On the first day of each week of the experiment, we supplied all chambers with conditioned leaf fragments in the same channel order followed during photo taking (see Appendix C for leaf conditioning methods). Eight insect chambers in each channel were assigned to each leaf species treatment (*Rhododendron* or *Acer*). We measured insect consumption rates in three week-long consumption trials (week 1: 12-13 March to 20-21 March, week 3: 27-28 March to 3-4 April, and week 5: 10 April to 17 April). In week 1, we randomly selected four insect chambers per leaf species per channel for consumption rate measurements. Due to logistical constraints, we reduced the number of insect consumption rate measurements to two per leaf species per channel in weeks 3 and 5. To account for leaf mass loss due to microbial decomposition, we incubated two additional chambers per leaf species in each channel that included leaf fragments but no insects. We

estimated the initial leaf mass supplied to each chamber by blotting fragments with a paper towel and weighing them to the nearest 0.1 mg (Halvorson et al. 2015). We supplied non-consumption trial chambers, as well as all chambers in weeks 2 and 4 (when feeding trials were not conducted), with un-weighed leaf fragments. *Acer* is inherently lower in mass than *Rhododendron*, so we supplied *Rhododendron* chambers with two leaf fragments and *Acer* chambers with three leaf fragments (mean initial blotted leaf mass, *Rhododendron* = 260 mg; mean initial blotted leaf mass, *Acer* = 170 mg).

At the end of each experimental week, we retrieved week-old leaf fragments from all chambers. For consumption trial chambers, we rinsed the fragments and stored them on ice for transport back to the laboratory. We discarded the week-old fragments from non-consumption trial chambers in weeks 1, 3, and 5, as well as all the week-old fragments in weeks 2 and 4. In the laboratory, we dried the post-consumption fragments for ≥48 h at 60°C, weighed them, combusted them in a muffle furnace at 500°C for 5.5 hours, and reweighed them to determine ash-free dry mass (AFDM).

Survivorship

Each week while refreshing leaf fragments, we recorded survivorship in every insect chamber and removed chambers in which insects had died from the experiment.

Fungal biomass

We estimated fungal biomass (mg g $AFDM^{-1}$) by quantifying ergosterol concentrations from one leaf-only chamber per leaf species per channel in experiment weeks 1 and 5 and a subset of temperature treatments (ambient, +2°C, and +4°C). At the end of the consumption trials, we placed leaf fragments from the selected leaf-only

chamber in pre-weighed scintillation vials. We transported these vials on ice back to the lab, freeze-dried them, and weighed them to determine leaf dry mass. We then extracted lipids and calculated ergosterol concentrations (Gulis and Bärlocher 2017, Appendix C). After excluding three samples with quality-control flags, we were left with 45 samples to determine patterns in fungal biomass across trials and treatments.

Data processing and analysis

Survival

We used survival regression to assess insect survival probability across temperature and leaf species treatments based on channel-level mortality data from each week of our experiment. We used the "Surv" function in the R package *survival* to prepare our data for analysis, formatting it as "interval-censored" because we monitored survival between known time intervals (R Core Team 2021, Therneau 2023). We analyzed the data with a parametric survival regression model with fixed effects for temperature treatment, leaf species, and the interaction between temperature treatment and leaf species. We constructed this model using the function "ic_par" in the package *icenReg*, which is specifically designed for analyzing interval-censored survival data (Anderson-Bergman 2017). We specified an accelerated failure time model with a Weibull distribution to account for the effect of time on survival probability. To account for repeated measures of survival at the channel level, we used the *icenReg* function "ic_clustBoot" for post-hoc adjustment of the standard error values.

Development

We characterized insect development at the end of the experiment based on the presence or absence of black wingpads in final insect photos. In hemimetabolous aquatic

insects such as *Tallaperla*, black wingpads appear in the final stages of larval development, shortly before the insect emerges as a winged adult (Merritt et al. 2019). To assess the effects of temperature treatment and leaf species on the probability of developing black wingpads by the end of the experiment, we conducted a binomial regression analysis using the function "glm" in R with temperature treatment and leaf species as fixed effects. After finding no effect of leaf species on the probability of developing black wingpads, we re-ran the model excluding leaf species.

Growth

We used ImageJ (Schneider et al. 2012) to measure initial (day 0) and final (day 36) insect lengths (mm) from photos of all insects that survived to the end of the experiment. We used these lengths to calculate initial and final insect dry masses (mg) using a length-mass regression (Benke et al. 1999) and determined instantaneous growth rates (IGRs) according to Equation 1:

$$IGR = \frac{log_e(final\ mass) - log_e(initial\ mass)}{Days\ in\ experiment} \quad \text{Eq. 1}$$

Insects for which photo quality was compromised (e.g., where body orientation was compromised or the photo was blurry such that accurate measurements were not possible, $n = 10$) and those that had developed black wingpads by the end of the experiment ($n = 10$) were excluded from growth rate analysis. Insects with black wingpads exhibited significant elongations in the initial stages of emergence, which are attributable to development and not true growth. Using a linear mixed-effects model, we modeled IGR as a function of temperature treatment, leaf species, and the interaction between temperature treatment and leaf species. Our linear mixed-effects model also included a random effect for channel identity. There were 54 negative IGR values in the final dataset

(*n*=182) which were likely attributable to measurement error associated with the challenges of measuring negligible changes in length from insect photos. We elected to keep these values in the dataset for modeling purposes, since we could not rule out that some insects may have truly lost length (e.g., in the process of molting) and to maintain the variation in the original dataset.

Consumption

We determined leaf AFDM for each chamber in each consumption trial using either a leaf species-specific blotted mass-to-AFDM regression (initial, Appendix C, Table C1) or the measured AFDM (final). We calculated insect consumption rates and microbial decomposition rates (mg d^{-1}) according to Equation 2:

$$Consumption\ or\ decomposition\ rate = \frac{AFDM_{initial} - AFDM_{final}}{Days\ in\ trial} \quad \textbf{Eq. 2}$$

To isolate insect contributions to leaf mass loss, we first subtracted the mean microbe-only decomposition rate at the trial, temperature treatment, and leaf species level from each individual insect consumption rate. We then mass-corrected the insect consumption rates by dividing each rate by an estimate of average insect mass during the consumption trial. We obtained these estimates of average insect mass during consumption trials by averaging insect mass on the first and last day of the trial, which we modeled based on insect beginning mass, days in experiment, and the global mean IGR. We modeled mass-specific consumption rate as a function of mean weekly channel temperature during corresponding consumption trials (range 9.3 - 16.9°C), leaf species, and the interaction between temperature and leaf species using a linear mixed-effects model with a random effect for trial number.

Fungal biomass

To determine temperature effects on detrital palatability and quality over the course of our experiment, we assessed the effect of mean weekly channel temperature (°C) on fungal biomass (mg $AFDM^{-1}$). To do this, we ran a linear mixed-effects model with an interaction for leaf species and a random effect for consumption trial (week 1 vs. week 5). We then conducted an ANOVA to assess the effect of consumption trial on fungal biomass with an interaction for leaf species. We performed post-hoc pairwise comparisons using the function "TukeyHSD" in R. In addition to testing differences in fungal biomass across temperatures and trials, we examined the relationship between fungal biomass and consumption rate in separate linear mixed-effects models for each leaf species, each with a random effect for trial number.

Results

Temperature and experimental conditions

Channels consistently maintained their target temperature elevations throughout the experiment (Appendix C, Table C2, Figure C1). The mean and range of temperatures in the five treatments were (10.7 (6.2 - 15.9), 11.8 (6.2 - 18.5), 12.6 (6.4 - 17.7), 13.6 (6.4 - 18.4), and 14.5 (6.0 - 19.3) for the ambient, +1°C, +2°C, +3°C, and +4°C treatments, respectively (Table C2). All treatments experienced diel temperature fluctuations and warmed steadily throughout the experiment due to seasonal temperature change. For example, the mean temperature in the +4°C channels was 12.8°C during the first week of the experiment (13-20 March), but during the last week of the experiment (10-17 April), the +4°C channels reached a mean temperature of 16.8°C. Ambient channels had a slightly higher (+0.5°C) mean and median temperature than the stream from which we

pumped water (Shope Fork) during the experiment, likely due to greater exposure of small volumes of water with large surface area to air (Table C2). Flow in each channel remained consistent throughout the experiment at an approximate rate of 0.1 L s^{-1}.

Survival

In a model with temperature treatment, leaf species, and the interaction between temperature and leaf species as fixed effects, temperature treatment had a marginally significant, negative effect on survival time (p=0.09). The coefficient associated with the temperature covariate in this model was -0.09 (SE = ±0.05), indicating that for each one-degree increase in temperature treatment, the logarithm of survival time in days decreased by 0.09. This translates to an approximately 8.7% decrease in survival time per degree increase in temperature treatment. Neither leaf species nor the interaction between temperature and leaf species had a significant effect on survival probability in this model (Figure 4.1b), so we re-ran the model without leaf species. In the model with only temperature as a fixed effect, there was a significant, negative effect of temperature on survival time, and the parameter estimate was unchanged (estimate = -0.09, SE = ±0.03, p = 0.003, Figure 4.1a).

Development

Though only 45 of 128 insects survived to the end of the experiment in the +3°C and +4°C treatments, 10 developed black wingpads. In contrast, only one of the 158 surviving insects in the ambient, +1°C, and +2°C treatments developed black wingpads by the end of the experiment. In our binomial regression model, temperature treatment had a significant, positive effect on the probability of developing black wingpads by the end of the experiment ($p < 0.001$, Figure 4.2). The temperature coefficient in this model

(log-odds ratio) was 1.3 (SE = ±0.3), indicating an odds ratio ($e^{1.3}$) of 3.7 and that, for every one-degree increase in temperature treatment, *Tallaperla* individuals were approximately 3.7 times more likely to develop black wingpads by the end of the experiment.

Growth

Instantaneous growth rates were highest in the +2°C treatment for insects fed *Rhododendron* leaves, while they peaked in the +3°C treatment for insects fed *Acer* leaves (Figure 4.3). We found no significant effects of temperature treatment or leaf species on insect IGR, including in exploratory models with a quadratic term for temperature.

Consumption rate

For insects fed *Rhododendron* leaves, there was a significant and positive relationship between mean weekly channel temperature (°C) and mass-specific consumption rate (slope = 0.04, SE = ± 0.01, $p < 0.001$, Figure 4.4). In contrast, for insects fed *Acer* leaves, there was no relationship between temperature and mass-specific consumption rate (Figure 4.4). The slope of the relationship between temperature and consumption rate was significantly higher for *Rhododendron*-fed insects than for *Acer*-fed insects ($p << 0.001$), but the intercept of the relationship was significantly higher for *Acer*-fed insects ($0.25 > -0.33$, $p << 0.001$, Figure 4.4).

Fungal biomass

Temperature (°C) had no significant effect on fungal biomass (mg g^{-1} AFDM) for *Rhododendron* or *Acer* leaf fragments in microbial chambers. However, there were significant effects of both leaf species and time on fungal biomass in our experiment.

Specifically, in week 5 of our experiment, fungal biomass on *Acer* leaves was significantly higher (+24.4 mg g^{-1} AFDM) than fungal biomass on *Rhododendron* leaves ($p << 0.01$, Figure 4.5). Fungal biomass on *Acer* leaves in week 5 was also significantly higher than fungal biomass on *Acer* leaves in week 1 (+21.2 mg g^{-1} AFDM, $p << 0.01$, Figure 4.5). For *Acer*-fed insects, there was no effect of fungal biomass on consumption rate. However, for *Rhododendron*-fed insects, there was a significant, negative relationship between fungal biomass and consumption rate (estimate = -0.02, $p = 0.01$, Figure C3).

Discussion

We observed variation in thermal responses among the parameters we measured, as well as in whether responses depended on food quality. Survival decreased and development stage increased with temperature, with the greatest effects at the highest temperature treatments. Contrary to our predictions, *Tallaperla* growth rate did not respond positively to temperature and instead peaked in intermediate temperature treatments. Consumption rate increased with temperature, but only for insects fed a higher-C:N leaf species. Our results suggest that organismal physiological parameters are not uniform in the direction and magnitude of their temperature response, with implications for the overall role that stream insect detritivores play in stream C processing as temperatures rise.

Survival probability, thermal stress, and the timing of temperature increases

Temperature substantially reduced survival probability for insects in our experiment, particularly during later weeks when temperatures in all treatments rose with

ambient stream temperatures. In a separate study, we measured temperature (°C) in the stream from which we collected insects (Carpenter Branch) at 15-min intervals for one year (22 September 2017 - 28 September 2018) and recorded a maximum daily temperature value of 18.1°C on 14 September 2018 (Appendix C, Table C2, Figure C1, Cummins et al. *in review*). However, during the timeframe that corresponded to our experiment (12 Mar - 17 Apr 2018), the maximum daily temperature we recorded in Carpenter Branch was only 13.6°C (Appendix C, Table C2, Figure C1). The maximum temperatures we recorded in the ambient and +4°C channels were 15.9°C and 19.3°C respectively; thus, even insects in ambient channels experienced higher temperatures than would be expected in Carpenter Branch during the time of our experiment, while the insects in higher (+3°C and +4°C) temperature treatments experienced temperatures that were close to or beyond the maximum annual temperature in the stream from which they were collected.

Based on initial insect lengths and black wingpad development, the insects in our experiment likely hatched in approximately November 2017 and were close to the end of their two-year life cycle when we began the experiment (O'Hop et al. 1984, Huryn and Wallace 1987). Thus, it is somewhat surprising that we observed such high mortality in higher temperature treatments given that the insects would have experienced temperatures of a similar magnitude during the summer of the previous year. However, stream habitats are more heterogeneous than our channels and chambers, providing more thermal refugia (e.g., shaded areas, groundwater input sites). These refugia may play an important role in the ability of stream organisms to persist at high temperatures, particularly for long-lived, temperate organisms like *Tallaperla* that experience a large

annual temperature range (Sheldon and Tewksbury 2014, Dallas and Ross-Gillespie 2015). Additionally, the timing of high temperatures in our experiment relative to the insects' life cycles may have contributed to high mortality. The final stages of hemimetabolous aquatic insect development involve substantial metabolic changes (e.g., molting), which can be more stressful at high temperatures (Camp et al. 2014). Thus, perhaps the insects in our experiment were more sensitive to high temperatures at the time when we conducted our experiment than they were earlier in their life cycles.

Growth and development

Tallaperla development stage was more advanced at higher temperatures in our experiment, which is consistent with previous studies that show shorter insect development times at higher temperatures (Sweeney and Vannote 1978, Sweeney et al. 2018). However, we did not observe a clear relationship between temperature and growth rate in either of our leaf species treatments. Instead, instantaneous growth rates (IGR) were highly variable in our experiment (range = -0.007 - 0.022 d^{-1}), and the mean IGR that we measured (0.002 d^{-1}) was relatively low compared to previous measurements for *Tallaperla* (e.g., ~0.014 d^{-1} O'Hop et al. 1984). Nonetheless, we observed a unimodal relationship between temperature and growth rate, a result which is consistent with previous studies (Bonacina et al. 2023). The clear increase in development rate with temperature that we observed indicates that temperature shortens the development window of a long-lived and abundant eastern North American stonefly species. Faster increases in development rate than growth rate for detritivores leading to shortened development windows and smaller body size at emergence could result in reduced routing of C to these organisms as stream temperatures rise.

Consumption rate, compensatory feeding, and the role of microbial biomass

Consumption rates were variable in our experiment and included negative values, indicating that some *Tallaperla* chambers exhibited leaf mass gain during consumption trials. Negative consumption rates likely arose from error in estimating the initial mass of leaf fragments and/or accumulation of fine particulate organic matter on leaf surfaces. Regardless, these negative values likely reflect very low consumption rates rather than true leaf mass gain. Despite the challenge of obtaining accurate detritivore consumption measurements, we observed a significant increase in consumption rate with temperature for *Rhododendron*-fed insects in our experiment. This pattern may suggest compensatory increases in feeding rate at high temperatures for insects fed lower-quality litter (Flores et al. 2014). The negative relationship we observed between fungal biomass and consumption rate for insects in the *Rhododendron* treatment is consistent with this hypothesis, as it indicates that insects consumed more when *Rhododendron* leaf fragments had the lowest level of microbial conditioning and thus were less palatable for detritivores (Marks 2019). In contrast, the weak negative relationship between temperature and consumption rate and the lack of relationship between fungal biomass and consumption rate in the *Acer* treatment indicate that *Tallaperla* fed a higher-nutrient leaf species did not exhibit compensatory feeding. Increases in fungal biomass likely generated increases in food quality (e.g., lower C:N or C:P; Manning et al. 2016) over time in the *Acer* treatment, potentially alleviating the need for increases in consumption rate as temperatures increased later in the experiment. Despite these patterns, our results suggest that neither compensatory feeding in the *Rhododendron* treatment nor increases in food quality in the *Acer* treatment led to differences in survival probability among leaf

species treatments. This indicates that factors other than food quality were more important for determining insect survival at high temperatures. These patterns shed light on the mechanisms that organisms use to cope with thermal stress (e.g., compensatory feeding) and the degree to which these mechanisms confer benefits to survival.

The importance of timing and duration in thermal physiology experiments

Based on previously published cohort production intervals for *Tallaperla*, the insects in our experiment were near the end of their two-year life cycle (O'Hop et al. 1984, Huryn and Wallace 1987). Aquatic insects grow and feed at a slower rate at the end of their larval life cycles (Merritt et al. 2019), so the timing of our experiment may explain our relatively low and variable growth and consumption rates. The timing of our experiment at the end of the insects' life cycles may also explain some mortality in high temperature treatments. In addition to the factors contributing to mortality discussed above, it is possible that some insects died during the process of trying to emerge. This mortality could have been caused either by the increased stress of undergoing emergence at high temperatures (Camp et al. 2014) or the inability to escape from submerged chambers.

While our experiment was long relative to many other laboratory experiments testing aquatic insect thermal physiology, it was short in the context of our insects' life cycle. Experiments conducted on short-lived taxa (e.g., mayfly or midge larvae; Sweeney et al. 2018) are well-suited to estimating temperature effects on physiological parameters over entire life cycles, but capturing these patterns for longer-lived taxa is more difficult. Additionally, while many studies have quantified temperature effects on discrete insect life stages (e.g., eggs, larvae; Brittain 1977, Chown et al. 2015), the extent to which

thermal sensitivity differs within life stages (e.g., early- vs. late-instar larvae) remains unclear. Future studies aimed at how thermal responses differ across aquatic insect life stages, as well as how temperature affects long-lived taxa over the course of their entire life cycles, are necessary to accurately predict organismal responses to future warming and consequent shifts in associated ecosystem processes.

Conclusions

Our results suggest that high temperatures decrease survival and increase development rate in a functionally important aquatic detritivore (Wallace et al. 1999, Cross et al. 2005). Such responses may lead to smaller body size at emergence, lower fecundity, and eventual population declines (Sweeney et al. 2018). Neither increases in food quality nor higher feeding rate appeared to confer benefits to insect survival, indicating that these mechanisms are not sufficient to alleviate thermal stress. Shifts in detritivore physiological rates such as those we measured may alter consumer-mediated C processing and the fate of stream C under climate change.

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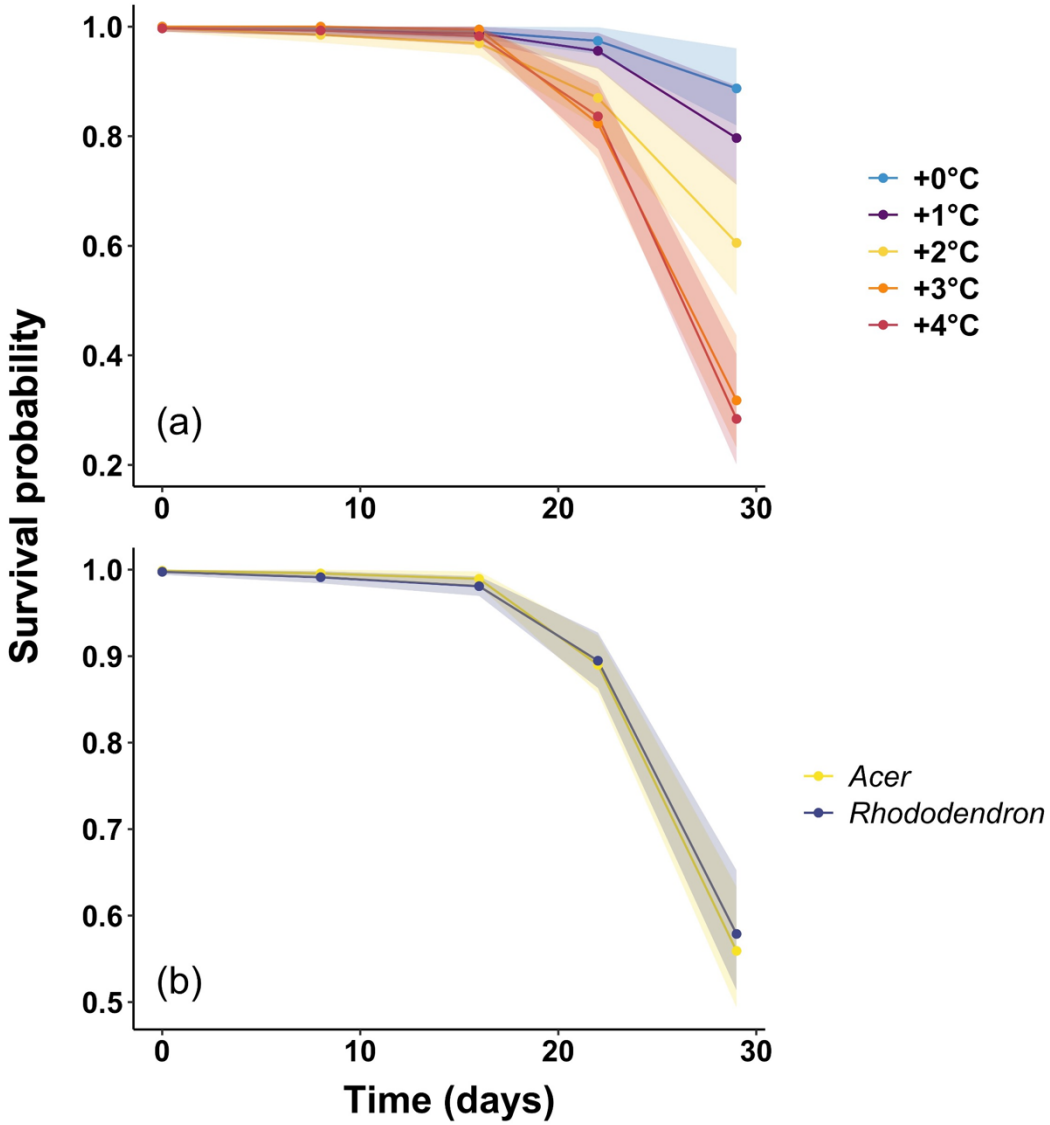

Figure 4.1. Survival probability vs. time (days) across (a) temperatures (ambient = blue, +1°C = purple, +2°C = yellow, +3°C = orange, and +4°C = red) and (b) leaf species (*Acer* = yellow, *Rhododendron* = blue). Shaded regions depict 95% confidence intervals. Survival curves were generated using the function "survfit" in the R package *survival* (Therneau 2023).

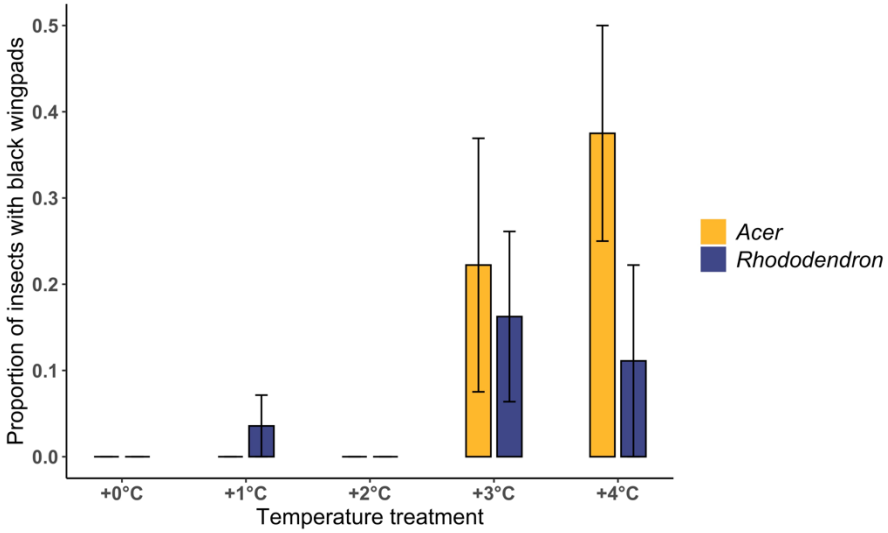

Figure 4.2. Proportion of insects with black wingpads at the end of the experiment as a function of temperature treatment (°C). Yellow bars represent the *Acer* treatment; blue bars represent the *Rhododendron* treatment. Error bars depict standard errors.

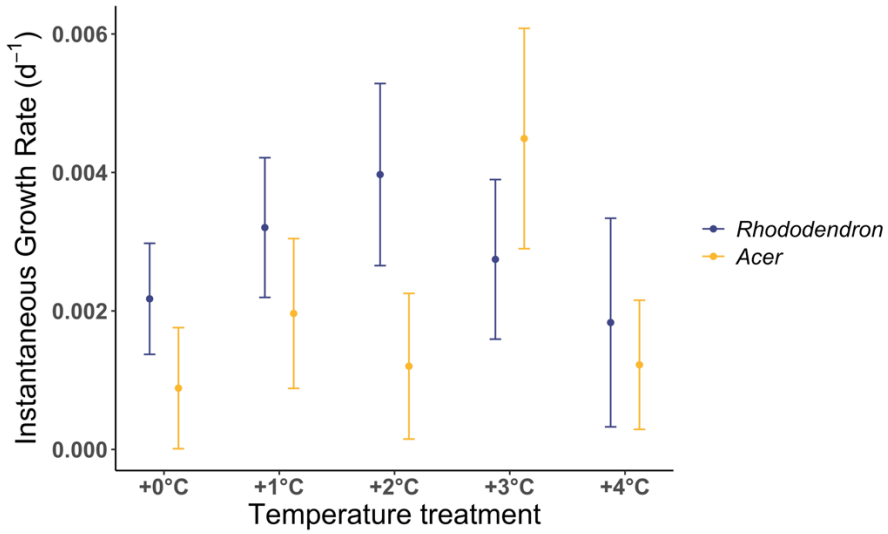

Figure 4.3. Average instantaneous growth rate (d^{-1}) as a function of temperature treatment (°C) for insects that survived to the end of the experiment and did not develop black wingpads. Yellow points represent the *Acer* treatment; blue points represent the *Rhododendron* treatment. Error bars depict standard errors.

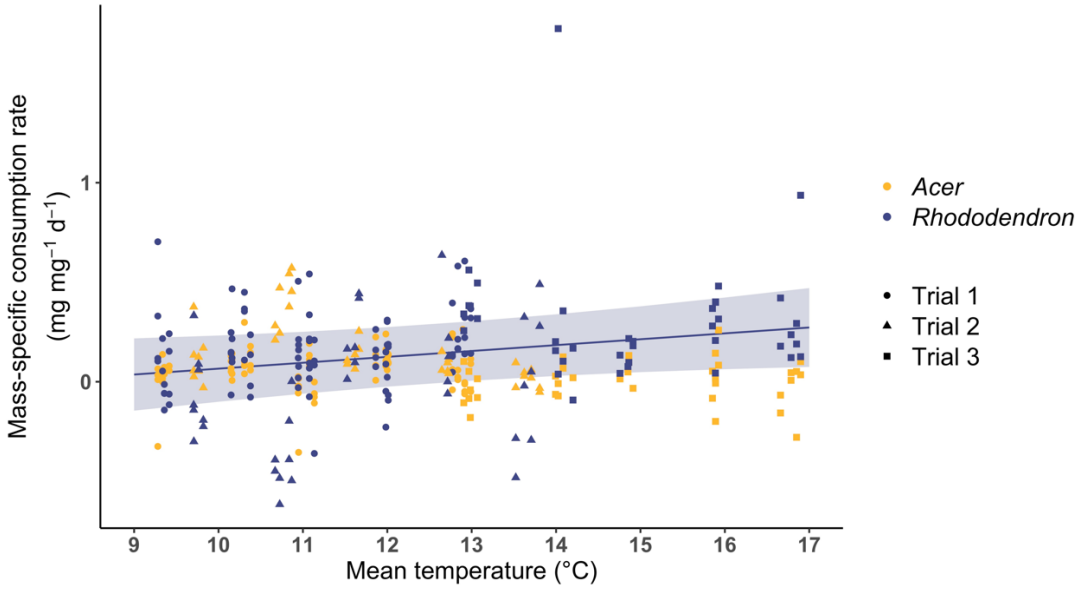

Figure 4.4. Mass-specific consumption rate (mg mg^{-1} day^{-1}) for individual insects as a function of mean channel temperature (°C) in each of our three consumption trials. Yellow represents the *Acer* leaf species treatment; blue represents the *Rhododendron* treatment. Trial number is denoted by point shape (circles = Trial 1, 12-20 March 2019; triangles = Trial 2, 27 March-3 April 2019; squares = Trial 3, 10-17 April 2019). Shaded area depicts the 95% confidence interval around the slope between temperature and mass-specific consumption rate for *Rhododendron*-fed insects.

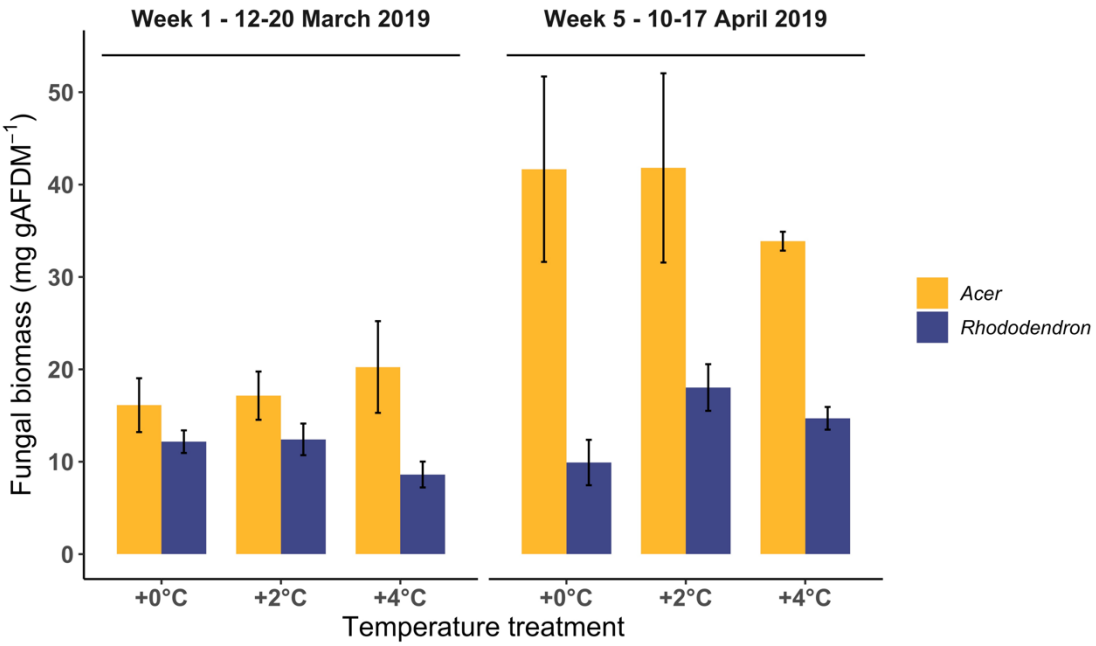

Figure 4.5. Fungal biomass on leaf fragments in microbial chambers as a function of temperature treatment during week 1 (12-20 March 2019) and week 5 (10-17 April 2019) of our experiment. Yellow represents the *Acer* leaf species treatment; blue represents the *Rhododendron* treatment. Error bars depict standard errors.

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CHAPTER 5

CONCLUSIONS

Organic matter processing in headwater streams is an essential part of the global carbon cycle (Aufdenkampe et al. 2011, Hotchkiss et al. 2015, Drake et al. 2018). However, questions remain about the relative thermal responses of the organisms responsible for stream C cycling, how organismal thermal responses scale up to affect community structure, and consequences for the rate of detrital C processing and the ultimate fate of terrestrially derived C in streams. The chapters of this dissertation address these knowledge gaps at the organismal, community, and ecosystem scales.

Observed temperature effects on organismal physiology, community structure, and ecosystem processes differ when measured at different spatial scales

My work highlights the importance of studies at the landscape scale, where patterns may differ from those measured across large spatial gradients. In Chapter 2, I demonstrated that detritivore breakdown had a higher temperature dependence than predicted by the MTE, contrasting studies across global- and regional-scale gradients which have measured a lower E_a for detritivore breakdown than MTE or even a negative relationship between detritivore breakdown with temperature (Boyero et al. 2011, Follstad Shah et al. 2017, Tiegs et al. 2019, Wilmot et al. 2021). In Chapter 3, I found that invertebrates in the scraper functional feeding group exhibited a positive response to temperature, in contrast to studies at broader spatial scales which have highlighted scraper thermal sensitivity (Pyne and Poff 2017, Jourdan et al. 2018). Studies of

temperature effects on stream organisms, communities, and ecosystems across large spatial extents have aided predictions about the effects of climate change at global scales. However, averaging thermal responses across large gradients inevitably integrates changes in consumer community structure, basal resource dynamics, and stream physicochemical characteristics, among other factors (Boyero et al. 2012, Wilmot et al. 2021). For this reason, studies at large spatial scales may not be reflective of site-specific responses to increasing temperatures, and studies across multiple spatial scales are needed for a holistic picture of how stream organisms, communities, and ecosystem processes will respond to global change. Integrating the results of local-scale studies to understand how increasing temperatures will affect headwater stream ecosystems differently in different biogeographic regions may be particularly useful.

Additional measurements of organismal thermal responses and the temperature dependence of stream organic matter processing in tropical regions should be prioritized, as there is a dearth of knowledge about these systems relative to their temperate counterparts (Bonacina et al. 2023).

Thermal responses depend on antecedent temperature conditions and the timing and magnitude of stream warming

The results of my dissertation chapters provide multiple lines of evidence that temperature effects on biological processes in stream ecosystems depend on the timing and magnitude of warming, as well as prior temperature conditions. For example, my results in Chapter 2 suggest that temperature effects on absolute amounts vs. relative proportions of C routed to detritivore and microbial fates depend on initial temperature conditions. Specifically, we observed that microbial breakdown was higher than

detritivore breakdown at low temperatures, but that the breakdown rates of these two organismal groups converged as temperatures rose. Our results in Chapter 2 imply that temperate stream detritivores likely rely on patchy sources of leaf litter in summer months when autumn-shed inputs are depleted, and that resource competition may be more intense in summer than in winter due to low background resource availability. These patterns suggest higher-than-expected leaf litter breakdown rates and routing of detrital C to detritivore fates during summer months in temperate headwater streams.

Season was also an important factor in determining temperature effects on aquatic invertebrate community structure and physiology. In Chapter 3, temperature was related to invertebrate community dissimilarity in summer months, but not in winter months. This pattern may be related to differences in baseline temperature conditions, where temperature variation across streams is more consequential when temperatures are higher overall. In addition, season-specific temperature effects may reflect differences in thermal sensitivity among aquatic invertebrate life stages. This phenomenon has been demonstrated in terrestrial insects (Ma et al. 2021) but less research has focused on variation in thermal sensitivity across aquatic insect life stages, particularly within the larval stage (i.e., early- vs. late-instar larvae). In Chapter 4, we found that survival of the common aquatic insect detritivore *Tallaperla* was compromised at temperatures which were within the range experienced over the course of their life cycle but relatively high for the time of the year we conducted our experiment. This corroborates the idea that aquatic insect thermal responses may vary throughout the larval life cycle and depend on initial temperature conditions.

Resource chemistry modulates the temperature dependence of biological processes

Building on previous studies in terrestrial and aquatic ecosystems (Fierer et al. 2005, Davidson and Janssens 2006, Sinsabaugh and Follstad Shah 2012, Flores et al. 2014, Ferreira et al. 2015, Follstad Shah et al. 2017), my work reiterates the importance of resource chemistry in modulating thermal responses across ecological scales. In Chapter 2, we found that the breakdown of a higher-C:N and slow-decomposing leaf species was more sensitive to temperature than the breakdown of a lower-C:N, fast-decomposing leaf species. Despite these patterns in the temperature dependence of leaf litter breakdown, our results in Chapter 2 also imply that the breakdown of low-C:N leaf litter will increase more on an absolute basis than that of high-C:N litter. As streams warm, high absolute increases in low-C:N litter breakdown combined with steep increases in breakdown for high-C:N litter with temperature may cause resource limitation for stream consumers that depend on detrital C.

In Chapter 4, I found that consumption rates of the stream insect detritivore *Tallaperla* increased with temperature when insects were fed a high-C:N leaf species. In contrast, there was no relationship between consumption rate and temperature for insects in our experiment that were fed a lower-C:N species, despite increases in fungal biomass on the lower-C:N species which corresponded to increases in temperature. These results suggest that the insects in our experiment that were fed the high-C:N leaf species may have attempted to compensate for low nutritional quality by increasing consumption rate (Flores et al. 2014). Insects in the lower-C:N leaf species treatment did not increase consumption rates with temperature, perhaps because feeding on the lower-C:N leaf species did not exacerbate energetic costs or thermal stress to the same degree as feeding on the higher-C:N leaf species. Interestingly, though these results suggest some

mechanisms that insects may use to cope with energetic costs at high temperatures, we found no differences in the thermal responses of survival probability, growth rate, or development rate among leaf species treatments. These results suggest that resource chemistry may modulate some physiological parameters more than others, that temperature effects on functional parameters (e.g., consumption rate) may depend more on resource chemistry than effects on other physiological parameters, and that higher nutritional quality may not be sufficient to alleviate thermal stress and confer benefits to survival at high temperatures.

Summary

As global climate change progresses, rising stream temperatures will affect organisms, communities, and ecosystems and the role these systems play in global C cycling. My research suggests that warming may increase the proportion of stream C routed to detritivore fates relative to current conditions (Chapter 2), but emergent patterns will depend on the effects of temperature on aquatic invertebrate community structure (Chapter 3) and physiology (Chapter 4). Specifically, my work demonstrates that temperature may affect invertebrate community structure to a greater degree in the summer when baseline temperatures are high, and that negative effects of temperature on detritivore physiology may compromise these organisms' role in headwater stream C processing, particularly if temperature spikes coincide with vulnerable life stages (Chapter 4). This work aids our understanding of how stream detrital processing may shift in a warmer world.

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APPENDIX A

SUPPLEMENTARY METHODS AND DATA: CHAPTER 2

I. Watershed area and elevation

We used a digital elevation model (USGS 2017) to extract elevation data given the latitude and longitude at the end of each of our stream reaches. We also used latitude and longitude to delineate the area of each watershed in hectares using StreamStats (USGS 2019).

II. Supplementary discharge methods and modeling

For the five streams in our study that were not monitored by the USFS or Coweeta LTER (Table A1), we continuously measured stage height using a pressure transducer. In three of these streams (HNCR, LCCR, USHF), we deployed HOBO water-level loggers. In the other two streams (TOWR and WS55) we used a Campbell Scientific CS451 connected to a Campbell data logger (CR800 or CR10) located in the flume at the downstream end of each study reach. We converted stage height to discharge using a rating curve generated from dilution-gauging or by filling a bucket at the V-notch of the flume (5-7 estimates per stream for the 3 HOBO sensor streams, Table A4; WS55 and TOWR total discharge estimates: TOWR $n = 31$; WS55 $n = 23$).

Due to sensor malfunction or power loss, there were short periods of lost data (primarily in TOWR). To fill in the missing data, we used observations from WS55 to predict TOWR discharge using a random forest model (model $R^2 = 0.99$) with the R package "randomForest".

III. Temperature modeling and gap-filling

Due to equipment malfunctions, there were some time periods of lost temperature data. We modeled the missing data based on either the closest and most similar stream or air temperature data from Climate Station 1 (CS01) at Coweeta. We performed all modeling and calculations in R (R Core Team 2020).

A. Temperature modeling - Year 1

In year 1 (YR1) of our study, we lost temperature data in all streams between 8 March and 6 April 2018 due to a shuttle malfunction. To fill in the missing data, we created files for each YR1 stream that contained all the data except the missing period, allowing us to create regressions based on as much available data as possible. We downloaded daily mean air temperature data from Climate Station 1 (CS01) at Coweeta and filtered for the dates that overlapped with the stream temperature data (21 September 2017 – 8 March 2018 and 7 April 2018 – 28 September 2018; Miniat et al. 2022). We also created a file with the air temperature data for the missing period (8 March 2018 - 6 April 2018; Miniat et al. 2022).

We created stream-specific linear regressions between stream temperature and CS01 air temperature. Stream temperature data were recorded at 15-min intervals, so we first summarized them to daily means to match the resolution of the air temperature data. For every stream, we used linear regressions to predict daily stream temperature from daily air temperature at CS01. We used these linear models to predict stream temperature for the missing period based on air temperature (Table A5). We repeated this process to model stream temperature in WS08 for 31 August 2018 – 28 September 2018. We were missing temperature data for this time for only WS08. We combined the modeled data

from all 12 YR1 streams, then combined the modeled data with the rest of the daily temperature data (see "C" below).

B. Temperature modeling – Year 2

In year 2 (YR2) of our study, we were missing temperature data from CWCR and WS09 for different time periods. WS09 is a tributary of CWCR and our sampling points for these two streams are close together, so we generated linear regressions for these streams based on one another to fill in the missing data. For CWCR, we were missing temperature data between 18 September 2018 and 5 December 2018. For WS09, we were missing temperature data between 7 January 2019 and 6 February 2019. To generate regressions, we used the longest time period for which we had data from both streams (7 February 2019 – 29 August 2019). Using the regression equations (Table A5), we gap-filled missing temperature data for both streams. We then appended the modeled values to the master temperature files for WS09 and CWCR. Since we had 15-min temperature data for both WS09 and CWCR, we used this data resolution to generate regression models and fill in the modeled data.

C. Temperature modeling - generating daily and deployment-level means

Since the temperature data we modeled for YR1 were daily means, we aggregated all temperature data to this resolution so that the data would be at a consistent resolution for generating deployment means. Once all the temperature data were aggregated at the daily mean level, we bound the modeled and non-modeled data for each stream in the correct order. We then bound the data for each stream to generate study year-specific master daily temperature files. Finally, we summarized the daily temperature data at the

deployment level, and these deployment-level temperature averages were used in all subsequent analyses.

IV. Supplementary methods - nutrient concentrations

The USFS or Coweeta LTER provided weekly nutrient concentration data for 15 of our study streams (Table A2, A3). For the five remaining streams, we collected monthly water samples from each stream at the location of the litterbags. We filtered stream water into acid-washed polypropylene vials using 0.45-µm nitrocellulose filters, transported the samples on ice back to the laboratory, and froze them. The nutrient samples we collected in the five streams not monitored by the USFS were analyzed for NH_4^+-N (automated phenate method), NO_3^--N + NO_2-N (automated cadmium reduction method), and PO_4 (automated ascorbic acid reduction method; APHA 2023) using colorimetry with an Alpkem RFA 300 Autoanalyzer (College Station, Texas, USA) at the Center for Applied Isotope Studies in Athens, Georgia, USA.

V. Leaf litter standing stock (LLSS)

We collected coarse benthic organic matter (CBOM) from each of our streams quarterly for one year. We delineated stream reaches into four blocks and randomly collected one core (0.038 m^2) from each block quarterly (January, April, July, October). We pushed the core into the stream bottom to ~10 cm and removed all CBOM by hand. In the laboratory, we rinsed the material and separated it into categories (leaves, wood, and other). We oven-dried the CBOM at 60°C for 48 hours, weighed it, ashed it at 500°C for 5.5 hours, and re-weighed it to determine ash-free dry mass. LLSS values for all streams in both YR1 and YR2 are presented in Table A8.

VI. Fungal biomass

In YR2, we obtained fungal biomass data (mg g^{-1} AFDM) from *Rhododendron* leaf fragments in fine-mesh bags by quantifying ergosterol, a biomarker for fungal biomass. Here, we provide additional information about how we obtained fungal biomass estimates. At the end of each YR2 deployment, we trimmed 6-8 leaf fragments from *Rhododendron* leaves in 3 of the 4 fine-mesh bags from each stream. We placed these leaf fragments into pre-weighed scintillation vials, froze them, stored them at -20°C, and then freeze-dried them. We re-weighed the vials containing the freeze-dried material and calculated ash-free dry mass (AFDM) by assuming ash content measured in corresponding litter samples.

Lipid extraction from leaf fragments was performed as described in Gulis and Bärlocher (2017). We extracted lipids with methanolic KOH and partitioned them into pentane, then removed the pentane layer and evaporated it to dryness under a stream of nitrogen. Lipids were then redissolved in methanol and filtered through a PTFE syringe filter into a vial for high-performance liquid chromatography (HPLC). A Shimadzu Prominence HPLC system equipped with a reverse phase Kinetex Core-Shell C18 column (Phenomenex) was run isocratically at 0.85 mL min^{-1} of methanol at 35°C. Ergosterol in 20 µL injections of lipid extracts was detected at 282 nm and eluted at ~4.5 min. We calculated the ergosterol concentration in each sample based on the area of the ergosterol peak and data from external ergosterol standards. A conversion factor of 5.5 mg ergosterol per g of fungal dry mass was used (Gulis and Bärlocher 2017).

Table A1. Summary of physicochemical data for the 20 streams in our study.

Stream	Year	Watershed area (ha)	Elevation (m)	Mean deployment temperature range (°C)	Mean deployment discharge range (L s^{-1})
WS02*	1	12	721	6.9 - 17.3	1 - 6
WS06*	1	9	690	3.3 - 18.2	1 - 4
WS07*	1	59	716	6.0 - 16.8	7 - 34
WS08*	1	760	688	4.5 - 17.1	159 - 597
WS13*	1	19	701	4.9 - 17.1	2 - 9
WS14*	1	61	695	4.0 - 17.6	8 - 37
WS18*	1	13	717	4.0 - 17.5	2 - 9
WS27*	1	39	1078	3.6 - 16.2	11 - 47
WS31*	1	34	863	6.6 - 16.4	8 - 28
WS32*	1	41	921	5.5 - 16.3	11 - 39
WS36*	1	49	1037	5.4 - 16.1	17 - 62
CWCR*	2	1577	679	7.6 - 18.1	240 - 1528
HNCR†	2	215	728	6.9 - 17.9	46 - 270
LCCR†	2	140	842	8.3 - 17.1	26 - 160
TOWR†	2	8	691	8.2 - 19.9	1 - 9
USHF†	2	479	733	7.3 - 17.7	63 - 439
WS09‡	2	724	682	7.2 - 18.1	105 - 733
WS34*	2	33	855	8.4 - 16.9	8 - 33
WS37*	2	44	1021	6.0 - 16.9	4 - 54
WS55†	2	8	796	10.6 - 17.3	0.2 - 5

*Streams monitored by the USFS.
†Streams for which we collected nutrient concentrations data.
‡Stream monitored by the Coweeta LTER.

Table A2. Range in deployment-level nutrient concentrations for the 20 streams in our study. All nutrient concentrations are in μg L^{-1}.

Stream	Year	NH_4^+-N (μg L^{-1})	NO_3^--N (μg L^{-1})	DIN (μg L^{-1})	PO_4 (μg L^{-1})
WS02*	1	2.4 - 5.0	1.3 - 9.7	3.9 - 12.7	5.3 - 11.8
WS06*	1	1.8 - 4.3	40.6 - 233.6	42.4 - 236.0	2.0 - 8.1
WS07*	1	2.0 - 4.8	6.9 - 70.5	8.9 - 75.0	2.0 - 4.7
WS08*	1	2.0 - 3.4	2.3 - 24.0	5.0 - 26.9	2.0 - 6.8
WS13*	1	2.0 - 3.3	1.7 - 8.2	4.3 - 10.7	1.9 - 3.2
WS14*	1	2.0 - 3.3	3.5 - 23.6	5.9 - 26.0	2.0 - 4.9
WS18*	1	1.8 - 2.8	1.9 - 22.0	3.9 - 24.0	2.0 - 4.1
WS27*	1	1.4 - 2.9	27.0 - 106.0	28.8 - 108.2	2.0 - 6.0
WS31*	1	1.6 - 2.6	0.1 - 2.1	1.9 - 4.1	2.0 - 5.1
WS32*	1	1.5 - 2.4	0.3 - 3.5	2.3 - 5.9	2.0 - 4.0
WS36*	1	1.5 - 3.3	5.9 - 36.8	8.1 - 39.3	2.0 - 4.4
CWCR*	2	0.3 - 4.6	4.0 - 57.3	7.4 - 61.9	1.8 - 8.1
HNCR†	2	0.1 - 4.7	2.7 - 52.1	3.6 - 54.1	1.2 - 3.9
LCCR†	2	0.7 - 12.7	2.0 - 33.7	3.8 - 46.2	1.6 - 3.0
TOWR†	2	0.7 - 10.4	1.4 - 25.4	2.2 - 35.8	1.3 - 15.7
USHF†	2	1.6 - 6.5	5.3 - 52.2	9.2 - 54.3	1.4 - 8.3
WS09‡	2	0.5 - 3.8	5.3 - 40.9	7.8 - 43.5	2.2 - 5.1
WS34*	2	1.9 - 2.9	1.0 - 13.9	3.2 - 16.1	1.9 - 9.2
WS37*	2	2.3 - 22.2	37.8 - 172.4	41.8 - 192.1	1.9 - 7.7
WS55†	2	2.1 - 17.0	0.5 - 31.7	3.0 - 44.8	1.7 - 3.1

*Streams monitored by the USFS.
†Streams for which we collected nutrient concentrations data.
‡Stream monitored by the Coweeta LTER.

Table A3. Coweeta Hydrologic Laboratory methods for measuring NH_4^+-N, NO_3^--N, and PO_4.

Analyte	Method	Analyzer
NH_4-N	Automated Phenate method	Astoria 2 Autoanalyzer, Astoria-Pacific, Clackamas, Oregon
NO_3-N	Micro-membrane Suppressed Ion Chromatography using a capillary AS 18 column	Thermo Scientific ICS 4000 capillary Ion Chromatograph, from Dionex, Sunnyvale, CA
PO_4	Micro-membrane Suppressed Ion Chromatography using a capillary AS 18 column	Thermo Scientific ICS 4000 capillary Ion Chromatograph, from Dionex, Sunnyvale, CA

Table A4. Stage height-discharge rating curve equations for the five streams not monitored for discharge by the USFS (for which we obtained discharge measurements).

Year	**Stream**	**Equation**	R^2
2	HNCR	$y = 665006x^{4.9862}$	0.93
2	LCCR	$y = 1259.4x^{1.1136}$	0.96
2	TOWR	$y = 0.061x^{1.9}$	0.99
2	USHF	$y = 4155.8x^{1.4143}$	0.99
2	WS55	$y = 0.102x^{1.854}$	0.98

Note: y is discharge in L s^{-1} and x is water depth in cm.

Table A5. Regression equations and R^2 values for temperature models.

Year	Stream	Regression equation	R^2
1	WS01	WS01 stream = CS01 air*0.50799 + 5.41235	0.88
1	WS02	WS02 stream = CS01 air*0.4432 + 7.0962	0.86
1	WS06	WS06 stream = CS01 air*0.65862 + 3.44360	0.89
1	WS07	WS07 stream = CS01 air*0.47779 + 6.164554	0.88
1	WS08	WS08 stream = CS01 air*0.56573 + 4.52781	0.89
1	WS13	WS13 stream = CS01 air*0.5326 + 5.1208	0.87
1	WS14	WS14 stream = CS01 air*0.59332 + 4.17439	0.88
1	WS18	WS18 stream = CS01 air*0.57982 + 4.35862	0.87
1	WS27	WS27 stream = CS01 air*0.53149 + 3.89423	0.83
1	WS31	WS31 stream = CS01 air*0.439256 + 6.52829	0.88
1	WS32	WS32 stream = CS01 air*0.477192 + 5.513446	0.87
1	WS36	WS36 stream = CS01 air*0.45853 + 5.59441	0.85
2	CWCR	CWCR stream = WS09 stream*0.9644186 + 0.7093227	0.99
2	WS09	WS09 stream = CWCR stream*1.0332395 - 0.6855092	0.99

Table A6. Data, structure, and purpose for each of our LME models.

Model structure	Breakdown data	Purpose
$\log_e(\text{mean\_k}) \sim \left[\frac{1}{k_B T} - \frac{1}{k_B T_0}\right] + Q_z + (1\|\text{stream}) + (1\|\text{date deployed})$	*Acer* coarse-mesh	Obtain *Acer* coarse-mesh E_a, 95% CI, and discharge-litter breakdown slope
	Acer detritivore	Obtain *Acer* detritivore E_a, 95% CI, and discharge-litter breakdown slope
	Acer microbial	Obtain *Acer* microbe E_a, 95% CI, and discharge-litter breakdown slope
	Rhododendron coarse-mesh	Obtain *Rhododendron* coarse-mesh E_a, 95% CI, and discharge-litter breakdown slope
	Rhododendron detritivore	Obtain *Rhododendron* detritivore E_a, 95% CI, and discharge-litter breakdown slope
	Rhododendron microbial	Obtain *Rhododendron* microbe E_a, 95% CI, and discharge-litter breakdown slope
$\log_e(\text{mean\_k}) \sim \left[\frac{1}{k_B T} - \frac{1}{k_B T_0}\right]$*leaf + Q_z*leaf + (1|stream) + (1|date deployed)	Coarse-mesh - *Acer*/*Rhododendron*	Test whether *Rhododendron* coarse-mesh E_a differs from *Acer* coarse-mesh E_a
	Detritivore - *Acer/Rhododendron*	Test whether *Rhododendron* detritivore E_a differs from *Acer* detritivore E_a
	Microbial - *Acer/Rhododendron*	Test whether *Rhododendron* microbial E_a differs from *Acer* microbial E_a
$\log_e(\text{mean\_k}) \sim \left[\frac{1}{k_B T} - \frac{1}{k_B T_0}\right]$*consumer + Q_z*consumer + (1|stream) + (1|date deployed)	*Acer* - microbes and detritivores	Test whether microbial *Acer* E_a differs from detritivore *Acer* E_a
	Rhododendron - microbes and detritivores	Test whether microbial *Rhododendron* E_a differs from detritivore *Rhododendron* E_a
$\log_e(\text{mean\_k}) \sim \left[\frac{1}{k_B T} - \frac{1}{k_B T_0}\right] + Q_z + (1\|\text{stream}) + (1\|\text{date deployed})$	*Rhododendron* microbial breakdown and fungal biomass, YR2	Obtain E_a for *Rhododendron*, microbial breakdown in YR2 to compare to E_a from the model with fungal biomass
$\log_e(\text{mean\_k}) \sim \left[\frac{1}{k_B T} - \frac{1}{k_B T_0}\right] + Q_z + [FB - FB_0] + (1\|\text{stream}) + (1\|\text{date deployed})$	*Rhododendron* microbial breakdown and fungal biomass, YR2	Obtain E_a for *Rhododendron*, microbial breakdown in YR2 to compare to E_a from the model without fungal biomass

Note: We ran models in R (R Core Team 2020) in the *'lme4'* package (Bates et al. 2015)

Table A7. For each of our LME models: $\log_e(k_0)$ (the model intercept), $1/k_BT_0$ (mean $1/k_BT$), Q_0 (mean discharge in L s^{-1}) and FB_0 (mean fungal biomass in mg g^{-1} AFDM).

Breakdown data	$\log_e(k_0)$	$\frac{1}{k_BT_0}$	Q_0	FB_0
Acer coarse-mesh	-3.77	40.71	86.03	
Acer detritivore	-5.30	40.65	83.42	
Acer microbial	-4.37	40.69	100.19	
Rhododendron coarse-mesh	-5.12	40.70	92.12	
Rhododendron detritivore	-6.76	40.65	88.40	
Rhododendron microbial	-5.60	40.69	94.66	
Coarse-mesh - *Acer*/*Rhododendron*	*Acer:* -3.78 *Rhododendron:* -5.11	40.70	89.11	
Detritivore - *Acer/Rhododendron*	*Acer:* -5.29 *Rhododendron:* -6.78	40.65	85.92	
Microbial - *Acer/Rhododendron*	*Acer:* -4.37 *Rhododendron:* -5.60	40.69	97.43	
Acer - detritivores/microbes	Detritivores: -5.26 Microbes: -4.36	40.67	92.50	
Rhododendron - detritivores /microbes	Detritivores: -6.75 Microbes: -5.58	40.67	91.78	
Litter breakdown/fungal biomass - *Rhododendron*, microbes, YR2	-5.48	40.59	177.09	32.17

Table A8. Mean leaf litter standing crop (g AFDM m^{-2}) for each study stream in January, April, July, and October of YR1 and YR2.

Stream	Year	January	April	July	October
WS02	1	359.4	93.4	22.4	8.6
WS06	1	101.4	49.2	25.0	40.1
WS07	1	88.1	20.8	27.5	23.1
WS08	1	24.1	5.6	0.7	2.4
WS13	1	169.7	156.3	18.4	14.8
WS14	1	154.0	27.4	7.0	4.7
WS18	1	207.1	45.1	14.1	24.7
WS27	1	20.7	59.0	8.2	16.5
WS31	1	NA	NA	NA	NA
WS32	1	142.4	52.5	31.9	68.2
WS36	1	58.0	9.5	21.3	4.3
CWCR	2	5.6	0.3	0.0	40.1
HNCR	2	12.7	4.6	0.12	28.1
LCCR	2	86.2	2.5	0.0	47.7
TOWR	2	36.2	62.6	8.4	76.5
USHF	2	4.6	1.2	0.0	55.9
WS09	2	13.5	1.8	5.0	16.2
WS34	2	103.4	26.1	10.1	63.4
WS37	2	2.0	2.3	18.4	263.7
WS55	2	58.5	131.4	11.7	33.5

Note: LLSC was not measured in WS31 in YR1.

Table A9. Summary of past manipulations in our study watersheds at the USFS Coweeta Hydrologic Laboratory.

Stream	Past manipulations	Citation
WS06	1958: WS clearcut and residue burned; 1959: WS planted with grass, limed, and fertilized; 1966-67: WS grass treated with herbicide, vegetation allowed to recover	Swank and Crossley 1988
WS07	1941-1952: WS cattle-grazed for ~5 months per year; 1977: WS clearcut	Swank and Crossley 1988
WS13	1939 and 1962: Woody vegetation in WS clearcut, residue left in place)	Swank and Crossley 1988
WS55	The top 170m of this stream was part of a 13-year litter exclusion study in which researchers excluded leaf litter (1993-1995), removed small- and large wood (1993-1995 and 1998-2000, respectively), added PVC pipe (2000-2001), and added leaf litter (fast-decomposing, slow-decomposing, and mixed in 2001-2003, 2003-2005, and 2005-2006, respectively).	Wallace et al. 2015

Table A10. Results of lmer models to test for significant differences among months for shredder abundance/biomass per leaf pack. All models included a random effect for stream.

Model	*n*	Pairwise comparisons (from *emmeans*)			
		Months	***estimate***	***SE***	***p***
lmer(detritivore biomass (mg) per leaf pack ~ month + (1|stream))	120	Jan-Jul	-31.28	8.65	0.0025
		Jan-Sep	-31.38	8.31	0.0015
		Jan-Dec	-7.84	8.38	0.7859
**Figure A4a		Jul-Sep	-0.10	8.72	1.0000
		Jul-Dec	23.44	8.83	0.0444
		Sep-Dec	23.54	8.44	0.0314
		Months	***estimate***	***SE***	***p***
lmer(detritivore abundance per leaf pack ~ month + (1|stream))	120	Jan-Jul	-25.08	2.36	<0.0001
		Jan-Sep	-19.79	2.27	<0.0001
		Jan-Dec	-1.48	2.29	0.9171
**Figure A4b		Jul-Sep	5.29	2.38	0.1238
		Jul-Dec	23.6	2.41	<0.0001
		Sep-Dec	18.32	2.3	<0.0001
		Months	***estimate***	***SE***	***p***
lmer($\log_e$(detritivore biomass (mg) per g AFDM ~ month + (1|stream))	117	Jan-Jul	-2.733	0.426	<0.0001
		Jan-Sep	-3.154	0.408	<0.0001
		Dec-Jan	0.554	0.412	0.5379
		Jul-Sep	-0.422	0.431	0.7620
		Dec-Jul	-2.179	0.439	<0.0001
**Figure A4c		Dec-Sep	-2.601	0.419	<0.0001
		Months	***estimate***	***SE***	***p***
lmer($\log_e$(detritivore abundance per gAFDM) ~ month + (1|stream))	117	Jan-Jul	-2.327	0.214	<0.0001
		Jan-Sep	-2.521	0.205	<0.0001
		Dec-Jan	0.394	0.207	0.2337
		Jul-Sep	-0.194	0.217	0.8067
**Figure A4d		Dec-Jul	-1.932	0.220	<0.0001
		Dec-Sep	-2.127	0.211	<0.0001

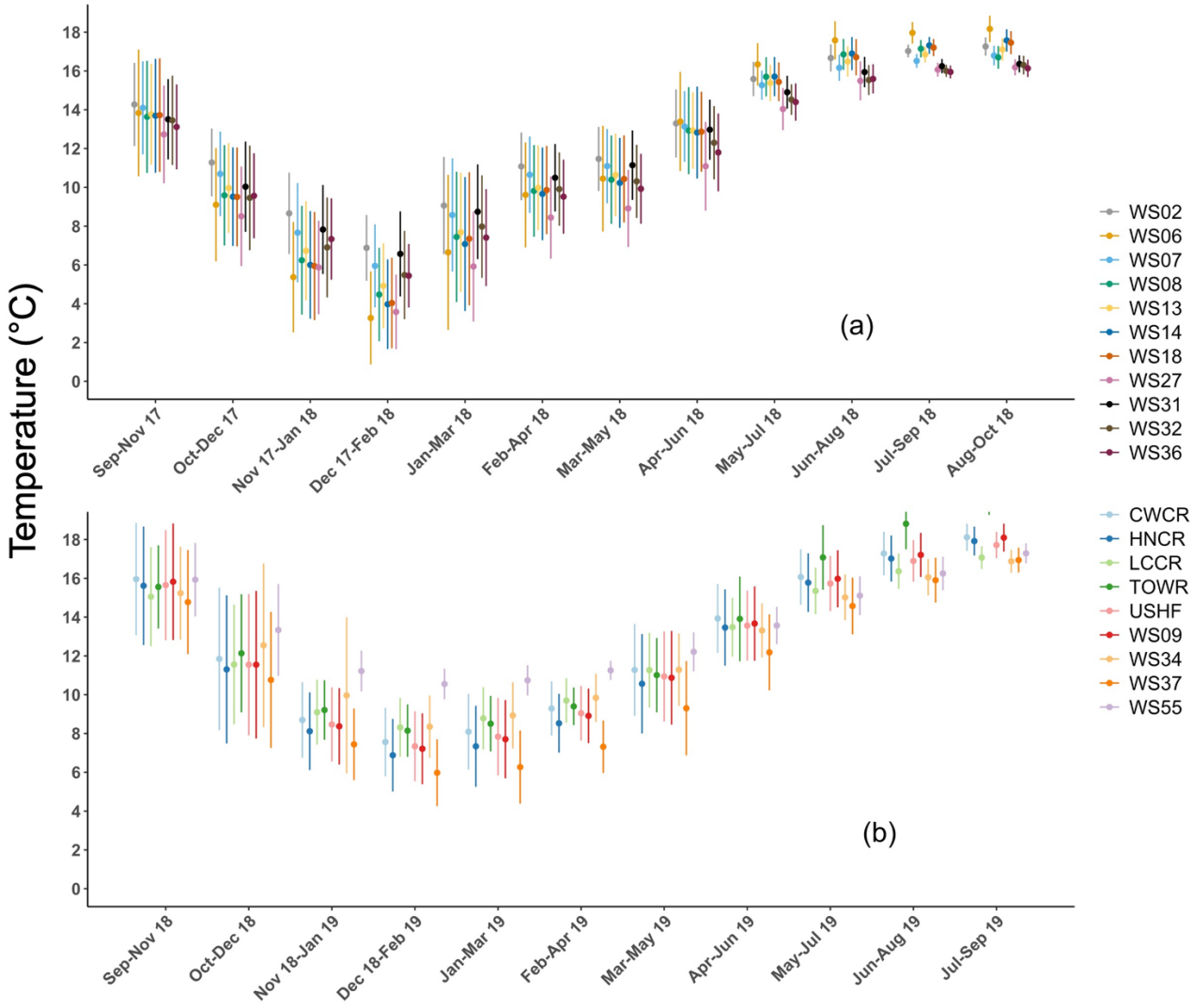

Figure A1. Mean temperature (°C, ± 1 standard deviation) for each stream and deployment in year 1 (a) and year 2 (b) of our study.

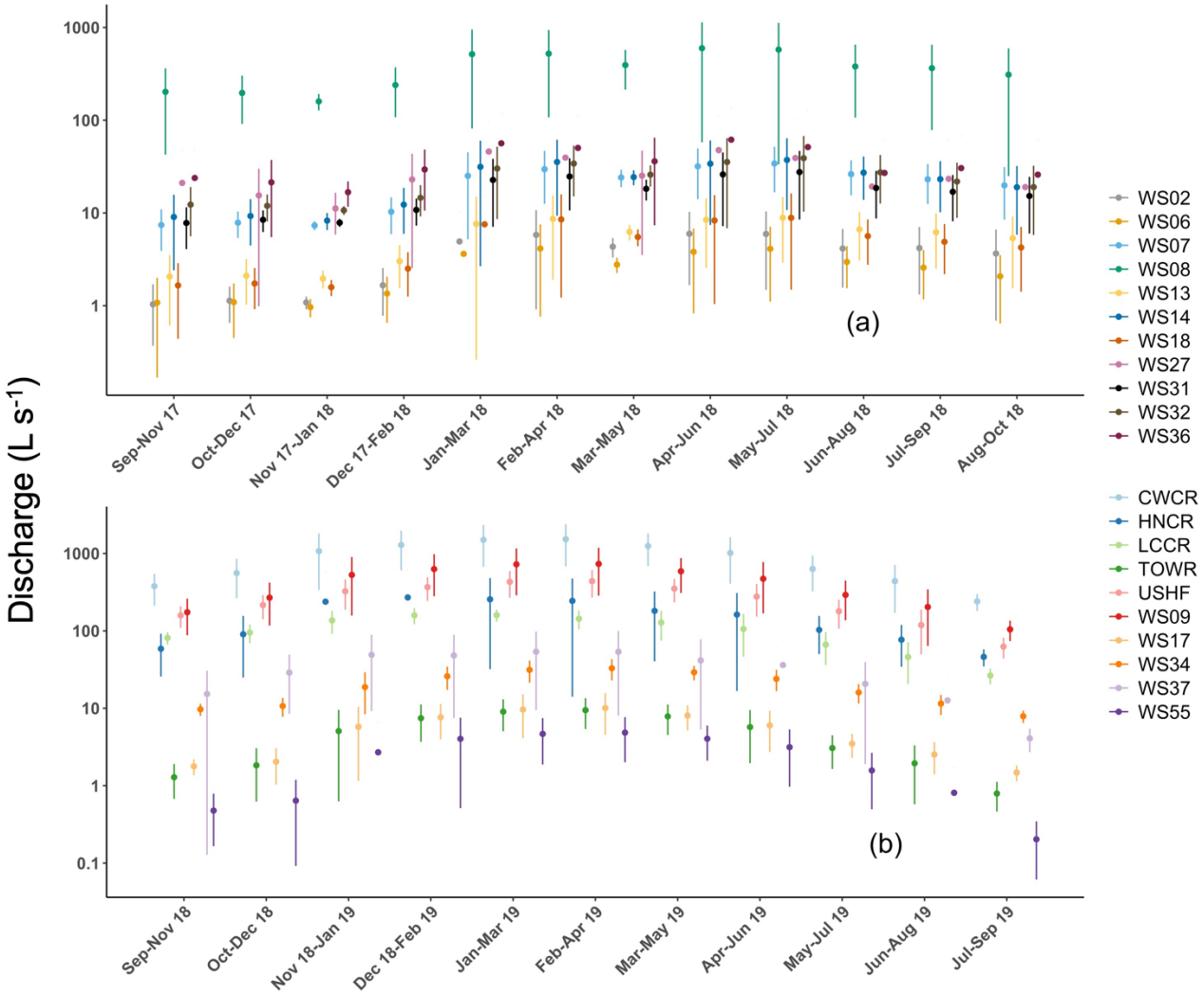

Figure A2. Mean discharge (L s^{-1}, ± 1 standard deviation) for each stream and deployment in year 1 (a) and year 2 (b) of our study

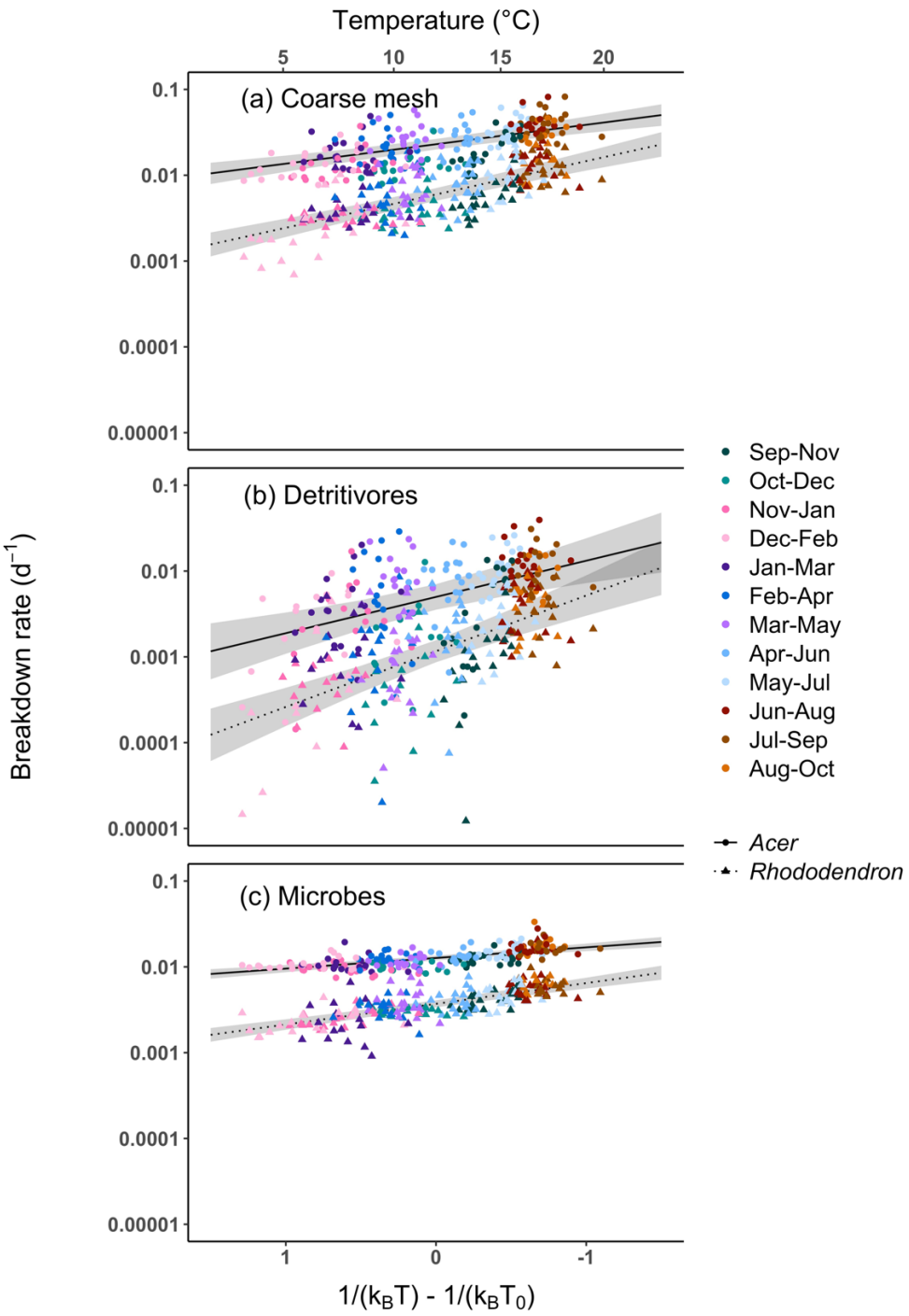

Figure A3. Breakdown rate (k, d^{-1}) vs. centered inverse Boltzmann temperature for coarse-mesh (a, *Acer* E_a = 0.52 eV, *Rhododendron* E_a = 0.89 eV), detritivore (b, *Acer* E_a = 0.97 eV, *Rhododendron* E_a = 1.49 eV), and microbial (c, *Acer* E_a = 0.29 eV, *Rhododendron* E_a = 0.56 eV) breakdown. Trendlines were generated using 'ggpredict' in the R package *ggeffects* (Lüdecke 2018) and reflect all fixed and random effects (Table 2.1). Shaded areas are 95% confidence intervals. y-axis is log$_e$-transformed and bottom x-axis is reversed.

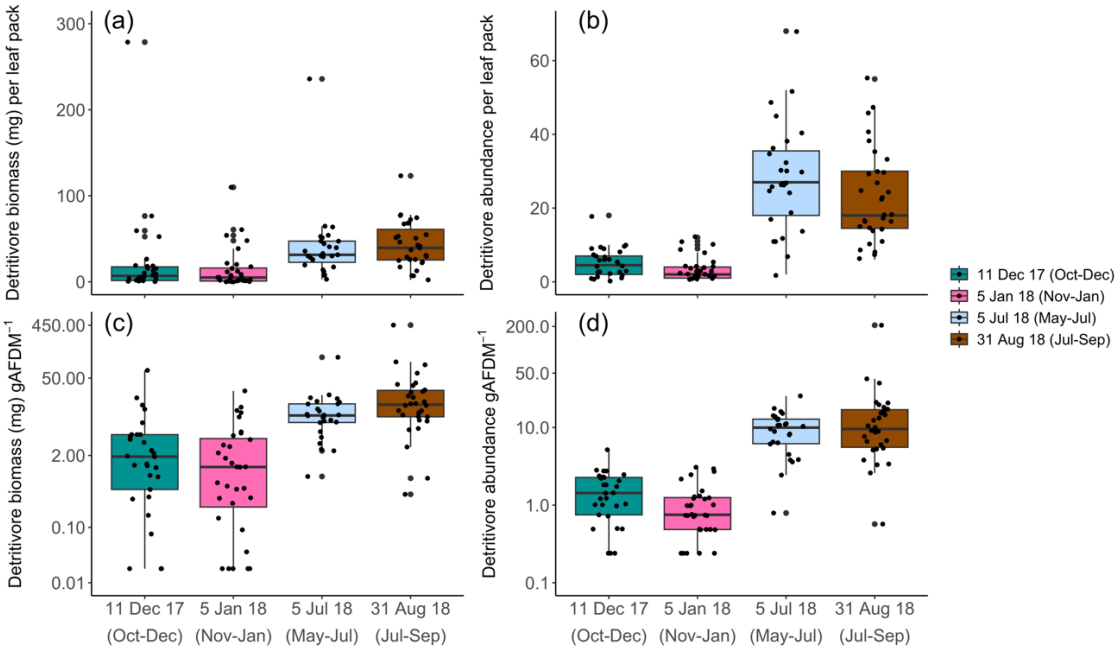

Figure A4: Detritivore biomass (mg) and abundance per leaf pack (a-b) and per gram of ash-free dry mass (AFDM) of leaf litter remaining in corresponding coarse-mesh litterbags (c-d). We collected invertebrate samples for this analysis from *Rhododendron* leaf bags at the end of the YR1 October-December, November-January, May-July, and July-September deployments (specific collection dates: 11 December 2017, 5 January 2018, 5 July 2018, and 31 August 2018). Points represent values from individual samples (2-3 samples per stream). Colors represent the day on which the sample was collected. Note that the *y*-axes of panels c and d are on a $\log_e$-scale.

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APPENDIX B

SUPPLEMENTARY METHODS AND DATA: CHAPTER 3

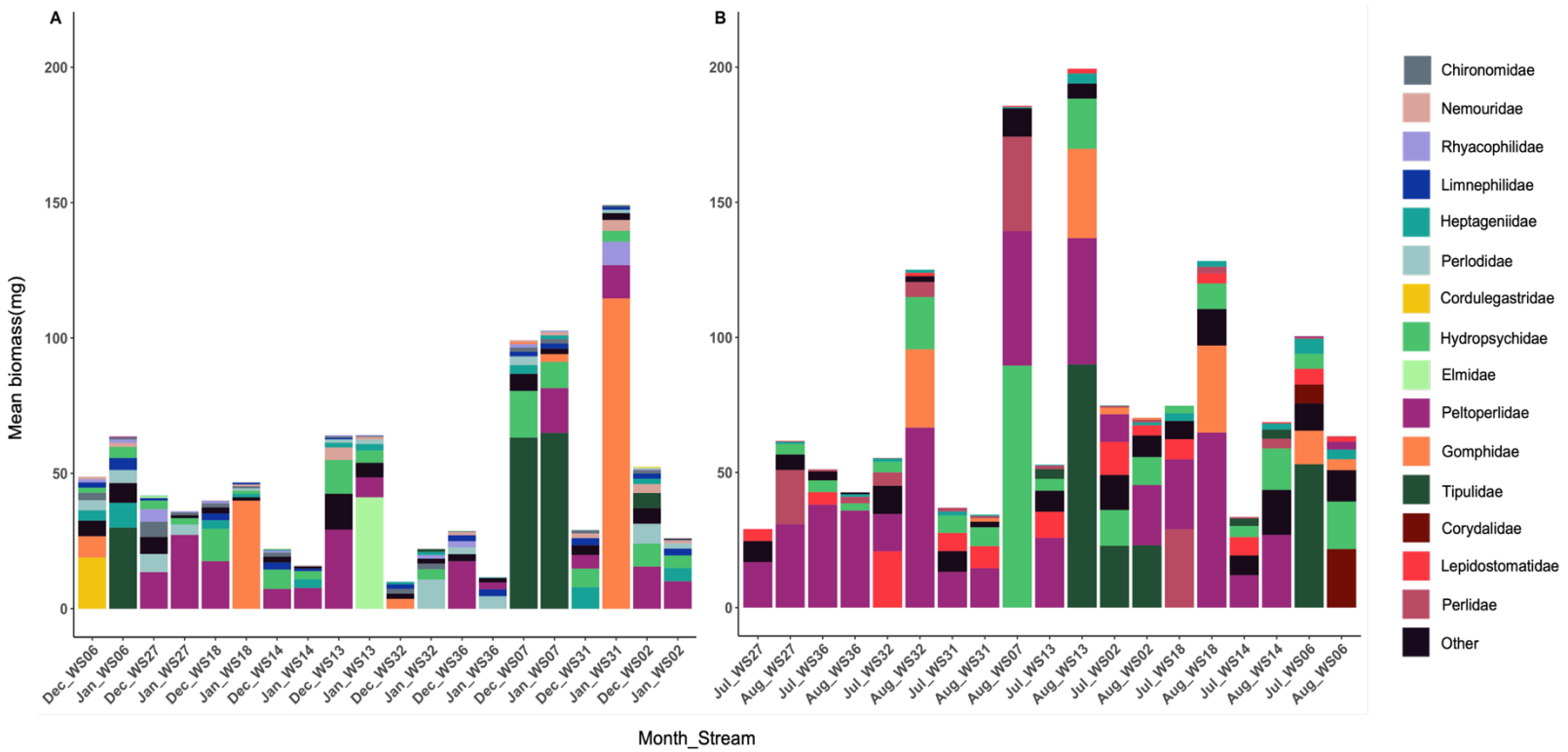

Figure B1. Stacked barplot based on family-level biomass at the stream-sampling date level for winter (A) and summer (B) invertebrate communities during our study period. Colors represent families that make up 90% of the overall biomass in a given season; all other families are grouped into the "Other" category. Streams are organized from left to right in order of ascending mean daily temperature (°C) for each season.

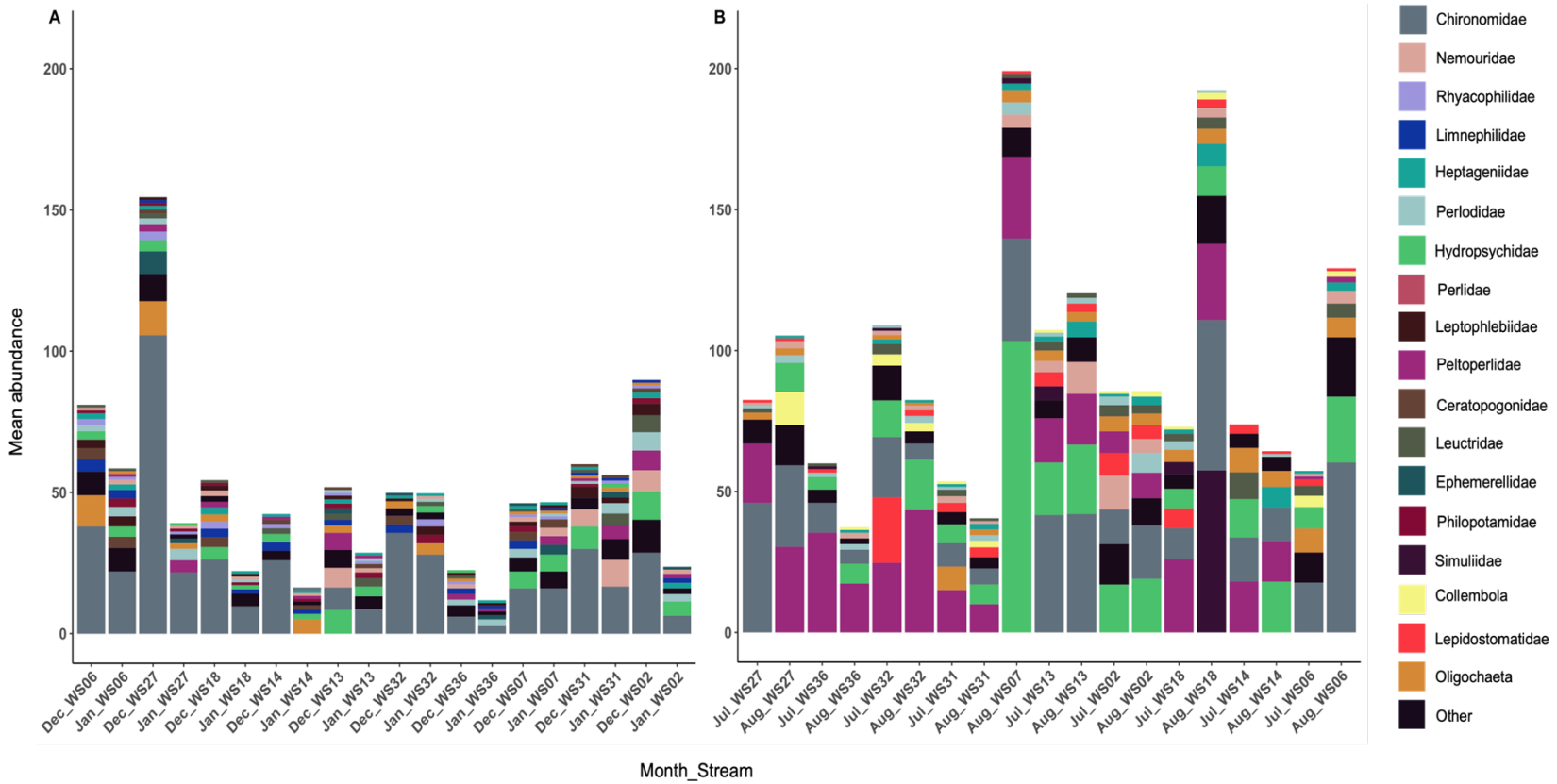

Figure B2. Stacked barplot based on family-level abundance at the stream-sampling date level for winter (A) and summer (B) invertebrate communities during our study. Colors represent families that make up 90% of the overall abundance in a given season; all other families are grouped into the "Other" category. Streams are organized from left to right in order of ascending mean daily temperature (°C) for each season.

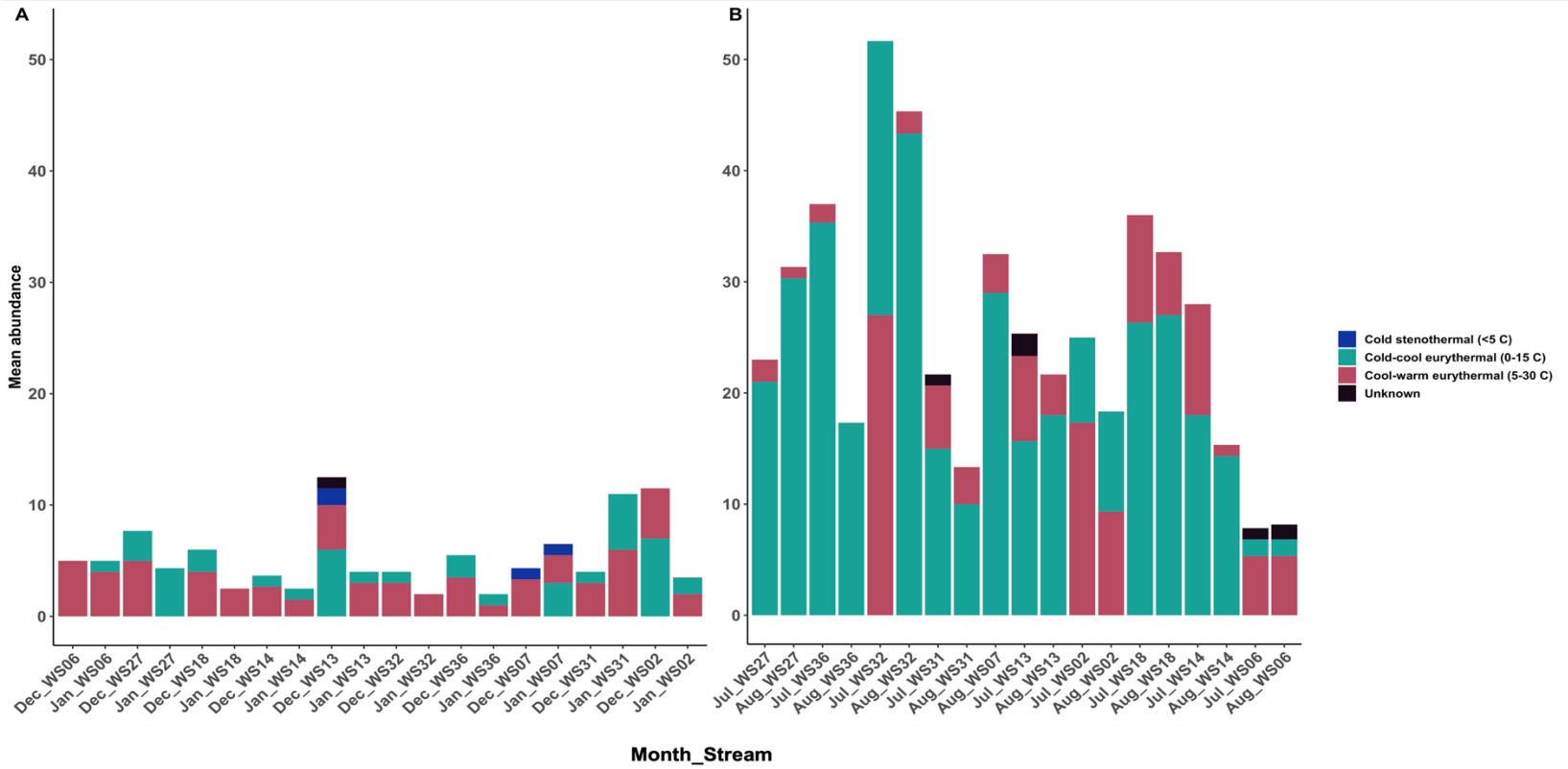

Figure B3. Stacked barplot based on the abundance of detritivore genera in four thermal preference groups based on designations from the Freshwater CONUS database (Twardochleb et al. 2021) (cold stenothermal (navy blue); cold-cool eurythermal (teal); cool-warm eurythermal (magenta) and unknown (black)) in winter (A) and summer (B) of our study period. Streams are organized from left to right in order of ascending mean daily temperature (°C) for each season.

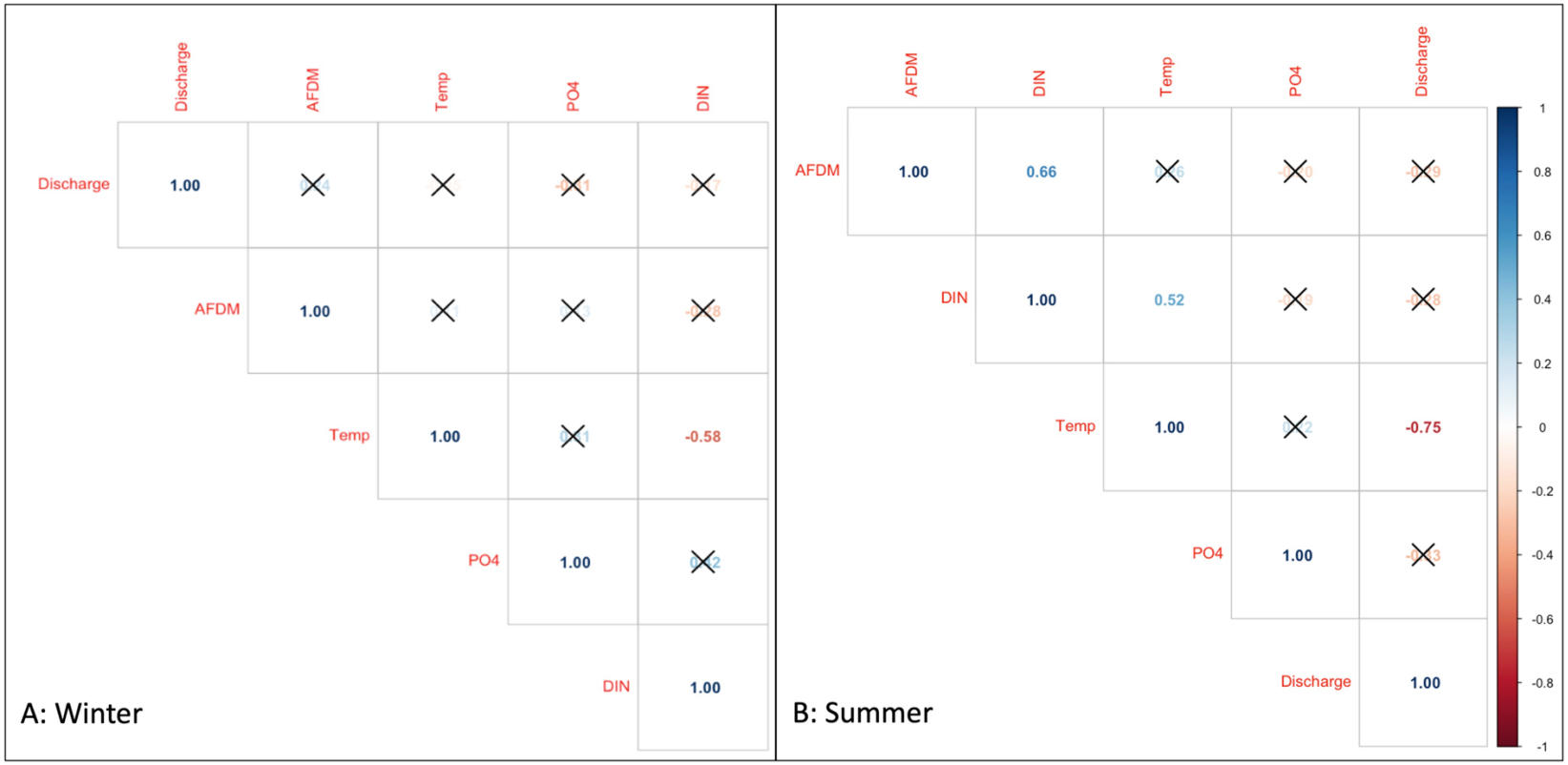

Figure B4. Correlation plots for the environmental variables in our winter (A) and summer (B) NMDS ordinations. Environmental variables were stream- and season-specific discharge (L s^{-1}), temperature (°C), dissolved inorganic nitrogen (DIN, mg L^{-1}), PO_4^{2-} (mg L^{-1}) and ash-free dry mass (AFDM) remaining in associated litterbags (g). Numbers are Pearson correlation coefficients. Red represents negative correlations, blue represents positive correlations, and the strength of the correlation is represented by color intensity. "X's" represent non-signifcant correlations. Plots were generated using the "corrplot" function in the R package *corrplot* (Wei and Simko 2021).

References

Twardochleb, L., E. Hiltner, M. Pyne, and P. Zarnetske. 2021. Freshwater insects CONUS: A database of freshwater insect occurrences and traits for the contiguous United States. Global Ecology and Biogeography 30:826–841.

Wei, T. and V. Simko. 2021. R package 'corrplot': Visualization of a Correlation Matrix. R Version 0.92.

APPENDIX C

SUPPLEMENTARY METHODS AND DATA: CHAPTER 4

I. Supplemental methods

A. Leaf conditioning

In autumn 2018, we collected and air-dried senescent *Rhododendron* and *Acer* leaves from the CHL. Four weeks prior to the start of the experiment (13 Feb 2019), we placed four 5-mm polypropylene mesh litterbags (22 × 40 cm, Cady Bag, Incorporated, Pearson, Georgia, USA) with ~20 g each of *Rhododendron* or *Acer* leaves (n = 2 bags per leaf species) into Shope Fork, where they incubated for three weeks to allow for initial microbial colonization and conditioning. Following the three-week incubation in Shope Fork, we collected and cut the leaves into approximately 1-inch fragments avoiding the midvein, which we placed into 250-µm mesh litterbags (6 × 12 cm, Industrial Netting, Maple Grove, MN, USA). We deployed two of these 250-µm bags per leaf species into each channel one week prior to the start of the experiment, allowing microbial communities to stabilize in each channel prior to insect feeding. We repeated the entire leaf conditioning process weekly during the experiment.

B. Supplemental fungal biomass methods

At the end of the consumption trials that took place in weeks 1 and 5, we saved leaf fragments from one leaf-only chamber per leaf species per channel for fungal biomass measurements. Following fragment collection, we stored the fragments in pre-weighed scintillation vials and transported them frozen back to the laboratory. We then stored the fragments at -20°C and freeze-dried them. We re-weighed the vials containing

the freeze-dried material and calculated ash-free dry mass (AFDM) by assuming the same ash content measured in all the microbe chambers in the whole experiment for the corresponding leaf species.

We extracted lipids and calculated ergosterol concentrations following the methods detailed in Appendix 1 of Cummins et al. *in review*. Briefly, we extracted lipids, re-dissolved them in methanol, and filtered them through a PTFE syringe filter into a vial for high-performance liquid chromatography (HPLC). We calculated ergosterol concentrations based on the area of the ergosterol peak and data from external ergosterol standards using a conversion factor of 5.5 mg ergosterol per g of fungal dry mass (Gulis and Bärlocher 2017).

C. Generating a blotted:AFDM regression

At the beginning of the experiment, we blotted and weighed 30 batches each of *Rhododendron* and *Acer* leaf fragments to generate a blotted:AFDM regression. We immediately stored these samples on ice and transported them back to the lab, where we dried them for at least 48 hours at 60°C and weighed the dried material. We then combusted these samples in a muffle furnace and re-weighed them to determine the ash-free dry mass. We regressed the blotted masses against the ash-free dry masses, forcing regressions through the origin. We used these blotted:AFDM regressions to estimate the initial ash-free dry mass of leaves fed to the insects based on blotted weights.

Table C1. Blotted:ash-free dry mass equations for *Rhododendron* and *Acer* leaves used to estimate the initial AFDM of leaf fragments fed to insects during consumption trials.

Species	Equation	*n*	R^2
Rhododendron	$AFDM = 0.399453(blotted\ mass)$	30	0.99
Acer	$AFDM = 0.209441(blotted\ mass)$	29	0.98

Table C2. Mean (± 1 standard deviation), median, and range of temperature values (°C) in Carpenter Branch (the stream from which we collected the insects) and in each temperature treatment during the artificial streams experiment. Values for Carpenter Branch are presented for both the period of time that corresponds to our experiment and the entire year for which we collected data in 2017-2018. Carpenter Branch values are based on daily mean temperature values, all of which were obtained using HOBO pendant temperature loggers except for those collected between 12 March and 6 April 2018, which were modeled based on air temperature data from CS01 at the Coweeta Hydrologic Lab (Miniat et al. 2022). Values for experiment temperature treatments are based on 30-minute temperature data from all four channels in each treatment.

	Mean (±SD)	Median	Range
Carpenter Branch (12 March – 17 April 2018)	10.2 (± 2.1)	9.8	5.7 - 13.6
Carpenter Branch (22 Sept 2017 – 28 Sept 2018)	12.2 (± 4.5)	13.0	0.7 - 18.1
+0°C (ambient)	10.7 (± 2.2)	10.7	6.2 - 15.9
+1°C	11.8 (± 2.2)	11.7	6.2 - 18.5
+2°C	12.6 (± 2.2)	12.5	6.4 - 17.7
+3°C	13.6 (± 2.3)	13.6	6.4 - 18.4
+4°C	14.5 (± 2.4)	14.6	6.0 - 19.3

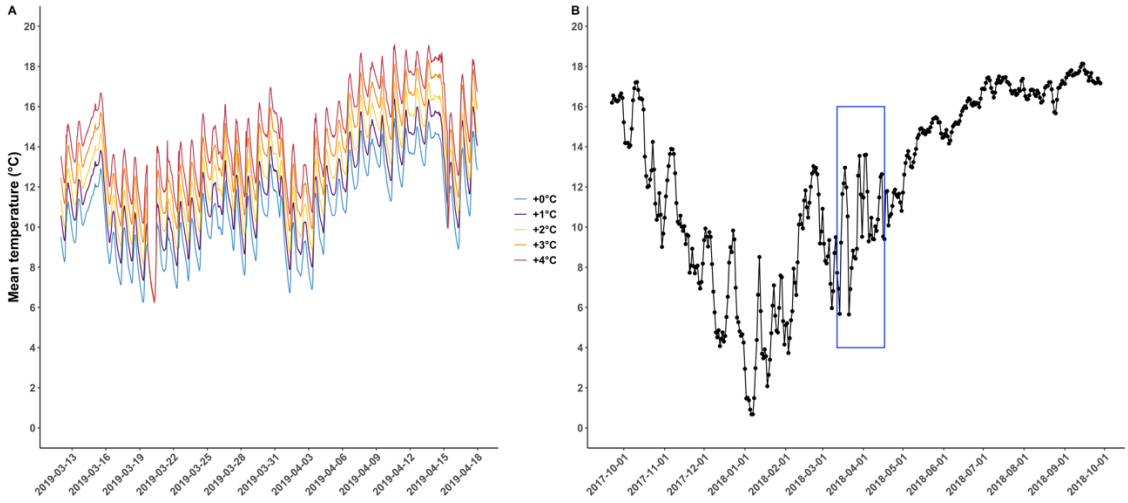

Figure C1. A: 30-minute temperature values (°C) in each temperature treatment in our experiment. B: Daily temperature values in Carpenter Branch, the stream from which the insects were collected, for 22 September 2017 - 28 September 2018. The blue box in panel B represents the same dates in 2018 as when our experiment took place in 2019. Carpenter Branch data was modeled based on air temperature at Climate Station 1 at Coweeta from 12 March to 6 April 2018 due to a temperature logger malfunction.

Figure C2. A photo of Block 1 of our streamside channels experiment with insect chambers.

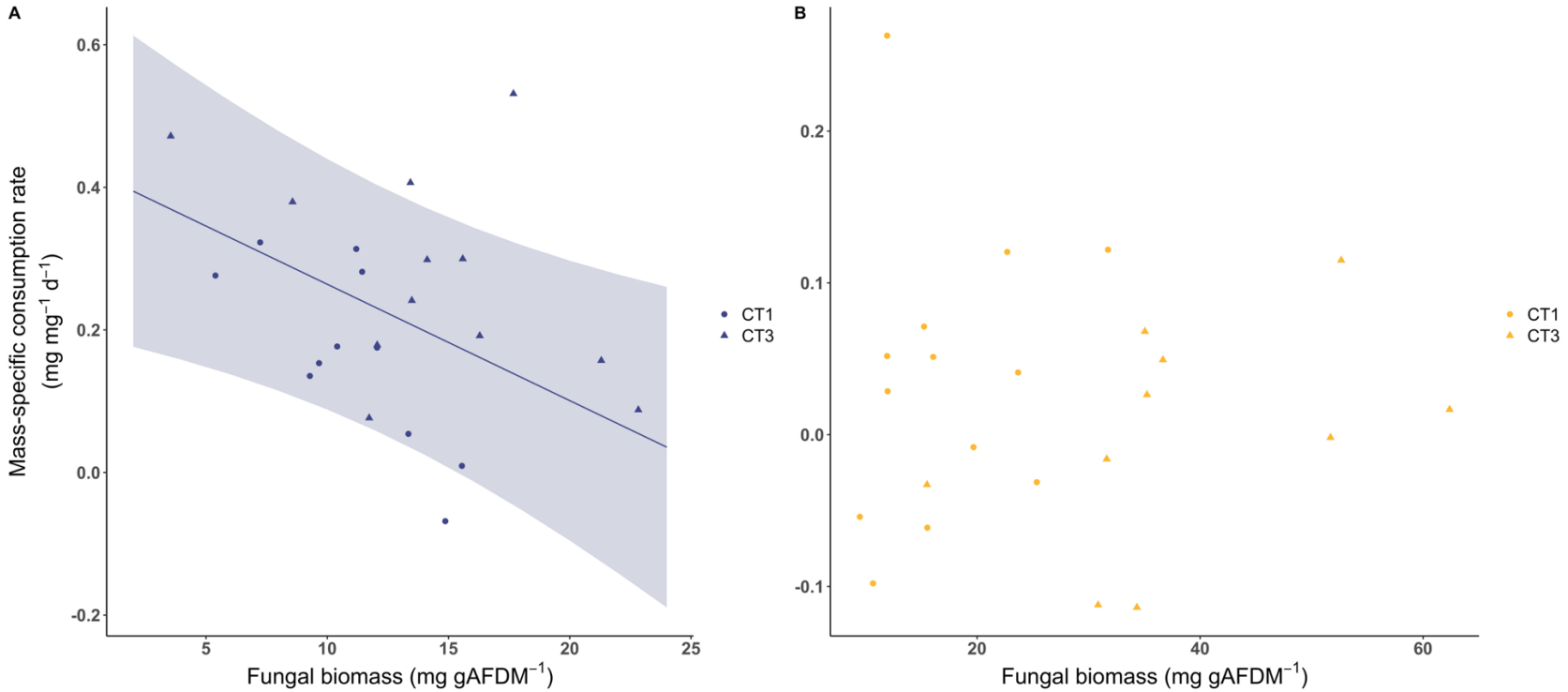

Figure C3. Relationships between fungal biomass (mg g^{-1} AFDM) and mass-specific consumption rate (mg mg^{-1} d^{-1}) for insects in consumption trials 1 ("CT1," week 1) and 3 ("CT3," week 5) in our experiment. Panel A represents this relationship for insects fed *Rhododendron* leaves, and the line indicates the linear fit of a mixed-effects model with temperature as a fixed effect and trial number as a random effect (shaded area represents 95% confidence interval). Panel B indicates the relationship for insects fed *Acer* leaves. Note the difference in the x-axis scales.

References

Gulis, V., and F. Bärlocher. 2017. Fungi: Biomass, Production, and Community Structure. Pages 177–192 Methods in Stream Ecology vol. 1: Ecosystem Structure. Third edition. Elsevier.

Miniat, Chelcy Ford; Laseter, Stephanie H.; Swank, Wayne T.; Swift, Lloyd W. Jr. 2015. Daily air temperature data, recorded by NWS thermometer, from climate station 01 at Coweeta Hydrologic Lab, North Carolina. Fort Collins, CO: Forest Service Research Data Archive. Updated 03 March 2022.