Ecology, 102(10), 2021, e03467

Decomposing decomposition: isolating direct effects of temperature from other drivers of detrital processing

Oliver J. Wilmot,[1,4] James M. Hood,[2] Alexander D. Huryn,[1] and Jonathan P. Benstead[1,3]

[1]*Department of Biological Sciences, University of Alabama, Tuscaloosa, Alabama 35487 USA*
[2]*Aquatic Ecology Laboratory, Department of Evolution, Ecology, and Organismal Biology, Translational Data Analytics Institute, The Ohio State University, Columbus, Ohio 43212 USA*

Citation: Wilmot, O. J., J. M. Hood, A. D. Huryn, and J. P. Benstead. 2021. Decomposing decomposition: isolating direct effects of temperature from other drivers of detrital processing. Ecology 102(10):e03467. 10.1002/ecy.3467

Abstract. Understanding the observed temperature dependence of decomposition (i.e., its "apparent" activation energy) requires separation of direct effects of temperature on consumer metabolism (i.e., the "inherent" activation energy) from those driven by indirect seasonal patterns in phenology and biomass, and by longer-term, climate-driven shifts in acclimation, adaptation, and community assembly. Such parsing is important because studies that relate temperature to decomposition usually involve multi-season data and/or spatial proxies for long-term shifts, and so incorporate these indirect factors. The various effects of such factors can obscure the inherent temperature dependence of detrital processing. Separating the inherent temperature dependence of decomposition from other drivers is important for accurate prediction of the contribution of detritus-sourced greenhouse gases to climate warming and requires novel approaches to data collection and analysis. Here, we present breakdown rates of red maple litter incubated in coarse- and fine-mesh litterbags (the latter excluding macroinvertebrates) for serial approximately one-month increments over one year in nine streams along a natural temperature gradient (mean annual: 12.8°–16.4°C) from north Georgia to central Alabama, USA. We analyzed these data using distance-based redundancy analysis and generalized additive mixed models to parse the dependence of decomposition rates on temperature, seasonality, and shredding macroinvertebrate biomass. Microbial decomposition in fine-mesh bags was significantly influenced by both temperature and seasonality. Accounting for seasonality corrected the temperature dependence of decomposition rate from 0.25 to 0.08 eV. Shredder assemblage structure in coarse-mesh bags was related to temperature across both sites and seasons, shifting from "cold" stonefly-dominated communities to "warm" communities dominated by snails or crayfish. Shredder biomass was not a significant predictor of either coarse-mesh or macroinvertebrate-mediated (i.e., coarse- minus fine-mesh) breakdown rates, which were also jointly influenced by temperature and seasonality. Unlike fine-mesh bags, however, temperature dependence of litter breakdown did not differ between models with and without seasonality for either coarse-mesh (0.36 eV) or macroinvertebrate-mediated (0.13 eV) rates. We conclude that indirect (non-thermal) seasonal and site-level effects play a variable and potentially strong role in shaping the apparent temperature dependence of detrital breakdown. Such effects should be incorporated into studies designed to estimate inherent temperature dependence of slow ecological processes.

Key words: activation energy; carbon; climate change; detritus; generalized additive mixed models; litter breakdown; metabolic theory; rivers; seasonality; temperature dependence.

Introduction

Organic matter decomposition plays a central role in mediating feedbacks in ecosystem and global responses to climate warming (Kirschbaum 2006). Thus, there is a critical need to understand the temperature dependence of this ecosystem process. The observed temperature dependence of litter breakdown (and of many other ecological processes) depends on timescale because it can be broken down most simply into two temporal components. The first component is the instantaneous and direct response of cellular metabolism to a change in temperature (i.e., the intrinsic or inherent activation energy), while the second comprises indirect factors that also affect the rate of litter breakdown and that change over longer than instantaneous timescales (Davidson and Janssens 2006, Yvon-Durocher et al. 2012). This second component

Manuscript received 23 May 2020; revised 16 November 2020; accepted 15 March 2021; final version received 25 June 2021. Corresponding Editor: Alexander S. Flecker.
[4]Present address: Natural and Environmental Sciences Department, Western Colorado University, Gunnison, Colorado, 81231 USA
[3] Corresponding Author. E-mail: jbenstead@ua.edu

includes different effects of what we refer to here as "seasonality," including physiological acclimation, phenological patterns, seasonal shifts in consumer biomass and identity, and changes in resource availability (Davidson and Janssens 2006, Follstad Shah et al. 2017). Together, these two components are usually integrated into an observed temperature dependence (i.e., an effective or apparent activation energy; Davidson and Janssens 2006) given that decomposition studies (and many other types of ecological study) routinely measure rates over relatively long timescales (Wall et al. 2008, Follstad Shah et al. 2017). The significance of combining these two groups of processes into one temperature dependence is underappreciated. Relatively long litter incubations, for example, result in estimates of temperature dependence that integrate multiple mechanisms, which may be directly or indirectly related to temperature, and therefore provide potentially misleading information about how breakdown rates respond to temperature directly.

Comparative studies using multiple sites along spatial gradients of temperature (related to latitude or altitude), as well as multi-site meta-analyses, also integrate an additional component: the long-term alignment of community structure with the thermal regime of ecosystems. Ecological communities are shaped by temperature via both evolutionary adaptation and community assembly. As a result, spatial studies can act as a proxy for potential change over relatively long timescales (i.e., decades to millennia), with the assumption that spatial differences in community structure accurately mimic long-term community responses to changing temperature regimes (Irons et al. 1994, Boyero et al. 2011*b*, 2012). These differences in community structure may also influence estimates of the apparent temperature dependence of litter breakdown and other ecological processes, due to taxonomic differences in phenology and relative contribution to processing rates.

Together, the various effects of seasonality, and of intersite differences in population and community structure that represent proxies for long-term shifts, can obscure the true, inherent temperature dependence of detrital processing. Parsing out an inherent temperature dependence from other drivers is important for accurate prediction of responses of the C cycle to future increments of warming, for example, but requires novel approaches to data collection and analysis. In this study, we examined leaf litter breakdown rates in nine headwater streams in the southeastern United States that lie along a latitudinal and altitudinal gradient and so experience different annual thermal regimes. These streams are in adjacent physiographic provinces and the same biogeographic region, decreasing the potentially confounding effects of large physical and regional species-pool differences among sites. Over a one-year period, we incubated red maple (*Acer rubrum* L.) litter in coarse- and fine-mesh bags (the latter excluding macroinvertebrates) in each stream for 12 successive ˜one-month increments. Here, we use these high-resolution, serial incubation data to separate the temperature dependence of litter breakdown into three components: the instantaneous temperature effects on decay rate; the effects of seasonal change not directly related to temperature; and the effects of differences driven by detritivore community structure. Two overarching questions framed our study. First, how does detrital processing by microbes and shredding macroinvertebrates vary across both seasonal and spatial gradients in temperature? Second, what is the relative importance of direct thermal effects vs. other drivers in affecting estimates of the temperature dependence of detrital processing rate?

Methods

Study sites

Fieldwork for this study was conducted in nine streams located along a natural temperature gradient from northern Georgia to central Alabama, USA (Appendix S1: Fig. S1). The streams ranged in both latitude (34.69° N to 32.98° N) and altitude (85–575 m above sea level) but were otherwise similar (Appendix S1: Table S1 and Fig. S2). All the streams were first- to third-order with relatively low levels of human disturbance (i.e., predominantly forested catchments, intact riparian vegetation, partially closed canopies, and mixed hard substrata) and low dissolved nutrient concentrations (Appendix S1: Table S2).

Litterbag deployment and sample processing

We quantified monthly detrital processing rates and shredder assemblages in each of the nine streams over one calendar year beginning on 7 March 2014 and ending on 9 March 2015. We collected red maple leaves (*Acer rubrum*) after abscission from a single stand in the Talladega National Forest in Hale County, Alabama, USA. during 7–11 November 2013. Red maple was chosen because it is relatively palatable and ubiquitous in the eastern United States (Whiles and Wallace 1997). Litterbags contained 5 ± 0.1 g (range) of litter enclosed in either fine-mesh (500-μm Nitex) or coarse-mesh material (nylon pecan bags, mesh size 8 × 3 mm). We incubated five fine-mesh and five coarse-mesh bags in each of the nine streams in serial approximately one-month deployments (i.e., litterbags were retrieved after 28–35 d and replaced with a new set; Benstead and Huryn 2011). On each deployment date, five additional litterbags were taken into the field as "breakage bags" to account for potential mass loss during handling and transportation. Water temperature was recorded in each stream using Hobo Pendant or Pro v2 temperature loggers (Onset Computer Corporation, Bourne, Massachusetts, USA) programmed to record temperature hourly.

Upon retrieval, litterbags were placed in plastic bags and transported on ice to the laboratory for processing within 24 h. Litter bag contents were elutriated in water

and the litter fragments dried (1 week at 60°C), weighed (±0.1 g), ashed (4 h at 500°C) and re-weighed to obtain estimates of ash-free dry mass (AFDM) loss over time. Remaining material from coarse-mesh litterbags was concentrated in a 500-μm sieve and preserved in a 5% formaldehyde solution. Macroinvertebrates from coarse-mesh litterbags were identified to the lowest practical taxonomic level (usually genus) then classified as shredders or non-shredders based on literature (Merritt et al. 2008) and our knowledge of the regional fauna. We measured the lengths of all shredders (±1 mm) and calculated their biomass using published length–mass regressions (Benke et al. 1999).

Data analysis

To examine how patterns of shredder community structure varied among streams in space and time, we used distance-based redundancy analysis (db-RDA; Legendre and Anderson 1999) using the function capscale in the R package vegan (Oksanen et al. 2019). In short, a Bray-Curtis dissimilarity matrix based on shredder biomass was entered into a principle coordinates analysis (PCoA) and those results were used as the response in a standard RDA to relate community structure to explanatory variables. Our community response data were mean shredder biomass for each stream-by-month combination aggregated at the family level for Insecta and the order level for Amphipoda, Isopoda, Gastropoda, and Decapoda (crayfish). For explanatory variables, we used mean annual temperature for each stream, as well as an interaction between monthly temperature anomaly (from each stream's mean temperature) and stream.

We used generalized additive mixed models (GAMMs) within an information-theoretic framework (Burnham and Anderson 2002) to characterize the relative dependency of fine- and coarse-mesh decomposition rates on temperature, seasonality, and shredder biomass. GAMMs are a nonparametric and nonlinear regression technique that does not require a priori specifications of the functional relationship between the response and predictor variables (Hastie and Tibshirani 1990, Wood 2017). Our GAMMs combined fixed and random effects splines and linear terms, as well as an autoregressive-moving-average (ARMA) model to characterize temporal autocorrelation. Briefly, our GAMM estimates two important terms: (1) a log-linear temperature effect and (2) a spline for month that characterizes all non-thermal variation related to time, which we refer to as seasonality. An analysis with simulated data indicates that this GAMM model accurately estimates a known log-linear temperature effect embedded within a seasonal pattern (see Appendix S2). For fine-mesh decomposition rates, we evaluated several candidate models that included combinations of a spline for month (characterizing seasonality) and a linear temperature term expressed as kT^{-1}, where k is the Boltzmann constant and temperature (T) is in Kelvin and centered at 288.15 K (15°C; the grand mean annual temperature across all sites). For coarse-mesh and coarse- minus fine-mesh decomposition rates, we evaluated candidate models that contained a spline for month, a linear temperature term (kT^{-1}), and a spline or a linear term for detritivore biomass and community structure (characterized by a single db-RDA axis; see *Results*). For all responses, we evaluated models with and without a random intercept and spline for each stream individually, as well as groups of streams. ARMA model parameters were determined by evaluating various time step (p) and smoothing (q) terms using the autocorrelation function (ACF) and selecting the most parsimonious option that effectively eliminated autocorrelation (Zuur and Ieno 2018). Prior to analysis, breakdown rates and shredder biomass were $\log_e$-transformed after adding a small value (0.001) to eliminate zeros. Models were ranked by Akaike information criterion corrected for sample size (AIC_c) scores and those with a $\Delta AIC_c < 2$ were considered the most likely models (where ΔAIC is the difference from the best model). GAMMs were conducted with the function gamm from the package mgcv with a cyclic cubic regression spline and a maximum of 12 knots (Wood 2017). Figures were made in R with the ggplot2 package (Wickham 2016).

Results

Temperature regimes

All streams showed unimodal annual temperature profiles with minima and maxima generally in February and July–August, respectively (Fig. 1). Mean annual temperature varied from 12.8°C to 16.4°C among streams and was negatively correlated with altitude, latitude, and longitude (Appendix S1: Table S1; altitude $P = 0.002$, $R^2 = 0.77$; latitude $P = 0.008$, $R^2 = 0.66$; longitude $P = 0.025$, $R^2 = 0.53$). Hendrick Mill and Little Schultz showed the smallest annual temperature variation, while Raccoon, Choccolocco, and Lick showed the greatest annual variation (Appendix S1: Table S1). Annual mean temperature showed no relationship to temperature variance (standard deviation of the annual mean) across the nine sites, so we ignored any effect of variance on estimating temperature dependence of litter breakdown (Tomczyk et al. 2020).

Shredder assemblages

Shredder biomass was highly variable and did not exhibit consistent seasonality across streams (Appendix S1: Fig. S3). Litterbag shredder assemblages also varied among streams but could be generalized into "warm" communities dominated either by crayfish (e.g., Stamp and Mayfield) or pleurocerid snails (e.g., Choccolocco, Hendrick, and L. Schultz) and "cold" communities dominated by peltoperlid and pteronarcyid stoneflies (Fig. 2 and Appendix S1: Table S3 and Fig. S3). The db-RDA model of these shredder communities contained mean

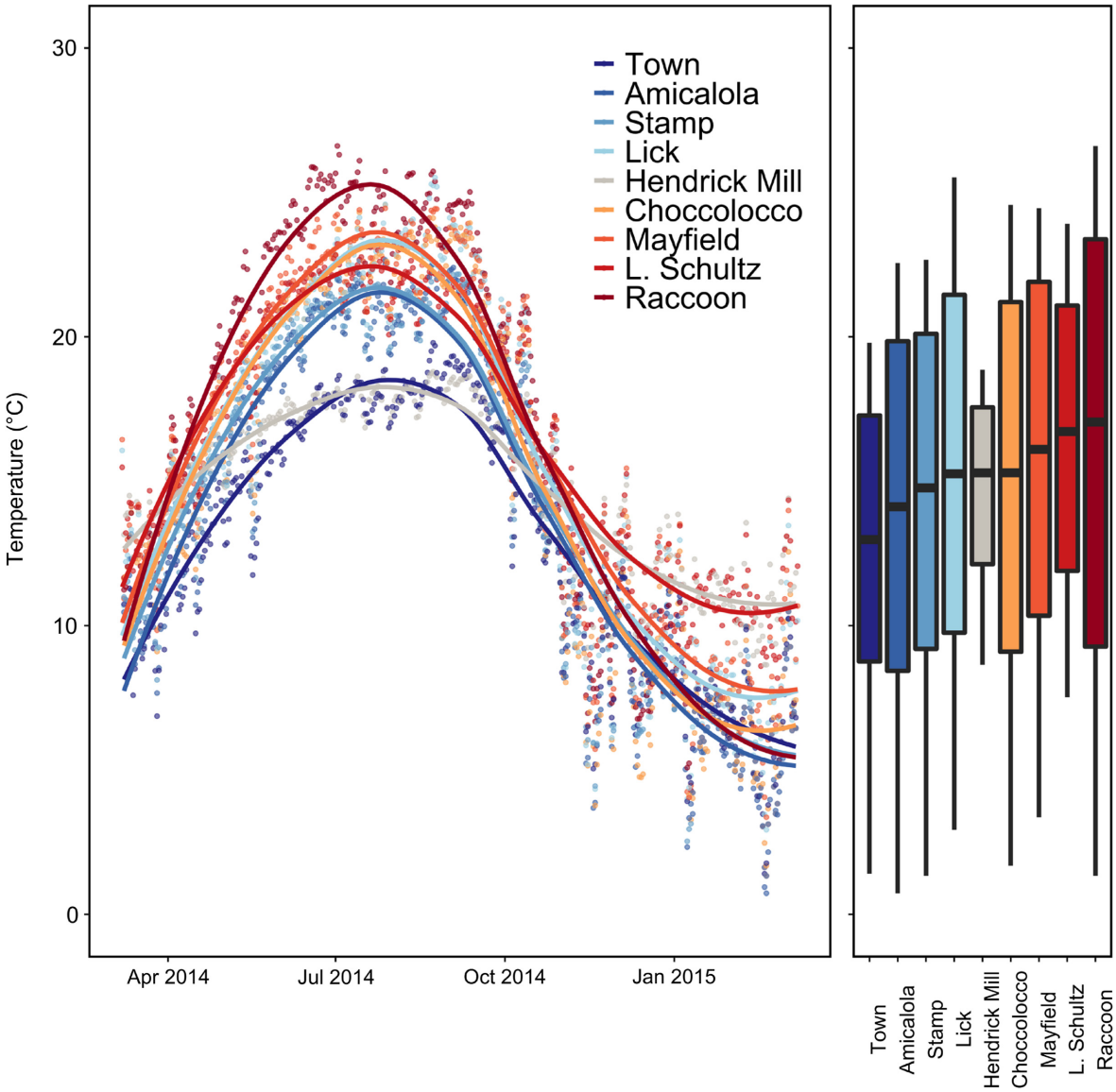

FIG. 1. Daily mean temperature of the study streams as a function of date and summarized with box plots (inset). Lines are LOESS fits. In the box plot, the central line is the median, the lower and upper hinges correspond to the 25th and 75th percentiles, and the whiskers represent the range in the data.

annual temperature ($P = 0.001$) and a monthly temperature anomaly × stream interaction ($P < 0.05$) and had two significant axes: CAP1 and CAP2 ($P = 0.001$, Fig. 2). Streams were distributed in a triangle within the ordination, with either crayfish, snails, and stoneflies at each vertex. Snail and crayfish streams were separated by CAP1 (Fig. 2), while CAP2 separated the crayfish/snail streams from the stonefly streams and was correlated with mean annual temperature ($r = 0.48$).

The db-RDA temperature anomaly × stream vectors indicated that temporal patterns of change in shredder community composition varied among streams (Fig. 2 and Appendix S1: Fig. S3). In all streams except Choccolocco ($r = -0.19$), CAP2 was positively correlated with the monthly temperature anomaly ($r = 0.29$–0.63). In six streams, winter or spring communities were generally characterized by stoneflies, while summer and early-autumn communities were characterized by either crayfish (Amicalola, Stamp, Lick, and Mayfield) or snails (Hendrick Mill, and L. Schultz). In Raccoon and Choccolocco, the shredder community transitioned between snails and crayfish during summer or autumn.

Decomposition rates in fine-mesh bags

Decomposition rates in fine-mesh bags, which varied in both seasonality and maximum decomposition rate among streams (Fig. 3), were jointly influenced by

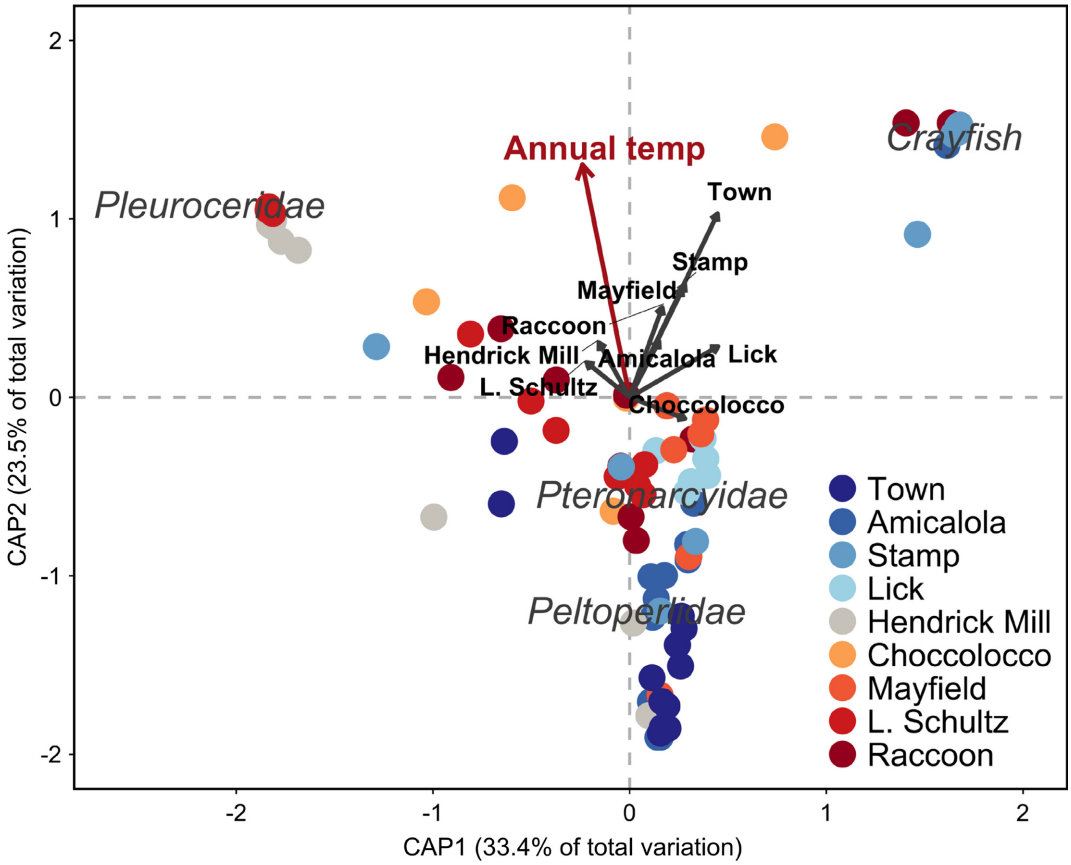

FIG. 2. Distance-based redundancy analysis (db-RDA) biplot of Bray-Curtis dissimilarity of shredder biomass. The best model included the covariates annual mean stream temperature (red arrow) and a monthly stream temperature anomaly × stream interaction (gray arrows). The model is significant ($P = 0.001$), both CAP1 and CAP2 are significant ($P < 0.001$), and all model terms are significant ($P < 0.05$). Gray text shows the four taxonomic groups most strongly correlated with CAP1 and CAP2. Biomass dynamics for these groups are shown in Appendix S1: Fig. S3.

seasonality (Fig. 4A) and temperature (Fig. 4D). The most likely GAMM for decomposition rate in the fine-mesh bags contained a term for temperature (kT^{-1}), a smoother for month for two distinct groups of streams, and a random intercept for each stream (Table 1). The next most likely model had very low support (based on AIC_c; Appendix S1: Table S4). Five streams showed a broad peak in decomposition rate during the spring and summer months, while the four other streams exhibited a sharp peak during July. After accounting for seasonality, the temperature dependence of decomposition rate was 0.08 eV (Table 1). Models that did not account for seasonality resulted in a much stronger apparent temperature dependence of decomposition: 0.25 eV (Table 1, Fig. 4D).

Breakdown rates in coarse-mesh bags

Breakdown rates in coarse-mesh bags were higher than loss rates in fine-mesh bags and were also co-influenced by seasonality (Fig. 4B) and temperature (Fig. 4E). There were four highly likely models ($\Delta AIC < 2$) for breakdown rate in coarse-mesh bags; all contained a term for temperature, a unique smoother for month by stream, and a random intercept for each stream. Three of these highly likely models also contained a term describing shredder biomass or community structure (Appendix S1: Table S5); however, these terms were statistically insignificant and resulted in minimal improvement to model R^2. Models that grouped the month smoothers had low support based on AIC_c and in the most likely model each stream had a unique smoother for month. However, these smoothers could be broadly categorized into three groups. Town, Amicalola, Hendrick Mill, and L. Schultz showed peaks in March or April. Lick, Mayfield, and Raccoon exhibited a trough in spring or early summer and a peak in July–August, and Choccolocco and Stamp showed minimal seasonality. The apparent temperature dependence of litter breakdown in coarse-mesh bags was 0.36 eV (Table 1; Fig. 4E) and did not differ between models with and without seasonality.

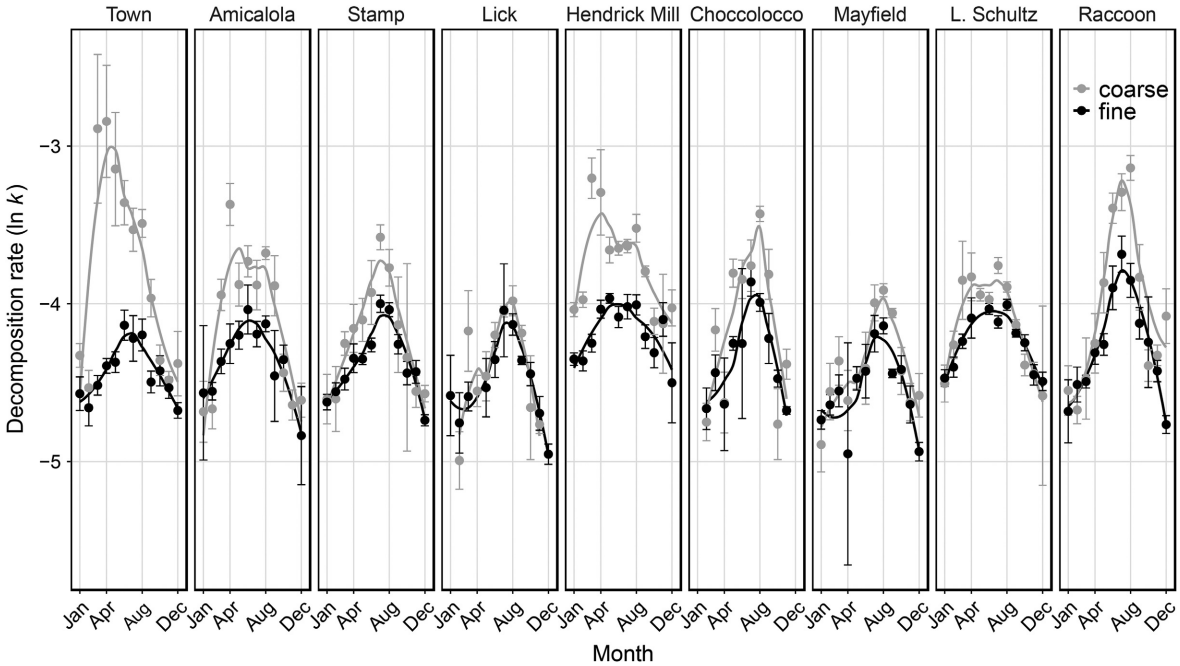

FIG. 3. Decomposition rate in coarse- and fine-mesh litterbags in the study streams ordered by annual mean temperature. The difference between these rates is considered macroinvertebrate-mediated decomposition. Error bars are ±SD. Lines are LOESS fits to ease visualization.

Macroinvertebrate-mediated breakdown

Macroinvertebrate-mediated decomposition rate (i.e., coarse-mesh rate minus fine-mesh rate) varied widely among streams, with the lowest rates observed in Lick and the highest observed in Town (Fig. 3). The best GAMM model for macroinvertebrate-mediated decomposition rate was very similar to the best model for decomposition rate in coarse-mesh bags in terms of predictor variables and the shape of smoothers for month. This model contained a term for temperature, a unique smoother for month for each stream and a random intercept for each stream. While each stream had a unique smoother for month, these smoothers could be broadly grouped as above for decomposition in coarse-mesh bags. The temperature dependence of macroinvertebrate-mediated decomposition rates was 0.13 eV and did not differ between models with and without the inclusion of seasonality (Table 1, Fig. 4F). Inclusion of shredder data in the GAMM models did not improve model fits, suggesting that neither total shredder biomass nor CAP2 were effective proxies for the functional role of macroinvertebrates in shaping breakdown rates (Appendix S1: Table S6). Indeed, correlations between macroinvertebrate-mediated breakdown rate and shredder biomass varied widely among streams, from −0.32 to 0.36 (Kendall rank correlation test).

DISCUSSION

Studies attempting to isolate the effect of temperature on detrital breakdown routinely incubate substrates over relatively long periods (weeks to months) that inevitably integrate indirect, seasonal drivers that affect breakdown rate (Karberg et al. 2008, Benfield et al. 2017). Other studies and meta-analyses have incorporated spatial temperature gradients that are partly confounded by indirect effects of temperature mediated by consumer adaptation and community assembly (Irons et al. 1994, Boyero et al. 2011*a*, Follstad Shah et al. 2017, Tiegs et al. 2019). Our study was motivated by the assumption that such indirect drivers could have important effects on estimates of the temperature dependence of detrital breakdown, the sensitivity of which represents a key feedback in the global climate system. We collected high-resolution data over one year in nine streams, tracking monthly changes in rates of litter breakdown attributed to microbial and macroinvertebrate activity. Our analysis revealed a consistent over-estimation of the temperature dependence of decomposition rate in fine-mesh bags, demonstrating an indirect effect of seasonality on microbial activity. The temperature dependence of litter breakdown attributed to macroinvertebrates showed no such universal effect of seasonality, however. Instead, the effects of seasonality were site-specific and highly idiosyncratic, complicating broad conclusions about the relative importance of indirect drivers for the temperature dependence of macroinvertebrate-mediated breakdown.

Using fine-mesh litterbags allowed us to isolate the activity of heterotrophic microorganisms that were presumably dominated by hyphomycete fungi, but also included bacteria and meiofauna. Analysis of our

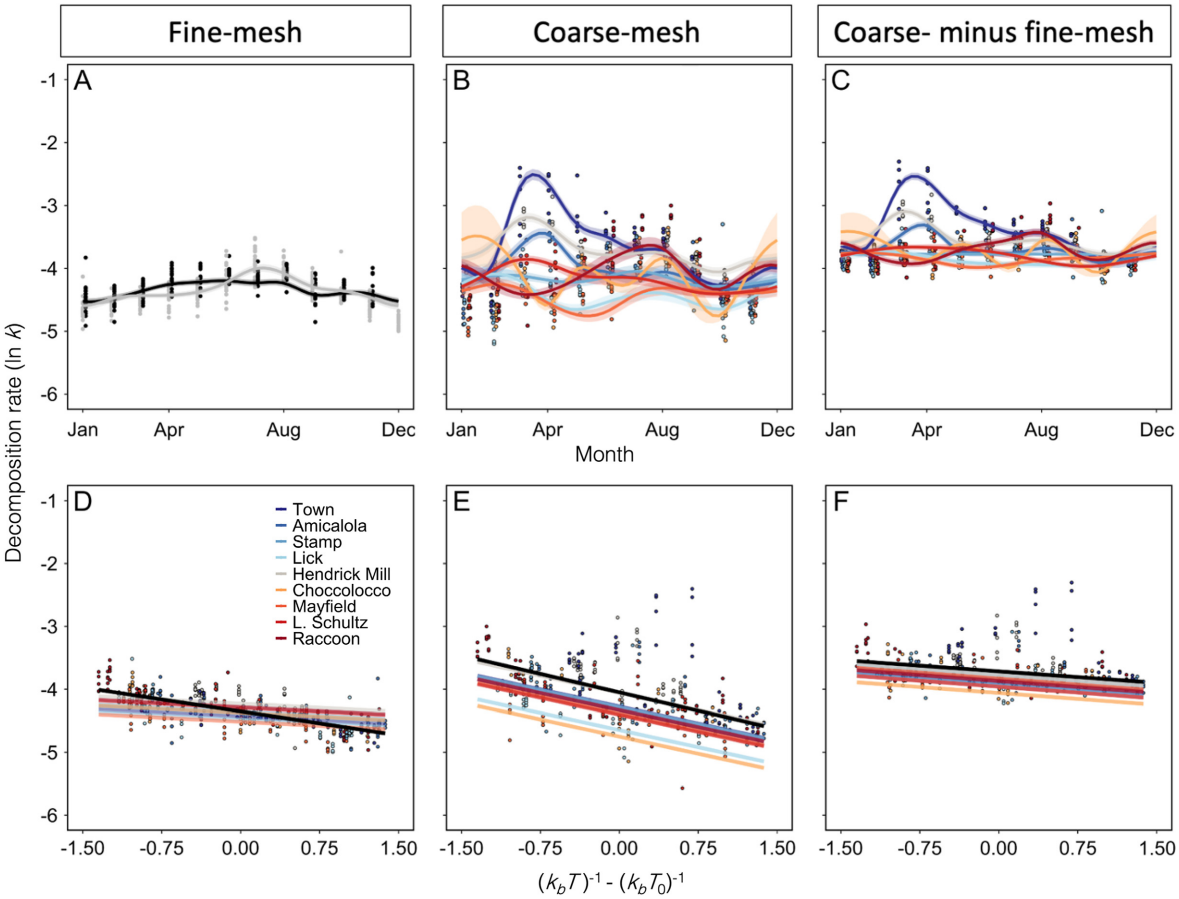

FIG. 4. Response of decomposition in fine-mesh, coarse-mesh, and coarse- minus fine-mesh litterbags in generalized additive mixed model (GAMM) (A–C) smooth curves to month and(D–F) linear fits to centered kT^{-1}. In panel A, the black line depicts Stamp, Lick, Choccolocco, Mayfield, and Raccoon sites; the gray line depicts the Town, Amicalola, Hendrick Mill, and L. Schultz sites. Black lines in panels D–F depict the relationship between decomposition rate and centered kT^{-1} estimated from a linear mixed effects model, ignoring any seasonality. For the purpose of visualization, we predicted ln k from the most likely GAMM model with the temperature term fixed to the mean stream temperature for A–C while D–F show model predictions for October (a "shoulder" season when effects of seasonality were intermediate).

monthly fine-mesh litter incubation data revealed clear patterns in decomposition related to seasonality, with lower observed decomposition rates in winter (and higher observed rates in summer) than would be expected based on temperature alone (see splines in Fig. 4A, which represent the temperature-corrected seasonality of decomposition rate). Consequently, the temperature dependence of decomposition rate was shallower than that predicted by an analysis that did not consider seasonality (in Fig. 4D, compare the black line that does not account for seasonality to the colored seasonality-corrected lines). Thus, a portion of the summer peak and winter trough in decomposition was attributed to seasonality, lowering the temperature dependence of decomposition. Our simulation study demonstrated that the GAMM could accurately extract a known temperature dependence from a unimodal seasonality pattern similar to that observed in Fig 4A (see Appendix S2). Removing the seasonality effect reduced the calculated activation energy of microbial decomposition from 0.25 eV to only 0.08 eV, providing an example of how indirect seasonal effects may bias estimates of temperature dependence. Unfortunately, our data cannot inform the mechanism driving the observed seasonality of decomposition. Potential explanations for this pattern include higher microbial biomass at higher temperatures, perhaps driven by a stimulatory effect of algal priming during high-light conditions (Guenet et al. 2010, Halvorson et al. 2019) or higher availability of other resources in summer (Mulholland et al. 1985, Roberts and Mulholland 2007, Rosemond et al. 2015). Another potential mechanism controlling relative microbial biomass is seasonal swings between resource limitation (i.e., low litter availability) in summer and saturation (i.e., high litter availability) in winter. Microbial activity linked to species composition could also have shifted in ways not necessarily correlated with temperature. Fungal community composition is known to

Table 1. Modeling results of the top-ranked linear and generalized additive mixed models (GAMMs) for factors affecting fine-mesh, coarse-mesh, and coarse- minus fine-mesh (C-F) rates of decomposition.

Term	β	edf	*F*	*P*
Fine; $n = 505$				
Linear model; $R^2_{adj} = 0.68$				
Intercept	−4.31 (0.02)***			
Centered kT^{-1}	−0.25 (0.01)***			
GAMM; $R^2_{adj} = 0.77$				
Intercept	−4.35 (0.03)***			
Centered kT^{-1}	−0.08 (0.04)*			
s(month): Group A		7.8	$16.9_{9,495}$	<0.001
s(month): Group B		8.4	$40.2_{9,495}$	<0.001
s(stream)		7.7	$31.9_{7,497}$	<0.001
Coarse; $n = 397$				
Linear model; $R^2_{adj} = 0.54$				
Intercept	−4.04 (0.05)***			
Centered kT^{-1}	−0.39 (0.02)***			
GAMM; $R^2_{adj} = 0.86$				
Intercept	−4.09 (0.09)***			
Centered kT^{-1}	−0.36 (0.08)***			
s(month): Ami		7.1	$9.8_{9,387}$	<0.001
s(month): Chocco		6.6	$168.6_{7,389}$	<0.001
s(month): Raccoon		6.6	$8.2_{9,387}$	<0.001
s(month): Hendrick		6.5	$8.7_{9,387}$	<0.001
s(month): Lick		4.8	$34.1_{8,388}$	0.004
s(month): Mayfield		5.0	$3.8_{9,387}$	0.002
s(month): Schultz		4.5	$4.9_{9,387}$	0.0042
s(month): Stamp		3.8	$1.1_{9,387}$	0.123
s(month): Town		8.9	$39.7_{9,387}$	<0.001
s(stream)		7.8	$97.7_{7,389}$	<0.001
C-F; $n = 397$				
Linear model; $R^2_{adj} = 0.32$				
Intercept	−3.68 (0.04)***			
Centered kT^{-1}	−0.12 (0.02)***			
GAMM; $R^2_{adj} = 0.76$				
Intercept	−3.70 (0.05)***			
Centered kT^{-1}	−0.13 (0.02)***			
s(month): Ami		6.9	$11.0_{9,369}$	<0.001
s(month): Chocco		6.5	$54.0_{7,371}$	0.008
s(month): Raccoon		6.8	$6.1_{9,369}$	<0.001
s(month): Hendrick		7.1	$9.7_{9,369}$	<0.001
s(month): Lick		2.6	$11.1_{7,371}$	0.004
s(month): Mayfield		3.2	$1.7_{9,369}$	0.003
s(month): Schultz		2.8	$8.4_{8,370}$	0.006
s(month): Stamp		4.5	$0.0_{9,369}$	0.222
s(month): Town		8.7	$38.9_{9,369}$	<0.001
s(stream)		7.4	$76.9_{7,371}$	<0.001

Notes:: Each model is represented together with the parameter estimate (β ± standard error in parentheses) for parametric terms and the effective degrees of freedom (edf), *F* statistics values (*F*), and corresponding *P* values for smoothing terms (*P*) as well as the number of samples (*n*) and model adjusted R^2 values (R^2adj). Group A: Choccolocco, Raccoon, Lick, Mayfield, Stamp; Group B: Amicalola, Hendrick Mill, L. Schultz, Town; Ami: Amicalola; Chocco: Choccolocco; Hendrick: Hendrick Mill; Schultz: L. Schultz.

* <0.05; ** <0.01; *** <0.001.

vary directly with temperature (e.g., Dang et al. 2009, Ferreira and Chauvet 2011) and with season (e.g., Nikolcheva and Bärlocher 2005), but growth responses of individual taxa to temperature often only partly explain seasonal patterns (e.g., Suberkropp 1984). Finally, our finding of a lower temperature dependence of microbial breakdown, once corrected for seasonality, also raises questions. One potential mechanism is the known temperature invariance in activity of some cellulolytic enzymes, which would tend to flatten the true

temperature dependence of microbial breakdown (Sinsabaugh and Follstad Shah 2010).

Unlike decomposition in fine-mesh litterbags, we found that accounting for seasonality did not influence estimates of the temperature dependence of coarse-mesh or macroinvertebrate-mediated decomposition rates The lack of synchrony in the seasonality of macroinvertebrate-mediated decomposition among streams distributed its temporal variation relatively evenly across our thermal gradient (see variation around black line in 4E), resulting in no seasonality-related bias in the temperature dependence of decomposition. Breakdown rates in coarse-mesh bags displayed a high degree of asynchrony across the nine streams, which is expected given the diverse shredder communities found in these streams, the complex impacts of temperature on shredder phenology, and how widely shredder impacts on decomposition vary among taxa (Huryn et al. 2002, Dangles and Malmqvist 2004). Such idiosyncratic behavior among streams confounded any consistent effect of non-thermal seasonality on the temperature dependence of breakdown in coarse-mesh bags.

Overall, the summed effect of temporal variation was to obscure a consistent seasonal signal in the coarse-mesh breakdown data, in contrast to what we observed in microbially mediated decomposition in fine-mesh bags. Microbial community structure has been shown to display seasonal synchrony among unconnected river systems, driven by extrinsic climatic factors such as temperature and discharge (e.g., Crump and Hobbie 2005). In contrast, breakdown rates in coarse-mesh bags displayed a high degree of asynchrony across the nine streams, suggesting that the structure and activity of their shredder communities were instead more subject to intrinsic, site-specific controls.

Relatively few studies have been designed to identify seasonal shifts in litter breakdown rates, preventing the parsing of direct temperature effects from indirect effects of seasonality. Of the existing studies in the literature, some have attempted to isolate the effects of temperature by calculating breakdown rates on a degree-day basis. For example, McArthur et al. (1988) incubated *Acer* litter for serial one-month periods over one year, finding breakdown that peaked in summer as per-day rates but in winter when temperature-corrected per degree-day. Similarly, Benstead and Huryn (2011) used serial 30-d incubations of *Salix* litter over two years to demonstrate the marked effect of the phenology of shredding macroinvertebrates in driving highest rates of breakdown rates (both per day and per degree-day) during winter in an arctic spring-stream. As in the present study, however, higher rates of breakdown are usually found during summer, suggesting the primacy of temperature in driving litter breakdown (Nikolcheva and Bärlocher 2005, Ferreira and Canhoto 2014, Griffiths and Tiegs 2016). Unfortunately, most seasonal studies have not attempted to isolate the effects of temperature from other indirect drivers.

Our estimates of the temperature dependence of detrital breakdown can be compared both to published estimates from other litter breakdown studies and to the canonical activation energy of cellular respiration (0.65 eV; Brown et al. 2004). Based on a meta-analysis of 1,025 published records of litter breakdown in streams and rivers, Follstad Shah et al. (2017) reported a mean activation energy of 0.34 ± 0.04 eV (mean ± 95% confidence interval), a value very close to the 0.36 eV that we estimated for breakdown in coarse-mesh bags. However, Follstad Shah et al. (2017) found no significant difference between the temperature dependence of breakdown rate of coarse- vs. fine-mesh bags (0.33 eV and 0.37 eV, respectively). Our estimate of fine-mesh breakdown that did not account for seasonality (0.25 eV) was similar to estimates from Follstad Shah et al. (2017), raising the possibility that at least the activation energy for fine-mesh breakdown they reported might be elevated due to indirect non-thermal effects.

Similar comparisons can be made to the activation energies reported from a coordinated global study of alder litter breakdown in 22 streams that spanned 91° of latitude (Boyero et al. 2011*a*). In that study, marked differences in patterns of breakdown were found between mesh sizes. Rates of microbial decomposition in fine-mesh bags increased along the temperature gradient with a calculated activation energy of 0.46 eV. In contrast, rates of breakdown in coarse-mesh bags, while higher than in fine-mesh bags, showed a flat relationship with temperature (Boyero et al. 2011*a*). The authors concluded that increases in microbial decomposition with climate warming could be offset by decreases in macroinvertebrate-mediated breakdown, with consequences for the fate of litter carbon (CO_2 evasion vs. particle storage) in freshwater ecosystems (Boyero et al. 2011*a*). Our study, which was designed around a comparatively regionally restricted temperature gradient, suggests that such a prediction might be problematic. Rates of breakdown in litterbags of both mesh sizes clearly increased with temperature in our study, while shredder assemblage structure shifted predictably across both site and season as taxonomic groups replaced each other along spatial and temporal temperature gradients. As such, our results likely mimic the consequences of long-term local warming within our study region more effectively than extrapolations based on very large latitudinal gradients such as those used by Boyero et al. (2011*a*).

As noted above, activation energies lower than the canonical value of 0.65 eV for cellular respiration (Brown et al. 2004) are routinely observed in litter breakdown studies focused on estimating temperature dependence (Boyero et al. 2011*a*, Follstad Shah et al. 2017). Our results were no different, with all estimates of activation energy, whether accounting for non-thermal seasonal effects or not, considerably lower than 0.65 eV. This difference was particularly marked for microbial decomposition in fine-mesh bags, especially after

accounting for indirect seasonal effects. Such large deviations from the canonical value of 0.65 eV suggest that the temperature dependence of breakdown processes was not dominated by cellular respiration. Instead, such low activation energies are usually attributed to a dominant role for extracellular enzymes in the breakdown process (Sinsabaugh et al. 2009). Typical activation energies for these exoenzymes range from ˜0.31 to 0.56 eV (Sinsabaugh and Follstad Shah 2012, Follstad Shah et al. 2017). Our estimates of activation energies were even lower than this range, especially in fine-mesh bags, suggesting that additional processes were lowering the temperature dependence of breakdown. Possible intrinsic mechanisms behind such an effect include seasonal changes in community composition and/or thermal acclimation or adaptation (Bradford 2013, Strickland et al. 2015). Extrinsic factors could also have played a role, such as the relative availability of detrital substrate. For example, relative scarcity of detritus during the summer months could have increased colonization and processing rates relative to winter months when detritus was more abundant and demand for substrates was saturated.

We also observed spatial differences in community structure that were related to temperature and so presumably partly reflect long-term differences in local thermal regime. As such, these community differences within a single biogeographic region represent an effective proxy for long-term shifts in the taxonomic composition of stream communities as temperature regimes change. Particularly striking was the transition from insect-dominated shredder assemblages at lower temperatures to non-insect assemblages (crayfish and snails) at higher temperatures. Shifts between insect and non-insect dominance were seen both across sites along the temperature gradient and within most of the sites across seasons. However, we found no strong relationship between community structure and breakdown rates, complicating assessment of the relative role of species identity in determining ecological function. Shredder assemblage structure also did not explain variation in seasonality effects observed in coarse-mesh litterbags. Instead, trajectories of seasonal shifts in breakdown rates driven by shredder presence were highly idiosyncratic across the nine stream sites. Such site-specific responses were likely driven by differences in community structure, biomass, and phenology across sites, as well as other potentially interacting site-level factors not quantified in our study.

In conclusion, collecting standardized litter breakdown rates from relatively short, serial incubations at sites along a natural temperature gradient allowed us to parse the direct effects of temperature from indirect effects associated with seasonal and site differences. Accounting for seasonality either lowered the resulting estimates of temperature dependence (i.e., for the largely microbial contribution in fine-mesh bags) or had no effect (i.e., coarse-mesh and macroinvertebrate-mediated). While the seasonality effect on fine-mesh decomposition was relatively consistent across our nine sites, seasonality signals in coarse-mesh bags were highly idiosyncratic and seemingly dependent on the temporal dynamics of resident macroinvertebrate communities (and possibly other factors, such as hydrology). Despite these site-specific effects, we observed consistent shifts from insect-dominated to snail- and crayfish-dominated macroinvertebrate communities along both spatial and temporal gradients in temperature. Such shifts underscore the importance of community reconfiguration in response to climate warming. However, we found no evidence that shifts in macroinvertebrate community structure will affect the temperature dependence of breakdown itself, perhaps due to the lack of synchrony in the seasonal component of decomposition, which was likely driven by spatiotemporal variation in macroinvertebrate community structure and physical factors such as discharge.

Understanding the various components of the temperature dependence of ecological processes is vital to modeling future responses to climate warming. In the case of detrital breakdown and many other slow processes, these components range temporally to include the instantaneous effect of temperature on enzymatic reactions (Sinsabaugh and Follstad Shah 2012), the mid-term effects of acclimation and seasonality (Suberkropp 1984, Nikolcheva and Bärlocher 2005), and the longer-term shifts associated with population adaptation and community alteration (Davidson and Janssens 2006, Follstad Shah et al. 2017, Padfield et al. 2017). While these overlapping responses are complex and challenging to parse, acknowledging them is a necessary first step to appreciating their potential effects on attempts to estimate the temperature dependence of ecological processes in any ecosystem type. Future studies designed to estimate temperature sensitivity should attempt to incorporate them from the outset.

Acknowledgments

This work was partly supported by the National Science Foundation (DEB-1354624 to J.P. Benstead and A.D. Huryn and DEB-1655956 to J.P. Benstead). We thank Alexandra, Maria, and Eugene Huryn for helping to collect the red maple leaves. We are also very grateful to Seth Wenger, who assisted with site selection and provided helpful input on an earlier version of the manuscript. Comments from two anonymous reviewers greatly improved the final manuscript.

Literature Cited

Benfield, E. F., K. M. Fritz, and S. D. Tiegs. 2017. Leaf-litter breakdown. Pages 71–82 *in* Methods in stream ecology, Volume 2. Ecosystem function. Third edition. Associated Press, San Diego, California, USA.

Benke, A. C., A. D. Huryn, L. A. Smock, and J. B. Wallace. 1999. Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States. Journal of the North American Benthological Society 18:308–343.

Benstead, J. P., and A. D. Huryn. 2011. Extreme seasonality of litter breakdown in an arctic spring-fed stream is driven by

shredder phenology, not temperature. Freshwater Biology 56:2034–2044.

Boyero, L., et al. 2011*a*. A global experiment suggests climate warming will not accelerate litter decomposition in streams but might reduce carbon sequestration. Ecology Letters 14:289–294.

Boyero, L., et al. 2011*b*. Global distribution of a key trophic guild contrasts with common latitudinal diversity patterns. Ecology 92:1839–1848.

Boyero, L., et al. 2012. Global patterns of stream detritivore distribution: implications for biodiversity loss in changing climates. Global Ecology and Biogeography 21:134–141.

Bradford, M. A. 2013. Thermal adaptation of decomposer communities in warming soils. Frontiers in Microbiology 4:333.

Brown, J. H., J. F. Gillooly, A. P. Allen, V. M. Savage, and G. B. West. 2004. Toward a metabolic theory of ecology. Ecology 85:1771–1789.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference. Springer-Verlag, New York, New York, USA.

Crump, B. C., and J. E. Hobbie. 2005. Synchrony and seasonality in bacterioplankton communities of two temperate rivers. Limnology and Oceanography 50:1718–1729.

Dang, C. K., M. Schindler, E. Chauvet, and M. O. Gessner. 2009. Temperature oscillation coupled with fungal community shifts can modulate warming effects on litter decomposition. Ecology 90:122–131.

Dangles, O., and B. Malmqvist. 2004. Species richness-decomposition relationships depend on species dominance. Ecology Letters 7:395–402.

Davidson, E. A., and I. A. Janssens. 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440:165–173.

Ferreira, V., and C. Canhoto. 2014. Effect of experimental and seasonal warming on litter decomposition in a temperate stream. Aquatic Sciences 76:155–163.

Ferreira, V., and E. Chauvet. 2011. Future increase in temperature more than decrease in litter quality can affect microbial litter decomposition in streams. Oecologia 16:279–291.

Follstad Shah, J. J., et al. 2017. Global synthesis of the temperature sensitivity of leaf litter breakdown in streams and rivers. Global Change Biology 23:3064–3075.

Griffiths, N. A., and S. D. Tiegs. 2016. Organic-matter decomposition along a temperature gradient in a forested headwater stream. Freshwater Science 35:518–533.

Guenet, B., M. Danger, L. Abbadie, and G. Lacroix. 2010. Priming effect: bridging the gap between terrestrial and aquatic ecology. Ecology 91:2850–2861.

Halvorson, H. M., S. N. Francoeur, R. H. Findlay, and K. A. Kuehn. 2019. Algal-mediated priming effects on the ecological stoichiometry of leaf litter decomposition: a meta-analysis. Frontiers in Earth Science 7:76.

Hastie, T. J., and R. J. Tibshirani. 1990. Generalized additive models. Chapman and Hall/CRC, London, UK.

Huryn, A. D., V. M. Butz Huryn, C. J. Arbuckle, and L. Tsomides. 2002. Catchment land-use, macroinvertebrates and detritus processing in headwater streams: taxonomic richness versus function. Freshwater Biology 47:401–415.

Irons, J. G., M. W. Oswood, R. Stout, and C. M. Pringle. 1994. Latitudinal patterns in leaf litter breakdown: is temperature really important? Freshwater Biology 32:401–411.

Karberg, N. J., N. A. Scott, and C. P. Giardina. 2008. Methods for estimating litter decomposition. Pages 103–111 *in* Field measurements for forest carbon monitoring. Springer, Dordrecht, The Netherlands.

Kirschbaum, M. U. F. 2006. The temperature dependence of organic-matter decomposition—still a topic of debate. Soil Biology and Biochemistry 38:2510–2518.

Legendre, P., and M. J. Anderson. 1999. Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. Ecological Monographs 69:1–24.

McArthur, J. V., J. R. Barnes, B. J. Hansen, and L. G. Leff. 1988. Seasonal dynamics of leaf litter breakdown in a Utah alpine stream. Journal of the North American Benthological Society 7:44–50.

Merritt, R. W., K. W. Cummins, and M. B. Berg. 2008. An introduction to the aquatic insects of North America. Kendall Hunt, Dubuque, Iowa, USA.

Mulholland, P. J., J. D. Newbold, J. W. Elwood, L. A. Ferren, and J. R. Webster. 1985. Phosphorus spiraling in a woodland stream: seasonal variations. Ecology 66:1012–1023.

Nikolcheva, L. G., and F. Bärlocher. 2005. Seasonal and substrate preferences of fungi colonizing leaves in streams: traditional versus molecular evidence. Environmental Microbiology 7:270–280.

Oksanen, J., et al. 2019. vegan: Community Ecology Package. R package version 2.5-6. https://CRAN.R-project.org/package=vegan

Padfield, D., C. Lowe, A. Buckling, R. Ffrench-Constant, Student Research Team, S. Jennings, F. Shelley, J. S. Ólafsson, and G. Yvon-Durocher. 2017. Metabolic compensation constrains the temperature dependence of gross primary production. Ecology Letters 20:1250–1260.

Roberts, B. J., and P. J. Mulholland. 2007. Instream biotic control on nutrient biogeochemistry in a forested stream, West Fork of Walker Branch. Journal of Geophysical Research 112:G04002.

Rosemond, A. D., J. P. Benstead, P. M. Bumpers, V. Gulis, J. S. Kominoski, D. W. P. Manning, K. Suberkropp, and J. B. Wallace. 2015. Experimental nutrient additions accelerate terrestrial carbon loss from stream ecosystems. Science 347:1142–1145.

Sinsabaugh, R. L., and J. J. Follstad Shah. 2010. Integrating resource utilization and temperature in metabolic scaling of riverine bacterial production. Ecology 91:1455–1465.

Sinsabaugh, R. L., and J. J. Follstad Shah. 2012. Ecoenzymatic stoichiometry and ecological theory. Annual Review of Ecology and Systematics 43:313–343.

Sinsabaugh, R. L., B. H. Hill, and J. J. Follstad Shah. 2009. Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. Nature 462:795–798.

Strickland, M. S., A. D. Keiser, and M. A. Bradford. 2015. Climate history shapes contemporary leaf litter decomposition. Biogeochemistry 122:165–174.

Suberkropp, K. 1984. Effect of temperature on seasonal occurrence of aquatic hyphomycetes. Transactions of the British Mycological Society 82:53–62.

Tiegs, S. D., et al. 2019. Global patterns and drivers of ecosystem functioning in rivers and riparian zones. Science Advances 5:eaav0486.

Tomczyk, N. J., A. D. Rosemond, P. M. Bumpers, C. S. Cummins, S. J. Wenger, and J. P. Benstead. 2020. Ignoring temperature variation leads to underestimation of the temperature sensitivity of plant litter decomposition. Ecosphere 11: e03050.

Wall, D. h., et al. 2008. Global decomposition experiment shows soil animal impacts on decomposition are climate-dependent. Global Change Biology 14:2661–2677.

Whiles, M. R., and J. B. Wallace. 1997. Leaf litter decomposition and macroinvertebrate communities in headwater streams draining pine and hardwood catchments. Hydrobiologia 353:107–119.

Wickham, H. 2016. ggplot2. Springer-Verlag, New York, New York, USA.

Wood, S. 2017. Generalized additive models: an introduction with R, Second edition. Chapman and Hall/CRC, London, UK.

Yvon-Durocher, G., et al. 2012. Reconciling the temperature dependence of respiration across timescales and ecosystem types. Nature 487:472–476.

Zuur, A. F., and E. N. Ieno. 2018. Beginner's guide to spatial, temporal, and spatial-temporal ecological data analysis with R-INLA Volume II: GAM and zero-inflated models. Highland Statistics, Newburgh, UK.

Supporting Information

Additional supporting information may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/ecy.3467/suppinfo

Open Research

Data and code are available on Zenodo: http://doi.org/10.5281/zenodo.4923472.