Contrasting activation energies of litter-associated respiration and P uptake drive lower cumulative P uptake at higher temperatures

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Abstract. Heterotrophic microbes play key roles in regulating fluxes of energy and nutrients, which are increasingly affected by globally changing environmental conditions such as warming and nutrient enrichment. While the effects of temperature and nutrients on microbial mineralization of carbon have been studied in some detail, much less attention has been given to how these factors are altering uptake rates of nutrients. We used laboratory experiments to simultaneously evaluate the temperature dependence of soluble reactive phosphorus (SRP) uptake and respiration by leaf litter-associated microbial communities from temperate headwater streams. Additionally, we evaluated the influence of the initial concentration of SRP on the temperature dependence of P uptake. Finally, we used simple simulation models to extrapolate our results and estimate the effect of warming and P availability on cumulative gross uptake. We found that the temperature dependence of P uptake was lower than that of respiration (0.48 vs. 1.02 eV). Further, the temperature dependence of P uptake increased with the initial concentration of SRP supplied, ranging from 0.12 to 0.48 eV over an 11 to 212 µg L^{-1} gradient in initial SRP concentration. Finally, despite our laboratory experiments showing increases in mass-specific rates of gross P uptake with temperature, our simulation models found declines in cumulative P uptake with warming, because the increased rates of respiration at warmer temperatures more rapidly depleted benthic carbon substrates and consequently reduced the biomass of the benthic microbial community. Thus, even though mass-specific rates of P uptake were higher at the warmer temperatures, cumulative P uptake was lower over the residence time of a pulsed input of organic carbon. Our results highlight the need to consider the combined effects of warming, nutrient availability, and resource availability/magnitude on carbon processing as important controls of nutrient processing in heterotrophic ecosystems.

1 Introduction

Microbial communities regulate ecosystem nutrient cycling and retention through their uptake and mineralization of nutrients (Burgin et al., 2011; Brookshire et al., 2011). Thus, any environmental factor that affects cell nutrient quotas, biomass,

or production of microorganisms can influence rates of ecosystem nutrient processing (Cross et al., 2005, 2015). Notably, increases in nutrient concentrations and temperature are both expected to increase rates of microbial metabolism and growth (Brown et al., 2004; Sterner and Elser, 2002), and such increases are being observed across human-influenced landscapes (Kaushal et al., 2010; Stets et al., 2020). Any increase in microbial community metabolism should be associated with higher demand for nutrients, as measured by gross nutrient uptake at the ecosystem level (Hall and Tank, 2003). In autotrophic systems, increases in temperature drive increases in gross primary production, resulting in predictably higher demand for nutrients (Rasmussen et al., 2011); however, in donor-controlled detrital systems, such as soils and forest streams, increased rates of metabolism stimulated by increases in temperature or nutrients can lead to reductions in pools of the dead organic matter that fuels metabolism, eventually reducing microbial biomass on an areal basis(Walker et al., 2018; Suberkropp et al., 2010). Thus, long-term responses of nutrient uptake to higher temperatures and nutrient concentrations are challenging to parse in detritus-dominated ecosystems, as mass-specific rates of uptake may increase even as total microbial biomass declines, complicating net ecosystem responses.

Mechanisms explaining the joint effects of temperature and nutrients on mass-specific rates of nutrient uptake (U) remain poorly resolved. Temperature may cause increases in nutrient demand that directly match increases in metabolism (Allen and Gillooly, 2009). Alternatively, increases in nutrient demand may deviate from metabolism for two reasons. First, temperature may influence the nutrient use efficiency of microbes. For example, algae can use nutrients more efficiently at higher temperatures, expressed as an increase in the ratio of carbon (C) to nitrogen (N) or phosphorus (P) in their biomass (Thrane et al., 2017; De Senerpont Domis et al., 2014; Yvon-Durocher et al., 2015). Bacteria and fungi can also exhibit variation in their demand for nutrients relative to their carbon demand (Gulis et al., 2017; Scott et al., 2012), but it is unknown whether their biomass stoichiometry varies systematically with temperature (Cross et al., 2015). If bacteria and fungi also increase their nutrient use efficiency in response to rising temperatures, temperature may increase metabolism and respiration more than U (Hood et al., 2018). Second, basal metabolic costs may increase with warming. As a consequence of increased basal metabolic costs, the carbon use efficiency (biomass produced relative to carbon assimilated) of heterotrophic microbes may decline with increasing temperature (Manzoni et al., 2012; Li et al., 2019; Doi et al., 2010). Decreased carbon use efficiency implies an increase in carbon use relative to nutrient demand if stoichiometry remains fixed. Despite differences in mechanism, both declines in carbon use efficiency and increases in nutrient use efficiency imply a greater increase in demand for carbon than for nutrients at higher temperatures.

Responses of nutrient uptake to higher nutrient concentrations are also potentially complex. Uptake of nutrients is often limited by the concentration of dissolved nutrients (Mulholland et al. 2008). As nutrient concentrations increase, uptake rates typically increases to a plateau (Dodds et al., 2002). At low nutrient concentrations, uptake is generally limited by the encounter rate between nutrient molecules and cell membranes; at high concentrations, uptake is instead limited by the rate of transfer of nutrients across cell membranes. These dynamics are generally described by Michaelis-Menten kinetics

(Weigelhofer et al., 2018). Consequently, the proportion of dissolved nutrients taken up by the microbial community may decline with increasing nutrient concentration (O'Brien et al., 2007). Organismal measurements of nutrient use efficiency have also demonstrated that increasing nutrient supply relative to carbon leads to less efficient use of nutrients, as demonstrated by lower biomass C:nutrient content (Godwin and Cotner, 2015). Lastly, the combined effects of nutrients and temperature on U may be additive or nutrient concentration and temperature may interact to determine growth and uptake rates (Cross et al., 2015; Davidson et al., 2012). However, to date there is little evidence that the effects of nutrients and temperature are strongly interactive, at least in detritus-based systems (Manning et al., 2018).

Regardless of temperature and nutrient availability, ecosystem-level nutrient uptake is also a function of substrate availability and total microbial biomass. Much of the benthic metabolism in forest streams and soils is supported by inputs of allochthonous organic matter, and particularly leaf litter from the terrestrial environment (Tank et al., 2018; Wallace et al., 2015). In temperate ecosystems with deciduous vegetation, there is strong seasonality in the input of senescent leaf litter. This finite supply of litter is subsequently depleted by the activity of microbial and animal consumers (Wallace et al., 2015; Webster and Tank, 2000; Marks, 2019). While increased temperature and nutrients stimulate mass-specific rates of metabolism, they also accelerate the loss of benthic carbon, which eventually reduces microbial biomass at the ecosystem level (Walker et al., 2018; Suberkropp et al., 2010). The importance of these dynamics for rates of ecosystem nutrient uptake and metabolism have been illustrated empirically; studies have found an apparent negative effect of temperature on nutrient uptake that is partially driven by seasonal changes in microbial biomass in forest streams (Hoellein et al., 2007; Valett et al., 2008), which tends to peak in the winter after leaf litter inputs have entered the stream, and then decline in the summer as the pulse of detrital carbon is depleted (Suberkropp et al., 2010). While these studies have illustrated the importance of carbon standing stocks as a control of ecosystem nutrient uptake, the consequences of increased temperature and nutrient concentration for cumulative nutrient uptake remain unexplored. Because the seasonal supply of carbon in forest stream ecosystems is finite within an annual cycle, the cumulative amount of nutrient uptake over the residence time of the detritus is important to consider, though challenging to evaluate empirically.

Here, we quantify how stream temperature and the concentration of soluble reactive P (SRP) affect gross uptake of P (U_{srp}) by leaf litter-associated microorganisms in forested headwater streams and evaluate whether increases in U_{srp} match warming-induced increases in metabolic rates (measured as respiration). We hypothesized that higher temperatures would drive increased respiration rates and U_{srp}, though we expected that U_{srp} would increase less with temperature than respiration due to changes in carbon or nutrient use efficiency. We also hypothesized that the temperature dependence of U_{srp} would vary based on the concentration SRP supplied, with low concentrations of SRP constraining the temperature dependence of U_{srp} (Cross et al. 2015). Further, we hypothesized that temperature would modify relationships between nutrient concentration and U_{srp}. Specifically, we expected that higher temperatures would increase maximum uptake rates while decreasing the half-saturation constants of Michaelis-Menten models (Cross et al. 2015). To test these hypotheses, we quantified the temperature

dependence of U_{srp} in laboratory experiments, tested whether this temperature dependence varied across nutrient concentrations, and compared it to the temperature dependence of respiration. Finally, we hypothesized that, if the temperature

95 dependence of respiration is greater than that of U_{srp}, the consequence would be a reduction in cumulative U_{srp} over the residence time of a pulsed leaf litter input, caused by faster loss of leaf-associated carbon at higher temperatures. To test this, we used simple simulation models to extrapolate our measured effects of temperature on carbon processing and U_{srp} and quantified the effect of warming on cumulative U_{srp} over the residence time of a single seasonal input of leaf litter.

2 Methods

100 ### 2.1 Comparing the temperature dependences of SRP uptake and respiration

We conditioned leaf litter for these experiments at the United States Department of Agriculture Forest Service Southern Research Station Coweeta Hydrologic Laboratory (CHL) in the southern Appalachian Mountains, Macon County, North Carolina, USA (see Swank and Crossley [1988] for site information). We incubated *Rhododendron maximum* (hereafter, *Rhododendron*) leaf litter to allow for microbial colonization in Watershed 5a in 5-mm mesh litterbags for 114 days beginning

105 on 17 November 2020. We removed a subset of the bags on 11 March 2021 and returned them to the laboratory, where we cut the leaves into fragments approximately 1.5cm × 1.5cm. We placed these fragments in 1-L bottles full of aerated stream water, which we incubated in water baths at five different temperatures (4, 8, 12, 16, 20°C). Each water bath contained three bottles, which we consider replicates, though we acknowledge the bottles are not fully independent. After we acclimated the microbial communities for 24 h, we removed leaf fragments from the bottles to measure either their gross SRP uptake or respiration rate

110 (see below). We repeated this procedure (only the 4°C to 16°C temperature treatments) on 18 March 2021 and pooled the results for analysis.

We used three subsamples from each replicate bottle to measure respiration rates. To estimate respiration rates, we filled 20-ml scintillation vials with stream water at the appropriate treatment temperature and measured the initial concentration of oxygen using a YSI 5100 Dissolved Oxygen Meter (YSI Inc, OH, USA). After measuring initial concentrations of oxygen,

115 we immediately replaced the water that was displaced during the initial measurement (~15% of the volume) with stream water from the same bottle that was initially used to fill the vial. Then, we added several leaf fragments (similar amounts among vials) to the vial and secured the cap such that no air remained in the vial. We prepared three blanks (water but no leaves added) along with the samples in each temperature treatment. We then returned the vials to the water bath to incubate in the dark for 2 to 7 h, giving the vials in colder temperatures more time to incubate to ensure meaningful changes in the

120 concentration of dissolved oxygen. After incubation, we recorded the final concentration of dissolved oxygen, removed the leaves, dried them to a constant mass, and weighed them. We calculated respiration rates (mg O_2 hr^{-1} mg^{-1}) based on the difference in the mass of oxygen in the vial before ($O_{2\text{-pre}}$, mg) and after the incubation ($O_{2\text{-post}}$, mg), minus the change in

oxygen in the blanks ($O_{2\text{-pre-blank}}$ and $O_{2\text{-post-blank}}$), divided by incubation time (T, h) and the dry mass of leaves in the vial (M, g, equation 1).

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$$Respiration\ rate = \frac{(O_{2-pre}-O_{2-post})-(O_{2-pre-blank}-O_{2-post-blank})}{T*M} \qquad (\qquad\qquad \text{(Equ. 1)}$$

We also used three different subsamples from each replicate bottle to measure rates of U_{srp} simultaneously with the measurements of respiration. We amended reservoirs of stream water at each temperature with nutrients to elevate concentrations from <5 ug L^{-1} to ~30 to 60 µg L^{-1} SRP. We then dispensed 40 mL of this nutrient-amended water into 50-mL centrifuge tubes, and added several leaf fragments. Three blanks (i.e., water with no leaves added) were prepared along with

130 each temperature treatment. After 2 to 7 hours of incubation, we removed a subsample of the water with a syringe and filtered it through an AE-grade glass fiber filter (nominal pore size 1.0-µm, Sterilitech, WA, USA), and immediately froze the sample for preservation. We determined SRP concentrations using an Alpkem Rapid Flow Analyzer 300 (Alpkem, College Station, Texas, USA). We retained leaf fragments, dried them to a constant mass, and weighed them. We calculated U_{srp} as the difference in the mass of SRP between the mean of the blanks (P_{blank}, µg P) and each subsample (P_{sample}, µg P), normalized to

135 the dry mass of leaves and the incubation time (equation 2).

$$U_{srp} = \frac{P_{sample}-P_{blank}}{T*M} \qquad\qquad \text{(Equ. 2)}$$

To quantify the effects of temperature on rates of respiration and $U_{srp,}$ we estimated their activation energies (E_a) using the Boltzmann-Arrhenius equation (equation 3, Brown et al., 2004), where the rate of the process (r_i) is a function of the rate at a reference temperature (r_{ref}), the activation energy (E_a), the temperature in kelvin (T), and the Boltzmann constant (k_B;

140 8.617×10^{-5} eV K^{-1}). We averaged the subsample measurements from each bottle and fit our data to the linearized version of the Boltzmann-Arrhenius equation, with temperature centered on a standard temperature (T_{12}, 12ºC), by regressing the $\log_e$-

transformed process rates against the standardized Boltzmann temperature (equation 3), and estimating the E_a based on the slope of this line (equation 4).

$$r_i = r_{ref} * e^{\frac{-Ea}{k_B*T}},$$ (Equ. 3)

$$\ln(r_i) = \ln(r_{12}) + \frac{1}{k_B*T_{12} - k_B*T} * -E_a,$$ (Equ. 4)

The two dates on which the experiment was run may have had different biological or environmental conditions, so we included a categorical effect of date in our statistical models to account for any differences. To evaluate whether the responses of respiration and U_{SRP} to temperature were different we used an ANCOVA-type linear model. To do this, we fit a linear model that described the $\log_e$-transformed rates of respiration and uptake rates as a function of the standardized Boltzmann temperature. The model included an interaction between temperature and a binary variable that indicated the type of rate (i.e., respiration or uptake). A significant interaction term in this model indicates that the slopes of the relationships between temperature and these rates differ. Finally, as an alternative way to evaluate relative differences in metabolism and P demand, we converted mass-based units of O_2 and SRP to their molar equivalents, and converted oxygen to units of C assuming a respiratory quotient of 0.85 (moles CO_2 produced per mole O_2 consumed; Bott 2006). Then, we calculated the molar ratio of C respired to U_{srp}, which we report as the C:P of respiration to uptake. We tested the effects of temperature on the $\log_e$-transformed molar ratio, using the centered inverse Boltzmann temperature as the predictor variable.

2.2 **Effect of nutrient concentration on temperature dependence of SRP uptake**

We conducted a separate experiment to test whether the initial concentration of nutrients affected the temperature dependence of nutrient uptake. We incubated *Acer rubrum* (hereafter, *Acer*) leaves in Lower Hugh White Creek at the CHL for approximately 30 d during summer 2019 and then returned the leaves to the laboratory. We used a shorter incubation time for *Acer* than for *Rhododendron* due to higher environmental temperatures and generally more rapid colonization of this more labile litter species. We added several whole leaves to 250-mL Nalgene bottles with 200 mL water and incubated them for approximately 3 h. Leaves were incubated at six temperatures ranging from 4ºC to 21ºC and eight initial SRP concentrations ranging from 11 to 217 µg L^{-1} that were created by adding a concentrated solution of KH_2PO_4 to the stream water. After incubation, we removed a subsample of water with a syringe, filtered it, and froze it immediately to preserve the sample. We then analyzed the water samples for SRP using a spectrophotometer (Shimadzu UV-1700) and the ascorbic acid method (APHA, 1995). Each temperature and concentration combination had two replicates and one blank that did not have leaves added. Leaf fragments incubated in each bottle were dried and weighed after the incubations as above.

We calculated U_{srp} in the same manner described above (equation 2). We then used two techniques to quantify how the initial concentration of nutrients and temperature interacted to affect rates of U_{srp}. First, we grouped the data based on the initial concentration and estimated the temperature dependence of U_{srp} at each initial nutrient concentration. We estimated the effect of temperature using the linearized version of the Boltzmann-Arrhenius equation (equation 4), by regressing the log_e-transformed U_{srp} rates against the centered inverse Boltzmann temperature, and estimated the E_a based on the slope of this line. Then, we evaluated the effect of the initial concentration of SRP on the temperature dependence of U_{srp} by estimating the slope of the relationship between initial SRP concentration and the activation energy of U_{srp} at each concentration, evaluating both a linear and saturation response of the activation energy of U_{srp} to temperature. In a second analysis of the same data, we grouped the data by temperature and estimated the effect of changes in initial nutrient concentration at different temperatures. We fit models of Michaelis-Menten kinetics to nutrient concentration and U_{srp} at each temperature, in which we modeled U_{srp} as a function of initial SRP concentration [SRP] and two parameters, the maximum uptake rate (U_{max}) and the half-saturation constant (k_m, equation 5):

$$U_{srp} = \frac{[SRP] * U_{max}}{[SRP] + k_m} \qquad \text{(Equ. 5)}$$

We then evaluated the influence of temperature on the Michaelis-Menten parameters using the framework of metabolic theory. We regressed log_e-transformed values of k_m and U_{max} against the standardized Boltzmann temperature to estimate the activation energy of each of these parameters.

2.3 Simulating the direct and indirect effects of temperature and enrichment on SRP uptake

We used a simple simulation model to evaluate how temperature and SRP concentration affect cumulative U_{srp} over the residence time of a pulsed leaf litter input. These simulations consider both the direct effects of SRP concentration and temperature on mass-specific U_{srp} and the indirect effects mediated through depletion of litter-associated carbon. These simulations were designed to illustrate the dynamic consequences of our laboratory measurements and inform a more comprehensive representation of carbon and nutrient cycles in forested streams. The simulated stream reach starts with 315 g leaf C m^{-2}, which is based on observations of leaf standing stocks in streams at CHL (Suberkropp et al., 2010). Mass-specific rates of leaf mass loss were estimated as a function of temperature and, in some scenarios, nutrient concentration (see below for details on scenarios). We estimated mass-specific rates of U_{srp} as a function of temperature and SRP concentration using data from our experiments or from the literature (see below). We then calculated areal rates of gross SRP uptake as the product of mass-specific U_{srp} and the areal mass of C remaining in the stream. For both rates of U_{srp} and respiration we converted mass-specific rates from units of dry-mass to units of carbon assuming an average leaf carbon content of 45%. We report cumulative U_{srp}, when 99% of the leaves were consumed by microbial metabolism.

We considered the effects of warming and nutrient enrichment on cumulative U_{srp} in four scenarios (Table 1); in each scenario we evaluated the effect of warming using a low temperature of 10°C and a high temperature of 14°C. First, we considered the effect of warming on cumulative U_{srp} when both respiration and uptake have the same temperature dependence

of 0.65 eV (Brown et al., 2004). In this model, we used estimates of r_{ref} of respiration from our first experiment and r_{ref} of U_{srp} from our 19 µg L^{-1} treatment (i.e., a low-to-moderate concentration). Second, we simulated U_{srp} and respiration using our measured temperature dependence values, using the temperature dependence of respiration from our first experiment and the measured temperature dependence of U_{srp} from the 19 µg L^{-1} treatment in our second experiment. Third, we simulated uptake at a higher nutrient concentration, using the temperature dependence of U_{srp} from the 111 µg L^{-1} treatment in our second experiment and the temperature dependence of respiration from our first experiment. Fourth, we simulated uptake with our estimates of U_{srp} at the high concentration of 111 µg L^{-1}, and included a factor to account for the effect of nutrient enrichment on respiration of 1.32× (Manning et al., 2018). We propagate uncertainty in our parameter estimates of temperature dependences by bootstrapping our estimates of cumulative U_{srp} 1000 times and compare outcomes of the simulations to estimate effect sizes. We do not include statistical analysis of the outcomes of these simulations.

3 Results

3.1 Comparing the temperature dependences of SRP uptake and respiration

We estimated an E_a of respiration during the laboratory experiment of 1.02 eV (SE 0.06), which is higher than the established canonical value for cellular respiration (0.60 - 0.70 eV; Brown et al. 2004, Figure 1a). We estimated an E_a of U_{srp} of 0.48 eV (SE 0.05), which was significantly lower than the E_a of respiration (estimated difference in E_a = 0.48, SE 0.09, $F_{1,48}$ = 28.22, P <0.0001, Figure 1a, b). Thus, there was a significant increase in the ratio of carbon respired relative to U_{srp} (Figure 1c, Table 2), which increased with an Ea of 0.54 eV (SE 0.08). In back-transformed units, this effect roughly translates to an increase in the C:P of respiration to uptake of 2.54 moles of C per mole of P with a one-degree increase in temperature (Figure 1c).

3.2 Effect of nutrient concentration on temperature dependence of SRP uptake

Temperature and the initial concentration of SRP both played an important role in determining rates of nutrient uptake (Figure 2). The E_a of U_{srp} was greater at higher concentrations (Figure 2, Table 2), though the rate of increase was greater at low concentrations and saturated at higher nutrient concentrations (Figure 2). We found that temperature influenced patterns of SRP uptake across nutrient concentrations (Figure 3, Table 2). Temperature increased U_{max}, with an Ea of 0.55 eV (SE 0.16) but did not have a detectable effect on k_m (Figure 3).

3.3 Simulating the direct and indirect effects of temperature and enrichment on SRP uptake

Across all simulations, warmer temperatures consistently reduced cumulative U_{srp} (Figure 4a). The reductions in cumulative U_{srp} were a direct consequence of the accelerated loss of leaf-associated carbon, which outweighed the effect of increased mass-specific rates of U_{srp} later in the simulations (Figure 4b). While warming reduced cumulative U_{srp} in each simulation, the magnitude of the reduction depended on both the nutrient concentration and the temperature dependence parameters we used to simulate mass-specific rates of U_{srp} and respiration. Our simulations that had the same activation energy

for both respiration and U_{srp}, projected 0.81× the cumulative U_{srp} in the warm scenario (14ºC) compared to the cold scenario (10ºC) (Figure 4a). However, when we simulated these processes using measured activation energies of respiration and U_{srp} measured at the low SRP concentration, we found that the effect of warming was greater, with cumulative U_{srp} in the warm scenario equal to 0.62× that in the cool scenario (Figure 4a). At the higher SRP concentration, the absolute effect of warming on cumulative P uptake was greater than at the lower concentration (i.e., absolute differences of 4.7 vs. 12.0 g SRP m^{-2}, Figure 4a). However, the relative effect of warming on P uptake was smaller at the higher nutrient concentration, with cumulative uptake in the warm scenario 24% lower than in the cool scenario regardless of the effect of enrichment on respiration (Figure 4a).

Increases in nutrient concentration increased cumulative U_{srp} in our simulations. In the cool scenario, cumulative U_{srp} was 4× higher at the higher SRP concentration (Figure 4a). Similarly, in the warm scenario, cumulative U_{srp} was 4.9× higher at the high compared to low nutrient concentration (Figure 4a). These differences in cumulative U_{srp} due to differences in concentration were somewhat smaller when we included the effect of nutrient enrichment on respiration, falling to 3× and 3.7×, respectively, at the low and high temperature (Figure 4a).

4 Discussion

We observed a lower activation energy for U_{srp} than for respiration in our experiment, indicating the potential for shifts in carbon and nutrient processing as temperatures increase in forest streams. Additionally, we found that the temperature dependence of U_{srp} increased as the concentration of SRP supplied increased. Simulated estimates of cumulative U_{srp} highlighted that, even though temperature increased instantaneous rates of U_{srp}, the indirect effect of temperature on benthic carbon standing stocks led to lower cumulative U_{srp} at higher temperatures. Together our results highlight that warming will likely alter rates of gross nutrient uptake in forest streams, but the magnitude and direction of these effects may depend on the spatial and temporal scale of interest, as well as the nature of the carbon resources available.

Our finding that U_{srp} increased less with temperature than microbial metabolism is in concordance with findings from other ecosystems. In a field experiment, Hood et al. (2018) found that stream warming increased primary production almost three-fold, while it had no measurable effect on rates of nutrient uptake. This was attributed to increases in the efficiency of nutrient recycling, mineralization, and N$_2$ fixation (Hood et al., 2018). In our leaf-microbe system, factors such as increased nutrient recycling, or an increasing proportion of nutrient demand being satisfied through "mining" of leaf nutrients, may explain the reduced temperature dependence of U_{srp} that we observed. Additionally, some of the increased respiration with temperature may be due to an increase in basal metabolic costs, which would not require a matched increase in nutrient demand (Manzoni et al., 2012; Li et al., 2019; Doi et al., 2010). Nutrient demands of heterotrophs may also shift with higher temperatures. In our litter-microbe system, fungi are of particular interest as they dominate leaf microbial communities (Findlay et al., 2002). A previous study found that the elemental content of *Agaricomycetes* fruiting bodies was correlated with environmental temperature, with temperature increasing their biomass C:P (Zhang and Elser, 2017). Additionally, the ratio of

C respired through respiration to P taken up in this study is low relative to the mean biomass C:P of fungi and bacteria (Godwin and Cotner, 2014; Zhang and Elser, 2017). This likely indicates some luxury uptake of SRP, which is known to occur at the ecosystem scale (Payn et al., 2005) and within fungal tissue (Gulis et al., 2017) when nutrient concentrations are temporarily elevated.

Nominally, our finding that temperature increases mass-specific U_{srp} is counter to previous examinations of the effect of temperature on rates of nutrient uptake in forest streams (Hoellein et al., 2007; Valett et al., 2008). However, while these previous studies found negative effects of temperature on nutrient uptake, their results highlighted the dominant role of microbial biomass as a control on nutrient uptake. In forest streams, biomass of the microbial community is tightly linked to the standing stock of detrital carbon, which varies inversely with seasonal temperatures in temperate forest streams (Hoellein et al., 2007; Valett et al., 2008; Suberkropp et al., 2010). While these studies potentially illustrate the role of heterotrophic microbial biomass in nutrient uptake, the observed winter peaks in nutrient uptake in these studies may be driven in part by increased autotrophic production allowed by a relatively open canopy during winter. The importance of both direct physiological and indirect biomass-mediated effects of temperature on ecosystem processes has been appreciated in detritus-based systems (e.g., Wilmot et al., 2021). However, our study is the first to separate the contribution of these two processes to patterns of long-term cumulative nutrient uptake. Specifically, when we considered only the direct effect of temperature on mass-specific rates of U_{srp}, we inferred that U_{srp} increases with temperature. However, when we incorporated the effect of temperature on respiration we found that this indirect effect of warming decreased cumulative U_{srp} (Figure 4a).

The aim of our simple simulation models was to isolate the dynamic consequences that our experimental results imply and explore their relevant long-term outcomes. As such, we ignored the dynamic process of biomass accumulation on leaves (Gulis et al., 2008), which is affected by both temperature and nutrients. Additionally, our simulations consider microbial respiration as the only mechanism of leaf mass loss. Under natural conditions, microbial fragmentation, physical abrasion, and consumption by macroinvertebrates can all drive meaningful amounts of leaf breakdown (Marks, 2019; Wilmot et al., 2021). Furthermore, our simulations were conducted at a constant temperature, which would lead to depressed rates of breakdown relative to simulations that include temperature variability (Tomczyk et al., 2020). Not including these processes in our model likely explains the high residence time of leaves in our simulations; at the low nutrient concentration and temperature our simulations had leaf residence times over 1000 days (Figure 4b), while field studies have found residence times of around two years for *Rhododendron* leaves in relatively pristine streams (Manning et al., 2015). Increasing rates of breakdown in our simulations to mimic residence times observed in the field leads to cumulative uptake in the cool scenario exceeding cumulative uptake in the warm scenario after only 139 days (Appendix S1). Furthermore, while our models did not include seasonal inputs of leaves, our general finding that cumulative uptake in cool scenarios is greater than in warm scenarios is robust to successive seasonal inputs of leaves (Appendix S2). Our treatment of the effect of nutrients on respiration is fairly simple, and comes from a study in which water was amended with both N and P, not just P as we consider throughout this study (Manning et al., 2018). While our simulation models do not incorporate all the complexity of stream ecosystems, the

consequence of the differences in the temperature dependence of carbon and nutrient cycle processes should persist in both more complex ecosystem models and in the natural environment.

Much like the carbon cycle processes of gross primary production, ecosystem respiration, and net ecosystem production, nutrient cycles in streams are comprised of positive and negative gross fluxes, the balance of which dictates net nutrient exchange between the water column and benthos (von Schiller et al., 2015; Brookshire et al., 2009). While we focus exclusively on the gross flux of nutrients from the water column to the benthos (i.e., U) in this analysis, relationships between temperature, gross nutrient release, and net nutrient exchange should also be examined to understand how nutrient cycling will change with warming. One detailed simulation of stream nutrient dynamics, which included the same temperature dependence for gross nutrient uptake and mineralization, predicted warming would cause declines in the net uptake of both N and P ranging from 0.9-4.3% (Webster et al., 2016). These modeled declines in net nutrient exchange were driven by the faster mineralization of organic matter that occurs at warmer temperature. Similarly, net mineralization of nutrients has been observed at higher temperatures in terrestrial systems; in one study, increased watershed nitrate export was linked to warming-induced increases in soil N mineralization (Brookshire et al., 2011). Periods of warming have also been linked to increases in *net* N mineralization and nitrate accumulation in agricultural soils (Liang et al., 2011), while experimental warming increased nitrate leaching in tundra soils (Harms et al., 2019). Thus, while our study focuses exclusively on U as a gross flux, mineralization and net nutrient exchange are important aspects of stream nutrient cycling that are also likely temperature dependent.

While much research has addressed the effects of warming on carbon cycle processes (Davidson and Janssens, 2006; Song et al., 2018), far less attention has been paid to how warming affects nutrient cycles, despite the importance of these processes for ecosystem function (Peterson, 2001; Conley et al., 2009). Much of the interest in the effects of temperature on nutrient cycling has been at the level of the individual organism, including surveys of the effects of temperature on organismal stoichiometry (Yvon-Durocher et al., 2015; Zhang and Elser, 2017; Woods et al., 2003; Yuan and Chen, 2015). While some studies have shed light on ecosystem-level changes in nutrient cycling caused by temperature (Brookshire et al. 2011, Liang et al. 2011, Hood et al. 2018, Harms et al. 2019), more work is needed to reveal the underlying mechanisms of temperature effects on carbon-nutrient interactions. The results of our study, although only a small step, highlight that nutrient uptake is dependent on temperature but uncoupled to increases in carbon demand, and that the direction of the effect of warming on nutrient uptake is sensitive to the time scale that is considered (i.e., instantaneous vs. over months or years).

5 Conclusions

In this study we compared the effects of temperature on rates of respiration and U_{srp} by leaf-associated microbial communities, as well as how SRP concentration altered the relationship between temperature and U_{srp}. Experimental changes in temperature increased mass-specific rates of both respiration and U_{srp}, though the increases in U_{srp} were smaller than increases in respiration. The relationship between temperature and U_{srp} changed with the concentration of SRP supplied, and the response to temperature was greater at high nutrient concentrations. However, despite the fact that our experiment found increases in mass-specific rates of U_{srp} with temperature, our simulation models predicted declines in cumulative U_{srp} over the

residence time of a single seasonal input of leaves, primarily as a consequence of more rapid depletion of leaf litter. The relative magnitude of this decrease may be greater in oligotrophic systems where increases in mass-specific U_{srp} are more constrained.

Microbial metabolism and nutrient processing are being altered by climate change (Brookshire et al., 2011; Song et al., 2018). This study highlights that changes in rates of metabolism may not perfectly predict changes in rates of gross nutrient demand, as simple stoichiometric models may predict (Cross et al., 2015). While our study highlights differences in the response of respiration and U_{srp} to temperature, further research is required to understand the cause of this divergence in process rates; we suspect changes in nutrient use efficiency and/ or carbon use efficiency with temperature drive the patterns we observed. Furthermore, our study highlights the dominant role that carbon supply plays in determining rates of nutrient cycling in detrital systems (Valett et al., 2008). Understanding general relationships between warming and nutrient cycling, with a particular consideration for the interconnectedness of the carbon and nutrient cycles (Schlesinger et al., 2011), will be important for understanding the future of nutrient cycling, including patterns of export from warming ecosystems.

Code and data availability: All data and result are included in the online repository for this paper: https://github.com/nathantomczyk/Temperature-Nutrient-Uptake

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Table 1: Summary of parameters used in different scenarios of simulations models. Reference rates of processes (r_{ref}) are
505 presented in units of mass of oxygen per gram of leaf ash-free dry mass per hour at 12°C. The temperature dependence of
processes is represented as the activation energy (E_a).

	Scenario 1	Scenario 2	Scenario 3	Scenario 4
Title on graph	Low P $E_a = 0.65$	Low P measured E_a	High P measured E_a	High P measured E_a P effect on respiration
r_{ref} of respiration (mg 0_2 g^{-1} hr^{-1})	0.04	0.04	0.04	0.04
r_{ref} of SRP uptake (µg P g^{-1} hr^{-1})	1.94	1.94	8.58	8.58
Ea of respiration (eV)	0.65	1.02	1.02	1.02
Ea of SRP uptake (eV)	0.65	0.19	0.52	0.52
Nutrient effect on respiration	none	none	none	1.32×

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Table 2: Parameter estimates and model fit from laboratory experiments. In the first experiment, *Rhododendron maximum*
530 leaves were incubated at five temperatures ranging from 4-20ºC and rates of soluble reactive phosphorus (SRP) uptake and
respiration were measured. In the second experiment, *Acer rubrum* leaves were incubated with different initial concentrations
of phosphorus at different temperatures. We report slopes of the models we evaluated, the model R^2, and the F-value and p-
value associated with the slope parameter.

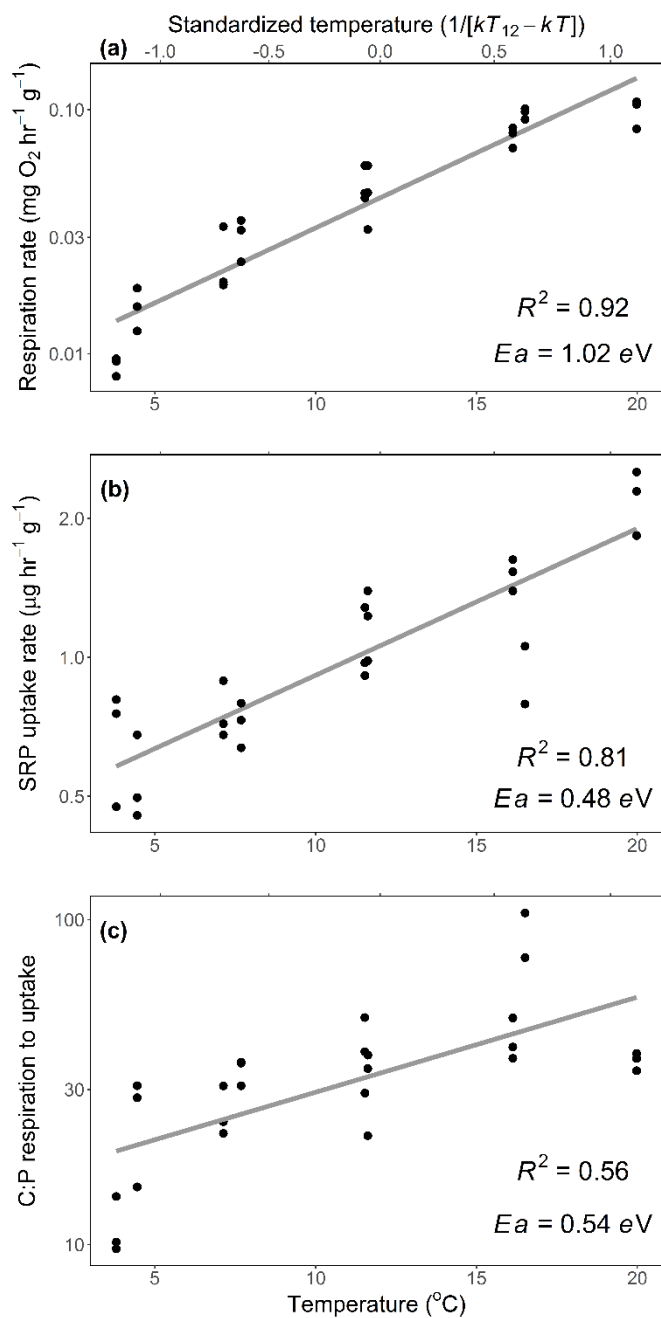
Model	Slope Estimate (SE)	R^2	F	p
Experiment 1				
Respiration vs. temperature	E_a=1.02 (0.06) eV	0.92	$F_{1,24}$=292	<0.0001
SRP uptake vs. temperature	E_a=0.48 (0.05) eV	0.81	$F_{1,23}$=102	<0.0001
C:P vs. temperature	E_a=0.54 (0.06) eV	0.69	$F_{1,23}$=39	0.0002
Experiment 2				
Uptake E_a vs. concentration	K_s = 61.4 (48.6) µg L^{-1}, U_{max}= 0.56 (0.17) eV	0.75	NA	NA
K_m vs. temperature	E_a=0.24 (0.24) eV	0.00	$F_{1,4}$=1.02	0.37
U_{max} vs. temperature	E_a=0.55 (0.15) eV	0.69	$F_{1,4}$=12.19	0.025

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Figure 1: Mass-specific respiration (**a**) and soluble reactive phosphorus (SRP) uptake rates (**b**) of *Rhododendron maximum* leaves with temperature and (**c**) the molar ratio of C respired to P uptake across different temperatures. Standardized Boltzmann temperature is presented on the secondary *x*-axis. Points represent measurements from replicate bottles and grey lines represent best fits. Slopes of lines represent activation energies (E_a), which are reported in units of eV. Note *y*-axes are $\log_{10}$-scaled. See

540 Table 2 (experiment 1) for information on model fit, slopes, and significance.
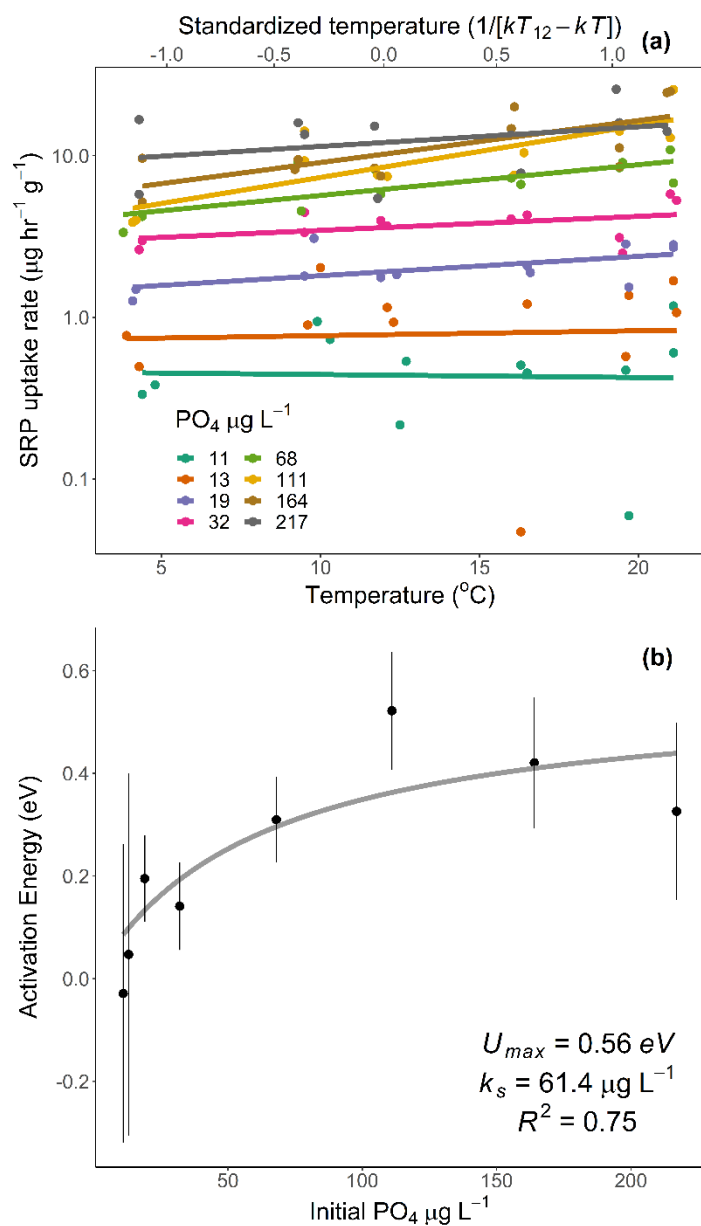
Figure 2: Rates of soluble reactive phosphorus (SRP) uptake compared to temperature for *Acer rubrum* leaves incubated at different temperatures and initial concentrations of SRP (**a**). The secondary *x*-axis in (**a**) represents the standardized Boltzmann temperature, and the *y*-axis is $\log_{10}$-scaled. Slopes of the lines in (**a**) represent the activation energy of SRP uptake at different SRP concentrations. Equations for lines of best fit in (**a**) are given in Table A1. The slope estimates and their standard errors are plotted in (**b**). See Table 2 (experiment 2) for information on model fit and significance for (**b**).
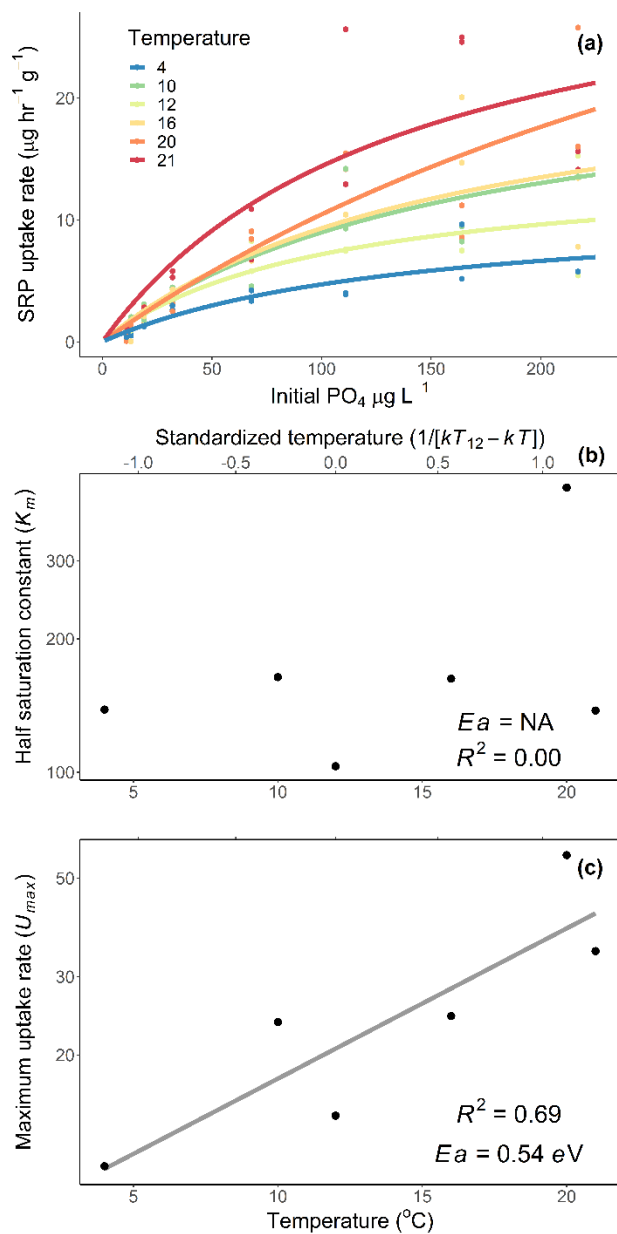
Figure 3: Uptake rates of soluble reactive phosphorus (SRP) across different initial concentrations of SRP grouped by temperature (**a**). The lines represent the best-fit Michaelis-Menten kinetics, and the effect of temperature on the Michaelis-Menten parameters, the half-saturation constant (k_m, **b**), and the maximum uptake rate (U_{max}, **c**) are represented in centered Boltzmann-Arrhenius plots. In (**c**) the grey line indicates the best fit, which represents the activation energy in units of eV. Equations and model fits for lines in (**a**) are given in Table A2. See Table 2 (experiment 2) for information on model fit and significance associated with (**b**) and (**c**).
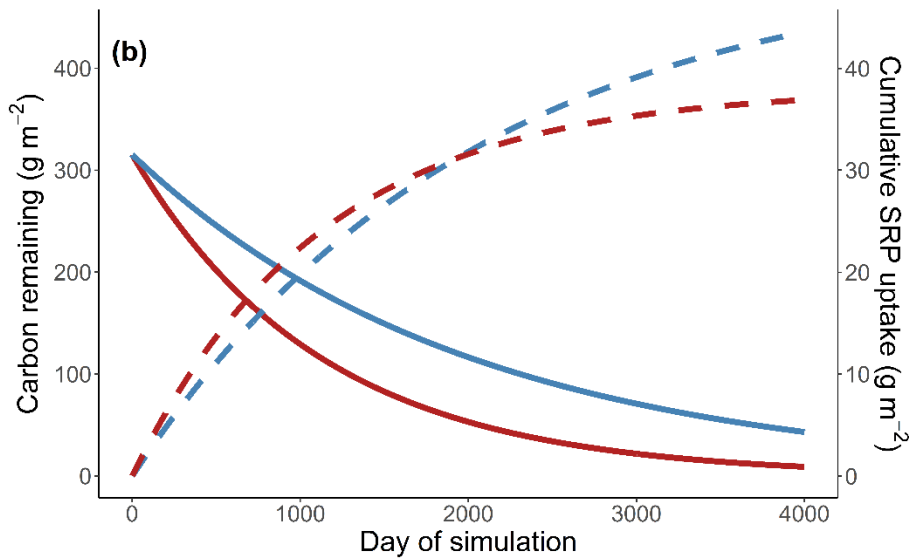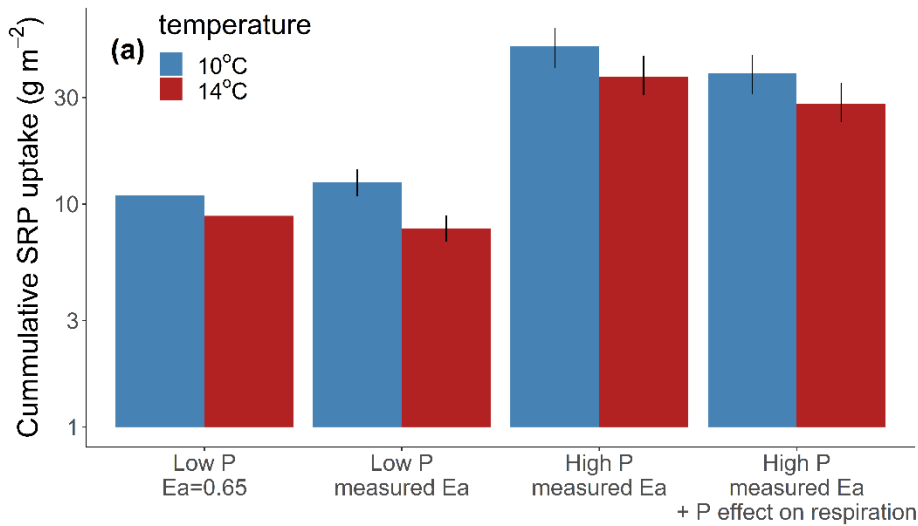
Figure 4: Simulations of the effect of temperature on cumulative soluble reactive phosphorus (SRP) uptake in four scenarios (**a**). First, we consider the effect of warming when the activation energy of respiration and uptake are both 0.65 eV (Low P, 0.65 eV). Second, we consider the effect of warming using the measured temperature dependence respiration and uptake at 19 µg L^{-1} (Low P, measured E_a). Third, we consider the effect of warming at a high initial concentration of 111 µg L^{-1} (High P, measured E_a). Finally, we considered the effect of warming at a high concentration where nutrients also affected the rates of respiration (High P, measured E_a + P effect on respiration). Note the *y*-axis in (**a**) is $\log_{10}$-transformed. We also include an example simulation from a cold and warm scenario using the temperature dependence from the high-SRP scenario (**b**). Mass of carbon (solid lines), and cumulative uptake of SRP (dashed lines), are presented over time for both temperatures, which are indicated by color. Model parameters used in each scenario are summarized in Table 1.