

Organic-matter decomposition along a temperature gradient in a forested headwater stream

Natalie A. Griffiths^{1,3} and Scott D. Tiegs^{2,4}

¹Climate Change Science Institute and Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831-6301 USA

²Department of Biological Sciences, Oakland University, Rochester, Michigan 48309 USA

Abstract: We used a natural temperature gradient in Walker Branch, a spring-fed forested stream in eastern Tennessee, USA, to examine the influence of temperature on organic-matter decomposition. In this stream, upstream sites are warmer than downstream sites in winter and are cooler than downstream sites in summer. We used a cotton-strip assay to examine breakdown of a substrate of uniform quality (95% cellulose) along the temperature gradient monthly for 2 y. We also used litter bags to examine the interactive effects of leaf-litter quality (labile red maple [*Acer rubrum*] and tulip poplar [*Liriodendron tulipifera*] and less labile white oak [*Quercus alba*]), invertebrates, and temperature on breakdown rates along the downstream temperature gradient for 90 d in winter. Cotton-strip tensile loss and leaf-litter breakdown rates were highly variable. Tensile-loss rates probably were driven by a combination of daily and diel temperature, discharge, and streamwater nutrients that varied seasonally and spatially along the temperature gradient. Leaf-litter breakdown rates tended to be faster in warmer upstream sites (red maple = 0.0452/d, tulip poplar = 0.0376/d, white oak = 0.0142/d) and slower in cooler downstream sites (red maple = 0.0312/d, tulip poplar = 0.0236/d, white oak = 0.0107/d), and breakdown rates were positively correlated with total invertebrate density. Temperature sensitivity of decomposition was similar among the 3 litter types. These results highlight the high degree of spatial and temporal heterogeneity that can exist for ecosystem processes and their drivers. Quantifying this heterogeneity is important when scaling functional metrics to stream and watershed scales and for understanding how organic-matter processing will respond to the warmer streamwater temperatures expected as a result of global climate change.

Key words: organic matter, decay, cotton-strip assay, litter quality, nutrients, shredders, snails, thermal gradient, diel temperature range, global change, Q_{10-q} , Metabolic Theory of Ecology

Anthropogenic activities are increasing streamwater temperatures across the globe. Long-term records show that stream temperatures have increased 0.009 to 0.077°C/y (Kausshal et al. 2010), and temperature tends to be elevated in anthropogenically affected streams (e.g., in urban and agricultural settings) because of riparian-canopy removal and altered flow regimes (LeBlanc et al. 1997, Poole and Berman 2001). Increasing temperatures can have cascading effects on temperature-dependent ecosystem processes and can lead to elevated rates of respiration (Sinsabaugh 1997, Yvon-Durocher et al. 2012), primary production (Rasmussen et al. 2011), nutrient cycling (D'Angelo et al. 1991, Rasmussen et al. 2011), and organic-matter decomposition (Buzby and Perry 2000, Ferreira and Chauvet 2011a, b). The temperature sensitivity of decomposition must be quantified to understand how stream C cycling may be altered under global-change scenarios.

Temperature can affect decomposition by influencing the metabolic rates of microorganisms and invertebrates

feeding on ecologically important organic matter, such as leaf litter (Cummins and Klug 1979). Microbial activity (Sand-Jensen et al. 2007, Acuña et al. 2008, Ferreira and Chauvet 2011a, b, Fernandes et al. 2012) and invertebrate consumption rates (Nolen and Pearson 1993, González and Graça 2003) tend to increase with temperature in laboratory studies. However, the effect of temperature on decomposition is more complex at larger spatial scales (Irons et al. 1994, Boyero et al. 2011b, Pozo et al. 2011). In a global-scale experiment, Boyero et al. (2011b) found no differences in the decomposition rate of black alder (*Alnus glutinosa*) leaves along a latitudinal gradient because the increase in microbial decomposition in warmer, tropical streams was countered by a decrease in shredding invertebrates, possibly because many shredding insects are cold-adapted species (Irons et al. 1994, Dobson et al. 2002, Boyero et al. 2011a). However, noninsects that consume detritus (e.g., crustaceans, tadpoles) can be important processors of leaf material in tropical streams (Crowl et al.

E-mail addresses: ³griffithsna@ornl.gov; ⁴tiegs@oakland.edu

DOI: 10.1086/685657. Received 30 September 2014; Accepted 13 July 2015; Published online 11 February 2016.
Freshwater Science. 2016. 35(2):000–000. © 2016 by The Society for Freshwater Science.

000

2001, Cheshire et al. 2005). At smaller spatial scales, leaf-litter decomposition rates can be faster in warmer streams than adjacent cooler streams (Friberg et al. 2009), and faster in warmer than cooler locations within a stream (Taylor and Dykstra 2005, Taylor and Chauvet 2014), unless abundance of shredding invertebrates is lower under warmer conditions (Taylor and Andrushchenko 2014).

The effect of temperature on decomposition also can vary across temporal scales. Decomposition rates can change seasonally such that breakdown is faster in warmer than colder months (McArthur et al. 1988, Graça et al. 2001, Ferreira and Canhoto 2014). Large diel temperature swings can also result in faster microbial decomposition rates because of shifts in fungal community structure (Dang et al. 2009). However, the effect of temperature variation on decomposition dynamics has not been as well characterized as the effect of temperature itself. The overall effect of temperature on leaf-litter decomposition in streams may depend on how temperature magnitude and variation affects the presence, composition, and activity of invertebrates relative to the activity and composition of the microbial community.

Temperature interacts with extrinsic (e.g., microorganisms, invertebrates) and intrinsic factors (e.g., litter quality) influencing decomposition. The quality of leaf litter (i.e., labile vs recalcitrant based on chemical composition such as C, N, P, % cellulose, % lignin) that enters streams varies among (Petersen and Cummins 1974, Ostrofsky 1997, Ardón et al. 2009) and within tree species (LeRoy et al. 2007, Axelsson et al. 2010) and among stands of trees because of differences in age or damage (Donaldson et al. 2006, Kominoski et al. 2008). Most investigators who have compared the temperature sensitivity of labile and recalcitrant organic matter to decomposition worked with terrestrial soils, in which sensitivity to temperature tends to be greater for recalcitrant than labile organic matter (Fierer et al. 2005, Conant et al. 2008). Temperature sensitivity pertains primarily to the microbial component of decomposition because enzyme kinetics dictate that a higher activation energy is required to decompose more-recalcitrant organic matter (Fierer et al. 2005). In stream microcosms, temperature sensitivity of microbial decomposition was greater for recalcitrant than labile organic matter (Fernandes et al. 2012, Gonçalves et al. 2013) or was not affected by organic-matter quality (Sand-Jensen et al. 2007, Ferreira and Chauvet 2011a). However, invertebrates play an important role in litter decomposition in stream ecosystems (Graça 2001), and the outcome of the interactions among temperature, invertebrates, microorganisms, and leaf-litter quality remains unclear.

We examined how temperature affects organic-matter decomposition along a natural temperature gradient in the West Fork of Walker Branch, a forested, spring-fed stream in eastern Tennessee, USA. In winter, upstream sites are

warmer than downstream sites because of groundwater input, whereas downstream sites are cooler because of stronger atmospheric influences. The reverse temperature gradient is present in summer. Diel temperature ranges are consistently smaller at upstream than downstream sites throughout the year, and diel temperature swings are largest in early spring before leaf out and in late autumn after leaf fall.

We carried out 2 experiments to examine the influence of temperature on organic-matter decomposition in Walker Branch. First, we measured cellulose decomposition (cotton-strip assay; Tiegs et al. 2007, 2013) along the temperature gradient monthly for 2 y to examine how small (i.e., diel and along the reach) and large (i.e., seasonal) differences in temperature influence cellulose breakdown. We predicted that variation in cotton-strip decomposition would be driven by seasonal changes in temperature and that site-to-site variation in cotton-strip decomposition (i.e., along the temperature gradient) would be driven by both mean daily temperature and diel ranges in temperature. Second, we examined how leaf-litter quality, temperature, and invertebrates interact to affect decomposition in large-mesh litter bags along the temperature gradient. During one period in winter, we measured breakdown rates, microbial respiration, and invertebrate density on leaf litter from 3 tree species that varied in quality. We predicted that leaf-litter decomposition would be faster at the warmer than the colder sites and that decomposition of less-labile litter would be more sensitive to temperature than decomposition of more-labile litter. Together, these experiments enabled us to evaluate the role of temperature on organic-matter decomposition dynamics in Walker Branch. Our results will contribute to efforts to predict the response of C cycling in this ecosystem to climate change.

METHODS

Study site

The West Fork of Walker Branch is a headwater stream draining a 38.4-ha forested watershed on the US Department of Energy's Oak Ridge Reservation (lat 35°57'32"N, long 84°16'47"W). Four perennial springs discharge to the 1st-order stream, which is ~300 m long. Two springs form the headwaters (S4, S5), and 2 springs discharge at ~160 m downstream (S3, S3A). Spring S3 supplies the greatest proportion of base flow to the West Fork compared with the other springs (Curlin and Nelson 1968, Genereux et al. 1993). The most upstream site at which cotton strips and litter bags were deployed was 10 m downstream from S3.

Walker Branch Watershed is vegetated by a 2nd-growth forest consisting primarily of oaks (*Quercus* spp.), tulip poplar (*Liriodendron tulipifera*), red maple (*Acer rubrum*), and American beech (*Fagus grandifolia*) (Johnson 1989). Trees shade the stream from mid-April to mid-October. Leaf fall occurs in October and November, when the mean

daily input rate to the stream is $6.17 \text{ g m}^{-2} \text{ d}^{-1}$ (Comiskey 1978). Large organic-matter standing stocks occur in autumn as a result of leaf fall and can decrease considerably after high-flow events (Comiskey 1978, Mulholland 1997).

The stream bed is composed primarily of exposed bedrock and areas of gravel and cobble, with some accumulations of fine and coarse benthic organic matter. Streamwater nutrient concentrations are low (Mulholland 2004, Lutz et al. 2012) and can limit organic-matter decomposition (Elwood et al. 1981). Long-term records for Walker Branch Watershed show that mean annual air temperature has increased 1.8°C over the past 40 y (Lutz et al. 2012).

Cotton-strip decomposition

We used cotton strips (Latter and Howson 1977, Harrison et al. 1988), a substrate of uniform quality (95% cellulose; Tiegs et al. 2007, 2013), to characterize seasonal and spatial variation in decomposition. Cotton-strip assays primarily assess microbial decomposition of cellulose, but some evidence indicates that invertebrate feeding does occur with this assay (Clapcott and Barmuta 2010). We occasionally observed the dominant snail in Walker Branch, *Elimia clavaeformis*, on the cotton strips, but we did not measure the snails' contribution to decomposition. We deployed and retrieved 21 cotton strips every ~ 35 d for 2 y (22 deployments from 10 August 2011 to 1 October 2013) at 7 sites in the West Fork of Walker Branch ($n = 3$ cotton strips/site). The locations (distance in meters downstream from the largest spring [S3]) of the 7 sites were: site 1 = 10 m, site 2 = 29 m, site 3 = 46 m, site 4 = 75 m, site 5 = 103 m, site 6 = 133 m, site 7 = 162 m. Each site had similar flow characteristics (i.e., all were shallow riffles).

We prepared cotton strips (80×25 mm) from bolts of unprimed 12-ounce, heavy-weight cotton fabric (style 548; Frederix, Lawrenceville, Georgia; Slocum et al. 2009, Tiegs et al. 2013). We pushed a pin between 2 cotton threads near the top of each strip and inserted a cable tie through the hole. We tied 3 cotton strips to a string and attached the string to a brick, which we placed in the stream in the direction of flow. After the ~ 35 -d incubation period, we retrieved the cotton strips, cleaned them with 80% ethanol, and air dried them before measuring tensile strength.

We measured tensile strength by placing the ends of each strip in the grips of a tensiometer (model MG100; Mark-10, Copiague, New York) and pulling the ends apart at a rate of 2 cm/min until the strip tore. We recorded the tensile strength for each strip and used it to calculate tensile strength loss, which we defined as the percentage of the initial tensile-strength lost per day:

$$\text{Tensile loss} = \frac{\left(\frac{\text{Tensile strength}_{\text{REF}} - \text{Tensile strength}_{\text{TRT}}}{\text{Tensile strength}_{\text{REF}}} \right) \times 100}{\text{Incubation time}} \quad (\text{Eq. 1})$$

where tensile strength_{TRT} is the tensile strength recorded for each cotton strip incubated in the field and tensile strength_{REF} is the mean tensile strength of 6 cotton strips that were not incubated in the field but were cleaned with 80% ethanol and air dried (Tiegs et al. 2013). To control for potential temperature differences, we also expressed tensile strength per degree day (dd; described below) by substituting degree days for incubation time in Eq. 1.

Leaf-litter decomposition

In the 2nd experiment, we used a litter-bag approach (Benfield 2006) to examine the influence of temperature on the decomposition of leaf litter that differed in quality. We used large-mesh litter bags so that the interactions among temperature, leaf-litter quality, and invertebrates could be examined. We selected red maple, tulip poplar, and white oak (*Quercus alba*) because they are common trees in Walker Branch Watershed and vary in lability. Dried tulip poplar and red maple leaves are more labile with lower C:N ratios (tulip poplar: 49.4, red maple: 52.5) and lower lignin contents (tulip poplar: 27.8%, red maple: 19.9%) than white oak leaves (C:N: 67.3, lignin: 36.7%) (Ostrofsky 1997). Our characterization of organic-matter quality is qualitative (i.e., more or less labile) because we did not measure the initial chemistry of the leaves.

We collected abscised, senescent leaves of tulip poplar, red maple, and white oak from Walker Branch Watershed in autumn 2011 and allowed leaves to air dry in the laboratory for 2 wk before constructing the litter bags. We placed dried leaves (12.0 ± 0.1 g) in 20.0×37.5 -cm nylon litter bags with 9-mm^2 (3×3 mm) apertures to allow invertebrate access. We deployed 12 litter bags of each litter type at 5 of the 7 sites used in the cotton-strip decomposition experiment (sites 1, 3, 5, 6, and 7). Two sites were not used because the number of litter bags was limited. We deployed litter bags on 15 December 2011 and retrieved 3 replicates of each type from each site 5, 26, 55, and 90 d after deployment (end date: 14 March 2012). We deployed only 4 litter bags with white oak leaves at site 6 because the number of white oak litter bags was limited. To account for handling losses, we brought 3 replicates of each litter type into the field and then immediately returned them to the laboratory for measurement of initial mass loss (day 0).

On each collection date, we retrieved litter bags from the field and placed them in pre-labeled plastic bags. We removed a subsample of ~ 0.1 g dry mass from each litter bag for a microbial respiration assay, then placed the litter bags on ice, and processed them immediately upon return to the laboratory. We washed leaf litter over nested 1-mm and 500- μm mesh sieves to remove inorganics and invertebrates. We used all litter collected on the 1-mm sieve to measure mass loss and counted invertebrates on the 1-mm and 500- μm sieves.

We placed the cleaned leaf litter in paper bags and dried it at 60°C for 48 h. We then weighed the leaf litter to measure dry mass and removed a subsample to estimate ash-free dry mass (AFDM). We put ~1 g of dry mass in a preweighed Al pan, weighed it, combusted it at 500°C for 4 h, and reweighed it to calculate AFDM. We calculated the breakdown coefficient (k ; /d) as $\ln(\text{AFDM remaining})$ over time for each litter type at each site. We also calculated a temperature-corrected breakdown coefficient (k ; /dd) by substituting degrees days for time.

Microbial respiration and invertebrates on leaf litter

We measured microbial respiration on all 3 litter types at each site in the field. We put the subsamples (0.1 g) of litter in 60-mL amber polypropylene tubes filled with stream water. We incubated samples in the stream at each site for 3 h and measured dissolved O_2 concentrations at the start and end of the incubation period with a handheld dissolved O_2 sensor (ProODO; Yellow Springs Instruments, Yellow Springs, Ohio). We also filled 3 control tubes at each site with stream water only and incubated them with the respiration samples to account for changes in background dissolved O_2 concentrations. Microbial respiration was expressed as $\text{mg O}_2 \text{ g}^{-1} \text{ AFDM h}^{-1}$.

We sorted all invertebrates that were captured on the 1-mm and 500- μm mesh sieves and stored them in 80% ethanol. In Walker Branch, the snail *E. clavaeformis* is a dominant invertebrate (can constitute up to 95% of total invertebrate biomass; Newbold et al. 1983, Rosemond et al. 1993), and *E. clavaeformis* was found on leaves in the litter bags. All snails and other invertebrates removed from each litter bag were counted separately, and their densities were expressed as the number of individuals [ind]/g AFDM of litter remaining on each collection date.

Environmental variables

We placed a temperature logger (TidbiT v2; Onset Computer Corporation, Bourne, Massachusetts; accuracy = 0.2°C) in the largest (S3) spring and at each site (1–7) in Walker Branch to measure water temperature hourly during the cotton-strip and leaf-litter decomposition experiments. We calculated mean daily streamwater temperature from the hourly data. Diel temperature range was calculated as the maximum hourly temperature measured on a given day minus the minimum hourly temperature measured on the same day. We calculated degree days as the sum of the mean daily temperatures for each incubation period.

To assess whether streamwater chemistry (NO_3^- -N, NH_4^+ -N, soluble reactive P [SRP]) changed along the downstream gradient during the leaf-litter decomposition experiment, we collected and filtered water samples at each site on days 55 and 90. To capture temporal variation, we measured nutrient concentrations at 1 downstream site weekly

throughout the leaf-litter and cotton-strip decomposition experiments. We measured NO_3^- -N concentrations with the Cd reduction method, NH_4^+ -N concentrations with the phenol-hypochlorite method, and SRP concentrations with the molybdate-antimony method (APHA 2005). See Mulholland (2004) and Lutz et al. (2012) for a detailed description of the nutrient analysis methods.

Streamwater discharge was measured at a v-notch weir every 15 min. All data from our experiment are available online (Griffiths and Tiegs 2015).

Calculations

We used 2 approaches to explore the influence of temperature on decomposition: Q_{10-q} (Conant et al. 2008) and the Metabolic Theory of Ecology (MTE) (Brown et al. 2004). Q_{10-q} is similar to the Q_{10} coefficient in that it estimates the rate of change of biological activity over a given temperature change, but unlike the Q_{10} , the Q_{10-q} can be calculated for a temperature change other than 10°C. The Q_{10-q} value accounts for differences in organic-matter quality because it incorporates the time required to decompose a given amount of organic matter (e.g., 50% of initial mass) (Conant et al. 2008). Therefore, Q_{10-q} can be used to compare the temperature sensitivity of organic matter differing in quality as it decomposes. We calculated Q_{10-q} for red maple, tulip poplar, and white oak in the leaf-litter decomposition experiment as:

$$Q_{10-q} = \left(\frac{t_c}{t_w} \right)^{\left(\frac{10}{(T_w - T_c)} \right)} \quad (\text{Eq. 2})$$

where t_c and t_w are the times (in d) taken to decompose 50% of the initial mass of leaf litter at the colder (downstream) and warmer (upstream) sites, respectively, and T_w and T_c are the mean temperatures (in °C) over the 90-d experiment at the warmer and colder sites, respectively (Conant et al. 2008).

The MTE mathematically describes the relationship between temperature and biological activity as the slope (in eV) of the natural logarithm of biological activity (leaf-litter breakdown rate and cotton-strip tensile loss) vs the inverse of the product of the absolute temperature (in K) and Boltzmann's constant (8.617×10^{-5} eV/K) (Brown et al. 2004). We calculated MTE slopes and 95% confidence intervals for each species in the leaf-litter decomposition experiment to examine whether the slopes varied among litter types and for the entire cotton-strip data set to examine the temperature dependence of tensile loss.

Statistical analyses

We used repeated measures analysis of covariance (rmANCOVA) to test whether tensile loss of cotton strips varied along the downstream gradient and over time. We treated site as a continuous variable (as distance in meters

from the S3 spring) and time as the repeated measures variable. We used a 2-way ANCOVA with litter type as a factor, site and time as covariates, and $\ln(\text{AFDM remaining})$ as the dependent variable to examine the influence of site and litter type on leaf-litter breakdown. We used 2-way rmANCOVAs with litter type as a factor and site as a covariate to examine changes in microbial respiration, invertebrate density, and snail density over time and 1-way rmANCOVA with site as a covariate to examine changes in water chemistry along the downstream gradient over time.

We used Pearson's correlation to examine the relationships between environmental and biological variables (i.e., mean daily streamwater temperature, diel temperature range, nutrient concentrations, invertebrate density) and breakdown rates of leaf litter and tensile loss of cotton strips. We used a nonlinear fit for the relationship of tensile loss and diel temperature range.

We examined the potential drivers of seasonal variation in cotton-strip tensile loss by testing for correlations between the mean tensile-loss rate across sites ($n = 7$) for each deployment period and streamwater nutrient concentration, stream discharge, and streamwater temperature (all calculated as the mean for each deployment period). We also used Pearson's correlation to examine the relationships between temperature and cotton-strip tensile loss along the temperature gradient, both by season (where seasons were defined by equinoxes and solstices) and for each deployment period.

We tested whether greater variation in temperature at the downstream vs upstream sites was correlated with increased variation in cotton-strip tensile loss based on Pearson's correlation between the coefficients of variation (CVs) of temperature and diel temperature range and the CV of tensile-loss rate across the 7 sites. We also used Pearson's correlation to examine whether CVs of temperature and diel temperature range were correlated with leaf-litter breakdown rates and tensile-loss of cotton strips.

We used $\ln(x)$ -transformations to normalize data to meet parametric assumptions when necessary. Statistical significance was designated when $p \leq \alpha = 0.05$. All statistics were done with SYSTAT (version 13; Systat Software, San Jose, California).

RESULTS

Streamwater temperature and chemistry

Mean daily water temperature varied seasonally in Walker Branch (summer mean \pm SD daily temperature across sites = $16.2 \pm 0.5^\circ\text{C}$, winter $11.4 \pm 1.0^\circ\text{C}$) (Fig. 1A). Mean daily water temperature also varied along the downstream gradient, with the largest difference between the most upstream and most downstream site in summer (mean daily difference = 1.1°C), smaller differences in winter (0.8°C) and autumn (0.7°C), and little difference in spring (0.2°C). The warmer sites switched throughout the

year, with upstream sites warmer than downstream sites in winter, and downstream sites warmer than upstream sites in summer (Fig. 1A).

Throughout the year, temperature varied less at upstream (CV = 0.12) than downstream sites (CV = 0.18) because temperature at upstream sites was influenced by the consistent temperature of the springs (mean \pm SD daily temperature of the S3 spring = $14.3 \pm 0.2^\circ\text{C}$) (Fig. 1A). Diel temperature swings also were consistently larger at the downstream than the upstream sites (Fig. 1B). The mean diel temperature range over the 2-y cotton-strip decomposition experiment was $1.0 \pm 0.6^\circ\text{C}$ at the most upstream site and $1.8 \pm 0.8^\circ\text{C}$ at the most downstream site. The largest diel temperature ranges occurred in early spring prior to canopy closure (Fig. 1B).

During the 90-d leaf-litter decomposition experiment, upstream sites were warmer than downstream sites (Fig. 1C), and diel temperature ranges were larger at the downstream sites (Fig. 1D). The mean daily streamwater temperature at the most upstream and most downstream sites during the experiment were $12.1 \pm 0.6^\circ\text{C}$ and $11.4 \pm 1.0^\circ\text{C}$, respectively, resulting in a mean daily difference of 0.6°C . The greatest difference in mean daily water temperature between the most upstream and most downstream sites was 2.1°C . The upstream (1085 dd) and downstream (1029 dd) sites differed by 56 dd during the 90-d leaf-litter decomposition experiment.

Water chemistry varied along the downstream gradient during the leaf-litter decomposition experiment. NO_3^- -N concentration was higher at upstream than at downstream sites on both dates (rmANCOVA, site: $F_{1,12} = 43.3$, $p < 0.0001$; Table 1). NH_4^+ -N concentration was higher at downstream than upstream sites on day 90 and was low and similar across all sites on day 55 (rmANCOVA, site \times time interaction, $F_{1,12} = 11.1$, $p = 0.006$; Table 1). Soluble reactive P (SRP) concentration was low and similar across sites (rmANCOVA, site: $F_{1,12} = 2.5$, $p = 0.14$; Table 1).

Cotton-strip decomposition: seasonal variation

Tensile loss of cotton strips averaged 1.24 %/d and ranged from 0.03 to 2.48 %/d over 2 y in Walker Branch. There was a temporal pattern in tensile loss that varied by site (rmANCOVA, site \times time interaction, $F_{21,294} = 2.8$, $p = 0.0001$; Fig. 2). The temporal trend in mean tensile loss across sites tended to follow the seasonal pattern in mean daily water temperature. Tensile loss was faster when water was warmer in summer (Fig. 2). When data from all cotton-strip deployments and sites were combined, tensile loss and streamwater temperature were positively correlated ($r = 0.65$, $p < 0.0001$, $n = 154$; Fig. 3A), and the slopes of the relationships between tensile loss and streamwater temperature did not differ among the 7 sites (ANCOVA, site \times temperature interaction, $F_{6,140} = 0.8$, $p = 0.56$). When tensile loss was expressed /dd, the

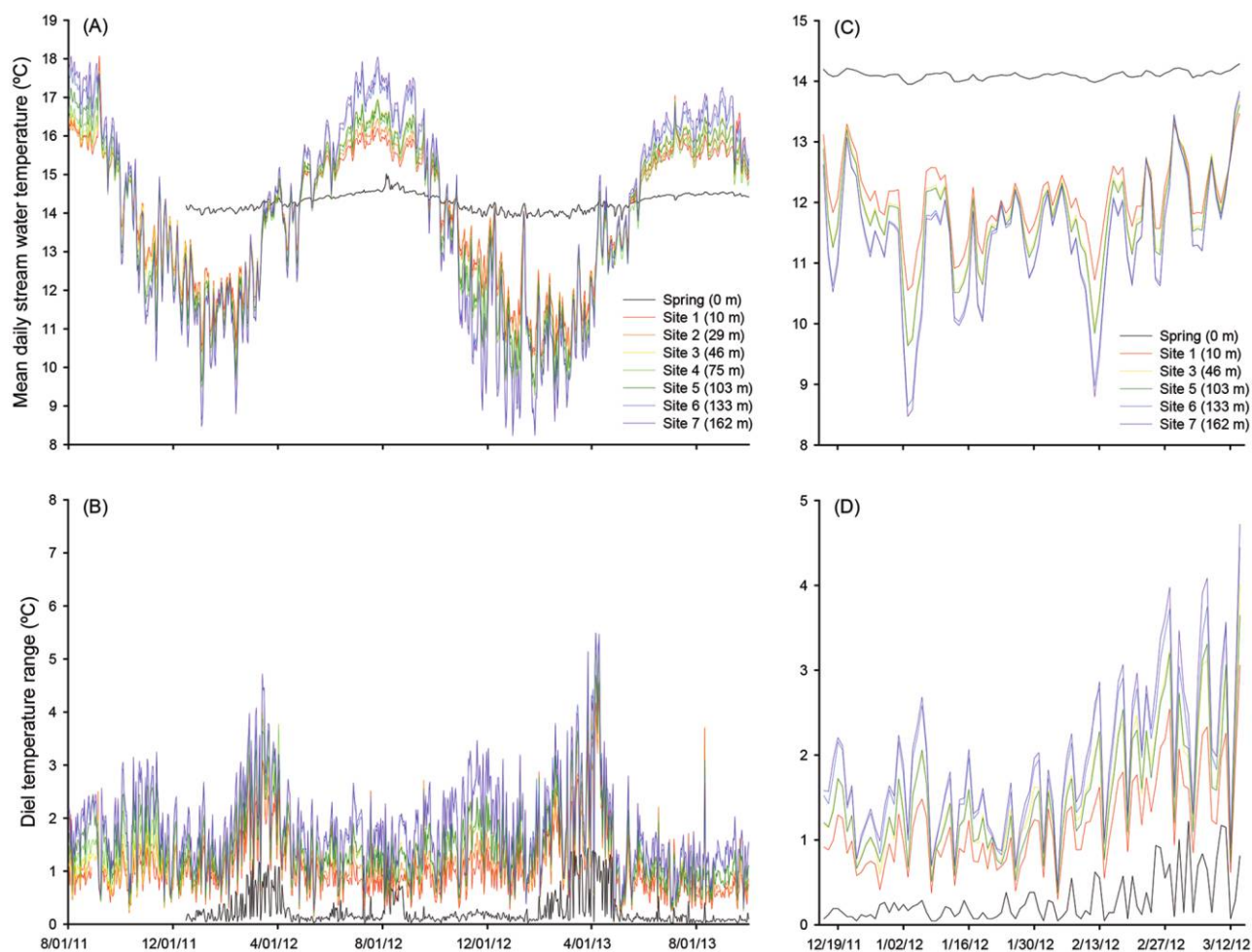


Figure 1. Mean daily streamwater temperature (A, C) and diel temperature range (B, D) of the upstream (S3) spring and 7 sites where cotton strips (A, C) and 5 sites where leaf litter bags (B, D) were deployed along the downstream temperature gradient. Site 1 is the most upstream site (closest to the largest [S3] spring feeding the West Fork of Walker Branch) and site 7 is the most downstream site. Distances (in m) are downstream from the S3 spring. Dates are formatted mm/dd/last 2 digits of the year.

correlation between streamwater temperature and tensile loss was positive but weaker ($r = 0.44$, $p < 0.0001$, $n = 154$). The slope of $\ln(\text{tensile loss}/d)$ vs the inverse of the product of absolute temperature and Boltzmann's con-

stant (per the MTE) was 1.41 eV (95% confidence interval [CI]: 1.07 to 1.74 eV, $r^2 = 0.31$, $p < 0.0001$, $n = 154$).

The seasonal variation in cotton-strip tensile loss (mean tensile loss across the 7 sites for each deployment) was

Table 1. Soluble reactive P (SRP), NH_4^+ -N, and NO_3^- -N concentrations on days 55 and 90 at 5 sites along the downstream temperature gradient in Walker Branch during the leaf-litter decomposition experiment. Site 1 is the most upstream site (closest to the largest [S3] spring feeding the West Fork of Walker Branch) and site 7 is the most downstream site. Distances (in m) are downstream from the S3 spring.

Site	SRP ($\mu\text{g P/L}$)		NH_4^+ -N ($\mu\text{g N/L}$)		NO_3^- -N ($\mu\text{g N/L}$)	
	Day 55	Day 90	Day 55	Day 90	Day 55	Day 90
1 (10 m)	2.2	3.2	<2.0	2.5	13.3	9.4
3 (46 m)	2.9	2.7	2.2	3.4	12.3	8.1
5 (103 m)	2.4	<2.0	2.5	3.5	10.8	7.1
6 (133 m)	<2.0	<2.0	2.0	4.6	9.7	6.4
7 (162 m)	2.3	2.7	2.6	5.9	10.4	6.3

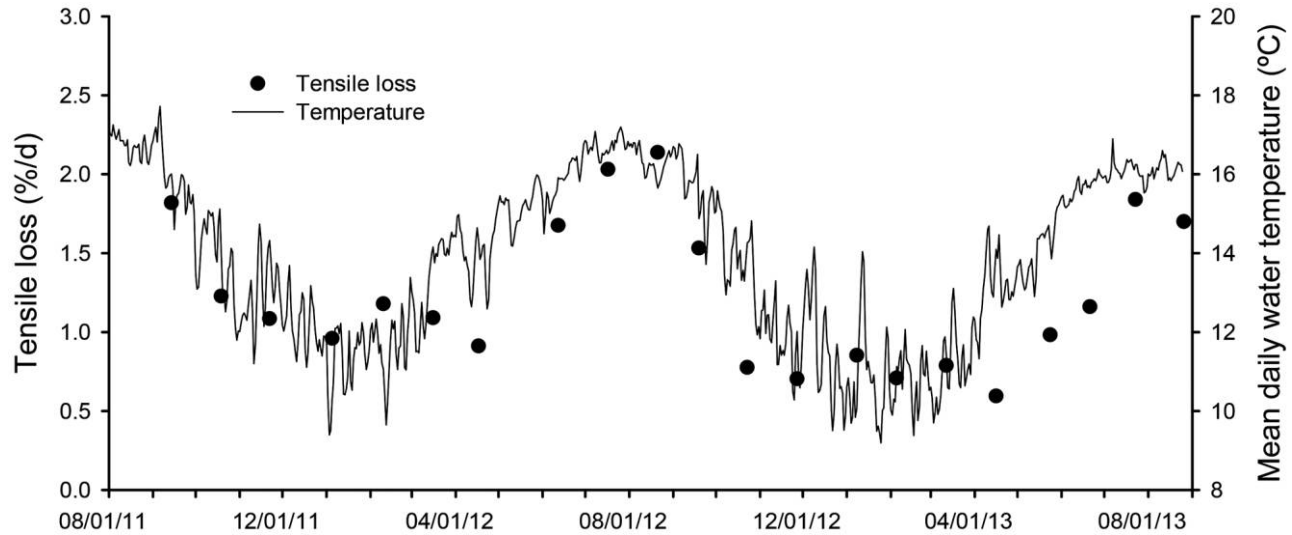


Figure 2. Mean ($n = 7$ sites/deployment period) tensile loss of cotton strips and mean daily stream water temperature over 2 y. Dates are the retrieval dates for each deployment and are formatted mm/dd/last 2 digits of the year.

correlated with mean daily water temperature ($r = 0.83$, $p < 0.0001$, $n = 22$), mean daily stream discharge ($r = -0.45$, $p = 0.04$, $n = 22$), and SRP ($r = 0.71$, $p = 0.0002$, $n = 22$) and NO_3^- -N concentrations ($r = 0.73$, $p = 0.0001$, $n = 22$). However, SRP, NO_3^- -N, and discharge were correlated with temperature (SRP: $r = 0.80$, $p < 0.0001$, $n = 22$, NO_3^- -N: $r = 0.67$, $p = 0.0006$, $n = 22$, discharge: $r = -0.61$, $p = 0.003$, $n = 22$). No significant correlations were found between tensile loss and NH_4^+ -N concentration or tensile loss and CV of mean daily temperature (all $p > 0.31$).

Cotton-strip decomposition: longitudinal variation

When tensile loss was examined along the downstream temperature gradient, the pattern between tensile loss and temperature was less clear potentially because the effect of the downstream temperature gradient varied at different temporal scales. On a daily time scale, diel temperature ranges were consistently larger at downstream than upstream sites, and a negative curvilinear relationship existed between tensile loss and mean diel temperature range ($r = 0.49$, $p < 0.0001$, $n = 154$; Fig. 3B), with a slower tensile loss corresponding to a larger daily range in temperature (i.e., at the downstream sites). The relationship was similar in shape when mean diel temperature range was corrected for mean streamwater temperature (mean diel temperature range/mean stream water temperature) during the deployment period ($r = 0.60$, $p < 0.0001$, $n = 154$).

On a seasonal time scale, tensile loss was faster at warmer upstream sites in winter (correlation between mean tensile loss and mean temperature in winter across the 7 sites: $r = 0.82$, $p = 0.02$, $n = 7$, Fig. 4A) but was not faster at the downstream sites when they were warmer in

summer ($r = -0.08$, $p = 0.87$, $n = 7$; Fig. 4C). Tensile loss was variable across all sites in spring when no strong downstream temperature gradient was present ($r = -0.12$, $p = 0.80$, $n = 7$; Fig. 4B). The temperature gradient in autumn was similar in magnitude to the gradient in winter, but tensile loss and mean temperature were not correlated in autumn ($r = 0.74$, $p = 0.06$, $n = 7$; Fig. 4D).

When all 22 deployments were examined together, tensile loss was faster at the warmer sites during 13 of 22 deployments and faster at the cooler sites during 9 of 22 deployments. The relationship of the difference between upstream and downstream tensile loss and the difference in temperature between the upstream and downstream sites ($r = 0.61$, $p = 0.002$, $n = 22$) suggests that when temperature was the same upstream and downstream, tensile loss was 0.30 %/d greater at the upstream site (i.e., intercept = -0.30 %/d; Fig. 5).

Variation in tensile loss was greater at the most downstream (CV = 0.62) than the most upstream site (CV = 0.31). CV of mean daily temperature and mean diel temperature range were positively correlated with CV of tensile loss across the 7 sites ($r = 0.76$, $p = 0.05$, $n = 7$, and $r = 0.78$, $p = 0.04$, $n = 7$, respectively). However, CV of mean daily stream temperature was strongly correlated with the mean diel temperature range at each site ($r = 0.99$, $p > 0.0001$, $n = 7$), so it was difficult to determine whether diel or seasonal variation in temperature at a given site increased variation in tensile loss. Neither mean daily temperature CV and tensile loss ($r = -0.45$, $p = 0.31$, $n = 7$) nor mean diel temperature range and tensile loss ($r = -0.48$, $p = 0.27$, $n = 7$) were correlated across the 7 sites. When individual 35-d-long deployments were analyzed, tensile loss and temperature were correlated ($p \leq 0.05$) on 5 of 22 deployments.

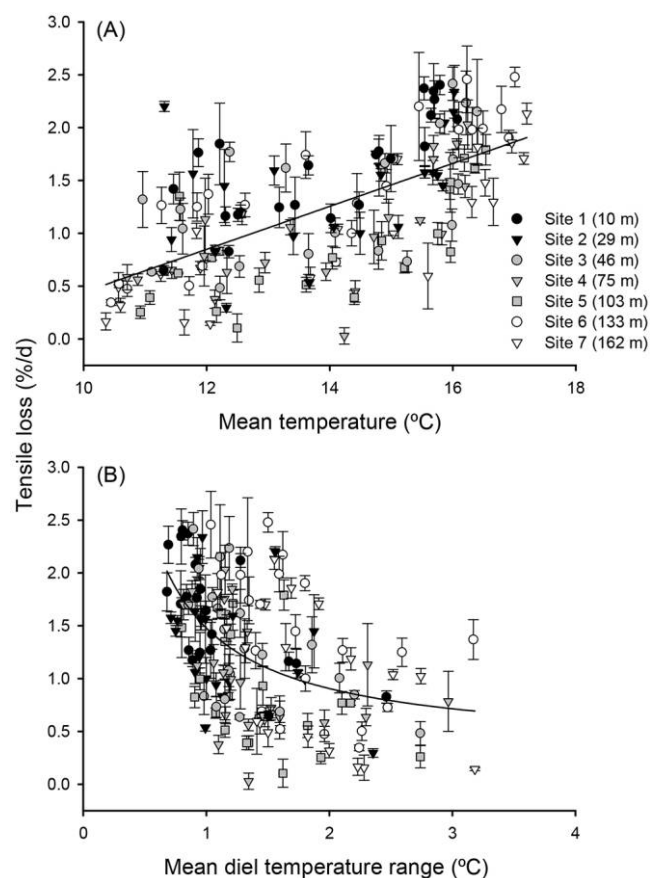


Figure 3. Scatterplots and trendlines for mean (± 1 SE) tensile loss of cotton strips vs mean stream water temperature ($r = 0.65$, $p < 0.0001$, $n = 154$) (A) and diel temperature range ($r = 0.49$, $p < 0.0001$, $n = 154$) (B) across all sites and deployment periods.

Leaf-litter decomposition: breakdown rates

Breakdown rates varied among litter types (ANCOVA, litter type \times time interaction, $F_{8,204} = 80.9$, $p < 0.0001$; Fig. 6). Red maple leaves broke down fastest (mean across sites: $k = 0.0359/\text{d}$), tulip poplar broke down at an intermediate rate ($k = 0.0259/\text{d}$), and white oak broke down slowest ($k = 0.0123/\text{d}$). Leaf-litter breakdown was faster at the warmer upstream sites than at the cooler downstream sites (ANCOVA, site \times time interaction, $F_{1,204} = 32.8$, $p < 0.0001$; Fig. 6), and the effect of site was consistent across litter types (ANCOVA, litter type \times site \times time interaction, $F_{2,204} = 2.8$, $p = 0.06$).

Leaf-litter decomposition: microbial respiration and invertebrates

Microbial respiration rates on litter showed no longitudinal pattern over time (rmANCOVA, site \times time interaction, $F_{3,108} = 1.2$, $p = 0.33$; Fig. 7A–C). Consistent with the patterns in breakdown rates observed among litter types, microbial respiration rate was highest on red maple and lowest on white oak over time (rmANCOVA,

litter type \times time interaction, $F_{6,108} = 2.7$, $p = 0.02$; Fig. 7A–C).

Total invertebrate density (all invertebrates including snails) increased over the decomposition period (range: 2.1–329.8 ind/g AFDM). Total invertebrate density was higher at the warmer upstream sites than the cooler downstream sites (rmANCOVA, site \times time interaction, $F_{3,108} = 4.9$, $p = 0.003$) and higher on red maple and tulip poplar than on white oak (rmANCOVA, litter type \times time interaction, $F_{6,108} = 12.1$, $p < 0.0001$) (Fig. 8A–C). The interaction between litter type and site over time was not significant (rmANCOVA, litter type \times site \times time interaction, $F_{6,108} = 1.0$, $p = 0.41$).

This temporal and spatial pattern in invertebrate density appeared to be driven in part by density of the snail *E. clavaeformis*. The density of *E. clavaeformis* was higher than that of all other invertebrates on the decomposing litter (range: 1.5–202.5 ind/g AFDM). Snail density differed among litter types (rmANCOVA, litter type \times time interaction, $F_{6,108} = 9.3$, $p < 0.0001$) and sites (rmANCOVA, site \times time interaction, $F_{3,108} = 5.4$, $p = 0.002$) through time in a way that mirrored the patterns observed for total invertebrate density. The interaction between litter type and site over time was not significant (rmANCOVA, litter type \times site \times time interaction, $F_{6,108} = 1.3$, $p = 0.28$).

The density of invertebrates other than snails was lower than the density of *E. clavaeformis* on litter (range: 0.2–127.3 ind/g AFDM) and nonsnail invertebrate density did not differ among sites over time (rmANCOVA, site \times time interaction, $F_{3,108} = 1.7$, $p = 0.18$). Nonsnail invertebrate density differed by litter type over the leaf-litter decomposition experiment (rmANCOVA, litter type \times time interaction, $F_{6,108} = 10.4$, $p < 0.0001$). Density was highest on red maple and lowest on white oak.

Leaf-litter decomposition: drivers

Leaf-litter breakdown rates (/d) and mean daily water temperature (red maple: $r = 0.78$, $p = 0.12$, tulip poplar: $r = 0.85$, $p = 0.07$, white oak: $r = 0.65$, $p = 0.24$, $n = 5$ for each correlation) or mean diel temperature range (red maple: $r = -0.71$, $p = 0.18$, tulip poplar: $r = -0.80$, $p = 0.11$, white oak: $r = -0.56$, $p = 0.33$, $n = 5$ for each correlation) were not correlated. Leaf-litter breakdown rates when expressed /dd and mean water temperature (red maple: $r = 0.75$, $p = 0.14$, tulip poplar: $r = 0.81$, $p = 0.10$, white oak: $r = 0.65$, $p = 0.23$, $n = 5$ for each correlation) also were not correlated. These results suggest that other factors affected breakdown rates along the downstream gradient. However, the low sample size ($n = 5$) may have precluded finding of a significant effect of temperature. Power analyses suggested that a sample size of 8 to 23 was needed to detect significant correlations in our study system (at $\alpha = 0.05$, power = 80%). Leaf-litter breakdown rates and CV of mean daily stream water tem-

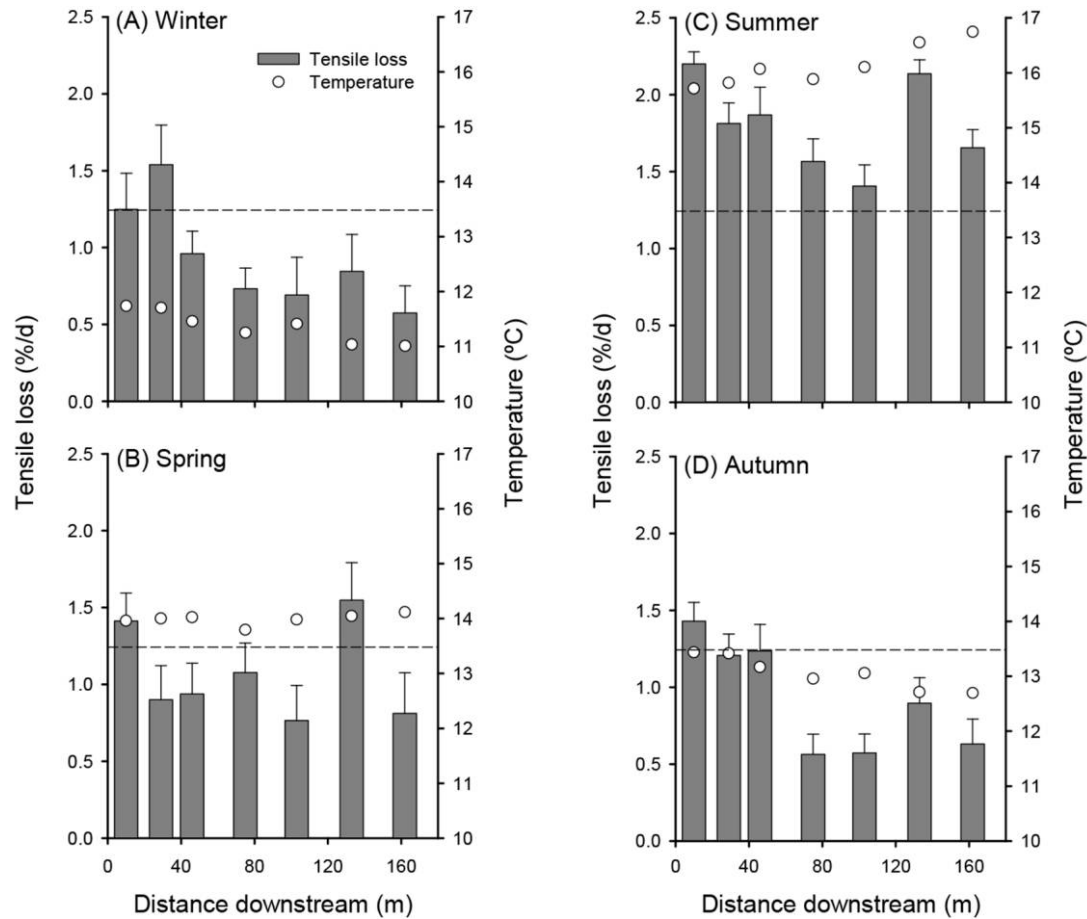


Figure 4. Mean (+1 SE) tensile loss of cotton strips and mean water temperature at 7 sites along the downstream temperature gradient (with distances downstream from the S3 spring) in winter ($n = 4$) (A), spring ($n = 5$) (B), summer ($n = 7$) (C), and autumn ($n = 6$) (D). Seasons were defined by day length (i.e., equinoxes and solstices). The dotted line represents the mean tensile loss rate (1.24%/d) across all sites and dates.

perature were not correlated (red maple: $r = -0.73$, $p = 0.16$, tulip poplar: $r = -0.80$, $p = 0.11$, white oak: $r = -0.60$, $p = 0.28$, $n = 5$ for each correlation).

Q_{10-q} values for red maple, tulip poplar, and white oak were 360, 1629, and 89, respectively. The 95% confidence intervals of the MTE slopes for red maple, tulip poplar, and white oak were large, and none of the MTE slopes were significantly different from 0 (red maple: 4.2 eV, 95% CI: -2.4 – 10.7 eV, $r^2 = 0.57$, $p = 0.14$; tulip poplar: 6.3 eV, 95% CI: -1.4 – 14.0 eV, $r^2 = 0.69$, $p = 0.08$; white oak: 3.3 eV, 95% CI: -3.9 – 10.5 eV, $r^2 = 0.42$, $p = 0.24$).

Breakdown rate and mean invertebrate density (all invertebrates including snails) were strongly correlated across all sites and litter types ($r = 0.97$, $p < 0.0001$, $n = 15$; Fig. 9). When litter types were considered separately, the relationship was significant for red maple and tulip poplar leaves (red maple: $r = 0.94$, $p = 0.02$, $n = 5$, tulip poplar: $r = 0.97$, $p = 0.007$, $n = 5$) and not significant for white oak leaves ($r = 0.79$, $p = 0.11$, $n = 5$). Mean invertebrate density was not correlated with mean streamwater temperature (red ma-

ple: $r = 0.86$, $p = 0.06$, tulip poplar: $r = 0.83$, $p = 0.08$, white oak: $r = 0.78$, $p = 0.12$, $n = 5$ for each correlation), CV of temperature (red maple: $r = -0.81$, $p = 0.10$, tulip poplar: $r = -0.80$, $p = 0.11$, white oak: $r = 0.74$, $p = 0.15$, $n = 5$ for each correlation), or mean diel temperature range (red maple: $r = -0.81$, $p = 0.10$, tulip poplar: $r = -0.82$, $p = 0.09$, white oak: $r = 0.67$, $p = 0.22$, $n = 5$ for each correlation).

DISCUSSION

Temperature is a fundamental driver of many stream ecosystem processes, but the effects of increased temperature on organic-matter decomposition are not straightforward. We found large variation in leaf-litter decomposition rates along a small thermal gradient in winter (0.6°C = mean temperature difference between upstream and downstream sites during the experiment), but temperature only partially explained this pattern. Invertebrates also were important because breakdown rates were posi-

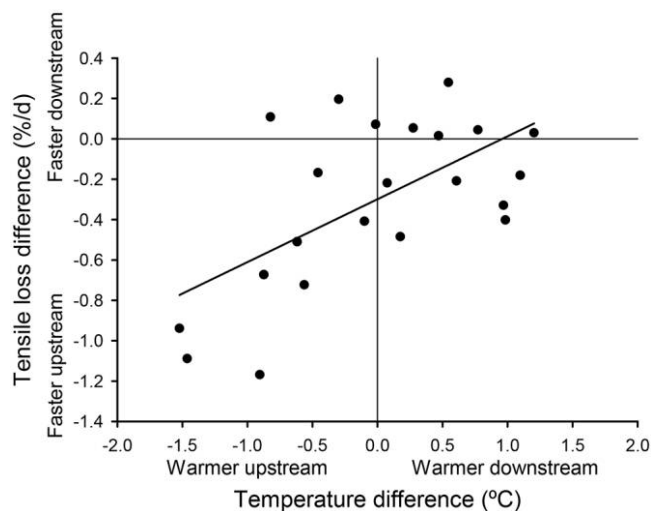


Figure 5. Scatterplot and trendline for the relationship of the difference in cotton strip tensile loss between the upstream and downstream sites vs the difference in temperature between the upstream and downstream sites. Difference in tensile loss and temperature was the difference between the mean tensile loss or temperature at the 2 most downstream sites (6 and 7) and the 2 most upstream sites (1 and 2) ($n = 22$ for the 22 deployments). Each data point on the figure represents one deployment and shows the difference in tensile loss or temperature between the upstream and downstream sites.

tively correlated with total invertebrate density. Leaf-litter types broke down as predicted, with faster decomposition of more-labile (red maple, tulip poplar) than less-labile (white oak) litter, but evidence of differential temperature sensitivity of decomposition among the 3 leaf-litter types was limited, probably because Q_{10-q} values and MTE slopes were calculated over a small temperature range. The rela-

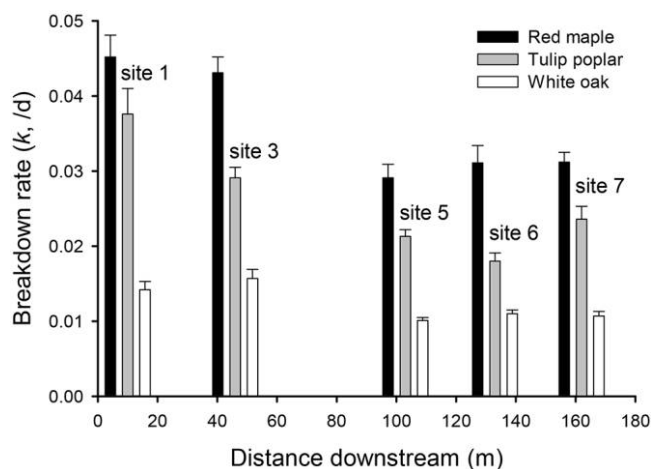


Figure 6. Mean (+1 SE) breakdown rate (k) of red maple, tulip poplar, and white oak at 5 sites along the downstream temperature gradient (with distances downstream [m] from the S3 spring) when the upstream sites were warmer than the downstream sites.

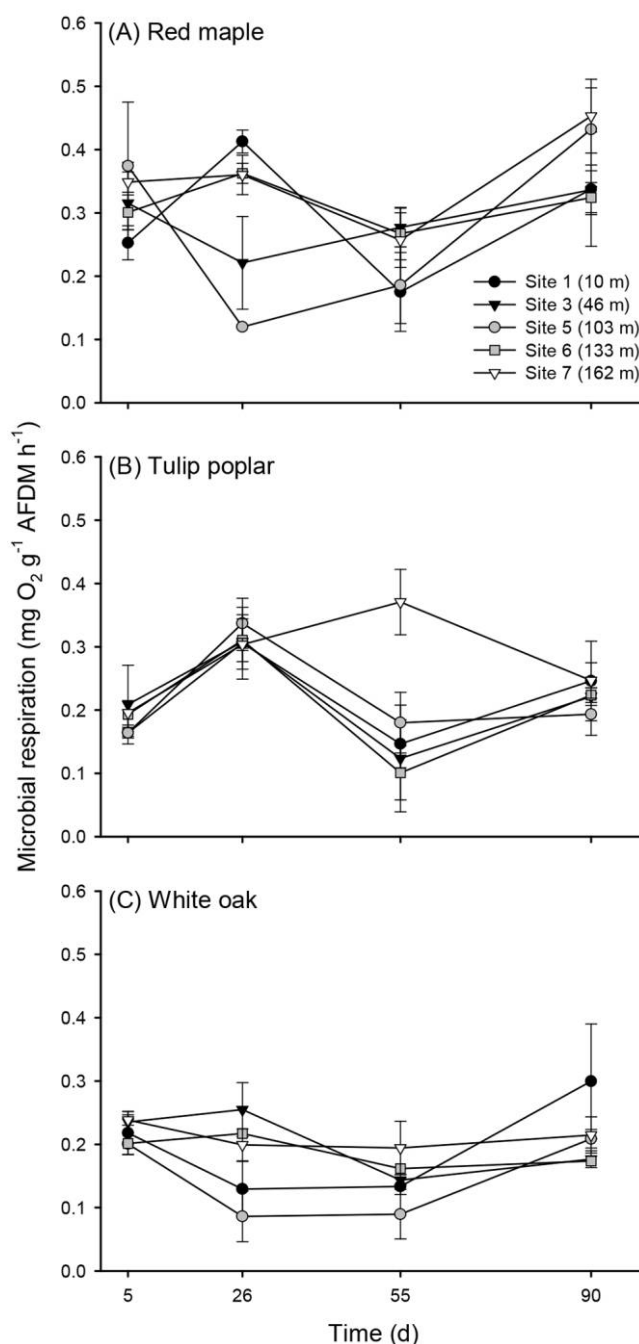


Figure 7. Mean (± 1 SE) microbial respiration on red maple (A), tulip poplar (B), and white oak (C) leaves incubated at 5 sites along the downstream temperature gradient over time. AFDM = ash-free dry mass.

tively short incubation times required for the cotton-strip assay allowed us to characterize the longer-term (i.e., 2 y) temporal dynamics of cellulose decay with high resolution, revealing patterns that tracked diel and seasonal fluctuations in temperature. These and other findings highlight the importance of assessing the effect of increased temperature on organic-matter decomposition in the context

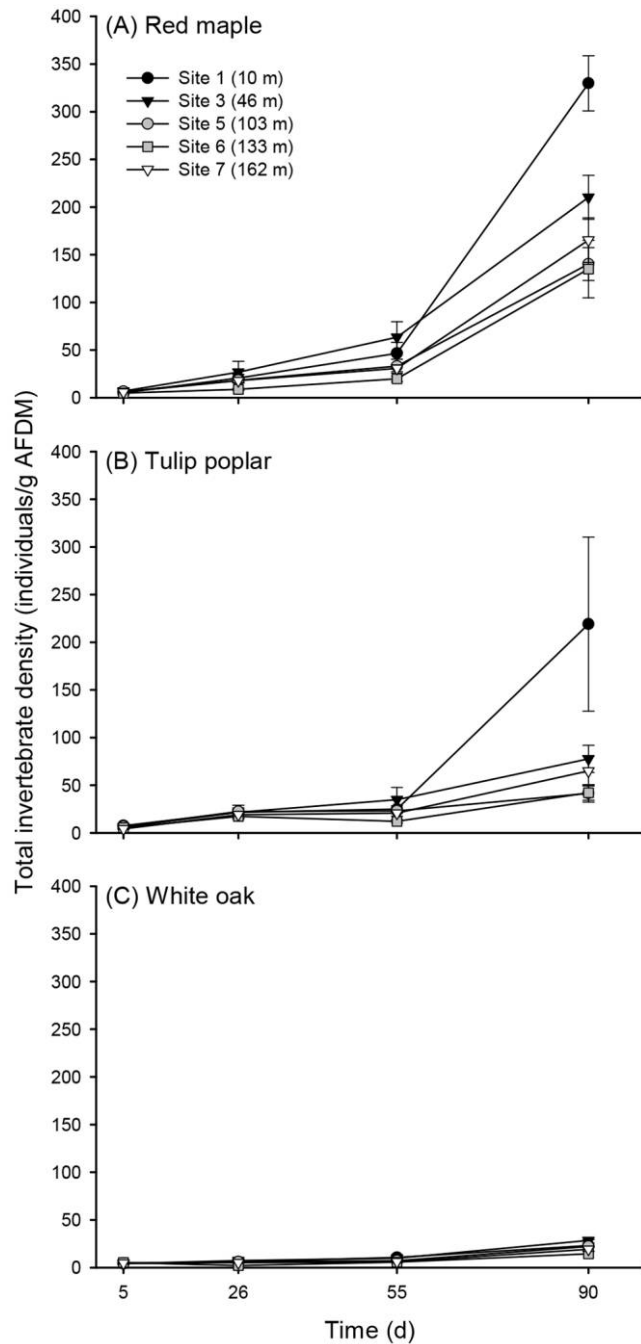


Figure 8. Mean (± 1 SE) total invertebrate density on red maple (A), tulip poplar (B), and white oak (C) leaves at 5 sites along the downstream temperature gradient over time. AFDM = ash-free dry mass.

of multiple extrinsic and intrinsic drivers and at multiple scales.

Cotton-strip decomposition

The response of cellulose decomposition to temperature varied depending on the scale being examined. A sea-

sonal pattern in tensile loss mirrored the seasonal changes in temperature, with faster tensile loss in summer when water temperature was warmer. Faster decomposition of cotton strips under warmer conditions is consistent with results from other studies focused on microbial decomposition of organic matter (Ferreira and Chauvet 2011a, b, Fernandes et al. 2012). However, additional factors appeared to increase cotton-strip decay to a higher rate at warmer temperatures than that predicted by the metabolism of microorganisms because our estimate of the activation energy (calculated from the MTE slope) was greater than expected for microbial decomposition (measured: 1.41 eV, 95% CI: 1.07–1.74 eV, predicted: 0.6–0.7 eV; Brown et al. 2004). One potential explanation for the faster decay rate is a synergistic effect of temperature and nutrient availability fueling microbial activity (Martinez et al. 2013) and, thus, cotton-strip breakdown. Previous investigators have shown that low nutrient concentrations in Walker Branch limit leaf-litter decomposition rates (Elwood et al. 1981, Grattan and Suberkropp 2001). Long-term measurements of streamwater nutrient concentrations in Walker Branch show a seasonal pattern, with higher NO_3^- -N and SRP concentrations in summer and lower concentrations in spring and autumn (Lutz et al. 2012). The higher nutrient concentrations in summer are caused by low whole-stream demand and uptake (Mulholland et al. 1985b, Roberts and Mulholland 2007). Thus, competition for nutrients may be lower during this time. Our examination of drivers of seasonality in cotton-strip tensile loss showed that temperature covaried with NO_3^- -N and SRP concentrations, so a combination of warmer temperatures and greater nutrient availability in summer may have accelerated cotton-strip decomposition or nutrients alone could have driven the seasonal patterns in cotton-strip ten-

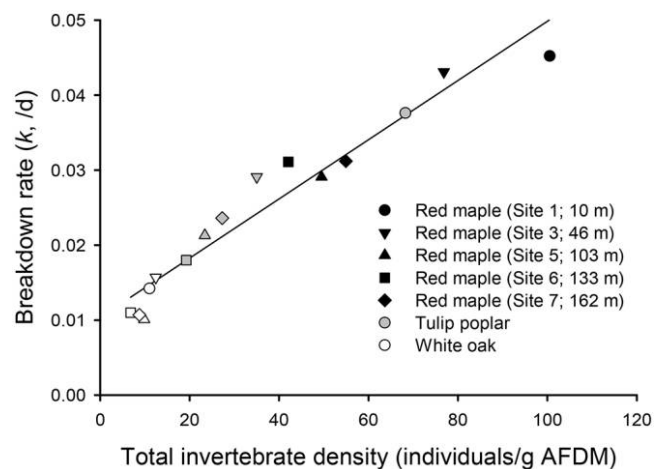


Figure 9. Scatterplot and trendline for leaf-litter breakdown rate vs total invertebrate density on litter across all sites (with distances downstream [in m] from the S3 spring) and litter types ($r = 0.97$, $p < 0.0001$, $n = 15$). AFDM = ash-free dry mass.

sile loss. Tensile loss also was negatively correlated with stream discharge, with higher discharge potentially decreasing biological activity on the cotton strips. Drivers of decomposition are difficult to separate in a natural stream that exhibits similar seasonal patterns in nutrients, discharge, and temperature. The accelerated cotton-strip breakdown rate compared with that predicted by the MTE also could have been a result of seasonality in feeding activity of snails or other invertebrates, but we did not measure these factors as part of the cotton-strip decomposition study.

The seasonal pattern in cotton-strip tensile loss appeared to be driven in part by temperature, but the pattern of cotton-strip tensile loss along the downstream temperature gradient was less apparent. When examined by season, tensile loss was faster in the warmer (upstream) sites in winter, but tensile loss was not faster in the warmer (downstream) sites in summer. Moreover, microbial respiration on leaf litter did not vary significantly in the downstream direction during the winter leaf-litter decomposition experiment, a result that does not correspond to the patterns of cotton-strip tensile loss in winter. This discrepancy may be a consequence of the different temporal scales that are captured by the microbial respiration and cotton-strip assays. Furthermore, significant relationships between cotton-strip tensile loss and temperature on only 5 of 22 deployment dates suggest that the microbial responses to the downstream temperature gradient were revealed only after multiple observations were analyzed together (i.e., examining tensile loss by season instead of during each deployment period).

Factors other than mean daily temperature probably influenced the downstream patterns in cotton-strip tensile loss because tensile loss was 0.3 %/d higher at the upstream than the downstream sites when temperature did not differ between the 2 locations. The smaller variation in temperature (at diel and seasonal time scales) at the upstream sites might have increased tensile loss if the microbial community was acclimated to the consistent temperature of the springs. Other investigators reported faster decomposition with greater temperature oscillations (Dang et al. 2009, Vyšná et al. 2014) because of a shift in fungal community structure (Dang et al. 2009). Diel temperature ranges in our study were smaller (mean = 1.0–1.8°C, maximum = 4.3–5.5°C) than in other studies carried out in stream microcosms (5–9°C; Dang et al. 2009) and in the Paddy River, Australia (1.5–18.1°C; Vyšná et al. 2014). The response of decomposition to small diel temperature swings has not been investigated, and whether the response of decomposition to diel temperature is linear is not known.

Nutrient concentrations probably played a role in the downstream decline in tensile-loss rate. However, our longitudinal nutrient measurements were limited to 2 sampling events during the leaf-litter decomposition study (Ta-

ble 1), so our conclusions regarding the importance of nutrient concentrations are based primarily on previously published data from this well-studied stream. In Walker Branch, NO_3^- -N and, to a lesser extent, SRP concentrations tend to decrease in the downstream direction throughout most of the year because of in-stream uptake along the reach (Mulholland and Rosemond 1992, Roberts and Mulholland 2007). Therefore, the consistently faster tensile-loss rate at the upstream sites may be a consequence of higher nutrient concentrations and availability to microorganisms. Together, the seasonal and longitudinal measurements of tensile loss suggest that microbial decomposition of cellulose was sensitive to both physical (temperature, discharge) and chemical (nutrient concentrations) variables across spatial and temporal scales in this forest stream.

Leaf-litter decomposition: litter chemistry

The cotton-strip decomposition experiment enabled us to examine the extrinsic factors influencing microbial decomposition of cellulose by means of a substrate of uniform quality. However, litter chemistry can strongly affect decomposition rates (Petersen and Cummins 1974, Webster and Benfield 1986, Irons et al. 1994). Therefore, the leaf-litter decomposition experiment was used to assess how extrinsic and intrinsic factors interacted to influence decomposition along a downstream temperature gradient.

Leaf-litter type was an important controlling factor of decomposition in Walker Branch. Breakdown rate, microbial respiration, and invertebrate density were all higher for more labile red maple and tulip poplar leaves than for less labile white oak leaves. Faster decay of more labile species is consistent with the findings of many studies of the role of litter chemistry in decomposition dynamics in streams (e.g., Irons et al. 1994, Ostrofsky 1997, LeRoy and Marks 2006, Ardón et al. 2009, Griffiths et al. 2012).

Rates of breakdown of these 3 litter types also varied along the downstream temperature gradient. All litter types broke down faster in the warmer upstream than the cooler downstream sites. However, breakdown rate and mean daily stream temperature were not correlated. Power analysis indicated that our sample size, which was limited by the number of available litter bags and temperature sensors, was low ($n = 5$ sites).

We were unable to measure the temperature sensitivity of the 3 litter types based on Q_{10-q} and MTE values because the large variation in MTE slopes (which did not differ significantly from 0) and large Q_{10-q} values (range: 89–1629; cf. values published by Conant et al. 2008: 3.3 [labile litter] and 4.9 [recalcitrant litter]) made inferences regarding the interaction between litter quality and temperature uncertain. Part of this uncertainty may stem from the small data set ($n = 5$ sites/litter type) and small temperature range over which Q_{10-q} values and MTE slopes were calculated.

Leaf-litter decomposition: invertebrates

Total invertebrate density on decomposing leaf litter was higher at the warmer upstream than the cooler downstream sites. Density of the dominant snail (*E. clavaeformis*) appears to have been the main source of this pattern because nonsnail invertebrate density did not differ significantly along the downstream gradient. We did not identify invertebrates other than snails, so we do not know whether density of a given species, functional feeding groups, or invertebrate community structure changed along the downstream temperature gradient.

Snails may play an important role in leaf-litter decomposition by fragmenting leaf litter and altering litter-associated microbial communities (Schaller 2013). In Walker Branch, *E. clavaeformis* skeletonize leaves, increase leaf fragmentation, stimulate microbial activity on litter, and increase coarse particulate organic matter export (Mulholland et al. 1985a). Thus, higher density of *E. clavaeformis* on litter at the warmer upstream sites suggests that these snails may have accelerated decomposition at those sites. The higher density of *E. clavaeformis* upstream might be a consequence of the more stable temperature regime at the upstream sites, but our data do not enable us to distinguish whether this density difference is a consistent spatial trend for Walker Branch. Other factors, such as resource availability and habitat structure, can influence invertebrate density, but these factors did not appear to change appreciably along the 152-m study reach during the winter decomposition experiment (NAG, personal observation).

Summary

Temperature, nutrients, discharge, and invertebrates all varied through time or space and influenced cellulose and leaf-litter decomposition in Walker Branch. Our study was conducted along a natural temperature gradient in one stream. Thus, we cannot isolate the effect of temperature or extrapolate findings to other stream ecosystems. However, even within a short reach (152 m), decomposition rates varied widely, suggesting that interactions with additional intrinsic and extrinsic drivers need to be considered at multiple scales when examining the effects of temperature on decomposition.

Ecological heterogeneity is a key factor in many conceptual models in stream ecology, including the Flood Pulse Concept (Junk et al. 1989) and the River Continuum Concept (Vannote et al. 1980). Heterogeneity has been described for physical habitats, community assemblages, and stocks of basal resources, such as biofilms and leaf litter (e.g., Downes et al. 1993), but less is known about the spatial heterogeneity of ecosystem-level processes. Tiegs et al. (2009) evaluated organic-matter decomposition among sites within a short stream reach and observed highly consistent decomposition rates, whereas we showed that organic-

matter decomposition varied considerably along a short distance of a single stream reach and that this variation tracked relatively modest changes in environmental conditions. These results add to a growing body of knowledge regarding how stream ecosystem processes vary across relatively small spatial scales (e.g., Langhans et al. 2008, Hoellein et al. 2009, Flores et al. 2013). Characterizing heterogeneity in ecosystem processes at small spatial scales will aid in scaling functional metrics to stream reach and watershed scales and will help evaluate the effect of climate change on stream ecosystem processes.

ACKNOWLEDGEMENTS

We thank D. Brice, M. Burchi, K. McCracken, and J. Smith for technical assistance. Comments provided by J. Smith, W. Hill, J. Follstad-Shah, B. Taylor, anonymous referees, and Associate Editors B. McKie and E. Chauvet greatly improved earlier versions of this manuscript. This research was part of the long-term Walker Branch Watershed project and supported by the US Department of Energy's Office of Science, Biological and Environmental Research. Oak Ridge National Laboratory (ORNL) is managed by UT-Battelle, LLC, for the US Department of Energy under contract DE-AC05-00OR22725. NAG was partially supported through the ORNL Postdoctoral Research Associates Program administered by Oak Ridge Associated Universities, and SDT was supported by an Oakland University URC Faculty Research Fellowship Award.

LITERATURE CITED

- Acuña, V., A. Wolf, U. Uehlinger, and K. Tockner. 2008. Temperature dependence of stream benthic respiration in an Alpine river network under global warming. *Freshwater Biology* 53:2076–2088.
- APHA (American Public Health Association). 2005. Standard methods for the examination of water and wastewater. 21st edition. American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, DC.
- Ardón, M., C. M. Pringle, and S. L. Eggert. 2009. Does leaf chemistry differentially affect breakdown in tropical vs temperate streams? Importance of standardized analytical techniques to measure leaf chemistry. *Journal of the North American Benthological Society* 28:440–453.
- Axelsson, E. P., J. Hjältén, C. J. LeRoy, R. Julkunen-Tiitto, A. Wennström, and G. Pilate. 2010. Can leaf litter from genetically modified trees affect aquatic ecosystems? *Ecosystems* 13:1049–1059.
- Benfield, E. F. 2006. Decomposition of leaf material. Pages 771–720 in F. R. Hauer and G. A. Lamberti (editors). *Methods in stream ecology*. 2nd edition. Elsevier, New York.
- Boyer, L., R. G. Pearson, D. Dudgeon, V. Ferreira, M. A. S. Graça, M. O. Gessner, A. J. Boulton, E. Chauvet, C. M. Yule, R. J. Albariño, A. Ramírez, J. E. Helson, M. Callisto, M. Arunachalam, J. Chará, R. Figueroa, J. M. Mathooko, J. F. Gonçalves, M. S. Moretti, A. M. Chará-Serna, J. N. Davies, A. Encalada, S. Lamothe, L. M. Buria, J. Castela, A. Cornejo, A. O. Y. Li, C. M'Erumba, V. D. Villanueva, M. del Carmen

- Zúñiga, C. M. Swan, and L. A. Barmuta. 2011a. Global patterns of stream detritivore distribution: implications for biodiversity loss in changing climates. *Global Ecology and Biogeography* 21:134–141.
- Boyero, L., R. G. Pearson, M. O. Gessner, L. A. Barmuta, V. Ferreira, M. A. S. Graça, D. Dudgeon, A. J. Boulton, M. Callisto, E. Chauvet, J. E. Helson, A. Bruder, R. J. Albariño, C. M. Yule, M. Arunachalam, J. N. Davies, R. Figueroa, A. S. Flecker, A. Ramírez, R. G. Death, T. Iwata, J. M. Mathooko, C. Mathuriau, J. F. Gonçalves, M. S. Moretti, T. Jinggut, S. Lamothe, C. M'Erimba, L. Ratnarajah, M. H. Schindler, J. Castela, L. M. Buria, A. Cornejo, V. D. Villanueva, and D. C. West. 2011b. A global experiment suggests climate warming will not accelerate litter decomposition in streams but might reduce carbon sequestration. *Ecology Letters* 14:289–294.
- 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.
- Buzby, K. M., and S. A. Perry. 2000. Modeling the potential effects of climate change on leaf pack processing in central Appalachian streams. *Canadian Journal of Fisheries and Aquatic Sciences* 57:1773–1783.
- Cheshire, K., L. Boyero, and R. G. Pearson. 2005. Food webs in tropical Australian streams: shredders are not scarce. *Freshwater Biology* 50:748–769.
- Clapcott, J. E., and L. A. Barmuta. 2010. Forest clearance increases metabolism and organic matter processes in small headwater streams. *Journal of the North American Benthological Society* 29:546–561.
- Comiskey, C. E. 1978. Aspects of the organic carbon cycle on Walker Branch Watershed: a study of land/water interaction. PhD Dissertation, University of Tennessee, Knoxville, Tennessee.
- Conant, R. T., R. A. Drijber, M. L. Haddix, W. J. Parton, E. A. Paul, A. F. Plante, J. Six, and J. M. Steinweg. 2008. Sensitivity of organic matter decomposition to warming varies with its quality. *Global Change Biology* 14:868–877.
- Crowl, T. A., W. H. McDowell, A. P. Covich, and S. L. Johnson. 2001. Freshwater shrimp effects on detrital processing and nutrients in a tropical headwater stream. *Ecology* 82:775–783.
- Cummins, K. W., and M. J. Klug. 1979. Feeding ecology of stream invertebrates. *Annual Review of Ecology and Systematics* 10:147–172.
- Curlin, J. W., and D. J. Nelson. 1968. Walker Branch Watershed project: objectives, facilities, and ecological characteristics. ORNL-TM-2271. Oak Ridge National Laboratory, Oak Ridge, Tennessee.
- 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.
- D'Angelo, D. J., J. R. Webster, and E. F. Benfield. 1991. Mechanisms of stream phosphorus retention: an experimental study. *Journal of the North American Benthological Society* 10:225–237.
- Dobson, M., A. Magana, J. M. Mathooko, and F. K. Ndegwa. 2002. Detritivores in Kenyan highland streams: more evidence for the paucity of shredders in the tropics? *Freshwater Biology* 47:909–919.
- Donaldson, J. R., M. T. Stevens, H. R. Barnhill, and R. L. Lindroth. 2006. Age-related shifts in leaf chemistry of clonal aspen (*Populus tremuloides*). *Journal of Chemical Ecology* 32:1415–1429.
- Downes, B. J., P. S. Lake, and E. S. G. Schreiber. 1993. Spatial variation in the distribution of invertebrates: implications of patchiness for models of community organization. *Freshwater Biology* 30:119–132.
- Elwood, J. W., J. D. Newbold, A. F. Trimble, and R. W. Stark. 1981. The limiting role of phosphorus in a woodland stream ecosystem: effects of P enrichment on leaf decomposition and primary producers. *Ecology* 62:146–158.
- Fernandes, I., C. Pascoal, H. Guimarães, R. Pinto, I. Sousa, and F. Cássio. 2012. High temperature reduces the effects of litter quality on decomposition by aquatic fungi. *Freshwater Biology* 57:2306–2317.
- 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. 2011a. Future increase in temperature more than decrease in litter quality can affect microbial litter decomposition in streams. *Oecologia (Berlin)* 167:279–291.
- Ferreira, V., and E. Chauvet. 2011b. Synergistic effects of water temperature and dissolved nutrients on litter decomposition and associated fungi. *Global Change Biology* 17:551–564.
- Fierer, N., J. M. Craine, K. McLauchlan, and J. P. Schimel. 2005. Litter quality and the temperature sensitivity of decomposition. *Ecology* 86:320–326.
- Flores, L., J. Ramón Díez, A. Larrañaga, C. Pascoal, and A. Elosegi. 2013. Effects of retention site on breakdown of organic matter in a mountain stream. *Freshwater Biology* 58:1267–1278.
- Friberg, N., J. B. Dybkjær, J. S. Olafsson, G. Mar Gislason, S. E. Larsen, and R. L. Lauridsen. 2009. Relationships between structure and function in streams contrasting in temperature. *Freshwater Biology* 54:2051–2068.
- Genereux, D. P., H. F. Hemond, and P. J. Mulholland. 1993. Spatial and temporal variability in streamflow generation on the West Fork of Walker Branch Watershed. *Journal of Hydrology* 142:137–166.
- Gonçalves, A. L., M. A. S. Graça, and C. Canhoto. 2013. The effect of temperature on leaf decomposition and diversity of associated aquatic hyphomycetes depends on the substrate. *Fungal Ecology* 6:546–553.
- González, J. M., and M. A. S. Graça. 2003. Conversion of leaf litter to secondary production by a shredding caddis-fly. *Freshwater Biology* 48:1578–1592.
- Graça, M. A. S. 2001. The role of invertebrates on leaf litter decomposition in streams: a review. *International Review of Hydrobiology* 86:383–393.
- Graça, M. A. S., R. C. F. Ferreira, and C. N. Coimbra. 2001. Litter processing along a stream gradient: the role of invertebrates and decomposers. *Journal of the North American Benthological Society* 20:408–420.
- Grattan, R. M., and K. Suberkropp. 2001. Effects on yellow poplar leaf decomposition and fungal activity in streams. *Journal of the North American Benthological Society* 20:33–43.

- Griffiths, N. A., J. L. Tank, S. S. Roley, and M. L. Stephen. 2012. Decomposition of maize leaves and grasses in restored agricultural streams. *Freshwater Science* 31:848–864.
- Griffiths, N. A., and S. D. Tiegs. 2015. Walker Branch Watershed: temperature response of organic-matter decomposition in a headwater stream. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, Tennessee, U.S.A. (Available from: <http://dx.doi.org/10.3334/CDIAC/ornlfa.003>)
- Harrison, A. F., P. M. Latter, and D. W. H. Walton. 1988. Cotton strip assay: an index of decomposition in soils. Institute of Terrestrial Ecology, Cumbria, UK.
- Hoellein, T. J., J. L. Tank, E. J. Rosi-Marshall, and S. A. Entrekin. 2009. Temporal variation in substratum-specific rates of N uptake and metabolism and their contribution at the stream-reach scale. *Journal of the North American Benthological Society* 28:305–318.
- Irons, J. G., M. W. Oswood, R. J. Stout, and C. M. Pringle. 1994. Latitudinal patterns in leaf litter breakdown: is temperature really important? *Freshwater Biology* 32:401–411.
- Johnson, D. W. 1989. Site description. Pages 6–20 in D. W. Johnson and R. I. Van Hook (editors). *Analysis of biogeochemical cycling processes in Walker Branch Watershed*. Springer-Verlag, New York.
- Junk, W. J., P. B. Bayley, and R. E. Sparks. 1989. The flood pulse concept in river–floodplain systems. *Canadian Special Publication of Fisheries and Aquatic Sciences* 106:110–127.
- Kaushal, S. S., G. E. Likens, N. A. Jaworski, M. L. Pace, A. M. Sides, D. Seekell, K. T. Belt, D. H. Secor, and R. L. Wingate. 2010. Rising stream and river temperatures in the United States. *Frontiers in Ecology and the Environment* 8:461–466.
- Kominoski, J. S., C. M. Pringle, and B. A. Ball. 2008. Invasive woolly adelgid appears to drive seasonal hemlock and carcass inputs to a detritus-based stream. *Verhandlungen der Internationalen Vereinigung für theoretische und angewandte Limnologie* 30:109–112.
- Langhans, S. D., S. D. Tiegs, M. O. Gessner, and K. Tockner. 2008. Leaf-decomposition heterogeneity across a riverine floodplain mosaic. *Aquatic Sciences* 70:337–346.
- Latter, P. M., and G. Howson. 1977. The use of cotton strips to indicate cellulose decomposition in the field. *Pedobiologia* 17:145–155.
- LeBlanc, R. T., R. D. Brown, and J. E. FitzGibbon. 1997. Modeling the effects of land use change on the water temperature in unregulated urban streams. *Journal of Environmental Management* 49:445–469.
- LeRoy, C. J., and J. C. Marks. 2006. Litter quality, stream characteristics and litter diversity influence decomposition rates and macroinvertebrates. *Freshwater Biology* 51:605–617.
- LeRoy, C. J., T. G. Whitham, S. C. Wooley, and J. C. Marks. 2007. Within-species variation in foliar chemistry influences leaf-litter decomposition in a Utah river. *Journal of the North American Benthological Society* 26:426–438.
- Lutz, B. D., P. J. Mulholland, and E. S. Bernhardt. 2012. Long-term data reveal patterns and controls on stream water chemistry in a forested stream: Walker Branch, Tennessee. *Ecological Monographs* 82:367–387.
- Martínez, A., A. Larrañaga, J. Pérez, E. Descals, and J. Pozo. 2013. Temperature affects leaf litter decomposition in low-order forest streams: field and microcosm approaches. *FEMS Microbiology Ecology* 87:257–267.
- 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.
- Mulholland, P. J. 1997. Organic matter dynamics in the West Fork of Walker Branch, Tennessee, USA. *Journal of the North American Benthological Society* 16:61–67.
- Mulholland, P. J. 2004. The importance of in-stream uptake for regulating stream concentrations and outputs of N and P from a forested watershed: evidence from long-term chemistry records for Walker Branch Watershed. *Biogeochemistry* 70:403–426.
- Mulholland, P. J., J. W. Elwood, J. D. Newbold, and L. A. Ferren. 1985a. Effect of a leaf-shredding invertebrate on organic matter dynamics and phosphorus spiralling in heterotrophic laboratory streams. *Oecologia (Berlin)* 66:199–206.
- Mulholland, P. J., J. D. Newbold, J. W. Elwood, L. A. Ferren, and J. R. Webster. 1985b. Phosphorus spiralling in a woodland stream: seasonal variations. *Ecology* 66:1012–1023.
- Mulholland, P. J., and A. D. Rosemond. 1992. Periphyton response to longitudinal nutrient depletion in a woodland stream: evidence of upstream–downstream linkage. *Journal of the North American Benthological Society* 11:405–419.
- Newbold, J. D., J. W. Elwood, R. V. O'Neill, and A. L. Sheldon. 1983. Phosphorus dynamics in a woodland stream ecosystem: a study of nutrient spiralling. *Ecology* 64:1249–1265.
- Nolen, J. A., and R. G. Pearson. 1993. Factors affecting litter processing by *Anisocentropus kirramus* (Trichoptera: Calamoceratidae) from an Australian tropical rainforest stream. *Freshwater Biology* 29:469–479.
- Ostrofsky, M. L. 1997. Relationship between chemical characteristics of autumn-shed leaves and aquatic processing rates. *Journal of the North American Benthological Society* 16:750–759.
- Petersen, R. C., and K. W. Cummins. 1974. Leaf processing in a woodland stream. *Freshwater Biology* 4:343–368.
- Poole, G. C., and C. H. Berman. 2001. An ecological perspective on in-stream temperature: natural heat dynamics and mechanisms of human-caused thermal degradation. *Environmental Management* 27:787–802.
- Pozo, J., J. Casas, M. Menendez, S. Mollá, I. Arostegui, A. Baraguren, C. Casado, E. Descals, J. García-Avilés, J. M. González, A. Larrañaga, E. López, M. Lusi, O. Moya, J. Pérez, T. Riera, N. Roblas, and M. J. Salinas. 2011. Leaf-litter decomposition in headwater streams: a comparison of the process among four climatic regions. *Journal of the North American Benthological Society* 30:935–950.
- Rasmussen, J. J., A. Baatrup-Pedersen, T. Riis, and N. Friberg. 2011. Stream ecosystem properties and processes along a temperature gradient. *Aquatic Ecology* 45:231–242.
- Roberts, B. J., and P. J. Mulholland. 2007. In-stream biotic control on nutrient biogeochemistry in a forested stream, West Fork of Walker Branch. *Journal of Geophysical Research* 112:G04002.
- Rosemond, A. D., P. J. Mulholland, and J. W. Elwood. 1993. Top-down and bottom-up control of stream periphyton: effects of nutrients and herbivores. *Ecology* 74:1264–1280.
- Sand-Jensen, K., N. L. Pedersen, and M. Søndergaard. 2007. Bacterial metabolism in small temperate streams under con-

- temporary and future climates. *Freshwater Biology* 52:2340–2353.
- Schaller, J. 2013. Invertebrate grazers are a crucial factor for grass litter mass loss and nutrient mobilization during aquatic decomposition. *Fundamental and Applied Limnology* 183:287–295.
- Sinsabaugh, R. L. 1997. Large-scale trends for stream benthic respiration. *Journal of the North American Benthological Society* 16:119–122.
- Slocum, M. G., J. Roberts, and I. A. Mendelssohn. 2009. Artist canvas as a new standard for the cotton-strip assay. *Journal of Plant Nutrition and Soil Science* 172:71–74.
- Taylor, B. R., and I. V. Andrushchenko. 2014. Interaction of water temperature and shredders on leaf litter breakdown: a comparison of streams in Canada and Norway. *Hydrobiologia* 721:77–88.
- Taylor, B. R., and E. E. Chauvet. 2014. Relative influence of shredders and fungi on leaf litter decomposition along a river altitudinal gradient. *Hydrobiologia* 721:239–250.
- Taylor, B. R., and A. N. Dykstra. 2005. Effects of hot ground water on a small swamp-stream in Nova Scotia, Canada. *Hydrobiologia* 545:129–144.
- Tiegs, S. D., P. O. Akinwale, and M. O. Gessner. 2009. Litter decomposition across multiple spatial scales in stream networks. *Oecologia (Berlin)* 161:343–351.
- Tiegs, S. D., J. E. Clapcott, N. A. Griffiths, and A. J. Boulton. 2013. A standardized cotton-strip assay for measuring organic-matter decomposition in streams. *Ecological Indicators* 32:131–139.
- Tiegs, S. D., S. D. Langhans, K. Tockner, and M. O. Gessner. 2007. Cotton strips as a leaf surrogate to measure decomposition in river floodplain habitats. *Journal of the North American Benthological Society* 26:70–77.
- Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell, and C. E. Cushing. 1980. The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences* 37:130–137.
- Vyšná, V., F. Dyer, W. Maher, and R. Norris. 2014. Cotton-strip decomposition rate as a river condition indicator: diel temperature range and deployment season and length also matter. *Ecological Indicators* 45:508–521.
- Webster, J. R., and E. F. Benfield. 1986. Vascular plant breakdown in freshwater ecosystems. *Annual Review of Ecology and Systematics* 17:567–594.
- Yvon-Durocher, G., J. M. Caffrey, A. Cescatti, M. Dossena, P. del Giorgio, J. M. Gasol, J. M. Montoya, J. Pumpanen, P. A. Staehr, M. Trimmer, G. Woodward, and A. P. Allen. 2012. Reconciling the temperature dependence of respiration across timescales and ecosystem types. *Nature* 487: 472–476.