

Nutrients and temperature additively increase stream microbial respiration

David W. P. Manning¹  | Amy D. Rosemond¹  | Vladislav Gulis² |
Jonathan P. Benstead³  | John S. Kominoski¹ 

¹Odum School of Ecology, University of Georgia, Athens, GA, USA

²Department of Biology, Coastal Carolina University, Conway, SC, USA

³Department of Biological Sciences, University of Alabama, Tuscaloosa, AL, USA

Correspondence

David W. P. Manning, School of Environment and Natural Resources, The Ohio State University, Columbus, OH, USA.
Email: manning.413@osu.edu

Present address

John S. Kominoski, Department of Biological Sciences, Florida International University, Miami, FL, USA.

Funding information

Division of Environmental Biology, Grant/Award Number: DEB-0918894, DEB-0918904, DEB-0919054; NSF, Grant/Award Number: DEB-0823293

Abstract

Rising temperatures and nutrient enrichment are co-occurring global-change drivers that stimulate microbial respiration of detrital carbon, but nutrient effects on the temperature dependence of respiration in aquatic ecosystems remain uncertain. We measured respiration rates associated with leaf litter, wood, and fine benthic organic matter (FBOM) across seasonal temperature gradients before (PRE) and after (ENR1, ENR2) experimental nutrient (nitrogen [N] and phosphorus [P]) additions to five forest streams. Nitrogen and phosphorus were added at different N:P ratios using increasing concentrations of N (~80–650 µg/L) and corresponding decreasing concentrations of P (~90–11 µg/L). We assessed the temperature dependence, and microbial (i.e., fungal) drivers of detrital mass-specific respiration rates using the metabolic theory of ecology, before vs. after nutrient enrichment, and across N and P concentrations. Detrital mass-specific respiration rates increased with temperature, exhibiting comparable activation energies (E , electronvolts [eV]) for all substrates (FBOM $E = 0.43$ [95% CI = 0.18–0.69] eV, leaf litter $E = 0.30$ [95% CI = 0.072–0.54] eV, wood $E = 0.41$ [95% CI = 0.18–0.64] eV) close to predicted MTE values. There was evidence that temperature-driven increased respiration occurred via increased fungal biomass (wood) or increased fungal biomass-specific respiration (leaf litter). Respiration rates increased under nutrient-enriched conditions on leaves (1.32×) and wood (1.38×), but not FBOM. Respiration rates responded weakly to gradients in N or P concentrations, except for positive effects of P on wood respiration. The temperature dependence of respiration was comparable among years and across N or P concentration for all substrates. Responses of leaf litter and wood respiration to temperature and the combined effects of N and P were similar in magnitude. Our data suggest that the temperature dependence of stream microbial respiration is unchanged by nutrient enrichment, and that increased temperature and N + P availability have additive and comparable effects on microbial respiration rates.

KEYWORDS

benthic organic matter, metabolic theory of ecology, microbial activity, nitrogen, phosphorus, rivers, temperature dependence

1 | INTRODUCTION

Temperature and nutrient availability are two key drivers of energy and material processing in ecosystems, and both are increasing worldwide (IPCC, 2013; Peñuelas, Sardans, Rivas-Ubach, & Janssens, 2012). In aquatic ecosystems, water temperatures are predicted to track rising air temperatures driven by climate change, in addition to the effects of modified thermal regimes resulting from changes in land use, such as urbanization and deforestation (Ferreira, Gulis, Pascoal, & Graça, 2014; Kaushal et al., 2010). Along with higher temperatures, nutrient availability in aquatic ecosystems has increased as a result of widespread anthropogenic nutrient inputs (Alexander & Smith, 2006; Woodward et al., 2012). However, these two important global-change drivers of ecosystem processes traditionally have been studied separately, with much less emphasis given to their combined effects. Thus, predicting how nutrients and temperature will interact to affect ecosystem functions is both critical and largely unresolved (Canhoto, Gonçalves, & Bärlocher, 2016; Cross, Hood, Benstead, Huryn, & Nelson, 2015).

Processing of terrestrial net primary production (NPP) in streams and rivers is increasingly recognized as a significant component of the global carbon (C) cycle, particularly in regard to the proportion of NPP that evades as CO₂ via respiration (Butman & Raymond, 2011; Cole et al., 2007; Hotchkiss et al., 2015). Processing of particulate organic matter such as leaf litter, wood, and fine particles (hereafter detritus) is a predominant energy pathway in streams and rivers, where fungal rather than bacterial decomposers control initial processing and respiration of coarse detrital inputs (Findlay et al., 2002; Gessner, Gulis, Kuehn, Chauvet, & Suberkropp, 2007; Suberkropp, Gulis, Rosemond, & Benstead, 2010; Tant, Rosemond, & First, 2013). Higher temperatures are expected to increase rates of microbial respiration in predictable ways, based on models that describe how the rate of most cellular reactions increases exponentially with temperature (metabolic theory of ecology [MTE], specifically the Van't Hoff–Arrhenius relationship; Brown, Gillooly, Allen, Savage, & West, 2004; Gillooly, Brown, West, Savage, & Charnov, 2001; Yvon-Durocher et al., 2012). Indeed, higher water temperatures in microcosm studies stimulated fungal biomass accrual and respiration rates on leaf litter (Ferreira & Chauvet, 2011a). Likewise, elevated nutrient availability also increased respiration of detritus in streams by stimulating microbial biomass accrual and activity (Gulis & Suberkropp, 2003; Suberkropp et al., 2010; Tant et al., 2013). However, respiration rates could increase more than would be predicted by either driver alone, because the combined effects of nutrients and temperature may be synergistic (Ferreira & Chauvet, 2011b). Thus, increased processing and loss rates of detrital C via respiration could occur when streams are warmer due to climate change, and when nutrients are elevated from anthropogenic sources, thereby altering the role of streams and rivers in global C budgets (Aufdenkampe et al., 2011).

Our objective was to test for changes in the temperature dependence of microbial respiration rates associated with different detritus types under ambient and nutrient-enriched conditions. We

quantified the average changes in respiration rates with temperature (i.e., the activation energy of stream microbial respiration: E , eV) before and after an experimental nutrient concentration gradient was applied to five streams for three detritus types: fine benthic organic matter (FBOM, settled particles <1-mm; Wallace, Hutchens, & Grubaugh, 2006), leaf litter, and wood. We predicted that nutrient enrichment would stimulate microbial biomass accrual and respiratory activity associated with decomposing detritus, with relatively greater increases for coarse particulate organic matter (CPOM, i.e., leaf litter and wood) than FBOM owing to lower initial nutrient content of CPOM (Stelzer, Heffernan, & Likens, 2003; Tant et al., 2013). Similarly, we predicted that the temperature dependence of respiration would be greater for relatively recalcitrant, nutrient-poor detritus (e.g., wood vs. leaves), given higher temperature sensitivity for enzymes that degrade complex C compounds such as lignin (Canhoto et al., 2016; Fernandes et al., 2012; Sinsabaugh & Follstad Shah, 2012). Finally, we hypothesized that the effects of nutrient availability would be dependent on temperature, with stronger, positive effects of nutrients at higher temperatures, because microbial (e.g., fungal) biomass and activity can reach saturation at lower nutrient concentrations when temperatures are elevated (Fernandes, Seena, Pascoal, & Cássio, 2014; Ferreira & Chauvet, 2011b). We tested these predictions at the ecosystem scale using multiyear experimental nutrient (nitrogen [N] and phosphorus [P]) additions to five forest streams. Since elevated nutrients and temperature have the potential to stimulate both microbial biomass accrual (and hence concentration per unit of detrital mass) and respiratory activity per unit of microbial biomass, we measured respiration rates and fungal biomass associated with decomposing CPOM (leaf litter and wood) and microbial respiration on FBOM (which is dominated by bacteria, Findlay et al., 2002). We quantified changes in respiration rates relative to pretreatment conditions during 2 years of nutrient enrichment across seasons, and then compared the strength of these effects across our nutrient concentration and seasonal temperature gradients. We modeled the temperature dependence of stream microbial respiration using the Van't Hoff–Arrhenius relationship and then tested for differences in activation energy of respiration (i.e., the magnitude of change in activity with temperature) across experimental N and P concentration gradients for each detrital substrate.

2 | MATERIALS AND METHODS

2.1 | Site description

Our study was conducted in five first-order streams at the Coweeta Hydrologic Laboratory (CHL), a USDA Forest Service site in Macon Co., North Carolina, USA. The CHL basin is characterized by mature hardwood forest and drained by a network of low-order streams that are heavily shaded year-round by *Rhododendron maximum*. Detailed descriptions of the study site can be found in Swank and Crossley (1988).

We identified five unnamed first-order stream reaches in the Dryman Fork subbasin at CHL (35°03'35"N, 83°25'48"W) for the

nutrient-addition experiment. All five 70-m reaches had similar chemical and physical characteristics, including low ambient nutrient concentrations (<0.2 mg/L dissolved inorganic nitrogen [DIN] and <0.005 mg/L soluble reactive phosphorus [SRP], Table S1). Detailed methods for nutrient additions can be found in Rosemond et al. (2015) and Manning et al. (2015) and are presented here in brief. Concentrated nutrient solutions (NH_4NO_3 and H_3PO_4) were added to the five streams using solar-powered metering pumps (LMI Milton Roy, PA, USA) that delivered the nutrients to stream-fed irrigation lines continuously for 2 years (ENR1, ENR2) following a year of pre-treatment data collection (PRE). Nutrients were delivered to the irrigation lines proportional to continuously measured discharge using pressure transducers (Keller America, VA, USA) and CR800 dataloggers (Campbell Scientific, UT, USA) as a defined volume of concentrated stock solution per 100 L discharge that flowed past the most upstream injection point. Treatment concentrations were targeted to reflect low-to-moderately elevated N and P concentrations that are common in the region (Scott, Helfman, McTammany, Benfield, & Bolstad, 2002). Streams received relatively more N or P in a design of target molar N:P ratios of 2:1, 8:1, 16:1, 32:1, and 128:1 and encompassed a range of DIN (~80–650 $\mu\text{g/L}$) and corresponding decreasing concentrations of SRP (~90–11 $\mu\text{g/L}$ range) across the five streams. Such opposing concentration gradients that skew N:P ratios have been observed in streams in the continental United States (Dodds & Smith, 2016) and can potentially occur in watersheds with differing land use (e.g., urban = low N:P vs. agricultural = high N:P; Alexander et al., 2008; Arbuckle & Downing, 2001). For streamwater concentrations of N and P in our analysis (see Methods: *Statistical analyses*), we used known amounts of N and P added to the stream (based on recorded volumes and concentrations of stock solution added to each stream and discharge, plus background concentrations) rather than measured concentrations, which were affected by concentration-dependent uptake rates (A. D. Rosemond, *unpublished data*; see also Manning et al., 2015; Rosemond et al., 2015). We refer to these as “added concentrations” hereafter, and these concentrations were comparable to measured concentrations in the stream (Rosemond et al., 2015). Streamwater nutrient concentrations were measured biweekly ~10 m upstream of the nutrient additions and in three distributed areas downstream of the nutrient addition in the 70-m treatment reach. Streamwater temperatures were monitored continuously using HOBO dataloggers (Onset Computer Corp., MA, USA) submerged at the midpoint of each study reach.

2.2 | Detritus sampling

We collected three types of detritus for analysis on a quarterly basis (July 2010–July 2013; typically around 1 July, 15 October, 1 January, and 15 April for summer, autumn, winter, and spring, respectively) for 1 year before nutrients were added (PRE) and during two consecutive years of nutrient enrichment (ENR1, ENR2). Leaf litter, wood (small sticks <2 cm in diameter), and FBOM were collected from randomly selected transects within four 17.5-m sections of each 70-m treatment reach. We collected five submerged leaves

from each transect without regard to litter type that were fully intact and amenable to cutting leaf disks; hence, most leaves were likely in early or intermediate stages of decomposition. We used a similar method for sampling wood, in which five submerged sticks were collected and reduced in size with a pipe cutter as needed. We collected and combined several surface-layer FBOM samples from obvious depositional areas within the same transect as leaf litter and wood. All samples were placed in individual plastic bags (Fisherbrand™ sterile sampling bags with round-wire closures; Fisher Scientific, CA, USA) with streamwater and transported to the laboratory on ice until analyses were conducted (see below).

2.3 | Microbial respiration rates and detrital ash-free dry mass

Microbial respiration rates associated with leaf litter, wood, and FBOM were measured using methods outlined by Gulis and Suberkrupp (2003), Gulis, Rosemond, Suberkrupp, Weyers, and Benstead (2004), and Tant et al. (2013) within 24 hr after collection. We measured microbial respiration a total of 14 times over 3 years (3 substrates \times 4 seasons \times 5 streams \times 4 transects \times 3 years = 720 respiration estimates, plus two additional sampling events immediately before the start of nutrient additions [July 2011] and in the first month after nutrients were added [August 2011] for an additional 120 measurements [total $n = 840$]). Thus, seasonal sample sizes were larger for summer vs. other seasons (summer $n = 300$, all others $n = 180$) and for the first 2 years of the study (PRE $n = 300$, ENR1 $n = 300$, and ENR2 $n = 240$). Microbial respiration rates were determined as the rate of oxygen (O_2) uptake associated with the leaf litter, wood, or FBOM normalized per gram ash-free dry mass (AFDM) of the sample ($\text{mg O}_2/\text{g AFDM/h}$). We placed two 12-mm diameter leaf disks from each of the five leaves in the sample into 30–50 ml of streamwater in glass respiration chambers in darkness. Streamwater was specific to a given stream from which each detrital sample was taken, so that detritus was incubated with ambient (PRE) or nutrient-enriched (ENR1, ENR2) streamwater with corresponding N and P concentrations. We then measured O_2 concentrations and temperature roughly every 5 min over a 30-min interval using YSI 5100 Dissolved Oxygen Meters (YSI Inc., OH, USA) or Orion Star Plus Dissolved Oxygen Benchtop Meters (Thermo Scientific, MA, USA) in a walk-in incubator set to stream temperatures measured at the time of sample collection. Incubation temperatures were occasionally slightly different than stream temperatures (e.g., to avoid issues with water freezing in the chambers during winter [see Table S2]). Respiration rates were estimated based on the slope of the decline in dissolved O_2 concentrations over time. Dissolved O_2 was also measured in chambers containing streamwater only (from each stream), and resulting slopes were used to correct for probe drift during incubations. Microbial respiration rates associated with five wood disks cut from the small sticks were measured in the same way. Respiration rates of FBOM were measured using longer (~2 hr) incubations of 100-ml subsamples of continuously agitated FBOM and 50 ml streamwater to fill 150-ml glass bottles, and were

computed using the difference in dissolved O₂ before and after the 2-hr incubation period (Tant et al., 2013).

After respiration rates were measured on leaf litter and wood, the samples were removed from the chambers, dried for 24 hr at 55°C, weighed to the nearest 0.001 g, combusted for 4.5 hr at 550°C, and reweighed to determine AFDM. We measured AFDM in the same manner for freeze-dried FBOM samples.

2.4 | Fungal biomass

We measured fungal biomass associated with leaf litter and wood. We did not measure fungal biomass associated with FBOM, due to the much greater relative importance of bacteria on this substrate (Findlay et al., 2002; Tant et al., 2013). We estimated fungal biomass by quantifying ergosterol concentrations associated with the detritus (Gulis & Suberkropp, 2006) and applying a standard conversion factor (5.5 µg of ergosterol per mg fungal dry mass; Gessner & Chauvet, 1993). Lipids were extracted from five leaf disks or five wood disks (~1–2 mm thick) that were preserved in the field with methanol and stored at 20°C. Ergosterol was quantified using HPLC (LC-10VP, Shimadzu, MD, USA) equipped with a Kinetex reverse phase C18 column (Phenomenex, CA, USA), a UV detector set at 282 nm and external ergosterol standards (Acros Organics, Geel, Belgium). Further details about this method can be found in Gulis and Suberkropp (2006).

2.5 | Statistical analyses

We used the linearized form of the Van't Hoff–Arrhenius equation (Arrhenius, 1915; Perkins et al., 2012; Yvon-Durocher et al., 2012) to estimate the temperature dependence of respiration associated with detritus:

$$\ln R(T) = E \left(\frac{1}{kT_c} - \frac{1}{kT} \right) + \ln[R(T_c)],$$

where $R(T)$ is the respiration rate at absolute temperature T , E is the activation energy, and k is the Boltzmann constant (8.617×10^{-5} eV/K, $1 \text{ eV} = 1 \times 10^{-19}$ J). Temperature values in our models were based on the temperature of the streamwater in the respiration chambers, which were similar to streamwater temperatures at the time of sampling (see Table S2). We centered our data using the approximate mean annual temperature for the study (T_c) in the five streams by subtracting observed temperatures from the reciprocal of absolute temperature at 10°C (i.e., $1/kT_c$), such that the intercept of the linear equation describes the mean respiration rate at this temperature. Temperature data were centered in this way to facilitate comparing mean respiration rates among streams at a consistent and biologically relevant temperature. The slope of this model is considered the activation energy (E) of respiration for a given substrate and year (Perkins et al., 2012; Yvon-Durocher et al., 2012). Given the scale of our nutrient manipulations, true replication was not feasible; therefore, we developed linear mixed-effects models with maximum likelihood to account for spatial and temporal

nonindependence of our samples (Gelman & Hill, 2007). We used a combination of likelihood-based metrics (e.g., Akaike's information criterion [AIC]), marginal, and conditional R^2 (variance explained by fixed or both fixed and random effects, respectively; Nakagawa & Schielzeth, 2013) to assess model fits. The general form of our models was the linearized Van't Hoff–Arrhenius equation described above that also included random intercepts for sampling time crossed with stream (i.e., 70 random intercepts for each stream/time combination). As the first step in our analysis, we tested a model with a categorical predictor for “year” (PRE vs. ENR1, ENR2) to evaluate the general effects of nutrient additions on microbial respiration rates and fungal biomass. This set of models also included a categorical predictor for each substrate (i.e., intercepts for FBOM, leaf litter, and wood) and centered temperature ($1/kT_c - 1/kT$), as well as interactions among substrates, years, and temperature (i.e., multiple linear regression/analysis of covariance [ANCOVA]). In this context, contrasts between regression coefficients were defined with FBOM as the baseline (this designation is arbitrary and arose because FBOM is first alphabetically) for comparison to leaf litter or wood coefficients, and the pretreatment year (PRE) was considered the baseline for year-specific coefficient comparisons. We note that sampling year in this model structure is confounded with the timing of nutrient additions (also see Kominoski, Rosemond, Benstead, Gulis, & Manning, 2017 for similar statistical approach). Hence, the effects of our nutrient additions technically cannot be separated from potential interannual differences in covarying natural drivers of microbial respiration.

To discern the relative influence of N and P on respiration, we tested another set of multiple regression models containing continuous, stream/time-level predictors of N and P concentrations. We used a combination of the mean measured [PRE] and added [ENR1, ENR2] N or P concentration of all biweekly measurements preceding a given sampling event to generate continuous N and P concentration gradients to use in the models (see Section 2.1). These continuous N and P models also contained categorical predictors for substrate (FBOM, leaf litter, and wood; as described for the first set of models above). Contrasts among substrate-specific regression coefficients were also between FBOM compared to leaf litter or wood coefficients. Continuous N and P concentrations were centered and scaled in the models using their means and standard deviations, respectively, to aid in the interpretation of potential interactions with temperature, and to allow us to compare the strength of their effects on respiration rates and fungal biomass (Gelman & Hill, 2007). The coefficients associated with these scaled parameters can be interpreted as the predicted increase in the response variable for a 1-standard deviation (SD) increase in N or P concentration. We then used this model structure to test for differences in E among substrates and for interactions of temperature with scaled N and P concentrations. Because N and P concentrations were centered at the mean and scaled, interactions between N, P, and temperature can be interpreted as the expected change in the slope E when N or P concentrations are increased by 1 SD.

We also compared a model with the interaction between N and P concentrations to a model without an interaction (i.e., $N \times P$ vs. $N + P$) using AIC, because our target N and P concentrations were related in our experimental design. The models that included $N + P$ were better supported based on a lower AIC value ($N \times P$ model AIC = 1,503 vs. $N + P$ model AIC = 1,493). We did not include N:P ratios in these models because N:P values spanned a similar range in pretreatment compared to enriched conditions (PRE N:P ratios ~15–138; Table S1). Instead, we used a separate model with N:P ratios that included data from enriched conditions only (Table 2). We also used the N:P ratio model as an additional line of evidence for N vs. P effects on microbial respiration rates. We used separate models for leaf litter and wood (fungal biomass was not measured on FBOM) when fungal biomass and fungal biomass-specific respiration were response variables, because we were interested in their individual responses to nutrient enrichment. Other assumptions of linear models, such as homoscedastic variance, were generally met based on visual inspection of residuals vs. fitted values. We ln-transformed N and P concentrations before scaling to meet assumptions of normality. All analyses were conducted using the statistical software R v. 3.3.0 (R Core Team, 2016). Linear mixed-effects models were run with the function “*lmer*” in the package “*lme4*” (Bates, Maechler, Bolker, & Walker, 2015). The package “*lmerTest*” was used to estimate significance probabilities for each parameter in the linear mixed-model; “*lmerTest*” uses the Satterthwaite approximation to assign degrees of freedom for specific *t*-tests and comparisons of model coefficients (Kuznetsova, Brockhoff, & Christensen, 2016). Significance probabilities <0.05 were considered evidence of a significant effect, and values <0.1 were considered evidence of a marginal effect.

3 | RESULTS

3.1 | Nutrient treatments and stream physical attributes

Mean daily temperatures during the study ($n = 1,099$ days) encompassed an 18.7°C range (minimum temperature = 0.8°C, maximum = 19.5°C; Fig. S1). Temperature regimes in the five study streams were similar across broad temporal scales (Fig. S1). For all possible comparisons of the five streams, the maximum observed difference in daily mean temperature was 3.3°C (Stream 2:1 vs. Stream 8:1), and the median difference in daily mean temperature was 0.65°C (Fig. S1). Therefore, daily temperature differences between any given stream were <0.7°C for the majority of the study. Our study streams had relatively consistent temperatures within each season; the largest differences in mean monthly temperature among streams were found during summer (July; range = 2.3°C; Table S2), and smallest differences were found in autumn (October; range = 0.5°C; Table S2). Mean temperatures among streams over the entire study period were unrelated to nutrient treatments, although the highest and lowest mean temperatures were found in the low N:P (2) and high N:P (128) treatment streams, respectively.

Median daily stream discharge across all streams and years was ~5 L/s; the median difference in discharge among all streams was

2.9 L/s. Highest median discharge (12.6 L/s) for the study was in our highest N:P treatment stream, while lowest median discharge (3.0 L/s) was in the lowest N:P treatment stream. Median discharge increased across all streams by 1.48× and 1.57× in ENR1 and ENR2 compared to PRE, respectively. Discharge did not change uniformly across streams from year to year and increased the most (~3.0× in both years) in the highest N:P treatment, followed by the lowest N:P treatment (~2.4× in both years). Discharge was more similar among years in the three other streams. Since these ranges in discharge were not predicted to affect respiration rates, discharge was not included in our models; however, antecedent flow conditions may be important in general for moving microbially colonized detritus to downstream locations (Webster et al., 1999).

Added N and P concentrations generally reflected target concentrations during the study (Table S1; also see Manning et al., 2015; Rosemond et al., 2015), and as a result, N and P concentrations differed among streams. Added nutrient concentrations in the streams spanned a gradient of 82–517 µg/L DIN and 9.7–93 µg/L SRP across the five streams and 2 years of nutrient additions (Table S1).

3.2 | Substrate-specific microbial respiration rates

Respiration rates normalized by AFDM were greatest for leaves and lowest for wood, with rates from FBOM being intermediate; leaf litter and wood (not FBOM) respiration rates tended to be higher, on average, under enriched conditions across all streams (Table 1; Figures 1a–c, S2a–c). The categorical “year” model showed higher intercepts in ENR1 and ENR2 for leaf litter and wood based on contrasts between year-specific coefficients (i.e., ANCOVA; Figure 1a–c). These higher intercepts corresponded to 1.36× and 1.28× higher respiration rates for leaf litter (ENR1, ENR2, respectively [mean = 1.32×]; Table 1), and 1.24× (marginally significant, $p = .059$; Table 1) and 1.51× increases in respiration rates for wood (ENR1, ENR2, respectively [mean = 1.38×]; Table 1), when compared at the same temperature (10°C) under nutrient-enriched conditions.

Respiration rates on detritus showed a positive relationship with temperature (i.e., $1/kT_c - 1/kT$; Figure 1a–c). Activation energies were statistically indistinguishable among all three substrates in both categorical year and continuous nutrient concentration models (ANCOVA; i.e., no difference in slopes among substrates, all $p > .05$, Tables 1 and 2; Figure 1a–c). We also found no evidence for differences in activation energies in ENR1 or ENR2 compared to PRE for any substrate, based on year-specific contrasts (Table 1). The estimated E from the categorical year model for FBOM was 0.43 eV, and the 95% confidence interval for this activation energy included the predicted E value of 0.62 eV based on metabolic reactions within the cellular respiratory complex (FBOM 95% CI = 0.18–0.69; Gillooly et al., 2001; Table 1). Leaf litter and wood activation energies were comparable to the estimate for FBOM (0.30 and 0.41 eV, respectively), and the upper bounds of their 95% CIs were also close to, or included, the MTE-predicted 0.62 eV activation energy value (95% CI = 0.07–0.54 [leaf litter], 0.17–0.62 [wood], Table 1).

TABLE 1 Fixed-effects parameter estimates, the difference among parameter estimates in ENR1 and ENR2 compared to PRE, and the exponentiated difference between coefficients ($e^{[ENR_x - PRE]}$ = predicted multiplicative increase in respiration rate for a given ENR vs. PRE comparison) from the categorical 'year' mixed-linear model for FBOM, leaf litter, and wood (Marginal- R^2 = 0.60, conditional- R^2 0.63)

Substrate	Parameter	Estimate	ENRx- PRE	$e^{(ENR_x - PRE)}$	SE	df	t-value	p-value	95% CI	
									Lower	Upper
FBOM	PRE	-2.832	n.a.	n.a.	0.078	327.7	-36.241	<.001	-2.985	-2.678
	ENR1	-3.004	-0.173	0.841	0.114	313.5	-1.518	.130	-0.397	0.051
	ENR2	-2.818	0.014	1.014	0.117	313.8	0.116	.907	-0.216	0.243
	PRE × T	0.433	n.a.	n.a.	0.131	337.5	3.319	.001	0.177	0.690
	ENR1 × T	0.657*	0.224	1.251	0.190	316.9	1.177	.240	-0.150	0.598
	ENR2 × T	0.239	-0.194	0.824	0.253	368.3	-0.766	.444	-0.695	0.303
Leaf litter	PRE	-2.221	n.a.	n.a.	0.076	309.6	-29.214	<.001	-2.370	-2.071
	ENR1	-1.915	0.305	1.357	0.116	334.2	2.625	.009	0.077	0.534
	ENR2	-1.977	0.243	1.275	0.114	296.2	2.136	.033	0.020	0.468
	PRE × T	0.304	n.a.	n.a.	0.118	321.2	2.573	.011	0.072	0.537
	ENR1 × T	0.624*	0.320	1.377	0.191	322.9	1.671	.096	-0.057	0.695
	ENR2 × T	0.134	-0.170	0.843	0.243	292.6	-0.701	.484	-0.650	0.307
Wood	PRE	-4.052	n.a.	n.a.	0.074	295.7	-55.059	<.001	-4.197	-3.907
	ENR1	-3.837	0.215	1.240	0.113	310.9	1.894	.059	-0.008	0.438
	ENR2	-3.643	0.409	1.505	0.114	301.5	3.599	<.001	0.186	0.632
	PRE × T	0.411	n.a.	n.a.	0.119	310.6	3.466	.001	0.177	0.644
	ENR1 × T	0.526*	0.115	1.122	0.193	321.9	0.598	.550	-0.263	0.494
	ENR2 × T	0.108	-0.303	0.739	0.238	297.4	-1.270	.205	-0.771	0.166

Parameters for YRx × temperature interactions (YRx × T) show the activation energy in a given year (Estimate), and the difference among coefficients in ENR vs. PRE conditions (ENRx – PRE). We report degrees of freedom (*df*; for *t* tests using Satterthwaite approximation), *t*-values, *p*-values, and 95% confidence intervals (95% CI) for coefficients corresponding to PRE (i.e., intercepts = mean log[AFDM-specific respiration rates]; PRE × T coefficients = activation energy in PRE), and for the coefficients comparing a given enrichment year (ENR1, ENR2) to pre-treatment conditions. Estimates of parameters with confidence intervals that exclude zero and with *p*-values <.05 are considered significant and are highlighted with bold text. Note that n.a. = not applicable, and *denotes where activation energy estimates for ENR1 were >0 at *p* < .001

Although there were no significant interactions between nutrient enrichment (as year) and temperature, the slopes (*E*, eV) appeared to be less steep in the second year of enrichment for all three substrates (Figure 1a–c).

We were unable to detect strong responses in terms of detrital mass-specific respiration rates as a function of N and P concentrations or N:P ratio, but the effects of N vs. P differed depending on the substrate (Table 2). Specifically, leaf litter and wood respiration rates responded more to P concentrations relative to FBOM, based on contrasts between substrate-specific P coefficients (*p* = .03 and .003 for leaf litter and wood P effects compared to FBOM P effects, respectively; Table 2). Activation energies (slopes) for all three substrates were unchanged across N or P concentrations and were comparable to those from the categorical year model (Tables 1 and 2). There was no effect of N:P ratio on the temperature dependence of respiration rates for any substrate (Table 2).

3.3 | Fungal biomass-specific respiration rates

Fungal biomass-specific respiration rates (i.e., per g fungal biomass) for leaf litter were positively related to temperature and had

activation energies that were higher than those observed for AFDM-specific respiration rates (leaf litter fungal biomass-specific *E* = 0.57 eV [95% CI = 0.05–1.10]; Figure 2a; Table S3). However, unlike AFDM-specific respiration rates, nutrient enrichment generally did not increase fungal biomass-specific respiration rates for leaf litter (Figure 2a; Table S3), when enrichment was considered either categorically (Figure 2a; Table S3) or as continuous N or P (Table S3).

Fungal biomass-specific respiration rates on wood were less sensitive to temperature compared to those on leaf litter (Figure 2a,b). For wood, activation energy was significantly higher in ENR1 (E_{ENR1} = 0.52 [95% CI = 0.27–0.86]; *p* = .048; Figure 2b; Table S3), and there was a significant interaction between temperature and P concentrations (*p* = .024; Table S3), such that temperature sensitivity of respiration was reduced at high P concentration.

3.4 | Fungal biomass and temperature

In assessing drivers of fungal biomass on leaf litter, we found no effects of temperature (Figure 3a; Table S3), or nutrient enrichment (when nutrients were considered on a categorical [Figure 3a;

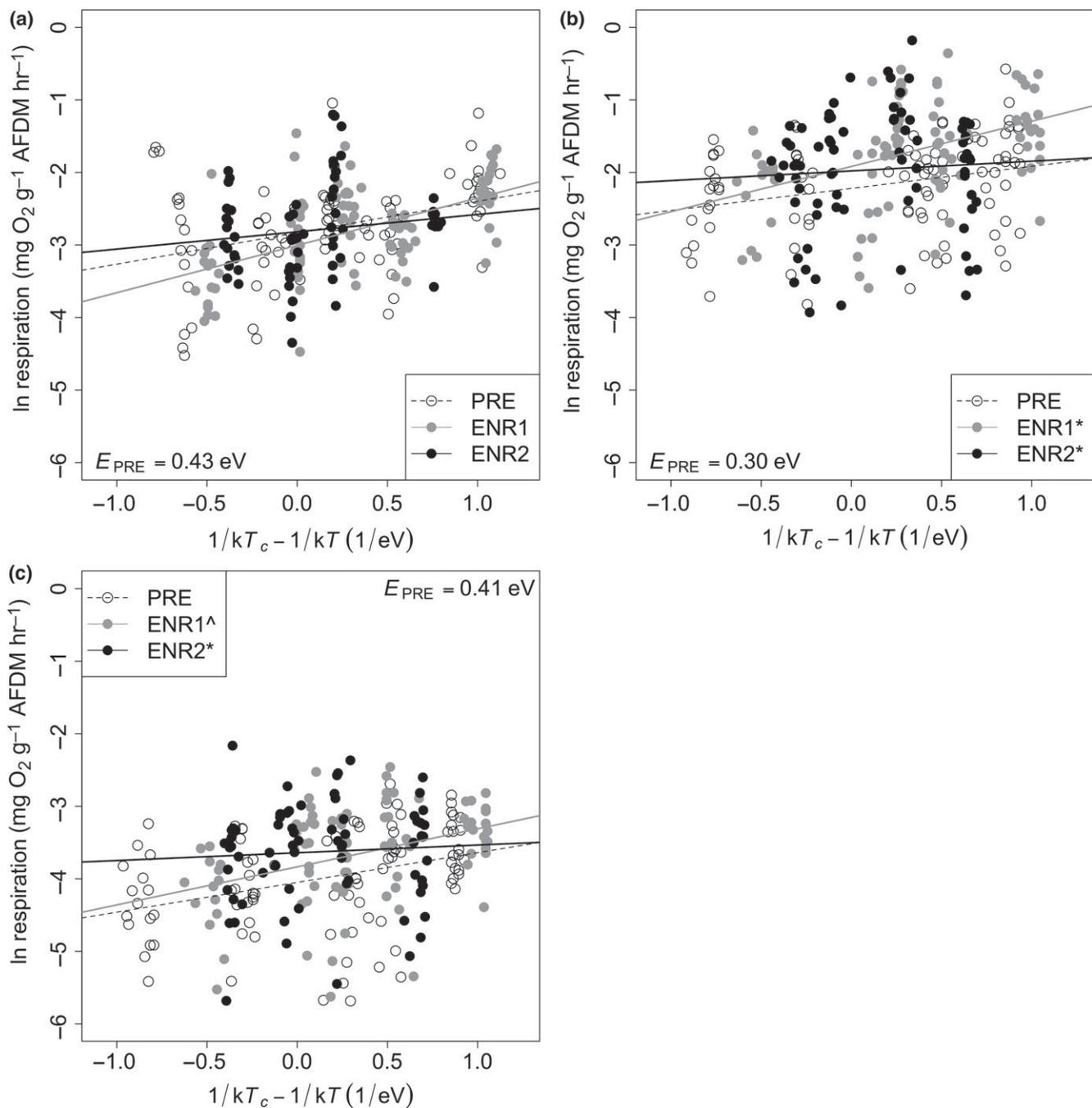


FIGURE 1 Temperature dependence of substrate-specific respiration rates for FBOM (a), leaf litter (b), and wood (c) (Conditional- $R^2 = .63$). Each point represents an individual respiration measurement from a given transect within a stream, season, year, and substrate. Temperature ($1/kT_c - 1/kT$, $k = \text{Boltzmann constant } 8.617 \times 10^{-5} \text{ eV/K}$) was centered at 10°C (mean temperature for the duration of the study period in all five study streams); hence, the intercept corresponds to this standard temperature (zero) on the plot. Open circles correspond to pretreatment conditions, and filled gray and black circles correspond to ENR1 and ENR2 of enrichment, respectively. Slopes of dashed black lines correspond to estimated activation energy for each substrate in PRE; solid gray and black lines correspond to intercepts and estimated activation energies ENR1 and ENR2, respectively (note that these slopes were not statistically different from PRE). Activation energies (E) in PRE for FBOM, leaf litter, and wood are shown ($E = 0.43, 0.30$ and 0.41 eV , respectively); these relationships were statistically similar for all three substrates (Table 2). Asterisks in the legend indicate years when mean respiration rates (i.e., intercepts) were significantly higher in nutrient-enriched conditions, and the \wedge indicates a marginally significant increase in mean respiration for wood in ENR1. Note that sampling year and nutrient treatments are confounded when “year” is considered categorically

Table S3] or continuous basis [Table S3]). In contrast, fungal biomass on wood was positively related to nutrients and temperature (but only under PRE conditions for the latter). Wood fungal biomass

increased by $1.16\times$ in ENR1 ($p = .089$; Table S3) and $1.22\times$ in ENR2 ($p = .024$; Table S3) relative to PRE (Figure 3b) and was positively related to temperature in PRE, but the slope was marginally

Substrate	Parameter	Estimate	SE	df	t-value	p-value	95% CI	
							Lower	Upper
(A) N and P concentration model								
FBOM	Intercept	-2.859	0.049	306.4	-58.949	<.001	-2.955	-2.764
	N	0.080	0.056	334.4	1.417	.157	-0.031	0.190
	P	-0.111	0.056	312.7	-1.991	.047	-0.220	-0.001
	T	0.438	0.088	318.2	4.990	<.001	0.265	0.610
	N × T	0.005	0.103	353.3	0.052	.958	-0.197	0.207
	P × T	0.092	0.096	292.6	0.958	.339	-0.078	0.313
Leaf litter	Intercept	*-2.052	0.048	307.5	-42.457	<.001	-2.147	-1.957
	N	0.067	0.054	323.8	1.240	.216	-0.039	0.174
	P	*0.043	0.055	315.5	0.771	.441	-0.066	0.151
	T	0.421	0.087	300.1	4.822	<.001	0.249	0.593
	N × T	0.052	0.097	321.5	0.540	.589	-0.138	0.243
	P × T	0.124	0.098	300.0	1.264	.207	-0.069	0.317
Wood	Intercept	*-3.847	0.048	299.8	-80.213	<.001	-3.941	-3.752
	N	0.019	0.051	284.8	0.370	.712	-0.082	0.120
	P	*0.145	0.053	284.9	2.744	.006	0.041	0.249
	T	0.346	0.088	303.0	3.952	<.001	0.174	0.518
	N × T	-0.119	0.092	295.0	-1.291	.198	-0.301	0.062
	P × T	0.092	0.096	292.6	0.958	.339	-0.097	0.282
(B) N:P ratio model								
FBOM	Intercept	-2.914	0.062	176.6	-47.024	<.001	-3.037	-2.792
	N:P ratio	0.073	0.056	174.6	1.290	.199	-0.046	0.184
	T	0.514	0.118	183.9	4.353	<.001	0.280	0.746
	N:P × T	-0.023	0.114	187.4	-0.203	.840	-0.250	0.202
Leaf litter	Intercept	*-1.948	0.063	186.5	-30.821	<.001	-2.073	-1.823
	N:P ratio	0.047	0.057	182.2	0.829	.408	-0.073	0.160
	T	0.502	0.125	178.3	4.031	<.001	0.256	0.748
	N:P × T	-0.196	0.124	186.8	-1.582	.115	-0.440	0.048
Wood	Intercept	*-3.739	0.063	184.9	-59.185	<.001	-3.864	-3.614
	N:P ratio	-0.064	0.056	167.0	-1.159	.248	-0.182	0.045
	T	0.352	0.124	185.3	2.833	.005	0.107	0.597
	N:P × T	-0.094	0.119	183.5	-0.795	.428	-0.328	0.139

Intercepts correspond to the mean respiration rate ($\ln R$, originally in $\text{mg O}_2/\text{g AFDM}/\text{h}$), expected at 10°C [T_c] for each substrate. The other parameters represent the effects of N, P, temperature (activation energy, E), and interactions between N, P, and T ($N \times T$, $P \times T$). Estimates of parameters with confidence intervals that exclude zero and with p -values $<.05$ are considered significant and are highlighted with bold text. Comparisons among substrate-specific coefficients were also performed: an asterisk indicates statistically significant ($p <.05$) differences between substrate coefficients (i.e., leaf litter P coefficient—FBOM P coefficient = 0.15, $df = 678$, $t = 2.1$, $p = .034$; wood P coefficient—FBOM P coefficient = 0.25, $df = 680$, $t = 3.6$, $p = .0003$). The N:P ratio model includes data from enriched conditions only.

lower and approached zero in ENR1 ($p = .089$; Figure 3b; Table S3). In a continuous N and P model, fungal biomass associated with wood tended to increase marginally with P concentrations ($p = .079$; Table S3). In this same model, wood fungal biomass was unrelated to temperature, and the relationship was unchanged across increasing N or P concentrations (Table S3).

3.5 | Comparing nutrient and temperature effects on stream microbial respiration

Temperature effects were similar for each detritus type, while N and P effects differed, according to the model that included scaled N, P, and temperature (Table S4). Nutrient concentration effects tended

TABLE 2 Fixed-effects parameter estimates, the standard error of each estimate, and associated degrees of freedom (df ; for t -tests using Satterthwaite approximation), t -values, p -values, and 95% confidence intervals (95% CI) from the mixed-linear models for N and P concentration effects (A) and N:P ratio effects (B) on microbial respiration rates for FBOM, leaf litter, and wood (Marginal- R^2 , conditional- $R^2 = .60$ and $.63$, respectively [N and P model], $.59$ and $.61$, respectively [N:P ratio model])

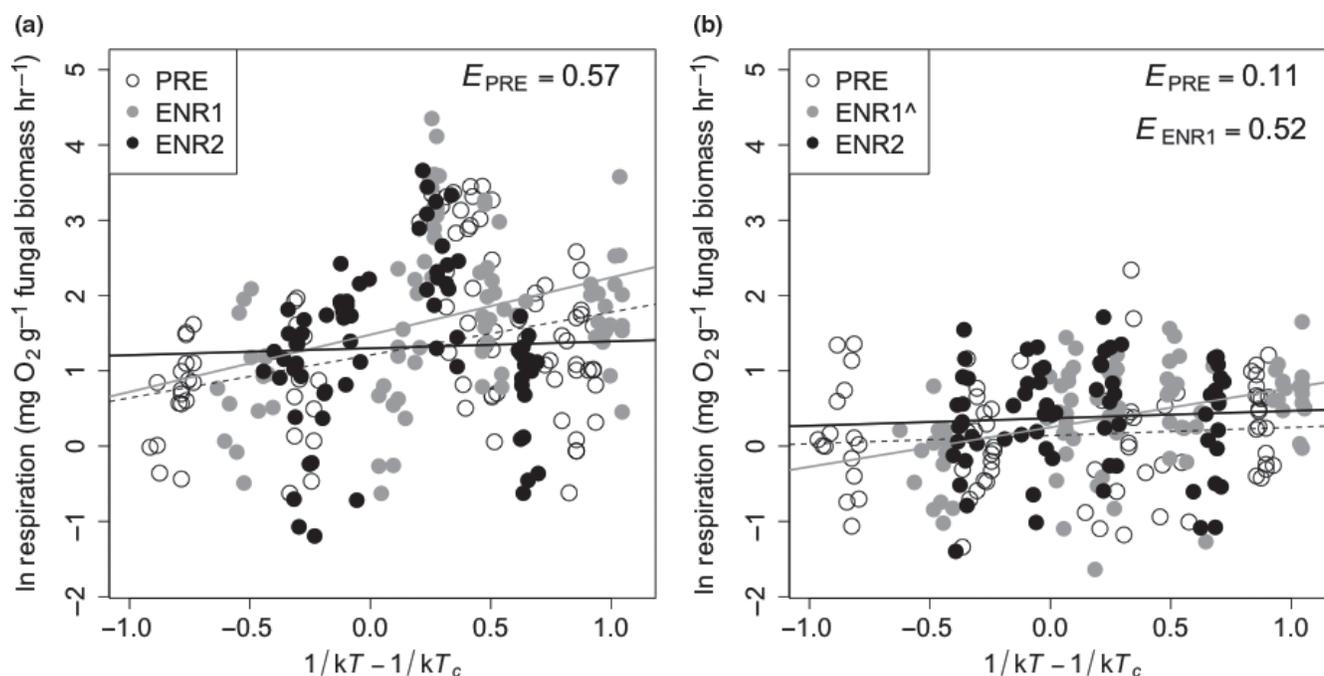


FIGURE 2 Fungal biomass-specific respiration rates as a function of temperature for leaf litter (a) and wood (b). Each point represents an individual fungal biomass-specific respiration measurement from a given transect within a stream, season, year, and substrate. Open circles correspond to pretreatment conditions; closed gray and black circles represent ENR1 and ENR2, respectively. Slopes of dashed black lines correspond to estimates of activation energies for both substrates in PRE, and solid gray and black lines correspond to activation energies in ENR1 and ENR2, respectively. A categorical year model showed no differences among intercepts or slopes for fungal biomass-specific respiration rates for leaf litter and a marginally higher intercept in ENR1 (denoted with ^ in legend, $p = .067$) and significantly higher activation energy in ENR1 compared to PRE for wood ($t = 2.015$, $df = 60.12$, $p = .048$). Note that sampling year and nutrient treatments are confounded when “year” is considered categorically

to be small (i.e., <0.1 *sensu* Nakagawa & Cuthill, 2007), except for P effects on wood (Table S4; Figure 4c), when considered separately. In general, combined N and P concentration effects were stronger for coarse detritus, compared to FBOM (Figure 4a–c). For leaf litter and wood, the strength of the combined effects of N and P was comparable to the strength of temperature effects alone (Figure 4b, c).

4 | DISCUSSION

Human-derived nutrient inputs and temperature are currently elevated above historical norms in streams and are expected to continue increasing, with relatively unknown combined effects on ecosystem functions such as C processing (Cross et al., 2015; Kaushal et al., 2010). Our study aimed to elucidate the potential interactive effects of these global-change drivers on heterotrophic microbial activity associated with three detrital resource pools that together contribute substantially to stream C budgets: leaf litter, wood, and FBOM (Webster et al., 1999). On the whole, our findings did not support our prediction of synergistic effects of temperature and nutrients on microbial respiration. Instead, our findings indicate that individual effects of nutrients and temperature can additively increase stream C processing rates. Thus, models incorporating both

nutrient and temperature effects on the contributions of river networks to global C cycles may adequately predict increases in C processing rates under the assumption that nutrient and temperature effects will be additive. An additional challenge to further resolving the role of river networks in the global C cycle is modeling the transport and fate of different forms of C in response to elevated nutrients or temperatures, including CPOM, fine particles, and dissolved forms (Hall, Tank, Baker, Rosi-Marshall, & Hotchkiss, 2016; Webster et al., 1999). Our findings indicate that these organic matter types could respond similarly to temperature, but differently to nutrient enrichment.

Our estimates of activation energies for naturally occurring detritus were slightly lower than predictions based on the MTE, while still within the range of those reported for respiration associated with several organism groups ($E = 0.41$ – 0.74 ; Gillooly et al., 2001), and comparable to the ranges reported for short-term respiration rates ($E = 0.44$ – 0.78 ; Yvon-Durocher et al., 2012) and microbially driven leaf litter breakdown ($E = 0.19$ – 0.56 ; Follstad Shah et al., 2017) in rivers. The lower values we observed for microbial respiration rates on detritus were also within the range of activation energies associated with microbial exoenzymes that degrade organic macromolecules, including lignin and cellulose ($E = 0.22$ – 0.62 ; Sinsabaugh & Follstad Shah, 2012). Taken together, these results imply that respiration rates associated with detritus in streams may be slightly less

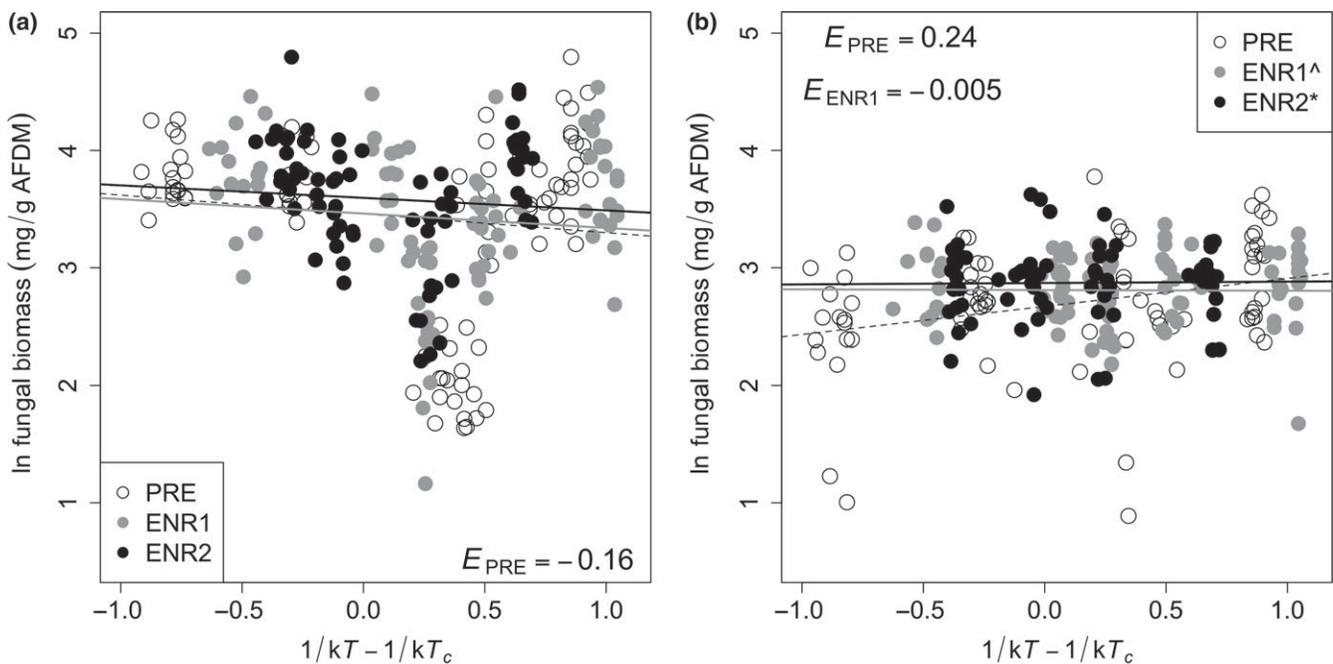


FIGURE 3 Fungal biomass as a function of standardized temperature for leaf litter (a) and wood (b). Each point represents an individual fungal biomass measurements from a given transect within a stream, season, year, and substrate. Open circles denote pretreatment values, and closed gray and black circles denote ENR1 and ENR2, respectively. Dashed lines in (a) and (b) indicate the slopes for fungal biomass as a function of temperature in PRE, and solid gray and black lines indicate the slopes for ENR1 and ENR2, respectively. A categorical year model showed no difference among intercepts or slopes for leaf litter fungal biomass (all $p > .05$), whereas there were marginally (ENR1; $t = 1.732$, $df = 59.93$, $p = .088$) and significantly (ENR2; $t = 2.31$, $df = 58.73$, $p = .024$) higher intercepts for wood, along with a marginally lower slope in ENR1 ($t = -1.723$, $df = 62.71$, $p = .089$) for wood fungal biomass. Note that sampling year and nutrient treatments are confounded when “year” is considered categorically

sensitive to temperature changes than those predicted by the MTE based on the activation energy of the cellular respiratory complex, matching more closely with the activation energies observed for related microbial-driven processes in streams.

Overall, our models describing the relationship between temperature and respiration suggest that respiration rates associated with naturally occurring detrital CPOM will increase by ~6% on average (95% confidence interval: 2.9%–9.1%) per 1°C increase in stream temperatures due to climate change or the thermal effects of land-use change. Although there was substantial uncertainty in our models describing N and P concentration effects on respiration rates (except for wood and P concentration), predictions from these models may still prove to be useful under the assumption that nutrients can increase respiration rates (as supported by our categorical year model and previous studies; e.g., Ferreira & Chauvet, 2011b; Stelzer et al., 2003). In this context, when temperature effects are combined with the effects of N and P concentrations, our models predict that FBOM, leaf litter, and wood respiration rates could increase by 1.09×, 1.24×, and 1.30×, respectively, given a 2.1°C increase in streamwater temperatures (median predicted increase in streamwater temperatures by the year 2100 from Kaushal et al., 2010) and a 1 SD increase in N and P concentration (164 μg/L N or 31 μg/L P) from concentrations that were low compared to river systems worldwide (Alexander & Smith, 2006; Woodward et al., 2012).

4.1 | Detritus type, nutrient enrichment, and temperature dependence of respiration

Nutrient enrichment and temperature generally increased respiration rates associated with coarse detritus; however, the effect of nutrients varied with detritus type, while the effect of temperature was relatively consistent. Several studies have demonstrated the importance of considering intrinsic substrate characteristics, such as C quality and nutrient content, in the response of respiration rates to nutrients (Ferreira, Gulis, & Graça, 2006; Ferreira et al., 2014; Stelzer et al., 2003) and temperature (Fierer, Craine, McLauchlan, & Schimel, 2005; Jankowski, Schindler, & Lisi, 2014). Our findings underscore the idea that respiration rates on nutrient-poor substrates respond to a greater extent to nutrient enrichment, as evidenced by strongest nutrient-induced stimulation of respiration rates for wood substrates compared to more nutrient-rich substrates (Ferreira et al., 2006; Gulis et al., 2004; Stelzer et al., 2003). In contrast, we found that FBOM, leaf litter, and wood responded comparably to temperature, despite marked differences in terms of the initial nutrient content and C quality among these three detritus types (Gulis et al., 2004; Stelzer et al., 2003; Tant et al., 2013).

There was no statistical evidence for an interaction between activation energy and nutrient enrichment according to our models, but there were trends to indicate potentially lower activation

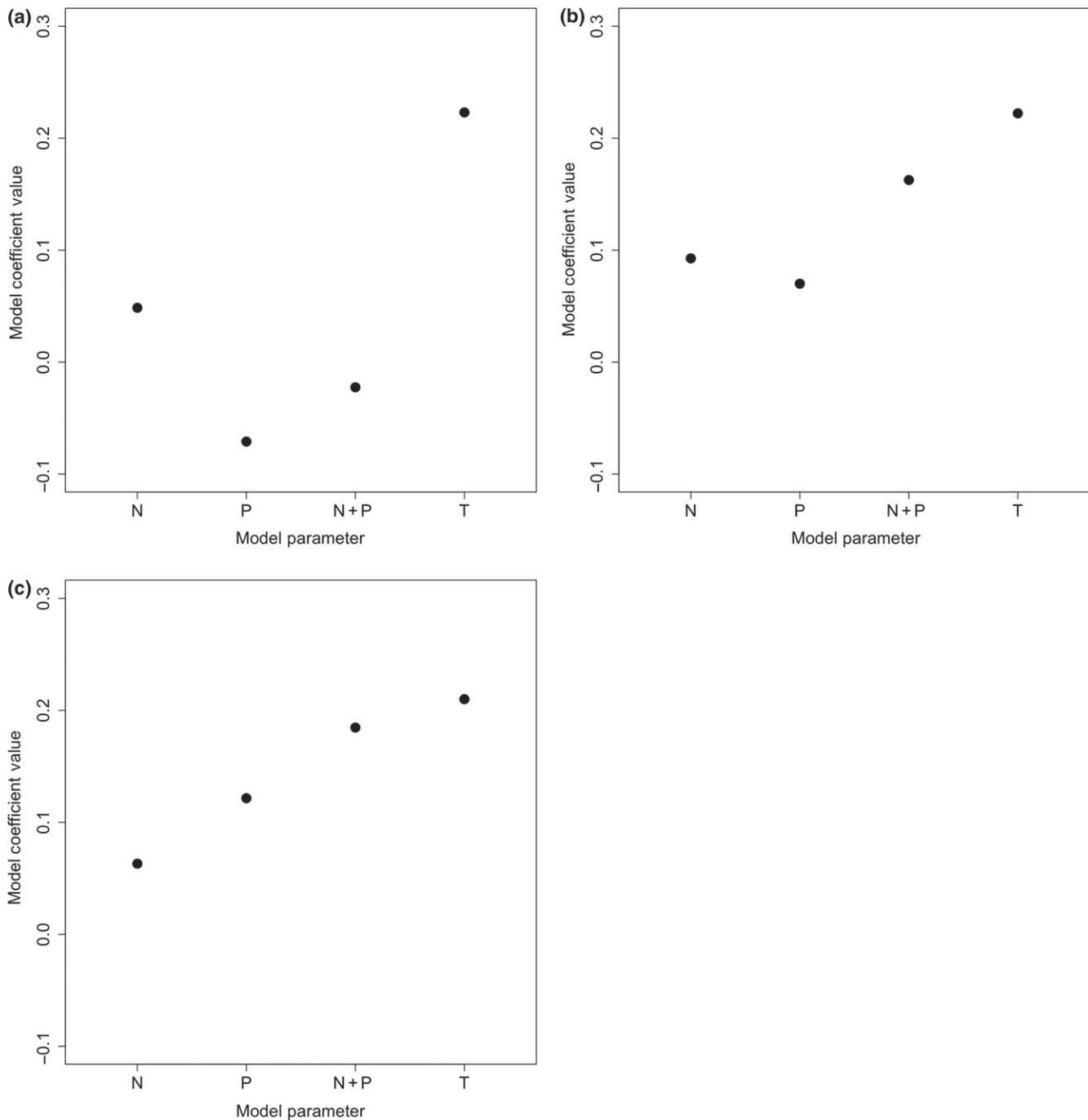


FIGURE 4 Model coefficient values from linear mixed-effects models comparing parameters that indicate N, P, additive (N + P) concentration effects, and seasonal temperature (T) effects on ln-transformed microbial respiration rates (Table S4). Coefficients were estimated in the model based on predictor variables that were centered and scaled using the mean and standard deviation, respectively (Gelman & Hill, 2007), and can be directly compared in terms of the strength of the effect on respiration rates. Standardized coefficients values are presented from a model that included all three detrital substrates (FBOM [a], leaf litter [b], and wood [c]). Note that when N and P are considered separately, coefficients were generally <0.1 except for P effects on wood respiration

energies with long-term enrichment. This trend is implied by less steep slopes describing temperature dependence of respiration rates in our second year of enrichment for leaf litter and wood (Figure 1b, c; we note that slopes increased slightly in ENR1). Consistent with this, slopes were statistically greater than zero in PRE and ENR1, but

were not different from zero in ENR2 (Table 1). We also found lower activation energy for fungal biomass-specific respiration on wood in ENR2 (Figure 2b). Long-term enrichment might result in lower activation energy if nutrients stimulate fungi most when water temperatures are cool.

The lack of evidence for synergistic effects of temperature and nutrients and comparable responses across detritus types contrasts with other studies that report synergistic effects on microbial activity in laboratory incubations (Fernandes et al., 2014; Ferreira & Chauvet, 2011b). Synergistic effects have been attributed to increased nutrient-use efficiencies at higher temperatures, as evidenced by lower nutrient thresholds (e.g., half-saturation constants of the Michaelis–Menten model, K_m) and higher maximum values (i.e., V_{max}) for microbial growth and activity when temperatures are elevated (Fernandes et al., 2014). Our nutrient concentrations were low compared to these microcosm studies (Fernandes et al., 2014; Ferreira & Chauvet, 2011b) and more eutrophic streams globally (e.g., Woodward et al., 2012), suggesting that the nutrient concentrations we used may not have been sufficiently high to elicit a synergistic effect. Indeed, in many cases presented here (e.g., FBOM, leaf litter respiration), we were unable to detect statistically significant increases in respiration rates as a function of N or P concentrations. On the other hand, reported half-saturation constants for fungal biomass and activity are comparable to, or lower than, the experimental nutrient concentrations we used in this study (e.g., ca. 20–300 $\mu\text{g/L}$ $\text{NO}_3\text{-N}$ [Ferreira et al., 2006; Fernandes et al., 2014;], ~ 13 $\mu\text{g/L}$ SRP [Rosemond, Pringle, Ramirez, Paul, & Meyer, 2002]), suggesting that many of our nutrient treatments probably stimulated fungal activity sufficiently to evaluate interacting effects of temperature and nutrients.

Other factors may have contributed to the lack of strong nutrient effects or nutrient \times temperature interactions, including those related to the inherent limitations of our experimental design that are common to most large-scale experiments (reviewed by Barley & Meeuwig, 2017). For example, our study relies on pretreatment data from each of the five streams (see also Kominoski et al., 2017; Manning et al., 2015; Rosemond et al., 2015); thus, sampling year and the nutrient treatments were confounded (particularly in the first step of our statistical analysis; see Section 2.5). The year-to-year differences that were unaccounted for remain a possible explanation for undetectable nutrient, N:P ratio, or nutrient \times temperature interactions in many cases (e.g., for FBOM, leaf litter, N:P ratio effects, and fungal biomass overall). As a result, our findings, particularly those that directly compare pretreatment and enriched conditions, should be interpreted within the context of this caveat. Nonetheless, as noted above, most studies of nutrient and temperature interactions have occurred in microcosms and employed factorial experimental designs to test discrete levels of nutrients and temperature (Fernandes et al., 2014; Ferreira & Chauvet, 2011a). Our multiyear study attempts to extend the questions addressed by these microcosm studies to entire stream reaches, by additionally treating N, P, and temperature as continuous variables, allowing for predictions related to their separate and combined effects (Cottingham, Lennon, & Brown, 2005).

Our sampling of conditioned detritus at different stages of fungal colonization (i.e., associated biomass) and our focus on fungal decomposers conceivably played a role in the lack of synergistic effects of nutrients and temperature. For instance, fungal metabolic

demands may decrease, and the contribution of bacterial decomposers may increase at later stages of decay (Hieber & Gessner, 2002). Further, the established pattern of higher fungal metabolic activity at early stages of colonization (Gessner et al., 2007) coincides with bulk leaf litter input and the coldest season (autumn–winter) in temperate streams (Gessner et al., 2007). Consistent with this, we found highest respiration rates per g fungal biomass during cooler autumn temperatures on leaf litter, but not on wood that enters streams sporadically (Webster et al., 1999). Thus, our measurements of leaf litter fungal activity at higher temperatures potentially overlapped with declining fungal metabolic rates and biomass accrual, or greater investment in reproduction vs. cellular respiration. The separate effects of nutrients and interactive effects of nutrients and temperature might have been stronger during the periods of greater metabolic demands that were possibly missed by measuring naturally occurring detritus (Ferreira & Chauvet, 2011b). Future studies examining nutrients and temperature in whole streams could also measure respiration rates on detritus incubated for known periods of time to capture the peak metabolic demand when stronger effects of nutrients and temperature could occur (Ferreira & Chauvet, 2011b).

4.2 | Comparing the strength of N, P, and temperature effects

The combined effects of increasing N and P concentrations on microbial respiration rates (AFDM-specific) in a model that included scaled temperature were strongest for the most nutrient-poor substrates (leaf litter, wood) compared to FBOM, consistent with our prediction (Table S4). There was little evidence for N or P effects on FBOM, whereas leaf litter responded more to N vs. P, and wood responded the most to P concentrations, consistent with wood being the most P-deficient relative to leaf litter and FBOM (Manning et al., 2016; Stelzer et al., 2003; Tant et al., 2013). The N:P ratio model supported these different N vs. P effects on the three detrital resources, as FBOM and leaf litter respiration rates tended to weakly increase (although not significantly), while wood respiration rates decreased slightly (also not significantly), as a function of N:P ratios observed under enriched conditions (Table 2). Differences in microbial communities associated with FBOM vs. CPOM could also explain their differential responses to nutrients. Fungi dominate microbial communities on leaf litter (95 to >99% of total microbial biomass), while bacterial biomass is much higher than fungal biomass on FBOM (Findlay et al., 2002; Gessner et al., 2007). At the same time, stimulation of fungal biomass and activity by nutrients on wood and leaves is well documented, while bacterial biomass and activity are largely unaffected by nutrients even on leaf litter (Gulis, Suberkropp, & Rosemond, 2008; Gulis et al., 2004; Suberkropp et al., 2010). In contrast to nutrient effects, temperature effects were comparable among the three detritus types, implying that microbial respiration on these different substrates responds similarly to increasing temperatures. These comparisons among N, P, and temperature effects demonstrate that the magnitude of responses to nutrient

enrichment (N + P) and increased temperatures can be comparable for respiration rates associated with coarse detritus.

4.3 | Seasonal vs. manipulated temperatures

Seasonal temperatures in our study varied more than the scope of projected changes to stream temperatures (i.e., 0.5–8°C in the next century; Kaushal et al., 2010), whereas our nutrient treatments were low-to-moderate compared to those presently observed in the continental United States (Alexander & Smith, 2006) and Europe (Woodward et al., 2012). Our seasonal temperature range (~19°C) was comparable to studies conducted in geothermal streams (Friberg et al., 2009; Perkins et al., 2012), but larger than those used in microcosm and whole-system manipulations (+3–10°C; Fernandes et al., 2012; Ferreira & Chauvet, 2011b; Ferreira & Canhoto, 2015; but see Welter et al., 2015). These differences suggest that some caution is required when comparing our findings with data from other studies, especially since we also introduced a nutrient gradient. Further, our findings must be interpreted under the assumption that seasonal dynamics of microbial community structure (which were not measured; also see Section 4 about sampling years and nutrient treatments above) were unchanged, and that microbial decomposers were adapted to seasonal temperatures, but not higher nutrient concentrations (e.g., warm-water “summer” species in July, cold-water “winter” species in January; Bärlocher, 2000; Canhoto et al., 2016; Nikolcheva & Bärlocher, 2005). Several experimental studies have documented temperature-driven shifts in aquatic fungal communities (including a greater relative abundance of warm-water fungal species; Dang, Christian, Schindler, Chauvet, & Gessner, 2009; Ferreira & Chauvet, 2011a; reviewed by Canhoto et al., 2016). Such shifts may drive synergistic responses to temperature and nutrient manipulations due to differences in species-specific temperature optima and nutrient requirements. However, the possibility that microbial communities underwent seasonal structural changes but remained functionally similar may be an explanation for the additive effects of nutrients and temperature observed in this study. The coincidence of the seasonal bulk leaf litter inputs (as discussed earlier) and the highest fungal activity on an areal basis in the coldest season (autumn–winter) in temperate streams may also have important implications for stream ecosystems under climate-change scenarios, especially if warming trends are higher in the winter (Dang et al., 2009; Duarte, Cássio, Ferreira, Canhoto, & Pascoal, 2016; Gulis et al., 2008, IPCC, 2014). Nonetheless, our findings suggest that for naturally occurring, seasonally adapted microbial communities decomposing different detritus types, metabolic activity is stimulated by nutrients. This response was largely independent of stream temperatures (Table 1), pointing to the potential for the effects of elevated nutrient concentrations to be as strong as the effects of temperature.

Understanding the combined effects of two dominant anthropogenic global-change drivers, nutrient enrichment and rising temperatures, on detrital processing in aquatic ecosystems is critical,

given the significant role of inland water networks in the global C cycle (Cole et al., 2007). Our study shows that across seasonal temperature gradients, nutrient enrichment consistently increased respiration rates of coarse detritus (leaf litter, wood), which was likely driven by stimulation of fungal activity via relaxation of nutrient limitation. Respiration rates responded similarly to low-to-moderate nutrient enrichment (relative to the global range of nutrient concentrations) and temperature increases predicted by climate-change scenarios. The temperature dependence of respiration on these substrates was unchanged by nutrient enrichment and was comparable to predictions based on the MTE (Gillooly et al., 2001). Thus, our findings imply that for coarse and fine detritus, respiration rates in stream ecosystems will increase additively in response to these two drivers, and with comparable sensitivity to nutrient enrichment and increased temperature.

ACKNOWLEDGEMENTS

We thank Jason Coombs and Katie Norris for help with maintenance and sampling of the study streams. Phillip Bumpers, Tim Burns, Jason Coombs, Emmy Deng, Jenna Martin, Tom Maddox, Katie Norris, and Louie Schoettle helped in the laboratory or in the field. This study received support from the NSF (DEB-0918894 to ADR, DEB-0918904 to JPB, and DEB-0919054 to VG). This study was also made possible through logistical support from the Coweeta LTER Program at the University of Georgia, which is supported by NSF award DEB-0823293 from the Long Term Ecological Research Program. Rob Case, Daniel Hutcheson, and Kevin Simpson of YSI Integrated Systems and Services constructed the infrastructure for the nutrient-dosing system. Aqueous ammonium nitrate was provided by The Andersons, Inc. through David Plank. We thank Alan Covich and Nina Wurzbarger for helpful comments that improved this manuscript.

CONFLICT OF INTEREST

All authors declare no conflicts of interest.

ORCID

David W. P. Manning  <http://orcid.org/0000-0003-1968-0707>
 Amy D. Rosemond  <http://orcid.org/0000-0003-4299-9353>
 Jonathan P. Benstead  <http://orcid.org/0000-0002-2845-1140>
 John S. Kominoski  <http://orcid.org/0000-0002-0978-3326>

REFERENCES

- Alexander, R. B., & Smith, R. A. (2006). Trends in the nutrient enrichment of US rivers during the late 20th century and their relation to changes in probable stream trophic conditions. *Limnology and Oceanography*, *51*, 639–654.
- Alexander, R. B., Smith, R. A., Schwarz, G. E., Boyer, E. W., Nolan, J. V., & Brakebill, J. W. (2008). Differences in phosphorus and nitrogen

- delivery to the gulf of Mexico from the Mississippi river basin. *Environmental Science & Technology*, 42, 822–830.
- Arbuckle, K. E., & Downing, J. A. (2001). The influence of watershed land use on lake N:P in a predominantly agricultural landscape. *Limnology and Oceanography*, 46, 970–975.
- Arrhenius, S. (1915). *Quantitative laws in biological chemistry*. London, UK: Bell.
- Aufdenkampe, A. K., Mayorga, E., Raymond, P. A., Melack, J. M., Doney, S. C., Alin, S. R., ... Yoo, K. (2011). Riverine coupling of biogeochemical cycles between land, oceans, and atmosphere. *Frontiers in Ecology and the Environment*, 9, 53–60.
- Barley, S. C., & Meeuwig, J. C. (2017). The power and pitfalls of large-scale, unreplicated natural experiments. *Ecosystems*, 20, 331–339.
- Bärlocher, F. (2000). Water-borne conidia of aquatic hyphomycetes: Seasonal and yearly patterns in Catamaran Brook, New Brunswick, Canada. *Canadian Journal of Botany-Revue Canadienne De Botanique*, 78, 157–167.
- Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67, 1–48.
- Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M., & West, G. B. (2004). Toward a metabolic theory of ecology. *Ecology*, 85, 1771–1789.
- Butman, D., & Raymond, P. A. (2011). Significant efflux of carbon dioxide from streams and rivers in the United States. *Nature Geoscience*, 4, 839–842.
- Canhoto, C., Gonçalves, A. L., & Bärlocher, F. (2016). Biology and ecological functions of aquatic hyphomycetes in a warming climate. *Fungal Ecology*, 19, 201–218.
- Cole, J. J., Prairie, Y. T., Caraco, N. F., McDowell, W. H., Tranvik, L. J., Striegl, R. G., ... Melack, J. (2007). Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget. *Ecosystems*, 10, 171–184.
- Cottingham, K. L., Lennon, J. T., & Brown, B. L. (2005). Knowing when to draw the line: Designing more informative ecological experiments. *Frontiers in Ecology and the Environment*, 3, 145–152.
- Cross, W. F., Hood, J. M., Benstead, J. P., Hury, A. D., & Nelson, D. (2015). Interactions between temperature and nutrients across levels of ecological organization. *Global Change Biology*, 21, 1025–1040.
- Dang, C., Christian, K., Schindler, M., Chauvet, E., & Gessner, M. O. (2009). Temperature oscillation coupled with fungal community shifts can modulate warming effects on litter decomposition. *Ecology*, 90, 122–131.
- Dodds, W. K., & Smith, V. H. (2016). Nitrogen, phosphorus, and eutrophication in streams. *Inland Waters*, 6, 155–164.
- Duarte, S., Cássio, F., Ferreira, V., Canhoto, C., & Pascoal, C. (2016). Seasonal variability may affect microbial decomposers and leaf decomposition more than warming in streams. *Microbial Ecology*, 72, 263–276.
- Fernandes, I., Pascoal, C., Guimarães, H., Pinto, R., Sousa, I., & Cássio, F. (2012). Higher temperature reduces the effects of litter quality on decomposition by aquatic fungi. *Freshwater Biology*, 57, 2306–2317.
- Fernandes, I., Seena, S., Pascoal, C., & Cássio, F. (2014). Elevated temperature may intensify the positive effects of nutrients on microbial decomposition in streams. *Freshwater Biology*, 59, 2390–2399.
- Ferreira, V., & Canhoto, C. (2015). Future increase in temperature may stimulate litter decomposition in temperate mountain streams: Evidence from a stream manipulation experiment. *Freshwater Biology*, 60, 881–892.
- Ferreira, V., & Chauvet, E. (2011a). Future increase in temperature more than decrease in litter quality can affect microbial litter decomposition in streams. *Oecologia*, 167, 279–291.
- Ferreira, V., & Chauvet, E. (2011b). Synergistic effects of water temperature and dissolved nutrients on litter decomposition and associated fungi. *Global Change Biology*, 17, 551–564.
- Ferreira, V., Gulis, V., & Graça, M. (2006). Whole-stream nitrate addition affects litter decomposition and associated fungi but not invertebrates. *Oecologia*, 149, 718–729.
- Ferreira, V., Gulis, V., Pascoal, C., & Graça, M. A. S. (2014). Stream pollution and fungi. In G. Jones, K. Hyde, & K.-L. Pang (Eds.), *Freshwater fungi and fungus-like organisms* (pp. 389–412). Riyadh, Saudi Arabia: De Gruyter.
- Fierer, N., Craine, J., McLauchlan, K., & Schimel, J. (2005). Litter quality and the temperature sensitivity of decomposition. *Ecology*, 86, 320–326.
- Findlay, S., Tank, J., Dye, S., Valett, H. M., Mulholland, P. J., McDowell, W. H., ... Bowden, W. B. (2002). A cross-system comparison of bacterial and fungal biomass in detritus pools of headwater streams. *Microbial Ecology*, 43, 55–66.
- Follstad Shah, J. J., Kominoski, J. S., Ardón, M., Dodds, W. K., Gessner, M. O., Griffiths, N. A., ... Zeglin, L. H. (2017). Global synthesis of the temperature sensitivity of leaf litter breakdown in streams and rivers. *Global Change Biology*, 23, 3064–3075.
- Friberg, N., Dybkjaer, J. B., Ólafsson, J. S., Gíslason, G. M., Larsen, S. E., & Lauridsen, T. L. (2009). Relationships between structure and function in streams contrasting in temperature. *Freshwater Biology*, 54, 2051–2068.
- Gelman, A., & Hill, J. (2007). *Data analysis using regression and multilevel/hierarchical models*. Cambridge: Cambridge University Press.
- Gessner, M. O., & Chauvet, E. (1993). Ergosterol-to-biomass conversion factors for aquatic hyphomycetes. *Applied and Environmental Microbiology*, 59, 502–507.
- Gessner, M. O., Gulis, V., Kuehn, K. A., Chauvet, E., & Suberkropp, K. (2007). Fungal decomposers of plant litter in aquatic ecosystems. In C. P. Kubicek & I. S. Druzhinina (Eds.), *The Mycota* (pp. 301–324). Berlin: Springer.
- Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M., & Charnov, E. L. (2001). Effects of size and temperature on metabolic rate. *Science*, 293, 2248–2251.
- Gulis, V., Rosemond, A. D., Suberkropp, K., Weyers, H. S., & Benstead, J. P. (2004). Effects of nutrient enrichment on the decomposition of wood and associated microbial activity in streams. *Freshwater Biology*, 49, 1437–1447.
- Gulis, V., & Suberkropp, K. (2003). Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. *Freshwater Biology*, 48, 123–134.
- Gulis, V., & Suberkropp, K. (2006). Fungi: Biomass, production, and sporulation of aquatic hyphomycetes. In F. R. Hauer, & G. A. Lamberti (Eds.), *Methods in stream ecology* (pp. 311–325). San Diego, CA: Academic Press.
- Gulis, V., Suberkropp, K., & Rosemond, A. (2008). Comparison of fungal activities on wood and leaf litter in unaltered and nutrient-enriched headwater streams. *Applied and Environmental Microbiology*, 74, 1094–1101.
- Hall, R. O., Tank, J. L., Baker, M. A., Rosi-Marshall, E. J., & Hotchkiss, E. R. (2016). Metabolism, gas exchange, and carbon spiraling in rivers. *Ecosystems*, 19, 73–86.
- Hieber, M., & Gessner, M. O. (2002). Contribution of stream detritivores, fungi, and bacteria to leaf breakdown based on biomass estimates. *Ecology*, 83, 1026–1038.
- Hotchkiss, E., Hall, R., Sponseller, R., Butman, D., Klaminder, J., Laudon, H., ... Karlsson, J. (2015). Sources of and processes controlling CO₂ emissions change with the size of streams and rivers. *Nature Geoscience*, 8, 696–699.
- IPCC. (2013). *Climate change 2013: The physical science basis*. In T. F. Stocker, D. Qin, G.-K. Plattner, M. M. B. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex, & P. M. Midgley (Eds.), *Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change*. Cambridge, UK: Cambridge University Press.

- IPCC. (2014). Climate change 2014: Impacts, adaptation, and vulnerability. In C. B. Field, V. R. Barros, D. J. Dokken, K. J. Mach, M. D. Mastrandrea, T. E. Bilir, M. Chatterjee, K. L. Ebi, Y. O. Estrada, R. C. Genova, B. Girma, E. S. Kissel, A. N. Levy, S. MacCracken, P. R. Mastrandrea, & L. L. White (Eds.), *Contribution of working group II to the fifth assessment report of the intergovernmental panel on climate change*. Cambridge, UK: Cambridge University Press.
- Jankowski, K., Schindler, D. E., & Lisi, P. J. (2014). Temperature sensitivity of community respiration rates in streams is associated with watershed geomorphic features. *Ecology*, *95*, 2707–2714.
- Kaushal, S. S., Likens, G. E., Jaworski, N. A., Pace, M. L., Sides, A. M., Seckell, D., ... Wingate, R. L. (2010). Rising stream and river temperatures in the United States. *Frontiers in Ecology and the Environment*, *8*, 461–466.
- Kominoski, J. S., Rosemond, A. D., Benstead, J. P., Gulis, V., & Manning, D. W. P. (2017). Experimental nitrogen and phosphorus additions increase rates of stream ecosystem respiration and carbon loss. *Limnology and Oceanography*, in press. <https://doi.org/10.1002/lno.10610>
- Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2016). lmerTest: Tests in linear mixed effects models. R package version 2.0-33. <https://CRAN.R-project.org/package=lmerTest>
- Manning, D. W. P., Rosemond, A. D., Gulis, V., Benstead, J. P., Kominoski, J. S., & Maerz, J. C. (2016). Convergence of detrital stoichiometry predicts thresholds of nutrient-stimulated breakdown in streams. *Ecological Applications*, *26*, 1745–1757.
- Manning, D. W. P., Rosemond, A. D., Kominoski, J. S., Gulis, V., Benstead, J. P., & Maerz, J. C. (2015). Detrital stoichiometry as a critical nexus for the effects of streamwater nutrients on leaf litter breakdown rates. *Ecology*, *96*, 2214–2224.
- Nakagawa, S., & Cuthill, I. C. (2007). Effect size, confidence interval and statistical significance: A practical guide for biologists. *Biological Reviews*, *82*, 591–605.
- Nakagawa, S., & Schielzeth, H. (2013). A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, *4*, 133–142.
- Nikolcheva, L. G., & Bärlocher, F. (2005). Seasonal and substrate preferences of fungi colonizing leaves in streams: Traditional versus molecular evidence. *Environmental Microbiology*, *7*, 270–280.
- Peñuelas, J., Sardans, J., Rivas-Ubach, A., & Janssens, I. A. (2012). The human-induced imbalance between C, N and P in Earth's life system. *Global Change Biology*, *18*, 3–6.
- Perkins, D. M., Yvon-Durocher, G., Demars, B. O. L., Reiss, J., Pichler, D. E., Friberg, N., ... Woodward, G. (2012). Consistent temperature dependence of respiration across ecosystems contrasting in thermal history. *Global Change Biology*, *18*, 1300–1311.
- R Core Team. (2016). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from <http://www.R-project.org/>
- Rosemond, A. D., Benstead, J. P., Bumpers, P. M., Gulis, V., Kominoski, J. S., Manning, D. W. P., ... Wallace, J. B. (2015). Experimental nutrient additions accelerate terrestrial carbon loss from stream ecosystems. *Science*, *347*, 1142–1145.
- Rosemond, A. D., Pringle, C. M., Ramirez, A., Paul, M. J., & Meyer, J. L. (2002). Landscape variation in phosphorus concentration and effects on detritus-based tropical streams. *Limnology and Oceanography*, *47*, 278–289.
- Scott, M. C., Helfman, G. S., McTammany, M. E., Benfield, E. F., & Bolstad, P. V. (2002). Multiscale influences on physical and chemical stream conditions across blue ridge landscapes. *Journal of the American Water Resources Association*, *38*, 1379–1392.
- Sinsabaugh, R. L., & Follstad Shah, J. J. (2012). Ecoenzymatic stoichiometry and ecological theory. *Annual Review of Ecology, Evolution, and Systematics*, *43*, 313–343.
- Stelzer, R. S., Heffernan, J., & Likens, G. E. (2003). The influence of dissolved nutrients and particulate organic matter quality on microbial respiration and biomass in a forest stream. *Freshwater Biology*, *48*, 1925–1937.
- Suberkropp, K., Gulis, V., Rosemond, A. D., & Benstead, J. P. (2010). Ecosystem and physiological scales of microbial responses to nutrients in a detritus-based stream: Results of a 5-year continuous enrichment. *Limnology and Oceanography*, *55*, 149–160.
- Swank, W. T., & Crossley, D. A. (1988). *Forest hydrology and ecology at Coweeta*. New York, NY, USA: Springer-Verlag.
- Tant, C. J., Rosemond, A. D., & First, M. R. (2013). Stream nutrient enrichment has a greater effect on coarse than on fine benthic organic matter. *Freshwater Science*, *32*, 1111–1121.
- Wallace, J. B., Hutchens, J. J., & Grubaugh, J. W. (2006). Transport and storage of FPOM. In F. R. Hauer, & G. A. Lamberti (Eds.), *Methods in stream ecology* (pp. 249–271). San Diego, CA: Academic Press.
- Webster, J. R., Benfield, E. F., Ehrman, T. P., Schaeffer, M. A., Tank, J. L., Hutchens, J. J., & D'Angelo, D. J. (1999). What happens to allochthonous material that falls into streams? A synthesis of new and published information from Coweeta. *Freshwater Biology*, *41*, 687–705.
- Welter, J. R., Benstead, J. P., Cross, W. F., Hood, J. M., Huryn, A. D., Johnson, P. W., & Williamson, T. J. (2015). Does N_2 fixation amplify the temperature dependence of ecosystem metabolism? *Ecology*, *96*, 603–610.
- Woodward, G., Gessner, M. O., Giller, P. S., Gulis, V., Hladysz, S., Lecerf, A., ... Chauvet, E. (2012). Continental-scale effects of nutrient pollution on stream ecosystem functioning. *Science*, *336*, 1438–1440.
- Yvon-Durocher, G., Caffrey, J. M., Cescatti, A., Dossena, M., del Giorgio, P., Gasol, J. M., ... Allen, A. P. (2012). Reconciling the temperature dependence of respiration across timescales and ecosystem types. *Nature*, *487*, 472–476.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Manning DWP, Rosemond AD, Gulis V, Benstead JP, Kominoski JS. Nutrients and temperature additively increase stream microbial respiration. *Glob Change Biol*. 2017;00:1–15. <https://doi.org/10.1111/gcb.13906>