Response to Reviewer 2

We appreciate the reviewer's careful assessment of our manuscript. We respond to the comments point by point below. It is noted that while addressing his comments, we also had taken the other review's suggestions into account, so that a balanced decision is made on the requested changes to the manuscript. We highlight the corresponding changes in the manuscript by quoting them in italic font.

Comment 1: The paper provides a critique of the MMRT model, concentrating on its underlying assumptions as stated by the authors. This makes for a useful contribution since MMRT has proved popular in several fields, such as soil science. Should the model be flawed or incomplete then these applications may have reduced value, particularly in the journal's subject area. The paper does not make a direct comparison with alternative models, though it refers to a number of them, collectively termed `chemical kinetics theory'. Certainly, empirical models would not be of interest here, but in any case, I do not consider such comparisons are needed since they can needlessly burden a paper with material that the interested reader can seek out.

Response: We appreciate the reviewer's sentiment on our paper, which fully captured our intent for the design and content of this manuscript. We followed the reviewer's suggestions as much as we can, and believe that the revised manuscript is now much more helpful to readers of biogeosciences.

Comment 2: In the introduction there is a brief coverage of publications that refer to MMRT. I found this could have been more comprehensive. It could have dealt with a number of alternative models and, as noted earlier, that would burden the paper.

Response: We now expand the introduction of MMRT, and also the alternative mechanistic models. We now included the comprehensive review by (Grimaud et al., 2017) and (Noll et al., 2020), and added the following paragraph

"Besides MMRT, a few other models with mechanistically interpretable parameters are also capable of equally well interpreting the non-monotonic temperature dependence of enzyme modulated reactions, including growth rates. Notably, Sharpe and Demichele (1977) proposed a model that incorporates the empirical observation of thermally reversible enzyme denaturation and the transition state theory (Eyring, 1935). Specifically, they considered that enzymes are in reversible transition between three states, one cold-induced inactive state, one heat-induced inactive state, and one active state which is able to carry out the catalysis. By assuming reactions to be substrate unlimited, they obtained a model with five thermodynamic parameters that is able to almost perfectly fit published temperature dependent growth rates of eight poikilothermic organisms (see their Figures 5 and 6). (The applicability of the Sharpe-Demichele model to growth rates of an organism is based on the assumed existence of control or master enzymes (Johnson and Lewin, 1946).) Motivated by the success of Sharpe and Demichele (1977) and the work on thermally reversible protein denaturation by Murphy et al. (1990), Ratkowsky et al. (2005) grouped the two inactive states into one, and, again assuming no-

substrate limitation, derived a model with two thermodynamic parameters and two enzyme informatic parameters, which was able to very accurately fit 35 sets of observed temperature dependent bacterial growth rates. The model by Ratkowsky et al. (2005) was later used by (Corkrey et al., 2012) and (Corkrey et al., 2014) to successfully interpret the temperature dependent growth rates of many more poikilothermic organisms. Ghosh et al. (2016) extended the model by Ratkowsky et al. (2005) to include the thermally reversible denaturation of many enzymes and proteins informed by proteomics, and were able to satisfactorily interpret the measured temperature-dependent growth rates of mesophiles and thermophiles. "

Comment 3: L20-25. The authors note that Hobbs et al. (2013), Schipper et al. (2014) claim MMRT is able to better than the Arrhenius-like functions for various ecological properties. I suggest that it may be worth adding here that while Schipper et al. (2014) compares MMRT to an Arrhenius model it does not do any more than this, in that it does not, for example, choose to compare to the Ratkowksy 2005, Corkrey 2012 models.

Response: We now acknowledge that models, e.g. by Sharpe and DeMichele (1977), Ratkowsky et al. (2005), Corkrey et al. (2012) can be equally well in terms fitting the pattern. We also acknowledged how the MMRT papers mis-criticized the Ratkowsky model. In particular, the important study by Oliveberg et al. (1995) who reported negative heat capacity of protein refolding was actually supporting the Ratkowsky model and the chemical kinetics theory here.

Comment 4: L25-30. Similarly, I would add that Alster et al (1016) does the same thing, but in addition, I would add that those authors unnecessarily dismiss other models as 'empirical' when, on examination, they do not appear be (e.g. Corkrey et al (2012), Peterson et al (2004), Daniel & Danson (2013)). This is a point of irritation since such claims are too easily repeated.

Response: We added this more balanced view throughout the manuscript. Also see response to comment 2.

Comment 5: L30-35. Examples (e.g., Ratkowsky et al (1983)) of papers as having parameters that are not biologically interpretable. This seems a little unfair since that particular model is explicitly empirical. I suggest adding in references to other models that do have interpretable parameters and/or are based on thermodynamic principles such as those listed above.

Response: We added references of other models, and annotated them fairly.

Comment 6: Section 2.3. I found this derivation somewhat hard to follow (though interesting). Perhaps a little more could be added to assist non-specialists?

Response: We added more verbal descriptions to mathematical derivations, so that they are easier to follow.

Comment 7: L130-135. I stumbled over the wording 'The motivating assumption ... many observations'. Perhaps tweak this to make it clearer.

Response: We rewrote this sentence:

" *Equation (16) or (17) can be used to analyze the motivating assumption and the two basic experimental assumptions that underlie MMRT. First, Hobbs et al. (2013) suggested that MMRT was motivated by noting that enzyme denaturation cannot satisfactorily explain the temperature dependence of catalysis rates. They then assumed that all enzymes are effectively in their active state to do catalysis, and attributed the decline in enzyme catalysis rate above an optimum temperature to the change of heat capacity associated with the enzyme catalysis. However, thermally reversible enzyme denaturation, as one type of enzyme denaturation, has been observed by many studies (Sizer, 1943;Alexandrov, 1964;Huang and Cabib, 1973;Maier et al., 1955;Weis, 1981), as well as by molecular dynamics simulations (McCully et al., 2008), and is ensured to occur by the thermal motion of molecules and ions in the enzyme solution.*"

Comment 8: Figure 2. Given the scale used it is difficult to closely examine the fits. But the majority of the fits appear excellent (except Barnase?). I was expecting comparisons to MMRT fitted lines since that is the point of the paper. For that matter, I speculate that the Ratkowsky 2005, Corkrey et al (2012) models and others would do as well and could be fitted. The fits are summarized as r2 values. The authors comment (L155-160) that uncertainties were not available, and that they were hindered by an 'ill-conditioned Hessian matrix', but I don't see why resampling could not be used to obtain 99% CIs bands (although they would be very narrow).

Response: We note that there were not many data points to extract from the original papers. Particularly, most of the data in those papers were plotted in log space, so we were not able to extract data uncertainty meaningfully. We thought about resampling, but with the few data points, it is problematic. Nonetheless, based on the excellent fitting we obtained, and the fact that Ratkowsky et al (2005) model is a special case of the chemical kinetics theory here, we think not presenting the uncertainty should not affect the conclusion of our analysis. In the past, we have also used the Ratkowsky model to fit similar data, and it worked well just as the reviewer pointed out.

Comment 9: L210-220. I found this enlightening. Please elaborate on what was flawed in La Mer (1933).

Response: We explain this below. Specifically, in that paper, La Mer suggested that the rate constant should be $k_f = Z_f \cdot exp(-\Delta G_f/RT)$ for forward reaction, and $k_b = Z_b \cdot exp(-\Delta G_b/RT)$ for backward reaction, so that the equilibrium constant is $K_e = k_f/k_b =$

$Z_f/Z_b \cdot exp(-\Delta G^o/RT)$, with $\Delta G^o = \Delta G_f - \Delta G_b$ (being a linear function of temperature). Because (La Mer, 1933) assumed a non-zero heat capacity for the free energy of activation, one will find $Z_f \neq Z_b$, whereas $Z_f = Z_b$ is needed to make $K_e = exp(-\Delta G^o/RT)$, so that it is consistent with the transition state theory and thermodynamics. However, per suggestion from the other reviewer, we removed this paragraph from the revised text.

Comment 10: L230-235. The point made about Ohm's law is well made. This an important point since several of the references made (e.g. Corkrey et al 2012) refer to organismal growth rates and not enzymic data. While it might be argued that the complexity of processes involved at the organism precludes such models being successful, this has been found to be incorrect, such in the above reference. Note that Corkrey et al (2014; 10.1371/journal.pone.0096100) may be relevant here since it also refers to multicellular strains. It is such extensions from the enzymic to large scale processes that makes the paper relevant to the journal.

Response: We are glad this point resonated with the reviewer's thoughts. We now related to more references, including Corkrey et al. (2012, 2014). We also highlighted that the success of Ghosh et al. (2016) using proteomic data to explain the temperature dependent growth rate of mesophilic and thermophilic bacteria suggests that the chemical kinetics theory is scalable.