https://doi.org/10.5194/bg-2022-146
Preprint. Discussion started: 9 August 2022

Biogeosciences Discussions

Contrasting activation energies of respiration and nutrient uptake drive lower ecosystem-level uptake at higher temperatures

Nathan J. Tomczyk[1], Amy D. Rosemond[1], Anna Kaz[2], and Jonathan P. Benstead[3].

1Odum School of Ecology, University of Georgia, Athens, Georgia 30606 USA

2Department of Oceanography and Coastal Sciences, Baton Rouge, Louisiana 70803 USA

3Department of Biological Sciences, University of Alabama, Tuscaloosa, Alabama 35487 USA

Correspondence to: Nathan Tomczyk (Nathan.tomczyk@gmail.com)

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 at the ecosystem level. 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 0.12-0.48 eV over a 11-212 µg L^{-1} gradient in initial concentrations. 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 ecosystem-level 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.

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,

https://doi.org/10.5194/bg-2022-146
Preprint. Discussion started: 9 August 2022

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 growth drive 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 (Walker et al., 2018; Suberkropp et al., 2010). Thus, responses of cumulative nutrient uptake to higher temperatures and nutrient concentrations are difficult to observe in detritus-dominated ecosystems, as mass-specific rates of uptake may increase even as total microbial biomass declines, distorting ecosystem responses.

Mechanisms explaining the 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 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 portion of dissolved nutrients taken up by the microbial community declines

https://doi.org/10.5194/bg-2022-146
Preprint. Discussion started: 9 August 2022


Biogeosciences Discussions

with 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). Nutrients and temperature can also combine additively or potentially interact to determine growth and uptake rates, with temperature altering both the initial slope and maximum rate of Michaelis-Menten relationships (Cross et al., 2015; Davidson et al., 2012). However, 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, there is strong seasonality in the input of senescent leaf litter, which mostly enters soil and stream ecosystems in the autumn. 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). Thus, while temperature and nutrients stimulate mass-specific rates of metabolism, they also stimulate 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 which is mainly 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 nutrient concentration 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 would respiration due to changes in carbon- or nutrient-use efficiency (H1). We also hypothesized that the temperature dependence of U_{srp} would vary based on the concentration of soluble reactive P (SRP) supplied, with low concentrations of SRP constraining the temperature dependence of U_{srp} (H2; 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

https://doi.org/10.5194/bg-2022-146
Preprint. Discussion started: 9 August 2022

across nutrient concentrations, and compared it to the temperature dependence of respiration. Finally, we hypothesized that, if the temperature 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 (H4). 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 cohort of leaves.

2 Methods

2.1 Comparing the temperature dependences of SRP uptake and respiration

We conditioned leaves 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. We removed a subset of the bags on 11 March 2021 and returned them to the laboratory, where we cut the leaves into smaller fragments. We placed these fragments in 1-L bottles of aerated stream water, which we incubated in water baths at five different temperatures (4, 8, 12, 16, 20ºC). Each water bath had 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 (see below). We repeated this procedure (only the 4-16ºC 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). Then, we added several leaf fragments 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 for 2-7 hours, giving the vials in colder temperatures more time to incubate to ensure meaningful changes in the 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 as the change in the mass of oxygen normalized to the dry mass of leaves and the incubation time, with a correction factor for the change in oxygen observed in the blanks.

We also used three different subsamples from each replicate bottle to measure rates of U_{srp} simultaneously with the measurements of respiration. We added several leaf fragments to 50-mL centrifuge tubes along with 40 mL of water that was amended with P (to elevate concentrations from <5 ug L^{-1} to ~30-60 µg L^{-1} SRP). Three blanks (i.e., water with no leaves

https://doi.org/10.5194/bg-2022-146
Preprint. Discussion started: 9 August 2022


Biogeosciences Discussions Open Access EGU

added) were prepared along with each temperature treatment. After 2-7 hours of incubation, we removed a subsample of the water with a syringe and filtered it through an AE-grade glass fiber filter (Sterilitech, WA, USA), and immediately froze the sample. 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 blanks and subsamples, normalized to the dry mass of leaves and the incubation time.

We estimated the activation energy (E_a) of both respiration and U_{srp} using the Boltzmann-Arrhenius equation (equation 1), 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; 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 of (T_{12}, 12ºC), by regressing the $\log_e$-transformed process rates against the standardized Boltzmann temperature, and estimating the E_a based on the slope of this line (equation 2).

$$\boldsymbol{r_i = r_{ref} * e^{\frac{-Ea}{k_B * T}}}, \quad (1)$$

$$\boldsymbol{\ln(r_i) = \ln(r_{12}) + \frac{1}{k_B * T_{12} - k_B * T} * -E_a}, \quad (2)$$

We included a categorical variable to account for the experimental batch, which we view as a block effect accounting for changes in the microbial communities or other factors between dates. To test whether the E_a of respiration and U_{srp} were significantly different from one another, we evaluated a model that included respiration, U_{srp}, and a categorical variable indicating the type of rate (respiration or uptake) to test their interaction. A significant interaction term in this model indicates that the E_a of respiration and U_{srp} are significantly different. 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

https://doi.org/10.5194/bg-2022-146
Preprint. Discussion started: 9 August 2022

for the *Acer* than for *Rhododendron* due to higher environmental temperatures and generally more rapid colonization of the more labile leaves. 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 4-21ºC and eight initial SRP concentrations ranging from 11-217 µg L^{-1}. 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.

We calculated U_{srp} in the same manner as described above. We then used two techniques to evaluate 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, 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 (equation 3) 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):

$$U_{srp} = \frac{[SRP]*U_{max}}{[SRP]+k_m} \quad (3)$$

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 input, considering the direct effects of SRP concentration and temperature on mass-specific loss rates and the indirect effects mediated through depletion of leaf associated carbon. These simulations were designed to illustrate the dynamic consequences of our laboratory measurements and provide insights that might inform more comprehensive representation of carbon and nutrient cycles in forested streams. The simulated stream reach starts with 250 g leaf C m^{-2} that was mineralized by microbial respiration. The mass-specific rates of leaf mass loss were estimated as a function

https://doi.org/10.5194/bg-2022-146
Preprint. Discussion started: 9 August 2022

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. 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 the cumulative U_{srp}, when 99% of the leaves were consumed by microbial metabolism.

We considered the effects of warming and nutrient enrichment on U_{srp} in four scenarios; in each scenario we evaluated the effect of warming using a low temperature of 10°C and a warmed 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 the 19 µg L^{-1} treatment (i.e., a low-to-moderate concentration). Second, we simulate 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 simulate 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 sensitivity of respiration from our first experiment. Fourth, we simulate uptake with our estimates of U_{srp} at the high concentration of 111 µg L^{-1}, and include 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 sensitivities by bootstrapping our estimates of cumulative U_{srp} 1000 times.

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 canonical value of respiration (0.60 - 0.70 eV, 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 1), 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, increasing by ~0.17 eV (SE 0.068) per 100 µg L^{-1} increase in initial SRP concentration (Figure 2, Table 1), though this increase was greater at low concentrations and saturated at higher nutrient concentrations. We found that temperature influenced patterns of SRP uptake across nutrient concentrations (Figure 3, Table 1). Temperature increased U_{max}, with an *Ea* of 0.55 eV (SE 0.16), but did not have a measurable effect on k_m.

https://doi.org/10.5194/bg-2022-146
Preprint. Discussion started: 9 August 2022


Biogeosciences Discussions

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 times the cumulative U_{srp} in the warm stream (14ºC) compared to the cold stream (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 treatment equal to 0.62× the cooler treatment (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 2.6 vs. 7.7 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 warmer stream 27% lower than the cooler stream regardless of the effect of enrichment on respiration (Figure 4a).

Increases in nutrient concentration increased cumulative U_{srp} in our simulations. At the cooler temperature, cumulative U_{srp} was 4.1 times higher at the higher SRP concentration (Figure 4a). Similarly, at the higher temperature, cumulative U_{srp} was 4.8 times 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 3.1and 3.6, respectively, at the low and high temperature (Figure 4a).

4 Discussion

We observed a much lower activation energy for U_{srp} than for respiration in our experimental studies, indicating the potential for shifts in carbon and nutrient processing as temperatures increase in forested 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 forested streams, but the magnitude and direction of these effects may depend on the spatial and temporal scale of interest and the carbon resource available.

Our finding that P uptake increased less with temperature than microbial metabolism is in concordance with previous findings from other types of systems. In a field experiment, Hood et al. 2018 showed stream warming increased primary production almost three-fold, while it had no measurable effect on rates of nutrient uptake (Hood et al., 2018). This was attributed to an increase in the efficiency of nutrient recycling, increases in 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 sensitivity 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

https://doi.org/10.5194/bg-2022-146
Preprint. Discussion started: 9 August 2022

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), and one 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 concentrations are temporarily elevated and may be further exacerbated by the fact that we did not amend the respiration trials with nutrients.

Nominally, our finding that temperature increases rates of 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 forested streams, biomass of the microbial community is tightly linked to the standing stocks 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). The importance of both direct physiological and indirect biomass-mediated effects of temperature on ecosystem processes has been appreciated in detritus-based systems (Wilmot et al., 2021). However, our study is the first to separate the contribution of these two processes on cumulative ecosystem-level nutrient uptake. Specifically, when we consider only the direct effect of temperature on mass-specific rates of U_{srp}, we infer that cumulative U_{srp} increases with temperature. However, when we incorporated indirect effects of temperature on respiration and its consequences for biomass, we find that warming decreases cumulative areal 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 ecosystem-level outcomes. As such, we ignore the dynamic process of biomass accumulation on leaves, which is affected by both temperature and nutrients (Gulis et al., 2008). Instead, our model implicitly assumes the quantity of microbial biomass per mass of leaf is constant, and that temperature and nutrients only affect the mass-specific rates of processes, not the amount of biomass per mass of leaf. 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 minimally impacted streams (Manning et al., 2015). Furthermore, 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

https://doi.org/10.5194/bg-2022-146
Preprint. Discussion started: 9 August 2022

of stream ecosystems, the consequence of the differences in the temperature dependence of carbon and nutrient cycle processes should persist in complex ecosystem models and 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-sensitive.

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 effect of temperature on nutrients 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 sensitive to temperature but uncoupled to increases in carbon demand, and this effect of warming on nutrient uptake is sensitive to the scale of observation.

5 **Conclusions**

In this study we compared the effect of temperature on rates of respiration and U_{srp} by leaf-associated microbial communities, and 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 experimental results found increases in mass-specific rates of U_{srp} with temperature our simulation models predict declines in U_{srp} at the ecosystem scale,

https://doi.org/10.5194/bg-2022-146
Preprint. Discussion started: 9 August 2022

primarily as a consequence of decreased leaf litter standing stocks. 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 (Song et al., 2018; Brookshire et al., 2011). This study highlights that changes in rates of metabolism may not perfectly predict changes in rates of gross nutrient demand, as a 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 – though we suspect changes in nutrient use efficiency and/ or carbon use efficiency with temperature drive this pattern. 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 and 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

Acknowledgements: This study received support from the National Science Foundation (DEB-1655789 to ADR and DEB-1655956 to JPB). This work was also supported by a small grant from the Odum School of Ecology at the University of Georgia. This manuscript benefited greatly from comments from Carolyn Cummins, Phillip Bumpers, Laura Naslund, Craig Osenberg, Seth Wenger, Vlad Gulis, Danielle Hare, and Erin Hotchkiss.

Author Contributions: NT developed the ideas for this manuscript with JB, AK, and AR. Data was collected by NT and AK. NT led the data analysis and wrote the first draft, and AK, AR and JB contributed to revisions of the manuscript.

Competing interests: No competing interests.

References

Allen, A. P. and Gillooly, J. F.: Towards an integration of ecological stoichiometry and the metabolic theory of ecology to better understand nutrient cycling, Ecol. Lett., 12, 369–384, https://doi.org/10.1111/j.1461-0248.2009.01302.x, 2009.

APHA: Standard Methods for the Examination of Water and Wastewater, 19th ed., American Public Health Association, 1995.

Bott, T.: Primary production and community respiration, in: Methods in Stream Ecology, edited by: Hauer, R. F. and Lamberti, G. A., Academic Press, Burlington, MA, 663–690, 2006.

Brookshire, E. N. J., Valett, H. M., and Gerber, S.: Maintenance of terrestrial nutrient loss signatures during in-stream transport, Ecology, 90, 293–299, 2009.

345 Brookshire, E. N. J., Gerber, S., Webster, J. R., Vose, J. M., and Swank, W. T.: Direct effects of temperature on forest nitrogen cycling revealed through analysis of long-term watershed records, Glob. Chang. Biol., 17, 297–308, https://doi.org/10.1111/j.1365-2486.2010.02245.x, 2011.

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M., and West, G. B.: Toward a metabolic theory of ecology, Ecology, 85, 1771–1789, https://doi.org/10.1890/03-9000, 2004.

350 Burgin, A. J., Yang, W. H., Hamilton, S. K., and Silver, W. L.: Beyond carbon and nitrogen: How the microbial energy economy couples elemental cycles in diverse ecosystems, Front. Ecol. Environ., 9, 44–52, https://doi.org/10.1890/090227, 2011.

Conley, D. J., Paerl, H. W., Howarth, R. W., Boesch, D. F., Seitzinger, S. P., Havens, K. E., Lancelot, C., and Likens, G. E.: Controlling eutrophication: Nitrogen and phosphorus, Science (80-.)., 323, 1014–1015,

355 https://doi.org/10.1126/science.1167755, 2009.

Cross, W. F., Benstead, J. P., Frost, P. C., and Thomas, S. A.: Ecological stoichiometry in freshwater benthic systems: recent progress and perspectives, Freshw. Biol., 50, 1895–1912, https://doi.org/10.1111/j.1365-2427.2005.01458.x, 2005.

Cross, W. F., Hood, J. M., Benstead, J. P., Huryn, A. D., and Nelson, D.: Interactions between temperature and nutrients across levels of ecological organization, Glob. Chang. Biol., 21, 1025–1040, https://doi.org/10.1111/gcb.12809, 2015.

360 Davidson, E. A. and Janssens, I. A.: Temperature sensitivity of soil carbon decomposition and feedbacks to climate change, Nature, 440, 165–173, https://doi.org/10.1038/nature04514, 2006.

Davidson, E. A., Samanta, S., Caramori, S. S., and Savage, K.: The Dual Arrhenius and Michaelis-Menten kinetics model for decomposition of soil organic matter at hourly to seasonal time scales, Glob. Chang. Biol., 18, 371–384, https://doi.org/10.1111/j.1365-2486.2011.02546.x, 2012.

365 Dodds, W. K., López, A. J., Bowden, W. B., Gregory, S., Grimm, N. B., Hamilton, S. K., Hershey, A. E., Martí, E., McDowell, W. H., Meyer, J. L., Morrall, D., Mulholland, P. J., Peterson, B. J., Tank, J. L., Valett, H. M., Webster, J. R., and Wollheim, W.: N uptake as a function of concentration in streams, J. North Am. Benthol. Soc., 21, 206–220, https://doi.org/10.2307/1468410, 2002.

Doi, H., Cherif, M., Iwabuchi, T., Katano, I., Stegen, J. C., and Striebel, M.: Integrating elements and energy through the

370 metabolic dependencies of gross growth efficiency and the threshold elemental ratio, 119, 752–765, https://doi.org/10.1111/j.1600-0706.2009.18540.x, 2010.

Findlay, S., Tank, J., Dye, S., Valett, H. M., Mulholland, P. J., McDowell, W. H., Johnson, S. L., Hamilton, S. K., Edmonds, J., Dodds, W. K., and Bowden, W. B.: A cross-system comparison of bacterial and fungal biomass in detritus pools of headwater\nstreams, Microb. Ecol., 43, 55–66, https://doi.org/10.1007/10.007/s00248-001-1020-x, 2002.

375 Godwin, C. M. and Cotner, J. B.: Carbon:phosphorus homeostasis of aquatic bacterial assemblages is mediated by shifts in assemblage composition, Aquat. Microb. Ecol., 73, 245–258, https://doi.org/10.3354/ame01719, 2014.

Godwin, C. M. and Cotner, J. B.: Aquatic heterotrophic bacteria have highly flexible phosphorus content and biomass stoichiometry, ISME J., 9, 2324–2327, https://doi.org/10.1038/ismej.2015.34, 2015.

Gulis, V., Suberkropp, K., and Rosemond, A. D.: Comparison of fungal activities on wood and leaf litter in unaltered and nutrient-enriched headwater streams, Appl. Environ. Microbiol., 74, 1094–1101, https://doi.org/10.1128/AEM.01903-07, 2008.

Gulis, V., Kuehn, K. A., Schoettle, L. N., Leach, D., Benstead, J. P., and Rosemond, A. D.: Changes in nutrient stoichiometry, elemental homeostasis and growth rate of aquatic litter-associated fungi in response to inorganic nutrient supply, ISME J., 11, 2729–2739, https://doi.org/10.1038/ismej.2017.123, 2017.

Hall, R. O. and Tank, J. L.: Ecosystem metabolism controls nitrogen uptake in streams in Grand Teton National Park, Wyoming, Limnol. Oceanogr., 48, 1120–1128, https://doi.org/10.4319/lo.2003.48.3.1120, 2003.

Harms, T. K., Cook, C. L., Wlostowski, A. N., Gooseff, M. N., and Godsey, S. E.: Spiraling Down Hillslopes: Nutrient Uptake from Water Tracks in a Warming Arctic, 22, 1546–1560, https://doi.org/10.1007/s10021-019-00355-z, 2019.

Hoellein, T. J., Tank, J. L., Rosi-marshall, E. J., Entrekin, S. A., and Lamberti, G. A.: Controls on spatial and temporal variation of nutrient uptake in three Michigan headwater streams, Limnol. Oceanogr., 52, 1964–1977, 2007.

Hood, J. M., Benstead, J. P., Cross, W. F., Huryn, A. D., Johnson, P. W., Gíslason, G. M., Junker, J. R., Nelson, D., Ólafsson, J. S., and Tran, C.: Increased resource use efficiency amplifies positive response of aquatic primary production to experimental warming, Glob. Chang. Biol., 24, 1069–1084, https://doi.org/10.1111/gcb.13912, 2018.

Kaushal, S. S., Likens, G. E., Jaworski, N. A., Pace, M. L., Sides, A. M., Seekell, D., Belt, K. T., Secor, D. H., and Wingate, R. L.: Rising stream and river temperatures in the United States, Front. Ecol. Environ., 8, 461–466, https://doi.org/10.1890/090037, 2010.

Li, J., Wang, G., Mayes, M. A., Allison, S. D., Frey, S. D., Shi, Z., Hu, X. M., Luo, Y., and Melillo, J. M.: Reduced carbon use efficiency and increased microbial turnover with soil warming, Glob. Chang. Biol., 25, 900–910, https://doi.org/10.1111/gcb.14517, 2019.

Liang, X. Q., Xu, L., Li, H., He, M. M., Qian, Y. C., Liu, J., Nie, Z. Y., Ye, Y. S., and Chen, Y.: Influence of N fertilization rates, rainfall, and temperature on nitrate leaching from a rainfed winter wheat field in Taihu watershed, Phys. Chem. Earth, 36, 395–400, https://doi.org/10.1016/j.pce.2010.03.017, 2011.

Manning, D. W. P., Rosemond, A. D., Kominoski, J. S., Gulis, V., Benstead, J. P., and Maerz, J. C.: Detrital stoichiometry as a critical nexus for the effects of streamwater nutrients on leaf litter breakdown rates, Ecology, 96, 2214–2224, https://doi.org/10.1890/14-1582.1, 2015.

Manning, D. W. P., Rosemond, A. D., Gulis, V., Benstead, J. P., and Kominoski, J. S.: Nutrients and temperature additively increase stream microbial respiration, Glob. Chang. Biol., 24, e233–e247, https://doi.org/10.1111/gcb.13906, 2018.

Manzoni, S., Taylor, P., Richter, A., Porporato, A., and Ågren, G. I.: Environmental and stoichiometric controls on microbial carbon-use efficiency in soils, New Phytol., 196, 79–91, https://doi.org/10.1111/j.1469-8137.2012.04225.x, 2012.

Marks, J. C.: Revisiting the fates of dead leaves that fall into streams, Annu. Rev. Ecol. Evol. Syst., 547–568, https://doi.org/10.1146/annurev-ecolsys-110218-024755, 2019.

O'Brien, J. M., Dodds, W. K., Wilson, K. C., Murdock, J. N., and Eichmiller, J.: The saturation of N cycling in Central Plains

streams: 15N experiments across a broad gradient of nitrate concentrations, Biogeochemistry, 84, 31–49, https://doi.org/10.1007/s10533-007-9073-7, 2007.

Payn, R. A., Webster, J. R., Mulholland, P. J., Valett, H. M., and Dodds, W. K.: Estimation of stream nutrient uptake from nutrient addition experiments, Limnol. Oceanogr. Methods, 3, 174–182, https://doi.org/10.4319/lom.2005.3.174, 2005.

Peterson, B. J.: Control of Nitrogen Export from Watersheds by Headwater Streams, Science (80-.)., 292, 86–90, https://doi.org/10.1126/science.1056874, 2001.

Rasmussen, J. J., Baattrup-Pedersen, A., Riis, T., and Friberg, N.: Stream ecosystem properties and processes along a temperature gradient, Aquat. Ecol., 45, 231–242, https://doi.org/10.1007/s10452-010-9349-1, 2011.

von Schiller, D., Bernal, S., Sabater, F., and Martí, E.: A round-trip ticket: the importance of release processes for in-stream nutrient spiraling, Freshw. Sci., 34, 20–30, https://doi.org/10.1086/679015., 2015.

Schlesinger, W. H., Cole, J. J., Finzi, A. C., and Holland, E. A.: Introduction to coupled biogeochemical cycles, Front. Ecol. Environ., 9, 5–8, https://doi.org/10.1890/090235, 2011.

Scott, J. T., Cotner, J. B., and LaPara, T. M.: Variable stoichiometry and homeostatic regulation of bacterial biomass elemental composition, Front. Microbiol., 3, 1–8, https://doi.org/10.3389/fmicb.2012.00042, 2012.

De Senerpont Domis, L. N., Van De Waal, D. B., Helmsing, N. R., Van Donk, E., and Mooij, W. M.: Community stoichiometry in a changing world: Combined effects of warming and eutrophication on phytoplankton dynamics, Ecology, 95, 1485–1495, https://doi.org/10.1890/13-1251.1, 2014.

Song, C., Dodds, W. K., Rüegg, J., Argerich, A., Baker, C. L., Bowden, W. B., Douglas, M. M., Farrell, K. J., Flinn, M. B., Garcia, E. A., Helton, A. M., Harms, T. K., Jia, S., Jones, J. B., Koenig, L. E., Kominoski, J. S., McDowell, W. H., McMaster, D., Parker, S. P., Rosemond, A. D., Ruffing, C. M., Sheehan, K. R., Trentman, M. T., Whiles, M. R., Wollheim, W. M., and Ballantyne, F.: Continental-scale decrease in net primary productivity in streams due to climate warming, Nat. Geosci., 11, 1–6, https://doi.org/10.1038/s41561-018-0125-5, 2018.

Sterner, R. W. and Elser, J. J.: Ecological stoichiometry : the biology of elements from molecules to the biosphere, Princeton University Press, Princeton, New Jersey, USA, https://doi.org/10.1111/j.1469-8137.2010.03214.x, 2002.

Stets, E. G., Sprague, L. A., Oelsner, G. P., Johnson, H. M., Murphy, J. C., Ryberg, K., Vecchia, A. V., Zuellig, R. E., Falcone, J. A., and Riskin, M. L.: Landscape Drivers of Dynamic Change in Water Quality of U.S. Rivers, Environ. Sci. Technol., 54, 4336–4343, https://doi.org/10.1021/acs.est.9b05344, 2020.

Suberkropp, K., Gulis, V., Rosemond, A. D., and Benstead, J. P.: Ecosystem and physiological scales of microbial responses to nutrients in a detritus-based stream: Results of a 5-year continuous enrichment, Limnol. Oceanogr., 55, 149–160, https://doi.org/10.4319/lo.2010.55.1.0149, 2010.

Swank, W. T. and Crossley, D. A.: Forest hydrology and ecology at Coweeta, Springer, New York, NY, https://doi.org/https://doi.org/10.1007/978-1-4612-3732-7, 1988.

Tank, J. L., Martí, E., Riis, T., von Schiller, D., Reisinger, A. J., Dodds, W. K., Whiles, M. R., Ashkenas, L. R., Bowden, W. B., Collins, S. M., Crenshaw, C. L., Crowl, T. A., Griffiths, N. A., Grimm, N. B., Hamilton, S. K., Johnson, S. L., McDowell,

W. H., Norman, B. M., Rosi, E. J., Simon, K. S., Thomas, S. A., and Webster, J. R.: Partitioning assimilatory nitrogen uptake in streams: an analysis of stable isotope tracer additions across continents, Ecol. Monogr., 88, 120–138, https://doi.org/10.1002/ecm.1280, 2018.

Thrane, J. E., Hessen, D. O., and Andersen, T.: Plasticity in algal stoichiometry: Experimental evidence of a temperature-induced shift in optimal supply N:P ratio, Limnol. Oceanogr., 62, 1346–1354, https://doi.org/10.1002/lno.10500, 2017.

Tomczyk, N. J., Rosemond, A. D., Bumpers, P. M., Cummins, C. S., Wenger, S. J., and Benstead, J. P.: Ignoring temperature variation leads to underestimation of the temperature sensitivity of plant litter decomposition, 11, e03050, https://doi.org/10.1002/ecs2.3050, 2020.

Valett, H. M., Thomas, S. A., Mulholland, P. J., Webster, J. R., Dahm, C. N., Fellows, C. S., Crenshaw, C. L., and Peterson, C. G.: Endogenous and Exogenous Control of Ecosystem Function: N Cycling in Headwater Streams, Ecology, 89, 3515–3527, https://doi.org/10.1890/07-1003.1, 2008.

Walker, T. W. N., Kaiser, C., Strasser, F., Herbold, C. W., Leblans, N. I. W., Woebken, D., Janssens, I. A., Sigurdsson, B. D., and Richter, A.: Microbial temperature sensitivity and biomass change explain soil carbon loss with warming, Nat. Clim. Chang., 8, 885–889, https://doi.org/10.1038/s41558-018-0259-x, 2018.

Wallace, J. B., Eggert, S. L., Meyer, J. L., and Webster, J. R.: Stream invertebrate productivity linked to forest subsidies: 37 stream-years of reference and experimental data, Ecology, 96, 1213–1228, https://doi.org/10.1890/14-1589.1.sm, 2015.

Webster, J. and Tank, J.: Effects of litter exclusion and wood removal on phosphorus and nitrogen retention in a forest stream, Verh Intern. Verein Limnol, 1995–1998, 2000.

Webster, J. R., Newbold, J. D., and Lin, L.: Nutrient spiraling and transport in streams: the importance of in-stream biological processes to nutrient dynamics in streams, in: Stream Ecosystems in a Changing Environment, edited by: Jones, J. B. and Stanley, E. H., Academic Press, Cambridge, Massachusetts, USA, 181–239, https://doi.org/10.1016/B978-0-12-405890-3.00005-1, 2016.

Weigelhofer, G., Ramião, J. P., Puritscher, A., and Hein, T.: How do chronic nutrient loading and the duration of nutrient pulses affect nutrient uptake in headwater streams?, Biogeochemistry, 141, 249–263, https://doi.org/10.1007/s10533-018-0518-y, 2018.

Wilmot, O. J., Hood, J. M., Huryn, A. D., and Benstead, J. P.: Decomposing decomposition: isolating direct effects of temperature from other drivers of detrital processing, Ecology, 102, 1–12, https://doi.org/10.1002/ecy.3467, 2021.

Woods, H. A., Makino, W., Cotner, J. B., Hobbie, S. E., Harrison, J. F., Acharya, K., and Elser, J. J.: Temperature and the chemical composition of poikilothermic organisms, Funct. Ecol., 17, 237–245, https://doi.org/10.1046/j.1365-2435.2003.00724.x, 2003.

Yuan, Z. Y. and Chen, H. Y. H.: Decoupling of nitrogen and phosphorus in terrestrial plants associated with global changes, Nat. Clim. Chang., 5, 465–469, https://doi.org/10.1038/nclimate2549, 2015.

Yvon-Durocher, G., Dossena, M., Trimmer, M., Woodward, G., and Allen, A. P.: Temperature and the biogeography of algal stoichiometry, Glob. Ecol. Biogeogr., 24, 562–570, https://doi.org/10.1111/geb.12280, 2015.

https://doi.org/10.5194/bg-2022-146
Preprint. Discussion started: 9 August 2022

Zhang, J. and Elser, J. J.: Carbon: Nitrogen: Phosphorus stoichiometry in fungi: A meta-analysis, Front. Microbiol., 8, 1–9, https://doi.org/10.3389/fmicb.2017.01281, 2017.

485

490

495

500

505

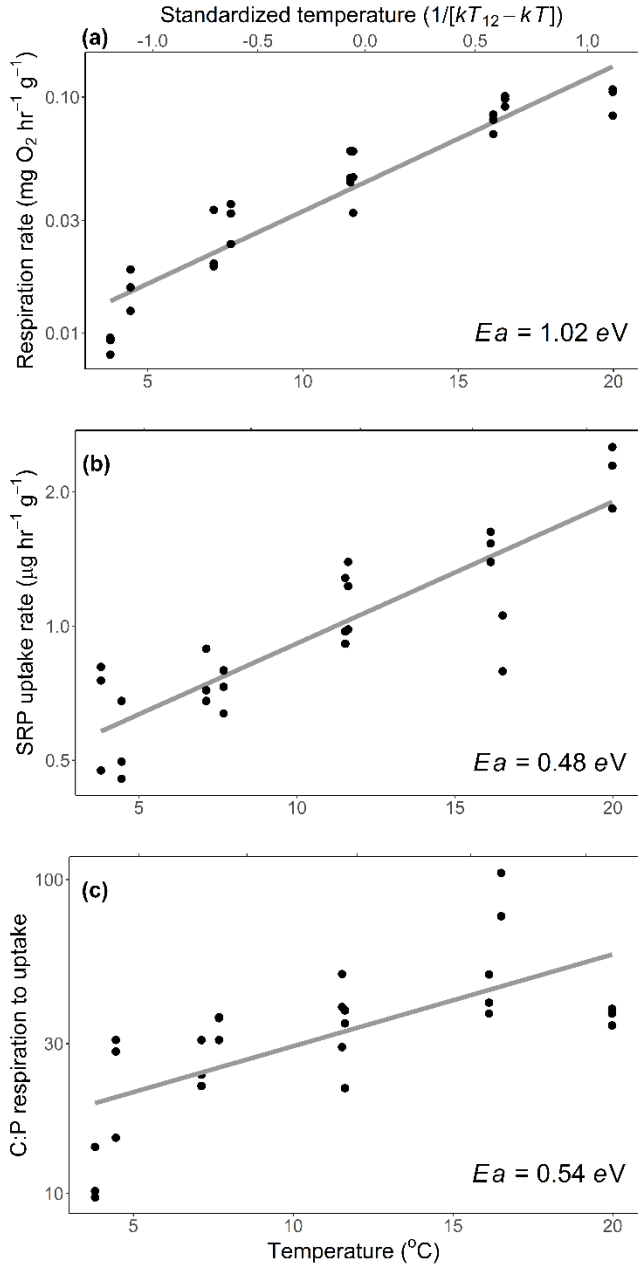
510

https://doi.org/10.5194/bg-2022-146
Preprint. Discussion started: 9 August 2022

Table 1: Parameter estimates and model fit from laboratory experiments. In the first experiment, *Rhododendron maximum* 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			
SRP uptake vs. temperature	*Ea*=0.48 (0.05) *eV*	0.81	$F_{1,23}$=102	<0.0001
Respiration vs. temperature	*Ea*=1.02 (0.06) *eV*	0.92	$F_{1,24}$=292	<0.0001
C:P vs. temperature	*Ea*=0.54 (0.06) *eV*	0.56	$F_{1,23}$=20	0.0002
	Experiment 2			
Uptake *Ea* vs. concentration	0.17 eV (0.068) per 100 µg L^{-1}	0.45	$F_{1,6}$=6.66	0.04
K_m vs. temperature	*Ea*=0.48 (0.05) *eV*	0.00	$F_{1,4}$=1.02	0.37
U_{max} vs. temperature	*Ea*=0.48 (0.05) *eV*	0.69	$F_{1,4}$=12.19	0.025

https://doi.org/10.5194/bg-2022-146
Preprint. Discussion started: 9 August 2022



520 **Figure 1:** Mass-specific respiration (A) and soluble reactive phosphorus (SRP) uptake rates (B) of *Rhododendron maximum* leaves compared to temperature and the molar ratio of C respired to P uptake across different temperatures (C). Standardized Boltzmann temperature is presented on the secondary *x*-axis. Points represent measurements from replicate bottles and grey lines represent best fits. Fits of lines represent activation energies (*Ea*) which are reported in units of eV. Note *y*-axes are $\log_{10}$-scaled. See Table 1 for information on model fit, slopes, and significance.

https://doi.org/10.5194/bg-2022-146
Preprint. Discussion started: 9 August 2022

Biogeosciences Discussions Open Access EGU

525

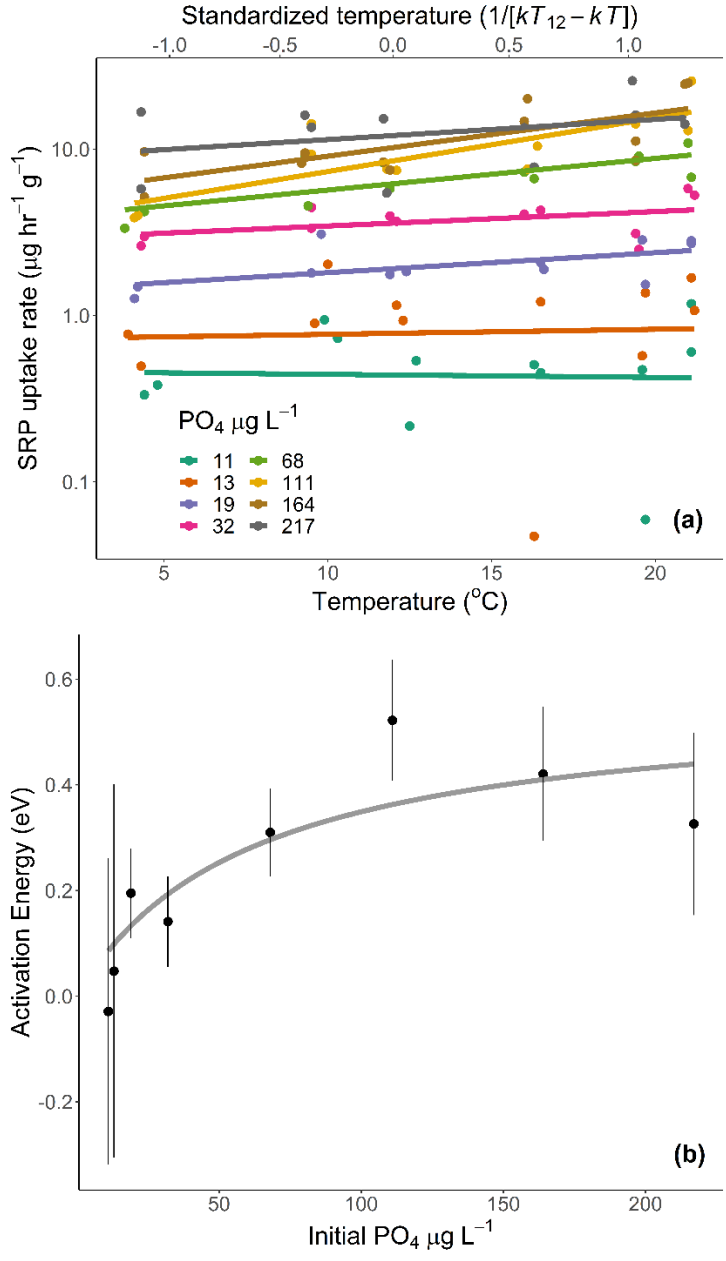


Figure 2: Rates of soluble reactive phosphorus (SRP) uptake compared to temperature for *Acer rubrum* leaves incubated at different temperatures and initial concentrations of SRP (A). The secondary *x*-axis represents the standardized Boltzmann temperature, and the *y*-axis is $\log_{10}$-scaled. Slopes of the lines in panel A represent the activation energy of SRP uptake at
530 different SRP concentrations. The slope estimates and their standard errors plotted in panel B. See Table 1 for information on model fit and significance.

https://doi.org/10.5194/bg-2022-146
Preprint. Discussion started: 9 August 2022

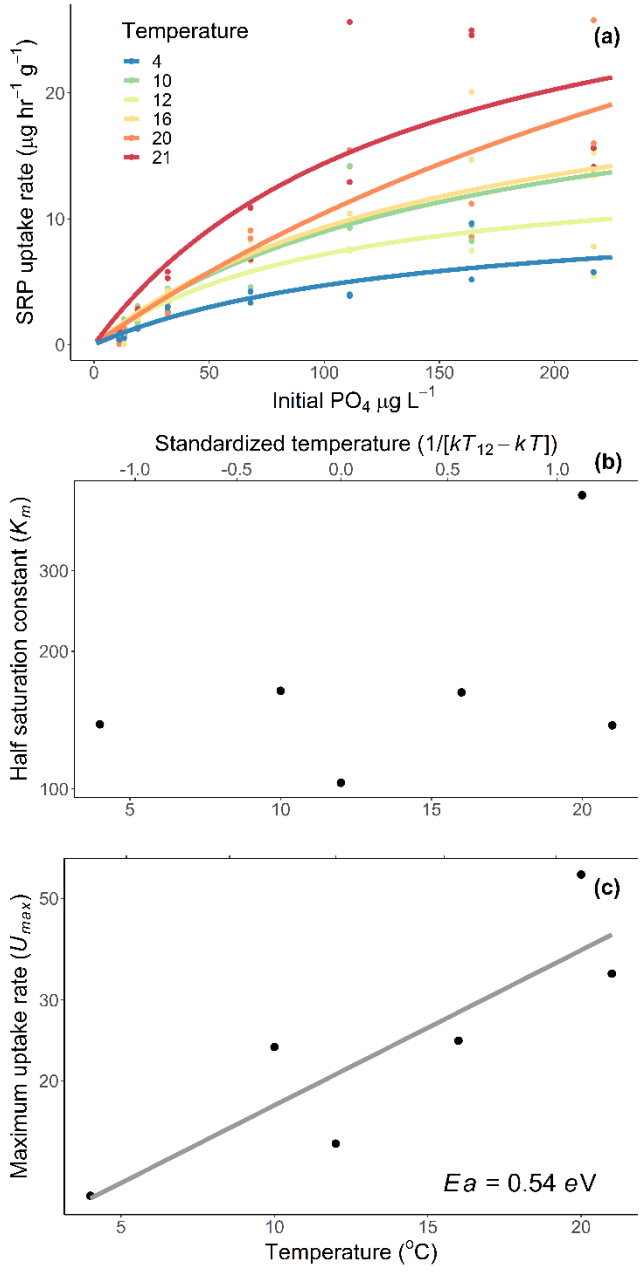
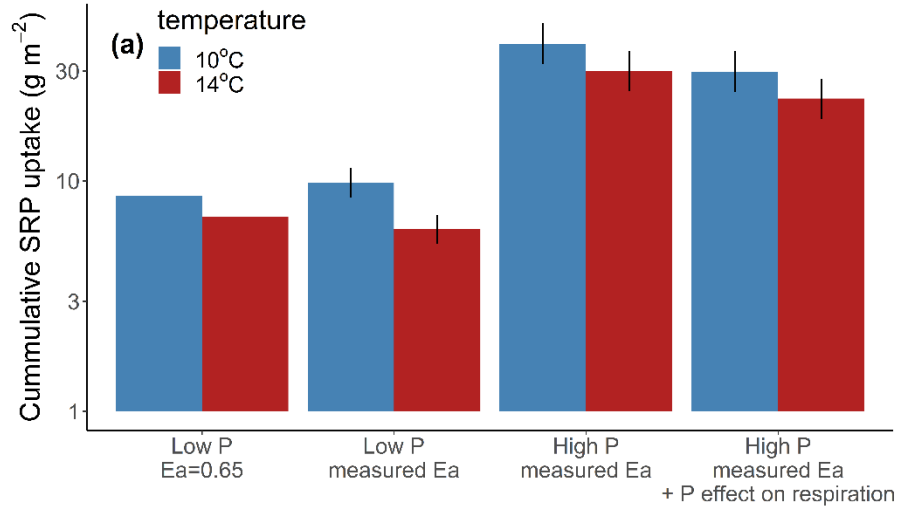


Figure 3: Uptake rates of soluble reactive phosphorus (SRP) across different initial concentrations of SRP grouped by temperature (A). The lines represent the best-fit Michaelis-Menten kinetics, and the effect of temperature on the Michaelis-Menten parameters, the half-saturation constant (k_m, panel B), and the maximum uptake rate (U_{max}, panel C) are represented in centered Boltzmann-Arrhenius plots. In panel C the blue line indicates the best fit, which represents the activation energy in units of eV. See Table 1 for information on model fit and significance.

https://doi.org/10.5194/bg-2022-146
Preprint. Discussion started: 9 August 2022



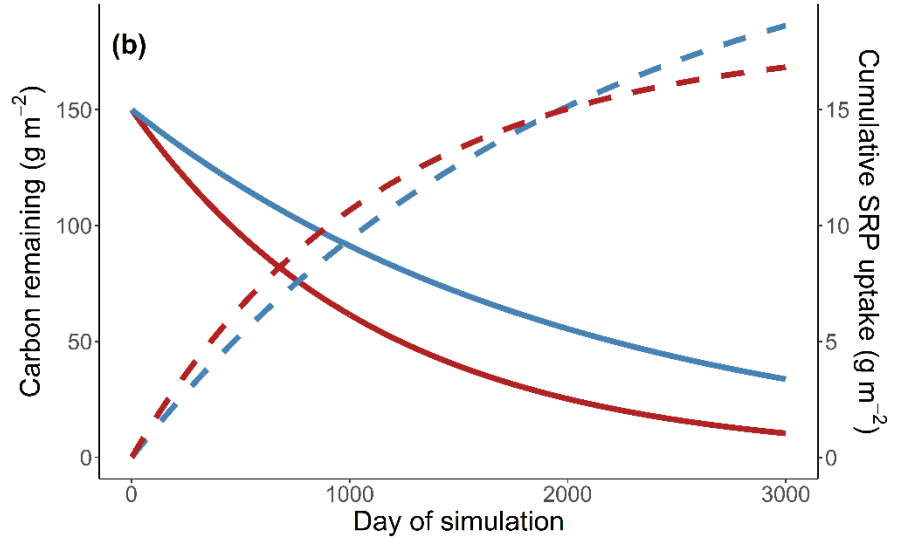


Figure 4: Simulations of the effect of temperature on cumulative soluble reactive phosphorus (SRP) uptake in four scenarios (A). First, we consider the effect of warming when the activation energy of respiration and uptake are both 0.65 eV (Low P, 0.65 eV). Second, we consider the effect of warming using the measured temperature dependence respiration and uptake at 19 µg L^{-1} (Low P, measured *Ea*). Third, we considered the effect of warming at a high initial concentration of 111 µg L^{-1} (High P, measured *Ea*). Finally, we considered the effect of warming at a high concentration where nutrients also affected the rates of respiration (High P, measured *Ea* + P effect on respiration). Note the *y*-axis in panel A is $\log_{10}$-transformed. We also include an example simulation from a cold and warm scenario using the temperature sensitivities 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.