DISSERTATION

TEMPERATURE SENSITIVITY AS A MICROBIAL TRAIT

Submitted by

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ABSTRACT

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Reaction rates in biological systems are strongly controlled by temperature, yet the degree to which temperature sensitivity varies for different enzymes and microorganisms is being largely reformulated. The Arrhenius equation is the most commonly used model over the last century that predicts reaction rate response with temperature. However, the Arrhenius equation does not account for large heat capacities associated with enzymes in biological reactions, thus creating significant deviations from predicted reaction rates. A relatively new model, Macromolecular Rate Theory (MMRT), modifies the Arrhenius equation by accounting for the temperature dependence of these large heat capacities found in biological reactions. Using the MMRT model I have developed a novel framework to assess temperature sensitivity as a biological trait through a series of experiments. This work provides evidence that microbes and enzymes can have distinct heat capacities, and thus distinct temperature sensitivities, independent of their external environment. I first assessed temperature sensitivity of soil CO_2 production from different soil microbial communities and then worked with pure cultures to examine temperature sensitivity of enzyme activities from soil microbial isolates. From these experiments I determined that temperature sensitivity varies based on genetic variation of the microbe and substrate type as well as examined the importance of using MMRT over the Arrhenius equation. Finally, I used a meta-analysis to analyze the distribution of temperature sensitivity traits to look across a variety of biological systems (e.g., the food industry, wastewater treatment, soils). I found that temperature sensitivity traits vary with organism type,

environment, process type, and biodiversity. Exploring temperature sensitivity as a trait allows for new insights of soil microbes from an ecological perspective as well has the potential to inform ecosystem climate models.

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Chapter 1: Introduction

The Arrhenius equation has been around for over a century and is the accepted way of modeling how reactions vary with temperature (Laidler, 1984). This equation predicts that reaction rates increase exponentially with increases in temperature. Although originally intended to describe simple chemical reactions, the Arrhenius equation is applied abundantly to biochemical reactions (Sierra, 2012; Schulte, 2015). As most biological reactions are unimodal, a consequence of applying this equation in biological settings is that the Arrhenius equation can only apply across a particular temperature range, above which it is assumed that denaturation of the enzymes cause declines in rate and thus deviance from the Arrhenius equation (Dell et al., 2011; Pawar et al., 2016). However many studies have noted that declines in reaction rates are within temperature ranges where enzyme denaturation is unlikely to occur (Ratkowsky et al., 2005; Knies and Kingsolver, 2010; Schipper et al., 2014; Pawar et al., 2016).

A relatively new model, Macromolecular Rate Theory (MMRT), explain this decline in rate as a function of the large heat capacities associated with enzyme-catalyzed reactions (Hobbs et al., 2013; Arcus and Pudney, 2015; Arcus et al., 2016). So, while the Arrhenius definition of temperature sensitivity is strictly a function of reaction thermodynamics, MMRT suggests that enzyme properties can also impact temperature response curves. Hobbs et al. (2013) show that minor mutations to an enzyme can change the heat capacity of the reaction, and thus significantly change the temperature dependence of the enzyme-catalyzed rate, in the absence of any enzyme denaturation. Thus, different isoenzymes may respond to temperature differently. Like the Arrhenius equation, MMRT has the potential to be applied not only to specific chemical or biochemical reactions, but also to whole organismal or community functions (e.g., growth rate or

respiration rate) as a measure of temperature sensitivity (Knies and Kingsolver, 2010), providing critical information about thermal performance of organisms (Schulte, 2015).

Soils are particularly pertinent in this discussion of temperature sensitivity. Soil microbial communities produce an immense amount of carbon dioxide and other greenhouse gases (Falkowski et al., 2000; Canfield et al., 2010; Conant et al., 2011; Lu et al., 2013). Small variations in microbial community composition and how they respond to environmental change may have enormous impacts for carbon and nitrogen budgets and subsequently global warming (Bradford, 2013; Karhu et al., 2014). Because biological reaction rates are fundamentally controlled by temperature, small changes in surface temperatures may significantly alter rates of greenhouse gas emissions as well as rates of nutrient cycling in soils (Crowther et al., 2016). Thus, using the best model to predict the response of soil microbes to changes in temperature is critical. Another facet of this inquiry is if different microbes and enzymes will have dissimilar temperature responses, convoluting our predictions. Although many studies support the notion that different microbes have dissimilar growth curves at different temperatures (Ratkowsky et al., 1982; Huang et al., 2011; Martiny et al., 2016), in the soils literature, differences in temperature sensitivity between different communities is predominantly attributed to differences in substrate and other abiotic characteristics (Conant et al., 2008; Haddix et al., 2011), and assumes that the importance of the microbes themselves in determining temperature sensitivity of a soil system to be static.

The objective of my dissertation is to improve understanding of temperature sensitivity of microbial communities. I address two major questions: 1) how does the activity of soil microbes vary with temperature? And 2) how should temperature sensitivity be characterized? I developed a novel framework to examine temperature sensitivity as a biological trait. I first identified that

soil microbial communities and microbial isolates do have distinct temperature sensitivities independent of their environment through two experiments (carbon dioxide flux of whole soil communities and enzyme assays of soil microbial isolates) that are better characterized by MMRT than by the Arrhenius equation. I then developed a temperature sensitivity framework introducing the concept of temperature sensitivity as a biological trait and characterized the distributions of those traits using a meta-analysis.

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Chapter 2: Temperature sensitivity of soil microbial communities: an application of macromolecular rate theory to microbial respiration[1]

Introduction

Heterotrophic soil respiration rates are one of the greatest sources of uncertainty in our current understanding of the earth's climate system (Mukhortova et al., 2015). While soil respiration rates are primarily driven by substrate availability, temperature, and moisture, roughly 30% of observed variation cannot be explained by these factors (Ayres et al., 2009; Cleveland et al., 2014; Keiser et al., 2013; Strickland et al., 2015). Microbial community composition sometimes explains part of this variation in rates of soil respiration (Cleveland et al., 2014; Strickland et al., 2009), indicating that differences in microbial physiology and kinetics among microbial communities may be important mediators of carbon (C)-climate feedbacks. For example, the extracellular enzyme produced by microbes to decompose C-rich organic matter can vary in their temperature sensitivity (Subke et al., 2006), resulting in different respiration kinetics among sites.

The thermal sensitivity of heterotrophic soil respiration rates can vary substantially among microbial communities due to thermal adaptation, changes in carbon use efficiency (CUE) (Frey et al., 2013; Steinweg et al., 2008), accelerated enzyme kinetics, and increased turnover (Bradford, 2013; Bradford et al., 2008). However, few studies have attempted to attribute variations in the temperature sensitivity of soil organic matter decomposition to variation in the physiology of soil microbial communities (Balser and Wixon, 2009).

[1] **Alster, C.J., Koyama, A., Johnson, N.G., Wallenstein, M.D., and von Fischer, J.C. (2016). Temperature sensitivity of soil microbial communities: An application of macromolecular rate theory to microbial respiration.** ***Journal of Geophysical Research-Biogeosciences***

Understanding if microbial communities have unique/differing temperature sensitivities, independent of the soil or soil organic matter, that impact rates of heterotrophic soil respiration is important not only for fundamental understanding of microbial enzyme dynamics, but also may have implications for global C modeling as small changes in respiration rates can correspond to major shifts in atmospheric CO_2 concentrations (Riley, 2015).

Most models predicting temperature sensitivity of microbial respiration rely on a version of the Arrhenius equation. This model predicts an exponential increase in reaction rate with temperature, but does not include mechanisms that might cause enzymatically-driven non-linearities with temperature (e.g., a temperature optimum before enzyme denaturation). However, numerous observations from both field and experimental studies indicate a temperature optimum of both soil enzymes and of soil microbial functions, such as respiration and nitrification, that happens at significantly lower temperatures than would be expected for enzyme denaturation (see Schipper et al. 2014). These optima often occur within a normal range of temperatures found *in situ* and contradict the reaction rate response predicted in the Arrhenius equation. A new model proposed by *Hobbs et al.* [2013] called Macromolecular Rate Theory (MMRT) explains this observed decline in rate that appears before true denaturation as a consequence of the change in the heat capacity ($\Delta C_P^{\ddagger}$) of the enzyme; heat capacity is the amount of thermal energy added (measured in Joules) that causes the temperature of the system to increase by 1°C. We hypothesize that, because different microbial communities may produce different isoenzymes (enzymes with different structures, but similar functions, (Wallenstein et al., 2011), communities differ in their temperature response because the various isoenzymes may have different $\Delta C_P^{\ddagger}$ values. While there are several semi-empirical theories that attempt to explain this nonlinear fit (Corkrey et al., 2014; Daniel and Danson, 2013; Del Grosso et al.,

2005; Peterson et al., 2004), MMRT provides theoretical justification for declines in temperature sensitivity as temperature increases, and has been shown to fit soil respiration data well (Schipper et al., 2014).

Our focus here is on an emerging and little addressed question: how important are differences in the microbial community for determining the temperature sensitivity of microbial respiration? This question falls under the more general realm of microbial community effects on decomposition dynamics. Here, we aim to 1) evaluate the relative importance of the source microbial community on individual components of soil microbial respiration, and 2) estimate the temperature sensitivity of different microbial communities. Our approach uses a reciprocal transplant design to measure rates of soil microbial respiration over time in microcosms at different temperatures. By setting up the experiment as a reciprocal transplant, we were able to isolate the effects of the microbial community from those of the soil and temperature. A complicating factor is that soil microbes draw upon disparate pools of soil organic C (SOC) that vary in size and reactivity (Davidson and Janssens, 2006; Haddix et al., 2011). To quantitatively account for this effect, we interpret our results in light of a two-pool model, divided into a labile and recalcitrant C pool. Unlike similar studies conducted by Strickland et al. (2009) and Cleveland et al. (2014) that examine the relative importance of microbial communities on soil respiration as a whole, we attempt to discern which aspects of heterotrophic soil respiration and temperature sensitivity are explained by differences in the microbial community. To calculate the temperature sensitivity of the different communities, we compared predictions of the Arrhenius and MMRT equations to find which was more consistent with our data. It should also be noted that this is also the first independent test of MMRT. We anticipate that understanding the relative importance of different microbial communities in predicting rates of soil respiration and how

changes in temperature could impact microbial communities and thus cause variation in rate of respiration is of fundamental and applied ecological importance.

Materials and Methods

Study Sites and Field Sampling

We collected soil from plots on three grassland sites that fall along a climatic gradient across the US Great Plains: Makoce Washte Prairie (MA) in South Dakota (43º32'N, 96º58'W); Shortgrass Steppe (SG) in Colorado (40º49'N, 104º43'W); and Sevilleta in New Mexico (34º21'N, 106º52'W). We chose these sites primarily because they vary in mean annual temperature, although they also differ in mean annual precipitation, vegetation, soil texture, and soil C:N (Table 2.1). Soils were sampled as cores 5 cm in diameter and 10 cm deep and were shipped overnight to Colorado State University. Upon receipt, the cores were homogenized and passed through a 2-mm sieve, then stored at 4°C until processing.

Reciprocal Transplant Incubation

We established a reciprocal transplant microcosm experiment in order to assess the relative importance of soil type, temperature, and source inocula on soil microbial respiration. We added 5 g (dry weight) of field-moist soil from each site to 60 mL glass vials and added water so that the soils were at 50% water holding capacity (WHC). After being covered with aluminum foil, the vials were autoclaved for 90 minutes at 121°C to sterilize the soil. We measured soil respiration rates after autoclaving to test for sterility and found that CO_2 production rates were below detection limits. To create the microbial inocula slurry, 4 g of unsterilized soil from each site was incubated at 10°C for 14.5 hours to acclimate the community

from the colder refrigerator temperature. We then added 45 mL of sterile DI water to each incubated soil and stirred for 2 minutes, followed by a 10 minutes settling period. This last step of stirring and settling was repeated and the supernatant was passed through a 1 mm mesh sieve to remove large organic debris, aggregates, and particulates from the inoculum (van de Voorde et al., 2012). We recognize that the water-extracted inocula may not be a representative sample of the in situ communities, but consider it a safe assumption that inocula from the different study sites contain different communities. Previous work in our laboratory has shown stark differences in methane-consuming microbial community composition at the 3 study sites (Johnson et al., 2014).

We then inoculated the microcosms by adding 0.25 mL of the inocula slurry to vials of sterilized soil, following our reciprocal transplant design. For example, inoculum slurry that originated from the MA site was added back to the sterilized MA soil, the sterilized SG soil, and the sterilized SV soil. This was done for each of the inoculum types onto each of the soil types, for a total of nine site × inocula combinations. Additional sterilized DI water was added to each microcosm to replace water lost during autoclaving. Afterwards, we incubated the microcosms containing the soil-inocula mixtures at 10, 20, and 30°C, for a total of 27 site × inocula × temperature combinations. Each set of the site × inocula × temperature combinations contained 6 replicates for a total of 162 microcosms in the experiment. Eighteen control microcosms for each soil type, with no microbial inocula added, were also created (6 for each soil type at each temperature) to check for contamination over the course of the incubation.

Rates of CO_2 production were measured at seven time points throughout the experiment (days 6, 16, 27, 37, 59, 89, and 124) through injections of 3 mL of the headspace gas from the vials into a continuously following stream of sample air into a Los Gatos DLT-100

Methane/Carbon Dioxide Analyzer using an autosampler. The resulting peak of CO_2 from the injections was measured and calibrated against known standards to estimate the concentration of CO_2 in each the vial. Vials were capped and sampled for an initial CO_2 concentration and then resampled four days later for a second CO_2 concentration measurement. We calculated rates of CO_2 production from these two time points. Respiration rates were not measured until day 6 to allow for acclimation of the microbes to the environmental conditions and for biomass to reach stable levels. Throughout the experiment the control vials produced CO_2 at rates 1-3 orders of magnitude lower than the experimental vials, although by the last month of the experiment, two of the 54 control vials produced CO_2 at rates closer to those of the sample vials. Therefore, although contamination was possible there is not evidence that it was a dominant factor driving variation throughout the experiment.

In between sampling dates, the vials were uncapped and covered with a thin polyethylene film (12.5 μm thick) to allow oxygen to enter the microcosms and to limit evaporation or movement of microbes, and additional sterile DI water was periodically added to maintain vials at 50% WHC.

Data Analysis: Two-Pool Model

It has long been recognized that temporal patterns in rates of soil respiration can be modeled as microbial consumption of organic matter pools that span from more quickly to more slowly depleted (e.g., Parton et al. (1980)). For the 124-day timescale of this experiment, quantifying this effect was important because recalcitrant versus labile organic matter pools can be differently temperature sensitive (Davidson and Janssens, 2006). To characterize this effect, we applied a two-pool model, assuming that CO_2 production from the more rapidly depleted

(hereafter "labile") pool could be modeled as a first-order process, while respiration dependent on the slower pool ("recalcitrant") could be treated as constant over time (Figure 2.1a). Equation 1 is the predicted microcosm CO_2 production rate over time.

$$Respiration(t) = L_0\, e^{-kt} + R, \quad (1)$$

where L_0 is the initial size of the labile C pool, k is the first order CO_2 production rate from the labile pool, R is the asymptotic rate of respiration, and t is time. In this model, a large value of L_0 would indicate a large initial labile C pool, a large R would indicate higher rates of respiration from the recalcitrant C pool, and a large k would indicate faster depletion of the labile C pool. An idealized temporal pattern is illustrated in Figure 2.1b.

To emphasize the importance of using Equation 1 in our analysis we illustrate in Figure 2.2 how the percent of respiration derived from the labile C pool changes over time among temperature treatments. This figure, which is developed from mean parameter values from a sample combination in this experiment (MA soil with SG inocula), illustrates the intuitive result that labile pools are more quickly depleted at warmer temperatures. If we had simply fit a temperature model (either MMRT or Arrhenius) to the respiration rate at some time point, Figure 2.2 shows that the respiration rates would also differ due to varying depletion of the labile pool. Thus, use of Equation 1 allows us to avoid this potentially confounding bias.

Parameter values L_0, k, and R were determined for each microcosm vial using JMP Pro 11 using an analytic Gauss-Newton solution to the non-linear model. We then assessed the relative importance of temperature, site, and microbial community for explaining variation in L_0, R, and k. We used Grubbs' test for outliers to remove anomalous parameter values from the data

set using the package outliers (Komsta, 2011) in R (R 3.1.1 GUI 1.65). Each parameter was examined using a linear model with a full factorial design (temperature, soil source, inoculum, and their interactions) and tested with an ANOVA using the package lmerTest (Kuznetsova et al., 2014) to determine which effects were significant, and to quantify the explanatory power from the sum-of-squares (SS) statistic. We also used the parameter values to integrate for the cumulative C respired from each vial over the course of incubation and then conducted another ANOVA with a full factorial design to determine significance of effects and their SS for the percent of the total C respired from initial SOC in each vial.

Comparison of Temperature Models

We used the k parameter values to estimate the activation energy and the heat capacity (temperature sensitivity) for each site × inocula combination. Activation energy was estimated using the Arrhenius equation:

$$\ln(k) = \ln(A) - \frac{E_A}{RT}, \quad (2)$$

where k is the rate constant, A is a pre-exponential factor, E_A is the activation energy, R is the universal gas constant, and T is temperature. The Arrhenius model assumes that activation energy is a barrier to the rate of the reaction, such that reactions with greater activation energies are more temperature sensitive (Davidson and Janssens, 2006). However, the Arrhenius equation assumes that the rate of reaction increases exponentially with temperature. Empirical observations of declines in rate as temperature increases, which by definition do not correlate with Arrhenius predictions, are attributed to enzyme denaturation.

We also examined the explanatory power of MMRT (Hobbs et al., 2013; Schipper et al., 2014), which incorporates additional principles from physical chemistry; that is, enzyme activity has a parabolic response to temperature, due in part to the heat capacity of the enzyme (C_P) (Arcus et al., 2016). The C_P of the system is simply the temperature dependence of enthalpy (H) of the system. Unlike in reactions with small molecules where the temperature dependence of Gibbs Free Energy ($\Delta G^{\ddagger}$) can be assumed to be negligible, biological reactions involve enzymes, which are relatively large and thus have large $\Delta C_P^{\ddagger}$ values making $\Delta G^{\ddagger}$ a function of temperature, allowing for an inaccurate estimate of E_A (Hobbs et al., 2013). The MMRT equation is:

$$\ln(k) = \ln\left(\frac{k_B T}{h}\right) - \frac{\Delta H^{\ddagger}_{T_0} + \Delta C^{\ddagger}_P (T - T_0)}{RT} + \frac{\Delta S^{\ddagger}_{T_0} + \Delta C^{\ddagger}_P (\ln T - \ln T_0)}{R}, \quad (3)$$

where k_B is Boltzmann's constant, h is Planck's constant, R is the universal gas constant, T is temperature, S is entropy, and ‡ indicates that it is the transition state. When $\Delta C_P^{\ddagger}$ is large and negative, the temperature response will deviate significantly from what is predicted in the Arrhenius equation, but when $\Delta C_P^{\ddagger}$ is zero, the temperature response will follow the predicted Arrhenius temperature response. Both E_A and $\Delta C_P^{\ddagger}$ were determined by fitting these equations to the site × inocula combinations in JMP Pro 11 using an analytic Gauss-Newton solution and a numerical Gauss-Newton solution to the linear and non-linear models, respectively. Following Hobbs et al. (2013), the T_0 value in the MMRT model was set to approximately 10º below average temperature at the maximum rate. Adjusted R^2 values for each of the Arrhenius and MMRT models were calculated in Excel. We compared Arrhenius and MMRT model fits using Akaike Information Criterion values corrected for a finite sample size (AICc). We calculated the differences between the $\Delta C_P^{\ddagger}$ values using a two-sampled approximate Z-test. In order to

calculate the point of maximum temperature sensitivity (TS_{max}) and temperature optima (T_{opt}) of the MMRT equation, we took the derivative with respect to temperature (dk/dT). The point of maximum temperature sensitivity occurs at the peak of the parabola, where variation in temperature has the largest effect on reaction rate, and the temperature optima occurs when dk/dT is zero, at the temperature where the reaction rate is at its maximum. For the Arrhenius equation, temperature sensitivity rises exponentially with temperature, thus there is no T_{opt} and TS_{max}.

Results

Exponential Decay Model

Our exponential decay model for respiration rates over time (Equation 1) gave a strong fit for the overwhelming majority of the CO_2 production data from each of the microcosms. The median R^2 value for the model fit was 0.988, with a range of 0.934 to 1. One vial was excluded from analyses due to a high CO_2 flux at the last time point, and 23 out of the 161 vials analyzed had a single data point excluded, usually because the first CO_2 production rate was lower than subsequent measurements. Example respiration data with corresponding fits are given in Figure 2.3. It should be noted that the majority of the cases (19 out of 22) where the first time point was excluded was at the lowest temperature (10°C) and in 12 of these cases, contained the SV-derived inocula.

The range of estimates for each of the three parameters, k, L_0, and R, varied considerably (Table 2.2). Parameter k, which corresponds to the rate that the labile C pool is depleted, ranged from 0.230 day^{-1} in SV soil with MA-derived inocula at 30°C to 0.007 day^{-1} in MA soil with SV-derived inocula at 10°C. Parameter L_0, corresponding to the initial labile C pool, also varied two

orders of magnitude, from 12.0 mg CO_2-C g soil C^{-1} in SG soil with SG- derived inocula at 30°C to 0.377 mg CO_2-C g soil C^{-1} in SV soil with MA- derived inocula at 10°C. Lastly parameter *R*, the asymptotic rate of CO_2 production, varied less than the other two parameters and ranged from 0.655 mg CO_2-C g soil C^{-1} day^{-1} in SG soil with SG- derived inocula at 30°C to 0.008 mg CO_2-C g soil C^{-1} day^{-1} in MA soil with MA- derived inocula at 10°C.

Sums of Squares Analysis for k, L_0, and R

In examining the relative explanatory power for *k*, L_0, and *R*, we found that all three of these response variables were well explained, with overall R^2 values of 0.635, 0.868, and 0.936, respectively. We found that temperature was the most important factor in explaining variation in *k*, but that additional important predictors exist as well. To compare the relative explanatory power of temperature, soil type, and inocula type, we calculated a combined contribution of each factor. For example, we computed the importance of temperature as the individual sum of squares (SS) contribution for temperature (i.e., just the SS for the main effect of temperature) plus SS for all interactions where temperature is present (i.e., the main effect of temperature plus the two-way interactions and three-way interaction of temperature with inocula and soil). Thus, while temperature is the most important factor explaining variation in *k*, we found that inocula type is nearly as important as soil type in explaining variation in *k* according to the combined SS results for inocula (Figures 2.4a and 2.4b). Because two-way and three-way interactions dominate in explaining variation in *k*, the total explanatory power of inocula in determining the rate of CO_2 flux in this experiment was 32%. Variation in L_0 is best explained by temperature, followed by soil type, although several higher order interactions are also significant (Figures 2.4c and 2.4d). Lastly, both the individual SS and combined SS analysis for *R* indicate that

temperature and soil type are most important and explain roughly equal amounts of variation in *R*, although inocula type and two two-way interactions are also significant (Figures 2.4e and 2.4f). Interestingly, the combined SS analysis for inocula type for L_0 and *R* were relatively small at 6.4% and 6.1%, respectively.

Cumulative C Respired

In addition to comparing the relative importance of temperature, soil type, and inocula type for parameters *k*, L_0, and *R*, we also examined the relative significance of each of these factors for the cumulative C respired. Although investigating each of the effects of temperature, soil type, and inocula type on aspects of soil microbial respiration separately provides greater resolution, we conducted this additional analysis in order to compare our results to similar studies as well as to assess the relationship between each of the parameters and total C respired. In our calculations, cumulative C respired is presented as a percentage of the total soil C. On average, 5.2% of soil C was respired away during the 124-day incubation (range 1.3% to 14%). This fraction was highest for SG soil with SG-derived inocula at 30°C and lowest for SV soil with SV-derived inocula at 10°C. The majority of variation in the cumulative C respired can be explained by soil type (55%), followed by temperature (40%) (data not shown). All of the inocula terms from the combined SS analysis explain less than 1.5% of the variation in C respired. The largest percent of C respired came from the incubations with the SG soil type with an average of 12% at the 30°C to an average of 5.0% at the 10°C (P<0.001). SV soil type had the smallest percent of C respired with an average of 3.9% at the 30°C to an average of 1.5% at the 10°C (P <0.001).

Arrhenius Versus MMRT Models Fits

Arrhenius and MMRT model fits to data can be evaluated on two criteria: do they give thermodynamically plausible results and does one model give a statistically superior fit as compared to the other. The Arrhenius equation predicted thermodynamically plausible results for all nine soil × inocula combinations (Figure 2.5), but MMRT predicted thermodynamically plausible results for only six of the nine combinations. Concave up curve fits occur under the thermodynamically impossible situation when the model fit yields a positive value for $\Delta C_P^{\ddagger}$.

When both models provided plausible fits to the data, MMRT was statistically superior to Arrhenius in all six combinations. AICc statistics found the most parsimonious model to be MMRT, with all probabilities ranging from 0.942 to 1. Our experiment utilized only three unique temperature points (note: each point was replicated). However, since our model comparisons were based on AICc, which penalizes model fit using the number of parameters in the model, we can conclusively state that MMRT was superior to Arrhenius in the six soil × inocula combinations tested. Thus, heat capacity values derived from successful MMRT model fits were chosen for further analysis.

Variation in the MMRT Model

Heat capacity values, derived from the MMRT equation, varied among soil × inoculum combinations (Figure 2.6a). Values were least negative for the MA-derived inoculum in the SG soil, and most negative for two of the SV-derived inocula. When averaging the $\Delta C_P^{\ddagger}$ of inocula across soils (Figure 2.6b), we found that the MA-derived microbial community has a heat capacity value that is significantly smaller in magnitude than SG or SV-derived microbial communities (P<0.0001). Following Figure 2.5 from Schipper et al. (2014), we also plotted

dk/dT as a function of temperature to illustrate the sensitivity of reaction rate (k) changes to variation in temperature (Figure 2.7), and to understand variation in $\Delta C_P^{\ddagger}$ at a more intuitive level. We found that T_{opt} ranged from 19.5°C to 37.2°C and TS_{max} ranged from 9.1°C to 15.4°C with the high T_{opt} and TS_{max} occurring in the SG soil with MA inocula and the low T_{opt} occurring in the SG soil with SV inocula, but the low TS_{max} occurring in the MA soil with SV inocula.

Discussion

Soil respiration comprises between 50-80% of total ecosystem respiration (Bradford and Ryan, 2008) with microbial respiration contributing an average of 54% annually to that total (Ryan and Law, 2005), yet much is still unknown about the key roles and mechanisms that differences in the microbial community play in global C cycling and how much it is impacted by changes in temperature. While the framework of Arrhenius kinetics underlies most current models of soil C, many studies have pointed out the need for increased sophistication for modeling temperature sensitivity of soil C stocks (Conant et al., 2008; Davidson and Janssens, 2006; Liang et al., 2014; Sierra et al., 2015). Our study provides new insights into the temperature sensitivity of soil microbial respiration and reaffirms the need for improved model representation of this process.

Although a few studies have noted moderate explanatory power of the microbial community in predicting rates of microbial respiration from soil and litter (e.g., Balser and Firestone (2005), Cleveland et al. (2014)), here we show that differences in temperature sensitivity of the microbial community are most pronounced in the rate that labile C pools are depleted (parameter k), and that temperature sensitivity varies with different soil × microbial

community combinations. Furthermore we find that the MMRT model is better than Arrhenius at explaining temperature sensitivity of microbial communities.

Importance of the Microbial Inocula

The importance of microbial community composition for predicting rates of CO_2 flux from soils is not well established. The few studies to date have focused on microbial respiration from litter and have not elucidated the components of respiration where differences in microbial community composition may have an effect (e.g., Strickland et al. (2009), Cleveland et al. (2014)). Temporal trends in soil respiration are complex and emergent features of soils that we characterized using a three-parameter equation (i.e., Equation 1). Although we found statistically significant effects of the microbial inocula for all parameters, only in *k*, the rate that the labile C pool is depleted, did the microbial inocula explain a large proportion of the variation. As illustrated in Figure 2.4b, the microbial inoculum was almost as important as soil type for explaining variance in *k*. Our experimental design did not allow us to determine the mechanisms for why the microbial inoculum type notably influenced variation in *k* (Figure 2.4b), while not strongly influencing variation in L_0 and *R*. One possible explanation for the insensitivity of L_0 to different microbial inocula is that it truly represents the size of the labile SOC pool, and this property is invariant with differences in the microbial community. Similarly it appears that the microbial inocula was also not an important determinant of *R*, the combined size and reactivity of the recalcitrant SOC pool, over the time scales of our experiment (Davidson and Janssens, 2006; Townsend et al., 1997). However for both L_0 and *R*, temperature was highly important and did drive increases in L_0, *R*, and cumulative C respired. Interestingly, this suggests that microbes perceive a larger pool of C at warmer temperatures and that temperature has a direct effect on

how much the microbial community perceives as available C, perhaps due to increased microbial activity and kinetics at the warmers temperatures.

Microbial inoculum type and its interactions with temperature and soil type may cause variation in *k* for a number of reasons such as differences in microbial biomass, CUE, genetic suite of enzyme production, and microbial physiology. Changes in microbial biomass over time could lead to changes in rate of respiration because a larger number of microbes could result in a larger value of *k* (Huang et al., 2015). However, it is unlikely that changes in biomass were the driving factor for variation in *k* because of the following observations. In 84% of the cases, there was a monotonic decline in respiration rate over time, suggesting that the incubation was immediately substrate limited. In the few instances where the second respiration rate time point (i.e., 16 days) was higher than the first, we infer that microbial biomass, as opposed to substrate, was the rate-limiting factor during the first 6 days of the incubation. Once respiration rates began to decline, which in most cases was immediately, we can assume that the microbial biomass had reached a maximum, and so variation in *k* was attributable to differences between communities.

Independent of microbial biomass, *k* may also vary due to differences among communities in CUE. CUE manifests as the interaction between specific inocula and organic matter quality at a particular temperature (Frey et al., 2013; Steinweg et al., 2008) and can be a function of both the community's ability to find and access physically protected substrates and the chemical suitability of the enzyme produced for the particular substrate (Cotrufo et al., 2013; Six et al., 2006). Microbial growth rates generally increase with increasing CUE (Sinsabaugh et al., 2013). Thus, when microbes are in an environment where their enzymes are more efficient at processing the dominant substrates, it is possible that they would exhibit a lower rate of respiration, and consequently a lower relative *k*. Over time, shifts in microbial community

composition to favor organisms that produce more suitable enzymes may cause an increase in efficiency (Allison, 2014), lowering respiration rates and impacting the overall value of k. However, the ability of the community to adapt throughout the course of the experiment may be confounded by insufficient genetic variation in the seed inoculum community (Aguilar et al., 2013) or by nutrient depletion (Sinsabaugh et al., 2013).

Along with differences in microbial CUE, other aspects of microbial physiology likely play an important role in predicting parameter *k*. Changes throughout the course of the incubation experiment, such as adaptation of the microbial inocula to the incubation temperatures and depletion of labile substrates, might affect the physiological state of microbial communities by changing their level of activity and consequently affecting their rate of respiration over time. Some of these changes in physiology could include shifts from the dormant state to active state or vice versa, increased metabolic costs with sustained warming, and energy spilling (Allison et al., 2010; Bradford, 2013; Curiel Yuste et al., 2007; Stenström et al., 2001). While our experiment was relatively short, 124 days, microbial communities have short generation times resulting in many generations throughout the course of the experiment and allowing for some level of adaptation at both the organismal and community scale. Understanding which, if any, of these mechanisms explain variation in *k* is certainly worth further inquiry in order to better predict how differences in microbial communities will influence soil CO_2 production.

Scope of Model Inference

We recognize that features of our laboratory incubations may limit extrapolation back to field conditions. In natural field sites, the sources of labile C are likely to vary widely and

include litter inputs, root exudates, dissolved organic C flows, and turnover of microbial biomass. In contrast, our experimental system likely has a larger initial pool of necromass than field systems because autoclaving of soils produces an abundance of microbial biomass that would be available for decomposition, thus leading to a larger value of L_0 for the laboratory than would be expected for the field. The first order decay constant, k, may differ between field and lab if early-phase microbial respiration is limited by factors other than labile C availability, or if the community composition in the inoculum differs from that of the natural soils. Likewise, sieving and autoclaving of soils likely altered the physical and chemical structures that influence rate of respiration from the recalcitrant pool (Berns et al., 2008). Even though autoclaving likely changed the relative proportions of the labile and recalcitrant C pools from field conditions, changes in the total organic C pool are should be relatively small (Berns et al., 2008; Shaw et al., 1999).

Despite the potential for differences between lab and field behaviors, we expect that the model described in Equation (1) characterizes structural similarities that are shared between natural and field systems. We took advantage of these shared structures to make comparisons of respiration patterns (from extracted model parameters) between treatments, even if such comparisons cannot be made between the incubations and natural systems.

Heat Capacity Varies with the Microbial Inocula

Our study is, to our knowledge, the first to observe that different microbial communities produce enzymes with different heat capacity values. Larger $\Delta C_P^{\ddagger}$ values indicate more degrees of freedom (i.e., an increased physical flexibility) of the enzyme (Hobbs et al., 2013). Increased values of $\Delta C_P^{\ddagger}$ lead to significant temperature dependence of $\Delta G^{\ddagger}$, which corresponds to

deviations from the Arrhenius equation and is accounted for in the MMRT equation. The interaction between $\Delta C_P^{\ddagger}$ and $\Delta G^{\ddagger}$ drives the curvilinearity that we see in Figure 2.5. Interestingly, microbial inocula from the same source environment often had similar trends in curvature and magnitude in their respiration-temperature response curves (e.g., SV inocula in Figure 2.5), independent of the soils in which they were incubated (Figure 2.6b), giving rise to similar $\Delta C_P^{\ddagger}$ values. This suggests that different microbial communities produce different enzymes that, in turn, respond differently to changes in temperature. Our study is the first to show the indirect effect of the microbial community on respiration via a heat capacity effect. Previously, studies have shown that microbial communities and their interaction with particular OM can directly affect the rate of soil respiration under different temperatures (Frey et al., 2013; Karhu et al., 2014). Here, we show that microbial community type also has an indirect effect on respiration due to a difference in heat capacity.

Temperature Sensitivity

As others have previously noted (e.g., Sierra (2012), Liang et al. (2014)), the notion of temperature sensitivity is deceptively complex. The comparison of Arrhenius and MMRT model fits (this study, and Schipper et al. (2014)) indicates that the temperature response of many soil microbial communities and their functions are not linear. The implication is that apparent temperature sensitivity, when defined as the slope of the respiration-temperature response line, changes depending on the range of temperatures observed. Interestingly, MMRT captures an important natural pattern: the rate of reaction is most variable with temperature at common environmental temperatures (Figure 2.7). This peak in temperature sensitivity is not captured in

the Arrhenius model, which instead predicts an exponential rise in temperature sensitivity with increasing temperature.

Variation in TS_{max} observed in this study is consistent with the idea that history shapes microbial community composition and thus "selects" for enzymes that are less temperature sensitive over a given temperature regime. However, the lack of a monotonic response to temperature complicates discussion of temperature "sensitivity" by requiring that the temperature range be specified. We therefore suggest that discussions of temperature sensitivity become more nuanced, considering dk/dT and the associated quantitative features like the temperature of maximum rate and the temperature of maximum temperature sensitivity.

Patterns in Temperature Sensitivity

Although extrapolation back to the original microbial communities and field conditions is beyond the scope of this study, we consider a few interesting aspects of the data for further inquiry based on geographic location of source inocula. We found that the microbial community derived from the MA site had a $\Delta C_P^{\ddagger}$ closest to zero, indicating the lack of a thermodynamic temperature optimum and generally less variation in dk/dT with temperature. In contrast, the microbial community derived from the SV site, at the other end of the temperature gradient, appeared to have a strong thermodynamic optimum and wide variation in dk/dT with temperature. The microbial community with the most prominent temperature optimum is from the warmest and driest site, while the community found to lack a temperature optimum is from the coolest and wettest site (Table 2.1). This is consistent with results found in Strickland et al. (2015), which found that microbial inocula from their coolest site showed least variation in respiration with moisture and temperature manipulations and the microbial inocula from their

warmest site showed most variation in respiration rates. We speculate that at the MA site there is not a competitive advantage for enzymes to be optimized for temperature, but at the SV site, where temperature and moisture are more extreme, a competitive advantage for enzymatic temperature optimization may exist. The mechanisms causing differences in $\Delta C_P^{\ddagger}$ between communities deserves further attention.

Conclusions

Overall our results provide the first outside evidence of MMRT outperforming Arrhenius in explaining the temperature sensitivity of soil microbial respiration. Moreover, we found wide variation in $\Delta C_P^{\ddagger}$ values with different soil × inoculum combinations. What drives this variation in $\Delta C_P^{\ddagger}$ warrants further investigation since *a priori* knowledge of temperature sensitivity of microbial communities has potential implications for modeling of SOC dynamics. Because the importance of the microbial community has not been well understood, the microbial component of soil microbial respiration has largely been left out of models until very recently (Treseder et al., 2012; Wieder et al., 2013; Wieder et al., 2015). We anticipate that significant improvements in theory, simulation modeling, and lab and field experiments, can come from focusing on factors that cause variation in enzyme activation energy that gives rise to the phenomenon of thermodynamic temperature optima.

Table 2.1. Site characteristics. Mean annual precipitation (MAP), mean annual temperature (MAT), grassland type, soil texture, gravimetric % carbon, and molar C:N ratio.

Site	MAP (mm)	MAT (°C)	Grassland Type	Soil Texture (%)			%C	C:N
				Sand	Silt	Clay		
Makoce Washte (MA)	627	7.8	Tall	17	49.3	33.8	6.2	11.6
Shortgrass Steppe (SG)	320	8.6	Short	50.8	22.8	26.3	1.8	10.6
Sevilleta (SV)	244	13.3	Desert	61	20.4	18.7	1.4	6.5

Table 2.2. Mean values for parameters k, L_0, and R ± SEM.

	k			L_0			R		
	10°C	20°C	30°C	10°C	20°C	30°C	10°C	20°C	30°C
MA soil, MA inocula	0.056±0.006	0.048±0.005	0.066±0.013	0.875±0.133	1.256±0.141	2.329±0.393	0.031±0.006	0.067±0.009	0.250±0.046
SG soil, MA inocula	0.056±0.002	0.092±0.004	0.120±0.007	1.592±0.060	4.269±0.244	6.778±0.508	0.199±0.015	0.297±0.011	0.501±0.023
SV soil, MA inocula	0.055±0.006	0.083±0.006	0.206±0.009	0.587±0.056	1.535±0.072	4.399±0.361	0.041±0.003	0.073±0.007	0.137±0.010
MA soil, SG inocula	0.051±0.011	0.105±0.007	0.118±0.007	0.923±0.192	2.022±0.164	4.462±0.326	0.042±0.009	0.128±0.007	0.327±0.016
SG soil, SG inocula	0.055±0.004	0.150±0.009	0.148±0.009	1.634±0.177	7.848±0.481	8.824±0.763	0.164±0.006	0.356±0.018	0.586±0.014
SV soil, SG inocula	0.074±0.012	0.065±0.013	0.145±0.009	0.880±0.126	0.861±0.219	3.062±0.151	0.042±0.004	0.066±0.010	0.118±0.010
MA soil, SV inocula	0.067±0.004	0.110±0.008	0.091±0.006	0.957±0.061	2.012±0.195	3.221±0.313	0.064±0.005	0.159±0.006	0.404±0.009
SG soil, SV inocula	0.073±0.004	0.135±0.008	0.094±0.008	2.032±0.149	5.068±0.287	4.914±0.326	0.150±0.004	0.267±0.010	0.474±0.020
SV soil, SV inocula	0.073±0.004	0.150±0.011	0.100±0.008	0.576±0.022	2.029±0.139	2.131±0.103	0.042±0.001	0.108±0.003	0.157±0.010

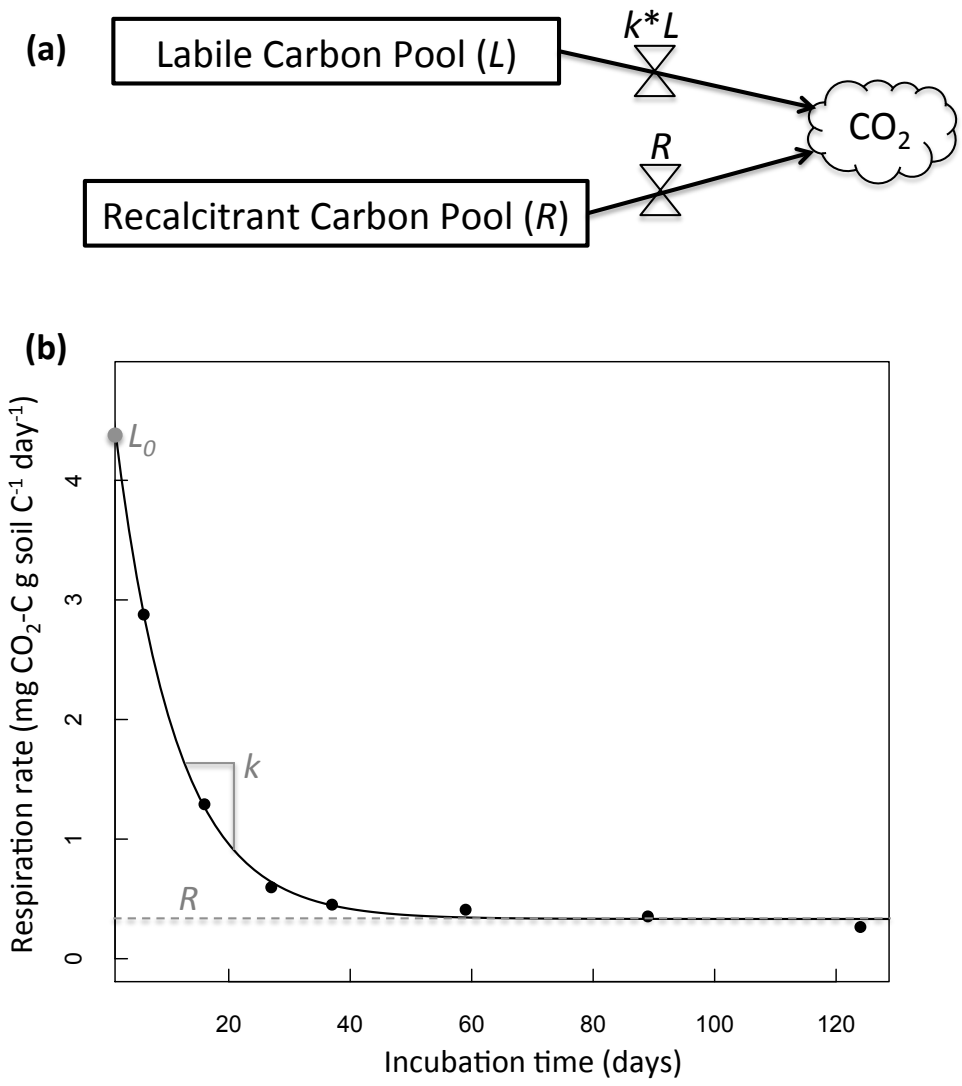

Figure 2.1. (a) Conceptual model for CO_2 produced from soil divided into two organic matter pools, *L* and *R*. The CO_2 produced from the labile pool changes over time, so it is multiplied by a constant, *k*. (b) Sample exponential decay curve (Equation 1) with fitted parameters. L_0 is SOC pool; *R* is asymptotic rate of respiration dominated by C from recalcitrant pools; and *k* is first order CO_2 production rate.

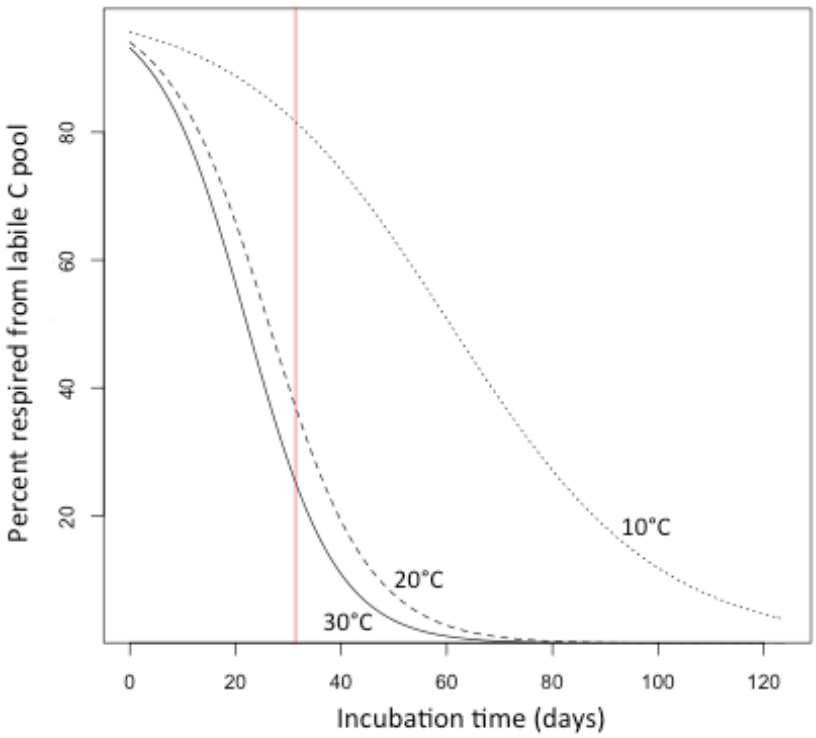

Figure 2.2. Percent of the respired CO2 that is derived from the labile pool for an example incubation (MA soil × SG-derived inocula) at 10, 20, and 30°C over time. The red vertical line indicates that a comparison of respiration rates at one month would confound the direct effect of temperature and the indirect effect of lost labile carbon. We implement a multi-pool analysis framework to overcome this potential source of bias.

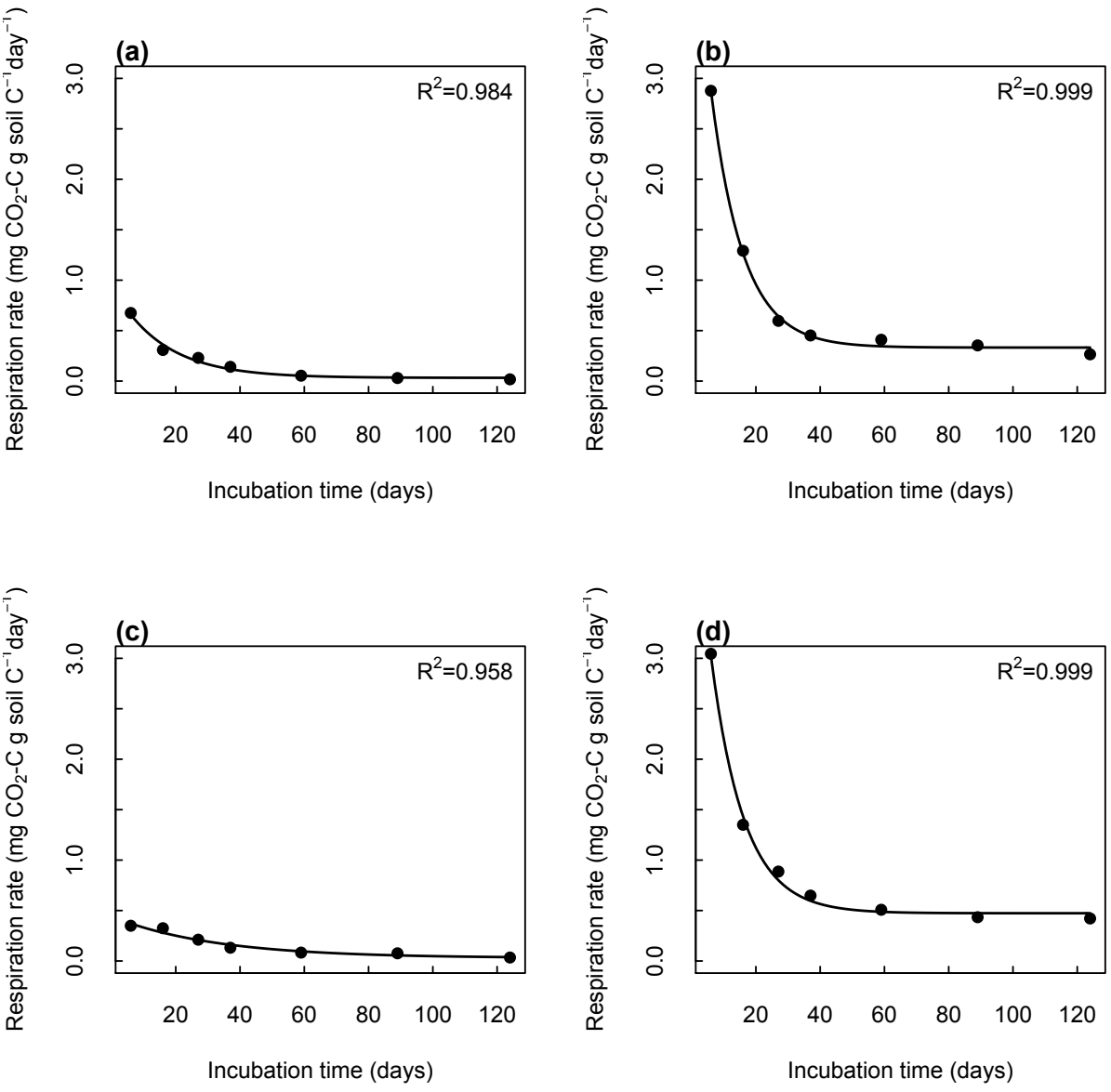

Figure 2.3. Example decay curves for individual incubation vials with fitted exponential decay curve from (a) MA soil with MA inocula at 10°C, (b) SG soil with MA inocula at 20°C, (c) SV soil with SG inocula at 20°C, and (d) SG soil with SV inocula at 30°C. These four vials illustrate the diverse types of kinetics exhibited among the set of incubations.

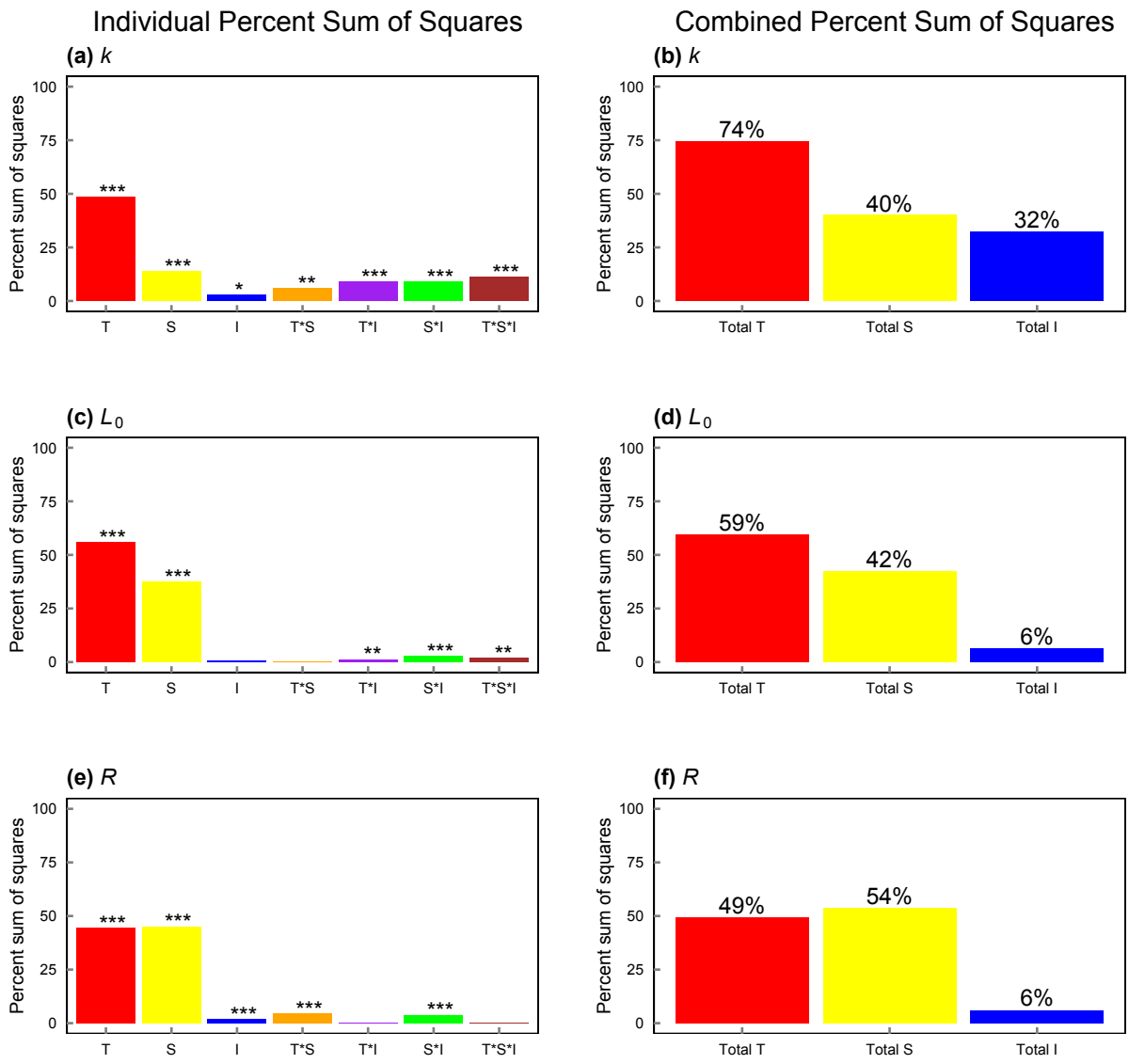

Figure 2.4. Sum of squares analysis for extracted parameters *k*, L_0, and *R*. The left side panel, (a), (c), and (e), represent the percent sum of squares for temperature (T), soil (S), inocula (I), temperature × soil (T*S), temperature × inocula (T*I), soil × inocula (S*I), and temperature × soil × inocula (T*S*I). (*) indicates $P<0.05$, (**) indicates $P<0.01$, and (***) indicates $P<0.001$. The right side panel, (b), (d), and (f), represent the percent sum of squares for the sum of all of the sum of squares relating to T, S, or I. Total percentages are indicated above each bar.

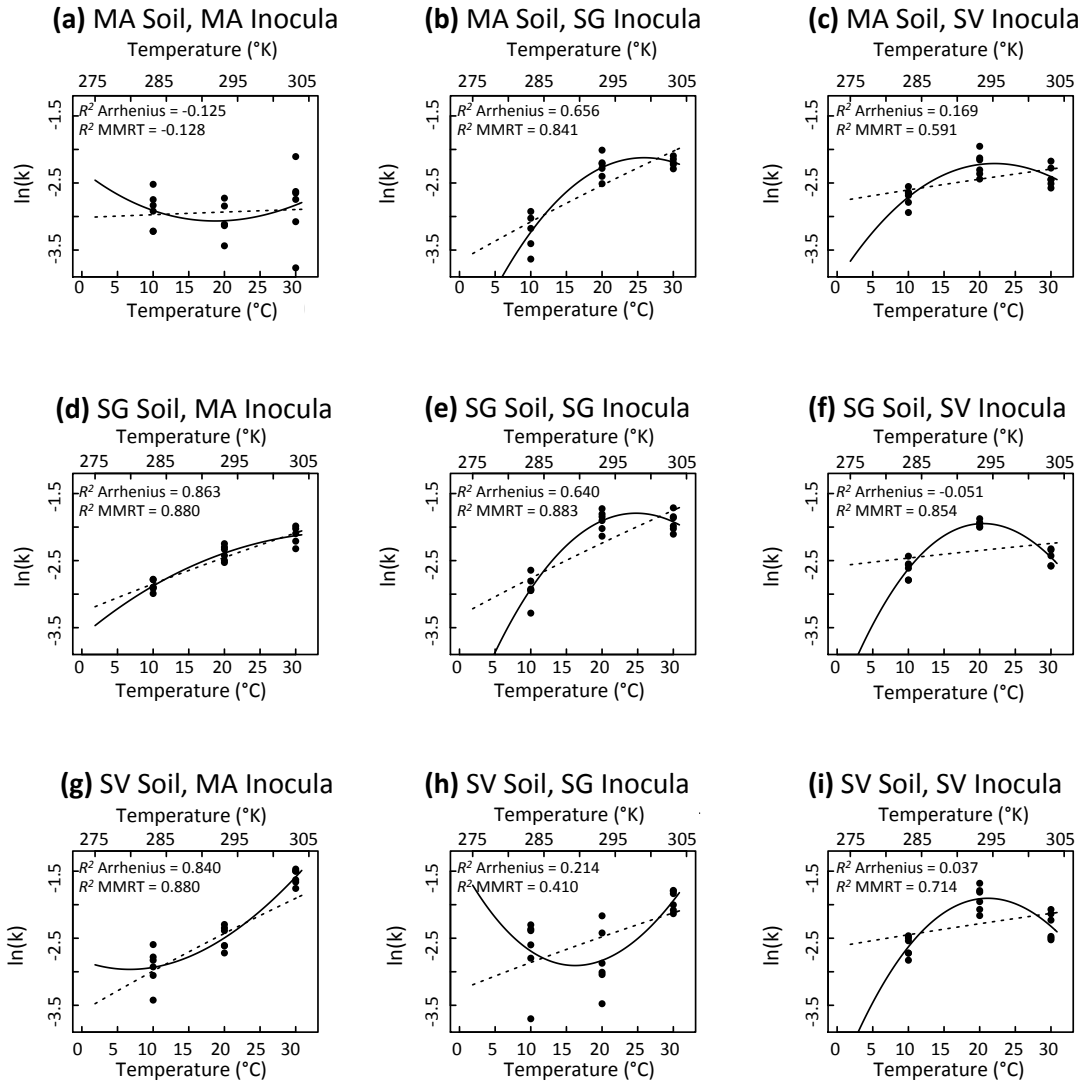

Figure 2.5. The fit of MMRT (solid line) and Arrhenius (dashed line) equations for each soil x inocula combination. Adjusted R^2 values for the Arrhenius and MMRT models are given in the top left corner.

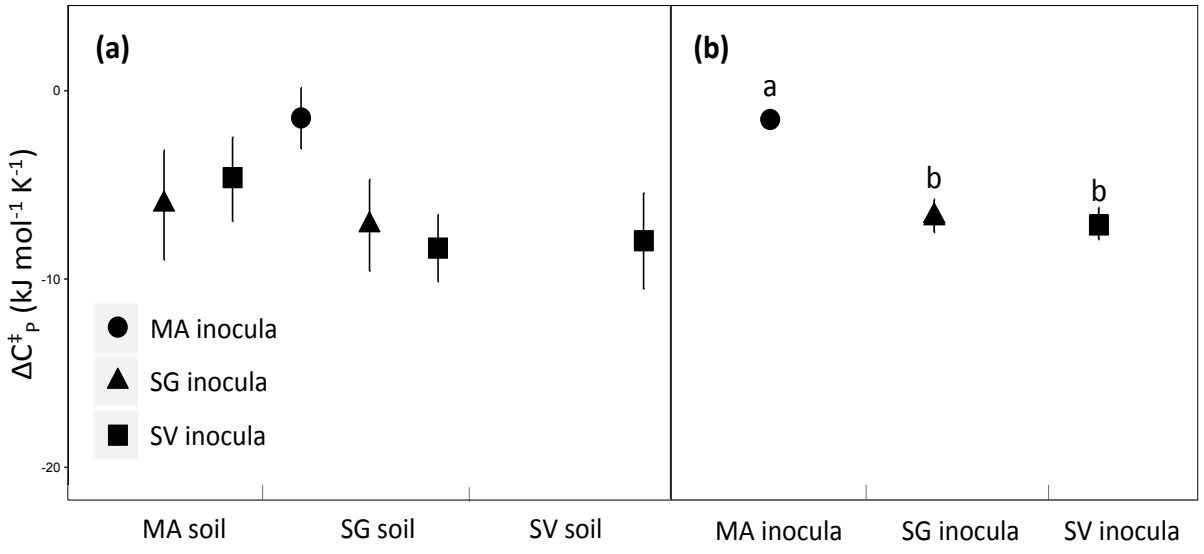

Figure 2.6. (a) Heat capacity values for each soil × inocula combination where MMRT was successful, thus heat capacity values that are >0 are not included; error bars (±2SE) represent uncertainty in the model fit. (b) Average heat capacity values for each microbial inoculum where MMRT was successful; error bars represent two standard errors above and below the mean and letters group similar means at $P<0.05$.

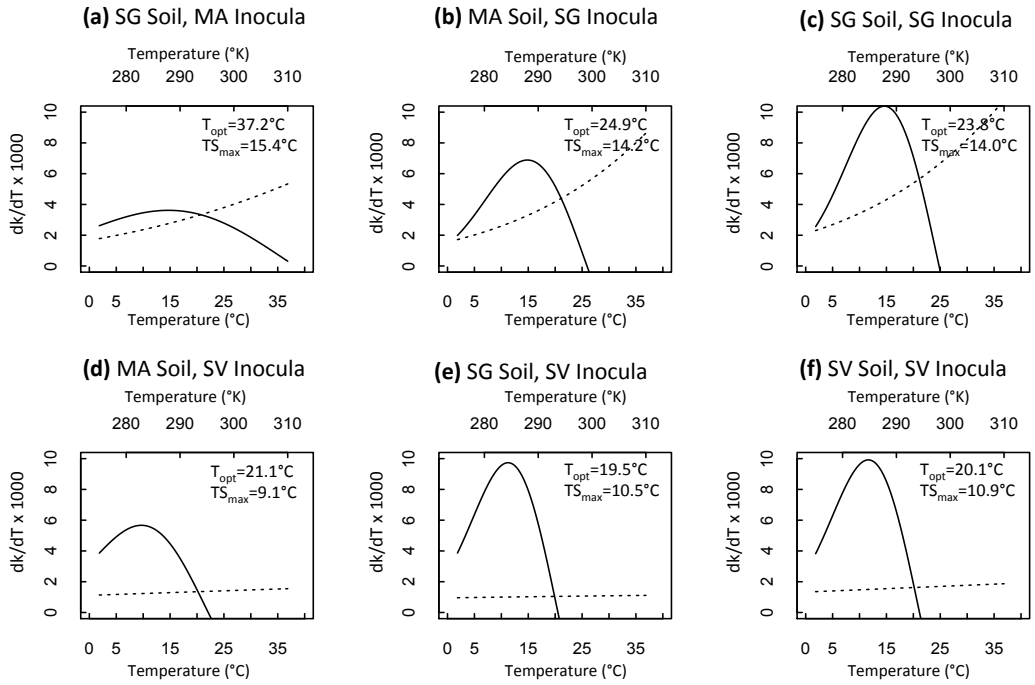

Figure 2.7. Temperature sensitivity, defined as the first derivative of reaction rate with respect to temperature (i.e., dk/dT), is a more complex feature of the MMRT model, when compared with Arrhenius. Panels a-f illustrate dk/dT of the MMRT equation (solid line) and Arrhenius equation (dashed line) for each of the soil × inocula combinations where MMRT was successful. T_{opt} and TS_{max} for MMRT are given in the top right corner.

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Chapter 3: Temperature sensitivity as a microbial trait using parameters from macromolecular rate theory[2]

Introduction

The activities of extracellular enzymes, a rate-limiting step in decomposition and important component in biogeochemical cycles (Burns and Dick, 2002), are strongly controlled by *in-situ* temperatures (Davidson and Janssens, 2006; Wallenstein et al., 2011). Although the importance of enzyme temperature sensitivity is widely recognized, the degree to which temperature sensitivity is an inherent property of the enzymes versus a response to environmental conditions (Davidson and Janssens, 2006) is largely unknown. It has been difficult to parse the mechanisms underlying observations of enzyme temperature responses in part because assays are typically conducted at the community level where the contribution of isoenzymes produced by individual taxa cannot be isolated (Bradford, 2013; Karhu et al., 2014). In addition, relative and absolute measures of temperature sensitivity using the same, simple models often produce contradictory results (Sierra, 2012). Understanding if these soil extracellular enzymes have inherent temperature sensitivity is critical for accurate predictions of soil carbon (C) and other nutrient dynamics in changing environments. In this study we attempt to determine the degree to which soil enzymes are responsive to temperature. In addition, we focus on clearing up some of the definitional confusions regarding temperature sensitivity in soils.

Over most of the last decade, the debate on if and how temperature sensitivity differs among enzymes has used parameters from two models: the Q_{10} temperature coefficient and

[2] Alster, C.J., Baas, P., Wallenstein, M.D., Johnson, N.G., and von Fischer, J.C. (2016). Temperature Sensitivity as a Microbial Trait Using Parameters from Macromolecular Rate Theory. *Frontiers in microbiology* 7(1821). doi: 10.3389/fmicb.2016.01821.

activation energy, E_A, derived from the Arrhenius equation. Q_{10} is a unitless measure of the change in rate with a ten-degree increase in temperature,

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{10/(T_2-T_1)}, \quad (1)$$

where R is reaction rate and T is temperature. The Q_{10} of biological systems is generally thought to be ~2 or 3, although it has been found to be substantially higher in some soils and enzymes (Lloyd and Taylor, 1994; Chapin III and Matson, 2011; Elias et al., 2014). The Arrhenius equation describes temperature response as

$$\ln(k) = \ln(A) - \frac{E_A}{RT}, \quad (2)$$

where k is the reaction rate constant, A is a pre-exponential factor, E_A is the activation energy, R is the universal gas constant, and T is temperature. According to the Arrhenius model, temperature sensitivity is compared using E_A as the parameter of interest instead of or in addition to Q_{10}. Reactions with higher Q_{10} values require a larger "push" or activation energy (E_A) to initiate the reaction (Davidson and Janssens, 2006).

Previously, studies have drawn varying conclusions about how Q_{10} and E_A vary with enzyme structure and function. From an evolutionary perspective, selection should generate an adaptive fit of enzyme kinetics to their thermal environment (Allison et al., 2011; Bradford, 2013). For example, thermophilic enzymes tend to have increased conformational rigidity (Zavodszky et al., 1998), while psychrophilic enzymes have improved flexibility, particularly at the active site (Feller, 2003; Struvay and Feller, 2012), impacting temperature sensitivity. Many soil studies have observed significant differences among Q_{10} and E_A values at a range of spatial and temporal scales (Koch et al., 2007; Trasar-Cepeda et al., 2007; Wallenstein et al., 2009; Brzostek and Finzi, 2012; Steinweg et al., 2013). Despite the predicted and observed differences between these different types of enzymes and isoenzymes, an analysis conducted by (Elias et al.,

2014) found no statistical difference across enzyme classes in Q_{10} values from 150 enzymatic reactions.

One reason why patterns in Q_{10} and E_A are not easily explained is that they may not be the most appropriate parameters to evaluate temperature sensitivity from soil microbial enzymes (Schipper et al., 2014). It has long been recognized that Arrhenius and Q_{10} do not always accurately describe the relationship between temperature and reaction rates in soil systems (Lloyd and Taylor, 1994), yet they have continued to be used out of convenience, convention, or perhaps due to lack of a better alternative model. The most conspicuous disparity between these models is the empirical data commonly observed showing negative curvature (i.e., a concave-down parabolic response) in rate versus temperature, which is not explained by either Q_{10} or E_A. This negative curvature is typically ascribed to enzyme denaturation even though the pattern is sometimes observed at relatively low temperatures. We hypothesize that this negative curvature causes estimated Q_{10} and E_A values to vary with the temperature range where they are measured, thus making them more phenomenological parameters than fundamental system properties (Pawar et al., 2016). A second issue is that thermodynamic principles indicate that the Arrhenius models are missing a key term when applied in biological systems: for large macromolecules like enzymes, it is not appropriate to assume that the transition state of Gibbs Free Energy ($\Delta G^{\ddagger}$) is constant across temperatures (Hobbs et al., 2013).

A relatively new model, Macromolecular Rate Theory (MMRT), accounts for both the physical and biological components of reaction rate with temperature (Hobbs et al., 2013). MMRT is defined as,

$$\ln(k) = \ln\left(\frac{k_B T}{h}\right) - \frac{\Delta H^{\ddagger}_{T_0} + \Delta C^{\ddagger}_P (T - T_0)}{RT} + \frac{\Delta S^{\ddagger}_{T_0} + \Delta C^{\ddagger}_P (\ln T - \ln T_0)}{R}, \quad (3)$$

where k is the rate constant, k_B is Boltzmann's constant, h is Planck's constant, R is the universal gas constant, T is temperature, H is enthalpy, S is entropy, C_P is heat capacity, and ‡ indicates that it is the transition state. Thus, we propose that the idea of "temperature sensitivity" when described by MMRT emerges as three fundamental components: the heat capacity of the enzyme ($\Delta C_P^{\ddagger}$), the temperature optimum (T_{opt}), and the point of maximum temperature sensitivity (TS_{max}). The heat capacity of the enzyme describes the degree of curvature in the parabolic response of reaction rate with temperature; more parabolic curves have larger, negative values of $\Delta C_P^{\ddagger}$ that arise when enzymes are more rigid at the transition state. The temperature optima denote the point at which the reaction rate is greatest, with lower reaction rates at higher temperatures not necessarily indicating enzyme denaturation. The point of maximum temperature sensitivity is calculated from the first derivative of k with temperature (dk/dT), and indicates the temperature where the rate of change is greatest.

Our proposed concept of temperature sensitivity could enable use of traits-based approaches for understanding how community-level patterns in temperature sensitivity are related to thermal responses of the many isoenzymes produced by diverse microbes. Under this definition of temperature sensitivity, $\Delta C_P^{\ddagger}$, T_{opt}, and TS_{max} are measurable properties of individual organisms, or enzymes from organisms, and one that clearly would influence organismal performance, falling under the ecological definition of a functional trait (McGill et al., 2006). Including temperature sensitivity in a traits-based framework could enable the linking of the microbial community with soil ecosystem functioning (Green et al., 2008), as well as allow for a stronger quantitative approach to integrate temperature sensitivity into models for improved predictive power (Webb et al., 2010).

If temperature sensitivities of enzymes are in fact traits and exhibit variation based on genetic and environmental variation, we hypothesize that different enzymes will demonstrate distinct temperature sensitivities, as defined by the terms $\Delta C_P^{\ddagger}$, T_{opt}, and TS_{max}. Because the difference in $\Delta C_P^{\ddagger}$ is impacted by the physical flexibility of the enzyme (Hobbs et al., 2013), which we hypothesize is a result of genetic variation among communities and/or from interaction with substrate type, we predict that temperature sensitivity of soil extracellular enzymes will vary by the microbe from which the enzyme was derived or by the enzyme type. Because most enzymes are substrate specific, different enzyme-substrate complexes can have a wide range of $\Delta C_P^{\ddagger}$ values. Moreover, different microbes produce different isoenzymes, so temperature sensitivity may also vary among microbes.

We measured extracellular enzyme activity from seven soil isolates and three different enzymes at six temperatures, in order to advance the study of temperature sensitivity as an intrinsic microbial trait. In a previous study (Alster et al., 2016), we found that temperature sensitivity varied among soil microbial communities. We applied both Arrhenius and MMRT to our data to compare the effectiveness of each of these models and demonstrate how Arrhenius estimates of temperature sensitivity may not be sufficient, even within *in situ* representative temperature ranges.

Material and methods

Experimental design

Extracellular enzymatic assays were performed for three enzymes, β-glucosidase (BG), leucine aminopeptidase (LAP), and phosphatase (PHOS) on seven soil isolates, each from a different genera—*Acinetobacter*, *Bacillus*, *Citrobacter*, *Comamonas*, *Enterobacter*,

Flaviobacterium, and *Pseudomonas*. The isolates were derived either from soil or worm castings and kept at -80°C with 20% glycerol until use. The isolates were revived from storage and grown in nutrient broth over a 2-3 day period at 25°C. We added 3-(*N*-morpholino)propanesulfonic acid buffer to maintain a pH of 7.2, which is the same pH as the microbes were originally isolated at. Before we began the enzyme assays, the isolate solution was plated on nutrient broth agar and total incubation time was determined by when cultures reached between 10^5-10^7 colony-forming units per mL.

The isolates were incubated in 96-well microplates with substrates at six temperatures: 4, 11, 25, 35, 45, and 60°C. We chose a large initial temperature range in order to capture the most accurate temperature response curve. The enzyme assay was modified from Bell et al. (2013). Forty microliters of 200 mM fluorometric substrate—4-MUB-β-D-glucopyranoside for BG, L-leucine-7-amido-4-methylcoumarin hydrochloride for LAP, and 4-MUB phosphate for PHOS—was added to 160 μL of a 1 isolate mixture: 15 acetate buffer solution. For each isolate x substrate combination, there were eight replicates for each temperature (7 isolate x 3 enzymes x 6 temperatures x 8 replicates). Standards ranging from 2.5 μM to 100 μM were used to calibrate the enzyme activity from each enzyme. 4-methylumbelliferone (MUB) was used to calibrate BG and PHOS and 7-amino-4-methylcoumarin (MUC) was used to calibrate LAP. The plates were incubated between 1 and 23 hours depending on the temperature and scanned on a Tecan Infinite M200 plate reader at optimal florescence as determined by the MUB and MUC standards. Reaction rates were linear regardless of incubation time, as determined by preliminary experiments. The MUB and MUC standard curves were used to calculate the raw florescence of the samples using the slope and y-intercept, as described in Bell et al. (2013) and converted into

units of nmol activity L culture^{-1} hour^{-1}, so that samples were comparable across temperatures with varying incubation times.

Calculating temperature sensitivity

In order to quantitatively characterize temperature responses of each of the 21 isolate × enzyme combinations, we plotted the natural log of the reaction rate against temperature and fitted both the Arrhenius and MMRT equations using an analytic Gauss-Newton for Arrhenius and a numerical Gauss-Newton for MMRT in JMP Pro 11 (Alster et al., 2016; Schipper et al., 2014). Parameters E_A and $\Delta C_P^{\ddagger}$, along with their uncertainty, were reported by the software. The optimum temperature (T_{opt}) and point of maximum temperature sensitivity (TS_{max}) from the MMRT curve fits were calculated by taking the derivative of the MMRT equation with respect to temperature (Alster et al., 2016). We used a Monte Carlo Simulation to estimate the standard error for T_{opt} and TS_{max}.

Analysis of variances (ANOVA) were performed using the software R version 3.2.1 (Core Team, 2015) to determine the relative importance of substrate type and species type in explaining variation in the parameters from each of the models ($\Delta C_P^{\ddagger}$, $\Delta S_{T_0}^{\ddagger}$, $\Delta H_{T_0}^{\ddagger}$, E_A, and A) as well as for T_{opt} and TS_{max}. We also used R to run pairwise comparisons with a Holm multiple testing adjustment to examine differences between each of the model parameters and T_{opt} and TS_{max} from each of the isolate × enzyme combinations. Differences in $\Delta C_P^{\ddagger}$ were calculated using a two-sampled approximate Z-test.

Comparison of MMRT and Arrhenius equations

We used adjusted R^2 and Akaike information criterion corrected for a finite sample size (AICc) to determine the most parsimonious model between the Arrhenius and MMRT model fits for the full temperature range (4-60°C). Additionally, we re-ran the Arrhenius model fit for each isolate × enzyme combination, but only using temperatures 4-25°C and 4-35°C to evaluate if, under more biologically relevant temperatures, the Arrhenius model fits were accurate predictors of reaction rate. To assess this, we calculated the percentage that each of the three models (MMRT from 4-60°C, Arrhenius from 4-35°C, and Arrhenius from 4-25°C) over or underestimated the reaction rate as compared to the actual experimental values and conducted corresponding lack-of-fit (LOF) tests. The importance of each of these models for predicting the percent error was examined with a linear model and tested with an ANOVA using the lmerTest package in R (Kuznetsova et al., 2014).

Results

Temperature sensitivity differs for isolate × enzyme combinations

Out of the 21 isolate × enzyme combinations we tested, we present here the results from the 19 that worked. BG activity in *Bacillus* and PHOS activity in *Comamonas* were below detection limits. Thus, these two combinations were eliminated from the analysis. We plotted the reactions rates of the remaining 19 isolate × enzyme combinations versus temperature and fit both the MMRT and Arrhenius equations to the data (Figure 3.1). These model fits for MMRT give temperature sensitivity parameters $\Delta C_P^{\ddagger}$, T_{opt} and TS_{max}, while Arrhenius gives E_A as a parameter.

We found that $\Delta C_P^{\ddagger}$ differed for some, but not all of the isolate × enzyme combinations. The $\Delta C_P^{\ddagger}$ differed among microbial isolates in BG and LAP enzymes (P<0.05; Figure 3.2a, b). However, in PHOS, the isolates did not differ in $\Delta C_P^{\ddagger}$ (P>0.05; Figure 3.2c). When comparing if $\Delta C_P^{\ddagger}$ differed between the same isolate for different enzymes we found significant differences in $\Delta C_P^{\ddagger}$ for *Acinetobacter*, *Citrobacter*, and *Enterobacter* (P<0.05), but not for *Bacillus*, *Comamonas*, *Flaviobacterium*, or *Pseudomonas*. Patterns in statistical differences were identical for $\Delta C_P^{\ddagger}$, $\Delta S^{\ddagger}$, and $\Delta H^{\ddagger}$. Overall 70.9% of variation in $\Delta C_P^{\ddagger}$ was explained by the microbial isolate type, compared with 29.1% of the variation explained by the enzyme type.

The temperature optima (T_{opt}) and point of maximum temperature sensitivity (TS_{max}) also varied with the isolate × enzyme combinations (Table 3.1). Despite the 25ºC conditions used during initial culturing of the inoculum isolates, T_{opt} ranged from 33.5°C in BG for the *Enterobacter* isolate to 60.7°C in LAP for the *Bacillus* isolate and TS_{max} ranged from 18.2°C in BG for the *Acinetobacter* isolate to 40.3°C in LAP for the *Bacillus* isolate. Pooling the T_{opt} and TS_{max} values across the same microbial isolate and enzyme type, we found differences for some of the values across both microbial isolate and enzyme type (P<0.05). While differences between these pooled values for T_{opt} and TS_{max} were similar, they were not identical. Furthermore, similar to $\Delta C_P^{\ddagger}$, variation in T_{opt} and TS_{max} are best explained by the microbial isolate type, with SS values of 86.7% and 80.0%, respectively.

The three metrics of temperature sensitivity, $\Delta C_P^{\ddagger}$, T_{opt} and TS_{max}, each have unique statistical patterns of similarity across inocula × enzyme combinations. In this paper, we do not deeply examine the basis for groupings but focus instead on identifying if patterns of similarity and difference exist or if all enzymes behave similarly. For conciseness, we illustrate only patterns of differences for $\Delta C_P^{\ddagger}$ in Figure 3.2, and provide T_{opt} and TS_{max} findings in Table 3.1.

For PHOS we found no differences in $\Delta C_P^{\ddagger}$ among the different microbial isolates (Figure 3.2). For TS_{max} of PHOS there were no differences, but for T_{opt} we found several significant differences among isolates (Table 3.1). Likewise for BG, there were quite a few differences among isolates in $\Delta C_P^{\ddagger}$ (Figure 3.2). However, there were no significant differences between different microbial isolates in TS_{max} for BG, and while there are differences in T_{opt}, they are not the same as the differences identified for $\Delta C_P^{\ddagger}$. Interestingly, patterns in significant differences among isolates for LAP are the same for T_{opt} and TS_{max}, but show a different pattern for $\Delta C_P^{\ddagger}$. Some of these patterns likely emerge because T_{opt} and TS_{max} are positively correlated (R^2 = 0.84), while $\Delta C_P^{\ddagger}$ does not correlated with T_{opt} or TS_{max} (R^2 = 0.23 and R^2 = 0.01, respectively).

MMRT provides better statistical fit than Arrhenius

For the temperature range of 4-60°C, we found that MMRT gave vastly superior fits to the data as compared to Arrhenius according to both AICc and R^2 criteria (Table 3.2). MMRT was also superior when the Arrhenius model was fit to the more linear part of the temperature range (4-35°C, and 4-25°C) for 13 of the 19 isolate × enzyme combinations (see example, Figure 3.3a and Table 3.2). For the 6 combinations where MMRT was not superior, AICc analysis found MMRT and Arrhenius to have equivalent explanatory power; in no case was Arrhenius the superior model. Phosphatase was the only enzyme where MMRT was significantly better in all isolates tested.

Despite the statistically improved fit of MMRT as compared to Arrhenius, when comparing the overall error produced from each of the model predictions, the results were less striking. We found 3.8% of the total variation was explained by LOF from the model in MMRT compared with 6.2% for Arrhenius from 4-35°C and 10.3% for Arrhenius from 4-25°C. This

means that by using Arrhenius instead of MMRT we are introducing 1.6 and 2.7 times more error into our predictions for the Arrhenius 4-35°C and 4-25°C models, respectively. ANOVA results for the percent error of the models compared to the experimentally observed results at 4, 11, 25, and 35°C, revealed a significant difference between MMRT and the Arrhenius 4-25°C model ($P \leq 0.05$) and between the two Arrhenius models ($P = 0.02$). However, there was no significant difference in percent error between MMRT and the Arrhenius 4-35°C model fits when compared to original activity values at 4, 11, 25, and 35°C**.**

Comparison of activation energy values

To examine the value of using activation energy as a trait, we compared activation energies derived from different temperature ranges to see if they vary. In a 3-way ANOVA examining the E_A values from all three of the Arrhenius model temperature ranges tested, we found that the temperature range explained 68.5% of variation in the data, compared to 12.7% explained by enzyme type and 11.0% explained by isolate type. Overall, we found that as the temperature range increased E_A values decreased (Figure 3.3b, c, d). Not only did the absolute values of E_A vary based on temperature range, but the relative E_A values also differ (Figure 3.3b, c, d) leading to different groupings of similarity among assays. In comparisons of E_A values for the different inocula × enzyme combinations that shared either the same enzyme or same isolate, 25% of the relationships changed between the Arrhenius 4-25°C and 4-35°C estimates, and 36.8% of the relationships changed between the Arrhenius 4-35°C and 4-60°C estimates. A similar analysis capturing different temperature ranges was not needed for MMRT since MMRT captures the peak.

Discussion

Heat Capacity Differs Significantly Among Enzymes

This study advances efforts to understand how temperature sensitivity of extracellular enzymes varies by substrate and isolate type. Such an effect has long been speculated, based on assays conducted at the community level with whole soils (e.g. Steinweg et al., 2013 and Trasar-Cepeda et al., 2007). We found that $\Delta C_P^{\ddagger}$ differed significantly among isolates for the BG and LAP enzymes, across the different isolates measured (Figure 3.2). Furthermore, heat capacity of different enzymes varied within the same isolate for three out of the seven isolates measured. While this study was not designed to elucidate the mechanisms behind why heat capacity varied between some enzymes and isolates but not others, here we provide a few possible explanations. One broad explanation for why we see these differences is that microbes adapt to their environment and more efficient enzymes are selected for in accordance to the thermodynamic conditions in that environment (Bradford, 2013); thus, microbes will adapt to produce isoenzymes with varying degrees of flexibility and consequently different heat capacities values depending on what is most advantageous for the microbial cell's survival. The idea that isoenzymes have distinct temperature sensitivities is not particularly groundbreaking if comparing enzymes derived from thermophilic, psychrophilic, and mesophilic conditions (Zavodszky et al., 1998; Lonhienne et al., 2000; Feller and Gerday, 2003) or even across the same soil microbial community throughout seasons (Koch et al., 2007; Trasar-Cepeda et al., 2007; Wallenstein et al., 2009). However, in this study all of the isolates measured were derived from a mesophilic environment and raised in culture at the same temperature (25°C). Thus, we found that temperature sensitivity varies even among organisms raised under the same temperature conditions.

In contrast to BG and LAP, the heat capacity of PHOS was invariant with isolate type, suggesting that perhaps this enzyme did not undergo a similar type of adaption over evolutionary history or that there are simply fewer isoenzymes. Although the PHOS enzyme is ubiquitous across different types of organisms, the genomic region encoding for the active site is highly conserved and fairly homologous across plants, animals, and bacteria, at least for the acidic version of the enzyme (Anand and Srivastava, 2012). As opposed to aminopeptidases in which relatively few homologies have been observed despite their high abundance (Taylor, 1993), gene conservation of PHOS might explain the lack of variation in heat capacity. Thermodynamic constraints of the enzyme or active site may also limit adaptation if there is a fundamental evolutionary tradeoff between the structure and function of the enzyme that is specific to the catalytic properties of PHOS (Bradford, 2013).

It is also worth noting that because these estimates of heat capacity were not necessarily of individual enzymes, but of the all of the isoenzymes produced by the isolates under the incubation conditions of this experiment. While it is unclear if multiple enzymes acting on the substrate impacted the results, it is worth highlighting that these results might be the average of one or more isoenzymes. It is also possible that given our sample size (seven isolates and three enzymes), more differences in heat capacity may have been observed if we had increased the diversity and number of the isolates and enzymes in the experiment. Furthermore, specific experimental conditions, such as pH, could potentially alter the temperature-response curve. Determining how heat capacity varies phylogenetically for different enzymes is an important avenue for future research.

Exercising caution for Arrhenius estimates of temperature sensitivity

Despite clear evidence of MMRT's statistical superiority to Arrhenius in this experiment, we found that at the lower temperature ranges (i.e., 4-25°C and 4-35°C) Arrhenius estimations were not necessarily poor. However, we still recommend that future estimations of temperature sensitivity for soil microbial enzymes that apply the Arrhenius equation use caution for the following reasons. First, we found that E_A values varied significantly with the range in which they were evaluated, making them an unreliable metric to use for comparisons across studies. These results are corroborated by Pawar et al. (2016), who tested 1,085 temperature-response curves from a variety of organisms and systems and determined that the calculated E_A value is an artifact of the temperature range, spread of temperatures measured, and where the temperature range falls. In order for temperature sensitivity to be used as a common currency of discussion and incorporated as a microbial trait, relationships should not be a function of different measurement methods. Second, even if Arrhenius is comparable to MMRT in a narrow temperature range, E_A fails to capture key phenomenological features of temperature sensitivity in soil biological systems. Other nonlinear models have also been shown to give suitable empirical fits to the temperature dependence of enzyme activity (Peterson et al., 2004; Del Grosso et al., 2005; Daniel and Danson, 2013; Corkrey et al., 2014), but MMRT not only fits well empirically, but is derived from thermodynamic theory and thus has an underlying theoretical basis. Thus, even if E_A continues to be used in the future, E_A values should not be

Conceptual framework

For nearly a decade, scientists have recognized the importance of using microbial traits as a framework for predicting ecosystem response to climate change (Green et al., 2008;

Wallenstein and Hall, 2012). Many of these studies make predictions about how microbial traits (e.g., nutrient use efficiency) respond across a gradient of temperatures (Rinnan et al., 2009; Dell et al., 2011; Wallenstein and Hall, 2012). In this study we argue that temperature sensitivity is not only a measure of how biological traits respond across a gradient of temperatures, which is how it is typically characterized, but also that temperature sensitivity is an inherent biological trait. In light of this interest and our results, we developed a new conceptual model that develops a more precise definition of temperature sensitivity and organizes the factors that can lead to variation in temperature sensitivity itself.

In our framework, we first consolidated the Arrhenius and MMRT definitions of temperature sensitivity. Under the Arrhenius equations, activation energy is the singular factor driving the apparent temperature response (Figure 3.4). But, this violates laws of thermodynamics with regards to biological systems because of the large molecular size of enzymes characterized by large heat capacities impacting the temperature response (Arcus et al., 2016). MMRT expands thermodynamic theory initiated with Arrhenius by incorporating heat capacity as part of the temperature response (Figure 3.4). Implicit within the MMRT theory is that the $\Delta C_P^{\ddagger}$ is a function of enzyme flexibility and thus $\Delta C_P^{\ddagger}$ varies among enzymes (Schulte, 2015; Arcus et al., 2016). Given existing evidence for substrate type influencing activation energy (Davidson and Janssens, 2006), our outline of temperature sensitivity includes potential for enzyme flexibility to be a product of the substrate upon which the enzyme acts as well as the genetic variation among different enzymes (Figure 3.4). In this experiment, we tested if these additional factors (i.e., substrate type and genetic variation) impacted the temperature sensitivity by measuring heat capacity as a proxy for enzyme flexibility and found strong evidence for heat capacity varying by both enzyme and isolate type.

Based on this expanded framework of temperature sensitivity, we propose that use of $\Delta C_P^{\ddagger}$ and TS_{max} will give a more comprehensive basis to describe "temperature sensitivity" than Q_{10} or E_A. Q_{10} gives a false sense that a single constant can characterize the temperature sensitivity of a system (Davidson and Janssens, 2006). In order to overcome this obvious discrepancy authors using Q_{10} often present multiple temperature sensitivity values at different temperature ranges for a given system, leading to results that are often difficult to compare. Conceptually, we consider temperature sensitivity to be the change in velocity per change in temperature (dV/dT). Unlike Q_{10}, E_A can be used as a summary term to capture temperature sensitivity of a system; this is effective because Arrhenius predicts a monotonic increase in rate with temperature. Since MMRT captures the inherently non-monotonic response of enzyme-catalyzed reactions, a single variable cannot fully capture the temperature sensitivity from the MMRT curve as is done by E_A in the Arrhenius equation. Thus the use of TS_{max} and T_{opt} provide two practical metrics to characterize this non-linear response of temperature sensitivity to temperature, although for modeling purposes $\Delta C_P^{\ddagger}$ and other thermodynamic parameters (i.e., $\Delta S_{T_0}^{\ddagger}$ and $\Delta H_{T_0}^{\ddagger}$) are sufficient to explicitly predict reaction rates with temperature. Temperature optimum values are also commonly reported in the literature for extracellular enzymes (Huston et al., 2000; Daniel et al., 2001; Peterson et al., 2004; Eijsink et al., 2005), but are typically quite high and perhaps not biologically relevant. We argue that TS_{max} is actually a more important term to consider than T_{opt} because TS_{max} describes where the greatest change in rate occurs and it typically falls within environmentally relevant temperature ranges (Table 3.1). Consequently, by focusing on TS_{max} we capture the area of the temperature-reaction curve that will have the greatest impact on rates of nutrient cycling and greenhouse gas production. Characterizing

temperature sensitivity with these unifying parameters gives us an avenue to incorporate temperature sensitivity into traits-based microbial models.

Our new framework suggests a number of future lines of inquiry. One immediate question is: how broadly does temperature sensitivity vary under this new definition of temperature sensitivity? If temperature sensitivity of different enzymes, microbes, or communities exhibit vastly different $\Delta C_P^{\ddagger}$ and TS_{max} values then this might impact current calculations of soil C and N dynamics. As the climate warms, does this inherent temperature sensitivity adapt or acclimate? What are the evolutionary constraints on rate of evolution and how is the overall temperature sensitivity value impacting by different groups of organisms? What other factors besides enzyme type and the microbe from which it was produced might impact enzyme flexibility? We hope that future research will be conducted in many of these avenues to elucidate mechanisms controlling temperature sensitivity of enzymes and determine what impact this has on communities, ecosystems, and nutrient cycling in soils.

Table 3.1. Mean temperature optima (T_{opt}) and point of maximum temperature sensitivity (TS_{max}) for each isolate and enzyme combination ± SEM. Pooled estimates are averages either across enzymes for a given isolate or across isolates for a given enzyme.

	BG		LAP		PHOS		**Pooled Estimate**	
	Topt	TSmax	Topt	TSmax	Topt	TSmax	Topt	TSmax
Acinetobacter	36.0±1.9	18.2±2.4	37.7±0.6	24.45±0.6	37.55±2.1	18.85±2.6	37.1±2.9	20.5±3.6
Bacillus	NA	NA	60.7±2.5	40.25±1.5	48.05±1	27.95±0.7	54.2±3.3	34.1±3.0
Citrobacter	38.1±0.7	22.6±0.8	39.7±0.6	24.55±0.6	47.95±0.9	27.95±0.7	41.7±1.3	25.0±1.2
Comamonas	39.1±2.5	19.0±3.1	40.6±0.7	24.55±0.8	NA	NA	39.8±2.6	21.8±3.2
Enterobacter	33.5±0.6	19.8±0.7	40.0±0.4	25.25±0.5	46.85±0.5	28.25±0.4	40.1±0.9	24.4±0.9
Flaviobacter	38.4±0.5	23.2±0.6	39.4±0.8	23.85±0.9	41.85±2.2	23.25±2.3	39.9±2.4	23.4±2.5
Pseudomonas	39.0±2.6	19.1±3.2	41.4±1.1	23.25±1.1	40.45±2.7	18.55±3.1	40.3±3.9	20.3±4.6
Pooled Estimate	37.3±4.2	20.3±5.2	42.6±3.1	26.6±2.4	43.8±4.3	24.1±4.8	--	--

Table 3.2. Akaike information criterion corrected for a finite sample size (AICc) and R^2 values for each model × isolate × enzyme combination. The MMRT values reported are for the full 4-60°C temperature range, while all three temperature ranges are reported for Arrhenius.

		MMRT		Arrhenius 4-60°C		Arrhenius 4-35°C		Arrhenius 4-25°C	
Enzyme	Isolate	AICc	R^2	AICc	R^2	AICc	R^2	AICc	R^2
BG	Aci	63.54	0.62	97.38	0.22	10.36	0.40	7.45	0.39
	Cit	-0.71	0.94	105.69	0.47	41.32	0.82	58.69	0.81
	Com	65.62	0.58	88.42	0.32	65.42	0.40	79.04	0.38
	Ent	3.42	0.94	118.95	0.14	-13.52	0.97	-6.32	0.97
	Fla	-30.38	0.97	105.27	0.51	14.30	0.92	20.72	0.92
	Pse	73.32	0.55	94.03	0.30	70.72	0.34	98.53	0.33
LAP	Aci	11.87	0.96	129.19	0.45	-2.28	0.97	-2.06	0.97
	Bac	24.69	0.97	526.13	0.90	-25.26	0.99	-23.92	0.99
	Cit	-4.67	0.96	110.94	0.54	14.02	0.95	25.17	0.95
	Com	4.62	0.95	96.74	0.60	-57.48	0.99	-52.69	0.99
	Ent	-29.40	0.98	108.18	0.59	22.37	0.93	37.94	0.93
	Fla	14.13	0.93	102.20	0.53	38.37	0.87	54.45	0.86
	Pse	14.16	0.91	77.15	0.60	12.22	0.91	13.92	0.90
PHOS	Aci	65.40	0.62	94.15	0.30	57.43	0.59	75.01	0.58
	Bac	-38.32	0.97	93.07	0.81	4.56	0.93	21.31	0.93
	Cit	-31.69	0.97	55.21	0.80	6.98	0.92	21.43	0.92
	Ent	-85.80	0.99	64.58	0.80	-21.68	0.97	0.56	0.97
	Fla	67.49	0.72	97.18	0.49	67.79	0.57	93.49	0.56
	Pse	56.54	0.59	77.34	0.37	55.42	0.48	83.56	0.47

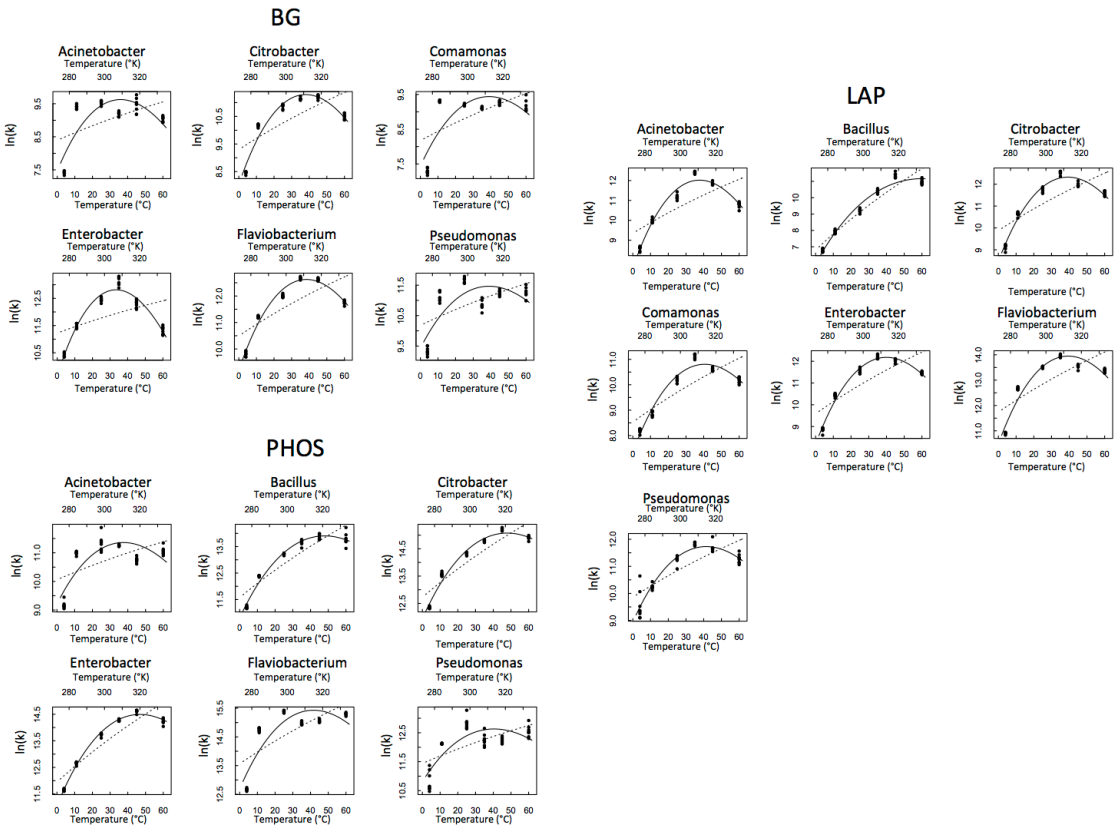

Figure 3.1. Temperature response of each isolate and enzyme combination. MMRT is represented by the solid line and Arrhenius is represented by the dashed line.

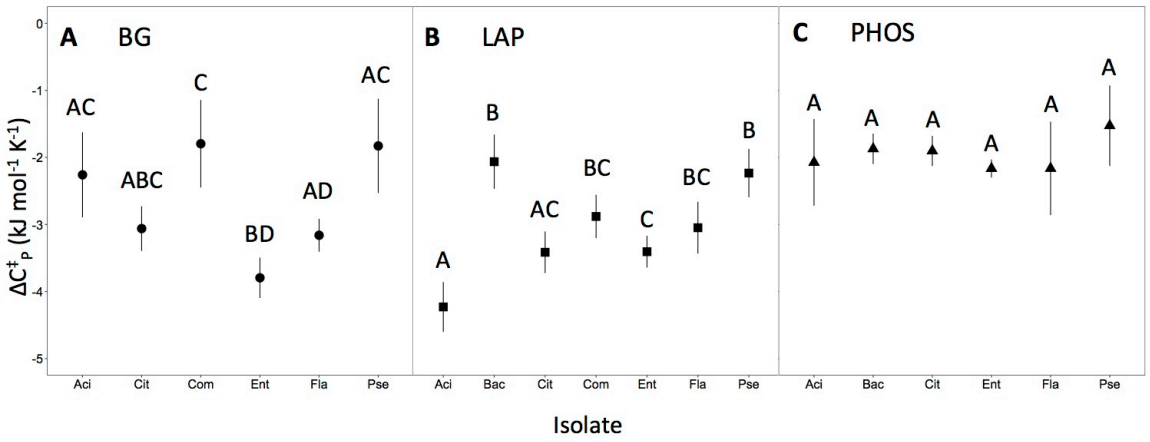

Figure 3.2. Heat capacity for each isolate and enzyme combination. Error bars (±2SE) represent uncertainty in the model fit. Letters represent significant differences (P<0.05) between isolates of the same enzyme (i.e., within panels), but not across the different enzyme types (i.e., not between panels).

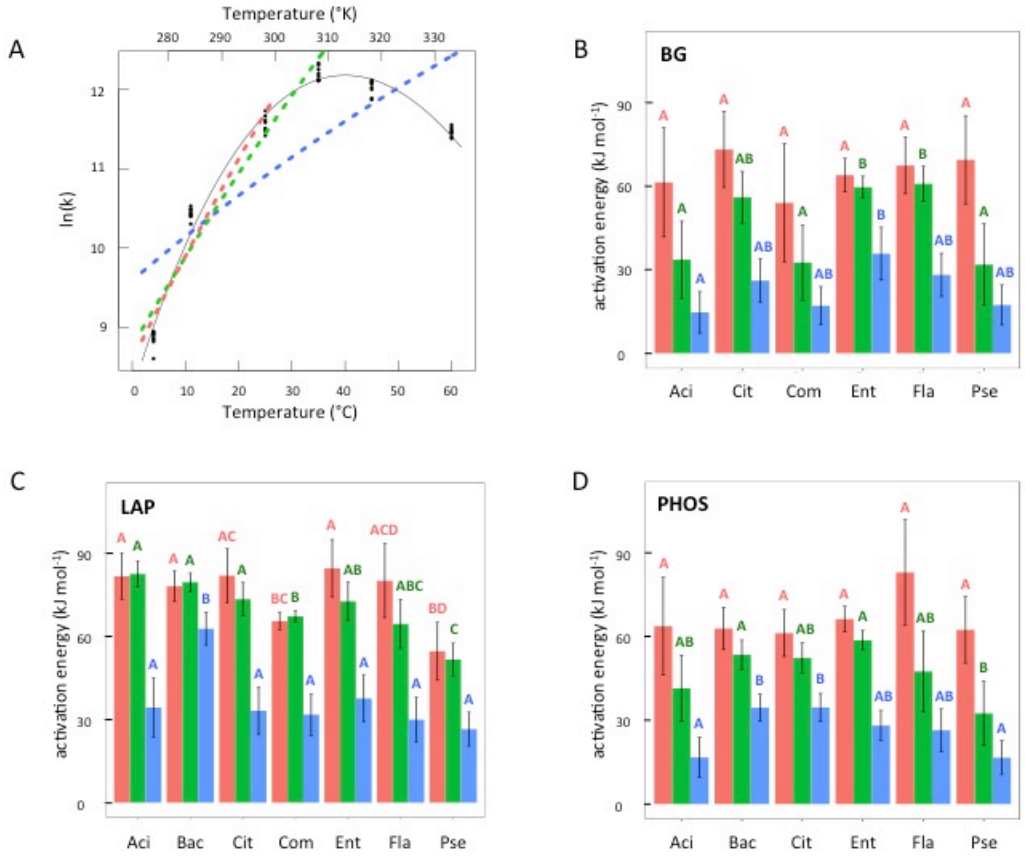

Figure 3.3. (a) Example temperature response plot (LAP for *Enterobacter*) showing fits for MMRT, Arrhenius with temperature range 4-60°C (blue, dashed line), Arrhenius with temperature range 4-35°C (green, dashed line), and Arrhenius with temperature range 4-25°C (pink, dashed line). (b), (c), and (d) Activation energy estimates from the three Arrhenius fits with bars corresponding to Arrhenius 4-25°C (pink), Arrhenius 4-35°C (green), and Arrhenius with temperature range 4-60°C (blue). Error bars (±2SE) represent uncertainty in the model fit. Letters represent significant differences (P<0.05) between different isolates for the same Arrhenius fit (i.e., 4-25°C, 4-35°C, or 4-60°C, not between different fits for the same isolate) and within the same enzyme types (i.e., not between panels). Only compare same colors within the same panels.

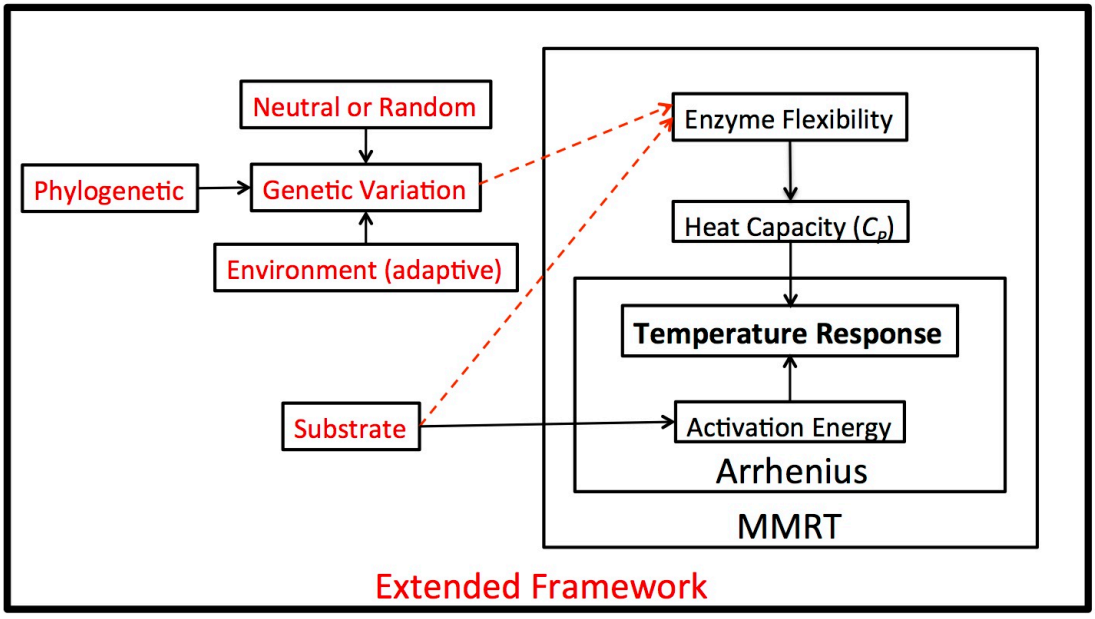

Figure 3.4. Theoretical framework and hypotheses. In this figure, we demonstrate how the Arrhenius and MMRT frameworks fit together conceptually and how that impacts our view of the temperature response, which in our case refers to the reaction rate of extracellular enzymes. Under the Arrhenius framework, activation energy is the singular factor driving the apparent temperature response. MMRT expands upon this framework, suggesting that the temperature response is a function of the heat capacity of an enzyme, which is related to the enzyme's flexibility. While there is already evidence for substrate type influencing activation energy (solid arrow connecting substrate to activation energy), in this experiment (red lettering) we extend this framework and hypothesize that the enzyme flexibility is also a product of the substrate type and genetic variation among different enzymes (dashed arrows). We posit that this overarching framework could be applied in a variety of situations relating to the temperature response.

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Chapter 4: A meta-analysis of temperature sensitivity as a microbial trait

Introduction

Understanding distributions of ecological communities is one of the central themes in ecology. Distributions are particularly difficult to measure in microbial communities where the species concept is difficult to define and communities are constantly changing (Antwis et al., 2017). Although a common approach in plant and animal ecology, trait-based ecological research is starting to gain traction in microbial ecology as a way to assess the biogeography of microbial communities and overcome the constraint of species diversity and ambiguity (Green et al., 2008; Crowther et al., 2014). Traits-based microbial ecology is also valuable in that it is a way to measure the impact of ecological response to environmental change and the importance of individual organisms in a community.

In light of present and future anthropogenic changes in climate, there is rising interest for predicting the response of microbial processes to temperature, and theoretical motive to use a traits-based approach in this effort. The response of microbial metabolism to temperature is of particular interest because 1) virtually all metabolic reactions are highly dependent on temperature, and 2) with global warming, small changes in temperature have the potential to severely impact organismal performance and consequently ecosystem functioning (Dell et al., 2014). Recently, we (Alster et al., 2016a) introduced the concept of temperature sensitivity as a microbial trait. We find in our previous work that temperature sensitivity can be characterized as a microbial trait itself with distinct temperature sensitivity trait values, influenced by genetic and environmental variation (Alster et al., 2016a; Alster et al., 2016b). Defining temperature sensitivity as a microbial trait with measurable and intercomparable characteristics adds another

technique to predict microbial community assemblage and creates an important tool to evaluate microbial response to climate change and impact on ecosystem function.

We have defined three meaningful temperature sensitivity traits (heat capacity, point of maximum temperature sensitivity, and the temperature optimum) derived from Macromolecular Rate Theory (MMRT). This theory, proposed by Hobbs et al., 2013, expands the Arrhenius equation to account for temperature dependence of activation energy found in enzyme-catalyzed reactions due to negative changes in heat capacity. The consequence of incorporating these thermodynamic principles into the Arrhenius equation is a concave down response in rate to temperature, instead of an exponential increase in rate. MMRT uses three parameters to characterize temperature sensitivity traits including the change between the ground state and transition state for enthalpy, entropy, and heat capacity ($\Delta H^{\ddagger}_{T0}$, $\Delta S^{\ddagger}_{T0}$, and $\Delta C^{\ddagger}_{P}$), which can be directly estimated from the MMRT equation,

$$\ln(k) = \ln\left(\frac{k_B T}{h}\right) - \frac{\Delta H^{\ddagger}_{T_0} + \Delta C^{\ddagger}_{P}(T - T_0)}{RT} + \frac{\Delta S^{\ddagger}_{T_0} + \Delta C^{\ddagger}_{P}(\ln T - \ln T_0)}{R}, \quad (1)$$

where k is the rate constant, k_B is Boltzmann's constant, h is Planck's constant, R is the universal gas constant, T is temperature, and T_0 is the reference temperature (Hobbs et al., 2013). Enthalpy and entropy are very closely related and reflect the intercept location of the temperature response curve. Heat capacity directly reflects the degree of negative curvature in the temperature response curve where more negative $\Delta C^{\ddagger}_{P}$ values correspond to a more narrow temperature response curve (i.e., more temperature sensitive) and less negative $\Delta C^{\ddagger}_{P}$ values correspond to a flatter temperature response curve (i.e., less temperature sensitive). From these parameters, we derived two temperature sensitivity traits in addition to $\Delta C^{\ddagger}_{P}$, the temperature

optimum (T_{opt}) and the point of maximum temperature sensitivity (TS_{max}), which can be estimated by taking the derivative of MMRT and provides perhaps a more practical measure of temperature sensitivity. The temperature optimum denotes the temperature with the greatest rate value, while the point of maximum temperature sensitivity denotes the point where the change in rate is greatest. While T_{opt} and TS_{max} are functions of $\Delta C_P^{\ddagger}$ and $\Delta H_{T0}^{\ddagger}$, the relationships between these traits have not yet been reported. Furthermore, although the T_{opt} and TS_{max} do not theoretically need to be correlated, it is likely that higher T_{opt} corresponds to higher TS_{max}. Together, these three traits ($\Delta C_P^{\ddagger}$, T_{opt}, and TS_{max}) provide the fundamentals to examine the ecological ramification of temperature sensitivity of enzymes and organisms.

While the concept of temperature sensitivity as a trait could be applied using other types of temperature response models besides MMRT, we chose this approach for several reasons. First, our previous work (Alster et al., 2016a), along with work of others (Schulte, 2015; Pawar et al., 2016), shows that parameters from the commonly used Arrhenius equation are inappropriate and misleading for traits-based approaches. Unlike most biological processes, the Arrhenius equation predicts monotonically increasing reaction rate with temperature, whereas biological rates are unimodal functions with distinct rate maxima (DeLong et al., 2017). Secondly, even if applied within a biologically relevant temperature range, activation energy estimated from the Arrhenius equation is strongly contingent on the experimental temperature range, producing vastly different parameter estimates depending on incubation conditions (Schulte, 2015; Alster et al., 2016a; Pawar et al., 2016). Several models besides MMRT have modified the Arrhenius equation to better fit unimodal temperature response in biological reactions including Johnson-Lewsin (Johnson and Lewin, 1946), Sharpe-DeMichele (Sharpe and DeMichele, 1977), Schoolfield (Schoolfield et al., 1981), Ratkowsky (Ratkowsky et al., 2005),

Equilibrium model (Daniel and Danson, 2010), and enzyme-assisted Arrhenius (DeLong et al., 2017) which could also be a basis for the temperature-traits approach. However, except for MMRT and enzyme-assisted Arrhenius, these other models assume an unlimited substrate supply and that reactions would occur in the absence of enzymes, which ignores the purpose of incorporating enzymes in the first place (DeLong et al., 2017).

Under this innovative approach identifying temperature sensitivity as a biological trait, we are now able to address a new suite of ecological questions and their relevant applications. Because temperature is a foundational property for regulating biological reaction rates (Cossins, 2012), comparing temperature sensitivity traits has the potential to provide nuance into organismal competition, biogeography, and adaptation at various thermal regimes and environmental conditions. Furthermore, assessing temperature sensitivity, as a microbial trait is pertinent as it has the potential to improve modeling of organismal and ecosystem response to climate change. For example, differential thermal response of organisms is not currently accounted for in ecosystem climate models (e.g., Wieder et al., 2013). Incorporating temperature response as a trait would allow easy incorporation into existing trait-based ecosystems models such as the Decomposition Model of Enzymatic Traits (DEMENT) model or the Microbial-Mineral Carbon Stabilization (MIMICS) model (Allison, 2012; Wieder et al., 2015). Delving into temperature sensitivity traits may also give us a better understanding of functional biodiversity of microbial communities and how we might expect individual taxa and microbial communities to adapt to warming.

In this meta-analysis we explore how temperature sensitivity varies as a biological trait. We aim to identify how broadly temperature sensitivity varies under this new definition of temperature sensitivity traits and assess the relationships between these temperature sensitivity

traits. We also begin identifying which characteristics of organismal type and environmental conditions are most important for predicting temperature traits. In this experiment we synthesize results from 113 studies by analyzing nearly 400 different temperature response curves from bacteria and fungi from a diverse array of systems including natural systems such as soils and oceans, wastewater treatment plants, and a variety of food sources in order to being filling in these gaps in understand of how temperature sensitivity varies as a trait. We hypothesize that organisms from a more variable thermal environment will produce enzymes that are less temperature sensitive (less negative $\Delta C_P^{\ddagger}$) than organisms from a less variable thermal environment due to thermal adaptation, but there will likely be trade-offs involving resource availability and competition, as well as gene conservation (Wallenstein and Hall, 2012; Alster et al., 2016a), and that T_{opt} and TS_{max} will reflect the average thermal environment from which the organisms originate. We also hypothesize that measurements of temperature sensitivity of larger groups of organisms or enzymes will result in lower temperature sensitivity, due to those estimates being a summation of a variety of individual temperature responses.

Methods

Literature survey

We searched Web of Science and Agricola databases for published papers reporting reaction rates by temperature interactions for bacteria and fungi. Each search contained at least one organismal term: microbes, "microbial communities", bacteria, bacterium, fungi, or microorganism*; at least one rate term: "respiration rate", "microbial growth", "carbon use", “ammonia oxidation”, “denitrification”, or “nitrification”; and at least one temperature term: “temperature sensitivity”, Arrhenius, MMRT, "activation energy", “ratkowsky model”, or

"macromolecular rate theory". Our search yielded 263 total studies, however 149 were eliminated after initial review based on preliminary criteria. The majority of data sets were excluded because the studies measured less than four separate temperatures and/or the temperature range measured spanned less than 15°C. We chose these criteria because they minimize the opportunity for thermodynamically implausible values once run through the MMRT model. Furthermore, we chose four temperature points for statistical purposes because it is one fewer than the number of parameters in the MMRT model. Several studies were eliminated because they did not measure an explicit rate (e.g., total growth or percent of activity) or did not measure bacteria and/or fungi (e.g., virus or algae). A few papers were also excluded if they were generated from a model as opposed to experimental data, if English versions of the papers could not be obtained (four studies), or if the paper could not be found by the Colorado State University Library services (four studies). In eight of the papers, the analyzed data were from previous studies, so in those cases we used the data from the original source paper (Supplementary Table S4.1).

Data acquisition

After initial review, we extracted data from the remaining 113 studies using WebPlotDigitizer, manually entering data from tables, or emailing the authors. After emailing, we were unable to obtain the specific data needed from the authors from eight papers that matched the experimental criteria. A total of 625 temperature response curves (rate vs. temperature) were obtained from the 134 papers we extracted data from (including the original data papers). Data from each temperature response was then fitted to the MMRT equation using a numerical Gauss-Newton approach in JMP Pro v.11. MMRT model parameter values ($\Delta H^{\ddagger}_{T0}$,

$\Delta C_P^{\ddagger}$, $\Delta S_{T0}^{\ddagger}$) and associated standard errors were estimated for each curve. After fitting each temperature curve, we eliminated temperature response curves that were concave up as thermodynamically implausible, and remained with MMRT parameter estimates of 531 temperature response curves. We calculated temperature trait values (T_{opt} and TS_{max}) for each of the successfully fitted temperature response curves using the first derivative of the MMRT equation and estimated the standard errors for these traits using a Monte Carlo simulation in R version 3.2.1.

For each of the temperature trait estimations, we also gathered additional meta-data from each of the curves, including: average native pH, average experimental pH, organismal type (i.e., bacteria, fungi, or a mix of bacteria and fungi), bacteria type (i.e., gram-positive or gram-negative), assortment of organisms measured (i.e., isolate, group of similar organisms, or community of organisms), microbial source, average native thermal environment, pre-incubation temperature, thermally/anthropogenically managed or unmanaged system, aquatic or terrestrial source (if from an unmanaged system), and rate type (Supplementary Table S4.1). It is important to note that not all of the trait values have all of the meta-data, either because the information was not reported in the paper or because the information was not relevant for that particular study (e.g., bacteria type when the paper measured fungal temperature response).

Statistical analysis

Temperature curves were removed as outliers if any of the standard errors of the parameter or trait values ($\Delta H_{T0}^{\ddagger}$, $\Delta C_P^{\ddagger}$, $\Delta S_{T0}^{\ddagger}$, T_{opt}, and TS_{max}) fell above 1.5 of the interquartile range of the upper quartile. The final number of temperature response curves analyzed after outlier removal was 381. Summary statistics for the mean, range, standard deviation, and

weighted mean (using the standard error) were calculated with their associated histograms. Correlations between each of the temperature traits were estimated. We ran a random effects model (REM) to estimate the difference between the means for each of the parameter values for each of the meta-data that we collected. In groups with categorical variables, we only report results where there were at least ten temperature response curves in a particular category. We checked the normality of the residuals for every distribution of temperature traits values that we examined and took the natural log of $\Delta C_P^{\ddagger}$ in order to normalize in all cases. All of these statistics were conducted in R 3.2.1.

Results

Summary statistics

One of the main goals of this meta-analysis was to characterize the frequency distributions of the MMRT parameters and temperature traits ($\Delta H_{T0}^{\ddagger}$, $\Delta C_P^{\ddagger}$, $\Delta S_{T0}^{\ddagger}$, $T_{opt,}$ TS_{max}), in order for this temperature sensitivity framework to be more widely incorporated into other studies and analyses. Overall, we found that the type of distribution varied based on the parameter estimated and the ranges of possible trait values were diverse. The temperature optimum and point of maximum temperature sensitivity have approximately normal distributions, with $T_{opt,}$ skewed slightly right with a mean of 29.5°C, a standard deviation of 10.8°C, and a range of 4.6 to 96.1°C (Figure 4.1a). TS_{max} is skewed slightly left with a mean of 17.8°C, a standard deviation of 9.5°C, and range of -33.4 to 61.6°C (Figure 4.1b). Heat capacity has a roughly bimodal lognormal distribution with a mean of -8.4 kJ mol^{-1} K^{-1}, a standard deviation of 6.5 kJ mol^{-1} K^{-1}, and a range of -33.8 to -0.328 kJ mol^{-1} K^{-1} (Figure 4.1c). The values closer to zero indicate a more linear temperature response (or the rate is less sensitive to

changes in temperature) whereas the more negative $\Delta C_P^{\ddagger}$ values indicate a more curved temperature response and thus more sensitive to changes in temperature. Lastly, enthalpy and entropy both have approximately normal distributions with means of 36.5 and -0.111 kJ mol^{-1} K^{-1}, standard deviations of 58.1 and 0.190 kJ mol^{-1} K^{-1}, and ranges of -172 to 385 and -0.887 to 0.998 kJ mol^{-1} K^{-1}, respectively (Figures 4.1d, e). Weighted means vary slightly from the reported means with a TS_{max} of 17.5°C, T_{opt} of 26.1°C, $\Delta C_P^{\ddagger}$ of -4.58 kJ mol^{-1} K^{-1}, $\Delta H_{T0}^{\ddagger}$ of 36.0 kJ mol^{-1} K^{-1}, and $\Delta S_{T0}^{\ddagger}$ of -0.110 kJ mol^{-1} K^{-1}.

Temperature trait correlations

Correlations among the MMRT parameters and temperature traits varied considerably. There was a nearly perfect positive correlation between $\Delta H_{T0}^{\ddagger}$ and $\Delta S_{T0}^{\ddagger}$ (r = 0.98). We also found a strong positive correlation between T_{opt} and TS_{max} (r = 0.89, Figure 4.2a) with an offset of T_{opt} approximately 8.6°C higher than TS_{max}, which has not been reported previously. We observed no correlation with $\Delta C_P^{\ddagger}$ for $\Delta H_{T0}^{\ddagger}$ and $\Delta S_{T0}^{\ddagger}$ (r = -0.09 and r = -0.05). Correlations between $\Delta H_{T0}^{\ddagger}$ and $\Delta S_{T0}^{\ddagger}$ with T_{opt} and TS_{max} were positive with correlation coefficients between 0.73 and 0.81. The relationships between $\Delta C_P^{\ddagger}$ and T_{opt} and TS_{max} (Figure 4.2b, c) had a T-shaped or right-angled type of curve where a variety of T_{opt} and TS_{max} values are possible when $\Delta C_P^{\ddagger}$ is less than about -10 kJ mol^{-1} K^{-1}, but values for higher negative $\Delta C_P^{\ddagger}$ values T_{opt} and TS_{max} fall within a very narrow temperature range.

Variation in temperature sensitivity traits

Using a random effects model, we compared how $\Delta C_P^{\ddagger}$, T_{opt}, and TS_{max} varied for different organism types and environmental factors. We found that bacteria had a higher T_{opt} and TS_{max} than fungi or than the mix of organisms (Figure 4.3a), however we did not find any difference in $\Delta C_P^{\ddagger}$ between those three groups (Figure 4.a). We also found considerable variation in temperature sensitivity within bacteria as a group. Gram-positive and gram-negative bacteria were found to differ in all of their temperature sensitivity traits, with gram-positive bacteria having a higher TS_{max}, T_{opt}, and more negative $\Delta C_P^{\ddagger}$ (Figures 4.3b and 4.4b).

Environmental conditions also varied with temperature sensitivity traits. We found weak, but significant positive correlations with average native thermal temperature (Figure 4.5a, b, c) and lower T_{opt} and TS_{max} values in sources with where we might expect lower average temperatures, like marine water and ice, and marine sediments. We found higher T_{opt} and TS_{max} values in sources where we might expect to have high average temperatures, like wastewater and sludge and food animal products (Figures 4.3d and 4.4d). Aquatic systems were found to have a higher T_{opt} and TS_{max} values than terrestrial systems (Figure 4.3c). We find that in thermally managed systems (i.e., wastewater and sludge and food animal products) $\Delta C_P^{\ddagger}$ is more negative than in unmanaged systems (Figure 4.4d). In contrast we find that in aquatic and unmanaged systems $\Delta C_P^{\ddagger}$ is less negative, with marine water and ice being the lowest, followed by soil (Figure 4.4c, d, e). We found only weak correlations between temperature sensitivity and pH (Figure 4.5g-l).

Temperature traits also varied with the type of rate measured and the assortment of organisms measured. The assortment of organisms measured (i.e., isolate, selected group of similar organisms, and community) resulted in significantly different temperature trait values.

TS_{max} and T_{opt} were both lowest for the community, followed by the isolate, and then by the group of similar organisms (Figure 4.3f). Heat capacity was also least negative for the community, followed again by the isolate and group of similar organisms (Figure 4.4f). Similarly, we found the type of rate measured in the analysis to make a difference in the temperature traits. Carbon dioxide flux and enzyme activity had the least negative $\Delta C_P^{\ddagger}$ values, but carbon dioxide flux had the largest TS_{max} and T_{opt} values whereas enzyme activity had one of the lowest TS_{max} and T_{opt} values (Figures 4.3g and 4.4g). Growth rate and nitrogen-related rates had more intermediate temperature sensitivity trait values (Figures 4.3g and 4.4g).

Discussion

Relationships among temperature traits

This is the first study, to our knowledge, to document the distribution of these temperature sensitivity traits, and explore how they diverge for different organisms, environments, and biological processes. Information about these distributions is necessary in order to accurately parameterize models. Moreover, establishing functional relationships between temperature sensitivity traits are necessary to further ecological theory (McGill et al., 2006). The relationships we observed between $\Delta C_P^{\ddagger}$ and T_{opt} and TS_{max} (Figure 4.2b, c) were not as expected based on data from Hobbs et al., 2013. Instead of a gradually sloping and saturating type of curve, we find a T-shaped or right-angled type of curve. The many more data points we have in our meta-analysis might explain the different relationship observed in the Hobbs et al., 2013 study. Another possibility for this discrepancy is that we included all types of metabolic rates in our analysis instead of rates for only individual enzyme activities. Regardless, these

relationships suggest a tradeoff between $\Delta C_P^{\ddagger}$ and T_{opt} and TS_{max} making it is unusual to have high temperature sensitivity with very high T_{opt} or very high or low TS_{max} values.

Temperature sensitivity traits vary among organism

In this meta-analysis we primarily explore how temperature sensitivity varies as a microbial trait. The differences expressed in temperature sensitivity traits among the different categories measured could be a function of variation in organismal physiology and adaptation, competition between organisms, thermal regime and other environmental trade-offs, or measurement approaches. The fact that more differences exist for T_{opt} and TS_{max} than for $\Delta C_P^{\ddagger}$ among different organisms, suggests that temperature sensitivity of bacteria, fungi, and a mix of organisms is largely driven by enthalpy as opposed to heat capacity. Similarities in $\Delta C_P^{\ddagger}$ between these different groups of organisms are consistent with the literature. Both Pietikainen et al., 2005 and Bárcenas-Moreno et al., 2009 found overall temperature response to be similar between bacteria and fungi. Furthermore, fungal to bacterial ratios have been found not to change with temperature (Allison and Treseder, 2008; Strickland and Rousk, 2010), suggesting similarities in temperature sensitivity. However, higher T_{opt} and TS_{max} in bacteria than fungi or the mix of organisms, contrasts Immanuel et al., 2006 which found higher T_{opt} values for fungal enzymes than for bacterial enzymes, although they only surveyed a few microbial strains. However, many thermophilic bacteria exist with high temperature optima due to highly thermally stable macromolecules (Zeikus, 1979).

Although both gram-positive and gram-negative bacteria are ubiquitous, with exceptionally high and low temperature optima found in both types of organisms (Pask-Hughes and Williams, 1975; Huston et al., 2000), gram-negative bacteria are more commonly known for

their ability to withstand extreme temperatures and pH levels due to their strong, but elastic outer membranes (Beveridge, 1999). However, this pattern is in contrast with our results. It is possible that thicker cell walls in gram-positive bacteria or other features of their cell wall allow them to have the higher TS_{max} and T_{opt} values observed in our study. The result of gram-negative bacteria having a less negative $\Delta C_P^{\ddagger}$ is also surprising. Both Schwab et al., 2014 and Eber et al., 2011 report higher densities of gram-negative bacterial pathogens in warmer months as opposed to cooler months, but little change in densities of gram-positive bacteria throughout the year. Thus, gram-negative bacteria appear to be more temperature sensitive in these studies, while our meta-analysis reveals that gram-negative bacteria are less temperature sensitive. We hypothesize that this difference could be due to the fact that the range of temperatures experienced by these pathogenic gram-negative bacteria were more in the range close to their TS_{max} where small changes in temperature can result in large changes in growth rate, while if the gram-positive bacteria had a higher TS_{max}, as our results suggest, then the apparent temperature sensitivity would appear negligible, even if the actual temperature sensitivity (i.e., $\Delta C_P^{\ddagger}$) is higher.

It is also possible that the differences in the temperature traits observed between gram-positive and gram-negative bacteria in our study are an artifact of the meta-analysis itself. All gram-positive bacteria in our meta-analysis were derived from thermally managed (mostly food systems), where the temperature was likely higher and more constant. The environmental source for gram-negative bacteria was much more varied, but included sources where the native thermal temperature was likely lower and more variable, e.g., soil. However, we do not presume this to be the dominant cause of the differences we observed since these results do not correspond with the TS_{max}, T_{opt}, and $\Delta C_P^{\ddagger}$ values found for the microbial source data.

Temperature sensitivity traits vary among environments

The source environment proved to be an exceptionally strong predictor of variation in temperature sensitivity traits. At the outset of this study, we expected temperature sensitivity to be highly dependent on the source environment—a function of the thermal regime (average temperature and stability of temperature), substrate quality and quantity, and pH. Specifically, we predicted that T_{opt} and TS_{max} would follow average temperature from the source environment, while $\Delta C_P^{\ddagger}$ would more strongly reflect the temperature variation. Unfortunately, our dataset did not include sufficient information about temperature variance to allow us to directly test this hypothesis. However, T_{opt} and TS_{max} were correlated with environmental temperature (Figure 4.5a, b, d, e). Based on our hypothesis, $\Delta C_P^{\ddagger}$ should be most negative (greatest temperature sensitivity) for environments that experience large changes in temperature (Wallenstein and Hall, 2012), have more recalcitrant substrate (Fierer et al., 2005; Davidson and Janssens, 2006; Conant et al., 2008; Craine et al., 2010), or that have low substrate availability (Nedwell, 1999; Pomeroy and Wiebe, 2001). Our findings indicate that thermally managed systems have more negative $\Delta C_P^{\ddagger}$ than in unmanaged systems, which perhaps indicates a greater thermal stability in these managed systems and increased substrate availability, contrasting with aquatic and unmanaged systems where $\Delta C_P^{\ddagger}$ is less negative. Low temperature sensitivity in aquatic systems is somewhat surprising, as we would expect temperature sensitivity to be higher because of greater temporal stability, thus selecting for microbial communities composed of more temperature specialists as opposed to generalists (Wallenstein and Hall, 2012). However, Yvon-Durocher et al., 2012 did find lower apparent activation energies for respiration in aquatic systems compared with terrestrial systems, which is roughly in line with our results that terrestrial systems are more temperature sensitive (i.e., more negative $\Delta C_P^{\ddagger}$). Higher temperature sensitivity in terrestrial

systems may also reflect a narrower range of temperature environments recorded in this meta-analysis compared to aquatic systems, thus providing a mean aquatic $\Delta C_P^{\ddagger}$ with a less sensitive temperature response even if some temperature response curves for individual organisms or communities may be more sensitive than in terrestrial.

The discrepancy in our results for aquatic and terrestrial systems could also be due to tradeoffs in temperature sensitivity with substrate or difficulties in disentangling nutrient availability from temperature response. Membrane structure and function exhibit a tradeoff between resource acquisition and thermal adaptation, and as temperature decreases, substrate affinity also decreases. Therefore, cold-adapted organisms might be less temperature sensitive in order to increase their competitive advantage for resources at colder temperatures (Nedwell, 1999). Thus, in marine water and ice lower average temperatures may restrict substrate uptake ability, which would result in minimal temperature sensitivity in order to compete for substrates at these low temperatures. In resource poor environments, a tradeoff may also exist between temperature response and nutrient acquisition, where temperature sensitivity must be lower to compete for resources (Hall et al., 2008; Manzoni et al., 2012). Lastly, a tradeoff between substrate type and temperature sensitivity has been widely documented in the literature where increasing temperature sensitivity corresponds to increasing substrate complexity or recalcitrance (Fierer et al., 2005; Conant et al., 2008; Craine et al., 2010). However, it is beyond the scope of this study to disentangle the constraints on temperature sensitivity due to substrate complexity and total substrate availability beyond speculation. Greater detail on substrate availability and recalcitrance may provide further insight into predicted temperature response of organisms from different environments. We also expected temperature and pH to be major drivers of microbial temperature sensitivity. Although pH is a major driver of microbial community composition

(Fierer et al., 2009) and its intrinsic relationship with temperature suggests a potential relationship with temperature sensitivity (increase in temperature corresponds to a decrease in pH), we did not find strong support for this in our analysis, which corroborates Craine et al., 2010 that found no relationship between pH and activation energy.

Temperature sensitivity traits vary based on measurement types

We found some interesting relationships between temperature sensitivity trait values and the type of rate measured and the assortment of organisms measured. The results supported our hypothesis that $\Delta C_P^{\ddagger}$ would be less negative in communities than isolates, suggesting that temperature responses of a community are the summation of individual temperature response curves. This effect lowers the $\Delta C_P^{\ddagger}$ and effectively flattens the temperature response curve (see Schipper et al., 2014 , Fig. 2). Heat capacity may also be less negative in communities versus individual isolates because microorganisms often have altered phenotypic expression in communities than as individuals (Paul, 2014; Wagg et al., 2014). Persisting in a community versus individually could lessen the apparent temperature sensitivity due to reducing other environmental constraints (i.e., resource acquisition). In contrast to these findings, the $\Delta C_P^{\ddagger}$ values for groups of similar organisms were the most negative (or most temperature sensitive). This is consistent with the hypothesis that increased competition between very similar organisms drives some sort of "niche" response (Crowther et al., 2014), resulting in large, negative $\Delta C_P^{\ddagger}$ values as microbes adapt to specific temperature regions where they outperform others. However, it is also conceivable that the temperature response we see in the group of similar organisms category (as opposed to the community and isolate category measurements) is due to the majority of this group coming from wastewater and food animal product studies where

temperature is highly managed compared to the other sources, driving the similar organism group to have a higher than natural temperature sensitivity.

The type of rate measured also explains variation in the observed temperature traits. Interestingly, the heat capacity results did not fully match our hypotheses, which predicted that rates involving a broader range of activities (i.e., carbon dioxide flux) would be less temperature sensitive than rates involving singular enzymes (i.e., enzyme activity rates). Consistent with our hypothesis, we found carbon dioxide flux to be the least temperature sensitive (Figure 4.4g) as it involves many different enzymes, which might combine to produce an overall less temperature sensitive response. However, we predicted that as enzyme activity is a more specific rate function, $\Delta C_P^{\ddagger}$ would be most negative, which was not the case (Figure 4.4g). This inconsistency might be due to the fact that enzymes are known to have different temperature sensitivities (Koch et al., 2007; Trasar-Cepeda et al., 2007; Steinweg et al., 2013; Alster et al., 2016a), so grouping them into one large group in the meta-analysis may have misconstrued the results. The rate with the most negative $\Delta C_P^{\ddagger}$, or most temperature sensitive, was denitrification, which follows reports of the Q10 of denitrification exceeding that of carbon dioxide flux due to the tight coupling of the microbial C and N cycle (Butterbach-Bahl et al., 2013). Denitrification also takes place under very specialized conditions (i.e., high nitrate availability, low oxygen concentrations, and electron donor availability) compared with the other metabolic functions (Seitzinger et al., 2006), perhaps restricting their ability to adapt to different temperature continuums.

It is widely assumed that reaction rates of all kinds will increase exponentially as temperature increases (Davidson and Janssens, 2006), unless limited by other factors such as water or other nutrients (Bouletreau et al., 2014) until reaching a very high temperature. The

results found in this study indicate that the temperature at which reaction rates might be expected to decline is lower than previously assumed, or at least the temperature where exponential growth in the rate term is lower than expected (Figure 4). Although a plethora of studies exist on how different rates vary with temperature, how temperature sensitivity varies between those different rates within the same system is not commonly studied. In agreement with our results, Pietikainen et al., 2005 found that the T_{opt} for respiration rate in soils was higher than the T_{opt} for growth rate. However, this study was included in our meta-analysis. Furthermore, many respiration studies do not capture the T_{opt}, which implies that the T_{opt} might be quite high (e.g., Lloyd and Taylor, 1994), which is consistent with our data. What we find most insightful about this analysis is the knowledge about which types of microbial functions might be most impacted by global warming and impact ecosystem models the greatest. Microbial transformations involving nitrogen appear to have the greatest temperature sensitivity as defined by a highly negative $\Delta C_P^{\ddagger}$, as well as moderate TS_{max} and T_{opt} values. Thus, modest increases in temperature could strongly affect how much nitrous oxide could be released from our soils and water and modeling of those changes could be largely inaccurate (Butterbach-Bahl et al., 2013).

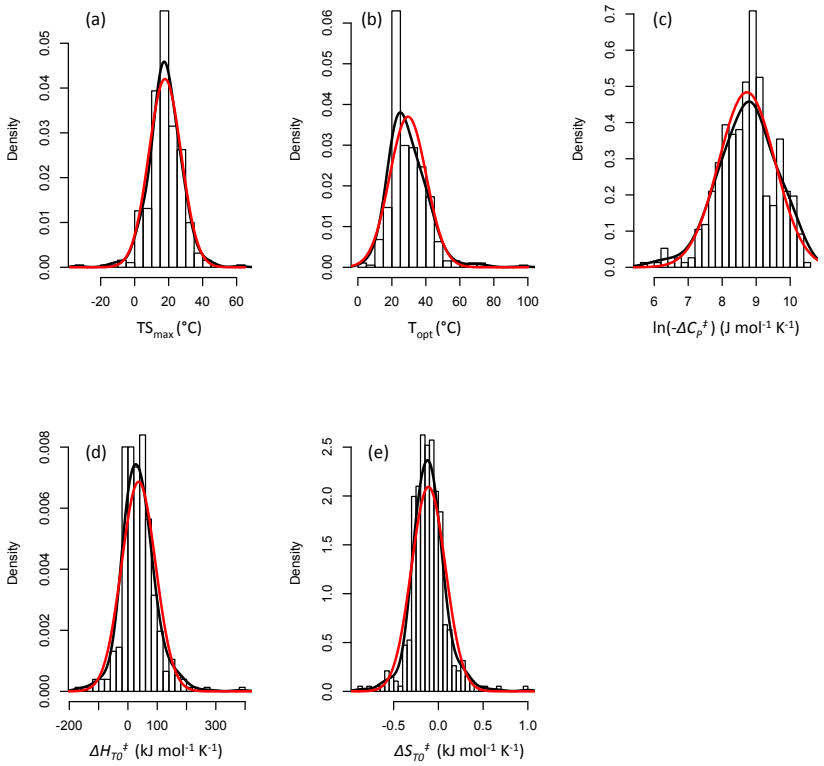

Figure 4.1. Histograms of TS_{max} (a), T_{opt} (b), ln(-$\Delta C_P^{\ddagger}$) (c), $\Delta H_{T0}^{\ddagger}$ (d), and $\Delta S_{T0}^{\ddagger}$ (e). The black line represents true shape of the distribution, while the red line indicates what the distribution would be if it was normally distributed (or lognormally distributed for $\Delta C_P^{\ddagger}$).

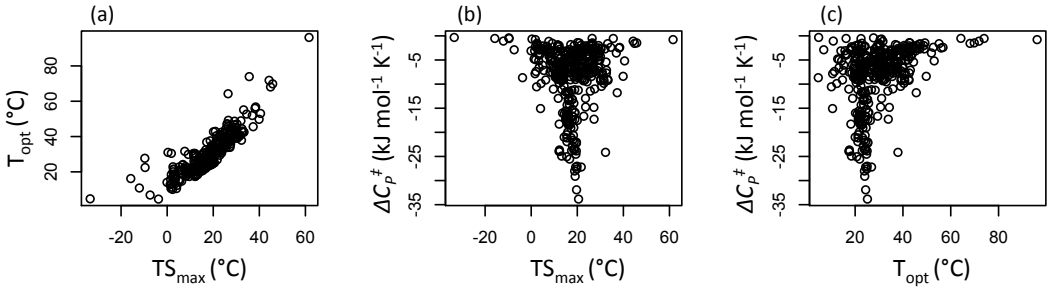

Figure 4.2. Correlations among temperature sensitivity traits.

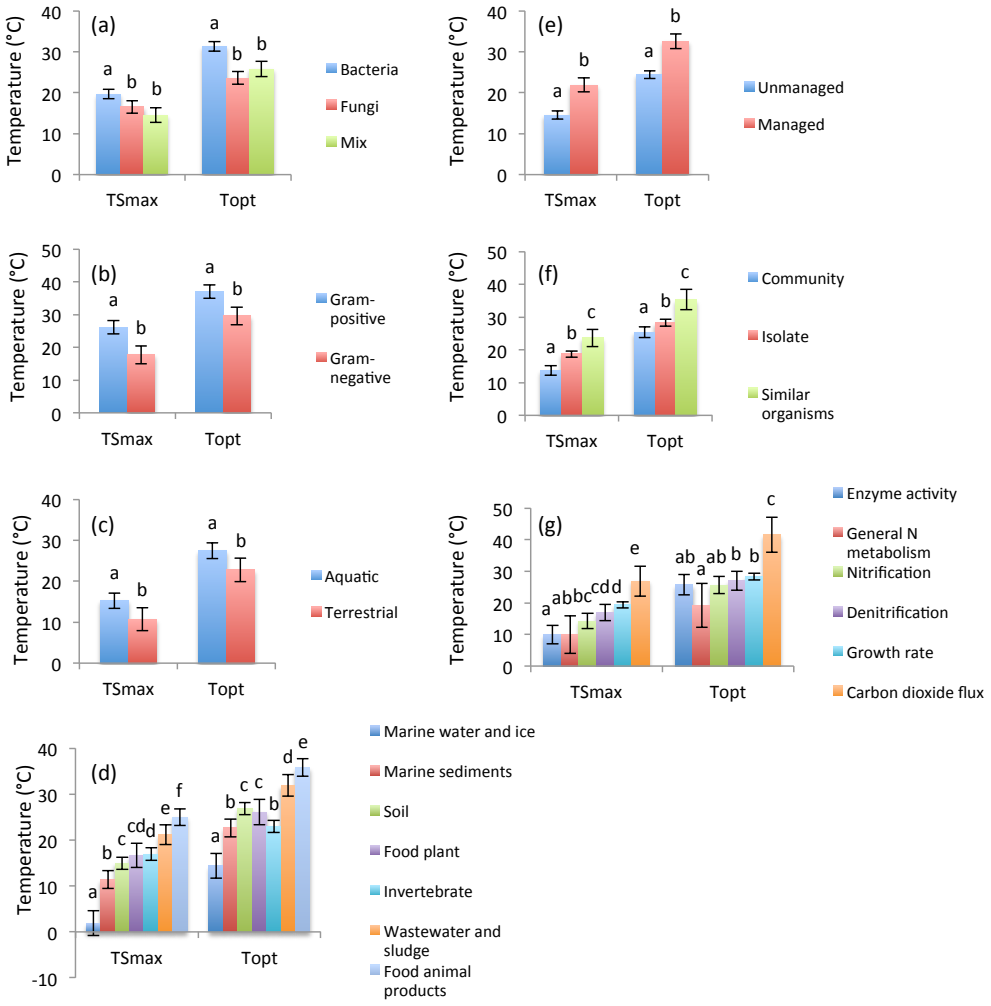

Figure 4.3. Mean TS_{max} and T_{opt} for organismal type (a), bacterial type (b), aquatic or terrestrial source (c), microbial source (d), thermally/anthropogenically managed or unmanaged system (e), assortment of organisms measured (f), and rate type (g). Error bars represent ±2 standard errors above and below the mean. Letters indicate significant differences (P <0.05) between groups within TS_{max} or T_{opt} (not between the two temperature traits).

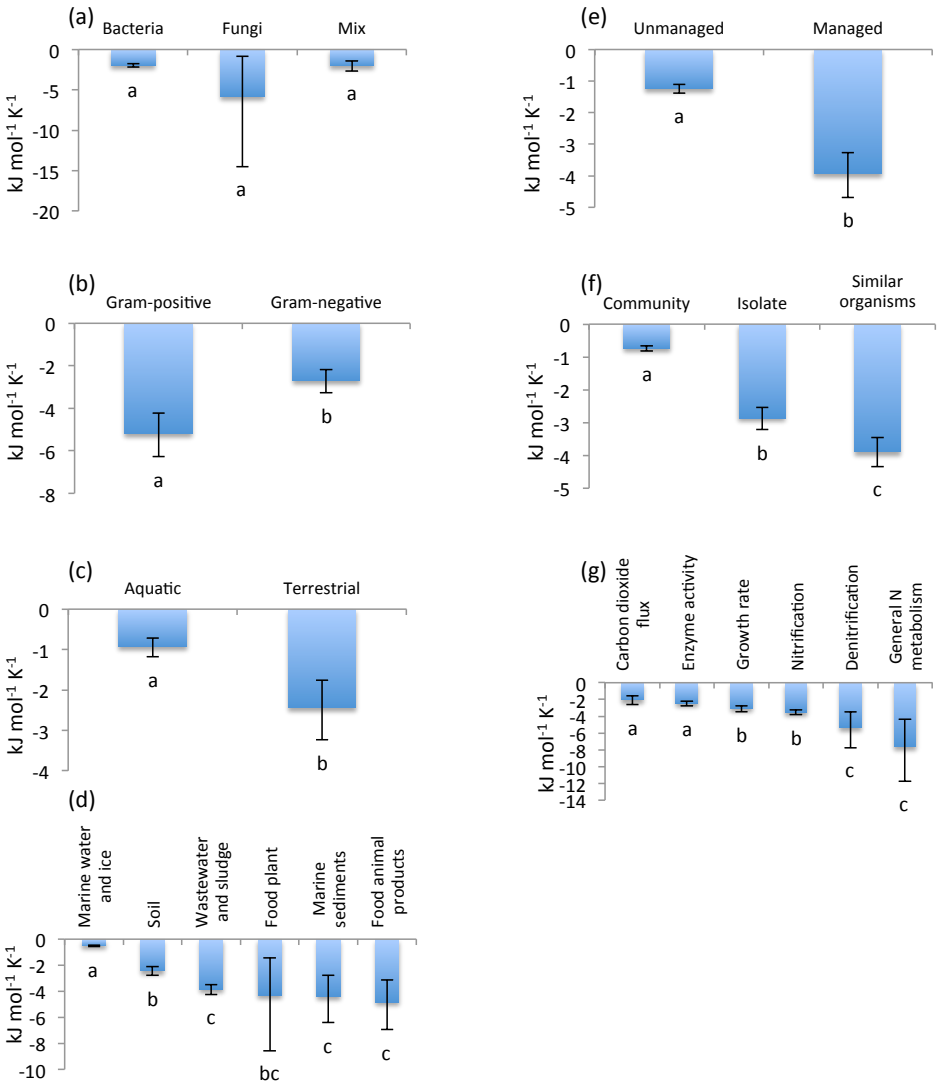

Figure 4.4. Mean $\Delta C_P^{\ddagger}$ for organismal type (a), bacterial type (b), aquatic or terrestrial source (c), microbial source (d), thermally/anthropogenically managed or unmanaged system (e), assortment of organisms measured (f), and rate type (g). Error bars represent ±2 standard errors above and below the mean. Letters indicate significant differences (P <0.05) between groups.

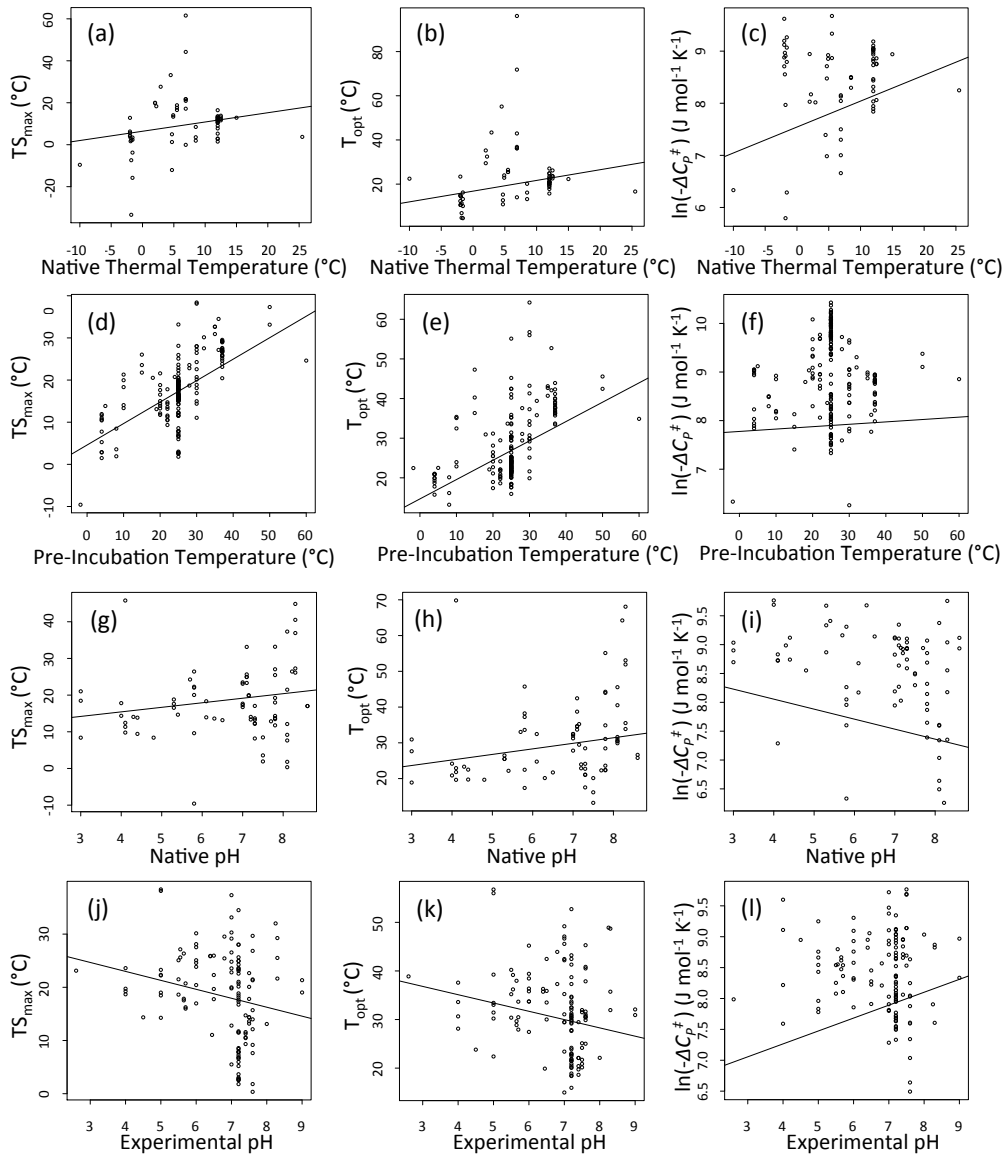

Figure 4.5. Correlations for temperature traits (TS_{max}, T_{opt}, and $\Delta C_P^{\ddagger}$) and native thermal temperature, pre-incubation temperature, native pH, and experimental pH.

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Chapter 5: Summary and Conclusions

The primary objective of my dissertation was to improve understanding of temperature sensitivity of soil microbial communities. I addressed two main questions: 1) how does the activity of soil microbes vary with temperature? And 2) how should temperature sensitivity be characterized? In chapters two and three I addressed question one by comparing the Arrhenius equation with Macromolecular Rate Theory (MMRT) and determined that MMRT is a better estimate of temperature response for both carbon dioxide flux from soil microbial communities and also for enzyme activity from soil microbial isolates. These results are some of the first empirical studies testing MMRT. I also determined here that microbes have distinct temperature sensitivities that can vary independent of their environment. Chapters three and four address question two and develop a comprehensive way to examine temperature sensitivity for soil ecology and potentially other biological systems and organisms. Using MMRT, I developed the idea of using temperature sensitivity as a biological trait in order to more meaningfully evaluate temperature sensitivity across different types of organisms and enzymes. I identify in chapter three that this traits-based approach cannot be supported using the Arrhenius equation since parameter estimates are so heavily dependent on methodology. In chapter four, I evaluated the distributions and correlations of these temperature sensitivity traits using a meta-analysis and show that they vary with organism type, environment, process, and biodiversity, further developing an understanding of how temperature sensitivity should be characterized.

Importance of this work is multi-faceted. First, it highlights the importance of differences among microbial communities. This work supports the notion that not everything is everywhere (even functionally), and that microbes and communities of microbes should be expected to have

differential responses to changes in temperature. Secondly, this work demonstrates that the Arrhenius equation is not the best approach to abstract temperature sensitivity for soil microbes and offers an alternative approach. Using MMRT, I developed the novel concept of temperature sensitivity as a microbial trait, which allows for integration of temperature sensitivity into other areas in ecology and climate science in new ways. Operating under this traits-based approach, it is easier to compare temperature sensitivities from soil microbial communities (and other organisms) across locations and to begin making ecological inferences about competition, adaptation, microbial community assemblage, etc., as it relates to temperature sensitivity. The traits-based approach might also be a practical method to discreetly incorporate differential temperature responses into trait-based ecosystem models to more accurately predict ecosystem functions. The meta-analysis performed in this dissertation provides the necessary data to begin parameterizing these models. Lastly, this work provides new insights into how temperature response of organisms might impact global climate change. For example, I found in the meta-analysis that processes related to nitrogen metabolism have higher temperature sensitivities than does respiration. This inspires new lines of inquiry as to what functions or organisms might be most sensitive to global warming.

While I believe that this body of work has made a contribution to advancing understanding of temperature sensitivity in soil microbial communities, there are still many unanswered questions. One major question is how temperature sensitivity traits respond to stress both on an individual level and as a community. Are temperature traits highly conserved or prone to adaptation? Better understanding of how these traits will adapt to climate change will allow us to improve predictions of both organismal and ecosystem responses to climate change. Secondly, what are the interactions between temperature sensitivity traits and resource availability? Finally,

how can we continue to scale up from organisms to communities and enzyme activities to large processes in the most realistic ways?