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Embracing a new paradigm for temperature sensitivity of soil microbes

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1 **Title:** Embracing a new paradigm for temperature sensitivity of soil microbes

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3

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21 **Key words:** Activation energy, Arrhenius, Macromolecular Rate Theory, Soil

22 microbes, Temperature sensitivity, Thermal adaptation,  $Q_{10}$

23

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25

26 **Abstract**

27 The temperature sensitivity of soil processes is of major interest, especially  
28 in light of climate change. Originally formulated to explain the temperature  
29 dependence of chemical reactions, the Arrhenius equation, and related  $Q_{10}$   
30 temperature coefficient, has a long history of application to soil biological  
31 processes. However, empirical data indicate that  $Q_{10}$  and Arrhenius model  
32 are often poor metrics of temperature sensitivity in soils. In this opinion  
33 piece, we aim to (1) review alternative approaches for characterizing  
34 temperature sensitivity, focusing on Macromolecular Rate Theory (MMRT),  
35 (2) provide strategies and tools for implementing a new temperature  
36 sensitivity framework, (3) develop thermal adaptation hypotheses for the  
37 MMRT framework, and (4) explore new questions and opportunities  
38 stemming from this paradigm shift. Microbial ecologists should consider  
39 developing and adopting MMRT as the basis for predicting biological rates as  
40 a function of temperature. Improved understanding of temperature  
41 sensitivity in soils is particularly pertinent as microbial response to  
42 temperature has a large impact on global climate feedbacks.

43

## 44 **1. Introduction**

45       The temperature sensitivity of soil biological processes under climate  
46 change is of major interest because of the major consequences for soil  
47 carbon dynamics (Bradford *et al.*, 2016). Although definitions vary in the  
48 literature, most simply, temperature sensitivity can be defined as the rate of  
49 change with respect to temperature (Sierra, 2012); or mathematically,  
50 temperature sensitivity is the first derivative of the temperature response.  
51 Historically, the most common metrics for measuring temperature sensitivity  
52 of soil processes have been the Arrhenius model or the  $Q_{10}$  temperature  
53 coefficient. It is important to note that  $Q_{10}$  is not the rate of change with  
54 respect to temperature, but rather the ratio between two rates. The  
55 Arrhenius model and  $Q_{10}$  temperature coefficient have been used to  
56 characterize soil temperature sensitivity since the 1920s (Singh & Gupta,  
57 1977). However, over the past several decades, a growing body of literature  
58 now clearly demonstrates that  $Q_{10}$  and Arrhenius models are ineffective and  
59 sometimes misleading models for characterizing temperature sensitivity in  
60 soils (e.g. Lloyd & Taylor, 1994; Davidson *et al.*, 2006; Hamdi *et al.*, 2013;  
61 Schipper *et al.*, 2014; Tang & Riley, 2015; Alster *et al.*, 2016a; Robinson *et*  
62 *al.*, 2017).

63       We argue that the  $Q_{10}$  temperature coefficient and the Arrhenius model  
64 are ill suited for soil biological systems. First, the Arrhenius equation was not  
65 originally intended for biological reactions, but instead to describe the  
66 thermal dependence of reaction rates in physical chemistry. Applying it in

67 biological systems neglects enzyme catalysis of the reaction by assuming  
68 that enzyme tertiary structure is not temperature sensitive (DeLong *et al.*,  
69 2017). Second, these equations assume that biological reaction rates rise  
70 monotonically with warming (i.e., only increase with increasing temperature).  
71 In reality, these rates are typically unimodal—they peak at intermediate  
72 temperatures, and decline at higher temperatures (Dell *et al.*, 2011). Third,  
73 Arrhenius and  $Q_{10}$ -modeled rates are dependent on the temperature range  
74 measured (Kirschbaum, 1995; Sierra, 2012; Schulte, 2015; Alster *et al.*,  
75 2016b; Pawar *et al.*, 2016). Therefore, the same data fit to the  $Q_{10}$   
76 temperature coefficient and the Arrhenius model can yield different  
77 parameter estimates for different temperature ranges, meaning that these  
78 model parameters can be inconsistent metrics of temperature sensitivity.  
79 Moreover, the parameters can be misleading when comparing results  
80 between studies. Even more problematic with  $Q_{10}$ , realistic values can be  
81 generated when using randomly generated data as a consequence of the  
82 mathematical formulation (Sierra, 2012). We therefore caution against the  
83 use of the  $Q_{10}$  temperature coefficient and the Arrhenius model in  
84 biogeochemical modeling.

85         Here, we advocate for broader adoption of an alternative model of  
86 temperature sensitivity for soil microbial processes. Recent studies provide  
87 feasible alternatives to the  $Q_{10}$  temperature coefficient and the Arrhenius  
88 model that provide technical advancement, empirical validation, and  
89 improved theoretical understanding of temperature sensitivity (Schipper *et*

90 *al.*, 2014; Pawar *et al.*, 2016; Dobri & Bååth, 2018). Despite these advances,  
91 of the papers published in *Global Change Biology* in 2017 and 2018  
92 regarding temperature sensitivity in soil systems, 25 out of 31 (81%) only fit  
93 data to the  $Q_{10}$  temperature coefficient or Arrhenius model. To move beyond  
94 these measures of temperature sensitivity, we provide (1) a review of  
95 alternative approaches, focusing on Macromolecular Rate Theory (MMRT), (2)  
96 strategies and tools to overcome potential barriers of transitioning to a new  
97 temperature sensitivity framework, (3) hypotheses for incorporating MMRT  
98 into thermal adaptation theory, and (4) exploration of new questions and  
99 opportunities stemming from these new approaches.

100

## 101 **2. Alternative approaches**

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

112 Several non-monotonic, unimodal equations have been proposed to  
113 describe temperature response in biological systems. Four of these have  
114 been applied to soil processes: the Johnson and Lewin model (Jing *et al.*,  
115 2014), the square root model (Ratkowsky equation) (Pietikäinen *et al.*, 2005;  
116 Rinnan *et al.*, 2009, 2011; Birgander *et al.*, 2013; van Gestel *et al.*, 2013;  
117 Taylor *et al.*, 2017; Duan *et al.*, 2018), the equilibrium model (Menichetti *et*  
118 *al.*, 2015), and macromolecular rate theory (MMRT) (Schipper *et al.*, 2014;  
119 Alster *et al.*, 2016a, 2016b; Robinson *et al.*, 2017; Taylor *et al.*, 2017; Duan  
120 *et al.*, 2018). Each has its own merits and shortcomings. However, all  
121 improve upon the  $Q_{10}$  temperature coefficient and the Arrhenius model by  
122 capturing the unimodality typical of biological enzymatic reactions. DeLong  
123 *et al.* (2017) review the assumptions of these models from a thermodynamic  
124 perspective (see Box 1 of DeLong *et al.*, 2017). There are no studies directly  
125 comparing all four approaches for soils, although Taylor *et al.* (2017)  
126 compared the square root model and MMRT for nitrification by soil bacteria  
127 and archaea. They noted that the two models did not differ in their  
128 effectiveness (Taylor *et al.*, 2017).

129 We propose the adoption of MMRT (Box 1) to represent temperature  
130 responses of soil biological systems for two reasons. First, in contrast to the  
131 square root model, MMRT is not strictly empirical but rather based on  
132 underlying thermodynamic theory. Second, in contrast to the equilibrium  
133 model, MMRT does not assume unlimited substrate supply (DeLong *et al.*,  
134 2017). Since substrates for soil enzymatic reactions are typically limiting



135 (Schimel & Weintraub, 2003), temperature models that assume substrate  
136 saturation may not be as accurate.

137

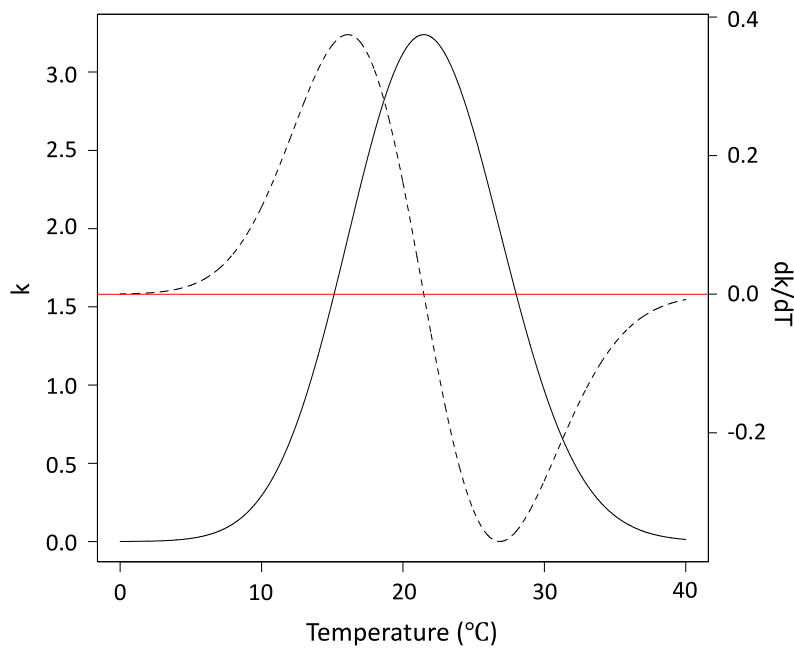
### **Box 1: Overview of Macromolecular Rate Theory**

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 is 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:

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

where  $k$  is the rate,  $T$  is 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  $\ddagger$  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 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 *et al.*, 2016a; Robinson *et al.*, 2017; Taylor *et al.*, 2017; Duan *et al.*, 2018; Liu *et al.*, 2018). Parameters from MMRT have also been used to define temperature response traits for microbes (Alster *et al.*, 2016b, 2018). These traits include  $\Delta C_p^\ddagger$ , which describes the steepness of the temperature response curve,  $T_{opt}$ , which 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):

$$T_{opt} = \frac{\Delta H_{T_0}^\ddagger - \Delta C_p^\ddagger T_0}{-\Delta C_p^\ddagger - R}, \quad (2)$$

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):

$$TS_{max} = \frac{\Delta H_{T_0}^\ddagger - \Delta C_p^\ddagger T_0}{-\Delta C_p^\ddagger \pm \sqrt{-\Delta C_p^\ddagger R}} \quad (3)$$

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.

138

### 139 **3. Potential barriers and solutions**

#### 140 **3.1 Additional model parameters**

141 One of the potential disadvantages of switching to MMRT is the  
142 additional parameters in the model. More complex models can be prone to  
143 overfitting and they require more data. The Arrhenius equation requires two  
144 fitted parameters (i.e., activation energy and the pre-exponential factor),  
145 while  $Q_{10}$  is based on a single slope parameter. More complex temperature  
146 models may involve three or four fitted parameters (DeLong *et al.*, 2017).  
147 Several studies comparing a three-parameter MMRT model and a two-  
148 parameter Arrhenius model found a superior goodness of fit for MMRT even  
149 when accounting for the additional parameter (Alster *et al.*, 2016a, 2016b;  
150 Robinson *et al.*, 2017; Liang *et al.*, 2018). Additionally, with MMRT some  
151 model parameters can be fixed using empirical information. The MMRT  
152 equation includes four parameters,  $T_0$ ,  $\Delta H_{T_0}^\ddagger$ ,  $\Delta S_{T_0}^\ddagger$ , and  $\Delta C_p^\ddagger$ . However,  $T_0$  can  
153 be set empirically to 4 to 10°C below the experimental  $T_{opt}$ , because  $T_0$  does  
154 not strongly affect overall model fit (Schipper *et al.*, 2014; Alster *et al.*,  
155 2016a). Due to the phenomena of enthalpy-entropy compensation (Sharp;

156 Chodera & Mobley, 2014),  $\Delta H_{T_0}^\ddagger$  and  $\Delta S_{T_0}^\ddagger$  are also typically interdependent  
157 (Mills & Plotkin, 2015; Alster *et al.*, 2018). Therefore, the effective number of  
158 model parameters is closer to two (Arcus *et al.*, 2016; Robinson *et al.*, 2017).

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

165

### 166 **3.2 Comparing temperature response with prior studies**

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

173

### 174 **3.3 Solutions for experimental limitations**

175 For optimal model parameterization, MMRT requires sufficient  
176 measurements across a broad temperature range. In an analysis of the  
177 sample size needed for fitting soil respiration data to the MMRT model,  
178 Robinson *et al.* (2017) demonstrate that model fits continue to improve up

179 until roughly 20 measurements at different temperatures. It is also important  
180 to capture the  $T_{opt}$  within the range of temperatures measured (Alster *et al.*,  
181 2018). These requirements (i.e., large number of independent temperature  
182 measurements and a large temperature range) may be an obstacle to fitting  
183 the MMRT model to existing empirical data or new data from small  
184 experiments. To overcome this problem in new experiments, we suggest use  
185 of multiple incubators or staggered time points to expand the range and  
186 number of temperature points. Sample sizes need not increase, though.  
187 Added temperature points across the experimental range could be offset by  
188 decreasing replicate number at each point (O'Brien *et al.*, 2009; Sefer *et al.*,  
189 2016). Total sample size could remain the same.

190 A temperature gradient block is another option. Common in  
191 microbiology experiments, temperature gradient blocks are made of  
192 aluminum set in a circulating water bath that is heated on one side and  
193 chilled on the other (detailed in Konishi *et al.*, 2006). This approach has been  
194 used to incubate soils and sediments at multiple temperatures with minimal  
195 additional effort (Fey & Conrad, 2000; Yao & Conrad, 2000; Canion *et al.*,  
196 2014; Robinson *et al.*, 2017). For experiments on soil microbial enzymes or  
197 isolates, temperature gradients in thermal cyclers—often found in microbial  
198 laboratories—are another option.

199 For field experiments, several solutions already exist to overcome  
200 these obstacles. Natural temperature and elevation gradients are commonly  
201 used for warming experiments (e.g., Bradford *et al.*, 2019; Dacal *et al.*, 2019)

202 and provide access to many temperature points. For manipulative field  
203 experiments, measurements could be collected near and far from the  
204 heating source because warming decreases with distance (Peterjohn *et al.*,  
205 1993). Field experiments also include temporal fluctuations in temperature.  
206 Such variation can be used to fit the model, as well as compare between  
207 warmed and control treatments (Carey *et al.*, 2016; Li *et al.*, 2019).  
208 Additionally, variation in environmental temperatures poses an interesting  
209 question for field studies: are organisms from more stable thermal  
210 environments more or less temperature responsive? In other words, do they  
211 display more or less negative  $\Delta C_p^\ddagger$ ? Overall, we urge scientists to consider  
212 incorporating more temperature levels into future research projects.

213         While we strongly encourage incorporation of additional temperature  
214 points, another logistical consideration is how temperature interacts with  
215 moisture and oxygen availability. High temperatures typically decrease  
216 moisture availability. The interaction of these three variables (i.e.,  
217 temperature, moisture, and oxygen availability) may confound the  
218 temperature-reaction rate relationship in soil systems (Sierra *et al.*, 2017).  
219 We therefore recommend controlling for these other variables when  
220 estimating soil temperature response, for example by adding water, to  
221 minimize misleading results.

222

### 223 **3.4 Development of thermal adaptation theory**



224 Another potential barrier in applying MMRT is a lack of conceptual  
225 theory on thermal adaptation (Allison *et al.*, 2018), or how temperature  
226 response curves adapt to changes in temperature. Particularly, how should  
227 respiration rate, or enzymatic  $V_{max}$ , the maximum reaction velocity, adapt or  
228 acclimate to temperature change? Enzyme catalyzed reactions are typically  
229 characterized through Michaelis-Menten kinetics,

230

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

231

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

244 Still, the empirical support for the Arrhenius-based theory of thermal  
245 adaptation is contradictory. Some studies find that warm-adapted enzymes

246 are more temperature sensitive (Koch *et al.*, 2007; Allison *et al.*, 2018; Tang  
247 *et al.*, 2019), while other studies find the opposite (Koch *et al.*, 2007;  
248 Wallenstein *et al.*, 2009; Brzostek & Finzi, 2012; Nottingham *et al.*, 2016;  
249 Razavi *et al.*, 2017; Tang *et al.*, 2019). This discrepancy could be due to  
250 confusion in defining the term “temperature sensitivity.” Some studies  
251 measure temperature sensitivity of  $V_{max}$ ,  $K_m$ , or both. Additionally, studies  
252 can measure *intrinsic* versus *apparent* temperature sensitivity. These details  
253 should be clearly reported.

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

265

#### 266 **4. Hypotheses for temperature sensitivity and thermal adaptation**

267         To build a conceptual theory on how  $V_{max}$  should adapt to temperature  
268 change under MMRT, we focus on two temperature response traits,  $\Delta C_p^\ddagger$  and

269  $T_{opt}$ . A more negative  $\Delta C_p^\ddagger$  corresponds to a steeper temperature response  
270 curve whereas a less negative  $\Delta C_p^\ddagger$  corresponds to a flatter curve. A steeper  
271 temperature response curve would indicate larger changes in rate with  
272 temperature compared with a flatter curve. Here, thermal adaptation refers  
273 to changes in temperature response traits (e.g.,  $\Delta C_p^\ddagger$ ,  $T_{opt}$ ) of an enzyme in  
274 response to shifts in the temperature or temperature regime experienced by  
275 an organism. Below we propose three hypotheses derived from biochemical  
276 and physiological mechanisms to describe how thermal adaptation might  
277 occur within the MMRT framework (Figure 2B, C, and D).

278         Under the *Enzyme Rigidity Hypothesis* (Figure 2B), cooling causes  $\Delta C_p^\ddagger$   
279 to become more negative (i.e., steeper curve) and  $T_{opt}$  decreases. A more  
280 negative  $\Delta C_p^\ddagger$  should be expected if cold-adapted enzymes have decreased  
281 rigidity compared with warm-adapted enzymes (Fields & Somero, 1998;  
282 Zavodszky *et al.*, 1998; Fields, 2001). With cold-adapted enzymes, increasing  
283 vibrations in the enzyme-substrate complex help compensate for declining  
284 activity at lower temperatures (Wallenstein *et al.*, 2011). A more negative  
285  $\Delta C_p^\ddagger$  (and a steeper curve) results when enzyme rigidity decreases through  
286 an increased number of enzyme-transition-state species (Arcus *et al.*, 2016).  
287 With the Enzyme Rigidity Hypothesis, the entire temperature response curve  
288 may also shift upwards with increasing temperature (Feller & Gerday, 2003;  
289 Arcus *et al.*, 2016). This directional shift and flattening of the temperature  
290 response curve are in line with empirical findings of thermophilic versus

291 psychrophilic enzymes (Struvay & Feller, 2012; Arcus *et al.*, 2016), but this  
292 upward shift is not always observed (Struvay & Feller, 2012). Additionally,  
293 several studies have found that a more negative  $\Delta C_p^\ddagger$  corresponds to a lower  
294  $T_{opt}$  (Hobbs *et al.*, 2013; Arcus *et al.*, 2016; Alster *et al.*, 2018), which is  
295 actually an expected consequence of the mathematics (Arcus *et al.*, 2016).

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

307 Lastly, the Thermal Breadth Hypothesis predicts that changes in  $\Delta C_p^\ddagger$   
308 are related to the temperature range of the environment (Figure 2D).  
309 Enzymes exposed to more temperature variation would have flatter  
310 temperature response curves (less negative  $\Delta C_p^\ddagger$ ) to maintain more constant  
311 rates across varying temperatures. We previously found that multiple  $\Delta C_p^\ddagger$

312 values are also possible with the same  $T_{opt}$  (Alster *et al.*, 2018). This result  
313 may be explained by enzymes experiencing different temperature regimes,  
314 but similar mean temperatures. Temperature regime is thought to play a role  
315 in determining temperature sensitivity, but few studies have examined this  
316 relationship (Zhu & Cheng, 2011; Bai *et al.*, 2017).

317

## 318 **5. New questions and opportunities**

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

328 Another set of key questions centers on scaling enzyme MMRT. Prior  
329 studies have already demonstrated MMRT's validity for higher level biological  
330 processes (Alster *et al.*, 2016a; Robinson *et al.*, 2017; Duan *et al.*, 2018;  
331 Liang *et al.*, 2018); however, what this theory means at scales beyond the  
332 enzyme level is still unknown. What is the relationship between single  
333 enzymatic reactions versus reactions involving a suite of enzymes (e.g.,

334 microbial respiration) (Alster *et al.*, 2018)? Can we expect changes in  
335 thermal adaptation to vary by ecosystem, microbe, or enzyme? How will  
336 production of different isoenzymes and changes in microbial community  
337 composition be reflected in the thermal adaptation of temperature sensitivity  
338 of a microbe or community? We previously hypothesized that reactions  
339 involving multiple enzymes would reflect the summation of the temperature  
340 response curves and thus have a less negative  $\Delta C_p^\ddagger$  (Alster *et al.*, 2018).  
341 However, we did not find that the data were entirely consistent with this  
342 hypothesis. Additionally, soils are composed of both organic and inorganic  
343 elements. These inorganic elements lack enzyme catalysts and follow  
344 Arrhenius-type kinetics for chemical reactions (e.g., sorption, desorption, or  
345 diffusion processes). Schipper *et al.* (2019) argue that soil processes should  
346 thus be determined by combining the MMRT and Arrhenius models based on  
347 substrate supply in the system. However, how to determine the relative  
348 contribution of each model and how to vary each of the model parameters  
349 for this mixed-model requires attention.

350

## 351 **6. Conclusion**

352 We have several recommendations to facilitate testing and application  
353 of MMRT. We advise researchers to design experiments with as many  
354 independent temperatures as possible, up to 20, and across a range that is  
355 biologically relevant and includes  $T_{opt}$ . This approach is important to secure  
356 adequate data for model fitting. We also encourage researchers to collect

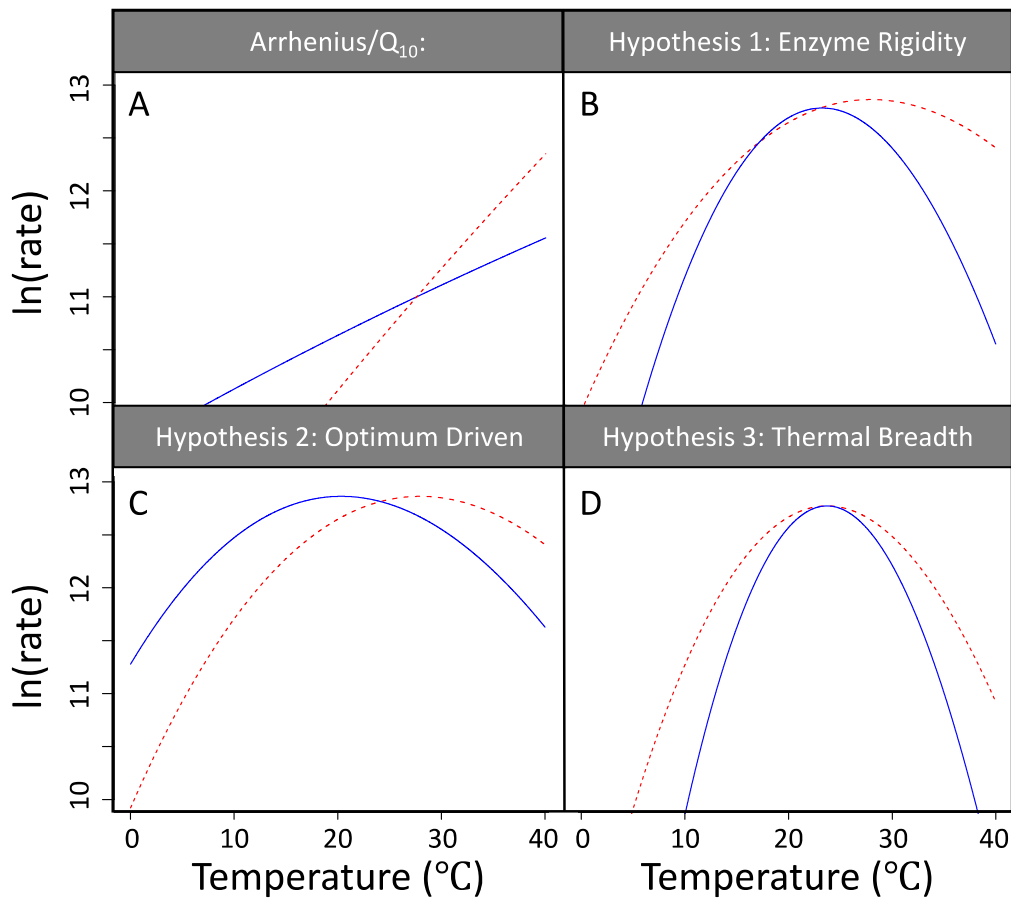
357 data that can be used to fit and compare alternative models. Macromolecular  
358 Rate Theory offers universal metrics for comparing temperature sensitivity  
359 across microbes and systems. Measurements that are only analyzed using a  
360  $Q_{10}$  temperature coefficient or the Arrhenius equation represent a missed  
361 opportunity to test MMRT. Using MMRT, we can examine more biochemically  
362 relevant parameters, which could provide insights into how enzymes and  
363 organisms adapt to temperature. Therefore, we suggest MMRT as a powerful  
364 tool for representing the biochemical mechanisms operating in soil systems.  
365 In doing so, we may improve predictions of microbial temperature responses  
366 to climate change.

367

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375



376

377 **Figure 2.** Hypotheses for thermal adaptation of temperature response  
 378 curves for cold-adapted (blue lines) and warm-adapted (red, dashed lines)  
 379 biological reactions. Panel A corresponds to the thermal adaptation  
 380 hypothesis generated from the Arrhenius equation and panels B-D  
 381 correspond to hypotheses for thermal adaptation developed for the MMRT  
 382 framework. With all hypotheses, the magnitude and direction of the change  
 383 in rate will depend on how much the temperature response curve shifts, and  
 384 at what temperature the reaction occurs. See table S1 for the example  
 385 parameter values corresponding to each plot.

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387

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