We thank the reviewer for providing us with helpful comments, which are addressed below (in blue font). Line numbers refer to the revised manuscript, in which our changes are in Track Changes.

Response to the reviewer

Comment: This work extends the theory published in 2005 by Rathowsky et al to take into account the effect of substrate availability on the temperature response of enzyme activities. The theory proposed here, called the chemical kinetics theory, combines the law of mass action, von Smoluchowski's diffusion limited reaction and Eyring transition state theory. The proposed theory is confronted against data from 12 series of enzymatic assays extracted from the literature. The main result is to show that the temperature-dependent affinity parameter of enzymes to their substrate controls the response of enzymatic reaction rates to temperature. In particular, reaction temperature optimums shift towards higher temperatures as substrate availability increases. This significant scientific advance deserves to be published in Biogeosciences.

However, this work has important limitations that would require modifications to the article before publication.

Response: We appreciate your positive appraisal of our study. When revising the manuscript, we have carefully followed your suggestions, and believe that they have helped improve the manuscript significantly.

Comment: First of all, the article risks missing its objective if it doesn't make a greater effort of pedagogy. You are addressing a fairly generalist readership interested in many processes from different disciplines (biogeochemistry, ecology, agronomy, soil science...), not just chemists specializing in chemical kinetics. You therefore need to make a greater effort to introduce the various concepts and equations that could perhaps be basic for chemists. For example, the differences between Gibbs energies, enthalpies and the link with the heat capacity of protein unfolding/refolding, and the linear and non-linear responses that follow. The introduction of several equations is done simply by quoting another study without any real explanation. This makes it very difficult to follow the paper without reading twenty or so articles in parallel.

Response: We now write out and explain the equations of those basic relationships explicitly to improve the readability of our revised manuscript. For example, (1) we revised the caption of Figure 1 to reflect the relationship between Gibbs free energy, enthalpy and entropy involved in the transition state theory applied to the forward conversion of enzyme-substrate complex into products. The same information is also highlighted at Lines 130-131. (2) We revised equation (10) to show the relationship between the heat capacity of enzyme unfolding with the enthalpy and entropy involved in enzyme unfolding, and explained in Line 142 that the heat capacity of enzyme unfolding is computed as the partial derive of enthalpy with respect to temperature.

Comment: Furthermore, in my opinion, it is necessary to create a table summarizing all the variables and parameters, including definitions and units. The Ent variable is introduced in the equation, but this variable, which seems important, no longer appears in subsequent equations. I was wondering whether the presentation of gross equations before their versions with a standard (reference) temperature would be necessary to better understand the demonstration.

Response: We have created a nomenclature table in the revised appendix. We also carefully checked the presentation of equations, and made updates that help improve clarity and readability.

Comment: It would also be very useful to construct a table summarizing the parameters that have been adjusted on the basis of experimental data, and their values.
Response: We have reported the inferred parameters (T_H, T_S , ΔH_V, and ΔC_p) for each dataset in Figure 2.

Comment: In the end, how many parameters are needed to model these results? I'm amazed at the impossibility of obtaining parameter uncertainties, despite what appears to be a substantial data set.
Response: The model requires inferring four parameters for each enzyme essay data (as shown in each panel of Figure 2) and this information is noted at Line 150. As we discuss in section 2.3 (Lines 159-181), without uncertainty information from the original data, and due to the ill-condition of the Hessian matrix of the inference problem, we are not able to make a meaningful uncertainty estimation.

Comment: The concept of quasi-steady-state-approximation for the equation is unclear. What have you done mathematically? What are the "biological" assumptions behind this choice?
Response: We added an explanation to the revised manuscript (Line 95) along with an equation that illustrates the concept: i.e., $k_1^+ E_n S = (v_{max} + k_1^-) C$, with C being the concentration of enzyme-substrate complex $E_n S$. Basically, this assumption means the concentration of enzyme-substrate complex is in rapid equilibrium during its formation and destruction. This assumption places some constraint on the kinetic parameters. Quasi-steady-state-approximation (QSSA) is the standard assumption in deriving Michaelis-Menten kinetics. QSSA was used by (Michaelis and Menten, 1913), and its rich content was discussed thoroughly in (Borghans et al., 1996).

Comment: I think a limitation of this work is to consider only temporary and reversible inactive forms of enzymes. However, the incessant movement of molecules inexorably leads to the definitive denaturation of enzymes. This denaturation is very often rapid (within a few hours) with important consequences for the temperate effect on enzymatic activities and living organisms (see Alvarez et al. 2018). This limit should be discussed.
Response: We now highlight the importance of irreversible enzyme denaturation by citing (Alvarez et al., 2018) in the introduction (Line 77) and discussion sections (Lines 226-227). We also note that (1) a dynamic model should consider both production and destruction of enzymes, and (2) the ReSOM model (Tang and Riley, 2015) applies the chemical kinetics theory, and considers irreversible enzyme denaturation.

Reference

Alvarez, G., Shahzad, T., Andanson, L., Bahn, M., Wallenstein, M. D., and Fontaine, S.: Catalytic power of enzymes decreases with temperature: New insights for understanding soil C cycling and microbial ecology under warming, Global Change Biol, 24, 4238-4250, 10.1111/gcb.14281, 2018.

Borghans, J. A. M., DeBoer, R. J., and Segel, L. A.: Extending the quasi-steady state approximation by changing variables, B Math Biol, 58, 43-63, Doi 10.1016/0092-8240(95)00306-1, 1996.
Michaelis, L., and Menten, M. L.: The kinetics of the inversion effect, Biochem. Z., 49, 333-369, 1913.
Tang, J. Y., and Riley, W. J.: Weaker soil carbon-climate feedbacks resulting from microbial and abiotic interactions, Nat Clim Change, 5, 56-60, 10.1038/Nclimate2438, 2015.