UC Irvine

UC Irvine Previously Published Works

Title

Embracing a new paradigm for temperature sensitivity of soil microbes.

Permalink

<https://escholarship.org/uc/item/1x7662p2>

Journal

Global change biology, 26(6)

ISSN

1354-1013

Authors

Alster, Charlotte J
von Fischer, Joseph C
Allison, Steven D
et al.

Publication Date

2020-06-01

DOI

10.1111/gcb.15053

Peer reviewed

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

Running title: Temperature response of soil microbes

Charlotte J. Alster¹, Joseph C. von Fischer², Steven D. Allison^{1,3}, Kathleen K. Treseder¹

¹Department of Ecology and Evolutionary Biology, University of California, Irvine, Irvine, CA, 92697, USA

²Department of Biology and Graduate Degree Program in Ecology, Colorado State University, Fort Collins, CO 80523, USA

³Department of Earth System Science, University of California, Irvine, Irvine, CA 92697, USA

Corresponding Author:

Charlotte J. Alster
Department of Ecology and Evolutionary Biology
University of California, Irvine
Irvine, CA
+0019498249423
Charlotte.Alster@uci.edu

Key words: Activation energy, Arrhenius, Macromolecular Rate Theory, Soil microbes, Temperature sensitivity, Thermal adaptation, Q_{10}

24 **Paper Type:** Opinion

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).

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) (Pietikäinen *et al.*, 2005; Rinnan *et al.*, 2009, 2011; Birgander *et al.*, 2013; van Gestel *et al.*, 2013; Taylor *et al.*, 2017; Duan *et al.*, 2018), the equilibrium model (Menichetti *et al.*, 2015), and macromolecular rate theory (MMRT) (Schipper *et al.*, 2014; Alster *et al.*, 2016a, 2016b; Robinson *et al.*, 2017; Taylor *et al.*, 2017; Duan *et al.*, 2018). 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

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.

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 *et al.*, 2016a, 2016b; Robinson *et al.*, 2017; Liang *et al.*, 2018). Additionally, with MMRT some model parameters can be fixed using empirical information. The MMRT 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 be set empirically to 4 to 10°C below the experimental T_{opt} , because T_0 does not strongly affect overall model fit (Schipper *et al.*, 2014; Alster *et al.*, 2016a). Due to the phenomena of enthalpy-entropy compensation (Sharp;

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

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

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)

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 *et al.*, 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 *et al.*, 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

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

368 **Acknowledgements**

369 We thank L.A. Schipper, V.L. Arcus, and an anonymous reviewer for their
370 helpful comments on this manuscript. We would also like to thank P.E.
371 Brewer for feedback on an earlier version on this manuscript and E.E. Alster
372 for help with the figures. This study was also funded by grants from NSF
373 (DEB 1912525) and the Department of Energy Office of Biological and
374 Environmental Research (DE-SC0016410).

375

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 MMRT 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.

387

388

389 **References**

- 390 Allison SD, Romero-Olivares AL, Lu Y, Taylor JW, Treseder KK (2018)
- 391 Temperature sensitivities of extracellular enzyme Vmax and Km across
- 392 thermal environments. *Global Change Biology*, **24**, 2884–2897.
- 393 Alster CJ, Koyama A, Johnson NG, Wallenstein MD, von Fischer JC (2016a)
- 394 Temperature sensitivity of soil microbial communities: An application of
- 395 macromolecular rate theory to microbial respiration. *Journal of*
- 396 *Geophysical Research G: Biogeosciences*, **121**, 1420–1433.
- 397 Alster CJ, Baas P, Wallenstein MD, Johnson NG, von Fischer JC (2016b)
- 398 Temperature sensitivity as a microbial trait using parameters from
- 399 macromolecular rate theory. *Frontiers in Microbiology*, **7**.
- 400 Alster CJ, Weller ZD, von Fischer JC (2018) A Meta-Analysis of Temperature
- 401 Sensitivity as a Microbial Trait. *Global change biology*.
- 402 Arcus VL, Prentice EJ, Hobbs JK et al. (2016) On the Temperature
- 403 Dependence of Enzyme-catalyzed Rates. *Biochemistry*.
- 404 Bååth E (2018) Temperature sensitivity of soil microbial activity modeled by
- 405 the square root equation as a unifying model to differentiate between
- 406 direct temperature effects and microbial community adaptation. *Global*
- 407 *Change Biology*, **24**, 2850–2861.
- 408 Bai Z, Xie H, Kao-kniffin J, Chen B, Shao P, Liang C (2017) Shifts in microbial
- 409 trophic strategy explain different temperature sensitivity of CO₂ flux
- 410 under constant and diurnally varying temperature regimes. *FEMS*
- 411 *microbiology ecology*, **93**, 1–12.

412 Birgander J, Reischke S, Jones DL, Rousk J (2013) Soil Biology & Biochemistry
 413 Temperature adaptation of bacterial growth and mineralisation in a
 414 laboratory study C-glucose. *Soil Biology and Biochemistry*, **65**, 294–303.
 415 Bradford MA, Wieder WR, Bonan GB, Fierer N, Raymond PA, Crowther TW
 416 (2016) Managing uncertainty in soil carbon feedbacks to climate change.
 417 *Nature Climate Change*, **6**, 751–758.
 418 Bradford MA, Mcculley RL, Crowther TW, Oldfield EE, Wood SA, Fierer N
 419 (2019) Cross-biome patterns in soil microbial respiration predictable from
 420 evolutionary theory on thermal adaptation. *Nature Ecology & Evolution*,
 421 **3**, 223–231 |.
 422 Brzostek ER, Finzi AC (2012) Seasonal variation in the temperature
 423 sensitivity of proteolytic enzyme activity in temperate forest soils.
 424 *Journal of Geophysical Research: Biogeosciences*, **117**.
 425 Canion A, Kostka JE, Gihring TM, Huettel M, Beusekom JEE Van, Gao H, Lavik
 426 G (2014) Temperature response of denitrification and anammox reveals
 427 the adaptation of microbial communities to in situ temperatures in
 428 permeable marine sediments that span 50 ° in latitude. *Biogeosciences*,
 429 **11**, 309–320.
 430 Carey JC, Tang J, Templer PH et al. (2016) Temperature response of soil
 431 respiration largely unaltered with experimental warming. *Proceedings of*
 432 *the National Academy of Sciences*, **113**, 13797–13802.
 433 Chodera JD, Mobley DL (2014) Entropy-enthalpy compensation: Role and
 434 ramifications in biomolecular ligand recognition and design. *Annual*

435 *Review of Biophysics*, **42**, 121–142.

436 Dacal M, Bradford MA, Plaza C, Maestre FT (2019) temperature in global
437 drylands. *Nature Ecology & Evolution*, **3**.

438 Davidson EA, Janssens IA (2006) Temperature sensitivity of soil carbon
439 decomposition and feedbacks to climate change. *Nature*, **440**, 165–173.

440 Davidson EA, Janssens IA, Luo Y (2006) On the variability of respiration in
441 terrestrial ecosystems: moving beyond Q₁₀. *Global Change Biology*, **12**,
442 154–164.

443 Dell AI, Pawar S, Savage VM (2011) Systematic variation in the temperature
444 dependence of physiological and ecological traits. *Proceedings of the*
445 *National Academy of Sciences of the United States of America*, **108**,
446 10591–10596.

447 DeLong JP, Gibert JP, Luhring TM, Bachman G, Reed B, Neyer A, Montooth KL
448 (2017) The combined effects of reactant kinetics and enzyme stability
449 explain the temperature dependence of metabolic rates. *Ecology and*
450 *Evolution*.

451 Dobri S, Bååth E (2018) Temperature affects lag period and growth of
452 bacteria in soil according to a Ratkowsky (square root) model after a
453 drying / rewetting episode. **124**, 32–37.

454 Duan P, Wu Z, Zhang Q, Fan C, Xiong Z (2018) Thermodynamic responses of
455 ammonia-oxidizing archaea and bacteria explain N₂O production from
456 greenhouse vegetable soils. *Soil Biology and Biochemistry*, **120**, 37–47.

457 Feller G, Gerday C (2003) Psychrophilic enzymes: hot topics in cold

458 adaptation. *Nature Reviews Microbiology*, **1**, 200–208.

459 Fey A, Conrad R (2000) Effect of Temperature on Carbon and Electron Flow
 460 and on the Archaeal Community in Methanogenic Rice Field Soil. *Applied*
 461 *and Environmental Microbiology*, **66**, 4790–4797.

462 Fields PA (2001) Review : Protein function at thermal extremes : balancing
 463 stability and flexibility. *Comparative Biochemistry and Physiology Part A*,
 464 **129**, 417–431.

465 Fields PA, Somero GN (1998) Hot spots in cold adaptation: Localized
 466 increases in conformational flexibility in lactate dehydrogenase A4
 467 orthologs of Antarctic notothenioid fishes. *Proceedings of the National*
 468 *Academy of Sciences*, **95**, 11476–11481.

469 van Gestel NC, Reischke S, Bååth E (2013) Soil Biology & Biochemistry
 470 Temperature sensitivity of bacterial growth in a hot desert soil with large
 471 temperature fluctuations. *Soil Biology and Biochemistry*, **65**, 180–185.

472 Hamdi S, Moyano F, Sall S, Bernoux M, Chevallier T (2013) Soil Biology &
 473 Biochemistry Synthesis analysis of the temperature sensitivity of soil
 474 respiration from laboratory studies in relation to incubation methods and
 475 soil conditions. *Soil Biology and Biochemistry*, **58**, 115–126.

476 Hobbs JK, Jiao W, Easter AD, Parker EJ, Schipper LA, Arcus VL (2013) Change
 477 in heat capacity for enzyme catalysis determines temperature
 478 dependence of enzyme catalyzed rates. *ACS chemical biology*, **8**, 2388–
 479 2393.

480 Jing X, Wang Y, Chung H (2014) No temperature acclimation of soil

481 extracellular enzymes to experimental warming in an alpine grassland
 482 ecosystem on the Tibetan Plateau. 39-54.
 483 Kirschbaum MUF (1995) The temperature dependence of soil organic matter
 484 decomposition, and the effect of global warming on soil organic C
 485 storage. *Soil Biology & Biochemistry*, **27**, 753-760.
 486 Koch O, Tscherko D, Kandeler E (2007) Temperature sensitivity of microbial
 487 respiration, nitrogen mineralization, and potential soil enzyme activities
 488 in organic alpine soils. *Global Biogeochemical Cycles*, **21**.
 489 Konishi T, Yamashiro T, Koide M, Nishizono A (2006) Influence of
 490 Temperature on Growth of *Legionella pneumophila* Biofilm Determined
 491 by Precise Temperature Gradient Incubator. *Journal of Bioscience and*
 492 *Bioengineering*, **101**, 478-484.
 493 Li J, Frey SD, Wang G, Mayes MA, Allison SD, Melillo JM (2019) Reduced
 494 carbon use efficiency and increased microbial turnover with soil
 495 warming. 900-910.
 496 Liang LL, Arcus VL, Heskell MA et al. (2018) Macromolecular rate theory
 497 (MMRT) provides a thermodynamics rationale to underpin the convergent
 498 temperature response in plant leaf respiration. *Global Change Biology*,
 499 **24**, 1538-1547.
 500 Liu Y, He N, Wen X et al. (2018) The optimum temperature of soil microbial
 501 respiration : Patterns and controls. **121**, 35-42.
 502 Lloyd J, Taylor JA (1994) On the temperature dependence of soil respiration.
 503 *Functional Ecology*, 315-323.

504 Menichetti L, Ortigoza ALR, García N (2015) Thermal sensitivity of enzyme
505 activity in tropical soils assessed by the Q 10 and equilibrium model.
506 299–310.

507 Mills EA, Plotkin SS (2015) Protein Transfer Free Energy Obeys Entropy-
508 Enthalpy Compensation.

509 Nottingham AT, Turner BL, Whitaker J, Ostle N, Bardgett RD, Mcnamara NP,
510 Salinas N (2016) Temperature sensitivity of soil enzymes along an
511 elevation gradient in the Peruvian Andes. *Biogeochemistry*, **127**, 217–
512 230.

513 O’Brien TE, Chooprateep S, Funk GM (2009) Encouraging Students to Think
514 Critically: Regression Modelling and Goodness-of-Fit. *Journal of Data*
515 *Science*, **7**, 235–253.

516 Pawar S, Dell AI, Savage VM, Knies JL, Kearney M, Kalisz S (2016) Real versus
517 artificial variation in the thermal sensitivity of biological traits. *The*
518 *American Naturalist*, **187**, E41–E52.

519 Peterjohn WT, Melillo JM, Bowles FP, Steudler PA (1993) Oecologia and
520 preliminary flux results. *Oecologia*, **93**, 18–24.

521 Pietikäinen J, Pettersson M, Bååth E (2005) Comparison of temperature
522 effects on soil respiration and bacterial and fungal growth rates. *FEMS*
523 *Microbiology Ecology*, **52**, 49–58.

524 Qi Y, Xu M, Wu J (2002) Temperature sensitivity of soil respiration and its
525 effects on ecosystem carbon budget : nonlinearity begets surprises. **153**,
526 131–142.

527 Ratkowsky DA, Olley J, McMeekin TA, Ball A (1982) Relationship between
 528 temperature and growth rate of bacterial cultures. *Journal of*
 529 *bacteriology*, **149**, 1-5.

530 Ratkowsky DA, Lowry RK, McMeekin TA, Stokes AN, Chandler RE (1983)
 531 Model for bacterial culture growth rate throughout the entire biokinetic
 532 temperature Model for Bacterial Culture Growth Rate Throughout the
 533 Entire Biokinetic Temperature Range. *Journal of Bacteriology*, **154**, 1222-
 534 1226.

535 Razavi BS, Liu S, Kuzyakov Y (2017) Soil Biology & Biochemistry Hot
 536 experience for cold-adapted microorganisms: Temperature sensitivity of
 537 soil enzymes. *Soil Biology and Biochemistry*, **105**, 236-243.

538 Rinnan R, Rousk J, Yergeau E, Kowalchuk GA, Baath E (2009) Temperature
 539 adaptation of soil bacterial communities along an Antarctic climate
 540 gradient: predicting responses to climate warming. *Global Change*
 541 *Biology*, **15**, 2615-2625.

542 Rinnan R, Michelsen A, Bååth E (2011) Long-term warming of a subarctic
 543 heath decreases soil bacterial community growth but has no effects on
 544 its temperature adaptation. *Applied Soil Ecology*, **47**, 217-220.

545 Robinson JM, O'Neill TA, Ryburn J, Liang LL, Arcus VL, Schipper LA (2017)
 546 Rapid laboratory measurement of the temperature dependence of soil
 547 respiration and application to changes in three diverse soils through the
 548 year. *Biogeochemistry*, **133**, 101-112.

549 Schimel JP, Weintraub MN (2003) The implications of exoenzyme activity on

550 microbial carbon and nitrogen limitation in soil : a theoretical model. **35**,
551 549-563.

552 Schipper LA, Hobbs JK, Rutledge S, Arcus VL (2014) Thermodynamic theory
553 explains the temperature optima of soil microbial processes and high
554 Q10 values at low temperatures. *Global Change Biology*, **20**, 3578-3586.

555 Schipper LA, Petrie OJ, Neill TAO, Mudge PL, Liáng LL, Robinson JM, Arcus VL
556 (2019) Shifts in temperature response of soil respiration between
557 adjacent irrigated and non-irrigated grazed pastures. *Agriculture*,
558 *Ecosystems and Environment*, **285**, 106620.

559 Schulte PM (2015) The effects of temperature on aerobic metabolism:
560 towards a mechanistic understanding of the responses of ectotherms to
561 a changing environment. *The Journal of experimental biology*, **218**,
562 1856-1866.

563 Sefer E, Kleyman M, Bar-joseph Z (2016) Tradeoffs between Dense and
564 Replicate Sampling Strategies for High-Throughput Time Series Report
565 Tradeoffs between Dense and Replicate Sampling Strategies for High-
566 Throughput Time Series Experiments. *Cell Systems*, **3**, 35-42.

567 Sharp KIM Entropy - enthalpy compensation : Fact or artifact ?

568 Sierra CA (2012) Temperature sensitivity of organic matter decomposition in
569 the Arrhenius equation: some theoretical considerations.
570 *Biogeochemistry*, **108**, 1-15.

571 Sierra CA, Malghani S, Loescher HW (2017) Interactions among temperature,
572 moisture, and oxygen concentrations in controlling decomposition rates

573 in a boreal forest soil. *Biogeosciences*, **14**, 703–710.

574 Singh JS, Gupta SR (1977) Plant decomposition and soil respiration in
575 terrestrial ecosystems. *The botanical review*, **43**, 449–528.

576 Struvay C, Feller G (2012) Optimization to low temperature activity in
577 psychrophilic enzymes. *International journal of molecular sciences*, **13**,
578 11643–11665.

579 Tang JY, Riley WJ (2015) Weaker soil carbon-climate feedbacks resulting from
580 microbial and abiotic interactions. *Nature Climate Change*, **5**, 56–60.

581 Tang J, Bradford MA, Carey J, Crowther TW, Machmuller MB, Mohan JE (2019)
582 *Temperature sensitivity of soil carbon*. Elsevier Inc., 175–208 pp.

583 Taylor AE, Giguere AT, Zoebelin CM, Myrold DD, Bottomley PJ (2017)
584 Modeling of soil nitrification responses to temperature reveals
585 thermodynamic differences between ammonia-oxidizing activity of
586 archaea and bacteria. *ISME J*, **11**, 896–908.

587 Wallenstein MD, McMahon SK, Schimel JP (2009) Seasonal variation in
588 enzyme activities and temperature sensitivities in Arctic tundra soils.
589 *Global Change Biology*, **15**, 1631–1639.

590 Wallenstein M, Allison SD, Ernakovich J, Steinweg JM, Sinsabaugh R (2011)
591 Controls on the temperature sensitivity of soil enzymes: a key driver of in
592 situ enzyme activity rates. In: *Soil Enzymology*, pp. 245–258. Springer
593 Berlin / Heidelberg.

594 Wolfenden R, Snider MJ (2001) The depth of chemical time and the power of
595 enzymes as catalysts. *Accounts of Chemical Research*, **34**, 938–945.

596 Yao H, Conrad R (2000) Effect of temperature on reduction of iron and
597 production of carbon dioxide and methane in anoxic wetland rice soils.
598 *Biology and fertility of soils*, **32**, 135–141.

599 Zavodszky Pe, Kardos J, Svingor Á, Petsko GA (1998) Adjustment of
600 conformational flexibility is a key event in the thermal adaptation of
601 proteins. *Proceedings of the National Academy of Sciences*, **95**, 7406–
602 7411.

603 Zhu B, Cheng W (2011) Soil Biology & Biochemistry Constant and diurnally-
604 varying temperature regimes lead to different temperature sensitivities
605 of soil organic carbon decomposition. *Soil Biology and Biochemistry*, **43**,
606 866–869.

607