

## ***Interactive comment on “Interactions among temperature, moisture, and oxygen concentrations in controlling decomposition rates” by Carlos A. Sierra et al.***

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Comment on reviewer 4

As the author/co-author of the original papers on MMRT I would like to address a comment made by reviewer 4 on what MMRT is representing.

The reviewer makes the comment: “As a matter of fact, the MMRT theory is largely based on reversible enzyme denature (though its authors did not say so), which was known as early as in the 1980s (Murphy et al., 1990: C1 BGD Interactive comment Printer-friendly version Discussion paper Common features of protein unfolding and dissolution of hydrophobic compounds, Sciences).”

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This is not correct. We are aware of earlier work on reversible denaturation. In our early papers on MMRT, we concurrently measured unfolding rates (denaturation) and reaction kinetics and clearly show that unfolding rates (whether reversible or not) were very minor contributors to the initial reductions in enzyme rate as temperature increased (Hobbs et al. 2013). In many cases, enzyme rates decline at temperatures well below the temperature for unfolding and MMRT accounts for this in the absence of unfolding (reversible or irreversible). Denaturation, of course, does occur at higher temperature but is not responsible for the initial decline in temperature sensitivity. We have a specific section in one of our more recent papers that addresses denaturation and we make that point that MMRT is independent of denaturation/unfolding (Arcus et al. 2016). MMRT actually describes how the activation energy changes with temperature, driven by the change in heat capacity between the enzyme-substrate complex and the enzyme-transition state complex. MMRT is simply a theoretical extension of transition state theory (Eyring and Polanyi) as it is applied to enzyme kinetics (M. Garcia-Viloca, J. Gao, M. Karplus, D. G. Truhlar, How enzymes work: analysis by modern rate theory and computer simulations. *Science*. 303, 186–195 (2004)).

We also note that if denaturation was responsible for defining the temperature optimum then for many soil biological processes (e.g., methanogenesis, methane oxidation, soil enzymes) would have very high denaturation rates at temperatures less than 30–40 degrees (Schipper et al. 2014). In our recent paper describing MMRT, we make evolutionary arguments for low values of  $T_{opt}$  without invoking low denaturation temperatures for individual enzymes.

Other curved functions have certainly been developed but these curves are empirical equations and generally considers denaturation (reversible or not) as the mechanism, while MMRT is theoretically derived, is independent of denaturation and has recently been validated by direct measurements of enzyme heat capacity changes on the reaction coordinate (Firestone et al 2016).

References Arcus VL, Prentice E, Hobbs JK, Mulholland AJ, Vander Kamp MW, Pud-

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Sincerely Louis Schipper and Vickery Arcus

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