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OPINION

Embracing a new paradigm for temperature sensitivity of soil microbes

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Abstract

The temperature sensitivity of soil processes is of major interest, especially in light of climate change. Originally formulated to explain the temperature dependence of chemical reactions, the Arrhenius equation, and related Q_{10} temperature coefficient, has a long history of application to soil biological processes. However, empirical data indicate that Q_{10} and Arrhenius model are often poor metrics of temperature sensitivity in soils. In this opinion piece, we aim to (a) review alternative approaches for characterizing temperature sensitivity, focusing on macromolecular rate theory (MMRT); (b) provide strategies and tools for implementing a new temperature sensitivity framework; (c) develop thermal adaptation hypotheses for the MMRT framework; and (d) explore new questions and opportunities stemming from this paradigm shift. Microbial ecologists should consider developing and adopting MMRT as the basis for predicting biological rates as a function of temperature. Improved understanding of temperature sensitivity in soils is particularly pertinent as microbial response to temperature has a large impact on global climate feedbacks.

KEYWORDS
activation energy, Arrhenius, macromolecular rate theory, Q_{10}, soil microbes, temperature sensitivity, thermal adaptation

1 | INTRODUCTION

The temperature sensitivity of soil biological processes under climate change is of major interest because of the major consequences for soil carbon dynamics (Bradford et al., 2016). Although definitions vary in the literature, most simply, temperature sensitivity can be defined as the rate of change with respect to temperature (Sierra, 2012); or mathematically, temperature sensitivity is the first derivative of the temperature response. Historically, the most common metrics for measuring temperature sensitivity of soil processes have been the Arrhenius model or the Q_{10} temperature coefficient. It is important to note that Q_{10} is not the rate of change with respect to temperature, but rather the ratio between two rates. The Arrhenius model and Q_{10} temperature coefficient have been used to characterize soil temperature sensitivity since the 1920s (Singh & Gupta, 1977). However, over the past several decades, a growing body of literature now clearly demonstrates that Q_{10} and Arrhenius models are ineffective and sometimes misleading models for characterizing temperature sensitivity in soils (e.g., Alster, Koyama, Johnson, Wallenstein, & Fischer, 2016; Davidson, Janssens, & Luo, 2006; Hamdi, Moyano, Sall, Bernoux, & Chevallier, 2013; Lloyd & Taylor, 1994; Robinson et al., 2017; Schipper, Hobbs, Rutledge, & Arcus, 2014; Tang & Riley, 2015).

We argue that the Q_{10} temperature coefficient and the Arrhenius model are ill suited for soil biological systems. First, the Arrhenius equation was not originally intended for biological reactions, but instead to describe the thermal dependence of reaction rates in physical chemistry. Applying it in biological systems neglects enzyme catalysis of the reaction by assuming that enzyme tertiary structure is not temperature sensitive (DeLong et al., 2017). Second, these equations assume that biological reaction rates rise monotonically with warming (i.e., only increase with increasing temperature). In reality, these rates are typically unimodal—they peak at intermediate temperatures, and decline at higher temperatures (Dell, Pawar, & Savage, 2011). Third, Arrhenius and Q_{10}-modeled rates are dependent on the temperature range measured (Alster, Baas, Wallenstein, Johnson, & Fischer, 2016; Kirschbaum, 1995; Pawar et al., 2016; Schulte, 2015; Sierra, 2012). Therefore, the same data fit to the Q_{10} temperature coefficient and the Arrhenius model can yield different parameter estimates for different temperature ranges, meaning that these model parameters can be inconsistent metrics of temperature sensitivity. Moreover, the parameters can be misleading when comparing results between studies. Even more problematic with Q_{10}, realistic values can be generated when using randomly generated data as a consequence of the mathematical formulation (Sierra, 2012). We therefore caution against the use of the Q_{10} temperature coefficient and the Arrhenius model in biogeochemical modeling.

Here, we advocate for broader adoption of an alternative model of temperature sensitivity for soil microbial processes. Recent studies provide feasible alternatives to the Q_{10} temperature coefficient and the Arrhenius model that provide technical advancement, empirical validation, and improved theoretical understanding of temperature sensitivity (Dobri & Bååth, 2018; Pawar et al., 2016; Schipper et al., 2014). Despite these advances, of the papers published in *Global Change Biology* in 2017 and 2018 regarding temperature sensitivity in soil systems, 25 out of 31 (81%) only fit data to the Q_{10} temperature coefficient or Arrhenius model. To move beyond these measures of temperature sensitivity, we provide (a) a review of alternative approaches, focusing on macromolecular rate theory (MMRT); (b) strategies and tools to overcome potential barriers of transitioning to a new temperature sensitivity framework; (c) hypotheses for incorporating MMRT into thermal adaptation theory; and (d) exploration of new questions and opportunities stemming from these new approaches.

2 | ALTERNATIVE APPROACHES

Over the past several decades, many alternative approaches have been proposed to describe the temperature sensitivity of biological processes. Some stem from enzyme biochemistry or microbiology and have been applied to soils (Ratkowsky, Lowry, McMeekin, Stokes, & Chandler, 1983; Ratkowsky, Olley, McMeekin, & Ball, 1982; Schipper et al., 2014), while others derive from empirical modifications of existing equations that fit soil data (Bååth, 2018; Lloyd & Taylor, 1994; Qi, Xu, & Wu, 2002). Most of these approaches improve predictions of temperature sensitivity by modifying Q_{10} or Arrhenius to account for residual variation in the data. However, most of these modified models remain monotonic, so projected responses are not necessarily representative of biological processes (Alster, Baas, et al., 2016).

Several non-monotonic, unimodal equations have been proposed to describe temperature response in biological systems. Four of these have been applied to soil processes: the Johnson and Lewin model (Jing et al., 2014), the square root model (Ratkowsky equation; Birgander, Reischke, Jones, & Rousk, 2013; Duan, Wu, Zhang, Fan, & Xiong, 2018; Pietikäinen, Pettersson, & Bååth, 2005; Rinnan, Michelsen, & Bååth, 2011; Rinnan, Rousk, Yergeau, Kowalchuk, & Baath, 2009; Taylor, Giguere, Zoebelein, Myrold, & Bottomley, 2017; van Gestel, Reischke, & Bååth, 2013), the equilibrium model (Menichetti, Ortigoza, & García, 2015), and MMRT (Alster, Baas, et al., 2016; Alster, Koyama, et al., 2016; Duan et al., 2018; Robinson et al., 2017; Schipper et al., 2014; Taylor et al., 2017). Each has its own merits and shortcomings. However, all improve upon the Q_{10} temperature coefficient and the Arrhenius model by capturing the unimodality typical of biological enzymatic reactions. DeLong et al. (2017) review the assumptions of these models from a thermodynamic perspective (see box 1 of DeLong et al., 2017). There are no studies directly comparing all four approaches for soils, although Taylor et al. (2017) compared the square root model and MMRT for nitrification by soil bacteria and archaea. They noted that the two models did not differ in their effectiveness (Taylor et al., 2017).

We propose the adoption of MMRT (Box 1) to represent temperature responses of soil biological systems for two reasons. First, in contrast to the square root model, MMRT is not strictly empirical but rather based on underlying thermodynamic theory. Second, in contrast to the equilibrium model, MMRT does not assume unlimited substrate supply (DeLong et al., 2017). Since substrates for soil enzymatic reactions are typically limiting (Schimel & Weintraub, 2003), temperature models that assume substrate saturation may not be as accurate.

3 | POTENTIAL BARRIERS AND SOLUTIONS

3.1 | Additional model parameters

One of the potential disadvantages of switching to MMRT is the additional parameters in the model. More complex models can be prone to overfitting and they require more data. The Arrhenius equation requires two fitted parameters (i.e., activation energy and the pre-exponential factor) while Q_{10} is based on a single slope parameter. More complex temperature models may involve three or four fitted parameters (DeLong et al., 2017). Several studies comparing a three-parameter MMRT model and a two-parameter Arrhenius model found a superior goodness of fit for MMRT even when accounting for the additional parameter (Alster, Baas, et al., 2016; Alster, Koyama, et al., 2016; Liang et al., 2018; Robinson et al., 2017). Additionally, with MMRT, some model parameters can be fixed using empirical information. The MMRT equation includes four parameters, T_0, $\Delta H^{\ddagger}_{T_0}$, $\Delta S^{\ddagger}_{T_0}$, and $\Delta C^{\ddagger}_{P}$. However, T_0 can be set empirically to 4–10°C below the experimental T_{opt} because T_0 does not strongly affect overall model fit (Alster, Koyama, et al., 2016; Schipper et al., 2014). Due to the phenomena of enthalpy–entropy compensation (Chodera & Mobley, 2014; Sharp, 2001),

BOX 1 Overview of macromolecular rate theory

Macromolecular rate theory (MMRT) was first proposed by Hobbs et al. (2013). They provided a model that better accounts for observed declines in enzyme activity at temperatures below thermal denaturation temperatures. They hypothesized that the curvature in biological temperature response curves is a function of the change in the heat capacity ($\Delta C_P^{\ddagger}$) between the enzyme–substrate complex and the enzyme–transition state complex, not denaturation. Heat capacity describes how the temperature of an object increases with added energy. In the Arrhenius equation, the activation barrier is independent of temperature, which is generally true for reactions involving small molecules, such as water. However, biological reactions are typically mediated by large macromolecules, such as enzymes, which have large heat capacities (per mole), meaning that more energy is needed to raise their temperatures compared to their surrounding environment. Therefore, large $\Delta C_P^{\ddagger}$ values lead to temperature dependence of the activation energy. The assumption that the temperature sensitivity of activation energy is negligible and therefore not appropriate for biological reactions. Thus, biological reactions are expected to deviate from the Arrhenius model.

Macromolecular rate theory modifies the Arrhenius equation to account for the large change in heat capacity associated with the transition between the enzyme–substrate complex and the enzyme–transition state for macromolecules. The MMRT equation is as follows:

$$\ln(k) = \ln\left(\frac{k_B T}{h}\right) - \frac{\Delta H_{T_0}^{\ddagger} + \Delta C_P^{\ddagger}(T - T_0)}{RT} + \frac{\Delta S_{T_0}^{\ddagger} + \Delta C_P^{\ddagger}(\ln T - \ln T_0)}{R}, \quad (1)$$

where k is the rate, T is the temperature, T_0 is the reference temperature, k_B is Boltzmann's constant, h is Planck's constant, R is the universal gas constant, H is enthalpy, S is entropy, and ‡ indicates the transition state (Figure 1). With MMRT, the $\Delta C_P^{\ddagger}$ of the enzyme controls the temperature response of the reaction. Minor mutations in isoenzymes can change the $\Delta C_P^{\ddagger}$ and therefore result in reactions having different temperature responses (Hobbs et al., 2013).

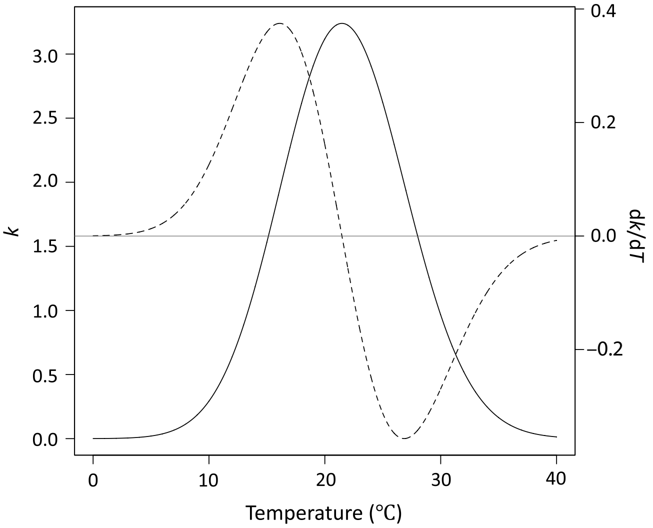

FIGURE 1 Example plot of k predicted by macromolecular rate theory (MMRT; solid, black line, y-axis, left-hand side) and the first derivative of k (dotted, black line, y-axis, right-hand side). The red line intersects dk/dT at the temperature optimum (T_{opt}). The positive peak of dk/dT is equal to the point of maximum temperature sensitivity (TS_{max}). Here, temperature dependence is the shape of the MMRT curve while the temperature sensitivity corresponds to dk/dT of the MMRT curve

Although originally intended to describe pure enzymatic response to temperature, MMRT has also been applied to soil microbial processes, at first by Schipper et al. (2014). They reported that MMRT is suitable for modeling soil enzymatic reactions and various ecosystem rates (i.e., respiration, nitrification, denitrification, and methane oxidation and production). Since then, MMRT has been further applied to soil biological reactions (Alster, Koyama, et al., 2016; Duan et al., 2018; Liu et al., 2018; Robinson et al., 2017; Taylor et al., 2017). Parameters from MMRT have also been used to define temperature response traits for microbes (Alster, Baas, et al., 2016; Alster, Weller, & Fischer, 2018). These traits include $\Delta C_P^{\ddagger}$, which describes the steepness of the temperature response curve, T_{opt}, which

(Continues)

BOX 1 (Continued)

describes the point of maximum activity, and TS_{max}, which describes the point of greatest positive change in the reaction rate (i.e., point of maximum temperature sensitivity). While $\Delta C_P^{\ddagger}$ can be estimated directly by fitting data to Equation (1), T_{opt} can be estimated by setting to zero the first derivative of that equation with respect to temperature and solving for T (Arcus et al., 2016):

$$T_{opt} = \frac{\Delta H_{T_0}^{\ddagger} - \Delta C_P^{\ddagger} T_0}{-\Delta C_P^{\ddagger} - R}, \tag{2}$$

and TS_{max} (also known as T_{inf}) can be estimated by setting to zero the second derivative of the MMRT equation with respect to temperature and solving for T (Schipper et al., 2019):

$$TS_{max} = \frac{\Delta H_{T_0}^{\ddagger} - \Delta C_P^{\ddagger} T_0}{-\Delta C_P^{\ddagger} \pm \sqrt{-\Delta C_P^{\ddagger} R}}. \tag{3}$$

These traits can replace activation energy or Q_{10} for describing and comparing the temperature response of different soil biological reactions. These traits also provide more intuitive and ecologically meaningful metrics for describing temperature response compared with the enthalpy and entropy parameters from MMRT.

$\Delta H_{T_0}^{\ddagger}$ and $\Delta S_{T_0}^{\ddagger}$ are also typically interdependent (Alster et al., 2018; Mills & Plotkin, 2015). Therefore, the effective number of model parameters is closer to 2 (Arcus et al., 2016; Robinson et al., 2017).

The MMRT traits provide a novel perspective on microbial and enzymatic responses to temperature. Determining the temperature at which the greatest change in rate occurs (TS_{max}) could help identify climate scenarios with large effects on nutrient cycling or greenhouse gas production. TS_{max} is particularly interesting because it typically falls within environmentally relevant temperature ranges (Alster, Baas, et al., 2016; Alster, Koyama, et al., 2016).

3.2 | Comparing temperature response with prior studies

Because researchers have long used Q_{10} and activation energy as measures of temperature sensitivity, there might be hesitation to adopt MMRT if its parameters are not comparable to previous models. Still, this hurdle could be overcome by fitting existing data to the MMRT model in a re-analysis. Here, we have included open-source tools in our supplement to facilitate MMRT parameter fitting, including T_{opt} and TS_{max}.

3.3 | Solutions for experimental limitations

For optimal model parameterization, MMRT requires sufficient measurements across a broad temperature range. In an analysis of the sample size needed for fitting soil respiration data to the MMRT model, Robinson et al. (2017) demonstrate that model fits continue to improve up until roughly 20 measurements at different temperatures. It is also important to capture the T_{opt} within the range of temperatures measured (Alster et al., 2018). These requirements (i.e., large number of independent temperature measurements and a large temperature range) may be an obstacle to fitting the MMRT model to existing empirical data or new data from small experiments. To overcome this problem in new experiments, we suggest the use of multiple incubators or staggered time points to expand the range and number of temperature points. Sample sizes need not increase, though. Added temperature points across the experimental range could be offset by decreasing replicate number at each point (O'Brien, Chooprateep, & Funk, 2009; Sefer, Kleyman, & Bar-Joseph, 2016). Total sample size could remain the same.

A temperature gradient block is another option. Common in microbiology experiments, temperature gradient blocks are made of aluminum set in a circulating water bath that is heated on one side and chilled on the other (detailed in Konishi, Yamashiro, Koide, & Nishizono, 2006). This approach has been used to incubate soils and sediments at multiple temperatures with minimal additional effort (Canion et al., 2014; Fey & Conrad, 2000; Robinson et al., 2017; Yao & Conrad, 2000). For experiments on soil microbial enzymes or isolates, temperature gradients in thermal cyclers—often found in microbial laboratories—is another option.

For field experiments, several solutions already exist to overcome these obstacles. Natural temperature and elevation gradients are commonly used for warming experiments (e.g., Bradford et al., 2019; Dacal, Bradford, Plaza, Maestre, & García-Palacios, 2019) and provide access to many temperature points. For manipulative field experiments, measurements could be collected near and far from the heating source because warming decreases with distance (Peterjohn, Melillo, Bowles, & Steudler, 1993). Field experiments also include temporal fluctuations in temperature. Such variation can be used to fit the model, as well as compare between warmed and control treatments (Carey et al., 2016; Li et al., 2019). Additionally, variation in environmental temperatures poses an interesting question for field studies: are organisms from more stable thermal environments more or less temperature responsive? In other words, do they display more or less negative $\Delta C_P^{\ddagger}$? Overall, we urge scientists to consider incorporating more temperature levels into future research projects.

While we strongly encourage incorporation of additional temperature points, another logistical consideration is how temperature interacts with moisture and oxygen availability. High temperatures typically decrease moisture availability. The interaction of these three variables (i.e., temperature, moisture, and oxygen availability) may confound the temperature–reaction rate relationship in soil systems (Sierra, Malghani, & Loescher, 2017). We therefore recommend controlling for these other variables when estimating soil temperature response, for example by adding water, to minimize misleading results.

3.4 | Development of thermal adaptation theory

Another potential barrier in applying MMRT is a lack of conceptual theory on thermal adaptation (Allison, Romero-Olivares, Lu, Taylor, & Treseder, 2018), or how temperature response curves adapt to changes in temperature. Particularly, how should respiration rate, or enzymatic V_{max}, the maximum reaction velocity, adapt or acclimate to temperature change? Enzyme catalyzed reactions are typically characterized through Michaelis–Menten kinetics:

$$V = V_{max}[S]/(K_m + [S]), \tag{4}$$

where V is the velocity, S is the substrate, and K_m is the half-saturation constant. V_{max} is thought to adapt to temperature through changes in the enzyme–substrate binding complex (Davidson & Janssens, 2006). According to the Arrhenius theory, cold-adapted enzymes should have lower activation energies to offset the lower kinetic energy of cooler systems, in comparison to higher activation energies of warm-adapted enzymes. Therefore, enzymatic adaptation to warming should entail increased activation energy (Figure 2a), which is controlled by a change in the enthalpy of activation (Wolfenden & Snider, 2001). A higher activation energy also implies a greater temperature response (and Q_{10}) because small changes in temperature have a larger effect on the reaction. Therefore, V_{max} is expected to become more temperature responsive with soil warming.

Still, the empirical support for the Arrhenius-based theory of thermal adaptation is contradictory. Some studies find that warm-adapted enzymes are more temperature sensitive (Allison et al., 2018; Koch, Tscherko, & Kandeler, 2007; Tang et al., 2019), while other studies find the opposite (Brzostek & Finzi, 2012; Koch et al., 2007; Nottingham et al., 2016; Razavi, Liu, & Kuzyakov, 2017; Tang et al., 2019; Wallenstein, McMahon, & Schimel, 2009). This discrepancy could be due to confusion in defining the term "temperature sensitivity." Some studies measure temperature sensitivity of V_{max}, K_m, or both. Additionally, studies can measure *intrinsic* versus *apparent* temperature sensitivity. These details should be clearly reported.

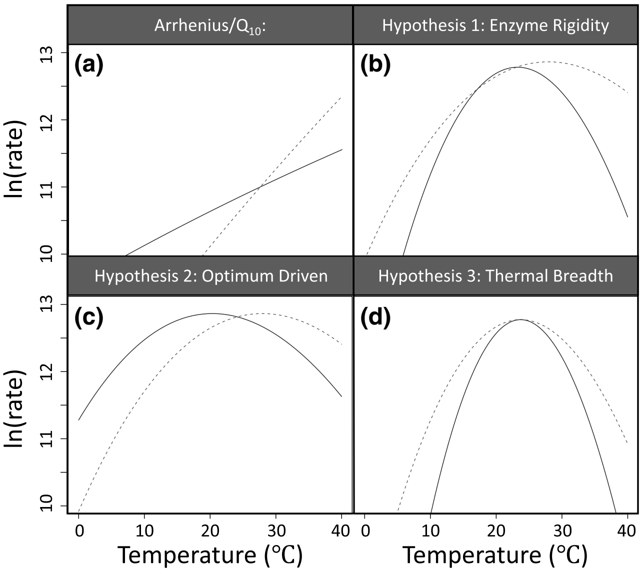

FIGURE 2 Hypotheses for thermal adaptation of temperature response curves for cold-adapted (blue lines) and warm-adapted (red, dashed lines) biological reactions. Panel (a) corresponds to the thermal adaptation hypothesis generated from the Arrhenius equation and panels (b)–(d) correspond to hypotheses for thermal adaptation developed for the macromolecular rate theory framework. With all hypotheses, the magnitude and direction of the change in rate will depend on how much the temperature response curve shifts, and at what temperature the reaction occurs. See Table S1 for the example parameter values corresponding to each plot

These inconsistencies could also result from limitations in the underlying reaction rate models. In particular, the assumption of a constant activation energy for the transition state in Arrhenius-based theory may not be valid, which could undermine thermal adaptation theory built on this assumption. In addition, poor fits of the Arrhenius model to rate data may lead to inaccurate estimates of the parameters used to test for thermal adaptation. As an alternative, in the next section, we propose new hypotheses about microbial and enzymatic temperature adaption that follow from MMRT and focus on V_{max}. Interpreting the temperature sensitivity of K_m is also important but requires more study of the thermal controls on enzyme–substrate binding.

4 | HYPOTHESES FOR TEMPERATURE SENSITIVITY AND THERMAL ADAPTATION

To build a conceptual theory on how V_{max} should adapt to temperature change under MMRT, we focus on two temperature response traits, $\Delta C_P^{\ddagger}$ and T_{opt}. A more negative $\Delta C_P^{\ddagger}$ corresponds to a steeper temperature response curve, whereas a less negative $\Delta C_P^{\ddagger}$ corresponds to a flatter curve. A steeper temperature response curve would indicate larger changes in rate with temperature compared with a flatter curve. Here, thermal adaptation refers to changes in temperature response traits (e.g., $\Delta C_P^{\ddagger}$, T_{opt}) of an enzyme in response to shifts in the temperature or temperature regime experienced by an organism. Below, we propose three hypotheses derived from biochemical and physiological mechanisms to describe how thermal adaptation might occur within the MMRT framework (Figure 2b–d).

Under the *Enzyme Rigidity Hypothesis* (Figure 2b), cooling causes $\Delta C_P^{\ddagger}$ to become more negative (i.e., steeper curve) and T_{opt} decreases. A more negative $\Delta C_P^{\ddagger}$ should be expected if cold-adapted enzymes have decreased rigidity compared with warm-adapted enzymes (Fields, 2001; Fields & Somero, 1998; Zavodszky, Kardos, Svingor, & Petsko, 1998). With cold-adapted enzymes, increasing vibrations in the enzyme–substrate complex help compensate for declining activity at lower temperatures (Wallenstein, Allison, Ernakovich, Steinweg, & Sinsabaugh, 2011). A more negative $\Delta C_P^{\ddagger}$ (and a steeper curve) results when enzyme rigidity decreases through an increased number of enzyme–transition state species (Arcus et al., 2016). With the Enzyme Rigidity Hypothesis, the entire temperature response curve may also shift upwards with increasing temperature (Arcus et al., 2016; Feller & Gerday, 2003). This directional shift and flattening of the temperature response curve are in line with empirical findings of thermophilic versus psychrophilic enzymes (Arcus et al., 2016; Struvay & Feller, 2012), but this upward shift is not always observed (Struvay & Feller, 2012). Additionally, several studies have found that a more negative $\Delta C_P^{\ddagger}$ corresponds to a lower T_{opt} (Alster et al., 2018; Arcus et al., 2016; Hobbs et al., 2013), which is actually an expected consequence of the mathematics (Arcus et al., 2016).

The second and third hypotheses, which we term the *Optimum-Driven Hypothesis* and the *Thermal Breadth Hypothesis*, respectively, take a physiologically driven approach (Figure 2c,d). For the Optimum-Driven Hypothesis, the T_{opt} increases with warming to more closely match the new thermal environment, but the $\Delta C_P^{\ddagger}$ remains the same (Figure 2c). We might expect this type of response if $\Delta C_P^{\ddagger}$ is a highly conserved property of that enzyme (Alster, Baas, et al., 2016). Furthermore, results from Alster et al. (2018; see figure 3c) suggest that multiple T_{opt} values are possible at the same $\Delta C_P^{\ddagger}$, particularly at less negative $\Delta C_P^{\ddagger}$ values. However, it might be that this relationship is not possible at more negative $\Delta C_P^{\ddagger}$ and that $\Delta C_P^{\ddagger}$ must increase with increasing T_{opt} as is predicted in the Enzyme Rigidity Hypothesis.

Lastly, the Thermal Breadth Hypothesis predicts that changes in $\Delta C_P^{\ddagger}$ are related to the temperature range of the environment (Figure 2d). Enzymes exposed to more temperature variation would have flatter temperature response curves (less negative $\Delta C_P^{\ddagger}$) to maintain more constant rates across varying temperatures. We previously found that multiple $\Delta C_P^{\ddagger}$ values are also possible with the same T_{opt} (Alster et al., 2018). This result may be explained by enzymes experiencing different temperature regimes, but similar mean temperatures. Temperature regime is thought to play a role in determining temperature sensitivity, but few studies have examined this relationship (Bai et al., 2017; Zhu & Cheng, 2011).

5 | NEW QUESTIONS AND OPPORTUNITIES

Several questions arise from these hypotheses, providing new opportunities for inquiry and development of thermal adaptation theory for soil biological systems. These questions include the following: Which of the proposed hypotheses, if any, are supported experimentally? Which enzyme thermal response traits change with long-term environmental warming (i.e., $\Delta C_P^{\ddagger}$ or T_{opt})? How much divergence, if any, does warming cause in the temperature response curves? How can we incorporate responses of K_m into this new thermal adaptation framework? How will adaptation of $\Delta C_P^{\ddagger}$ and T_{opt} vary with substrate availability or temperature variability?

Another set of key questions centers on scaling enzyme MMRT. Prior studies have already demonstrated MMRT's validity for higher level biological processes (Alster, Koyama, et al., 2016; Duan et al., 2018; Liang et al., 2018; Robinson et al., 2017); however, what this theory means at scales beyond the enzyme level is still unknown. What is the relationship between single enzymatic reactions versus reactions involving a suite of enzymes (e.g., microbial respiration; Alster et al., 2018)? Can we expect changes in thermal adaptation to vary by ecosystem, microbe, or enzyme? How will production of different isoenzymes and changes in microbial community composition be reflected in the thermal adaptation of temperature sensitivity of a microbe or community? We previously hypothesized that reactions involving multiple enzymes would reflect the summation of the temperature response curves and thus have a less negative $\Delta C_P^{\ddagger}$ (Alster et al., 2018). However, we did not find that the data were entirely consistent with this hypothesis. Additionally, soils are composed of both organic and inorganic elements. These inorganic elements lack enzyme catalysts and follow Arrhenius-type kinetics for chemical reactions (e.g., sorption, desorption, or diffusion processes). Schipper et al. (2019) argue that soil processes should thus be determined by combining the MMRT and Arrhenius models based on substrate supply in the system. However, how to determine the relative contribution of each model and how to vary each of the model parameters for this mixed model require attention.

6 | CONCLUSION

We have several recommendations to facilitate testing and application of MMRT. We advise researchers to design experiments with as many independent temperatures as possible, up to 20, and across a range that is biologically relevant and includes T_{opt}. This approach is important to secure adequate data for model fitting. We also encourage researchers to collect data that can be used to fit and compare alternative models. MMRT offers universal metrics for comparing temperature sensitivity across microbes and systems. Measurements that are only analyzed using a Q_{10} temperature coefficient or the Arrhenius equation represent a missed opportunity to test MMRT. Using MMRT, we can examine more biochemically relevant parameters, which could provide insights into how enzymes and organisms adapt to temperature. Therefore, we suggest MMRT as a powerful tool for representing the biochemical mechanisms operating in soil systems. In doing so, we may improve predictions of microbial temperature responses to climate change.

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SUPPORTING INFORMATION

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